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LESSON–1 : NORMAL FLORA OF HUMAN BODY

OBJECTIVE: To study the normal flora of human body and its significance

CONTENTS

1.1. Introduction

The environment in which man lives, food he takes, water he drinks and air he breathes is teeming with innumerable number of invisible microorganisms. The microorganisms which are in close contact with humans establish on and in the human body. The human body with its constant source of nourishment and moisture, relatively stable pH and temperature, and extensive surfaces for colonization, provides a favourable habitat for an abundance of microorganisms. In fact, it is so favourable that cell for cell, microbes outnumber human nucleated cells ten to one. It is estimated that an average human body is composed of 10^{13} nucleated cells while the magnitude of microflora it harbours is in the order of 10^{14} i.e. ten times more over the number of nucleated cells of the body.

The large and mixed collection of microorganisms that establish more or less permanent residence (=colonize) in the human body but do not produce disease under normal conditions is known as normal flora. It is variously described as normal resident flora, indigenous flora or normal microflora. Some microbiologists prefer to use the term "normal biota" instead of normal flora. The term normal flora implies that these microbes are harmless and for the most part they do not cause any disease and may even be beneficial. However, some microorganisms of normal flora may become pathogenic when the equilibrium is

disturbed or host defences are weakened. Such organisms are termed opportunistic pathogens.

The normal flora merits thorough investigation both as a natural phenomenon and also for a proper understanding of its role, beneficial or otherwise, on general health. Further, knowledge of normal flora of different organ systems is essential in diagnostic cultural studies to distinguish between the normal inhabitants and the probable pathogenic species.

1.2. Acquiring of normal flora: A healthy human foetus has no resident microbial population up to the time of its birth. It begins to acquire microbial populations from mother's birth canal. Just before a woman gives birth to a baby, lactobacilli in her vagina multiply rapidly. The newborn's first contact with microorganisms is usually with these lactobacilli and they become predominant in the newborn's intestine. More microorganisms are introduced to the newborn's body from environment with breathing, feeding and contact with relatives and so on. The bottle fed infants tend to acquire a mixed population of coliforms, lactobacilli, enteric streptococci and staphylococci, while the intestinal flora of breastfed infants is very simple consisting primarily of *Bifidobacterium* species. With age the normal flora of an individual gets established. Though basic flora persists, it is subject to constant change.

1.3. Composition of the normal flora: Bacteria makeup most of the normal flora of the human body. Fungi (mainly yeasts) and protozoa may also inhabit the body but their numbers are very low compared to the bacterial flora.

Some viruses like Orphan viruses [Enteric Cytopathic Human Orphan (ECHO) viruses; Respiratory Enteric Orphan (REO) viruses], coxsackie viruses and some adenoviruses are known to occur in human body without causing any apparent disease. Since viruses are obligate, intracellular pathogens, their occurrence is considered as cases of symptomless infections and they are not usually considered as part of normal flora.

Among the bacterial populations of various organs, some are considered as part of basic flora because of their universal occurrence. The components of basic flora are

Escherichia coli	---	Intestine
Streptococci	---	Nasopharyngeal region

Staphylococcus epidermidis	---	Skin
Lactobacilli (Doderlien bacilli)	---	Vagina

1.4. Characters of normal flora:

1.4.1. Stability: Of the seemingly limitless influx of microbes only some are capable of persisting on or in the human body and vast majority are removed or destroyed by host defenses. As such, the composition of normal flora is relatively stable, but fluctuations to a limited extent occur with seasons, age, variation in diet, hygiene, drug therapy, general health etc. The stability of normal flora is evident from the occurrence of a basic flora that occurs irrespective of variations in age, place, race, sex etc.

1.4.2. Attachment: Many species of normal flora organisms have the ability to adhere to the surface of host epithelial cells. Thus, they have a selective advantage over other microorganisms in colonizing the host. Some microorganisms may often adhere specifically to one body site. For example, *Streptococcus salivarius* adheres mainly to the surface of the tongue where as *S. mutans* selectively binds to the smooth surface of the teeth. Such selective adherence gives competitive edge over other microbes in particular body sites.

Detachment or shedding of host epithelial cells and their replacement by new cells is called desquamation. One result of desquamation is elimination of microorganisms that are attached to the epithelial cells. Normal flora members have advantage over others because they can selectively and specifically attach to new epithelial cells.

1.4.3. Competition for food and space: Microorganisms that comprise normal flora compete effectively for limited space and nutrients available in their environment, thus rendering it difficult for new microorganisms to colonize the host.

1.4.4. Antagonism: Microorganisms constituting normal flora often produce metabolic products that can inhibit other microbes. For example, in large intestine certain anaerobic bacteria produce various organic acids like acetic acid, butyric acid etc. as metabolic waste products which can inhibit the growth of other bacteria. Some strains of skin staphylococci have been shown to produce antibiotics that inhibit a wide variety of other bacteria. Many strains of *E. coli* produce toxic metabolites called colicins which may help to protect the intestinal tract from closely related pathogenic bacteria.

1.4.5. Variations in the composition: Normal flora, though often described as a single unit, is a complex mixture of hundreds of species differing qualitatively and quantitatively from one individual to another, and from one region of the body to another. The surfaces directly exposed to the environment usually harbour microbes extensively. Such surfaces include skin and mucous membranes, parts of the inner surface of alimentary tract, respiratory and urogenital tracts. Moist areas through which food passes (alimentary tract) tend to have the largest and most diverse populations. By contrast, organs and fluids inside the body cavity and central nervous system remain free of normal flora. Although microorganisms may transiently enter these sites, they do not normally become established there.

1.5. NORMAL FLORA OF SKIN:

Skin is the largest and most accessible of all organs. An average human adult has about 1.75 - 2.0 sq.m. area of skin surface (weight about 5 kgs). It is composed of an outer layer of epithelial cells (epidermis) and a thicker inner layer of connective tissue (dermis). The roots of hairs, sweat and sebaceous glands are embedded in the dermis. Fat deposition occurs below the dermis, and the amount of fat deposits varies with age, sex body type and other factors. The outer most surface layer is covered with a protective waxy cuticle that may help microbes to adhere.

The skin has two contrasting environments for microbial colonization (1) larger and more accessible but less hospitable surface and (2) smaller and less accessible but more hospitable skin glands (Fig. 1.1).

Figure 1.1. Anatomy of the human skin

1.5.1. Skin surface: The surface of the skin is hostile because it is relatively dry and salty. But even here certain components of normal flora, some gram positive cocci including *Staphylococcus epidermidis* and *Micrococcus* species proliferate. An important pathogen *S. aureus* is commonly found on the skin of healthy individuals. Other organisms commonly isolated from human skin are *Acinetobacter calcoaceticus* and enteric bacteria including *E. coli*. Personal hygiene greatly influences the surface flora.

1.5.2. Skin glands: Skin glands provide attractive site for microbial growth because the secretions of the skin glands are utilized by microbes as nutrients. The secretions of skin glands are rich in microbial nutrients. Urea, aminoacids, salts, lactic acid and lipids are present in considerable amounts. The pH of human secretions is almost always acidic, usual range being between pH 4 and 6. The flora associated with skin glands is regular and relatively stable and much less influenced by personal hygiene.

There are three types of skin glands viz. eccrine sweat glands, apocrine sweat glands and sebaceous sweat glands.

1.5.2.1. Eccrine sweat glands: These are not associated with hair follicles and unevenly distributed over the body with denser concentrations on palms, finger pads and soles of feet. They are responsible for perspiration associated with body cooling. These glands are relatively devoid of microorganisms, perhaps, because of the extensive flow of the fluid (sweat). When flow of an eccrine gland is blocked, bacterial invasion and multiplication occur rapidly.

1.5.2.2. Apocrine sweat glands: These are restricted in their distribution, being confined mainly to the under arm and genital regions. They are inactive in childhood and become fully functional only at puberty. Bacterial populations are relatively high in these areas. Underarm odour develops as a result of bacterial activity on the secretions of apocrines.

1.5.2.3. Sebaceous sweat glands: These are associated with each hair follicle and pour their secretions into the follicle. Hence, hair follicles provide an attractive habitat for microorganisms. The secretions of these glands, called sebum, is a lipid substance and lipophilic microorganisms such as *Propionibacterium acnes*, and yeasts like *Pityrosporum ovalis* are predominant in these glands, apart from streptococci, staphylococci and diphtheroids.

1.5.3. Microbial populations of skin:

The microorganisms of skin flora can be characterized into two types of populations 1. transients, and 2. residents.

1.5.3.1. Transient flora of the skin: The transient flora occurs mainly on skin surface because it is continuously bombarded by microbes from various sources. Most of the microbes that are deposited on the skin does not ordinarily grow there but cling to the skin surface. It varies markedly from person to person, from site to site and even moment to moment. The transient flora could include any microbe a person has picked up, and is greatly influenced by the hygiene of the individual. The death of the transients on skin surface is thought to result primarily from two factors viz. skin's low moisture content, and low pH (4-6) due to its organic contents. Further, the secretions of sweat and oil glands have antimicrobial properties.

1.5.3.2. Resident flora of the skin: The resident population of skin lives and multiplies in deeper layers of the epidermis and in glands and follicles. Because the resident flora is regular and relatively stable, it is more predictable and less influenced by hygiene. The normal skin residents consist primarily of *Staphylococcus (S. epidermidis, S. aureus), Corynebacterium (C. xerosis), Propionibacterium (P. acnes)* and yeasts (*Pityrosporum ovalis, P. orbiculare*). The occasional species include *Micrococcus luteus, Streptococcus anginosus, Acinetobacter calcoaceticus* etc.

Chronically moist skin folds, especially between the toes tend to harbour fungi, while lipophilic mycobacteria and staphylococci are prominent in sebaceous secretions of axilla (under arm), external genitalia and external ear canals. One species, *Mycobacterium smegmatis*, particularly lives in the cheesy secretion (or smegmum) on the external genitalia of both men and women. *Acinetobacter* is found particularly in the axillae and groin, its numbers increase in wet weather.

Most of the resident flora belongs to Gram positive group, and Gram negative bacteria are comparatively less. Further, the bacterial populations are comparatively very high in association with glands than on surface. The species associated with hair follicles are mostly anaerobic while the surface flora is aerobic. The anaerobes out number aerobes by 10:1 to 100:1, indicating the importance of hair follicles as niches for microbial flora. On the skin

surface, the bacteria are not uniformly distributed, but occur in isolated groups, as revealed by SEM studies.

Although normal skin flora usually plays a protective role, it plays a pathogenic one in the disease 'acne vulgaris', which commonly occurs during adolescence, when sebaceous glands become plugged. Normally these glands produce a substance termed sebum, which is formed when cells in the lining of the gland die, become released into the gland's internal cavity and lyse. These bacteria metabolize some of the cellular components producing sebum. In some individuals sebum triggers an inflammatory response, causing redness and swelling. Swelling blocks the gland's duct. Such a plugged sebaceous gland is called a black head or pimple. *Propionibacterium acnes* has been tentatively identified as the organism responsible for producing the substance in sebum that cause inflammation. This organism is extremely sensitive to the antibiotic tetracycline, a drug which in low doses often exerts a beneficial (curative) effect on the disease.

Yeasts are generally harmless, but in some cases species of *Pityrosporum* (= Melassezia) cause dandruff of skin as well as Tinea versicolor, and more serious rashes in AIDS patients.

Important normal flora of skin and their characters are given in the table 1.1

Table-1.1: Characters of normal flora of skin

Organism	Gram reacation	Morphology	Remarks
Group : Bacteria			
Staphylococcus epidermidis	+ve	cocci in bunches	Nonpathogenic
S.aureus	+ve	cocci in bunches	pathogenic, but uncommon
Corynebacterium xerosis	+ve	club shaped irregular rods	nonpathogenic
Propionibacterium acnes	+ve	club shaped irregular rods	anaerobic, mostly in skin glands

Micrococcus luteus	+ve	cocci in singles	less common
Streptococcus anginosus	+ve	cocci in singles	less common
Acinetobacter calcoaceticus	-ve	rods	associated with with hairs in humid conditions
Escherichia coli	-ve	rods	surface contaminant
Group : Yeasts			
Pityrosporum ovalis		small oval cells	lipophilic
P.orbiculare		small oval cells	lipophilic

1.5.4. NORMAL FLORA OF EYE: It is often included in the skin flora. A small number of microorganisms can be isolated from the normal conjunctiva. The delicate layer that covers the eye lids and eye ball is called conjunctiva. The conjunctival flora is very limited and include *Corynebacterium xerosis, Staphylococcus epidermidis, Branhamella catarrhalis* etc. Other bacteria include staphylococci and streptococci etc.

The relatively few bacterial flora of eye is attributed in part to the highly potent lytic enzyme, lysozyme, present in tears and continuous wetting of conjunctiva with tears. Tears had a strong bactericidal effect, and is capable of dissolving certain saprophytic cocci in dilution of 1 : 40,000.

1.6. ORAL FLORA:

Digestive system is the most heavily populated part of the human body. The different parts of the digestive tract are oral cavity, oesophagus, stomach, small intestine, large intestine (colon), rectum and anus. The different parts of the system show large differences in their normal flora both qualitatively and quantitatively. Of the different parts, mouth and colon are most heavily populated areas.

The mouth or oral cavity is warm and moist, and has regular supply of fresh food. Hence it is an ideal growth habitat for microorganisms. Even the fairly clean and healthy

mouth contains a considerable amount of detritus and other organic matter derived from food particles, desquamated epithelium, pharyngeal mucous and other sources. The assortment of organisms in the oral flora is relatively stable though subject to change with aging. In the early part of childhood the facultative anaerobic flora predominate but with emergence of teeth the balance shifts to anaerobic flora.

Bacteria are the most predominant group of microbes in the oral cavity and common groups are lactobacilli, staphylococci, streptococci and actinomycetes. The others include *Bacteroides*, *Borrelia*(spirochete), *Branhamella, Corynebacterium,* diphtheroids, *Fusobacterium, Mycoplasma, Neisseria, Nocardia* etc.

Of the fungi, the most commonly found one is *Candida*. It has been estimated that approximately 40% of normal mouth flora belongs to *Candida* alone. In healthy mouth, they make up only a small percentage of oral flora but in children and aged, they are of major importance. Oral *Candida* infections often follow the decrease of bacterial populations in the oral cavity.

The distribution of the oral flora show marked variation with different microenvironments in the mouth. For example

Streptococcus salivarius colonizes surface of the tongue

S. sobrinus colonizes smooth surface of teeth

S. mutans found predominantly in crevices of teeth

S. mitis inhabit mostly cheeks

Spirochetes, *Bacteroides* and *Fusobacterium* occur between gum and teeth

1.6.1. Microhabitats in mouth: In the oral environment, saliva and teeth provide most important microhabitats.

1.6.1.1. Saliva: Saliva is the most pervasive source of microbial nutrient in the oral cavity. Saliva contain about 0.5 percent dissolved solids, half of which are inorganic in nature (mostly chlorides, bicarbonates and phosphates of sodium, calcium, potassium and trace elements). The predominant organic constituents of saliva are proteins such as salivary enzymes, mucoproteins and some serum proteins. Small amounts of carbohydrates, urea, ammonia aminoacids and vitamins are also present. The microorganisms isolated from saliva

are quite varied and include species of *Staphylococcus, Streptococcus, Micrococcus, Lactobacillus, Corynebacterium, Actinomyces, Clostridium, Propionibacterium, Escherichia, Proteus, Psuedomonas, Klebsiella, Rothia, Bacterionema, Bacteroides, Pasturella, Fusobacterium* etc. The bacterial population of saliva may be as high as 10×5^9 cells per ml, under favourable conditions.

Even though saliva is rich in nutrients and support varied microbial flora, it is not an especially good microbial culture medium as it contain immunoglobulins, lysozyme, lactoferrin, lactoperoxidase etc. Lysozyme directly lyse the bacterial cells. Lactoferrin chelate free iron efficiently, thus making it unavailable for microbes. The lactoperoxidase enzyme, which is present both in milk and saliva kills bacteria by a reaction involving release of singlet oxygen from hydrogen peroxide (H_2O_2). Despite the activity of these antimicrobial substances, the presence of food particles and epithelial debris makes the oral cavity a very favourable microbial habitat.

1.6.1.2. Teeth: Teeth provide a surface for growth of microbes. The tooth consists of calcium phosphate crystals (enamel) within which the living tissue (dentin and pulp) is present (Fig. 1.2).

Figure 1.2. Section through tooth showing surrounding tissues that anchor tooth in the gum.

Bacteria found in mouth during first few months of life are predominantly aerotolerant anaerobes such as Streptococci and Lactobacilli along with some aerobes. When teeth appear there is a pronounced shift in the balance of microflora towards anaerobes. The activities of the anaerobic microflora of teeth result in dental plaques and dental caries (tooth decay).

1.6.2. Dental plaque: Certain streptococci like S. mutatans and S. sanguis form an insoluble dextran slime layer on the surface of the teeth. With in the dextran slime matrix anaerobic bacteria grows luxuriantly, the film of dextran slime layer on the teeth with its bacterial flora is called a plaque or dental plaque. The most common bacterial member in dental plaque is a strict anaerobe *Fusobacterium*. It is a gram negative, non spore-forming spindle shaped rod. The cells are closely packed, extending out perpendicular to the surface of the tooth. Associated with it are various species of streptococci, *Bacteroides*, spirochetes, diphtheroids and gram negative cocci etc. Plaque varies in its microbial content according the age.

Earliest colonizers -- Streptococci and Neisseriae
2nd phase colonizers -- Gram +ve rods, filaments, actinomycetes etc.
3rd phase colonizers -- Gram -ve rods, Spirochetes
Final phase colonizers -- Bacteroides and Fusobacterium

1.6.3. Dental caries: The role of oral flora in tooth decay (dental caries) has now been well established. The tooth surfaces and crevices, where food particles can be retained, are the sites where tooth decay predominates. Two organisms that have been implicated in dental caries are *Streptococcus mutans* and *S. sobrinus*. Both are lactic acid producing bacteria and the acid etches the enamel. *S. sobrinus* is able to colonize smooth tooth surface because of its specific affinity for salivary glycoproteins. *S. mutans* is found predominantly in crevices and small fissures and its ability to attach seems to be related to its ability to produce a dextran polysaccharide which is strongly adhesive.

1.7. Normal flora of gastrointestinal tract:

The human gastrointestinal tract, the site of food digestion, consists of stomach, small intestine and large intestine (Fig. 1.3.). Normal flora of the tract varies with different regions.

Figure 1.3. The gastrointestinal tract of humans

1.7.1. Stomach: The pH of the stomach fluids is very low, about 2. Hence, the stomach is not a very hospitable habitat for microorganisms and it can be viewed as a microbiological barrier against entry of foreign bacteria into the intestinal tract because of the bactericidal effect of hydrochloric acid and gastric juices. Although the bacterial count of stomach contents is generally low (often less than 10 per ml), the walls of the stomach are often heavily colonized with bacteria. These are primarily acid tolerant lactobacilli and

streptococci, and yeasts such as *Candida* spp. *Mycobacterium tuberculosis* and *Helicobacter pylori* resist acidity of the stomach, but only the latter is identified as gastric pathogen.

1.7.2. Small intestine: This part is about 25 feet in length and is divided into 3 parts viz. duodenum, jejunum and ileum.

Duodenum (one foot length) contains few microbes because of the combined effect of stomach's acidic juices and inhibitory effect of bile and pancreatic secretions. Of the bacteria present gram positive cocci and rods predominate.

Jejunum (5 feet length) contain increasingly more bacteria. Apart from gram positive bacteria, gram negative bacteria also make appearance. Lactobacilli, diphtheroids, *Enterococcus faecalis* and yeasts like *Candida albicans* are found.

In the ileum (19 feet length) pH becomes more alkaline. As a result anaerobic gram negative bacteria and members of enterobacteriaceae become established.

The distribution of microbial flora gradually increase in the small intestine. The duodenum may contain 100-1000 organisms per ml; the jejunum 1,000 -10,000; the upper ilem about 1,00,000 and lower ileum 10,00,000 per ml. However there is always much variation from sample to sample.

1.7.3. Large intestine (colon): It has the largest microbial population in the body. Many bacteria live with in the lumen it self probably using some products of digestion of food as nutrients. Microbial population approach 10^{12} per gram of colon contents (represented by faeces). Over 300 different species have been isolated from human faeces and the colon can be viewed as a large fermentation vessel. The microbiota consist primarily of anaerobic, gram negative, non spore-forming bacteria, and gram positive spore forming and non spore forming rods are comparatively less. The ratio of anaerobic to facultative anaerobes is 300:1. The activities of facultative anaerobes consume any oxygen present in the colon making it strictly anaerobic and favourable for profuse growth of obligate anaerobes. The important bacterial genera are *Bacteroides, Eubacterium, Peptostreptococcus, Fusobacterium, Bifidobacterium, Clostridium, Lactobacillus, Escherichia, Klebsiella, Proteus* etc.

Apart from bacteria, yeasts and protozoa also occur in the colon. Among the yeasts, *Candida albicans* is the most common species. Among protozoa important species that occur as commensals are *Entamoeba hartmanii, Endolimax* sp., *Iodamoeba* sp., *Trichomonas hominis* etc.

The typical bacterial flora of the human large intestine, as isolated from faces, is given in the table 1.2.

Table – 1.2. Typical bacterial flora of colon

Concentration per gram	Genus / species	anaerobe/ F.anaerobe	Gram reaction	Morphology
6 x 10^{10}	*Bacteroides fragilis*	strict anaerobe	-ve	rod
2 x 10^{10}	*Eubacterium aerofaciens*	"	+ve	rod
	Peptostreptococcus productus	"	+ve	cocci
1 x 10^{10}	*Fusobacterium* sp.	"	-ve	rod
5 x 10^{9}	*Bifidobacterium* sp	"	+ve	rod
	Coprococcus sp.	"	+ve	cocci
1 x 10^{9}	*Clostridium* spp.	"	+ve	rod
	Lactobacillus sp.	"	+ve	rod
	Ruminococcus sp.	"	+ve	cocci
1 x 10^{8}	*Escherichia coli*	F. anaerobe	-ve	rod
Less then 10^{8}	*Enterobacter aero-genes*	"	-ve	rod
	Proteus spp	"	-ve	rod
	Klebsiella pneumoniae	"	-ve	rod
	Streptococcus faecalis	"	+ve	cocci

From the table it is clear that *Bacteroides* is the most predominant genus of the intestinal flora. It is a gram negative, pleomorphic, nonsporeforming, obligatory anaerobic rod. About 20 species are present in the genus, and *B. fragilis*, which is the type species is the most common one.

Further, it is clear that *Escherichia coli*, which is often taken as representative of intestinal flora, contribute only about 0.1% of total population.

The population of large intestine is generally in the range of 10^8 to 10^{11} per gram material and it constitutes 10 to 30% of faecal matter by volume. A person on a long term fasting may pass faeces comprising primarily of bacteria and 99% of them are anaerobic.

1.7.3.1.Factors affecting normal flora of large intestine:

1.7.3.1.1.Effect of diet: The initial residents of colon of breast fed infants are members of the gram positive *Bifidobacterium* because human milk contains a disaccharide, amino sugar, that *Bifidobacterium* species require as a growth factor. In bottle fed infants *Lactobacillus* predominate because formula milk lacks the required growth factor. With the ingestion of solid food the initial flora is gradually replaced by more stable characteristic normal flora and the nature of diet influence the normal flora both qualitatively and quantitatively. A high carbohydrate diet favours growth of obligately anaerobic bacteria, particularly Bifidobacteria and other acid tolerants. Diet high in fat content encourages the proliferation of *Bacteroides* and represses the enterococci and bifidobacteria. Persons who consume more animal protein (meat) show a higher number of *Bacteroides* and lower numbers of coliforms and lactic acid bacteria than those taking vegetable food.

The normal flora of European people is qualitatively different from that of Asian or African people because in the West the people are omnivorus while in Asia and Africa people mainly take vegetarian food.

1.7.3.1.2. Effect of antibiotics: When an antibiotic is given orally it may inhibit the growth of the normal flora as well as pathogens, and thus cause virtual sterilization of the intestinal tract. In the absence of the normal flora opportunistic microorganisms such as antibiotic resistant staphylococci, *Proteus* or yeast *Candida albicans* establish in the intestine. Under normal conditions they cannot colonize the colon because they cannot compete with normal

flora. One effect of overgrowth of antibiotic resistant bacteria is that it may lead to diarrhoea, especially with resistant staphylocci.

The addition of antibiotics (for example: tetracycline) to the diet stimulate the growth, as shown in case of chicks, pigs and turkeys. In the case of experimental animals, not only they grow faster but in terms of weight gained per unit weight of food taken also increased. The positive effect has been attributed to 1) the more complete absorption of the nutrients, 2) increased production of microbial vitamins or other growth factors needed by the animal, 3) inhibition of bacteria that otherwise compete with the animal for nutrients in the gut and 4) inhibition of bacteria that have deleterious effect on the host.

1.7.3.1.3.Other factors: Apart from diet and antibiotics, the composition of the normal flora of the intestine can be influenced by various factors such as strong emotional stress, changes in air pressure due to altitude, starvation etc. The rate at which intestinal contents are passed through alimentary tract also influence the magnitude of normal flora. For example, the food material is passed through ileum (the major part of small intestine) at a speed 10 times more than that in colon. Hence, microbes are found in greatest numbers in colon.

1.7.4. ACTIVITIES OF INTESTINAL FLORA:

Two most common activities of intestinal flora are 1. helps the nutrition of host and 2. flatulence or gas production.

1.7.4.1. Nutrition: There is both suggestive and direct evidence of the role of normal flora of the intestine in host nutrition by production of various vitamins. For example, *E. coli* and *Klebsiella aerogenes* synthesize biotin, riboflavin, pantothenic acid, pyrodoxin and vitamin K, both in vivo and in vitro.

During antibiotic therapy, partial sterilization of the intestine occurs which leads to a condition of avitaminosis because the vitamin producing bacteria of normal flora is eliminated. It is an indirect evidence of vitamin production by normal flora. Hence, physicians prescribe vitamin intake along with antibiotics.

Studies with germ free animals showed that they need vitamin K, whereas conventional animals do not require any addition or external source of vitamin K. *E. coli* synthesize vitamin K.

There is evidence that *Klebsiella pneumoniae* fixes nitrogen anaerobically in the colon. In New Guinea, people eat mainly sweet potatoes, a food virtually devoid of nitrogen. *K. pneumoniae* is a constant member of intestinal flora of these people. Apparently sufficient nitrogen is fixed by *K. pneumoniae* and it passes through intestinal wall to the blood stream, so that these people can subsist on a diet that would be unsuitable otherwise.

1.7.4.2. Flatulence: Gas production (or flatus) is one of the common activities of intestinal flora. The phenomenon of producing gas is called flatulence (in polite circles gas production is known as flatus and the explosion of it as flatulence). Intestinal bacteria produce an average of 7 to 10 litres of gas daily, and only about one tenth of it is actually ejected as flatus.

Poorly digested food is fermented by anaerobic bacteria resulting in the production of hydrogen and carbondioxide. In many individuals methanogenic bacteria convert some of the hydrogen and carbondioxide into methane gas. Diet has major effect on the gas production. Vegetables such as cabbage, cauliflower, corn, onion and beans contain carbohydrate residues that are not attacked by human digestive enzymes, yet readily fermented by gas producing bacteria. If large quantities of beans are consumed total gas production increases about ten fold. Another common cause for gassiness in some individuals is poor absorption of milk sugar lactose. If individuals with this abnormality do not eliminate lactose from their diet, their intestinal bacteria are stimulated to produce gas.

1.8. NORMAL FLORA OF THE RESPIRATORY TRACT:

The respiratory tract can be recognized as two parts 1. upper respiratory tract comprising nasal passage, nasopharynx and oropharynx, and 2. lower respiratory tract comprising trachea, bronchi, bronchioles and alveoli (Fig. 1.4).

Figure 1.4. The respiratory tract of human beings

From birth to the last breath, humans breathe consciously and unconsciously without any interruption. More than 10,000 litres of air is inhaled every day and exhaled promptly. The air passing into the respiratory tract contains inorganic dust particles and organic particles including microorganisms. The microorganisms that enter the respiratory tract are rapidly eliminated from the upper respiratory tract itself, and if any microbes reach alveoli it usually takes very long time to eliminate them. It is estimated that the deposited particles are removed from nasal mucosa within 15 minutes, but takes 60 to 120 days to eliminate them from alveoli.

Of the various microorganisms that enter the respiratory tract, only a few are able to colonize the upper respiratory tract and very few reach lungs.

1.8.1. Normal flora of upper respiratory tract: In the upper respiratory tract microorganisms colonize the surfaces bathed with the secretions of the mucous membranes. The relatively low rate of flow and turbulence of inhaled air are ideal for particle deposition and few particles greater than l0 μm pass through the nasal passage. For this reason nasal secretions contain many protective proteins in the form of antibodies, lysozyme and interferon. In addition, the cilia of nasal epithelium move the mucous gel layer rapidly back

to the oropharynx, where it is swallowed and destroyed by the acid secretions of stomach. Despite these factors the nose and nasopharynx are colonized by numerous microorganisms, mainly by those which have ability to adhere to epithelial layer of mucous membrane. The common members of the normal flora of upper respiratory tract are species of *Staphylococcus, Streptococcus*, *Bacteroides, Haemophilous, Neisseria, Branhamella, Micrococcus*, diphtheroids etc. *Staph. epidermidis and Staph. aureus* preferentially resides in the nasal entrance and passage and anterior nasopharynx, *Neisseria* species occur on mucous membranes behind the soft palate, alpha streptococci including *S. pneumoniae* and species of *Haemophilous* occur in the lower nasopharynx in the vicinity of tonsils.

The common members of normal flora of upper respiratory tract and their characters are given in the table.

Table- 1.3.: Bacteria in the upper respiratory tract

Genus	Characters	Comments
Corynebacterium	Gram +ve, pleomorphic rods	Zon thogenic species collectively called diphtheroids
Staphylococcus	Gram +ve; cocci in clusters	commonly includes S.epidermidis and potentially pathogenic S. aureus
Branhamella	Gram –ve cocci	resemble Neisseria Spp
Haemophilous	Gram -ve, small	include potentially pathogenic H.influenzae strict anaerobes pleomorphic rods
Bacteroides	Gram -ve, small	pleomorphic rods
Streptococcus	Gram +ve, cocci in chains	alpha, beta and gama haemolytic streptococci; commonly include S. pneumoniae

1.8.2. Lower respiratory tract: The lower respiratory tract (trachea, bronchi, lungs) is essentially sterile, inspite of large numbers of organisms potentially able to reach this region during breathing. Dust particles and other relatively bigger particles like pollen and fungal spores are filtered out in the upper respiratory tract, its rate of movement decrease markedly and the microorganisms in the air settle on the walls of the passages. The walls of trachea and bronchi are lined with ciliated epithelium, and the cilia beating upwards push bacteria and particulate matter towards the upper respiratory tract where they are expelled in the saliva and nasal secretions. Only droplet nuclei smaller than 1.0 μm in diameter are able to reach lungs and they are promptly engulfed and eliminated by alveolar macrophages.

1.9. NORMAL FLORA OF URINARY TRACT:

The different parts of the urinary tract in humans are kidneys, upper urethra (ureters), urinary bladder and lower urethra (Figure 1.5).

Figure 1.5. Urinary tract of humans

Kidneys are normally sterile and highly resistant to bacterial invasion. Upper urinary tract and urinary bladder are also usually sterile, apparently because the urethral lining exerts antibacterial effect and also frequent flushing of the epithelial surface of the urethra. However, there are some reports of the occurrence of nonpathogenic strains of gram positive cocci and gram negative enterobacteria in these parts.

The lower urethra of both sexes is normally colonized by bacteria. The female urethra being shorter (about 3-4 cm) than male urethra, it is much more frequently and heavily colonized by bacteria.

The normal flora of male urethra comprise lactobacilli, nonhaemolytic streptococci, *Mycobacterium*, diphtheroids, and *Bacteroides*. Gram negative diplococci of *Acinetobacter* group is also present. A protozoan *Trichomonas vaginalis* often colinize male urethra without causing any symptoms.

The principal resident flora female urethra are nonhaemolytic streptococci, staphylococci, corynebacteria, *Mycobacterium* and occasionally coliforms. Unlike in males, the presence of *Trichomonas vaginalis* leads to vaginal infection termed as trichomoniasis.

1.10. NORMAL FLORA OF THE GENITAL TRACT:

The genital tract is essentially germ free except the distal parts viz. vulva and vagina in females and distal urethra in males.

The typical normal flora of vulva comprises *Staphylococcus epidermidis*, *Streptococcus faecalis*, corynebacteria, *E. coli* and other coliforms.

The changes in the normal flora of vagina present a clear example of how changes in physiology greatly influence the composition of normal flora. An important factor influencing these changes in women is hormone estrogen produced by ovarian activity. Estrogen normally stimulates the vaginal mucosa to secrete the carbohydrate glycogen. The glycogen on the vaginal mucosa is fermented by *Lactobacillus acidophilus*, often described as doderlein bacillus, producing the acid thus resulting in low pH of the vagina.

Before puberty, a girl produces little estrogen, little gycogen and has a vaginal pH of about 7. These conditions favour the establishment of diphtheroids, staphylococci,

streptococci and some coliforms. As the hormone levels rise at puberty the vagina begins to deposit glycogen and the flora shifts to the acid producing lactobacilli.

It is thought that the acidic pH of the vagina during the child bearing age is protective, preventing the establishment and invasion of microbes with potential for harming the developing foetus. The estrogen-glycogen effect continues, with minor disruption, throughout the child bearing years until menopause, when the flora returns to a mixed population similar to that of pre-puberty . These transitions are not abrupt but occur over several months to years.

In the distal part of the genitalia of both sexes *Mycobacterium smegmatis* and *Ureaplasma* are commonly found. The preputial cheesy secretion of genitalia is called smegmum and one species of *Mycobacterium* described as *M. smegmatis* preferentially thrives in smegmum. Occasionally smegmum contaminates urine, and such contaminated urine samples when cultured produce colonies similar to tubercle bacilli thus raising false alarm.

Strains of *Mycoplasma* called T-strains or *Ureaplasma,* which form minute colonies on culture media, are commonly present as part of the normal flora of both sexes.

Table- 1.4: Genital flora of male and females

Sex	Part	Bacteria
Female	Vulva	*Staphylococcus epidermidis* Corynebacteria *Escherichia coli* and other coliforms *Streptococcus faecalis*
	Vagina	*Lactobacillus acidophilus* *Bacteroides melaninogenicus* *Streptococcus faecalis* Corynebacteria Yeasts (*Candida albicans*)

Male & Female	Distal urethra	*Staphylococcus epidermidis* Corynebacteria *Mycobacterium smegmatis*

The overall picture of the distribution of normal flora of human body is shown in the figure 1.6.

Figure 1.6. Distribution of normal flora of the human body

1.11. SIGNIFICANCE OF NORMAL FLORA:

To know the significance of normal flora to the host, experiments were conducted with germ free animals. As the foetus is sterile in mother's womb, it is taken out aseptically by caesarian operation, immediately transferred to sterile incubators and nurtured with sterile nutrients. Once germ free parents are maintained, they will continue to produce germ free young ones without human intervention. Rats, mice, rabbits, guinea pigs, monkeys, dogs, cats etc. are raised in germ free state, and used for experimental studies. Such animals are called gnotobiotic animals. Normal animals are called conventional animals.

Studies with gnotobiotic animals have clearly established that normal flora is mainly beneficial to host, but they also cause some harmful effects.

1.11.1.Beneficial role of normal flora: The normal flora is beneficial to humans mainly in three respects: 1. protection against pathogen invasion, 2. host nutrition and 3. maintenance of host immune system

1.11.1.1. Protection against pathogen invasion:

The members of normal flora occupy their own niches in the body and inhibit foreign microorganisms invading from other parts of the body or from external environment. Such inhibition is brought about by

a) competition for food and space

b) production of antibiotic substances or other inhibitory substances

c) changes in the environmental conditions such as oxygen content, pH

d) secretion of mucin and laying down a mucous blanket that prevents attachment of pathogenic organisms.

The germ free animals are much more susceptible to bacterial infections than are the conventional animals. Organisms like *Bacillus subtilis* and *Micrococcus luteus* which are harmless to conventional animals are harmful to germ free animals.

Vibrio cholerae (cholera pathogen) and *Shigella dysenteriae* (dysentery pathogen) infect germ free animals more easily than conventional animals.

The ecological balance apparently prevents indigenous pathogens such as *Candida albicans, Streptococcus pneumoniae, Haemophilous influenzae* and *Staphylococcus aureus* from causing severe disease.

Certain diseases like pseudomembranous colitis caused by *Clostridium difficile*, staphylococcal enterocolitis and invasion of respiratory tract by coliforms and yeasts occur mainly when normal flora has been suppressed by over use of wide spectrum antibiotics.

Another example of protective role of normal flora is that of doderlein bacillus in adult vagina. The vaginal flora of an adult female before menopause is almost exclusively of lactobacilli which breaks down glycogen of the vaginal epithelium to lactic acid. The high acidity renders vagina highly resistant to invasion of other organisms.

1.11.1.2. Beneficial role in maintenance of immune system:

The normal flora contributes significantly to the development of immune system. When germ free animals are placed in contact with normal control animals, they gradually develop a flora similar to the controls. However, germ free subjects are less able to tolerate some of the colonists and may die from infections by relatively harmless species. This is due to the immature character of the immune system of the germ free animals. These animals have smaller lymph nodes, a reduced number and types of white blood cells and slower antibody response. From this it becomes clear that normal flora helps the host to develop normal immune system and lack of it result in immature immune system.

1.11.1.3. Beneficial role in host nutrition:

The normal flora members of the intestine like *Escherichia coli* and *Klebsiella aerogenes* can synthesize vitamins like biotin, riboflavin, pantothenic acid, pyridoxin and vitamin K in cultures. Of these, vitamin K is a cofactor for synthesis of clotting factors II, VII, X and XI. Without the contribution from the normal flora, the human clotting system would be grossly ineffective.

Klebsiella pneumoniae can fix nitrogen and that it will fix nitrogen in the intestine of New Guinea people who takes only carbohydrate food of sweet potatoes is deduced by indirect evidence.

1.11.2. Harmful activities of normal flora:

A dramatic characteristic of germ free animals is that they live longer and have fewer diseases than normal controls, as long as they remain in sterile environment. From this stand point, it is clear that the normal flora is not needed for survival and may even be the source of infectious agent (i.e. opportunistic pathogens).

Acne vulgaris, dental caries and intestinal problems are some of the harmful activities of the normal flora. Dental caries and amoebic dysentery are two pathological conditions that occur only in conventional animals.

1.11.2.1. Acne vulgaris: One of the most common skin infections is acne, a disease of sebaceous glands. In the normal sebaceous gland follicle, the skin scales are shed and carried out in a continuous flow. In acne, the dead cells and oil do not move smoothly out of the pore

but build up along the inside of the duct. Eventually the build up forms a plug or comedo (black head or pimple). This prevents the normal flow of sebum, and the anaerobic diphtheroid, *Corynebacterium acne*, found in the tissue is able to hydrolyze the sebum into fatty acids which begin to destroy the wall of the gland. The swollen gland then ruptures under the surface of the skin. Phagocytes are mobilized to attack the bacteria and pus is formed. The necrotic tissue accumulates to from the typical white head of pustule. Studies indicate that growth of the bacteria and development of acne is correlated with hormonal changes during puberty. As these changes subside the infections decrease in frequency and severity with little or no treatment.

1.11.2.2. Dental plaque and dental caries: *Streptococcus mutans* is a bacterium that is able to ferment sucrose and produce an insoluble, sticky polysaccharide known as dextran. Dextran molecules adhere to the surface of the tooth and serve as a hold fast for acid producing bacteria responsible for decay. The sticky mat of bacteria and dextran is known as dental plaque. *Streptococcus mutans* and a number of lactobacilli lodged in dental plaque metabolize sucrose and other sugars releasing lactic acid as an end product. Dental caries form in two stages. In the first stage, acid formed due to bacterial fermentation of food decalcifies the inorganic component of enamel, and in the second stage enzymes from these microbes hydrolyze enamel protein. This destructive process can eventually reach the inner living portion of the tooth and completes its decay. It has been established that children fed on high amounts of carbohydrates will be more susceptible to cavities than those on low sugar diets. Sucrose is the most cariogenic (cavity producer) carbohydrate while starch is the least. When sugar is lodged in plaque in the fissures and crevices of the teeth, bacterial fermentation will produce enough acid to initiate decay in only 10 to 15 minutes.

For a number of years, the precise involvement of microbes in dental caries had been ambiguous. Studies with germ free animals confirmed that caries development is influenced by heredity, a diet high in sugars and poor oral hygiene, but even when all these predisposing factors are present, germ free animals still remain free of caries unless they have been inoculated with specific bacteria.

1.11.2.3. Intestinal problems: Amoebic dysentery, constipation, flatulence and other intestinal problems mainly occur due to normal flora.

Entamoeba histolytica is more pathogenic in normal animals than in the germ free animals. One explanation for this phenomenon is that *E. histolytica* must feed on intestinal bacteria to complete its life cycle. Due to lack of bacterial flora in the intestine of germ free animals *E. histolytica* cannot complete life cycle in them.

In bottle fed infants constipation, intestinal cramping and pain may result from an imbalance in normal flora due to lack of stabilizing antagonists. Breast fed infants have fewer cases of constipation and less likely to have serious digestive upsets. Lactinex granules (concentrates of *Lactobacillus acidophilous* in a lyophilized tablet form are known as lactinex) or sweet acidophilous milk may serve to stabilize intestinal flora and relieve the problem.

Flatulence or gas production is mainly due to the activities of anaerobic microflora of large intestine which utilizes the partially digested food and produce carbondioxide, hydrogen, methane etc.

1.12. Summary:

A healthy human foetus has no resident microbial population up to the time of its birth. It begins to acquire microbial populations from mother's birth canal, and within hours of birth resident microbial populations establish on skin, in the mouth, intestine and so on. Bacteria make up most of the normal flora of human body. Escherichia coli in the intestine, streptococci in the nasopharynx, *Staphylococcus epidermidis* on the skin and doderlein bacilli in the vagina of women are considered as basic members of normal bacterial flora of human body as they are present universally. Skin surface, though extensive more accessible, is less colonized because it is inhospitable, while sweat glands, though less accessible are more hospitable for colonization by bacteria. In the oral cavity, saliva and surface of teeth are colonized by bacteria. Oral flora mainly comprises of streptococci, spirochetes, *Bacteroides* and *Fusobacterium*. Stomach is inhospitable for normal flora because of highly acidic gastric juices. Normal flora gradually increase in small intestine and large intestine has the largest microbial population in the body. Most of the colon bacteria are obligate anaerobes and

Escherichia coli the predominant among facultative bacteria. In the respiratory tract, upper part (nasal passage, nasopharynx and oropharynx) is heavily colonized, while lower part (trachea, bronchi and lungs) is essentially sterile. In the urogenital tract, the lower is colonized by normal flora while upper part is essentially sterile. Distal urethra of female is more heavily colonized by bacteria when compared to that of males. Doderlein bacilli is the predominant bacterium of vagina of female during child bearing age. The normal flora of human body is beneficial to the host in providing protection against pathogen invasion, in nutrition and in maintenance of immune system. The activities of normal flora cause acne vulgaris (in young adults), dental plaques, dental caries, flatulence and intestinal disorders, which are not very serious but nonetheless harmful.

1.13. Model questions:

Essay type questions

Give an account of normal flora of skin and respiratory tract and their importance

Discuss the normal flora of gastrointestinal tract and its significance

Discuss the composition, characters and significance of normal flora

Discuss the beneficial and harmful activities of normal flora

Short answer questions

Composition of normal flora

Characters of normal flora

Normal flora of skin

Normal flora of respiratory tract

Normal flora of oral cavity

Normal flora of small intestine

Normal flora of colon

Normal flora of urogenital tract

Significance of normal flora

Escherichia coli

Doderlein bacilli

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LESSON-2: NONSPECIFIC HOST RESISTANCE MECHANISMS (PART -1)

OBJECTIVE: To study the mechanical and chemical barriers to microbial infection

CONTENTS

2.1. INTRODUCTION

Immunity refers to the condition of being resistant to a disease and nonsusceptibility to the invasive or pathogenic effects of foreign microorganisms or to the toxic effect of antigenic substances. The immune system of higher vertebrates including humans respond to pathogenic agents in two ways and these two types are designated as 1) non specific immunity or innate immunity and 2) specific immunity or acquired immunity.

Nonspecific immunity refers to the natural immunity of the host determined by genetic constitution. It exists from the earliest of life and protects against all parasites. Hence, it is referred to as nonspecific host resistance. No previous exposure to the pathogen is required for development of this type of resistance as the host is born with the protective mechanisms and do not involve antibody production.

The nonspecific or natural or innate immunity is due to mechanical barriers to infection, chemical barriers to infection, phagocytosis or biological barrier, and may also due to host - pathogen interactions which result in inflammation.

2.2. MECHANICAL BARRIERS TO INFECTION

Skin and mucous membranes with their secretions act as mechanical barriers to infection and constitute the body's first line of defense.

2.2.1. SKIN: Intact skin is highly effective barrier against microbial invasion. Various factors that contribute to its effectiveness are as follows

i. It is the outer layer of the body and consists of thick, closely packed keratinized cells which the pathogens cannot enzymatically attack. Keratins are scleroproteins comprising the main component of hairs, nails and outer layer of epidermis of skin.

ii. The skin is relatively dry and hence not favourable for growth of many pathogens.

iii. Mild acidity (4-6 pH) of skin layer due to breakdown of lipids into fatty acids by normal skin microbiota, makes the habitat less hospitable for growth and establishment by many pathogens.

iv. Sebum liberated from sebaceous glands form a protective film over the surface of the skin.

v. The normal flora of skin acts antagonistically against many pathogens. It also occupies receptor sites on skin and compete for nutrients.

vi. Continuous shedding of outer epidermal cells, called desquamation, is a natural phenomenon and it removes the organisms regularly, except those that have specific ability for attachment.

vii. Regular bathing and maintaining body hygiene removes the microorganisms.

The importance of skin barrier can be realized by high frequency of superficial infections when its efficiency is impaired due to wounds or burning or vitamin deficiency. However, hair follicles and glands form comparatively weak points in defence.

2.2.2. MUCOUS MEMBRANES

The mucous membranes of the respiratory, digestive and urogenital tracts and eyes also act as mechanical barriers to infection. Mucous membranes of these organ systems produce copious amounts of mucous which form protective covering that resist penetration. The mucous secretions of the membranes trap many microorganisms until they can be disposed of mechanically or lose their infectivity.

In the respiratory tract, larger particles and spores in air are filtered out in the nose by impinging on hairs (vibrissiae) or on the sticky mucous membranes. The large and complex

normal flora of upper respiratory tract constitutes an important mechanical barrier to colonization by external microbes. In the lower respiratory tract mucous secretions and ciliary action of mucociliary epithelial cells provide efficient clearance system for inhaled microbes. The microorganisms are trapped on the sticky mucous where they are exposed to lysozyme and lactoferrin, and are swept upward by the mucociliary escalatory process to be coughed out or swallowed and dealt with by the gastric secretions. Toxic agents like cigarette smoke and also virus infections inhibit ciliary action and hence lower respiratory tract become susceptible to infections.

In the intestinal tract, peristaltic movements trap microorganisms in mucous. Peristalsis means progressive, rhythmic contraction and expansion of intestinal wall. Establishment of infection is also hindered by the rapid transit through intestine and by the need for potential pathogens to compete with the resident bacterial flora for attachment to epithelial cells.

The conjunctiva is a specialized mucous secreting membrane that lines the inner surface of the eyelid and exposed surface of the eye ball. The secretions of the lacriminal glands, called tears, keep the conjunctiva moist. The eyes are constantly washed by tears and wiped by the movement of eyelids. Tears contain an enzyme lysozyme which nonspecifically lyse the bacteria.

Mechanical actions like coughing and sneezing help in expelling the foreign particles that enter digestive and respiratory tracts.

2.3. CHEMICAL BARRIERS TO INFECTION

Chemical barriers to infection are mainly of three types. 1.chemicals which are preformed and present in the tissues or body fluids. 2. chemicals that present in the body that are activated due to the presence of invading microorganisms and act nonspecifically and 3. chemicals which are produced as a response to invading microbes and act nonspecifically.

2.4. PREFORMED CHEMICALS

2.4.1. Acidic pH as microbial barrier: Stomach and female vagina are natural barriers for microbial colonization and invasion because of high acidity of their contents.

2.4.1.1. Stomach: A natural barrier for microbial invasion in gastrointestinal tract is provided by stomach acids, with high pH (approximately 2). Most organisms are destroyed in this environment. Bile from the gall bladder enters the system at the duodenum and serves as an inhibitory substance. In addition, duodenal enzymes digest proteins, carbohydrates and other large molecules of microorganisms. Notable exceptions include typhoid pathogen, tubercle bacilli, protozoan cysts, polio and hepatitis A viruses.

2.4.1.2. Vagina: Resistance in the vaginal tract is enhanced by low pH. This develops when Lactobacillus species in the normal flora breakdown glycogen to various organic acids. In the urogenital tract slightly acidic pH of urine promotes resistance to parasites and flow of urine flushes microorganisms away.

2.4.2. Lysozyme: A chemical inhibitor of non specific nature is the enzyme lysozyme. This protein was described in early 1920s by Alexander Fleming, who later gained recognition for the discovery of penicillin.

Lysozyme is a thermostable enzyme present in several body fluids such as saliva, tears, perspiration and also in tissues. It disrupts the cell walls of Gram positive bacteria by digesting peptidoglycan layer. Low antibody concentrations on mucous membranes increase its effectiveness against several microorganisms.

2.4.3. Bacteriocins: Many of the normal flora bacteria synthesize and release plasmid encoded substances called bacteriocins, which are lethal to other closely related bacteria. Most bacteriocins that have been identified are proteins and are produced by Gram negative bacteria. Bacteriocins may give their producers an adaptive advantage against other bacteria. For example, coliform bacteria produce colicins.

2.4.4. Leukins: In 1891 Hankin observed that extracts of lymph nodes of animals had bactericidal activity against *Bacillus anthracis*. Schneider obtained active substance from leukocytes which he called leukins.

2.4.5. Phagocytin: It is a protein extracted from leukocytes, mainly polymorphonuclear leucocytes (PMNLs), and is active against Gram negative bacteria and also against staphylococci and streptococci. The substance is believed to function in conjunction with other tissue and body fluids such as lysozyme and histones.

2.4.6. Betalysin: It is a polypeptide released from blood platelets. It can kill some Gram positive bacteria by disrupting their plasmamembranes.

2.4.7. Fibronectin: It is a high molecular weight glycoprotein that can interact with certain bacteria like *Staphylococcus aureus* and streptococci. Fibronectin also covers the receptors of certain epithelial cells to block the attachment of many bacteria.

2.4.8. Protamine: It is a protein produced by spermatozoa and act against Gram +ve bacteria.

2.4.9. Spermine: It is a protein produced by prostate glands and pancreas and act against Gram positive bacteria. It was first isolated by Dubos and Hirsh in 1953.

2.4.10. Histones: They are proteins produced by lymphatic system and nonspecifically act against Gram positive bacteria.

2.4.11. Tranferrin and Lactoferrin: These are proteins which chelate or tie up available Iron in the body environment and thus limit its availability to invading microorganisms.

2.5. ACTIVATED CHEMICAL BARRIER

Among the chemicals that are activated in the presence of invading microorganisms and act nonspecifically, the most important one is COMPLEMENT.

2. 5. 1. COMPLEMENT

Complement refers to a group of nonspecific heat labile serum proteins that have enzymatic activity. It plays an important role in resistance against infections and is the principal mediator of inflammatory response. It was first discovered by Jules Bordet in 1895, who named it as alexin. Paul Ehrlich coined the term complement for it because its action complements that of antibody mediated reactions.

Complement comprises of a series of 11 enzymatic proteins which are capable of reacting with each other in a particular sequence as follows

C 1q C 1r C 1s C4 C2 C3 C5 C6 C7 C8 and C 9

The complement proteins remain in an inactive form in the blood and are activated by antigen - antibody complexes, aggregated antibodies like Ig G or Ig A, animal viruses, Gram

negative bacteria, bacterial endotoxins, yeasts etc. When one component is activated, other components are triggered in a sequence in a cascade pattern and brings about biological activities such as lysis, phagocytosis etc.

2.5.2. PATHWAY OF COMPLEMENT ACTIVATION:

When an antigen-antibody complex is formed, it is recognized by Clq component of complement protein and it attaches to the Fc (fragment crystallisable) portion of the antibody, and Ca++ ions are essential for effective Clq binding. Then other components of complement system are activated sequentially as given below

1. Attachment of C1q to the immune complex activates C1r.
2. Activated C1r functions as serine-histidine esterase and activates C 1s.
3. Cls activates C4 and C2 in the presence of Mg ions
4. Activated C4 splits into two fragments, a small fragment C4a and a large fragment C4b. Then C4b binds either to antibody - Cl complex or to the surface of the microbe.
5. Activated C2 also splits into a small fragment C2a and a large fragment C2b. Then C2a reacts with C4b to form C4bC2a complex.
6. The C4bC2a complex function as C3 convertase enzyme and it activates C3 component.
7. The activated C3 splits into a small C3a fragment and a large C3b fragment. The C3b binds with C4bC2a complex to form C4bC2aC3b complex.
   The C3b has binding sites for macrophages and phagocytes also, and hence C4bC2aC3b complex adheres to macrophages or phagocytes along with the microbe. Thus it promotes phagocytic activity.
8. The C4bC2aC3b complex activates C5 which splits into a small C5a fragment and a large C5b fragment. The C5b has two combining or binding sites. One site binds to antigen (microbe) and other to C6 and C7.
9. C5b binds with C6 and C7 to form a trimolecular complex called C5b,6,7.
10. C5b,6,7 then binds with C8 to form a tetramolecular complex C5b,6,7,8
11. C9 then binds with C5b,6,7,8 to form C5b,6,7,8,9 complex.

The final complex cause disruption of the lipid bilayer of the microbial membrane. As a result a hole is made on the microbe and through this hole the contents of the cell are released and the cell is lysed. The cascade of events in complement recognition and activation culminating in cell attack are diagrammatically shown in figure 2.1.

Figure 2.1. Model of complement assembly on the membrane of a bacterial cell in the sequence of events in complement recognition, activation and cell attack

The activation of complement proteins starting with Clq to attachment of C9 is called classical pathway. The classical pathway of complement activation can be represented as follows (Fig. 2.2).

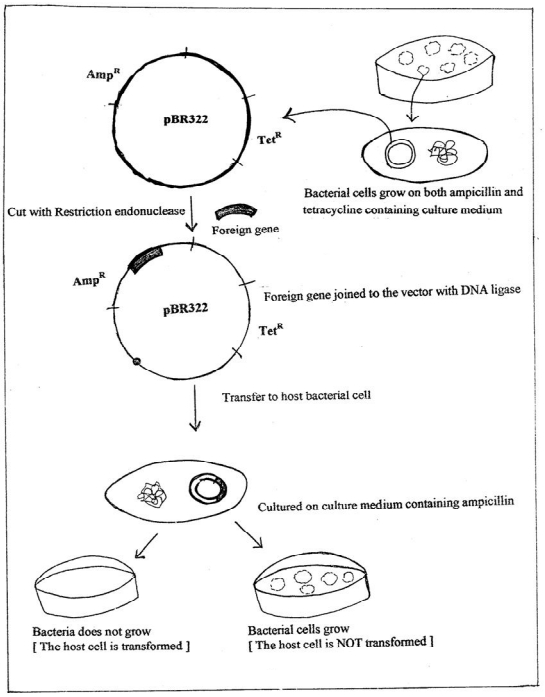

Fig-5. **Selection of transformed host cells containing recombinant vectors**

2.5.3. BIOLOGICAL ACTIVITIES OF COMPLEMENT COMPONENT

2.5.3.1. Opsonization: The complement system helps to adhere the microbe to the phagocytes (neutrophils and macrophages). This phenomenon is called Opsonization, which enhances ingestion of microbes by phagocytes. C3b component of complement is the main opsonizing factor as it has binding sites to both microbe and phagocytes.

2.5.3.2. Lysis: The attachment of complement proteins to Antigen-Antibody complex is called complement fixation. Mere attachment of a specific antibody to surface of the microbial pathogen do not result in lysis of the microbe, unless complement is also fixed. When complement (Cl-9) is fixed it causes lysis of viruses, virus infected cells, tumor cells, mycoplasma, bacteria and protozoa. Lysis of a body cell such as a tumor cell is called cytolysis. Lysis of a bacterial cell is called bacteriolysis and lysis of RBCs is called haemolysis.

When complement proteins bind to the microbial cell it causes disruption of lipid bilayer of the membrane of the microbe. As a result a hole is made on the microbe. Through the hole the contents of the cell are released and the cell dies. Cytolysis starts with formation of C5b,6,7,8 complex and is enhanced with the addition of C9.

2.5.3.3. Chemotaxis: Complement bound Antigen-Antibody complexes release chemotactic factors and attract leucocytes which in turn release lysosomal enzymes. This helps in localization and inactivation of infectious agents or may enhance tissue injury. Thus, complement is one of the important initiator of inflammatory changes. The component C5a is responsible for chemotaxis of neutrophils and eosinophils.

2.5.3.4. Cytotoxicity: Complement augments IgG mediated cell cytotoxicity.

2.5.3.5. Effect on endotoxins: Endotoxins released by the lysis of Gram negative bacteria are inactivated by complement proteins especially C1-C5.

2.5.3.6. Aanaphylactic reaction: The complements C3a and C5a are considered as anphylatoxins. They cause release of histamine from mast cells. They have kinin like activity and also chemotactic activity.

2.5.4. PROPERDIN PROTEINS

When sufficient quantities of specific antibodies are not available for activation of complement through classical pathway, a set of proteins called properdin proteins directly activate C3 component of complement which attaches to the microbial cell. This process was first discovered by Pillemer in 1954 and is called alternative pathway or properdin pathway of complement activation.

The properdin proteins consists at least 4 factors called B, D, P and H. Their combined activity result in release of C3 convertase enzyme which cleaves C3 into C3a and C3b fragments. Then C3b may activate C5 and enter classical pathway to produce C5b,6,7,8,9 complex which cause lysis of the microbial cell.

The substances that can activate alternate pathway include lipopolysaccharides of Gram negative bacteria, bacterial capsules, techoic acids of Gram positive bacteria, fungal cell walls, aggregated globulins high in carbohydrate content, inulin , dextran etc.

2.6. INDUCED CHEMICAL BARRIER - INTERFERONS

Alick Issacs and Jean Lindenmann of National Institute of Medical Research, London in 1957 during their studies on virus multiplication in chick embryo cultures discovered that, virus infected cells produce a soluble factor that protects other cells from infection by it and other viruses. The virus inhibiting principle is identified as a small protein and is termed as interferon. Later it was discovered that interferon is not a single substance but a group of over 20 substances. Three main types of interferons were identified and designated as α-IFN, β-IFN and γ- IFN. Each of these IFNs has several components and all appear to be proteins.

α-IFN : It is a product of leucocytes or lymphocytes that are stimulated by infection with viruses, bacteria and other agents. It is encoded by over 20 genes present on chromosome 9.

β-IFN : It is a product of fibroblasts, epithelial cells and macrophages, produced in response to virus infection. It is encoded by a single gene on chromosome 9.

γ-IFN : It is a product of T cells that function in immune regulation. It is produced by a subset of T - memory cells in response to stimulation by an antigen previously encountered. It is thus regarded as a type of lymphokine and different from IFN- α and β, which are mainly nonspecific inhibitors of viral multiplication. (Lymphokines are proteins released by

lymphocytes and are mediators of immune response). It is encoded by a single gene on chromosome 12.

2. 6. 1. INDUCERS OF IFN PRODUCTION

Viruses are the major inducers of IFN production. Among the viruses, RNA viruses are stronger inducers than DNA viruses. They are also induced by natural or synthetic dsRNA, some bacteria like Chlamydia and bacterial endotoxins.

2. 6. 2. PROPERTIES OF INTERFERONS

1. Interferons are proteins with mol. wt. 20,000
2. They are relatively stable at low pH (of about 2) except gamma interferon.
3. Interferons are not virus specific. The IFNs induced by one virus are effective against a number of other viruses.
4. IFNs are host species specific. Hence the IFNs produced by humans are effective in humans only. The IFNs produced by a guinea pig is ineffective in mouse or human cells.
5. IFNs are extremely potent and only small amounts are required for function. It has been estimated that less than 5- molecules of interferon per cell are sufficient to induce antiviral state.

2.6.4. FUNCTIONS OF ENTERFERONS

2.6.4.1. Prevention of viral replication: Development of antiviral state is the major effect of interferons. The IFNs appear within 48 hours after virus infection and play an important role in inducing antiviral state. They do not kill viruses nor act as antibodies. They do not interact with viruses but they protect the host cell from further infection by any viruses. For this reason they have been considered to have a broader inhibitory effect.

2.6.4.2. Inhibition of cell proliferation: IFNs can inhibit cell division and this property has been used to some effect in treating certain forms of cancer like leukemia, osteosarcoma in children etc.

2.6.4.3. Regulation of immune system: One of the most important activity of IFN is enhancement of the display on cell surfaces of histocompatibility antigens which are essential to antigen mediated activities of T cells. They also modulate both B and T cell activities.

2.6.4.4. Cytotoxicity: IFNs promote natural cytotoxicity of Natural Killer (NK) cells and cytotoxic T cells.

2.6.5. MODE OF ANTIVIRAL ACTION OF INTERFERONS

The interferons do not kill viruses or even interact with viruses but interact with host cells to produce antiviral state. The mechanism of action is complex and indirect. Different steps involved are as follows

1. When viruses attach to a host cell by specific receptors, they induce production of interferon molecules. They are produced in very low quantities and within 48 hours.
2. IFN molecules produced by virus infected cells diffuse out from the cell and induce antiviral state in neighbouring cells
3. IFN molecules attach to receptors on the nearby cells and induce them to release translation inhibiting principle (TIP) which specifically prevent virus multiplication. It is recognized that TIP comprise three enzymes namely a) Ribonuclease b) Protein kinase and c) 2-5 A synthetase.
4. Protein kinase and 2-5 A synthetase are activated by a double stranded RNA.
5. The activated 2-5 A synthetase in turn activate ribonuclease.
6. Ribonuclease degrade RNA
7. The activated protein kinase is capable of phosphorylating and thus inactivating a factor that initiates synthesis of viral proteins.
8. The end result is inhibition of viral replication, as RNA is degraded and viral polypeptides are not formed.

The different events in antiviral effect of interferons is shown in the figure 2.3.

Figure 2.3. Antiviral effect of interferon

2.6.6. IFN Synthesis: Interferons are produced in very low quantities by the virus infected cells and attempts to isolate from naturally infected cells in sufficient quantities are not successful. It impeded their use in experiments as well as antiviral drug. With the advent of recombinant technology, interferons are now synthesized in genetically modified bacteria.

The gene for human alpha interferon has been synthesized and inserted into Eschericihia coli through recombinant DNA technology, and it was mass cultured to produce alpha interferon.

2.6.7. Uses of IFN as a drug: In 1984 a Swiss biotechnology firm began marketing alpha interferon using the trade name Intron. IFNs are now used against leukemia, chronic hepatitis-B and genital herpes .

The interferons, though nontoxic, are not free from side effects, some of the side effects are psychiatric changes, fatigue, depression and even severe somnolence.

2.7. SUMMARY

Non specific immunity refers to the natural immunity of the host body that exists from the earliest of life and protects against invasion of all microorganisms. Skin and mucous membranes with their secretions act as mechanical barriers to infection and constitute the body's first line of defence. Intact skin is highly effective barrier against microbial invasion as it comprises outer epidermal layer of keratinized cells which cannot be enzymatically attacked by the pathogens. The skin surface is dry and acidic which is highly inhospitable for growth of bacteria. Mucous membranes of the respiratory, digestive and urogenital tracts also act as mechanical barriers and mucous layer on the membranes trap many microorganisms until they can be disposed of mechanically or lose their viability

Stomach and female vagina are natural barriers for microbial colonization because of high acidity of their contents. Lysozyme is a thermostable enzyme that is present in several body fluids, and it disrupts the cell walls of Gram positive bacteria. Chemical substances in various body fluids and tissue secretions like leukins, phagocytin, betalysin, fibronectrin, polypeptides, iron chelators like trasferrin and lactoferrin etc act against invading microorganisms nonspecifically destroy them.

Complement refers to a group of nonspecific proteins present in blood, which are activated when antibodies form a complex with surface antigens of invading microorganisms, and destroy the microbe by sequentially depositing on it.

Interferons are a group of proteins that induced in the body when viruses infect the host cells and they nonspecifically act against viral pathogens inducing an antiviral state. There are three main types of interferons viz. α-, β-, γ- interferons. Their main functions include prevention of viral replication, inhibition of cell proliferation, regulation of immune system and cytotoxicity. Interferons, produced by recombinant DNA technology, are now a days are used as drugs in treatment of cancer, chronic hepatitis and herpes infections.

2.8. MODEL QUESTIONS

Essay type questions

1. What is nonspecific immunity? Give an account of different types of nonspecific host resistant mechanisms against microbial infections
2. Discuss the role of skin and mucous membranes as mechanical barriers to infections
3. Discuss the chemical barriers to microbial invasion of host body
4. What is complement? Discuss the classical pathway of activation of complement
5. Discuss the importance of complement against invasion of microbial Pathogens
6. Give an account of interferons and their mode of antiviral action

Short answer type questions

Mechanical barriers to infections
Skin as mechanical barrier
Mucous membranes as mechanical barriers
Preformed chemical barriers
Lysozyme
Complement
Significance of complement
Properdin pathway
Interferons
Functions of interferons

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LESSON – 3. NON-SPECIFIC HOST RESISTANCE MECHANISMS (PART-II)

OBJECTIVE: To know about the biological barriers to infection and factors effecting nonspecific host resistance mechanisms

CONTENTS

3.1. INTRODUCTION

In the first part of this lesson the mechanical barriers and chemical barriers to infection are explained. In this lesson the biological barriers for infections, inflammation as a nonspecific host resistance mechanism and factors affecting nonspecific host resistance are explained. The major biological barrier to infection by microorganisms is the activity of phagocytic cells in the blood as well as in the tissue systems. Normal flora is also considered as a biological barrier to infection because they occupy the sites which pathogens cannot reach and thus prevent infection.

When the pathogens over come various barriers to infection and infect the host cells, the host cells react to the invasion of microbes resulting inflammation, which limits the spread of the invading microorganisms.

3.2. PHAGOCYTIC CELLS

Phagocytosis, the process of engulfing the invading microorganisms and killing them by digestion, is a major nonspecific defence mechanism of the human body. The cells involved are called phagocytes. Elie Metchnikoff, an associate of Louis Pasteur, was the first to observe this process and proposed the theory of phagocytosis in 1884 as a major defence mechanism against invading pathogen and received nobel prize for his contribution in 1908

along with Paul Ehrlich, who proposed humoral theory of immunity. In 1882, Metchnikoff observed that amoeboid cells (leukocytes) ingest cells of yeast (Monospora bicuspidata) in the water flea Daphnia. Apparently he was among the first investigators to recognise the important role played by leucocytic ingestion in protecting a host from disease. He recognized two kinds of phagocytic cells and named them microphages and macrophages. In the present day terminology, microphages are mainly polymorphonuclear leucocytes (PMNLs), and macrophages are monocytes.

The phagocytic cells include polymorphonuclear leucocytes and monocytes of circulatory system as well as cells of reticuloendothelial system. This system, also called mononuclear phagocytic system, is a collection of monocyte derived cells that leave the circulation and undergo modification. They include Kupffer cells of the liver, macrophages of spleen, bonemarrow, lymph nodes, brain and connective system.

Five types of phagocytic cells are found in the human body. They are 1). PMNLs 2). Monocytes or macrophages 3). Lymphocytes 4). Eosinophils and 5). Basophils (Figure 3.1). All these phagocytic cells are collectively called white blood cells (WBCs) or leukocytes.

Figure 3.1. Human leucocytes a) Neutrophils b). eosinophils c). basophils d). lymphocytes e). monocytes

The normal concentration of WBCs in blood is 5000 to 8000 per ml, and more than 25 million WBCs wander through circulatory system. The normal proportion of different phagocytes in WBCs is as follows

Polymorphonuclear neutrophil leucocytes (PMNLs)	---	60 to 70%
Basophils	---	0 to 1%
Eosinophils	---	1 to 4%
Large Lymphocytes	---	0 to 3%
Small Lymphocytes	---	25 to 30 %
Monocytes	---	4 to 8%

3.2.1. POLYMORPHONUCLEAR LEUCOCYTES (PMNLs): These are small (10 to 12 μm size) actively motile cells containing many distinctly staining membranous granules called lysosomes. These granules contain several bactericidal substances and enzymes such as lysozyme, proteases, phosphatases, nucleases, lipases and also hydrogen peroxide. The PMN leucocytes are short lived (2-4 days) cells that appear in blood in large numbers during acute phage of infection and migrate to the site of inflammation.

The PMNLs are recognized by the distinctive shape of their nucleus, which is divided into 5 segments connected by thin bridges of nuclear material. They stain orange with neutral dyes. They constitute 60 to 70% of total leucocyte count in blood.

3.2.2. MONOCYTES: These are the largest cells (15 to 20 μm in size) normally found in blood. They are distinctive in appearance because of their nucleus, which may be horseshoe shaped or kidney shaped. They constitute 4-8% of total leucocyte count of blood.

3.2.3. MACROPHAGES: The monocyte derived cells that leave the circulation and undergo modification in tissues are called macrophages. They include kupffer cells of the liver and macrophages of spleen, bone marrow, lymph nodes, brain and connective tissues. These are larger than monocytes and have more lysosomes and a longer life span. Some are called resting cells because they are stationary while others are called wandering cells because they are actively motile. They play an important role in both active and chronic phases of infection and also in antibody formation.

3.2.4. LYMPHOCYTES: They constitute 25 to 35% of total leucocyte count. They measure about 8-12 μm in size and their nucleus is complete and round. Their life span ranges from 3 weeks to 3 months or more. They exert their antimicrobial activity mainly in synthesis of antibodies and regulation of immune response. They also show phagocytic activity against foreign eukaryotic cells and cancer cells.

3.2.5. EOSINOPHILS: They stain intensely red with acidic dyes like eosin. They measure about 10-12 μm in size and their nucleus is bilobed with lobes connected at the anterior end. Their life span is about 10-13 days. They constitute 1-4% of total leucocyte count. They also show phagocytic activity but are less active than PMNLs and macrophages. They also migrate to the site of inflammation. They are particularly effective in eliminating the larvae of helminth parasites.

3.2.6. BASOPHILS: They are least common of all phagocytic cells being only 0-1% of total leucocyte count. They are 8-10 μm in size and their nucleus is bilobed. They have a life span of about 15 days. They stain blue with basic dyes. They along with a type of cell found in connective tissue termed mast cells, contain histamine, which when released causes inflammation, a fundamental process that protects against infection.

The neutrophils. eosinophils and basophils together are termed granulocytes because they possess higher concentration of cytotoplasmic granules. The rest of the WBCs are called agranulocytes because they do not possess cytoplasmic granules.

3.2.6. NATURAL KILLER CELLS: NK cells are a unique group of defensive cells that roam the body in blood and lymph and kill cancer cells and virus infected cells before the immune system is enlisted. The name natural killer cells (NK cells) reflect the nonspecific nature of the killing activity. Originally they were considered as lymphocytes but now recognized as a special group of killer cells in body fluids. They are considered as lymphocytes of uncertain origin and without immunological specificity. Their activity is greatly enhanced by interferons.

3.3. MECHANISM OF PHAGOCYTOSIS

Phagocytes are attracted chemotactically to invading microbes and accumulation of phagocytes is the first indication of the presence of infection. The attraction is mediated by

unidentified substances released by the parasite. The phagocytes work best when they can trap a microbial cell upon a solid surface such as a vessel wall, a fibrin clot or even particulate macromolecules. The interaction between the parasite and phagocyte is also enhanced by the presence of antibodies. These protein molecules attach to parasites and thereby increase adherence to the phagocytic cell at specific receptor sites. In some situations, components of complement system bind the parasite to the phagocyte. Enhanced phagocytosis is called opsonization, and the antibodies and complement proteins that encourage it are called opsonins. The opsonins are first described by Almroth Wright in 1903.

After the parasite adheres to phagocytic cell, an invagination of the phagocyte's plasmamembrane envelopes the parasitic cell, and the entire complex is pinched off and enter the cytoplasm of phagocyte as a vacuole and it is described as phagosome. The lysosomes generated from golgi apparatus then fuse with the phagosome and at this stage it is called phagolysosome. The lysosome release enzymes responsible for destroying or digesting the microbial cell. At this stage it is called digestive vacuole. The process is completed as waste materials are ejected from the phagocyte. The sequence of events in phagocytosis are shown in the figure 3.2.

Figure 3.2. Phagocytosis of a microbial cell

The initial act of phagocytosis conditions a phagocyte so that it is more efficient in subsequent phagocytic action. A phagocytic cell that has recently phagocytized can take up bacteria about 10 times more efficiently than a phagocytic cell that has not.

The destruction and digestion of the phagocytized bacterial cell or other pathogen is carried by one or more mechanisms.

3.3.1. Enzymatic action: The lysosomes are store houses of a number of digestive enzymes (over 60), which are released into the phagosome on fusion of lysosome with phagosome. These enzymes include lysozyme, phospholipase, ribonuclease, deoxyribonuclease, proteases etc. They bring about the enzymatic degradation of the trapped pathogen.

3.3.2. Acidic pH: During the process of phagocytosis the metabolism of the phagocytic cell changes from aerobic pathway to anaerobic pathway which results in formation of organic acids such as lactic acid. Hence pH of the cell drops. The acidic pH is partly responsible for the death of the trapped microbe.

3.3.3. Toxic forms of oxygen: The phagocytic cells also make use of toxic forms of oxygen in killing the trapped microbial cell. Super oxide (O2-) is generated upon phagocytosis. It is directly lethal to many microorganisms. Super oxide ions, hydrogen peroxide (H2O2), singlet oxygen (O1) and hydroxyl radical (OH) are also produced in phagocytic cell and help in killing the microbe.

$$NADPH + 2O_2 \xrightarrow{\text{NADPH oxidase}} 2\,O_2^{-}\text{ (super oxide)} + H^{+} + NADP$$

$$2\,O_2^{-} + 2\,H^{+} \xrightarrow{\text{Superoxide dismutase}} H_2O_2 + O_2$$

$$H_2O_2 + Cl \xrightarrow{\text{Peroxidase}} \text{HOC1 (hypochlorous acid)} + OH^{-}$$

$$ClO + H_2O_2 \longrightarrow {}^1O \text{ (singlet oxygen)} + OH$$

$$O_2 + H_2O_2 \longrightarrow OH + OH^{-} + O$$

These reactions occur in phagosomes and their increased oxygen utilization is termed "oxygen burst". It occurs even before the lysosomal fusion with phasome.

The oxygen burst is due to enhanced activity of hexose monophosphate shunt pathway of metabolism. Its pathway is shown in the figure 3.3.

Figure 3.3. The respiratory burst that produces bactericidal products

3.3.4. Reactive nitrogen intermediates (RNIs): Macrophages, neutrophils and mast cells have been shown to form "reactive nitrogen intermediates" when stimulated by interferons. These molecules include nitric oxide (NO), nitrite (NO2) and nitrate (NO3). These RNIs are very potent cytotoxic agents. Nitric oxide is most effective RNI. Macrophages produce it from aminoacid arginine when stimulated by cytokinins. Macrophage killing of Herpes Simplex Virus (HSV), Toxoplasma gondii, Leishmania major, Cryptococcus neoformans and tumor cells involve RNIs.

3.3.5. Action of defensins: Neutrophils produce a group of broad spectrum antimicrobial peptides called defensins. There are 4 human defensins called Human neutrophil proteins (HNPs) HNP-1,2,3 and 4. These defensins are formed during neutrophil formation in bone marrow, and are stored in cytoplasmic granules of mature cells. Hence nuetrophils are also called granulocytes. The release of these peptides occur as the granules disintegrate after phagocytosis and help in destroying the pathogen.

3.3.4 Evasion of phagocytic destruction by pathogens: Some pathogens evade the destruction by phagocytic cells after internalization and become intracellular pathogens. The intracellular pathogens employ diverse strategies to survive within macrophages

Mycobacterium tuberculosis, M. leprae, Legionella pneumophila and Toxoplasma gondii inhibit phagosome fusion with lysosomes, thereby preventing exposure to toxic lysosomal contents.

Trypanasoma cruzi, Listeria monocytogenes and Shigella flexneri lyse the phagosomal membrane and escape into cytoplasm.

Leishmania spp., Mycobacterium lepraemurium, Salmonella typhimurium etc. lie within the macrophage in a phagosomal compartment where they apparently resist inactivation by lysosomal factors.

3.4. NORMAL FLORA AS BIOLOGICAL BARRIERS:

The members of normal flora occupy their own niches in the body and inhibit foreign microorganisms invading from other parts of the body or from external environment. Such inhibition is brought about by a) competition for food and space b) production of antibiotic substances or other inhibitory substances c) changes in the environmental conditions such as oxygen content, pH and d) secretion of mucin and laying down a mucous blanket that prevents attachment of pathogenic organisms.

3.5. INFLAMMATION:

Inflammation is one of the most important non specific host defence mechanisms. It probably occurs throughout the body on a small unnoticed scale everyday, but is most easily recognized when it occurs on the surface of the skin. The tissues of the host react to infection or mechanical injury by an inflammatory response. The characteristic symptoms of inflammation are

1. Erythema (redness or rubor)
2. Edema (swelling or tubor)
3. Heat (calor)
4. Pain (dolor)

The infection or injury causes dilation of blood vessels and accumulation of blood which result in redness or rubor. The dilation of blood vessels is accompanied by an increased permeability which causes swelling (edema or tumor) as tissue fluid accumulates in the spaces surrounding the tissue cells. The increased diameter of the blood vessels increases the flow of warm blood to the injured area, thereby raising the temperature, resulting in a condition of calor (not fever). The pain (or dolor) experienced in an inflammatory reaction is due to the pressure exerted by the accumulated fluids on local sensory nerves or direct injury to the local nerves. Within the inflammed area, a fibrin clot is usually formed which localize the invading microbes. The pathogens that produce fibrinolytic enzymes may be able to escape and continue to invade the body.

The principal events of the inflammatory process and their effect are as follows

EVENT	EFFECT
Tissue injury	Release of kinins, histamines and other mediators to act on adjacent blood vessels
Blood vessels dilate and show increased permeability to plasma which may clot	Swelling of the tissues result from leakage of plasma, elevated temperature of the region may occur as a result of increased blood flow through the dilated vessel, redness may appear for the same reason. Pain result from increased fluid in the tissues and from direct effect of mediators on sensory nerve endings.
Circulating white blood cells adhere to the walls of altered blood vessels	The WBCs migrate chemotactically through the vessel walls and to the area of injury, They are responsible for phagocytosis of foreign material and tissue debris, and for initiating antibody formation.

3.5.1. TYPES OF INFLAMMATION

Depending upon the nature and morphology, various types of inflammation are recognized.

3.5.1.1. Acute inflammation: A relatively short lived inflammation characterized by exudation of fluid and plasma proteins and the emigration of leucocytes, predominantly neutrophils.

3.5.1.2. Chronic inflammation: It is of long duration type and is associated histologically with the presence of lymphocytes and macrophages, and with proliferating blood vessels and connective tissue. It is also described as granulomatous inflammation. Granuloma is nodular mass formed by fibroblasts, macrophages surrounded by lymphocytes.

3.5.1.3. Serous inflammation: It is marked by the outpouring of a thin fluid derived either from blood serum or the secretions of the mesothelial cells.

3.5.1.4. Fibrinous inflammation: With more severe injury and resulting greater vascular permeability, large fibrinous inflammatory exudates develop (exudates containing coagulated fibrin).

3.5.1.5. Suppurative or purulent inflammation: This form of inflammation is characterized by the production of large amounts of pus or purulent exudates. Certain pyogenic bacteria such as Staphylococci produce suppurative inflammation.

After the phagocytes have destroyed the microbial cells and engulfed the tissue debris, they become degranulated and die. In the involved area a central mass of fluid is formed by the remains of damaged cells, dead phagocytes and microbial casualties. This fluid is called pus.

3.5.1.6. Catarrhal inflammation: It is a form of inflammation mainly effecting mucous membranes with copious discharge of mucous and epithelial debris. Conjunctival inflammation is the best example for this type and it is hence called catarrhal inflammation.

3.5.1.7. Pseudomembranous inflammation: It is an acute inflammatory response to a powerful necrotizing toxin like diphtheria toxin and form a psuedomembrane. The best example is the formation of diphtheros or psuedomembrane in diphtheria.

3.5.1.8. Ulcers: An ulcer is a local defect of the surface of an organ or tissue that is produced by shedding of inflammatory necrotic tissue. Ulceration can occur only when inflammatory

necrotic area exists on or near the surface. Ulcers commonly occur in mouth, stomach, intestine or urogenital tract.

3.5.2. MECHANISM OF INFLAMMATION:

The inflammatory response is triggered by a complex set of events induced by pathogen invasion or tissue injury. Cells ruptured in the infected area release their cytoplasmic contents, which in turn raises the acidity in the surrounding extracellular fluid. This decrease in pH activates an extracellular enzyme called Kallikrein. It acts on a plasma glycoprotein termed high molecular weight kininogen (HMWK) and releases bradykinin from it. The bradykinin binds to the receptors on the blood capillary wall and also to the mast cells in the connective tissue associated with most small blood vessels.

Binding of bradykinin to the walls of blood vessels result in opening of the junction between the cells, which allows fluid and infection fighting leucocytes to leave the capillary and enter the infected tissue.

Binding of bradykinin to mast cells leads to active influx of calcium ions into the mast cells which result in degranulation and release of preformed mediators such as histamine, heparin, enzymes and chemotactic factors. The activities of these mediators are as follows

Histamine	:	cause dilation of blood vessel and as a result more fluid and leucocytes move out and cause edema and swelling.
Heparin	:	It is an anticoagulant
Proteolytic enzymes	:	They include tryptase and other proteolytic enzymes that activate C3 component of complement system
Chemotactic factors	:	Attract phagocytes like neutrophils, basophils, eosinophils monocytes etc.

A change in mast cell plasmamembrane permeability associated with activation stimulates phospholipase A2 to act on plasmamembrane to release arachidonic acid (AA), a poly unsaturated fatty acid that is present in large amounts in phospholipids of the cell membrane. The arachidonic acid thus released is metabolized by enzymes cyclooxygenase and lipooxygenase. When AA is metabolized by cyclooxygenase, two chemical mediators namely Thromboxane A2 and prostaglandins are released. Thromboxane causes vasodilation

and platelet aggregation which cause temporary clotting to obstruct loss of blood. Prostaglandins E2 and F2 promote swelling and also bind to free nerve endings starting a pain impulse. When AA is metabolized by lipooxygenase, leukotrienes (C4 D4 E4) and slow reactive substances are released. Leukotrienes are vasoactive and cause increased permeability. Slow reactive substances cause vasoconstriction.

The overall effect of the reactions or changes during inflammation are

1. increase in blood flow and capillary dilation which bring into the area more antimicrobial factors and leucocytes
2. the rise in temperature stimulate inflammatory response and inhibit microbial growth
3. a fibrin clot may be formed which limit the spread of invading microorganism
4. phagocytes collect in the inflammed area and phagocytose the pathogen. Neutrophils arrive first followed by macrophages.

The flow chart of biochemical events of inflammation are shown in the figure 3.4.

Figure 3.4. Biochemical events of inflammation

3.6. FACTORS AFFECTING NONSPECIFIC HOST RESISTANCE

Non-specific host resistance mechanisms are affected by a number of factors of which genetic factors are most important. Other factors include age, sex, nutrition, hormones and various physical and individual factors.

3.6.1. Genetic factors: These are clearly responsible for the innate resistance of a genus or species to infection by a particular pathogen, Genetic factors are also responsible for variation in resistance shown by races, families and individuals within a species.

3.6.2. Age: Infants are very susceptible to bacterial diseases because of insufficient development of resistance factors. Older people also become susceptible due to break down of resistance factors.

3.6.3. Sex: Even though both male and female are equally susceptible or resistant to most of the diseases, certain differences with respect to certain diseases are obvious. For example, women carry Salmonella typhi, the causal organism of typhoid, in their gall bladder much more frequently than men. Hence, recurrence of typhoid is more in female than in male.

The fungus Paracoccidioides brasiliensis infects both sexes equally but overt disease is over 10 times more common in men than in women.

The protozoan flagellate Trichomonas vaginalis, infect both male and female but it causes vaginitis in female while male are symptom less carriers.

3.6.4. Harmones: Hormone is a chemical substance produced in the body which has specific regulatory effect on the activity of the certain cells or a certain organ. Hormonal imbalance greatly affects the resistance of the individuals. Corticotrophin and corticosteroids inhibit the inflammatory reaction and lower resistance to bacterial and viral infections. Metabolic derangements due to lack of insulin, a hormone produced by pancreas and regulate blood glucose, may be responsible for susceptibility of diabetics to staphylococcal infections and tuberculosis.

3.6.5. Nutrition: Gross deficiencies of protein or vitamins A, B and C are usually associated with increased susceptibility to bacterial infections.

3.6.6. Other factors: Various other conditions that lower host resistance and their effect are as follows

Factor/ condition	Effect
Alcoholism	Possible depression of the inflammatory response to infection
Indiscriminate use of antibiotics	Elimination of normal flora, over growth of resistant microbial flora interference with digestive process and vitamin utilization
Immunosuppression (Immunotherapy)	Impairment of cell mediated immune mechanisms
Complement deficiency	Inability to inactivate or destroy certain infectious disease agents
Circulatory disturbances in tissues	Localized destruction of tissues, congestion, accumulation of fluid
Acute radiation injury	Alteration of cellular defences of the host
Atmospheric pollutants	Depressed immunological function of polymorphonuclear leucocytes. accumulation of eosinophils (a condition of eosinophilia)
Traumatic injury	Direct access of body tissues for opportunistic pathogens. Possible interference with immunity mechanisms and destruction of body drainage systems
Agranulocytosis	Reduction or absence of phagocytosis by neutrophils

3.7. SUMMARY:

Phagocytic cells and normal flora constitute the nonspecific biological barriers to microbial infection. Five types of phagocytic cells are found in the human body, especially in the blood. They are polymorphonuclear leucocytes (PMNLs), eosinophils, basophils, monocytes and lymphocytes. They are collectively called white blood cells. PMNLs

constitute 60 to 70% of the total WBCs in the blood and, they small actively motile cells that are first to act against invading microorganisms. Macrophages, which are relatively bigger cells, constitute 4-8% of total WBCs. They slowly accumulate around the invading cells but are more active phagocytes. Macrophages are cells similar to monocytes but are present in body tissues. Eosinophils, basophils and lymphocytes have relatively lesser phagocytic activity. The mechanism of phagocytosis is similar to the process of endocytosis in which protozoan cells take up particulate food. The phagocytized cells are destroyed by various mechanisms like enzymatic degradation and by the action of toxic forms of oxygen, formed during oxygen burst following phagocytiosis, and by the activity of reactive nitrogen intermediates and defensins. Those that escape phagocytic destruction become intracellular pathogens and cause chronic infections.

The normal flora of the human body also is a biological barrier to infection because normal flora organisms occupy the binding sites on host cells and also by competing with invading microorganisms for nutrients, and producing antimicrobial substances.

When microorganisms escape other non specific barriers and infect the host cells, the cells of the body are injured and inflammation occurs. Erythema, edema, heat and pain are characteristics of inflammatory reaction. During inflammation, blood capillaries dilate and increase the flow of blood to site of injury. The increased flow of blood brings into the area more antimicrobial factors and leucocytes. Fibrin clot may form which limit the spread of the invading microorganism. Phagocytes collect in the inflamed area and phagocytose the pathogen.

Nonspecific host resistance mechanisms are affected by a number of factors which include genetic factors, age, sex, hormones, nutrition etc. Other factors like alcoholism, indiscriminate use of antibiotics, complement deficiency; radiation injury, atmospheric pollution etc also decrease the nonspecific resistance mechanisms of human body.

3.8. MODEL QUESTIONS

Essay type questions

Give an account of phagocytic cells and phagocytosis

Discuss the mechanism of phagocytosis and destruction of phgocytized cells

What is inflammation? Give an account of mechanism of inflammation and its signigicance

Discuss the factors effecting nonspecific immunity

Short answer type questions

Biological barriers to infection

Phagocytic cells

Granulocytes

PMNLs

Macrophages

Phagocytosis

Digestion of phogocytized cells

Natural killer cells

Inflammation

Mechanism of inflammation

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LESSON – 4: VIRULENCE OF THE PATHOGENS

OBJECTIVE : To study about the concept of virulence and virulence characters exhibited by the pathogens for causing infection

CONTENTS

4.1. INTRODUCTION

Only a small percentage of tens of thousands of known microorganisms are capable of overcoming the defence mechanisms of the host to cause disease. The ability of a microorganism to cause disease is called its pathogenicity and the organism is called a pathogen. Pathogens vary greatly in their pathogenicity. Some pathogens such as cholera, plague and typhoid bacilli are well known for their ability to cause serious human disease, while some pathogens such as common cold viruses are considered as less pathogenic because they cause milder illness only. Some microorganisms like *Candida albicans*, though occur within the host usually do not cause disease but do so when the host defences or immunity is lowered. Thus, all pathogens (belonging to different groups or genera) are not having the same degree of pathogenicity.

Even within a pathogenic genus or species also all the members do not show same level of pathogenicity. For example, all the four species of *Shigella* cause bacterial dysentery but the one caused by *S. dysenteriae* is most severe followed by that caused by *S. flexneri* and *S. boydii*, and that caused by *S. sonnei* is very mild. In the same way malaria is caused by four species of *Plasmodium*, but among the four species *P. falciparum* cause perinicious form of malaria called brain fever malaria, while *P. malariae* cause mild disease or benign malaria.

Even within a species different strains show variation in their pathogenicity. For example, *Streptococcus pneumoniae* is a serious pathogen that cause primary pneumonia in

humans but only capsulated strains are pathogenic while noncapsulate strains are non pathogenic. In the same way only coagulase positive strains of *Staphylococcus aureus* are pathogenic and coagulase negative strains are nonpathogenic.

The degree of pathogenicity exhibited by microbial species or strains is expressed as their virulence.

4.2. CONCEPT OF VIRULENCE:

The term virulence is derived from latin word virulentus which means full of poison. Virulence is expressed in comparative terms and the pathogens that invariably cause serious disease are described as highly virulent, while the pathogens that cause mild disease are termed moderately virulent. The strains within a pathogenic species that lost their ability to cause disease are described as avirulent.

The virulence of a pathogen is usually measured by determining its LD_{50} (Lethal dose 50) value for a particular type of laboratory animal. The LD_{50} dose is defined as the number of organisms which when administered to a number of laboratory animals will kill 50 per cent of them within a specified time under standard conditions. The LD_{50} dose can be determined more precisely than other end points such as dose that kills 100 per cent of the test animals (i,e, LD_{100} dose some times also termed minimum Lethal dose MLD) because the rate of change in mortality versus changes in dose is greatest around the point of 50 percent mortality.

The virulence of a pathogen is not an absolute character but depends upon a number of factors, the most important being host resistance and inoculum dose.

Even when a host is susceptible to the pathogenic strain in general, the level of resistance or susceptibility is influenced by a number of factors such as general health, age, nutrition, prior exposure to the pathogen, underlying diseases etc.

Dose or inoculum density of a pathogen refers to the number of individual cells that must be taken into the body for the disease to be established. Experiments indicate that consumption of a mere million typhoid bacilli in contaminated water (at a concentration of 10^3 to $l0^8$ per ml) lead to the disease, where as many times that number of cholera bacilli (at the rate of 10^7 per ml) must be ingested if cholera is to be established. One explanation offered is the high resistance of typhoid bacilli to acidic conditions in the stomach in contrast

to the low resistance of cholera bacilli. When the host is exposed to low doses of a pathogen continuously immunity against the pathogen develops and high doses are needed to breach the resistance of the host.

The relationship between the virulence of the pathogen, inoculum dose and resistant state of the host to establish disease is expressed as

$$\text{Infections} = \frac{V \times D}{RS}$$

where V = virulence of the pathogen
D = dose or no. of organisms
RS = resistance of the host

The disease develops only when the value is more than one i.e. the value of virulence of the pathogen and inoculum density is more than the value of host resistance.

4.3. FACTORS EFFECTING VIRULENEE:

The virulence of a pathogen can either increased (exalted) or decreased (attenuated) by various factors like genetic changes, prolonged storage, growth conditions, passage through animals etc.

4.3.1. Effect of genetic changes: Classical experiments of Griffith showed that non capsulate strains of *Streptococcus pneumoniae* can become virulent by genetic transfer brought about by transformation.

Microorganisms may mutate and become virulent or avirulent. For example, *E. coli* was long considered as an avirulent commensal of humans, but toxin producing strains are now isolated during out breaks of human gastroenteritis and these are virulent strains. The mutated strains of *Neisseria gonorrhoea*e that lack pili for attachment to the host cells lose virulence.

4.3.2. Prolonged storage: The strains of *Pasteurella aviseptica*, which cause chicken cholera, become avirulent or attenuated by prolonged storage, as shown by classical experiments of Louis Pasteur.

4.3.3. Growth conditions: *Bacillus anthracis*, the causal organism of anthrax in sheep, become avirulent or attenuated when pathogen is cultured at temperature above 42 ^{0}C while those cultured at 37 ^{0}C retain virulence.

The pathogens also lose virulence when they are cultured in presence of an inhibitory substance. For example, bovine strains of *Mycobacterium tuberculosis* are attenuated by repeatedly growing them in medium containing bile salts.

4.3.4. Passage through animals: Virulence of a pathogen may be exalted by passing the strain through a series of individual animals of the same susceptible species, inoculating animals one from another in succession i.e. passage. In this way a mutant strain may develop that has increased virulence for that particular species. This was first demonstrated by Pasteur by repeated inoculation of rabies virus into the rabbit. The virulence of *Vibrio cholerae, Streptococcus pyogenes, S. pnuemoniae* also increase when passed successively through susceptible animals.

Attenuation usually results when organisms are passed through animals of a different species. For example, *Bacillus anthracis* when passed through guinea pigs or rats attenuation occurs.

4.4. BASIS OF VIRULENCE:

To cause disease in a host a pathogen must

a) attach to the specific tissues of the host after entry
b) multiply within the host tissues
c) resist host defence mechanisms and
d) damage the host.

All virulent pathogens are endowed with mechanisms to carryout all these steps and cause disease. The virulence mainly depends on two factors that may be largely independent of one another 1. invasive capacity and 2. toxigenicity.

4.5. INVASIVE FACTORS:

To establish a host parasite relation and cause disease, a pathogen must be able to colonize a surface and invade tissues of the host. For this purpose different pathogenic

microorganisms have evolved different mechanisms. They include adhesins, antiphagocytic factors, penetrating factors, spreading factors etc.

4.5.1. ADHESINS: In order to colonize host tissue, especially mucous membranes, pathogens must be able to adhere to the host tissue. In gastrointestinal, urogenital and respiratory tracts the surface of mucous membranes are recurrently washed with fluids that sweep away unattached organisms. Further, the organisms must compete with normal flora organisms for surface attachment. Bacteria and viruses have specific mechanisms for adhesion to host tissue, but precise means of attachment of pathogenic fungi and protozoa are not yet clearly understood,

4.5.1.1. Bacteria1 adhesins: Bacterial adhesins that bind bacteria to host cells are limited in type and include pili or fimbriae, lipoteichoic acid, surface proteins and antigens.

4.5.1.1.1. Fimbriae or Pili: Nonflagellar filamentous structures called pili or fimbriae are present on the surface of many gram negative bacteria. The pili are made up of repeating subunits and the basal unit that anchors pilus to the bacterial cell wall is similar in widely divergent bacteria (Eg.: *E. coli, Psuedomonas, Neisseria* etc.) but differ in the composition of the distal unit, with respect to minor protein components and it gives specificity to the pili. Most of the pili help in adherence of bacterium to the host cells with their distal proteins. The strains of *E.coli* associated with different infection types have antigenically different pili, which gives them specificity in attachment to particular type of cells.

E. coli Type I pili : bind mannose and cause lower urinary tract infections and intestinal infections

E. coli Type P pili : bind galactose and cause pyelonephritis of urinary tract infections

E. coli Type S pili : bind sialic acid and cause meningitis

Such specific attachment of pathogens to host tissue through pili is also observed in *Neisseria gonorrhoeae*. It adheres specifically to the epithelial cells of human urogenital tract. The strains that lose the ability to produce pili also lose ability to bind tightly to the host and hence lose ability to colonize. Hence, pili in *N. gonorrhoeae* is called virulence factor.

4.5.1.1.2. Lipoteichoic acids: The surface of gram positive cocci such as streptococci is covered with lipoteichoic acids. These lipoteichoic acids are hydrophobic and bind to the surface of all eucaryotes, although with a higher frequency or affinity to particular receptors on blood cells and oral epithelial cells.

4.5.1.1.3. Surface proteins: *Streptococcus pyogenes* attach to epithelial cells of throat by M - proteins, which are present on the surface of the cell wall. *Vibrio cholerae* adheres to epithelial cells of small intestine through a surface component protein. It may be a hemaglutinin protein since it also agglutinate RBCs in lab experiments.

4.5.1.1.4. Surface antigens: In case of *E. coli* majority of strains that produce diarrhoeal disease have specific pili, Type I pili, that strongly adheres to intestinal mucosa. The strains that donot possess these pili also some times cause diarrhoeal infection and such strains possess surface antigens termed colonization factor antigen Type I and II (CFA I and CFA II) and these also function as adhesins.

4.5.1.2. Viral adhesins: Many viruses, especially enveloped viruses, possess specific spikes on their envelope which are identified as adhesins. In influenza virus, two types of spikes are present on the envelope 1. Neuraminidase (N) spikes and 2. Hemagglutinin (H) spikes. Neuraminidase of N spikes have enzymatic activity and hydrolyse mucous of respiratory tract thus exposing the epithelial surface. The virus attaches to the epithelial cells by hemagglutinin spikes.

In HIV, glycoprotein (gp120) of the spike is a specific adhesin which attaches to CD_4 proteins of the T-lymphocytes.

4.5.2. ANTIPHAGOCYTIC FACTORS:

Non pathogenic bacteria that are deposited in a wound or able to enter other tissues are usually eliminated very rapidly by phagocytic cells of the host. However, invasive pathogens possess special properties that protect them from elimination by phagocytosis. Capsules, surface proteins, leucocidins etc. are important antiphagocytic factors exhibited by invasive pathogens.

4.5.2.1. Capsules: Many pathogens produce a capsule that confers resistance to phagocytosis, as was first demonstrated in studies on *Streptococcus pnuemoniae*. Virulent strains possess capsules and are resistant to phagocytosis whereas avirulent strains lack capsules and are easily phagocytized. In other bacteria like *Haemophilous influenzae, Klebsiella pneumoniae, Neisseria meningitidis, Bacillus anthracis, Yersinia pestis* also capsules act as antiphagocytic factor. The major antiphagocytic factor of these bacteria, except *B, anthracis*, is identified as a polysaccharide component of capsule. The polysaccharide can be extracted and purified. The purified polysaccharide can neutralize the phagocytosis promoting effect of specific antisera.

4.5.2.2. Surface proteins: Most pathogenic bacteria donot possess obvious capsules but possess surface proteins that have antiphagocytic function. For example, M-protein of *Streptococcus pyogenes* which not only helps the pathogens to attach to epithelial cells but also antiphagocytic in nature.

4.5.2.3. Leucocidins and Leucostatins: Some pathogens produce extracellular toxic substances that may kill phagocytic leucocytes and are called leucocidins. Species of staphylococci and streptococci produce leucocidins. Once liberated these leucocidins attaches the leucocyte membranes and trigger a series of changes leading to degranulation and death of the leucocytes. The leucocidins are usually most effective after phagocytes have engulfed the microbe and the chemical comes in direct contact with the interior of the phagocyte,

Leucostatins are extracellular proteins released by the pathogens that interfere with phagocyte's ability to engulf the pathogen.

4.5.2.4. Resistance to degradation by phagocytic cells: Some pathogens are relatively easily phagocytized but are not killed within the phagocytes, either by interfering with fusion of lysosome with phagosome or by lysing the membrane of the pathogen. Such pathogens produce chronic disease and include *Mycobacterium tuberculosis, M. leprae, Salmonella* etc. Some pathogens that survive within the host phagocytes

hogen	Disease
Mycobacterium tuberculosis	Tuberculosis
M. leprae	Leprosy
Salmonella typhi	Typhoid
Yersinia pestis	Bubonic plague
Brucella abortus	Brucellosis (undulating fever)
Listeria monocytogenes	Listeriosis
Nocardia asteroides	Nocardiosis
Francisella tularensis	Tularemia
Chlamydia trachomatis	Lymphogranuloma venereum

4.5.3. PENETRATION FACTORS: Tissue penetration by the pathogens that were able to adhere to specific host cell receptors is important for pathogenis in many diseases, except in case of such diseases like cholera, tetanus, pertussis etc. which are essentially caused by the release of the toxins produced by the pathogens. The ability to penetrate the host cells is a property of invasive pathogens.

Experiments reported by Stanley Falkow and his coworkers at Stanford University indicate that genes for cell penetration exist on the chromosome of certain bacteria. These genes appear to code for surface proteins that assist penetration. In the late 1980s Falkow's group successfully isolated penetration genes from *Yersinia psuedotuberculosis* and inserted them into *E. coli* which then displayed penetration. Penetration genes occur on plasmids in *Shigella flexneri*

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4.5.4. BACTERIAL ENZYMES IN PATHOGENESIS:

The virulence of a pathogen depends to some extent on its ability to produce a series of enzymes directed at host defences. The enzymes act on host cells and interfere with certain functions meant to retard invasion. Important bacterial enzymes that help in pathogenesis are haemolysins, leucocidins, fibrinolysin, coagulase, hyaluronidase, nucleases etc.

4.5.4.1. Haemolysins: Haemolysins are a series of enzymes that dissolve red blood cells. Studies show that haemolysins combine with the membranes of erythrocytes after which lysis takes place. A number of bacteria produce haemolysins and they are named after bacteria which produce them Eg. Streptolysin, Staphylolysin, tetanolysin, pneumolysin etc. The haemolysins are proteins in nature and are heat labile. They are antigenic and stimulate production of antihaemolysins when injected into rabbits.

In gasgangrene produced by *Clostridium perfringens*, haemolysins lead to substantial anemia. In pathogenic streptococci and staphylococci also haemolytic activity is associated with the virulence of the organisms. A number of saprophytic bacteria may also produce haemolysins Eg. *Bacillus subtilis*.

A single bacterial species may produce more than one haemolysin. For example, *Staphylococcus aureus* produce three types of haemolysins alpha (α), beta (β) and gamma (γ) haemolysins which differ in their action on RBCs. Streptococci are often grouped on their haemolytic actitivity on sheep blood RBCs.

α - Streptococci: Cause partial lysis of sheep RBCs producing discoloration zone around colonies growting on blood agar medium Eg. *Streptococcuc pneumoniae.*

β - Streptococci: Cause complete lysis of RBCs Eg. *S .pyogenes*

γ - Streptococci: Do not cause lysis of sheep RBCs and they are usually non pathogenic Eg. Enterostreptococci .

4.5.4.2. Leucocidins: Some organisms such as *Staphylococcus aureus* and *Streptococcus pyogenes* produce complex substances which kill leucocytes and these are called leucocidins. In streptococci haemolysins and leucocidins are identical, but in staphylococci they are different. Leucocidins contribute to the virulence of the pathogen by killing the leucocytes.

4.5.4.3. Fibrinolysin: Many streptococci produce a substance that had the power of dissolving fibrin of preformed human blood clot and it is called fibrinolysin. Fibrinolysin is protein in nature, thermostable and antigenic. It acts by activating plasmin present in human blood and thus causing dissolution of the clot which helps in spread of the pathogen. Fibrinolysin produced by streptococci is termed streptokinase.

4.5.4.4. Coagulase: Clotting of plasma is accelerated by an enzyme coagulase produced by pathogenic organisms like *Staphylococcus aureus* and it may be responsible for thrombosis

(= formation of clot) produced in this infection. Occasionally strains of other bacteria like *Pseudomonas aeruginosa, Bacillus subtilis* also exhibit this property.

Virulence of staphylococci is associated with their ability to produce coagulase, and only coagulase positive strains are pathogenic.

It is very difficult to evaluate the significance of the clotting of blood as a determining factor of virulence but it has been observed that this property may help the pathogen as defensive mechanism against the action of immune bodies of the patient.

4.5.4.5. Hyaluronidase: It is sometimes called the spreading factor because it enhances penetration of a pathogen through the tissues. The enzyme digests hyaluronic acid, a viscous mucopolysacharide that binds the cells together in a tissue. The term 'tissue cement' is occasionally applied to this polysaccharide. As hyaluronic acid is spilt up, the natural barrier is overcome by bacteria and they easily spread through the tissue.

Hyaluronidase is an important virulence factor in pneumococci and certain species of streptococci and staphylococci. In streptococci, hyaluronidase is present in their capsular substance and virulence is thus associated with the presence of capsule in them. The gasgangrene pathogen, *Clostridium perfringens*, also use it to facilitate its spread through the muscle tissue.

4.5.4.6. Deoxy ribonuclease (DNAase): It is produced by most streptococci of Group A and Group B, and it depolymerizes deoxyribinucleoprotein to simple polynucleotides. Its production is essential in some strains for pathogenicity.

4.5.5. OTHER FACTORS WHICH HELP MICROBIAL INVASION

Apart from the various factors described above other factors like the ability of bacteria to gather iron, evasion of immune system etc also help in pathogenisis.

4.5.5.1. Microbial Iron chelators (Siderophores) : Most bacteria grow poorly, if at all, in media that contain less than 10^{-8} M free iron and the concentration of free iron in most human tissue is less than this. The ability of aerobic microbial pathogens to compete with the host for available iron has considerable bearing on microbial virulence. Aerobic or aerotolerant organisms need iron for biosynthesis of iron containing enzymes such as cytochrome and catalase. Most of the iron that is available for aerobic or aerotolerant organisms is present in

the oxidized form, which is extremely insoluble. Organisms that can grow under anaerobic conditions have less difficulty in obtaining iron, since in the reduced environment iron is in ferrous form which is very soluble. Hence, most aerobic pathogens developed special mechanisms for acquiring iron. Many do so by secreting low molecular weight compounds called siderophores. These microbial siderophores compete with iron binding proteins of the host called transferrin and lactoferrin. The pathogen takes up iron while it is bound to a siderophore.

In *E. coli*, the siderophores termed enterochelin is secreted into the surroundings and it solubilizes polymeric ferric iron and forms complexes with the ferric ions. The ferric-enterochelin complex is then transported into the bacterial cell, where the complex is degraded and the iron is reduced to ferrous form.

Some pathogens directly obtain iron without producing siderophores. For example, some *Neisseria* species synthesize an outer membrane protein that removes iron directly from transferrin.

4.5.5.2. Antigenic mimicry: Some pathogens evade the host immune system by producing surface molecules that are antigenically similar or even identical to one of the host's macromolecules, a strategy termed antigenic mimicry. For example, some invasive strains of *E. coli* produce a capsule composed of a polysaccharide (designated as type K5) that is identical to a portion of the heparin molecules normally present in the host tissue. The mechanism (tolerance) that prevents the host's immune system from producing antibodies active against components of its own tissue, may act to protect these pathogens.

4.6. BACTERIAL TOXINS AS VIRULENCE FACTORS

Soon after the discovery that important human diseases like diphtheria and tetanus are caused by bacteria, it was demonstrated that the toxins produced by these bacteria are involved in pathogenesis. It was demonstrated that when cell free preparation made from a culture of pathogenic bacterium was injected into experimental animals, they developed a fatal disease nearly identical to the naturally acquired one. Later, a number of bacterial pathogens have been demonstrated to produce toxins. Toxigenicity of pathogenic strains is recognized as an important virulence factor. Some diseases like botulism, tetanus, cholera etc.

are identified as mainly toxigenic and disease develops even when the pathogens do not show invasive capacity. Diphtheria is caused by strains of the pathogen which show toxigenecity as well as invasiveness. Some pathogenic strains, especially of gram negative bacteria release toxins which play a secondary role in pathogenesis.

The bacterial toxins basing on their chemical properties and production are divided into 2 groups, namely 1. Endotoxins, and 2. Exotoxins.

4.6.1. ENDOTOXINS:

Most gram negative bacteria produce endotoxins. Chemically, endotoxin is lipopolysaccharide (LPS), which is the structural component of the outer cell wall of gram negative bacteria. This LPS is called endotoxin because it is bound to the bacterium and is released only when the microorganism lyses. Only the released LPS show toxic activity and the effect is essentially similar irrespective of the bacterial species. This is because LPS is composed of a long chain fatty acid anchor Lipid-A connected to a core sugar chain, both of which are the same in all gram negative bacteria. The toxin component of LPS is the lipid portion called Lipid-A, which is not a single macromolecule but appear to be a complex array of lipid residues (Fig.4.1). The lipid-A component exhibits all the properties associated with endotoxicity.

Figure 4.1. Diagrammatic structure of lipopolysaccharide showing he endotoxin complex

4.6.1.1. Properties of endotoxin:

1. Chemically lipid component of LPS
2. Heat stable
3. Poorly antigenic, do not stimulate immune response
4. Cannot be attenuated
5. Toxic only at high doses (mg per Kilogram amounts)
6. Generally similar despite source
7. Produce similar symptoms
8. Usually produce fever (pyrogenic), blood coagulation and vascular shock, intestinal haemorrhage
9. When administered at sublethal levels confer resistance to bacterial infections by triggering the release of interleukin-1 from host cells.

4..6.1.2. Organisms that produce endotoxins: Most gram negative bacteria release the endotoxin on lysis. Some is released during bacterial multiplication. Important examples of endotoxin producers are species of *Salmonella, Shigella, Brucella, Neisseria, Vibrio cholera, Escherichia coli, Psuedomonas aeruginosa* etc.

4.6.1.3. Symptoms caused by endotoxins: Endotoxins play a contributory role rather than primary role in pathogenesis. Important symptoms caused by endotoxin are fever, coagulation of blood and septic shock. Prodromal syndrome (symptoms of infection that initially occur before obvious symptoms of disease develop, or preclinical symptoms) of endotoxin activity are an increase in body temperature, substantial body weakness, body aches and general malaise.

4.6.1.3.1. Pyrogenicity: Endotoxins indirectly induce fever in the host by causing macrophages to release endogenous pyrogens that effect the thermoregulatory region of hypothalamus. Important endogenous pyrogen is the lymphokine Interleukin - 1. Other cytokines released by macrophages are tumor necrosis factor, TNF - α, and IL-6 also produce fever.

4.6.1.3.2. DIC of blood : Disseminated intravascular coagulation (DIC) is characterized by activation of coagulation sequence that leads to thrombi (clots) throughout the microcirculation. In gram negative bacterial sepsis, an important cause of DIC is initial activation of blood clotting factor XII called Hageman factor. Activated monocytes release IL-1 and TNF-α both of which increase expression of tissue necrosis factor on endothelial cell membranes and simultaneously decrease expression of thrombomodulin. The result is both activation of clotting system and inhibition of coagulation control.

4.6.1.3.3. Septic shock: Shock, often loosely called 'vascular collapse', is due to reduction or decrease of effective circulating volume of blood. It may be due to cardiac malfunction, severe haemorrhage, overwhelming bacterial infection or other causes. The shock due to bacterial infection is termed 'septic shock'.

The majority of the cases of septic shock are caused by endotoxin producing gram negative bacteria like *E. coli, Klebsiella pneumoniae, Proteus vulgaris, Psuedomonas aeriginosa, Serratia* and *Bacteroides*.

Endotoxin mediated activation of mononuclear phagocyte system and the consequent release of TNF-α is a key event in the pathogenesis of septic shock. The cytokine signals the synthesis and release of a whole array of secondary metabolites including prostaglandins and platelet activating factors. TNF-α also promote intravascular coagulation and capillary thromboses.

Bacterial endotoxins also cause direct injury to cells and tissues. Thus even the cells that are well supplied with blood fail to extract adequate oxygen from blood which leads to septic shock.

4.6.1.3.4. Acute respiratory distress (ARD): Endotoxins from gram negative bacteria that infect respiratory tract cause acute respiratory distress syndrome. The bacterial endotoxins trigger release of TNF-α from monocytes and alveolar macrophages, and also activate alternate pathway of complement to generate C5a. ARD syndrome is characterized by a) decreased arterial oxygen pressure and b) decreased lung compliance and c) development of diffuse pulmonary infiltrates.

4.6.2. BACTERIAL EXOTOXINS:

Exotoxins are soluble, heat labile proteins that are usually released into the surroundings as the pathogen grows. Often exotoxins may travel from site of infection to other body tissues or target cells on which they show their effect.

4.6.2.1. Character / properties of exotoxins :

1. They are synthesized by specific pathogens that often have plasmids or prophages bearing the exotoxin genes.

Toxin genes on chromosome	---	*Vibrio cholerae,*
		Pseudomonas aeruginosa,
		Shigella dysenteriae
On plasmids	---	*Clostridium tetani,*
		Bacillus anthracis,
		E.coli
Contributed by phages	---	*Clostridium botulinum,*
		Corynebaterium diphtheriae,
		Str. pyogenes.

2. They are heat labile proteins and are inactivated at 60 - 80 ^{0}C

3. Highly effective and toxic in very small doses (micrograms per kilogram amounts)

Botulinum toxin is the most toxic substance known and it is more than 100 times lethal than cobra snake poison. Its MLD for mouse is 2.5 x 10^{-5} µg. One mg of pure toxin is enough to kill one million guinea pigs.

Tetanus toxin is the second most lethal toxin and its MLD to mouse is 4 x 10^{-5}

MLD of Diphtheria toxin for guinea pig is 6 x 10^{-2} µg.

MLD of *Staphylococcus aureus* α-toxin for rabbits is 5 µg

4. They are associated with specific disease. Tetanus and botulism are essentially toxigenic diseases caused by tetanospasmin and botulinum toxins respectively. Cholera is caused by cholera toxin, and diphtheria by diphtheria toxin etc.

5. They are highly immunogenic and stimulate the production of neutralizing antibodies (antitoxins).

6. Easily inactivated by formaldehyde and other chemicals to form immunogenic toxoids.
7. They donot produce fever directly in the host.
8. Basing on the mode of their action exotoxins are mainly three types viz. neurotoxins, enterotoxins and cytotoxins.

4.6.2.2. Structural model: Although exotoxins occur in many forms, there is a general structural model to which they frequently conform - the AB model. In this model, each toxin is composed of an enzymatic subunit or fragment (termed A fragment) that is responsible for the toxic effect once inside the host cell, and a binding subunit or fragment (termed B fragment). Isolated A subunits are enzymatically active but lack binding and cell entry capability, whereas B subunits bind to target cells but are non toxic and biologically inactive.

4.6.2.3. Mechanism of entry: The A subunit of exotoxin enters the host cell either by (1) membranous pores or (2) by endocytosis.

1. When B subunit of the toxin inserts into the plasmamembrane, a pore is formed and through it the A subunit enters as shown below (figure 4.2)

Figure 4.2. Entry of exotoxin molecule through pores in the cellmembrane

2. When B subunit binds to the specific receptor site on the plasmamembrane, the entire unit is taken in by encytosis as shown below (figure 4.3).

Figure 4.3. Entry of exotoxin molecule through specific receptors

4.6.2.4. Mechanism of action: Basing on mode of action the exotoxins are recognised into three groups.

Sl.no.	Group	Toxin	Pathogen
1.	Neurotoxins	Botulinum toxin	Clostridium botulinum
		Tetanospasmin	Clostridium tetani
2.	Enterotoxins	Cholera toxin	Vibrio cholerae
		Diorrhoeal toxin	Escherichia coli
3.	Cytotoxins	Shiga toxin	Shigella dysenteriae
		cholera toxin	Vibrio cholerae
		Diphtheria toxin	Corynebacterium diphtheriae
		Whoopping cough toxin	Bordetella pertussis

4.6.2.5. Neurotoxins:

Botulinum toxin and tetanus toxin are neurotoxins and affect the normal operation of voluntary muscles.

In normal muscle contraction, nerve impulses from the brain travel through spinal cord and initiate a sequence of events at the motor end plate (the structure forming a junction between a muscle fibres and its motor nerve), which results in muscle contraction. When one set of muscles contract, the opposing set of muscles becomes stretched. A stretch sensitive receptor in the opposing muscles would cause neurons enervating these muscles to fire and oppose the stretching, but the firing is inhibited by impulses from an inhibitory nerve. Thus the opposing set of muscles relaxes. Nerve impulses are regulated by acetylcholine.

In botulism, the toxin binds to the nerve axon near the neuromuscular junction and prevents the release or secretion of acetylcholine, thus the muscles cannot contract. If this paralysis extends to the muscles of chest and diaphragm, death by respiratory failure can result.

Tetanus toxin binds specifically to the inhibitory motoneurons and blocks the inhibitory action resulting in contraction of both sets of muscles. If the muscles of the mouth are involved, the prolonged spasms restrict the mouth's movement, resulting in the condition known as lock-jaw. If the respiratory muscles are involved, death may be due to asphyxiation (impaired exchange of oxygen and carbondioxide).

The contrasting actions of botulinum and tetanus toxins are shown in the figure 4.4.

Figure 4.4. The action of neurotoxins

4.6.2.6. Enterotoxins: (Eg. Cholera toxin)

Enterotoxins are exotoxins that act on the small intestine, generally causing massive secretion of fluids into the intestinal lumen, leading to the symptoms of diarrhoea. Among the enterotoxins the cholera toxin produced by *Vibrio cholerae* has been studied extensively. Other enterotoxin producing pathogens include food poisoning organisms like *Staphylococcus aureus, Closridium perfringens* and *Bacillus cereus*, intestinal pathogens like *Escherichia coli, Salmonella enteritidis* etc.

The cholera toxin is a polypeptide having AB structure. The B sub unit is made of 5 parts arranged in a ring structure. The B subunit ring anchors itself to the epithelial cell's plasmamembrane and then insert small A subunit into the cell. The A sub unit activates adenyl cyclase enzyme in the affected cells. The activated adenyl cyclase causes the increase of cyclic AMP concentration in the intestinal cells. High cAMP causes the movement of massive quantities of water and electrolytes (as much as 20 litres per day) across the intestinal cells into the lumen of the gut. The genes for this enterotoxicgenicity reside on the *Vibrio cholerae* chromosome.

Cyclic AMP is a specific mediator of a variety of regulatory systems in the cells and its increase bring about active secretion of chloride and bicarbonate ions from mucosal cells into the intestinal lumen. This change in ionic balance leads to the secretion into lumen large amounts of water. In acute phase the rate of secretion of water into the small intestine is greater than the absorption of water by the large intestine, so that massive fluid loss occurs, and cholera victims die of dehydration (Figure 4.5).

Figure 4.5. Action of cholera toxin

4.6.2.7. Cytotoxins (Eg. Diphtheria toxin)

The toxin produced by *Corynebacterium diphtheriae*, the causal agent of diphtheria, was the first exotoxin to be discovered. It is a protein of m.w. 58,000. It has AB model.

When released from the bacterium, the toxin is inactive as the fragment A is masked. Fragment B is responsible for binding the toxin to the host cells and enters the host by endocytosis. With in the host cell cytoplasm, the disulphide bond of diphtheria toxin is reduced and broken releasing enzymatically active A fragment. The fragment A catalyzes covalent transfer of ADP ribose from NAD (Nicotinamide adenine dinucleotide) to EF_2 (elongation factor 2). EF_2 is an elongation factor in polypeptide synthesis. One toxin molecule can kill a cell by ADP-ribosylating more than 10^6 EF_2 molecules (Figure 4.6).

Figure 4.6. The mode of action of diphtheria toxin

The effect of the toxin is to create a layer of dead cells in the throat, on which *C. diphtheriae* out grow competing bacteria. Subsequently wide dissemination of diphtheria toxin causes neural and myocardial dysfunction.

A comparison of bacterial exo- and endo- toxins

Feature	Exotoxin	Endotoxin
Bacterial source	Secreted by living organisms, both G+ve and G-ve bacteria	Released from the cell walls of lysed G-ve bacteria
Chemical nature	Protein	Lipopolysaccharide
Heat tolerance	inactivated easily	Heat stable,can withstand autoclaving
Immune response	Highly immunogenic, produce antibodies called antitoxins	Poorly immunogenic antibodies are not formed
Toxoids	Can be converted to toxoids and readily neutralized by anti-toxins	Cannot form toxoids, neutralization with antitoxins not possible.
Mode of action	Each has a highly characteristic mechanism of action	All act similarly to cause their effects

Fever potential	do not produce fever	cause fever
Lethal dose	small, in µg quantities	Much larger, in mg quantities.

4.7. SUMMARY:

The ability of a microorganism to cause disease is called its pathogenicity, and the degree of pathogenicity exhibited by microbial species or strains is expressed as their virulence. The virulence of a pathogen is usually measured by determining its $LD_{50.}$ The virulence of a pathogen is influenced by host resistance and inoculum dose.

The virulence of a pathogen mainly depends upon two factors viz. 1. its invasive capacity and 2. ability to produce toxins.

The invasive factors exhibited by invasive pathogens include adhesins, antiphagocytic factors, penetration factors and spreading factors etc. The bacterial adhesins include pili, lipotechoic acids, surface proteins and surface antigens. The viral adhesins include glycoprotein spikes of the envelope. The antiphagocytic factors exhibited by bacterial pathogens are capsules, surface proteins and production of leucocidins. Bacterial pathogens produce various enzymes which help in spreading of the pathogen in the tissues and these include haemolysins, leucocidins, coagulase, fibrinolysin and hyaluronidase.

Various bacterial pathogens produce toxins which help in their ability to cause disease. Bacterial toxins include endotoxins and exotoxins. Most Gram negative bacteria produce endotoxins Chemically the bacterial endotoxin is lipopolysaccharide (LPS) which is the structural component of its cell wall. Hence it is released on the disruption of the cell wall. Bacterial endotoxin is poorly antigenic and act at relatively higher concentrations. It produces symptoms like pyrogenicity, disseminated intravascular coagulation (DIC), septic shock and acute respiratory disease, depending upon the amount of endotoxin released. The bacterial exotoxins are mainly three types viz. neurotoxins, enterotoxins and cytotoxins depending on their mode of action. Chemically they are proteins secreted during active growth of the pathogen. They are highly antigenic and act at relatively low concentrations.

4.8. MODEL QUESTIONS

Essay type questions

Discuss the concept of virulence and factors effecting virulence of the bacterial pathogens
Give an account of invasive factors exhibited by virulent pathogens
Discuss the bacterial enzymes as virulence factors
Discuss the importance of bacterial toxins in pathogenesis
Discuss the mechanisms of action of bacterial exotoxins

Short answer type questions

Concept of virulence
Factors effecting virulence
Invasive factors
Adhesions
Antiphagocytic factors
Spreading factor
Bacterial enzymes in pathogenesis
Siderophores
Bacterial endotoxin
Neurotoxins
Enterotoxins
Cytotoxins

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LESSON – 5: DISEASES CAUSED BY COCCOID BACTERIA

Objective: To study the diseases caused by Gram positive and Gram negative coccoid viz. *Staphylococcus aureus, Streptococcus pneumoniae* and *Neisseria meningitidis*

Contents

5.1. INTRODUCTION

Among the eubacteria, *Staphylococcus, Streptococcus* and *Neisseria* are important coccoid genera that contain some species which are pathogenic to humans. *Staphylococcus* is a Gram positive bacterium that is characterized by the arrangement of coccoid cells in grape like bunches, and among the three species in the genus *Staph. aureus* is the only pathogenic species causing very common pyogenic skin infections in humans, especially among children. *Streptococcus* is a very large genus characterized by arrangement of Gram positive coccoid cells in chains, and in the genus there are two species viz. *Strp. pyogenes* and *Strp. pneumoniae* (pneumococci) which are important human pathogens. *Neisseria* is the only important Gram negative coccoid genus that contains human pathogens. Among more than 30 species recognized in the genus, *N. gonorrhoeae* (gonococci) and *N. meningitidis* (meningococci) are pathogenic causing gonorrhoea and meningitis respectively. In this lesson the infections caused by *Staphylococcus aureus, Streptococcus pneumoniae* and *Neisseria meningitidis* are explained.

5.2. *STAPHYLOCOCCUS AUREUS*

Staphylococci are Gram positive coccoid bacteria that usually appear in grape like bunches. The genus *Staphylococcus* (staphyle = bunch of grapes; coccos = a berry) include three species viz. *S. aureus, S. epidermidis* and *S. saprophyticus*. Of these only *S. aureus* is pathogenic species.

5.2.1. Occurrence: Humans are the only natural sources.

5.2.2. Morphology: The cells of the bacterium are spherical and measure 0.7 – 1.0 μm diameter. They are arranged in bunches like grapes (Figure 5.1) The cells are capsulate, non spore forming, and Gram positive in staining property.

Figure 5.1. Staphylococcus

5.2.3. Cultural characters: It is aerobic or facultative anaerobic, and grows well on common media under aerobic conditions. Optimum temperature for growth is around 37 ^{0}C. On nutrient agar or on blood agar colonies of 2-4 mm diameter appear within 24 hours. Colonies are smooth, shining, opaque, golden yellow (sometimes yellow to white) domes resembling small drops of glass paint. It can grow in higher concentrations of sodium chloride (5 % in nutrient agar or 10% in nutrient broth) which are inhibitory to other genera, and this helps in isolating the species from faeces and other specimens which are likely to contain large number of other bacteria.

5.2.4. Biochemical characters: The pathogen produces a number of chemicals (enzymes and toxins) both in vivo and in vitro. These include

Coagulase: This enzyme helps in clotting of blood. Only pathogenic strains are coagulase positive while non pathogenic strains or species are coagulase negative.

Deoxyribonuclease: It produces DNase enzyme which hydrolyse the DNA of infected host cell.

Leucocidins: Polypeptides that kill the leucocytes.

Haemolysins: α-, β- and δ- haemolysins are produced, which are cytotoxic and lyse red blood cells.

Enterotoxin: Cause food contamination and diarrhoea.

α-toxin: It cause toxic shock syndrome (T.S.S.)

5.2.5. Antigenic characters: Staphylococci possess two types of antigens. 1) capsular antigens and 2) cell wall protein antigens.

5.2.6. Infections: *Staphylococcus aureus* is the most common cause of acute pyogenic (pus producing) infections in man. It has a marked predilection for skin and surface structures, causing acute but localized infections. Some times the pathogen invades the various body tissues causing systemic infection of inner body parts. Some strains are highly toxigenic and cause acute and general toxemia. Hence the staphylococcal infections can be grouped into 3 categories viz. 1) Localized cutaneous infections 2) systemic infections and 3) Toxigenic disease.

5.2.6.1. Localalized cutaneous infections: *Staphylococcus* usually invades the skin through wounds, hair follicles or skin glands. The most common infection is a mild, superficial inflammation of hair follicles (folliculitis) or glands (hiradenitis). Although these lesions are usually resolved with no complications, they may lead to infections of subcutaneous tissues.

Furuncle: A furuncle (boil) results when the inflammation of a single hair follicle or sebacious gland progresses into a large red and extremely tender abscess or pustule. Furuncles often occur in clusters (furunculosis) on parts of the body such as buttocks, breasts, axilla and back of the neck where skin rubs against other skin or clothing.

Carbuncle: A carbuncle is a larger (sometimes as big as a baseball) and deeper lesion created by aggregation and interconnection of a cluster of furuncles It is usually found in areas of thick, tough skin such as on the back of the neck. Carbuncles are extremely painful and can even be fatal in elderly patients when they give rise to systemic disease.

Impetigo: It is characterized by bubble like epidermal swellings that may break and peel away and as such, is considered a localized form of scalded skin syndrome.

Sepsis: Presence of pathogenic organisms or their toxins in tissues or blood is called sepsis. *S. aureus* is responsible for 30-40% of all cases of sepsis occurring in surgical wounds (hospital staphylococci).

5.2.6.2. Systemic infections: Most systemic staphylococcal infections begin as a local cutaneous lesion that seeds the blood and is carried to other sites.

Osteomyelitis: The pathogen is establishes in a variety of bones (often femur, tibia, ankle or wrist). Abscess formation in the affected areas result in an elevated, tender lump and necrosis of the bone tissue. Symptoms of osteomyelitis include fever, chills, pain and muscle spasms. This form of osteomyelitis is seen most frequently in growing children, adolescents and intravenous drug abusers. Another type, secondary osteomyelitis develops after traumatic injury or surgery in cancer and diabetic patients.

Staphylococcal Pneumonia: No organ can remains untouched by systemic staphylococcal infections. Because these bacteria inhabit the nasopharynx, they may be aspirated into the lungs and cause a form of pneumonia involving multiple lung abscesses and symptoms of fever, chest pain and bloody sputum. Although staphylococci account for only a small proportion of such pneumonia cases, their fatality rate is 50%. Most cases occur in infants and children, mainly as a serious complication of influenza and other lung diseases.

Bacteremia: The presence of bacteria in blood is described as bacteremia. Staphylococcal bacteremia causes a high mortality rate among hospitalized patients with chronic disease. Its primary origin is bacteria that have broken loose from cutaneous and lung infections. Circulating bacteria transported to the kidneys, liver, spleen, muscles etc. often form abscesses and contribute to a serious toxemia.

One consequence of staphylococcal bacteremia is a fatal form of endocarditis associated with the colonization of the heart's lining, cardiac abnormalities and rapid destruction of the valves.

Infection of the joints may produce a deforming arthritis (pyoarthritis). A severe form of meningitis (accounts for about 15% of cases) occurs when *S. aureus* involves meninges.

5.2.6.3. Toxigenic disease: Disorders due to the toxin production of *S. aureus* are a) food poisoning or intoxication b) Toxic shock syndrome (TSS) and c) scalded skin syndrome (SSS).

Food poisoning: Enterotoxin produced by some *S. aureus* strains is the most common type of food poisoning. It is associated with eating of foods such as custards, sauces, creams, meats, chicken salad etc. that have been contaminated by handling and left unrefrigerated for a few hours. When *S .aureus* grows in the food, toxin is released. It does not alter the taste or smell of food but when ingested toxin acts upon gastrointestinal lining with acute symptoms of cramping, nausea, vomiting and diarrhoea, that appear in 2 to 6 hours. Recovery occurs usually within 24 hours.

Toxic Shock Syndrome (TSS): It was first identified as a discrete clinical entity in 1978 in young women using vaginal tampons during menstrual period. The Harvard Medical school researchers discovered that ultra absorbent brands of tampons strongly bind magnesium ions, and that the resultant low magnesium concentration in the vaginal fluid can trigger TSS toxin production by *S. aureus* that is present sometimes as normal flora. TSS toxemia causes a series of reactions including fever, vomiting, rash and renal, liver, blood and muscle involvement that are some times fatal. The ultra absorbent tampons are banned and taken off from the markets in 1981 in USA.

Staphylococcal scalded skin syndrome (SSSS): Children with infection of the umbilical stump or eyes are susceptible to a toxemia called staphylococcal scalded skin syndrome (SSSS). Upon reaching the skin this toxin induces a painful, bright red flush over the entire body that first blisters and then cause desquamation of the epidermis. SSSS occur mainly in children under the age of four. The same toxin causes the local reactive impetigo which can afflict persons of all ages.

5.2.7. Transmission: The exact mode of transmission or origin of infection is always not clear, mainly because there is equal chance of spread from an external source or the organisms occurring in the body as normal flora commensals may cause infection. Hence, the source of infection may be exogenous or endogenous. Cross infections are common in hospitals and autogenous infections in general population.

5.2.7.1. Exogenous sources:

Contact with individuals having open infected wounds readily transmit the pathogen. Carriers who happen to be food handlers constitute important source for food poisoning. Hospital personnel serve as source for hospital staphylococcal infections

Fomites (inanimate objects used by the patients) can transmit the disease. Important fomites are clothing and bedding. Airborne infections also occur mainly when the nasal carriers or persons with lung infection emit the pathogen as droplet-nuclei or when the contaminated dust becomes airborne.

5.2.7.2. Autogenous sources: *S. aureus* is found in the anterior nasal mucosa of 40-50% of healthy adults, in the throat of many of them, in faeces of about 20% and on the skin of 5-10%. Newborn babies are rapidly colonized by *S. aureus* and, 90% or more of hospital borne babies

carry it in the nose and around umbilical cord within 2 weeks of birth. When opportunity arises in the form of decreased host resistance, it becomes pathogenic.

5.2.8. Diagnosis: Apart from morphological and cultural characteristics, coagulase test is the most important diagnostic test for identification of the pathogen.

Coagulase test: There are two types of tests viz. slide test and tube test.

Slide coagulase test is a rapid method but less sensitive. In this test, a part of the colony of *Staphylococcus* is placed on a slide in a drop of water mixed to make a milky suspension and then a loopful of plasma is added. If, further mixing results in visible clumping of bacteria, the test is positive. In this test coagulase present on the cells (described as bound coagulase) is responsible for the deposition of fibrin on the cells, thus causing clumping.

Tube coagulase test is more sensitive but time taking test. In this test a broth culture of the organism and diluted plasma are incubated in a test tube. Formation of a clot indicates that the organism is coagulase positive. In this test, enzyme secreted into the medium (called free enzyme) causes the clotting.

Serological tests are not commonly used, but may be useful in diagnosis of deep seated infections such as bone abscess.

5.2.9. Therapy: Staphylococcal infections can be controlled by using antibiotics. Penicillin was the first antibiotic used extensively. However, extensive use of penicillin resulted in the appearance of penicillin resistant strains. 50% of domicillary and 80% or more of hospital strains are now penicillin resistant. Resistance is due to the production of beta-lactamase enzyme by the pathogen. This enzyme splits β-lactam ring of penicillin.

Penicillin resistant strains may be treated with cephalosporins or vancomycin. Other effective antibiotics are methicillin, erythromycin, clindamycin, cloaxacillin etc.

In many hospitals, the over use of antibiotics lead to a buildup of multiple resistant strains. Hence, sensitivity testing is essential in the choice of an appropriate drug for therapy rather than 'best guess' method.

5.2.10. Prevention: The principal reservoir of the pathogenic staphylococci can never be eliminated. As long as there are humans, there will be carriers and infections. It is difficult to block the colonization of the human body. Hence to minimize the risk, prophylactic measures that can be taken are maintenance of personal and environmental hygiene.

5.3. *STREPTOCOCCUS PNEUMONIAE*

Pneumonia (Pneumono = Gr = lung) is a condition of inflammation of lungs with exudation and consolidation leading to serious consequences. Pneumonia is caused by a number of bacteria and viruses. Among the bacterial species that cause pneumonia, *Streptococcus pneumonia*e is the most the important one accounting for about 60-75 % bacterial pneumonia cases. The pathogen is generally described as pneumococci.

Pneumococci were first noticed by Louis Pasteur and Sternberg, independently in 1881. The relationship between pneumococci and pneumonia was established by Albert Fraenkel and Weichselbaum independently in 1886.

5.3.1. Pathogen: The pathogen belongs to α- haemolytic group of streptococci. It is an exclusively human pathogen, and it is a common inhabitant of human nasopharyngeal region.

5.3.2. Morphology: The cells are typically present in pairs in vivo (in pus, sputum etc) or in short chains in culture, and are surrounded by a large conspicuous capsule. Each coccoid cell is some what elongated and pointed at one end but rounded at the other (i.e. lanceolate type) and the two members of the pair are arranged with their long axes in line with each other, approximately 1 µm long and the pair point away from each other. The pair of cells is surrounded by a prominent polysaccharide capsule (Figure 5.2), which enables the cells to resist phagocytosis. Basing on the type of capsule, more than 40 types were recognized but only 10-12 are widespread. The organism is gram positive, non spore forming and nonmotile.

Figure 5.2. *Streptococcus pneumoniae* – Conspicuous capsules in Indian ink preparation

5.3.3. CULTURAL CHARACTERS:

The pathogen is normally cultured on blood agar medium. The optimum temperature for growth is 37 ^{0}C and the range is 25 – 42 ^{O}C. The thermal death point is 52 ^{O}C. The optimum pH for growth is 7.8 and the range 6.5 to 8.3. The organism is aerobic or facultative anaerobe, and special requirement for growth is 5% CO_2 in the culture vessel.

On blood agar medium, after incubation for 18 hrs, the colonies are small (0.5-1 mm), dome shaped, glistening with an area of greenish discoloration around them, indicating that the pathogen produces α –haemolysis which result from partial lysis the RBCs.

On prolonged incubation, the dome shaped colonics gradually became flat with raised edges and central depression or umbonation. It is because the aged cells in the centre of the colony undergo autolysis. The autolytic enzymes cleave the bond between alanine and muramic acid in the peptidoglycan layer. The surface active agents such as bile or bile salts enhance the activity of autolytic enzyme amidase.

On repeated subculturing, pneumococci undergo a smooth to rough (S →R) variation. In R form the colonies are rough and cocci are noncapsulate and avirulent.

The pathogen is very sensitive to optochien (Ethyl hydro cuprein) which in hibits the growtn even at 2 ppm concentration, and it is used as a test to distinguish pneumococci from others.

5.3.4. ANTIGENIC PROPERTIES:

Capsular antigens: Capsular polysaccharide is an important antigen and more than 80 serotypes were recognized basing on it. serotypes 1-8 are major pathogens indults, while serotypes 6,14,19,23 mainly infect in children.

Somatic antigens: M-antigen is a protein antigen of cell wall of the pathogen but it is not a virulent factor.

Acute phase proteins: An abnormal protein (Beta globulin) that precipitates with C-antigen (carbohydrate antigen) of pneumococci appears in the serum during acute phage of infection. It is described as acute phage protein (ADP) or C-reactive protein (CRP).

5.3.5. INFECTIONS:

The invasion of the lungs and bronchi result in two types of pneumonia Viz. lobar pneumonia and bronchopneumonia. Chronic infections lead to systemic spread of the pathogen to various other parts of the body. Hence infections can be categorized into three types: a) Primary infection leading to lobar pneumonia b) Secondary infections causing bronchopneumonia and c) systemic infections.

5.3.5.1. Primary infection or Lobar pneumonia: Primary infection leading to Lobar pneumonia develop when encapsulated pneumococci are inhaled into the alveoli of a susceptible host. Incubation period is 1-3 days. Multiplication of the pathogen in the lungs induce an over whelming inflammatory response marked by release of copious amounts of edematous fluid. In lobar pneumonia, this fluid accumulates in the alveoli along with RBCs and WBCs and spread rapidly through the lung. When this mixture of exudates, cells and bacteria solidify in the air sacs, a condition known as consolidation occurs. Consolidation leads to difficulty in breathing and production of sputum.

The inflammation can involve nerve endings, causing pain. The bacteria commonly enter the blood stream from inflammed lung leading to systemic spread.

The symptoms of lobar pneumonia include cough, fever, rust colored sputum, shortness of breath and chest pain.

5.3.5.2. Bronchopneumonia: It is almost always a secondary infection and commonly follows viral infection of the respiratory tract. The damage to respiratory epithelium and excessive broncheal secretions caused by the primary infection facilitate the invasion of pneumococci along the bronchial tree. The infecting cocci are found in the upper respiratory tract and this is an endogenous infection, frequently occurring as a terminal event in aged and debilitated patients.

5.3.5.3. Systemic infections: The cells of the organism can spread from the focus of infection as bacteremia resulting in infection of various other organs, such as meninges, middle ear, heart, nose eyes etc.,

Streptococcal meningitis: It is the most serious of pneumococcal infections. It is usually secondary to other pneumococcal infections. Systemic spread result in infections of meninges about 17-20% of all cases of bacterial meningitis is due to pneumococci.

Endocarditis: It results from infection of the heart valves.

Otitismedia: It is the condition of middle ear infection.

Conjunctivitis: It results from infection of conjunctiva.

Sinusitis: Inflammation of nasal passage.

5.3.6. Spread of the pathogen: Lobar pneumonia or primary illness is a communicable disease occurring particularly in the age range of 10-50 years. Epidemics occur in semi closed communities (barracks, institutions, factories etc). *S. pneumoniae* is essentially a human pathogen, and patients and carriers are main sources of infection. Disease spread either by contact or the bacteria may become air borne as droplet-nuclei during sneezing, coughing etc. Patients convalescing from lobar pneumonia may continue to harbour the organism for considerable periods. Contact carriers who have never suffered clinically apparent infection, may also become carriers of epidemic pneumococci. The disease is at its highest in spring (early summer).

Bronchopneumonia, in which pneumococci are commonly involved as secondary bacterial pathogens, occur most often at the extremes of life (very young or old) or after primary virus infections like influenza, measles etc. The infecting pneumococci are found in the upper respiratory tract and this is an endogenous, not a communicable infection. The infections occur mostly in the winter months.

5.3.7. Predisposing factors: Since 40-70% of humans are carriers of virulent pneumococci, the normal respiratory mucosa must possess great natural resistance to the pneumococcus. Among the factors that lower the resistance, and thus predispose to pneumococcal infections are a). Abnormalities of the respiratory tract- viral and other infections that damage surface cells, abnormal accumulations of mucus which protect pneumococci from phagocytosis, respiratory dysfunction, b). Alcohol or drug intoxication which depresses phagocytic activity, depresses, c). cough reflexes and facilitates aspiration of foreign material, d). Abnormal circulating dynamics eg: pulmonary congestion, e). General debilitating conditions like malnutrition, anemia etc.

5.3.8. DIAGNOSIS

5.3.8.1. Specimens: Sputum, throat and nasal swabs, aspirants from lung.

5.3.8.2. Microscopy: Direct examination of sputum after gram staining.

5.3.8.3. Quellung reaction: Capsular swelling within homologous antiserum is known as quellung reaction. *S. pneumoniae* exhibits easily visible quellung reaction.

5.3.8.4. Culture: The pathogen is cultured on blood agar medium, α- haemolysis is indicative of *S. pneumoniae*.

5.3.8.5. Bile solubility: The pathogen shows the characteristic autolysis. Bile and bile salts enhance autolysis. Adding of bile salt to broth containing bacteria, completely lyse the cells within 30 minutes. It is a confirmatory test for *S. pneumoniae*.

5.3.8.6. Optochin sensitivity: The pathogen is highly sensitive to optochin (ethyl hydro cuprein). By placing the discs of optochin on seeded plates a clear zone occur around the discs.

5.3.9. CHEMOTHERAPY: The infections of *S. pneumoniae* can be controlled effectively by penicillin, amoxycillin, vancomycin erythromycins, tetracyclin sulphonilamide and other antibiotics.

5.3.10. PROPHYLAXIS: A vaccine containing 23 most common capsular antigens is now available commercially and used for high risk group. However vaccination is not always successful because of the presence of a number of serotypes.

5.4. *NEISSERIA MENINGITIDIS*

Inflammation of the meninges is called meningitis. Meninges are the three membranes viz. dura, arachnoid and pia, that cover the brain and spinal cord. The outer layer dura is tough fibrous membrane and closely adheres to skill and vertebrae. It provides an effective barrier for spread of infection from cranial bones and vertebrae. The inner two membranes, arachnoid and pia, are mainly involved in meningitis. Cerebrospinal fluid flows between these two membranes.

Meningitis may be caused by viruses or bacteria, and *Neisseria meningitidis*, commonly called meningococci, is the most important one that causes severe spinal meningitis, mainly in children aged 6-24 months. It is greatly feared because it can sometimes progress very rapidly leading to fatality within a few hours.

5.4.1. PATHOGEN: The genus *Neisseria* belongs to the family Neisseriaceae. The genus comprises about 30 species and they are Gram negative, aerobic, non sporulating cocci that are

typically arranged in pairs. Meningococcus was first isolated and described by Weichselbaum in 1887. It is a normal inhabitant of the human nasopharynx with of 5-10% carriage rates normally but as high as 44% in some populations. It causes menigococcal meningitis or cerebrospinal fever in susceptible persons.

N .meningitidis is gram negative, aerobic, nonspore forming, non motile, Oxidase positive coccoid form. The cocci are oval or semispherical, measure 0.6-0.8 μm and typically arranged in pairs with adjacent sides flattened (Fig. 5.3). The long axis of the coccus is at right angles to the line joining the cocci in a pair. In the host they are generally intracellular.

Figure 5.3. Meningococci in cerebrospinal fluid. Inset – enlarged view to show flat adjacent sides of the cocci

Meningococci have exacting growth requirements and do not grow on ordinary media. Blood agar, chacolate agar, starch-casein hydrolysate agar are commonly used for culturing the pathogen. Optimum temperature for growth is 35 to 36 ^{0}C. No growth occurs below 30 ^{0}C. Optimum pH for growth is 7.4-7.6. Special requirement for growth is presence of 5-10% CO_2 in the environment. On agar medium the colonies are small 1mm in diameter, translucent, round, convex, bluish grey in color with a smooth glistening surface and smooth (entire) margin. Weak haemolysis occurs on blood agar medium.

It is oxidase positive and also catalase positive. The oxidase reaction helps in identification of Neisseria colonies in mixed cultures. When freshly prepared 1% solution of oxidase reagent (Tetra methyl paraphenylene diamine hydrochloride, TPDH) is poured on the culture plate, the *Neisseria* colonies turn deep purple. Subculturing should be made immediately, as the organism dies on prolonged exposure to the reagent.

13 serogroups of meningococci have been identified on the basis of immunological specificity of capsular polysaccharides. They are A B C D XYZ W-135 29E H 1 K and L . Of these A B C W-135 and Y are wide spread. Strain-A cause epidemics and strain-C causes localized out breaks.

Besides capsular antigens, meningococci possess carbohydrate and protein somatic antigens also but they have not been fully characterized.

Capsular antigens are virulence factors in serotypes A and C, while in serotype B surface antigens are virulent factors.

Meningococci are very delicate organisms being highly susceptible to heat, desiccation, alterations in pH and to disinfectants. They are susceptible to sulphonamides, penicillin, streptomycin and many other antibiotics.

The organism produces a heat stable endotoxin but it disappears easily due to quick autolysis.

5.4.2. PATHOGENESIS: Infection is acquired by inhaling airborne droplet nuclei released from the respiratory tract of a patient or carrier of a virulent strain. The incubation period for pathogenesis is one week. However, most infections are asymptomatic and it becomes a component of normal flora in most of the healthy persons . In some cases, the deposition of the meningococci in the nasal passage or throat leads to rhinitis or pharyngitis. The bacteria attach to the mucous membranes by their pili and multiply. Then they enter the epithelial cells of the respiratory tract, pass through them and then invade blood stream. The presence of meningococci in blood is referred to as Meningococcaemia. The symptoms of meningococcaemia are acute fever with chills, malaise and prostration. A typical petechial rash involving skin and mucosa occurs in early stages. Minute red spots on skin and mucosa due to escape of small amount of blood are called petechia. Meningococci can be isolated from the petechial lesions. The petechial development is attributed to the presence endotoxin in the blood. It may cause a drop in blood

pressure that can lead to shock. The tendency of meningococci to autolyse may enhance the release of endotoxin.

It is observed that severe meningococcaemia is a consequence of complement deficiency.

5.4.2.1. Meningitis: The pathogen reaches the meninges mainly through hematogenous Spread. It may also reach meninges along the perineural sheath of the olfactory nerve. It is also believed that in certain cases the site of entry may be conjunctiva, especially in the cases of purulent meningococcal conjunctivitis.

On reaching the central nervous system, the pathogen multiplies rapidly on the meninges and in cerebrospinal fluid. As they multiply the neutrophil phagocytes engulf and destroy them. When the multiplication occur faster than the rate of their elimination by phagocytosis by neutrophils, suppurative lesions are formed on meninges involving the surface of the spinal cord as well as the base and cortex of the brain. The inflammatory response with its formation of pus and clots may cause brain swelling. It may cause death of the tissue due to obstruction of blood supply. It can also lead to obstruction of normal out flow of CSF, and increase of internal pressure leads to squeezing of the brain flat against the skull. The infection may damage the nerves of hearing or vision or motor nerves, which may lead to blindness, deafness and paralysis. The fatality rate is variable but may be as high as 80% in untreated cases.

5.4.2.2. Systemic infections: Metastatic involvement of joints, ears, eyes, lungs and adrenals may occur. Meningococcal conjunctivitis may result from direct infection or through systemic spread.

The symptoms associated with meningeal infections are extensive nasal secretions sore throat, severe headache, malaise, fever, vomiting, photophobia, convulsions, irritability and drowsiness progressing to unconsciousness.

In neonates the major symptom is failure to feed and often vomiting.

In elderly and immunoocompromised, mental confusion is a prominent feature.

5.4.3. SPREAD OF THE PATHOGEN: The human nasopharynx is the only reservoir of the meningococcus. The carriage rates may be as high as 44% in some populations, but its presence in contacts of a patient has high risk of developing the disease.

Carriers rarely contact illness but serve to infect the susceptible contacts. The disease spreads as droplet nuclei from individual to individual. Fomite transmission occurs rarely.

During the inter epidemic periods the carrier rate is about 5-10%. An increase in carrier rate indicates the on set of an epidemic. During epidemics, carrier rates in closed communities may go up to 90%.

Meningitis is more common in children below the age of 5 years and in males. Epidemics usually occur in semi closed communities that live crowded together as in jails, ships, army camps etc. Group B strains caused epidemics in UK while A and C groups are common in other countries. Epidemic meningitis caused by group A meningococcus affected Delhi and surrounding areas in early 1985.

5.4.4. DIAGNOSIS:

For the purpose of diagnosis specimens may be collected from CSF, blood, nasopharyngeal region and petechial lesions.

Nasopharyngeal swabs are useful for detection of carriers.

Blood: In meningococcaemia and in early cases of meningitis, blood culture is often positive. Cultures should be incubated for 4-7 days.

Petechial lesions: Meningococci may be demonstrated in petechial lesions by microscopy and culture.

CSF: The most useful specimen in true cases of meningitis is cerebrospinal fluid. CSF is aspirated aseptically and is divided into 2 portions. One portion is centrifuged and gram stained smears are prepared from the deposit. The meningococci are seen mainly in side the polymorphs but often extracellularly also. The supernatant will contain meningococcal antigen, which may be demonstrated by precipitation with polyvalent or monovalent antimeningococcal serum.

The second portion is inoculated on blood agar and chocolate agar plates and incubated at 35^0 to 36 ^{0}C under 5-10% CO_2. The colonies appear after 18-24 hours and may be identified by morphology and biochemical reactions.

Oxidase test is an important test for neisseriae. To the colonies on agar medium, the oxidase reagent viz. (Tetrameyl paraphenylene diamine hydrochloride) is added. If the colonies turn purple red, it is positive for neisseriae.

5.4.5. THERAPY: Because untreated meningococcal disease has a mortality rate of up to 85%, it is vital that chemotherapy begin as soon as possible with one or more drugs. It may even be given while tests for the causative agent are underway. Penicillin-G is the most potent of the drugs

available for meningococcal infections. It is generally given in high doses intravenously. If the patient cannot tolerate penicillin, intravenous chloramphenicol is the second choice.

5.4.6. PROPHYLAXIS: Meningococcal vaccines that contain specific purified capsular antigens are available to protect high risk groups, especially during epidemics. Group A vaccine protects persons of all ages but group C vaccine is useful only for individuals over two years of age and group B vaccine is not yet available.

5.5. SUMMARY

Steptococcus aureus is one of the most common skin pathogen causing pyogenic infections in humans, especially children. It is an exclusively human pathogen. It is characterized by Gram positive coccoid cells arranged in grape like bunches. It is an aerobic/facultatively anaerobic, non spore forming, non capsulate, non motile organism that can grow on ordinary bacteriological media. Biochemically, the pathogenic strains are characterized by the production of coagulase enzyme. It produces pyogenic skin infections like boils, furuncles, carbuncles and impetigo. Systemic infections may lead to osteomyelitis pneumonia and bacteremia. The strains that produce toxins cause toxic shock syndrome (TSS) and staphylococcal scalded skin syndrome (SSSS). The pathogen is characterized by cultural, morphological and biochemical characters. Coagulase test is the confirmatory test. The infections can be controlled by antibiotics and penicillin is the drug of choice.

Streptococcus pneumoniae is the most important cause of bacterial pneumonia in humans. Morphologically it is a diplococcus as the cells are typically present in pairs surrounded by a large conspicuous capsule. It is mainly cultured on blood agar medium on which it produces α-haemolysis. The cultures undergo autolysis on prolonged incubation. The pathogen spread by droplet nuclei and primary infections produce lung infection causing pneumonia. In elderly people the pathogen present in the nasopharyngeal region often invades bronchial tree and cause bronchopneumonia. The infections can be controlled by antibiotics and penicillin is the drug of choice.

Neisseria meningitidis is a Gram negative diploccus characterized by arrangement of two coccoid cells in pairs with their adjacent sides flattened and surrounded by a conspicuous capsule. Infection is acquired by inhalation of droplet nuclei emanated from active patients. The primary multiplication occurs in the nasopharyngeal region and then the pathogen enters blood stream

resulting in a stage called meningococcaemia characterized by fever and petechial lesions. From the blood it enters the central nervous system and infects meninges, the layers that cover the brain and spinal cord. Infection leads to inflammation of meninges, a condition described as meningitis. In susceptible children the infection is often fatal. Penicillin and chloramphenicol are the drugs of choice for treatment of meningitis.

5.6. MODEL QUESTIONS:

Essay type questions

Give an account of the characters of Staphyloccus aureus and infections caused by it.

Give an account of pathogen, pathogenesis, epidemiology and control of streptococcal pneumoniae

Give an account of pathogen, pathogenesis, epidemiology and control of meningococcal meningitis

Short answer type questions

Staphylococcus aureus

Staphylococcal skin infections

Toxic shock syndrome

Coagulase test

Streptococcus pneumoniae

Lobar pneumonia

Neisseria meningitidis

Meningitis

Meningococcaemia

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LESSON–6: DISEASES CAUSED BY GRAM NEGATIVE ROD SHAPED BACTERIA-1

Objective: To study the diseases of humans caused by three important species of Gram negative bacteria viz. *Salmonella typhi, Shigella dysenteriae* and *Escherichia coli*

Contents:

6.1. INTRODUCTION

Among the bacteria that cause human infections, Gram negative rod shaped bacteria are very important and a large number of genera of this cause serious diseases. The genera belonging to the family enerobacteriaceae commonly occur in the large intestine and some species especially those belonging to *Salmonella* and *Shigella* cause serious infections. *Salmonella typhi* is the most serious pathogen causing typhoid characterized by progressively rising fever. Species of *Shigella*, especially *S. dysenteriae* is the common cause of bacterial dysentery in tropics. *Escherichia coli* is one of the very common normal flora constituents of colon bacteria. The genus is generally considered as a non pathogen, but some strains of *E. coli* cause intestinal infections. Typhoid caused by *Salmonella typhi*, dysentery caused by *Shigella dysenteriae* and intestinal infections caused by pathogenic strains of *E. coli* are discussed in this lesson.

6.2. TYPHOID CAUSED BY *SALMONELLA TYPHI*

Salmonella typhi, the causal agent of typhoid, was first observed and described by C.J. Eberth in 1880 from mesenteric lymph nodes and spleen of fatal cases of typhoid fever. It was isolated into pure culture by Gaffky in 1884.

6.2.1. Occurrence: *S. typhi* is an exclusively human pathogen, either causing enteric fever or establishing as a carrier.

6.2.2. Morphology: It is a Gram negative, non spore forming, rod shaped bacterium measuring 2-4 x 0.5 μm. It is actively motile with numerous long peritrichous flagella, and does not possess a capsule.

6.2. 3. Cultural Characters: It is an aerobic or facultative anaerobe, growing readily on simple media over a range of pH 6-8 and temperature 15-41 ^{0}C. Optimum temperature is 37 ^{0}C and thermal death point (TDP) is 56 ^{0}C.

On nutrient agar medium colonies are greyish white, circular, dome shaped, smooth, translucent and 2-3 mm diameter in 24 hours.

Apart from nutrient agar medium, a number of other differential and selective media are also used for isolation and culturing of *S. typhi.*

McConkey agar medium is a differential medium containing lactose as sole carbon source and lactose fermenters appear pink colored while non lactose fermenters form colourless colonies. *S. typhi* is a non lactose fermenter and colonies appear colourless.

SS agar (*Salmonella* and *Shigella* agar) is a selective medium, and it contains bile salts and sodium citrate. *S. typhi* colonies are colourless with black centre, while *Shigella* colonies are colourless.

Bismuth sulphite agar is a selective medim for *S. typhi*. Sulphite is reduced to sulphide and colonies appear black.

Sodium deoxy cholate citrate agar (DCA) is a differential medium, contain three times more bile salt than in McConkey agar. *S. typhi* colonies appear red.

McConkey agar medium is commonly used for culturing.

6.2. 4. Biochemical characters: It ferments carbohydrates to produce gas and in IMVC tests, it is

I : -ve MR : +ve VP : -ve and C : +ve

It does not hydrolyse urea and do not ferment lactose

6.2. 5. Antigenic properties: *S. typhi* possess H (flagellar), O (somatic) and Vi antigens.

H-antigen is a heat labile protein present on flagella and is called flagellar antigen. It is strongly immunogenic and induces antibody formation rapidly and in high titre.

O – antigen is a phospholipid – protein – polysaccharide complex which forms an integral part of the cell wall. It is identical with endotoxin. It is called somatic antigen. A number of serotypes

were recognized basing on the presence of characteristic O-antigen. It is less immunogenic than H-antigen.

Vi-antigen is a surface antigen. Fresh isolates of *S. typhi* generally carry a surface layer that completely masks 'O' antigen. This surface layer is also antigenic and is called vi-antigen or virulence antigen. Strains possessing vi-antigens cause clinical disease more consistently than those lacking it. It may act by coating the bacterial surface thus preventing the anti bacterial and opsonic effect of O- antibodies.

The Vi antigen is poorly antigenic and only low titers of antibodies are produced following infection. On repeated subculturing Vi-antigen is lost.

6.2. 6. Infections:

Man is the only natural host of *S. typhi* and infection is by ingestion of contaminated water, milk or food. ID_{50} was found to be as low as 10, normally it is 10^3-10^5. The incubation period is usually 14 days but may range from 5-20 days and appears to be related to the dose of infection.

The clinical picture in the first week of illness usually consists of progressively mounting fever, headache and severe malaise. Diarrhoea is not common at this stage and the patient may often constipate. In the second week characteristic rose coloured spots appear on the abdomen of fair skinned people. They may not be found in dark skinned people. Spleen enlargement and soft abdominal swelling and the onset of profuse diarrhoea occur in the second week. Ulceration of Peyer's patches may lead to intestinal perforation and haemorrhage which are common causes of death in untreated patients.

After ingestion, in the intestine, the bacilli attach themselves to the epithelial cells of the intestinal villi and penetrate to the lamina propria and submucosa. They are phagocytosed there by PMNLS and macrophages. The ability to resist intracellular killing and to multiply within these cells is a measure of their virulence. From the intestine, they enter the mesenteric lymph nodes via lymphatics. During this first and transient bacteremic phase, the bacilli spread to liver, gall bladder, spleen, lungs, kidneys and bone marrow. This first bacteremic phase occurs in the first 7-10 days after infection.

By the beginning of 2nd week, there occurs a massive bacteremia as the bacilli multiply in different organs and reenter blood stream. Gall bladder is the main source from which bacteria enter intestine in large numbers, as the bile is a good culture medium for the pathogen. The bacteria in the intestine mainly colonize Peyer's patches and lymphoid follicles. It results in inflammation, necrosis, sloughing and formation of characteristic typhoid ulcers. Haemorrhage of varying degrees may occur which complicate the illness. The events in the pathogenesis of typhoid fever are shown in the figure 6.1.

Figure 6.1. Pathogenesis of typhoid fever

Convalescence is slow. In about 5-10% of cases, relapse may occur during convalescence. In 2-5% of convalescents, the typhoid bacilli persists in the body, some times for indefinite period. In such chronic carriers, the bacilli are most commonly present in gall bladder and rarely in the urinary tract. The development of carrier state is more common in females than in males.

Infections with typhoid bacilli usually confer a certain degree of immunity. Reinfection may occur but is often milder than the first infection. Circulating antibodies to O and Vi antigens are related to resistance to infections. However, relapses may occur in 2-3 weeks after recovery in spite of antibodies because of short duration of resistance and less degree of immunity. Vaccination procedures are of little significance.

6.2. 7. Epidemiology: Typhoid fever occurs in two epidemiological types. The first one is endemic type that occurs almost through out the year. The second is an explosive epidemic which occurs mainly due to contamination of general source of water, milk or food supplies.

Waterborne epidemics which are so common previously became rare, especially in developed countries, but still are a problem in under developed countries. Milk borne epidemics also became rare due to general use of pasteurization.

Food handlers or cooks who became carriers are particularly dangerous source of contamination of food (as exemplified by the case of Typhoid Mary).

In epidemic out breaks, unsuspected carriers and convalescent carriers are important source of contamination rather frank clinical cases that are promptly isolated. Carrier state may be due to growth of bacteria in gall bladder (faecal carriers) or in kidney (urinary carriers). Urinary carriage is less frequent than faecal carriage. Even though, in pure waters it lose viability very rapidly, it can retain viability up to 4 weeks in contaminated waters, as the bacilli are protected by organic matter.

6.2. 8. Diagnosis: Culture and serological tests are employed in diagnosis

6.2. 8.1. Blood Culture: It is the most conclusive diagnostic method and should be employed in all suspected cases during the first 7-10 days. Blood culture rapidly becomes negative on treatment with chloramphenicol and other antibiotics.

The blood sample is first inoculated into a bile broth and incubated at 37^0C overnight and then subcultured on McConkey agar. The identity can be confirmed by biochemical tests or by agglutination test using 'O' – antiserum.

6.2. 8.2. Faeces culture: Salmonellae are shed in the faeces throughout the course of disease and even in convalescence. A positive faecal culture is useful to detect the active patients as well as for identifying convalescent carriers. As samonellae are greatly outnumbered in faeces, selective and enriched media should be used. McConkey agar, Deoxycholate citrate agar (DCA) and Wilson Blair media are commonly used.

6.2. 8.3. Widal test: It is a commonly employed serological test in diagnosis of typhoid. The test is a measurement of H and O antibodies against *S. typhi* and *S. paratypthi* in patient's serum. The patient's serum is mixed with commercially available H and O antigens separately and incubated

in a water bath at 37 ^{0}C overnight. H- agglutination leads to the formation of loose, cottony woolly clumps, while O-agglutination is seen as a disc like pattern at the bottom of the tube. In both, the supernatant fluid is clear.

6.2. 9. Therapy: Chloramphenicol is the drug of choice in treatment of typhoid. S. typhi is susceptible in vitro to many antibiotics like streptomycin, tetracycline etc. but they are ineffective in vivo. Apart from chloramphenicol, ampicillin, amoxicillin, furozolidine and cotrimoxazole have been found to be useful in treatment.

However, multiple drug resistance, transmitted genetically by plasmids is a problem in Salmonella. As many as 25% of *Salmonella* strains are resistant to ampicilin, and 5% to chloramphenicol. Hence, sensitivity testing is essential.

6.2. 10. Prophylaxis: Typhoid fever can be effectively controlled by general measures, such as improvement in sanitation and provision of protected water supplies.

As a specific prophylactic measure, a triple vaccine TAB vaccine containing killed bacilli of threee species (*S.typhi, S. paratyphi* A and *S. paratyphi* B). It has been later replaced by a divalent vaccine, TA vaccin eliminating paratyphi B which is generally rare.

However, typhoid vaccines are not in general use because the typhoid bacilli are primarily intracellular parasites and cell mediated immunity (CMI) rather humoral immunity is relevant in providing long standing protection and the heat killed vaccines does not stimulate CMI.

6.3. DYSENTERY CAUSED *SHIGELLA DYSENTERIAE*

The genus *Shigella* was established by Castellani and Chalmers in 1919 and named it after K.Shiga, the Japanese bacteriologist who first discovered the dysentery bacillus. Dysentery is a clinical condition characterized by the passage of loose motion mixed with blood and mucus.

6.3.1. Characters:

6.3.1.1. Morphology: *Shigella* are gram negative, non spore forming, non motile, non capsulate, aerobic/facultative anaerobic, rod shaped bacteria that measure 1-3 X 0.5 µm. Fimbriae may be present.

6.3.1.2. Cultural characters: It grows on ordinary media forming small 2 mm diameter colonies on agar, which are smooth convex, circular and translucent. Optimum temperature is 37^{0}C, range

15-42^{0}C, Thermal death point is 56^{0}C. Optimum pH 7.4. In broth medium it produces uniform growth with mild turbidity.

6.3.1.3. Biochemical characters: It does not ferment lactose. It form acid from carbohydrates and rarely produce gas.

Shigella produces both endotoxin and exotoxin. In experimental animals, the exotoxin acts as (a) neurotoxin causing paralysis (b) enterotoxin causing enteritis and (c) cytotoxin damaging the cells. It is not established whether these activities are manifested by a single toxin or by separate toxins. The toxin is described as shiga-toxin.

6.3.1.4. Antigenic properties: *Shigella* possess a number of somatic (O) antigens. They are lipopoly saccharides. There are more than 40 serotypes. In general the antigenic structure of *Shigella* is simple and there is some degree of antigenic similarity between *Shigella* and *E. coli*. Hence, identification of *Shigella* should be made by a combination of antigenic and biochemical properties, and not by serology alone. *Shigella* converts tryptophan to indole. It helps to distinguish it from *Salmonella*.

6.3.2. Infections:

Infection occurs by ingestion and the pathogen is limited to gastrointestinal tract, and rarely enters blood stream. The pathogen is highly communicable, minimum infective does is as few as 10-100 bacilli. In general less than 10^3 organisms cause disease. Pathogenesis is essentially an invasive process, though exotoxin produced by the pathogen may also involve in the process. It was observed that nontoxigenic strains can still cause dysentery but not the non-invasive ones. The two may act in sequence, the toxin producing an early nonbloody voluminous diarrhoea and invasion of the large intestine resulting in later dysentery with blood and pus in stools. The classic symptoms of shigellosis are frequent liquid stools containing blood and pus, abdominal cramps and a painful but unproductive urge to defecate.

The pathogen invades mucosal epithelium of large intestine and microabscesses are formed in the wall of large intestine. It leads to necrosis of the mucous membrane, superficial ulceration, bleeding and formation of a psuedomembrane on the ulcerated area. As the process subsides, granulation tissue fills the ulcers, and scar tissue forms.

Infection of large intestine often interferes with absorption and stimulates peristalsis and diarrhoea. Peristalsis is wave like contraction of intestinal wall that normally moves in proper direction, but can result in painful abdominal cramps during intestinal infections.

Uncomplicated shigellosis is a self limiting condition. After a short incubation period of 1-2 days there is a sudden on set of abdominal pain, fever and watery diarrhoea, which is attributed to the activity of the exotoxin. A day or so later, as the infection involves mucosal invasion, the number of stools increase. The stools are less in fluid but often contain mucous and blood. In more than half of the adult cases fever and diarrhoea subside spontaneously in 2-5 days. However, in children and elderly people, loss of water and electrolytes may lead to dehydration, acidosis and even death. On recovery most persons shed bacilli only for a short period but a few remain chronic carriers and may have recurrent bouts of disease.

6.3.3. Spread: Bacterial dysentery is mainly a disease that flourishes under poor hygienic conditions. Contamination of drinking water supplies often cause explosive epidemics. In 1969 in Gautemala, a small central American country, there were 1,10,000 cases and 8,000 deaths and the epidemic is attributed to the appearance of a virulent strain and contamination of water supplies.

Faecal-oral route through contaminated fingers is considered as an important cause of spread in children. Fomite transmission is also an important mode of spread. Flies are important vectors in mechanically transmitting the bacilli from faeces to the exposed food stuffs.

6.3.4. Immunity: Infection is followed by a type specific antibody response but it fails to prevent recurrent infections. The attempts to develop vaccine against the disease are not successful.

6.3.5. Diagnosis: Diagnosis is made by isolating the pathogen from faceces. Selective media used are McConkey agar, EMB (Eiocene Methylene Blue) agar or DCA (Deoxycholate citrate agar). Identification is confirmed by slide agglutination tests with specific sera.

Demonstration of antibodies in the sera of patients is of no value in diagnosis, because they are of little significance in disease process.

6.3.6. Therapy: The pathogen can be inhibited by a number of antibiotics but development of multiple during resistance is also common. Since, the disease is a self limiting one, oral rehydration is adequate in most cases, and antibiotics should be reserved for severe toxic cases only. Ampicillin and sulfa drugs (Trimethoprim) are drugs of choice for treatment.

6.3.7. Prophylaxis: Maintenance of hygienic conditions.

6.4. INFECTIONS CAUSED BY *ESCHERICHIA COLI*

Escherichia coli a normal flora member of large intestine of humans, and generally considered as nonpathogenic organism. However, some strains of the organism cause infections of intestine, urinary tract and parts of the body.

6.4.1. Occurrence: *E. coli* is generally a normal flora member occurring only in the intestine of only humans and animals.

6.4.2. Characters:

6.4.2.1. Morphology: *E. coli* is a Gram negative, non-spore forming, straight rod measuring 1-3 x 0.4-0.7 μm, arranged singly or in pairs. It is motile by peritrichous flagella, but some strains are non motile. Capsules and fimbriae are found in some strains.

6.4.2.2. Cultural characters: It is an aerobe and facultative anaerobe. Optimum temperature for growth is 37 ^{0}C and the range 10-40 ^{0}C. It can be easily cultured on ordinary bacteriological media. On nutrient agar medium, the colonies are large, thick, grayish white, moist, smooth, opaque or partially translucent. On MacConkey agar medium, which is most commonly used for isolation of enterobacteriaceae members, the colonies are pink due to lactose fermentation, On blood agar medium, the colonies shows a zone of β-haemolysis. In broth medium it shows uniform turbidity.

6.4.2.3. Biochemical characters: It can utilize a number of carbon sources including lactose producing acid and gas. It is positive for indole production and methyl red reduction tests, but negative for Voges-Proskauer test and citrate utilization test. Some strains of *E. coli* produce haemolysins and exotoxins. The exotoxins are usually enterotoxins acting on intestinal epithelium. The cell wall component is endotoxin.

6.4.2.4. Antigenic properties: *E. coli* possess a number of somatic (O), capsular (K) and flagellar (H) antigens. A large number of serotypes have been identified based on the variations in the antigenic nature. The antigenic pattern of a strain is recorded as number of particular antigen it carries, e.g. *E. coli* O_{157} H_7, E. coli O_{111} K_{58} H_2 etc.

6.4.3. Infections:

E. coli is generally considered as nonpathogenic, some strains are involved in causing infections. The etiological role of a specific strain of *E. coli* in causing childhood diarrhea was first recognized in 1945. Since then a number of different strains, with varied properties, were diagnosed as enteric pathogens. Apart from enteric infections, strains of *E. coli* are also recognized as causing urinary tract infections, pyogenic infections and septicaemia.

6.4.3.1. Intestinal infections: At least five types of *E. coli* (EC) are recognized as causing intestinal infections and they are described as EPEC, ETEC, EIEC, EHEC and EAEC basing on their mode of causing infection and other characters.

6.4.3.1.1. Enteropathogenic *E. coli* (EPEC): The strains of *E. coli* that attach to the epithelial cells of the intestine and cause damagae to the membranes of microvilli resulting in diarrhea are referred to as strains of EPEC. They are mainly involved in causing diarrhea in infants and young children. Over 25 strains were identified, e.g. O_{26}:B_6, O_{55}:B_5, O_{86}:B_7 etc.

6.4.3.1.2. Enterotoxigenic *E. coli* (ETEC): The strains of *E. coli* that produce heat labile and heat stable enterotoxins which act on intestinal epithelial cells resulting in diarrhea are called ETEC. They generally cause diarrhea in children and in adults who frequently undertake traveling to various places. Hence, the diarrhea caused by ETEC is also described as 'traveller's diarrhea'. Most strains of ETEC belong to O serotypes 6, 8, 15, 25 etc. These strains possess fimbriae or colonization factor antigens (CFA) which help in adhesion, thus contributing to their virulence. The toxin gene is plasmid borne and can be transferred to any strain, but only a limited number of strains carry the toxin gene.

6.4.3.1.3. Enteroinvasive *E. coli* (EIEC): The strains of *E. coli* that invade intestinal epithelium and cause diarrhea and dysentery are referred to as EIEC. They resemble shigellae in many respects, and show O antigen cross reaction with species of *Shigella*. Most of these strains are nonmotile, and do not ferment lactose or do so very lately, and hence are often described as 'atypical *E. coli*'. They belong to serogroups O28 ac, O112 ac, O124, O143 etc.

6.4.3.1.4. Enterohemorrhagic *E. coli* (EHEC): They were identified in 1983 following food-borne outbreaks of hemorrhagic colitis caused by *E. coli* O157:H7. The infection is characterized by marked hemorrhage with little or no fever. It mainly occurs in young children and elderly

people due to food poisoning by *E. coli* strains. The strains of EHEC produce a cytotoxin referred to as 'verotoxin' because of its affects on vero cells in culture.

6.4.3.1.5. Enteroaggregative *E. coli* (EAEC): These strains are so named because they appear aggregated in a 'stacked brick' formation. They have been associated with persistent diarrhea. Antigenically they are O-untypical. They produce a low molecular weight heat stable enterotoxin called EAST 1 (enteroaggregative heat stable enterotoxin-1), which is responsible for diarrhea.

6.4.3.2. Urinary tract infections: *E. coli* is one of the common causes of urinary tract infections, and the serotypes involved belong to O group 1, 2, 4, 6, 7 etc. The infections of the lower urinary tract (cystitis) seem to be due to 'ascending infection' caused by fecal coliforms while pyelonephritis is due to hematogenous infection. The strains having K antigen are responsible for pyolonepritis, while most isolates from cystitis lack K antigen. The symptoms of urinary tract infections by *E. coli* include dysuria, hematuria and pyuria. These are general symptoms of urinary tract infections, and involvement of *E. coli* must be confirmed by cultural, biochemical and serological tests.

6.4.3.3. Pyogenic infections: *E. coli* form the most common cause of intra-abdominal infections, such as peritonitis and abscesses resulting from spillage of bowel contents. They also cause pyogenic infections in the perianal area, meninges and wounds.

6.4.3.4. Septicaemia: *E. coli* is one of the commonest causes of septicaemia. The manifestations are fever, hypertenstion, disseminated intravascular coagulation etc. All these symptoms are due to release of endotoxin released by lysis of the cells. Mortality due to *E. coli* septicaemia may be significantly high.

6.4.4. Diagnosis:

Laboratory diagnosis involves isolation and identification of *E. coli* strains from the disease specimens. The specimens are likely to contain a mixed bacterial flora, and hence differential media such as MacConkey agar medium for isolation, and biochemical tests for identification. Serological tests are important for identification of serotypes.

6.4.5. Treatment:

E. coli isolated from community acquired infections are usually sensitive to most antibiotics, but, multiple drug resistant strains have been obtained from hospital acquired

infections. Since there is no set pattern for sensitivity of the organism to the antibiotics, therapy has to be based upon the results obtained by in vitro determination.

6.5. SUMMARY

Salmonella typhi causes typhoid, a very important and wide spread febrile disease. It is an exclusively human pathogen. It is a Gram negative, nonspore forming, noncapsulate, actively motile rod shaped bacterium. It can grow readily on ordinary bacteriological media. It is positive for methyl red reduction and citrate utilization, but negative for indole production and Voges-Proskauer test. It does not ferment lactose. It possesses both flagellar antigens and somatic antigens. Infection is acquired by ingestion of contaminated food or water, and incubation period is about two weeks. The initial multiplication occurs in the small intestine and then the pathogen enters blood stream enters various organs including gall bladder. The secondary invasion of small intestinal leads to severe infection of peyer's patches. The clinical picture includes mounting fever and severe intestinal haemorrhage. Diagnosis is made by culturing of the pathogen from blood or faeces. Widal test is a common serological test used for diagnosis. Chloramphenicol is the drug of choice for treatment of typhoid. Typhoid vaccine contained killed cells of the pathogen is available for prophylactic use for high risk cases, but it is not in general use because it gives protection for only short duration.

Shigella dysenteriae is the most common cause of bacterial dysentery. The pathogen is a Gram negative, nonmotile, noncapsulate, nonspore forming rod shaped bacterium. It possesses somatic antigens similar to those of *Escherichia coli.* On autolysis endotoxin is released, and actively growing cells produce an exotoxin described as shiga toxin. The infection is acquired by ingestion of contaminated food. Pathogen mainly multiplies in the intestine and rarely enters blood. The incubation period is 3-4 days, and the classic symptoms of shigellosis are frequent liquid stools containing blood and pus, abdominal cramps and a painful but unproductive urge to defecate. Uncomplicated shigellosis is self resolving disease and the patient recovers within a weak. Faecal-oral route through contaminated fingers is considered as an important cause of spread in children. Fomite transmission is also an important mode of spread. Flies are important vectors in mechanically transmitting the bacilli from faeces to the exposed food stuffs. Diagnosis

is made by isolating the pathogen from faceces. Ampicillin and trimethoprim are drugs of choice for treatment.

Escherichia coli is common normal flora member of large intestine of man, and often considered as a commensal. However, a number of strains of *E.coli* cause infections, especially of intestinal tract. The strains that attach to intestinal epithelium and cause diarrhea are called enteropathogenic strains; those that produce toxin to cause infection are described as enterotoxigenic strains; those that invade intestinal epithelium and cause infection are called enteroinvasive strains; the strains that cause hemorrhagic symptoms are called enterohemorrhagic strains; some strains appear aggregated in a 'stacked brick' formation, and they are described as enteroaggregative strains. Apart from the intestinal tract infections, *E. coli* also cause urinary tract infections, pyogenic infections and septicaemia. The pathogenicity of E. coli has to be carefully confirmed by cultural, biochemical and serological tests. Antibiotic sensitivity testing is necessary for selecting the antibiotics for treatment.

6.6. MODEL QUESTIONS:

Essay type questions

Give an account of pathogen, pathogenesis, epidemiology and control of typhoid

Give an account of pathogen, pathogenesis, epidemiology and control of bacterial dysentery

Give an account of epidemiology, diagnosis and control of typhoid and shigellosis

Give an account of pathogenic strains of Escherichia coli and infections caused by them

Short answer type questions

Salmonella typhi
Typhoid
Diagnosis of typhoid
Shigella dysenteriae
Bacterial dysentery
Pathogenic strains of Escherichia

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LESSON–7: DISEASES CAUSED BY SPECIES OF GRAM NEGATIVE BACTERIA-2

Objective: To study the diseases caused by three species of Gram negative rod shaped and spiral bacteria viz. cholea caused by *Vibrio cholerae*, plague caused by *Yersinia pestis* and syphilis caused by *Treponema pallidum*

Contents:

7.1. INTRODUCTION

A large number of Gram negative rod shaped bacteria cause human infections, and the infections caused by three species belonging to the family enterobacteriaceae are explained in the lesson – 6. Another genus in the family enterobacteriaceae is *Yersinia* which is not a normal flora member but comprise some species that cause minor infections of intestine. One of the species in the genus *Yersinia, Y. pestis* causes plague, the dreaded disease of mankind. *Vibrio cholerae* is a Gram negative slightly curved rigid rod shaped bacterium belonging to the family vibrionaceae, and it causes one of the very common epidemic disease cholera. *Treponema pallidum* is a Gram negative, flexible spiral bacterium belonging to the group spirochetes, and it causes one of the very wide spread sexually transmitted disease, syphilis.

Cholera caused by *Vibrio cholerae*, plague caused by *Yersinia pestis* and syphilis caused by *Treponema pallidum* are explained in the lesson.

7.2. CHOLERA CAUSED *VIBRIO CHOLERAE*:

Vibrios are among the most common bacteria in surface waters world wide and their transmission through drinking water supplies is well established in epidemic out breaks of the disease through the classical work of John Snow in 1849-55. The pathogenicity of *V. cholerae* was established by Robert Koch in 1883. Cholera is a classic example of a very severe form of

diarrhoea, and is characterized by sudden onset of acute gastroenteritis, often running a rapid and fatal course. All clinical manifestations and complications of cholera result from massive water and electrolyte depletion. The patient becomes acutely dehydrated, acidotic and shocked within a few hours.

7.2.1. Characters:

7.2.1.1. Morphology: The cells of the organism are typically curved or comma shaped rods with round or slightly pointed ends (Figure 7.1). They measure about 1.5 – 3.0 μm x 0.5 μm in size. The cells of *Vibrio* are actively motile by a single long terminal flagellum. In liquid cultures the *Vibrio* occur singly or in pairs forming S forms or in chains end to end with curves alternating i.e. presenting a spiral arrangement. In mucous flecks of faecal matter from infected patients, vibrios are seen arranged in parallel rows described by Robert Koch as 'fish in stream' appearance.

The cells are Gram negative, non capsulate, non spore forming strongly aerobic/weakly facultative anaerobic, rigid curved rods.

Figure 7.1. Vibrio cholerae

7.2.1.2. Cultural characters: The organism grows on ordinary media. Blood agar medium or McConkey agar medium is commonly used for isolation. On nutrient agar medium the colonics are moist, translucent, 1-2 mm in diameter, showing a characteristic bluish colour in transmitted light. Optimum temperature for growth is 37 ^{0}C and the range is 16-40 ^{0}C. The optimum pH is 8.5 and the range is 6.4-9.6.

7.2.1.3. Antigens: *V. cholerae* possess flagellar (H) and somatic (O) antigens. Heat labile flagellar antigen is similar to such H-antigen in other vibrios also, but 'O' antigens of *V. cholerae* are distinct. There are more than 100 O-antigens. *V. cholerae* strains of O-group I cause classic cholera.

7.2.1.4. Biochemical properties: Important biochemical character of *V. cholerae* is production of a heat labile enterotoxin described as cholera toxin or choleragen. It is a polypeptide of M.W. 84,000 having sub units A (MW 28,000) and B (MW 52,000). The B subunit is made up of 5 parts arranged in a ring like structure. The subunit helps in attaching the toxin molecule to the mucosal receptors. Subunit A enters the cell and activates a number of biochemical reactions leading to pathogenesis. Hence, toxin is the virulence factor. The genes for the enterotoxin are on bacterial chromosome. The enterotoxin is antigenically active and antibodies against it neutralize the toxin.

Some strains of *V. cholerae* produce soluble haemolysins, and others digest red blood cells without liberating a soluble haemolysin. But most strains are non haemolytic but may produce a greenish clearing in blood agar media probably due to a chemical alteration of haemoglobin.

The classical isolate is non fermentor of lactose. It is positive for indole production, negative for Voges-Proskauer test, positive for methyl red reduction test and negative for citrate utilization test.

The El-Tor biotype of the pathogen is positive for Voges-Proskauer test and also haemolytic.

7.2.2. Infection: Under natural conditions, *V. cholerae* is pathogenic to only humans. A person may have to ingest 10^8-10^{10} organisms to become infected and develop disease.

Cholera is not an invasive infection. The organisms do not reach the blood stream but remain localized within the intestinal tract. There they may multiply, invade superficial epithelium and liberate cholera toxin, which stimulates hyper secretion of water and salts (bicarbonates and chlorides) in all parts of the small intestine while inhibiting absorption of sodium. The attachment of toxin is irreversible and fragment A activates enzyme adenylate cyclase which converts ATP to cyclic Adenosine monophosphate (cAMP). cAMP activates protein kinases and ca^{2+} ions which act on cell membrane to cause chloride ion secretion and inhibition of Na^+ absorption. Although colon (large intestine) is not affected by the toxin, it cannot absorb huge volume of fluid that rushes through it and diarrhoea results. The normal shedding of intestinal cells eventually gets rid of toxin.

The incubation period is 1-4 days, and there is a sudden onset of nausea and vomiting, profuse diarrhoea with abdominal cramps. A typical cholera patient excretes 10-20 litres of stools. The stools resemble 'rice water' and contain mucous, epithelial cells and large numbers of vibrios. Great loss of water leads to severe dehydration, thickening of blood. The patient's eyes become gray and sink into their orbits. The skin is wrinkled, dry and cold. Muscular cramps occur in the arms and legs. Despite continuous thirst, patients cannot hold fluids. The blood thickens, urine production ceases, and sluggish blood flow to the brain leads to shock and coma. The mortality rate without treatment is between 25 to 50%.

7.2.3. Epidemiology: Cholera occurs in many forms viz. sporadic, endemic, epidemic and pandemic. Cholera is considered as endemic in the Indian subcontinent for many centuries, and during 19^{th} and early 20^{th} centuries it spread around the world in a series of devastating pandemics. There have been 7 cholera pandemics since early 1800. The last and 7^{th} pandemic began in 1961 in Indonesia spreading to south Asia, Middle east, parts of Europe, Africa and South America.

Up to 1961, cholera was considered to be caused by classical biotype, but in 1960s cholera was found to be caused by another biotype which causes milder disease, called El-Tor biotype or El-Tor vibrio. The Eltor biotype of *V. cholerae* was named after Tor-quarantine station in the Sinai Peninsula of Indonesia where it was first isolated in 1905 from six Haj pilgrims who died of dysentery. It was identical to classical vibrio in all respects except that it is VP-positive and haemolytic.

In 1961 El-Tor cholera was reported from various other parts of south east Asia. This marked the beginning of seventh pandemic which has yet to subside, being active even now in many regions. El-Tor Vibrio was first found in India in 1964. Its rapid spread is attributed to the fact that those infected are only mildly ill or symptomless but are vigorous excreters of the organism. In 1966 it was reported from middle East, in 1970 Eastern Europe. In 1970s outbreaks occurred in Queensland, Australia and Gulf-coast in USA because of special environmental foci in the coastal waters, but they were localized and soon controlled. It appeared in the western hemisphere in 1991.

The spread of cholera to western hemisphere was the consequence of a common shipping practice. Large ships take on ballast water when sailing without cargo. The ballast water is then discharged in the bay of the port where the ship is to pick up its cargo. The South American cholera out break began when a Chinese freighter discharged its ballast water into a Peruvian harbor, inoculating the bay with the *Vibrio*. It had taken on ballast from waters of its previous port. The *V. cholerae* discharged by the ship into the bay was concentrated by shell fish, which filters large quantities of water to extract plankton for food. The cholera bacteria concentrated in shell fish, which were then eaten raw, a popular appetizer. The low levels of chlorination used in Lima water supplies allowed the organism to multiply and spread. Cases of cholera caused by the same strain are now seen throughout Central America and Mexico.

Approximately 45 countries, mostly in Asia, Africa and South America are currently affected by cholera.

In late 1992, a new strain of *V. cholerae* (O-139) appeared in India and spread rapidly across south Asia attacking even the people with immunity to 7^{th} epidemic strain. The new strain belongs O-group (O-139), and its spread suggests that it could initiate another pandemic.

7.2.5. Control measures:

7.2.5.1. Therapy: The most important part of therapy consists of water and electrolyte replacement to correct the severe dehydration and salt depletion. Immediate use of oral rehydration solution (ORS) is strongly recommended for cases of suspected cholera. Two types of ORS viz. ORS bicarbonate and ORS citrate are used

Composition of ORS bicarbonate: glucose or sucrose: 20.g, Sodium chloride : 3.5g Sodium bicarbonate: 2.5g and Potassium chloride 1.5g , and Water (boiled and cooled) : 1 litre

Composition of ORS citrate: Sodium chloride: 3.5 g, Trisodium citrate: 2.5 g, Potassium chloride: 1.5 g, Glucose/sucrose: 20.0 g, and Water (boiled and cooled) : 1 litre

The glucose is essential because it stimulates uptake of sodium and subsequent absorption of water. The uptake of water in the absence of glucose is insufficient to replace fluid loss.

Intravenous infusion or rehydration is usually required for initial rehydration of severely dehydrated patients.

Many antimicrobial drugs are effective against *V. cholerae*. Oral tetracycline tends to reduce stool output in cholera and shortens the period of excretion of vibrios.

7.2.5.2. Prophylaxis: Control of cholera mainly rests on improvement of sanitation particularly of food and water. Patients should be isolated, their excreta disinfected.

Two types of vaccines are available against cholera. For antimicrobial immunity, killed vaccines (containing heat killed vibrios) are used, and for immunity against the toxin, formalin treated inactivated toxin (toxoid) is used.

Repeated injections of vaccine containing either lipopolysaccharide extract from vibrios or dense *Vibrio* suspension can confer limited protection to heavily exposed persons, but not effective as an epidemic control measure. The WHO vaccination certificate for cholera is valid for only 6 months.

7.2.6. Diagnosis:

7.2.6.1. Specimens: Specimens for observation and culture consists of mucous flecks from stools. Vomitus is not commonly used. Polluted waters and suspected food samples are also used for isolation of *Vibrio.*

7.2.6.2. Microscopy: The microscopic appearance of smears made from stool samples is not distinctive. Dark field or phase contrast microscopy may show rapidly motile *Vibrio* cells.

7.2.6.3. Culture: Peptone agar or blood agar is commonly used for culture of *Vibrio*. pH near 9.0 and temperature of 37 ^{0}C is optimum for bacterial growth. Typical colonies develop in 18 hours.

7.2.6.4. Agglutination test: Specific test for identification of *Vibrio* is slide agglutination test using anti O-group1 antiserum.

7.2.6.5. Biochemical tests: Two biochemical tests are important in classification of *Vibrio* biotytes. 1) Voges-proskauer test and 2) Haemolytic activity. Classical *Vibrio* is VP negative and non haemolytic while Eltor *Vibrio* is VP positive and haemolytic.

7.3. PLAGUE CAUSED BY *YERSINIA PESTIS*:

Plague is an ancient dreaded disease of mankind. Ancient civilizations of Asia were quite aware of the disease. The causal organism of plague was first isolated independently by French bacteriologist AEJ Yersin and Japanese Bacteriologist Kitosato in 1894. Though the pathogen was called by various names earlier, it is now called *Yersinia pestis* and is placed in the family Enterobacteriaceae.

7.3.1. Occurrence: *Y .pestis* naturally occurs mainly in wild rodents and transmitted through one to the other by rat fleas. Man is only an accidental collateral host.

7.3.2. Characters:

7.3.2.1. Morphology: *Y .pestis* is a Gram negative rod that exhibits striking bipolar staining (Fig. 7.2). Cells are pleomorphic, may be coccoid, oval or slender rods, measure about 1.5 x 0.7 μm. It is non motile and non spore forming. It is surrounded by a thin slime layer (envelop or capsule).

Figure 7.2. *Yersinia pestis*

7.3.2.2. Cultural characters: It grows as a facultative anaerobe on many bacteriological media. It ferments glucose, maltose, mannitol etc., but not lactose, and produce acid but not gas. On nutrient agar medium colonies are small, delicate, transparent discs becoming opaque on continued

incubation. In broth medium, it is a slow grower and does not produce turbidity. In older cultures there is pellicle formation. Optimum temperature for growth is 27^0C range 22 to 45^0C. Optimum pH for growth is 7.2 and range 5 - 9.6. It produces coagulase enzyme, which is implicated in virulence of the strains.

7.3.2.3. Toxins: *Yersinia* possesses lipopolysaccharides that have endotoxic activity, when released. In addition to it, it produces an exotoxin which is a protein. The term plague toxins refer to both, and they are called murine toxins as they are active in rats. The role of plague toxins in man in not known.

7.3.2.4. Antigens: Antigenically *Y. pestis* is homogenous and serotypes do not exist. At least 20 antigens are detected and some are regarded as virulence factors. The pathogen is covered by a protein envelope, when cultured at 37^0C and it inhibits phagocytosis and is generally present only in virulent strains. Antibodies formed against the protein envelope antigen gives protection against infection (at least in mice). Virulent wild type *Y. pestis* possess two antigens designated as V and W, always produced together. They are considered to be virulence factors as they inhibit phagocytosis.

7.3.3. Infections: Plague is a disease of rats and other rodents. Rats constitute the main reservoir of infection for man (Fig. 7.3).

Figure 7.3. Pathogenesis of plague

The pathogen is transmitted from rat to rat by fleas. *Xenopsylla cheopis, X. astia* and *Ceratophyllus fasciatus* are the major rat fleas that transmit the disease.

When a flea feeds on an infected rodent, pathogen enter the flea stomach and multiply extensively and helped by the enzyme coagulase causes mechanical gut blocking. When such a blocked flea bites another healthy rodent, it can not suck blood because bacterial mass blocks the passage mechanically. The blood mixed with bacteria reenter the bite and the rodent becomes infected. The blocked hungry fleas bite ferociously and actively transmit the pathogen.

7.3.4. Types of plague: The victims of flea transmission are usually rats but occasionally man. In man plague occurs mainly in three forms viz. bubonic plague, pneumonic plague and septicemic plague. Cutaneous plague is considered as a fourth type.

7.3.4.1. Bubonic plague: It is the most common type following the bite of an infected flea, after an incubation period of 2-5 days, the regional lymph nodes enlarge. The inflated lymphnodes are called Buboes. Hence the name bubonic plague. The buboes enlarge and suppurates. The pathogen enters the blood stream and produce septicemia. This form of disease is not readily transmitted from person to person. The case fatality in untreated patients varies from 30-90%.

Pneumonic plague: In the course of bubonic plague, a focus of infection is set up in the lungs, resulting in rapidly spreading haemorrhagic branchopneumonia. Thus preumonic plague do not start the epidemic, but once the stage occurred, the patient is highly infections. The bloody mucoid sputum coughed out by patients contains enormous numbers of pathogenic cells, and transmission occurs by droplet nuclei. Rat fleas are not involved in the spread of pneumonic plague. In untreated cases fatality is almost cent percent.

7.3.4.3. Septicaemic plague: This is usually the terminal event in bubonic or pneumonic plague. In highly susceptible person early invasion of blood may occur with obvious involvement of lymph nodes and lung, but it is a rare event.

7.3.4.4. Cutaneous plague: When the organism gains entrance into the skin by the bite of an infected rat flea, it multiplies and may produce a local lesion in the form of a vesicle containing pure culture of the organism. The carbuncle gradually turns into an ulcer.

7.3.5. Epidemiology:

Central Asia is believed to be original place from where it spread to various countries causing epidemics and pandemics resulting in great human loss. Historians of plague identify 41 epidemics before Christ and 109 in the first 15 centuries AD. Between 1500 and 1720 AD 45 pandemics were recorded. The pandemic that started in 542 AD, termed great plague, was bubonic plague and responsible for death of over 100 million people in 50 years. The pandemic that occurred during 14th century in Europe, termed 'black death', was the worst catastrophe that struck man kind, and it decimated an estimated 1/3 of world population. The name 'black death' was coined because of severe cyanosis (blue or purple color of the skin) that developed during the terminal stages of the disease. The disease was quiescent during 18th century and most of 19th centering, and last great epidemic started in central Asia (Hong Kong) in 1894 and spread throughout the world. India was one of the countries worst hit by this pandemic. It reached Bombay in 1896 and spread all over the country, causing more than 10 million deaths by 1918. It gradually subsided thereafter. During 1958-77 plague occurred in 29 countries involving 45,000 cases but relatively few deaths. It is in news once again during 1994 when plague broke out in Surat, Gujarat, after a massive earth quake.

Plague is essentially a disease of wild rodents and infection is transmitted by rat fleas. Man is only an occasional host. Plague epidemics generally occur in the cool humid seasons that favour multiplication of fleas, leading to a high flea index (i.e. mean number of fleas per rat). In hot, dry weather fleas do not survive and transmission of infection is interrupted.

Governmental plague commission in Bombay, which probed in to the nature of epidemics in India revealed that plague produced epizootics first in *Rattus norvegicus* (sewer rat). When their number dwindled the disease passed to the domestic rat (*Rattus rattus*). It was from the domestic rats that infection spread to man.

Two natural cycles of plague exist, the domestic and wild plague. The term domestic plague refers to the one transmitted through house rats, wild plague (called sylvatic plague) occurs in nature in wild rodents, independent of man. The rodents involved vary in different regions. Over 200 species and subspecies are involved. In USA, prairie dogs, ground squirrels, wood rats and mice have infection. Pet cats also found infected.

Geographical distribution and efficiency of rat flea species involved in transmission also play an important role in epidemics. *X cheopis*, an efficient transmitter is a prevalent rat flea in North India while in South India *X. astia*, a relatively mild transmitter is the major species. Hence, plague epidemics are more prevalent in North than in South India.

7.3.6. Diagnosis:

In bubonic plague the pathogen may be readily demonstrated in buboes by microscopy, and blood samples are taken for culture. In pneumonic plague sputum is used for microscopy and culturing. Smears are observed for rods with typical bipolar staining. For culturing blood specimens, MacConkey agar medium is commonly used. Serological tests are positive during active infection.

7.3.7. Treatment:

Tetracycline is the recommended drug for both bubonic and pneumonic plague. Streptomycin, chloramphenicol, gentamicin and kanamycin are also effective.

7.3.8. Preventive measures:

Control of fleas and rodents are of great importance in prevention of plague epidemics. Garbage is the breeding place of rats, and public health measures to keep the environment clean is an essential measure.

In India a formalin killed vaccine prepared by Halfkine Institute, Bombay was in use and it gives protection against infection up to 6-12 months.

When a human case is diagnosed or suspected, patient should be isolated, especially in case of pneumonic plague, which is highly contagious. Contacts of patients with suspected plague pneumonia should receive tretracycline 0.5/g/d for 5 days as chemoprophylaxis.

7.4. *TREPONEMA PALLIDUM*

Syphilis is one of the most common venereal diseases through out the world. It was first recognized in Europe near the end of the 15th century. It is thought to be of New World origin, and Christopher Columbus (1451-1506) and his crew acquired it in the West Indies and introduced

into Spain after their return from their historic voyage. In 1838 Phillppe Ricord recognized different stages of the syphilis through his observations on more than 2,500 human inoculations. In 1905 Fritz Shaudinn and Erich Hoffmann discovered the causative organism *Treponema pallidum*, a spirochete.

7.4.1. OCCURRENCE: Man is the only natural host for *T. pallidum*.

7.4.2. CHARACTERS:

7.4.2.1. Morphology: *T. pallidum* is an extremely slender, unicellular long filamentous spiral organism measuring 5-20 μm in length and less than 0.2 μm in thickness. It has 4-14 coils (spiral) which are uniform and about 1 μm in length. The ends are pointed and tapering. The tapered terminals are often described as terminal flagella. Each cell has three axial filaments inserted at each end of the protoplasmic cylinder and overlapping in the middle of the cell. The axial filaments (also called endoflagella or periplasmic flagella) coil around the protoplasmic cylinder. Covering the protoplasmic cylinder and axial filament, an outer sheath or periplast is present.

7.4.2.2. Staining: It is gram negative in staining character but takes the stain very pale or poorly. As they are very slender and take the stain poorly they are best observed under dark field microscopy (Fig. 7.4). In this, light is reflected rather than passed through the field. Hence the background is dark, and the organism that reflects the light and appears bright.

Figure 7.4. *Treponema pallidum* – dark ground illumination

7.4.2.3. Culture: The pathogen can be propagated in vivo in laboratory animals, but only poorly. It is very difficult to culture in vitro.

7.4.2.4. Antigens: Characterization of treponemal antigens has been greatly impeded by the inability to grow them in culture. A specific antigen of *T. pallidum* was detected by *T. palladium* immobilization (TPI) test. In the test, treponemes obtained from syphilitic lesions in rabbit are mixed with patient serum and after incubation for 18 hrs at 35^0C anaerobically, the organisms were immobilized if the serum contain specific antibodies.

Employing Radioimmunoassay (RIA) and western blot techniques a *T. pallidum* specific protein antigen has been recognized.

7.4.3. INFECTIONS:

Syphilis occurs only in humans and is transmitted by sexual contact, causing venereal syphilis. It enters the body through mucous membranes or minor breaks or abrasions of the skin. Apart from sexual contact, another mode of spread is through placental transfer from an infected mother to the foetus during first 4 months of pregnancy. It results in congenital syphilis in new born babies.

Venereal syphilis is in adults and congenital syphilis in new born babies progress differently and can be studied separately.

7.4.4. VENEREAL SYPHILIS: It progresses through three different recognizable stages 1. primary stage, 2. Secondary stage and 3. Tertiary stage.

7.4.4.1. Primary stage: This stage is also called chancre stage. After initial incubation of about 10-20 days or more, a small, painless, reddened ulcer with hard margin develops on genitals, the initial site infection. The chancre begins as a papule and breaks down to form a superficial ulcer with a firm base. The predominant inflammatory cells in the lesion are lymphocytes and plasma cells. The pathogen is present in the chancre. Contact with the chancre during sexual intercourse may result in disease transmission. During the primary stage the patient does not feel ill and chancre usually heals within 20-40 days.

Although the chancre heals spontaneously, organisms escaping from it at an early stage invade the regional lymph nodes forming satellite buboes and eventually reach the blood stream and establish a systemic infection.

7.4.4.2. Secondary stage: It is characterized by a skinrash, 2-12 weeks after the primary lesion appeared. It results from systemic spread of the pathogen. Mucous membranes of the body may also involved and the patient may have lymphoadenopathy (=swollen lymph nodes) malaise (= a vague feeling of bodily discomfort), slight fever and headache. The pathogen may be present in lesions but are often hard to demonstrate. In highly susceptible individuals lesions may develop in bones, liver, kidneys, CNS and other organs also. Usually the skin and mucous membrane lesions disappear after 3-12 weeks and the disease becomes latent. The patient is highly infectious during skin-rash stage, as the pathogen is present in the lesions. The other symptoms of secondary phage are the loss of hair in patches, malaise and mild fever.

7.4.4.3. Tertiary stage: In approximately 75% of the untreated patients enough treponemes persist in tissues in latent form. The latent stage may continue for a few to many years (up to 10 years) and reactivation occurs in about one third of the persons with latent infections.

Tabes dorsalis or general paresis of CNS and gummas on skin are the major symptoms of tertiary stage. The tertiary lesions often contain very few organisms but frequently result in necrosis, scar formation and extensive damage, probably involving a delayed hypersensitivity response to products of the small number of persisting organisms.

General paresis (=slight or incomplete paralysis) or tabes dorsalis is the main symptom. It results from degeneration of posterior roots and ganglions of the spinal cord. Damage to the nervous system results in uncoordinated movements and loss of reflexes, uncontrolled urination, loss of perception and sensation, impotency, lightning pains, loss of hearing, blindness etc. In the initial stages the symptoms include failing memory insomnia and delusion. Shuffle walk and insanity may occur.

Disfiguring granulomatous lesions on the skin are called gummas. They appear on various parts of the body. Gummas are rubbery masses and are not very painful, but disfiguring. When they develop on eyes or ears blindness of deafness may result.

Severe tertiary stage is very difficult to treat and final stage is inevitable. Even though tertiary syphilis is mild patients usually have 5 to 10 years taken off their life span and exhibit symptoms of senility (i.e. being old before one's time) such as blindness, difficulty in hearing etc.

7.4.5. TREATMENT: Penicillin is the drug of choice in treatment of syphilis. Tetracycline is also effective. In early stages, the control can be accomplished with a single dose of long acting benzathine penicillin G or aqueous procaine penicillin. Later stages of syphilis are more difficult to treat with drugs and require much larger doses over a longer period.

7.4.6. DIAGNOSIS: Since the pathogen cannot be cultured, microscopy and serological tests are used for diagnosis.

7.4.6.1. Microscopy: In its primary and secondary stages, syphilis can often be diagnosed by dark field examination of fresh exudates fluid obtained from open lesions. *T. pallidum* can be differentiated from other spiral organisms on the basis of its characteristic morphology and motility. Negative dark field examinations do not necessarily exclude the diagnosis of syphilis, as lesions in the later stages of the disease may contain relatively few organisms.

Treponema pallidum may also be identified in fluid from active lesions by immunofluorescent staining. Monoclonal or polyclonal antibodies to surface component of the pathogen are conjugated with fluorescent dyes and used for staining. If *T. pallidum* is present, it fluoresces because fluorescent antibodies attach to it and it can observed under fluorescent microscope.

7.4.6.2. Serological tests: A number of serological tests have been developed for diagnosis of syphilis

7.4.6.2.1.Wasserman test: It is compliment fixation test. In this test, patients serum is heated to 56 ^{0}C to inactivate compliment in the serum (but antibodies retain activity) and mixed with

antigen and external source of compliment is added. After 24 hours of incubation, sheep red blood cells and antibodies to them are added and incubated for the second time. If the compliment is not fixed in the original system, it will be available for lysing the sheep red blood cells. If the compliment is fixed in the original system, the sheep RBCs do not lyse because compliment is not available.

1. antigen + patient's serum + external compliment ------ Incubation for 24 hrs
(heated to 56 ^{0}C) (from sheep)

After 24 hours

2. First set up + Sheep RBCs + Antibodies to sheep RBCs ----- Incubation for 24 hrs

Observation		**Result**
1. Lysis of sheep	-----	Patient's serum do not contain syphilis antibodies. Patient is negative for syphilis
2. No lysis	-----	Patients serum contain antibodies to syphilis pathogen Patient is positive for syphilis

7.4.6.2.2. Kahn test: It is a tube flocculation test. The antibodies to *T. pallidum* can cross react with cardiolipin, a lipid extract from healthy animal heart. Hence, it is used as antigen to detect antibodies against syphilis pathogen. Cardiolipin + patient's serum is incubated in test tubes over night. Flocculation indicates that patient is positive for syphilis.

7.4.6.2.3. VDRL test: It is the simplest and most commonly used serological test for syphilis. It is a slide flocculation test developed by venereal disease research laboratory. Hence, it is called VDRL test. In this test, a few drops of antigen preparation (cardiolipin) and serum from patient are mixed on a slide. For thorough mixing, a VDRL shaker is used. The slides are observed for flocculation under microscope.

7.4.7. CONGENITAL SYPHILIS:

During pregnancy *T. pallidum* readily cross the placenta and infects the foetus. This occurs at any stage of pregnancy but damage to the foetus does not generally develop until 4 month stage. Therefore, if mother's syphilis is diagnosed and treated before the 4^{th} month of pregnancy, the foetus will be treated too and will not develop disease. Without treatment, the risk to foetus depends partly on the stage of the mother's infection. More than 75% foetuses become infected if mother has primary or secondary syphilis. Significant infections also occur during latent period. About 40% of fetuses are lost through miscarriage or still birth, and the remaining borne with congenital syphilis and frequently appear normal at the time of birth. However, some of these infants will develop characteristic deformities on their face, teeth and other body parts later in the child hood. Affected children suffer poor bone formation, meningitis and/or Hutchinson's triad, a combination of deafness, impaired vision and notched peg like teeth. This triad was first described by Joseph Hutchinson of London Hospital in 1861. The tertiary syphilis that develops in children is very difficult to cure.

7.5. SUMMARY:

Vibrio cholerae causes cholera, an acute form of diarrhea characterized by sudden onset and often running a rapid and fatal course. The pathogen is a Gram negative, non spore forming, noncapsulate, actively motile curved or comma shaped bacterium that naturally occurs in surface waters. It grows on ordinary bacteriological media, and optimum temperature for growth is 37 ^{O}C and optimum pH 8.5. Cholera is a toxigenic diseases, and only the strains that produce toxin are pathogenic. Cholera toxin is an enterotoxin and when it enters the epithelial cells of small intestine it stimulates hypersecretion of water and electrolytes from the infected cells resulting severe diarrhoea and dehydration. The pathogen spreads mainly by contaminated waters. Since 1800 AD seven pandemics were recorded and the 7^{th} pandemic started in 1961 by a strain different from that causes classical cholera and is called El-Tor Vibrio. This strain causes less severe diarrhoea but the pathogen is vigorously excreted in the faeces of the patients. The most important therapy for cholera is immediate replacement of water and electrolytes in the form of oral rehydration

solution (ORS). Cholera vaccines are available for prophylactic use and they provide protection for only a limited period of about six months.

Plague caused by *Yersinia pestis* is an ancient dreaded disease of mankind. The pathogen naturally Infects mainly wild rodents and man is only an accidental collateral host. *Y .pestis* is a Gram negative rod that exhibits striking bipolar staining. It grows on many bacteriological media. Optimum temperature for growth is 27 ^{0}C range 22 to 45 ^{0}C. Optimum pH for growth is 7.2 and range 5 - 9.6. The pathogen is transmitted from rat to rat by fleas. *Xenopsylla cheopis, X. astia* and *Ceratophyllus fasciatus* are the major rat fleas that transmit the disease. The victims of flea transmission are usually rats but occasionally man. In man plague occurs mainly in three forms viz. bubonic plague, pneumonic plague and septicemic plague. Cutaneous plague is considered as a fourth type. Central Asia is believed to be original place from where it spread to various countries causing epidemics and pandemics resulting in great human loss. Tetracycline is recommended drug for treatment.

Treponema pallidum, a spirochete, caused syphilis, one of the most common venereal diseases. The pathogen is an extremely slender, unicellular long filamentous spiral organism measuring 5-20 μm in length and less than 0.2 μm in thickness. It has 4-14 coils (spirals) which are uniform and about 1 μm in length. Each cell has three axial filaments inserted at each end of the protoplasmic cylinder and they coil around the protoplasmic cylinder. It is gram negative in staining character but takes the stain very pale or poorly. It is very difficult to culture in vitro. The disease occurs in two forms viz. venereal syphilis is in adults, and congenital syphilis in new born babies. Venereal syphilis is a sexually transmitted disease and it progresses through three stages viz. primary, secondary and tertiary stages. The primary stage is also called chancre stage and it is characterized by formation of a small, painless, reddened ulcer with hard margin develops on genitals. The secondary stage is characterized by a skin-rash, 2-12 weeks after the primary lesion appeared. It results from systemic spread of the pathogen. The tertiary stage is a reactivation disease that appears after a latent period of 5 to 10 years. Tabes dorsalis or general paresis of CNS and gummas on skin are the major symptoms of tertiary stage. Penicillin is the drug of choice in treatment of syphilis. Tetracycline is also effective. The congenital syphilis is a disease of new born babies or young children. During pregnancy *T. pallidum* readily cross the placenta and infects

the foetus. More than 75% foetuses become infected if mother has primary or secondary syphilis. About 40% of fetuses are lost through miscarriage or still birth, and the remaining borne with congenital syphilis and frequently appear normal at the time of birth but develop characteristic deformities later in the child hood. Wasserman's complement fixation test, Kahn's tube precipitation test and VDRL test, a slide precipitation test, are common serological tests for diagnosis of syphilis and of these, VDRL is most commonly used.

7.6. MODEL QUESTIONS:

Essay type questions

Give an account of pathogen, pathogenesis, epidemiology and control of cholera

Give an account of pathogen, pathogenesis, epidemiology and control of plague

Give an account of pathogen, clinical symptoms and control of syphilis

Give an account of diagnosis and control of cholera and syphilis

Describe the pathogens and clinical symptoms of plague and syphilis

Short answer type questions

Vibrio cholerae
Cholera toxin
Symptoms of cholera
Treatment for cholera
Yersinia pestis
Bubonic plague
Types of plague
Control of plague
Treponema pallidum
Venereal syphilis
Congenital syphilis
Diagnosis of syphilis

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LESSION–8: BACTERIAL DISEASES CAUSED BY GRAM POSITIVE RODS AND FILAMENTS

Objective: To study the diseases caused by Gram positive rod shaped bacteria and filamentous bacteria

Contents

8.1. INTRODUCTION

Tetanus, diphtheria and tuberculosis are very serious diseases of humans caused Gram positive bacteria. Tetanus is caused by *Clostridium tetani*, an obligately anaerobic, spore forming, rod shaped bacterium. Diphtheria is a serious paediatric disease occurring mostly in the children below five years of age, and it is caused by *Corynebacterium diphtheriae*, a Gram positive, irregularly rod shaped bacterium. Tuberculosis is the most important killer disease of humans throughout the world, and it is caused by *Mycobacterium tuberculosis*, a filamentous bacterium previously placed in the group actinomycetes, but now treated as a separate group along with Gram positive rod shaped bacteria. The characters of the pathogens, pathogenesis, clinical symptoms, epidemiology, diagnosis and control of tetanus, diphtheria and tuberculosis are discussed in this lesson.

8.2. *CLOSTRIDIUM TETANI*

Clostridium tetani was first isolated by Nicolaier in 1884 from a tetanus patient, and it was described by Flugge *et al*. in 1886 and final proof of its pathogenicity was provided by Kitasato in 1889.

8.2.1. Occurrence: *C. tetani* is present in the intestinal tract of herbivores particularly cattle, horses and is widely distributed in the soil, especially when the land has been manured and cultivated. It may also be present in the dust of streets, houses and hospitals. It has occasionally

been found in human faeces. In soil and other areas the pathogen is present in the form enodospores, which are very resistant to adverse conditions and can remain viable for a number of years.

8.2.2. Characters:

8.2.2.1. Morphology: *C. tetani* cells are gram positive, slender straight rods measuring 2.5-5 x 0.5-1 μm in size. The cells are motile with peritrichous flagella. They possess no capsules. The organism is a strict anaerobe.

C. tetani produces conspicuous and highly resistant endospores. The endospores are spherical and formed at the end giving the cell a 'drumstick' appearance (Figure 8.1).

Figure. 8.1. *Clostridium tetani* – some with spores and some without

8.2.2.2. Culture: It easily grows on simple media under anaerobic conditions but commonly cultured on blood agar medium show α-haemolysin and later β-haemolysin. On solid media the colonies are thin, translucent, spreading with finger-like projections. Optimum temperature for growth is 37 ^{0}C and optimum pH 7.4. In broth medium shows uniform turbidity.

8.2.2.3. Biochemical: It do not attack any sugar and weakly proteolytic. The most important biochemical character of *C. tetani* is production of exotoxin called tetanospasmin. It is the most potent neurotoxin. The toxin is a polypeptide of M.W. 1,60,000, and its production is under the

control of a plasmid gene. In the receptor cells the toxin splits into 2 fragments by the action of proteolytic enzymes, and these fragments show increased toxicity. Minimum lethal dose (MLD) for humans is about 130 nanograms. Mammals are highly susceptible but, birds, reptiles and amphibians are resistant.

8.2.2.4. Antigens: 10 serotypes were identified (I to X) based an agglutination tests. All produce same type of toxin. Several flagellar (H) antigens are present and same type of somatic (O) antigens. These structural antigens are not virulence factors. The toxin produced by the pathogen is a potent antigen and it is the virulence factor. Only the toxigenic strains are pathogenic.

Normally no immunity develops after recovery from tetanus presumably because the amount of toxin is too small to elicit immune response.

8.2.3. Infections: *C. tetani* is not an invasive organism. The infection remains strictly localized in the area into which the spores have been introduced. The infections are mainly associated with wounds contaminated with soil and foreign matter, deep puncture wounds, wounds involving extensive tissue destruction and burns. The infections are particularly common in regions where bare footed persons often with multiple chronic infected lesions on the feet live in close association with horses, cattle, goats and other domesticated animals. When the spores are introduced into the tissues of man, bacterium grows and cause disease only when certain conditions are fulfilled. Interference with blood supply to at least a small amount of tissue, together with multiplication of other organisms which result in depletion of oxygen, appears to be necessary for the production of an anaerobic environment in which the bacterium can grow. Presence of soil particles in the lesion increases the risk of tetanus probably because the calcium salts stimulate germination of spores.

The incubation period may range from one week to more than one week. The pathogen do not spread from the place of its entry or growth, but release a neurotoxin, which cause the disease. The toxin does not harm locally but travels along the peripheral nerves to the central nervous system where it inhibit the transmission of nerve impulses. The toxin also spread by blood sheaths.

The disease is characterized by convulsive contraction of voluntary muscles. Muscular spasms often involve first the area of injury and infection. Next the muscles of jaw are affected and it becomes very difficult to open the mouth, hence it is called ‘lock-jaw’. In the third phase,

voluntary muscles are involved resulting in generalized spasms and bending of the body backwards like a bow (Fig. 8.2).

Figure 8.2. Painting of a patient dying of tetanus. Extreme bending of the back like a bow is is the characteristic symptom of the severe form of tetanus

Increased tension of muscles with exaggeration of deep reflexes and some loss of muscle control is called spasm. The patient is fully conscious and pain may be intense. The final stage (death) reached in about 50% of untreated cases and it results from respiratory paralysis due to the effect on thoracic muscles.

8.2.4. Types of tetanus: Depending upon the nature of infection, tetanus is of several types.

8.2.4.1. Acute tetanus: Incubation period is less than 10 days and symptoms are acute, progressive and often fatal.

8.2.4.2. Chronic tetanus: Incubation period is about a month and symptoms are less severe. The incubation period may be longer when patient receives prophylactic injection of antitoxin.

8.2.4.3. Delayed tetanus: The organism remains latent in the wound for long period and tetanus is produced when the wound is reopened.

8.2.4.4. Idiopathic tetanus (Idiopathic= without evident reason): In this, the wound heals and the host does not develop tetanus until the general or local resistance is lowered by some other

infection or local trauma. Microscopic trauma or absorption of toxin from intestinal tract may also induce tetanus.

8.2.4.5. Local tetanus: It occurs around the initial wounds in patients who have received anti-toxin.

8.2.4.6. Uterine tetanus: It may occur due to septic abortion.

8.2.4.7. Surgical tetanus: Use of contaminated surgical instruments may cause the disease.

8.2.4.8. Tetanus neonatorum: Due to infection in new born babies through the wound left after the separation of the umbilical cord. It is one of the most under reported notifiable disease. This disease occurs mainly in areas with poor access to health care.

8.2.5. Spread: Tetanus is not a contagious disease, and infections do not spread from person to person. All infections arise independently.

8.2.6. Therapy: Penicillin and other antibiotics are effective against C. tetani. They can stop further production of toxin by inhibiting the growth of the organism but cannot cure established disease. But, the use of antibiotics control associated pyogenic infections. ATS (anti tetanus serum) is given for therapy against circulating toxin.

8.2.7. Immunization: Immunization is against the toxin, not against the organism it self. Active immunization, sufficiently far in advance and adequately maintained prevents tetanus. Three injections comprise initial course of immunization followed by another dose after one year and third dose at the age of 5 years. In young children, tetanus toxoid is often combined with diphtheria toxoid and pertussiss vaccine, thus making it a triple vaccine called DPT. Boosting of active immunity at the time of any injury is given by tetvac injection.

8.2.8. Diagnosis: Diagnosis rests on clinical picture or symptoms. Anaerobic culture from contaminated wounds may yield *C. tetani* but neither preventive nor therapeutic use of antitoxin should be withheld, pending diagnosis. In many typical cases also the isolation of the organism is often difficult. It may not be possible to identify the relevant lesion and the bacilli may be present only in small numbers. They are often out numbered by other organisms. Proof of isolation of *C. tetani* rest on production of toxin and its neutralization by specific antisera.

Animal inoculation tests are usually employed for confirmation of toxicigenicity by the isolated strain. The inoculation is generally made on tail, and symptoms develop within 24 hrs starting with tail-stiffness. The animal dies within 2 days.

8.3. *CORYNEBACTERIUM DIPHTHERIAE*

Corynebacterium diphtheriae is the causal organism of diphtheria, an important paediatric disease. Diphtheria is known since ancient times but first recognized as a clinical entity in 1826 by French pathologist Pierre Bretonneau, who described it as 'diphtherite' infection because of the formation of a tough leathery pseudomembrane (Gr.diphtheros=leather, membrane). The pathogen was first described by Klebs in 1883 and first isolated by Loeffler in 1884. Roux and Yersin (1888) discovered that the pathogen produces a cytotoxic exotoxin (diphtheria toxin) which is responsible for the manifestation of characteristic pathological symptoms. Emil von Behring, a German physician, in 1890 discovered that the serum of artificially inoculated animals acts as antitoxin, and this paved the way for 'passive immunization' against diphtheria. For the discovery of antitoxin against diphtheria, Behring received Nobel Prize in 1901, and he was the first microbiologist honoured with Nobel Prize in Medicine and physiology.

8.3.1. Occurrence: *C. diphtheriae* is essentially a human pathogen. Patients, either with active infection or convalescent carriers are main source of the pathogen. It mainly occurs in the nasopharyngeal region.

8.3.2. Characters:

8.3.2.1. Morphology: It is gram positive, aerobic/facultative anaerobic, non spore forming, noncapsular, nonmotile, straight or curved rod that tapers with characteristic club shaped swelling (Gr. Coryneum= a club) at one or both ends. They measure 3-6 μm in length and 0.6.-0.8 μm in width. Granules composed of polymetaphosphate are seen in the cells. They are more strongly gram positive than the rest of the cell. The granules are called metachromatic granules or volutin granules or polar bodies.

The bacilli are arranged in a characteristic fashion in smears. They are usually seen in pairs or small groups, the bacilli being at various angles to each other, resembling the letters V or L or like Chinese letters. This is due to incomplete separation of the daughter cells after binary fission (Fig. 8.3).

Figure 8.3. *Corynebacterium diphtheriae*. Unseparated cells with irregular arrangement (left), and typical club shaped cells with strongly stained parts (right)

8.3.2.2. Culture: Growth is scanty on ordinary medium. Blood agar is commonly used. Telleurite blood agar is selective medium. Colonies are small 1-2 mm greyish black on telleurite agar. Optimum temperature is 37^0C and the range 15-40 ^{0}C. Optimum pH is 7.2. Potassium telleurite inhibits the growth of many other bacterial species. It reacts with *C. diphtheriae* causing the colonies to appear gray or black.

Basing on colony morphology on telleurite medium and other properties, especially pathogenicity, *C .diphtheriae* is divided into 3 types viz.

Gravis: colonies brittle, pellicle form in broth, no turbidity, cells straight, no metachromatic granules, cause most severe infection, cause epidemics.

Intermedius: intermediate between gravis and mitis in all characters.

Mitis: colonies soft and buttery, cells long curved pleomorphic with prominent metachoromatic granules, ferment glucose, galactose, maltose and dextran produce acid and but not gas, donot ferment lactose, sucrose or mannitol.

8.3.2.3. Diphtheria toxin: Many strains produce a powerful toxin called diphtheria toxin. Only lysogenized strains attain genetic capability to produce toxin. Further, the toxin production is

stimulated when iron availability decrease in the media. The pathogenic effects of the pathogen are due to the toxin.

Diphtheria toxin is a protein and has been crystallized. It has a molecular weight of about 62,000. Lethal dose to a 250 gram guinea pig is 0.0001mg. The toxin molecule consists of 2 fragments A and B of 24,000 and 38,000 M.W. When released by the bacterium, the toxin is inactive site on the fragment A is masked. Fragment B is responsible for binding the toxin to the host cells and enters the host cell with in the host cell cytoplasm the disulphide bond of diphtheria toxin is reduced and broken, releasing enzymatically active fragment A of the toxin that catalyzes the covalent transfer of ADP-ribose from NAD to EF-2 inactivating it. EF2 is an elongation factor in polypeptide synthesis. One toxin molecule can kill a cell by ADP-ribosylating more than 106 EF-2 molecules.

The toxin is attached to a specific receptor on the host cell. The cells that lack appropriate receptor do not take up toxin and are unaffected by it. This receptor specificity explains why some tissues of the body are not affected while some tissues such as those in heart and nerves are severely damaged.

8.3.2.4. Antigenic characters: Diphtheria bacilli are antigenically heterogeneous. By agglutination tests gravis has been classified into 13 types intermedius into 4 types and mitis into 40 serotypes. Toxin is a powerful antigen and is the major virulence factor.

8.3.3. Infections: Diphtheria is essentially a child hood disease. Human beings either carriers or persons with active infection are the source of *C. diphtheriae*. The pathogen spread by droplet nuclei. The organisms are inhaled and establish infection in the upper respiratory tract. The incubation period varies from 2-7 days, commonly 3-4 days. They have very little invasive ability and mainly confine to the site of entry and produce a powerful toxin. Diphtheria is a toxemia and the toxin causes local necrotic changes and the resulting fibrinous exudate together with disintegrating epithelial cells, leucocytes, erythrocytes and bacteria, constitute a grayish white pseudomembrane (= diphtheros) which is characteristic of diphtherite infections. The membrane may come loose and obstruct the airways so that the patient may be some times choked to death. Absorption of toxin by body cells result in cessation of cellular protein synthesis, the effect occurring at the stage of activated amino acyltransfer from tRNA to the growing peptide chain of a

protein. Thus the mechanical complications of diphtheria are due to the membrane while systemic effects are due to the toxin.

Diphtheria usually begins with a mild sore throat and slight fever accompanied by a disproportionately great amount of fatigue and malaise.

The pathogen mainly occurs in the pharynx or throat and the infection is referred to as pharyngeal diphtheria or simply diphtheria. The infection that occurs in larynx is called laryngeal diphtheria. Infection of nose is nasal diphtheria, and infection of skin is called cutaneous diphtheria.

8.3.3.1. Pharyngeal diphtheria: It is the most common type of infection and is characterized by marked tonsillar and pharyngeal inflammation and formation of a tough, greyish white membrane (diphtheros). The membrane is formed by fibrin, bacteria, epithelial cells, mononuclear cells and polymorphs, and is firmly adherent to the underlying tissue. Regional lymphoadenopathy is prominent and produces bull neck (cervical adenitis).

8.3.3.2. Laryngeal diphtheria: The upper part of the trachea or the space between trachea and base of the tongue is known as larynx. The laryngeal infection is not a primary infection but is due to extension of the membrane from the pharynx.

A husky voice, a brassy cough and later dyspnoea (=difficulty in breathing) and cyanosis (=a bluish discoloration of skin and mucous membranes) due to respiratory obstruction are common features.

One of the major complications of pharyngeal or laryngeal diphtheria is myocarditis, due to absorption of the bacterial exotoxin by the myocardium, the muscle that form the wall of heart. Acute circulatory failure due to myocarditis may occur in untreated patients or/and convalescent individuals around the 10th day of illness and is usually fatal.

Bleeding from the edge of the membrane, haemorrhage is another complication.

8.3.3.3. Nasal diphtheria: It is characterized by the presence of a unilateral serosanguinous nasal discharge that crusts around the external nares.

8.3.3.4. Cutaneous diphtheria: It is associated with burns, and individuals with poor personal hygiene. Typically the ulcer is punched out with undetermined edges and is covered with a greyish white to brownish adherent membrane. Constitutional symptoms are uncommon.

8.3.4. Spread: Humans are the primary reservoir for *C. diphtheriae*. Sources of infections include carriers who have recovered from infections, new cases not exhibiting symptoms and people with

active disease .The pathogen is emitted as droplet nuclei and inhalation of air borne droplet nuclei start infection. As the aerial spread of the droplet nuclei is the main mode of transmission, the disease is more severe in semiclosed communities. It is typically a disease of schools where children of susceptible age are herded together.

8.3.5. Epidemiology: Diphtheria is an important pediatric disease world over, but now it is almost eradicated in most of the advanced countries. In poor and developing countries it is still endemic in many areas. In endemic areas it is mainly a disease of child hood. It is rare in the first year of life, reaches peak between 2 and 5 years, falls slowly between 5 and 10 years and rarely between 10 and 15 years, and the incidence above 15 years is almost insignificant.

Infection is rare in early infancy because of passive immunity obtained from the mother, and in adults due to active immunity acquired by repeated sub clinical infections.

The disease is common in rural areas than in urban areas. In rural endemic areas there may be 100 carriers for every clinical case. The high risk group is non immunized children below ten years of age living in crowded, unsanitary conditions.

8.3.6. Treatment: Specific treatment of diphtheria consists of antitoxic antibodies against the toxin. It must be administered immediately when a case is suspected of diphtheria without waiting for the proof of diagnosis since a delay of several days needed to obtain confirmation can be fatal.

The pathogen is susceptible to a number of antibiotics like penicillin, erythromycin etc. These antibiotics kill the pathogen but show no effect on the toxin that has been absorbed already. Hence, even with treatment about one out of 10 patients die.

8.3.7. Prophylaxis: Since the disease results primarily from toxin absorption rather than from microbial invasion, its control can be accomplished most effectively by immunization with toxoid (=toxin inactivated by treating with chemicals). It is given usually as part of a triple vaccine DPT. Three doses at six week intervals to the children, 4th dose after one year, booster dose at school entry.

8.3.8. Diagnosis:

8.3.8.1. Specimens: The throat swabs, swabs from infected region.

8.3.8.2. Culture: The pathogen is cultured on telleurite blood agar medium. Morphology and cultural characters are of preliminary diagnostic value to determine the pathogen.

8.3.8.3. Serology: Elek's test is a common serological test to identify the toxigenic strains of *C. diphtheriae* In this test a rectangular strip of filter paper impregnated with diphtheria antitoxin is placed on the surface of a 20% normal horse serum agar in a petridish while the medium is still fluid and slightly pressed. When the agar has set and the surface is dried, narrow streaks of the strains are made at right angles to the filter paper strip. A positive and negative controls are also set up. The plate is incubated at 37 ^{O}C for 24 – 48 hrs. Toxins produced by the bacterial growth will diffuse in the agar, and where it meets the antitoxin at optimum concentration produce lines of precipitation which appear like arrow head line (Fig. 8.4). The presence of the arrow head like lines of precipitation indicates that the strain is toxigenic one, while no precipitate will form in case of nontoxigenic strain.

Figure 8.4. Elek's test. A. Filter paper strip impregnated with diphtheria antitoxin, B. toxigenic strain, C. Nontoxigenic strain, D. test strain showing toxigenicity

8.3.8.4. Skin test: Schick (1913) developed a test to determine the susceptibility of the children for the purpose of immunization. In the test a small amount of toxin (1/50 concentration of MLD)

is injected into the fore arm, and observed for the reactions over a period of one week. In susceptible individuals erythema develops in 24-48 hrs and slowly increases reaching a maximum between 4 and 7days. In resistant individuals no erythema develops.

8.4. *MYCOBACTERIUM TUBERCULOSIS*

Mycobacterium tuberculosis is one of the major bacterial pathogens of mankind which causes the dreaded killer disease tuberculosis or TB. The disease is of ancient origin and there is evidence that some Egyptian mummies dated 1500-1000 BC had spinal tuberculosis. Great German Microbiologist Robert Koch in 1882 isolated the pathogen and proved its pathogenicity, and received Nobel Prize in 1905 for his studies on the disease.

8.4.1. Occurrence: *M. tuberculosis* is an exclusive human pathogen and can be transmitted to experimental animals through artificial infection.

8.4.2. Morphology: The cells of *M. tuberculosis* are straight or slightly curved delicate long rods that measure 3.0 x 0.3 µm. They occur singly or in pairs or as fragile filaments. The filaments in virulent strains appear as coiled bundles called 'serpentine cords'. The cord formation is due to a glycolipid of cell wall identified as 'Trehlose-6, 6-dimycolate'. The cord factor in bacterial cells can disrupt the respiration of mitochondria in phagocytes and tissue cells. Hence, it is considered as a virulence factor and strains lacking it are avirulent. In cultures the cord factor is indicated by rough looking colonies due to growth of bacteria in cable like arrangement (cords).

The organism is a strict aerobe and cells are non capsulate, non motile and non spore forming.

8.4.3. Staining property:

8.4.3.1. Gram staining: *M. tuberculosis* is Gram positive but do not easily takes up the stain in normal staining procedures. It is due to the presence of large amounts of lipid substance, mycolic acid in cell wall. However, when stained for long time (10 minutes) it takes up the stain, even without mordant iodine, and once stained, retain it even after treating with decolorizing agents.

8.4.3.2. Ziehl-Neelsen (Z-N) staining: It is also called Acid fast staining. It was first developed by Ziehl in 1882 later modified by Neelsen. It is the most commonly used staining method for mycobacteria. It is also a differential staining technique. Carbolfuschin is the primary stain. The cells take up the stain and appear red. When the smear is treated with acid alcohol (20% H2SO4 and absolute alcohol) for 10 minutes mycobacteria retain the stain. Hence they are called acid

alcohol fast or simply Acid fast bacilli (AFB). Acid fastness is ascribed to the presence of mycolic acid. Staining may be uniform or granular. Other than mycobacteria lose the stain by Acid-alcohol treatment, and for observing them counter stain is used. Methylene blue or some times malachite green are used as counter stain.

8.4.3.3. Fluorescent staining: Auramine and Rhodamine are fluorescent dyes specific to mycobacteria. The stained cells fluoresce under fluorescent microscope.

8.4.4. Cultural characters: *M. tuberculosis* has no exacting growth requirement but are highly susceptible to even traces of toxic substances. The most commonly used medium is Lowenstein-Jensen (LJ) medium which is an enriched medium with egg yolk together with mineral salt solution, asparagine and malachite green. The last one acts as selective agent inhibiting other bacteria.

Even on suitable medium, the bacilli grow slowly, the generation time in vitro being 14-15 hours. The colonies are usually very small and appear after 10-14 days or more. On solid media, the pathogen forms dry rough, raised, irregular colonies with wrinkled surface. They are at first creamy white later becoming yellowish or buff colored. They do not spread but tend to heap up. They do not easily emulsify. Optimum temperature for growth is 37 OC. They do not grow below 25^{O}C or above 40^{O}C. Optimum pH is 6.4 – 7.0. It is an obligate aerobe.

In liquid media, without dispersing agent (Tween 20 or T-80), the growth begins at the bottom, creeps up the sides and forms a prominent surface pellicle which may extend along the sides above the medium. Virulent strains tend to form large serpentine cords.

8.4.5. Biochemical characters: Important biochemical reactions commonly studied for characterization are

8.4.5.1. Niacin production: *M. tuberculosis* produces large amounts of niacin (nicotinic acid) when grown on egg yolk medium. *M. bovis* and other atypical mycobacteria are negative for niacin production.

8.4.5.2. Nitrate reduction: *M. tuberculosis* grows readily on medium containing nitrates and reduces them, while *M. bovis* is negative for nitrate reduction.

8.4.5.3. Catalase–peroxidase activity: To the cullture, if a mixture of equal volumes of H_2O_2 and 0.2% catechol in distilled water is added and allowed for a few minutes, effervescence indicate catalase production and browning indicates peroxidase activity.

M. tuberculosis is weakly positive for catalase while atypical mycobacteria are strongly positive. *M. tuberculosis* is peroxidase positive while atypical mycobacteria are peroxidase negative.

8.4.5.4. Neutral red reaction: Virulent strains of *M. tuberculosis* bind to neutral red in alkaline buffer solution while avirulent strains do not.

8.4.6. Antigenic properties: *M. tuberculosis* possesses a number of polysaccharide, protein and lipid antigens and antigenically homogeneous. Hence no serotypes are present. The antibodies readily form against surface antigens but they have not been found useful in diagnosis or relevant in immunity.

The infection stimulates cell mediated immunity. T-cells mediate delayed type hypersensitivity, and cause tissue destruction. The hypersensitivity develops on exposure to the pathogen and it is detected by tuberculin test developed by Robert Koch.

8.4.7. Infections: Two types of infections of *M. tuberculosis* are recognized 1. Primary infection, and 2. Post primary infection.

Primary infections are the first infections usually acquired in the early child hood. Majority of primary infections do not develop into clinical cases but the infected persons become hypersensitive and pathogen remains dormant in the healed but not resolved lesions or tubercles. Post primary infections are the infections that occur in the later part of the life. They may be due to the reactivation of latent infections or acquiring a virulent strain by inhalation. Since the nature of adult infection is not always clearly known they are described as post primary infections instead of reactivation or secondary infections.

8.4.7.1. Primary infections: Primary infections are acquired in the early childhood by the inhalation of droplet nuclei. Sputum, 'coughing sprays' and droplets released by sneezing of infected persons are common sources of the disease. Contaminated dust may also be important source mainly in hospitals and sanitoria.

Other ways by which mycobacteria may enter susceptible individual include ingestion and direct inoculation. Pathogen may be swallowed by children when they place the contaminated objects in their mouth or consume food containing the bacteria. The danger of acquiring tuberculosis from contaminated milk and milk products has largely became eliminated in many countries by introduction of pasteurization of milk and milk products.

8.4.8. Pathogenesis: When the droplet nuclei of *M tuberculosis* cells are inhaled, the cells pass through the respiratory tract and enter alveoli as they are very slender. As cells are deposited in alveoli, PMNLs appear at the site of infection within 24 hrs. They engulf the bacilli and help their localization, but they are unable to destroy the engulfed cells. Within 2 to 3 days PMNLs are replaced by mononuclear cells which are either monocytes or pulmonary macrophages. They are highly phagocytic and engulf the bacilli and also PMNLs containing bacilli. Most of the engulfed bacilli are destroyed but some may still survive. Then the mononuclear cells are transformed into epithelioid cells after 2 weeks of infection. The epithelioid cells are specific and characteristic of TB by their larger, vesicular, pale stained, elongated or reniform nuclei with abundant cytoplasm. The surface processes of epithelioid cells form a reticulum by anastomosis with their neighbouring cells. Some of the epithelioid cells may fuse together and form large plasmodial masses with cytoplasmic processes and contain a large number of nuclei arranged in the periphery giving the appearance of a giant cell. These are called giant cells of Langhan's type.

8.4.8.1. Tubercle formation: The giant cells of Langhan's type form the centre of developing tubercle. Lymphoid cells appear around the giant cells. These are small cells with compact nuclei and identical with lymphocytes. The lymphoid cell layer is surrounded by young connective tissue frame work containing the reticulum fibers. Thus the tubercle comprise 3 layers (1) central zone of pale giant cells of langhan's type (2) middle layer of deeply stained lymphocytes (3) outer layer of fibrous tissue.

Small tubercles may fuse to form a bigger tubercle. Lesions or tubercles are two types (1) exudative type and (2) productive type.

Exudative type lesion is an accute inflammatory reaction with accumulation of edematous fluid. The central part of the lesion may undergo necrosis and cheesy fluid oozes out. Such lesions are called caseous lesions. This is particularly seen in lungs, and resembles bacterial pneumonia. It may heal by resolution so that the entire exudate becomes absorbed. It may lead to massive necrosis of tissue, or may develop into second type of lesion (productive lesion).

Productive type lesion when fully developed forms typical tubercle. Central area of the fully developed lesion undergoes caseation necrosis. A caseous lesion may break in a bronchus, empty its contents there and form a cavity. They may heal by calcification and such lesions are called Ghon complexes and appear prominently in a chest X-ray.

8.4.9. Healing of the lesions: In more than 90% of cases of primary infection, the development of lesions usually do not progress further and slowly heal up. Healing of the tuberculous lesions may occur either by calcification or fibrosis.

8.4.9.1. Calcification: Healing of the lesions by deposition of calcium within the semisolid (caseous) centres of tuberculosis lesions is called calcification. Lesions usually heal by this process over a long period extending up to 2 years. The healed lesions appear rough.

8.4.9.2. Fibrosis: Healing of the lesions by deposition of collagen (fibrous protein of connective tissue) is called fibrosis or scarring.

8.4.9.3. Resolution: Complete disappearance of lesions either by calcification or fibrosis is called resolution.

8.4.10. Progress of primary infection: In about 5 to 10% of cases of highly susceptible individuals the infection may progress further resulting in clinical disease. The symptoms of TB are fever, fatigue and chronic cough. As the disease progresses, the patient begins to exhibit loss of appetite, weight loss, night sweat and a persistent worsening cough. If a blood vessel is eroded in the lungs, the sputum coughed up by the patient may become stained with blood. Death ultimately results, in untreated patients, when sufficient damage has occurred in the lungs or other vital organs.

8.4.11. Spread of infection: In progressive primary infections, the infection spread by direct extension, lymphatic system or through blood.

8.4.11.1. Direct extension: The spread of infection to surrounding tissues occur commonly by local extension forming satellite lesions. Phagocytes containing bacteria are responsible for their transport in the neighbour hood of the original focus. Secondary lesions occur in trachea, larynx and intestine.

8.4.11.2. Lymphatic spread: Spread through lymphatic system is the most important mode. TB is a disease of lymphoid tissue. The pathogen pass through the lymphatics to the regional lymph nodes where they multiply. In pulmonary infection tracheo-bronchial lymph nodes are involved. In intestinal infection, mesenteric lymph nodes are affected.

8.4.11.3. Hematogenous spread: A caseous lesion may erode a blood vessel and discharge the contents into it, or through lymph nodes the bacilli may enter blood stream. In either case hematogenous spread results in development of tubercular lesions in various parts of the body.

8.4.12. Types of infections: Depending upon the focus of infection and spread, different types of TB are recognized.

8.4.12.1. Tuberculosis broncho-pneumonia: It is an acute, diffuse extension of infection throughout the lung due to discharge into bronchial tree, and characteristic inflammatory lesions develop. Production of thick cheesy material consisting of pus cells and necrotic tissue, called caseation, occur and the lesions are called exudative lesions.

8.4.12.2. Miliary tuberculosis: It is characterized by development of small tuberculous foci (tubercles) disseminated widely throughout the body as a result of bloodborne spread of infections. Many small tubercles, resembling millet seeds, form in spleen, liver and various other organs.

8.4.12.3. Tuberculous meningitis: Bloodborne spread of infection may involve meninges also. This infection is uniformly fatal before antibiotic era, and still is a serious disease, with considerable mortality and disabling sequale in some survivors.

8.4.12.4. Bone and Joint tuberculosis: Spread of infection can affect many different sites, the most common being spinal cord. In spinal tuberculosis, vertebrae may collapse and patient becomes bedridden.

8.4.12.5. Renal tuberculosis: Urinary tract infection often leads to painless haematuria and pyuria. Presence of blood in urine is called haematuria and presence of pus cells in urine is called pyuria. Genital tract may also be infected.

8.4.12.6. Intestinal tuberculosis: Previously it used be primary infection due to ingestion but now it is more due to secondary spread.

8.4.13. Post primary infections: In tuberculosis positive individuals, the post primary tuberculosis infection can occur. When renewed pulmonary infection occurs, they are usually chronic infections that involve destruction of lung tissue followed by partial healing and slow spread of the lesions with in the lungs. In many cases, post primary infections are a result of reactivation and growth of bacteria that have remained alive and dormant in the lungs for long period i.e. endogenous infection. Exogenous infection from inhalation of infected respiratory secretions from a case of open tuberculosis can also occur. Malnutrition, overcrowding, stress and hormonal imbalance often are the factors predisposing an individual to secondary infections.

8.4.14. Epidemiology: TB is a great epidemic disease, worldwide in distribution with devastating morbidity and high rate of mortality. It is estimated that about 1/3 of world population i.e. about 2 billion people are positive for TB infection, with 8 to 9 million new cases and 3 million deaths every year.

TB is essentially a respiratory tract disease and inhalation of droplet nuclei emanated from active patients is main source of spread. Before the introduction of pasteurization of milk, ingestion of contaminated milk from cattle (*M. bovis*) used to be a major source of infection, but now it is not a major one.

Some occupations used to be associated with high rate of mortality for TB. These are mainly quarry and mine industries in which workers are exposed to the inhalation of stone or metal dust. Doctors and other healthcare personnel are also prone to occupational infections.

Tuberculosis is a disease of associated with poverty and malnutrition and over crowding. Improving the living standards reduces the incidence. The disease is common in black people in America and Asian immigrants in UK, and environmental and economic factors may be responsible for this.

Immunocompromised patients suffering from diseases which affect the functions of the immune system or on immune suppressive therapy are extremely susceptible to tuberculosis. Recent resurgence of TB in the world is attributed to prevalence of AIDS, and 15-30 % of all AIDS patients developed TB.

8.4.15. Therapy: Long term multi drug therapy is suggested for control of TB. Penicillin and sulfa drugs are inactive against the pathogen, and penicillin in particular has been found to have stimulating effect on growth of the pathogen. Streptomycin is the first effective drug used but it soon became ineffective. The real success in treatment of TB came with the discovery of isonicotinic acid hydrazide or isoniazid (INH). It is free from toxicity, highly effective and also inexpensive. However, the pathogen is acquiring resistance to INH. Hence use of multiple drugs is suggested. Commonly used anti-tuberculosis drugs are

Bactericidal --- streptomycin, isoniazid, pyrazinamide, rifampicin

Bacteristatic --- ethambutol, paraaminosalicylic acid, cycloserine

Bactericidal and bacteristatic drugs are suggested in combination. Major problem in chemotherapy is development of drug resistance. Resistance may be primary (pretreatment) or secondary (acquired during treatment). Inadequate or irresponsible chemotherapy is responsible for development of drug resistant strains endangering the health of both patient and the community at large.

8.4.16. Prophylaxis: A live attenuated vaccine called BCG vaccine was developed by French bacteriologists Calmette and Guerin (hence name) by using *M. bovis* which was subcultured serially for 239 times over 13 years. It was released in 1921. Now the cultures are grown in glycerin-bile- potato medium for not more than 14 days.

However, the use of BCG was not found to be ineffective, and usage of it stopped in USA in 1965. In India also it was reported to be ineffective by the expert committee appointed ICMR.

8.4.17. Diagnosis: Tuberculin test cannot prove the presence of active disease. Only isolation of the pathogen gives such proof.

8.4.17.1. Specimens: The specimens for diagnosis of tuberculosis include fresh sputum (in case of pulmonary TB), gastric washings (intestinal TB), urine (renal TB), or spinal fluid (Tuberculous meningitis, spinal TB).

However most common specimen is sputum since pulmonary TB is the most common type of the disease.

8.4.17.2. Microscopy: Smears are prepared from the specimens and examined for Acid fast bacilli by ZN- Staining or by fluorescent microscopy using auramine-rhodamine stain.

8.4.17.3. Culturing: For culture, sputum or other specimens, are diluted with saline and treated with 2% sodium hydroxide or other agents bactericidal for contaminating microorganisms but less so for tubercle bacilli. Then liquified sputum is neutralized, centrifuged and sediment inoculated into appropriate medium, usually Lowenstein – Jensen medium. Incubation is continued for up to 8 weeks. Isolated mycobacteria are identified by biochemical tests, and tested for drug susceptibility. The pathogenicity of the isolates is tested by animal inoculation.

8.4.17.4. Pathogenicity test: For Animal inoculation tests Guinea pig is the most suitable animal. The presence of even a few bacilli in the material will kill the guinea pig in 6 to 8 weeks time. A smear from spleen, liver or lymph node of the dead experimental animal, stained by Z-N method, shows numerous bacilli.

8.5. SUMMARY

Clostridium tetani is the causal organism of tetanus. It is a common constituent of the intestine of herbivores and present in the soil as highly resistant endospores. *C. tetani* cells are obligately anaerobic, sporeforming, motile, gram positive, slender straight rods. The endospores are spherical and formed at the end giving the cell a 'drumstick' appearance. The most important biochemical character of *C. tetani* is production of exotoxin called tetanospasmin. It is the most potent neurotoxin, and accounts for all the major symptoms of tetanus. The infections are mainly associated with wounds contaminated with soil. The incubation period may range from one week to more than one week. The pathogen do not spread from the place of its entry or growth, but release a neurotoxin, which cause the disease. The toxin does not harm locally but travels along the peripheral nerves to the central nervous system where it inhibit the transmission of nerve impulses. The disease is characterized by convulsive contraction of voluntary muscles. Muscular spasms often involve first the area of injury and infection. Next the muscles of jaw are affected and it becomes very difficult to open the mouth, hence it is called 'lock-jaw'. In the third phase, voluntary muscles are involved resulting in generalized spasms. The final stage (death) reached in about 50% of untreated cases and it results from respiratory paralysis due to the effect on thoracic muscles. Depending upon the nature of infection, tetanus is of several types like acute tetanus, chronic tetanus, delayed tetanus, idiopathic tetanus, local tetanus, uterine tetanus, surgical tetanus, tetanus neonatorum etc. Tetanus is not a contagious disease, and infections do not spread from person to person. All infections arise independently. Penicillin and other antibiotics are effective against *C. tetani*. They can stop further production of toxin by inhibiting the growth of the organism but cannot cure established disease. Immunization is against the toxin, not against the organism it self. Diagnosis rests on clinical picture or symptoms.

Corynebacterium diphtheriae is the causal organism of diphtheria, a serious pediatric disease. It is a gram positive, non spore forming, non capsular, non motile, straight or curved rod with characteristic club shaped swelling at one or both ends. Telleurite blood agar medium is a selective medium for growth of the organism. Lysogenized strains of the pathogen produce a toxin called diphtheria toxin which is a powerful cytotoxin, and responsible for formation a pseudomembrane in the infected throat region. Infections are acquired by inhalation of droplet nuclei released by active patients or carriers. The organism establish in the upper respiratory tract

and incubation period is 2-7 days. The toxin released by the pathogen cause necrosis of the cells in the throat region forming a pseudomembrane. The mechanical complications of diphtheria are due to the membrane, while systemic effects are due to the toxin. Depending on the area of infection diphtheria is recognized as pharyngeal diphtheria, laryngeal diphtheria, nasal diphtheria and cutaneous diphtheria. Specific treatment of diphtheria consists of antitoxic antibodies against the toxin and must be administered immediately when a case is suspected of diphtheria. The pathogen is susceptible to a number of antibiotics like penicillin, erythromycin etc. The infections can be prevented by vaccination with diphtheria vaccine which is available as a triple vaccine DPT. The diagnosis rests on culturing and serological identification.

Tuberculosis is one of the dreaded diseases of humans caused by *Mycobacterium tuberculosis*. The cells of the organism are slender, long, delicate rods that occur as fragile filaments. The pathogen is stained with Ziehl-Neelsen staining method in which carbol fuschin is the primary stain and methylene blue is the second stain. The pathogen is cultured on Lowenstein-Jensen medium. It grows very slowly and form small, dry, rough, heaped up buff coloured colonies. The pathogen do not produce any toxin. It possess a number of antigens but antibodies formed against them are not protective in nature. It causes T-cell mediated hypersensitivity which can be recognized by tuberculin skin tests. The primary infections are acquired by inhalation of the droplet nuclei and produce characteristic tubercles in the lungs. Usually the tubercles heal up over a long period, but the pathogen remains dormant in the lesions and may cause reactivation disease in the later part of life. Progressive primary infections susceptible individuals may spread to various other parts of the body and cause infections. Depending upon the focus of infections and spread, different types of TB are recognized. They are tuberculosis bronchopneumonia, military tuberculosis, tuberculous meningitis, bone and joint tuberculosis, renal tuberculosis and intestinal tuberculosis. The post primary infections usually occur in adults and are chronic. Long term multidrug therapy is suggested for the control of tuberculosis. Rifampicin, isoniazid, ethambutol are drugs of choice for treatment. BCG vaccine is used extensively for prevention and it is not very effective. The diagnosis rests on isolation and culturing of the organism and identification by biochemical tests and pathogenicity tests using animals.

8.6. MODEL QUESTIONS:

Essay type questions

Give an account of pathogen, pathogenesis, clinical symptoms and control of Tetanus

Give an account of pathogen, pathogenesis, clinical symptoms and control of Diphtheria

Give an account of clinical symptoms and control of tetanus and diphtheria

Give an account of pathogen, pathogenesis, epidemiology and control of Tuberculosis

Give an account of tubercle formation, progressive primary infection and diagnosis of tuberculosis

Short answer type questions

Clostridium tetani
Tetanus toxin
Types of tetanus
Corynebacterium diphtheriae
Clinical symptoms of diphtheria
Diphtheria toxin
Control of diphtheria
Tubercle
Resolution of tubercle
Post primary tuberculosis
Control of tuberculosis

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LESSON–9: FUNGAL INFECTIONS

Objective: To study the fungal infections of humans

Contents

9.1. INTRODUCTION

Fungi are eukaryotic, nonchlorophyllous, unicellular or filamentous organisms. They are the major cause of plant diseases, but less than 100 species of an estimated 1,00,000 species cause disease in man and animals.

Morphologically, the medically important fungi are four types

1. Yeasts: Unicellular fungi which occur mainly as single spherical or ellipsoidal cells and reproduce by budding. Among the yeasts, *Cryptococcus neoformans* is the major pathogen.

2. Yeast like fungi: They grow partly as yeast and partly as long filaments joined end to end to form pseudomycelium. Eg. *Candida albicans*, which is a common member of normal flora, cause opportunistic infections.

3. Filamentous fungi: They grow as long filamentous hyphae which form extensive mycelium, and reproduce by formation of various types of spores. Dermatophytic fungi viz. *Trichophyton, Microsporum* and *Epidermophyton* are important examples of pathogenic filamentous fungi.

4. Dimorphic fungi: Fungi which occur as yeast like form in the infected tissue and in cultures incubated at 37 °C, but occur in filamentous form in their natural habitat (soil) and in cultures incubated at 25 °C are called dimorphic fungi. They mainly cause deep seated mycotic infections. Eg. *Histoplasma, Blastomyces, Coccidioides, Paracoccidioides* etc.

9.2. TYPES OF FUNGAL INFECTIONS:

Infection of humans by a fungus is called mycosis (plural= mycoses). Depending on the area of infection and nature of infection, mycoses can be grouped into 5 types.

9.2.1. Superficial mycoses: Fungal infections limited to the outer surface of skin and hair are called superficial mycoses Eg. Piedras.

9.2.2. Dermatomycoses: Fungal infections confined to keratinized tissue, viz. skin, hair and nails, but do not invade deeper tissue Eg. skin infections commonly described as ring worm or tinea.

9.2.3. Subcutaneous mycoses: Fungal infections that penetrate the skin and establish in the subcutaneous tissue. Eg. Mycetoma.

9.2.4. Deep mycoses or systemic mycoses: Fungal infections that are usually acquired by inhalation of fungal spores and start as pulmonary infection but later spread to various other organs of the body through blood stream. Eg. Histoplasmosis, Blastomycosis, Cryptococcosis etc.

9.2.5. Opportunistic mycoses: Fungal infections caused by members of normal flora or common saprophytic fungi, when an individual's innate immunity is lowered are called opportunistic infections Eg. Candidiasis, Aspergillosis etc.

In addition to the above types of infections, fungi also cause other ailments in humans like mycotoxicoses, ergotism and allergies, which are not infectious or contagious in nature.

Mycotoxicoses are result of eating mouldy food in which the fungus has produced toxic metabolites called mycotoxins. Aflatoxins produced by Aspergillus flavus are very potent mycotoxins that can kill lower animals and act as carcinogens in humans.

Ergotism is a complication that arises due to consumption of mouldy food contaminated with Claviceps purpurea. The sclerotia of the fungus contain a number of ergotoxins and ergotamines which effects central nervous system and also cause constriction of blood vessels with serious consequences.

Allergic reactions are due to inhalation of fungal spores, notably those of Aspergillus fumigatus, which provoke hypersensitive reaction. Some times antigenic stimulus is prolonged because the fungal hyphae grow in the lumen of bronchi.

9.3. SUPERFICIAL MYCOSES:

Fungal infections confined to the outer surface layer of skin and hair are described as superficial mycoses. The superficial hair infections are collectively termed as piedras. In Spanish piedra means stone, which refer to the hard nodules formed by mycelium. They are also called tineas. In Latin tinea means worm. They are called tinea because before the fungal nature of infection is diagnosed, people thought that they are caused by small worms. In English they are commonly described as ring worm, because infection usually spreads in concentric rings.

9.3.1. Piedras:

Black piedra and White piedra are important examples of superficial infections caused by fungi. These are world wide in distribution but more common in tropics.

9.3.1.1. Black piedra: An ascomycete fungus *Piedraia hortai* form dark black nodules on the shaft of infected scalp hair. The nodules contain oval asci with two to eight ascospores. The fungus belongs to the order Myriangiales and family Saccardinulaceae of Loculoascomycetes.

9.3.1.2. White piedra: The fungus that cause white piedra is *Trichosporon cutaneum*. It produces soft, pale white to light brown nodules on scalp hair, and also on beard. The nodules consist of hyphae and oval arthrospores.

9.4. DERMATOMYCOSES:

Fungal infections that spread through keratinized tissues of the skin, hair and nails are called dermatomycoses. Keratin is a fibrous scleroprotein that is deposited in the outer layers of skin, hair and nails. Dermatophytic fungi have a predilection for it. Dermatophytic infections are caused by a group of closely related fungi classified into three genera.

1. ***Trichophyton*** - various species infect skin, hair and nails
2. ***Microsporum*** - various species infect skin and hair but rarely nails
3. ***Epidermophyton*** - a single species, infect skin and nail but never hair

9.4.1. Characters of Deratophytic fungi: All the three fungi are conidial fungi classified in deuteromycotina. The perfect stages are reported for *Trichophyton* (*Arthroderma*) and *Microsporum* (*Nannizzia*) but not for *Epidermophyton*. The perfect stages belong to Plectomycetes of Ascomycotina. They produce ascigerous stages in culture and the ascocarps do not have a clear

peridium but the clusters of asci are covered by weft of sterile hyphae. The characters of the dermatophytes are shown in the figure 9.1.

Figure 9.1. Dermatophytic fungi a) *Trichophyton* species b) *Epidermophyton floccus* c) *Microsporum* species

9.4.1.1.*Trichophyton*: Filamentous fungus which produce abundant microconidia in cultures. The perfect stage is an ascomycetous fungus *Arthroderma*. The fungus can be cultured easily on Sabourad's agar medium from the infected specimens. Important species in the genus are *T. mentagrophytes, T. rubrum, T. tonsurance* etc.

***T. mentagrophytes*:** colonies are white, granular to powdery, hyphae long septate and some are coiled. Abundant microconidia are formed in grape like clusters. The microconidia are small spherical to oval and measure 3-4 µm.

***T. rubrum*:** colonies are white but produce red pigment into the medium. Tear shaped (oval) microconidia are produced along the sides of hyphae.

***T. tonsurans*:** colonies yellow or cream coloured, powdery, produce numerous microconidia.

9.4.1.2. *Microsporum*: Filamentous fungus which produce both microconidia and macroconidia in culture. Macroconidia are predominant conidial form in cultures. The perfect stage is an ascomycetous fungus *Nannizzia*. The important species are

***M. canis*:** forms numerous thick walled, 8-l5 celled macroconidia that have a curved or hooked spiny tips, micrcconidia form on short pegs from hyphal cells.

***M gypseum*:** produce abundant thick walled, 4-6 celled macroconidia, and single celled microconidia.

M. audounii: rarely form conidia in cultures but many thick walled chlamydospores are formed. The hyphae are characteristically comb shaped.

9.4.1.3 *Epidermophyton floccosum*: The genus *Epidermophyton* is monotypic with a single species *floccosum*. It forms greenish - yellow colonies on agar medium. Produce 1-5 celled club shaped macroconidia. Perfect stage is not known so far.

9.4.2. INFECTIONS (Tineas): In the host the fungi occur in only two forms 1. as vegetative mycelium which grows through keratinised tissues and 2. as chains of arthrospores formed by septation of the hyphae into short cylindrical or rounded segments which become widened and thick walled. The arthrospores are disseminating propagules and are capable of infecting intact skin on which they are deposited, but they more readily infect skin subjected to minor injury, scratching or prolonged moistening. They germinate and give rise to hyphae which spread as mycelium through the whole depth of horny layer and extend radially into adjoining areas of skin. The infected areas show characteristic inflammation. The mechanism where by this superficial

growth causes inflammation of the skin is uncertain, perhaps liberation of toxic products from digested keratin or an allergic response to fungal antigen may be responsible.

In hair infections, hyphae grow first from epidermis into hair follicle and then into hair shaft. Hair infections are mainly two types. 1. Endothrix and 2. Ectothrix infections. In endothrix type of infection, the hyphae grow within the hair shaft, where they form long parallel rows of arthrospores. In ectothrix type, hyphae grow both within and on the external surface of the hair shaft. After 2 to 3 weeks of growth, the infected part of the hair above the skin surface become so weakened that it breaks leaving a short stump.

In nail infections, growth of the fungus in the nail and surrounding tissue results in discoloration and thickening of the nail, which raises from the surface. It loses its natural luster and becomes brittle.

Dermatomycoses are mainly recognized as 6 types basing on the area of infection.

9.4.2.1. Tinea pedis (L Pedis=foot): It is also commonly known as athlete's foot and is the most prevalent of all dermatophytoses. The toe webs are infected with a *Trichophyton* species (*T. rubrum, T. mentagrophytes*) or with *Epidermophyton floccosum*. Initially, there is itching between the toes and the development of small vesicles that rupture and discharge a thin fluid. The skin of the toe webs becomes macerated and peels, whereupon cracks appear, that are prone to secondary bacterial infections. When fungal infection becomes chronic, peeling and cracking of the skin are principal manifestations. Nail infections follow prolonged tinea pedis. In persons affected with tinea pedis, such symptoms also occur on hand, starting with itching between fingers. It is usually called Tinea manuum (L mannus=hand).

9.4.2.2. Tinea corporis (L corpus=body): It is the common ring worm infection. It is the infection of the non-hairy skin of the body that gives rise commonly to circular lesion called ring worm. In the lesion, scaly centre is surrounded by a red advancing border that often contains vesicles. It is commonly caused by *T. rubrum, T. mentagrophytes* or *Microsporum canis*. Transmission is by direct contact with infected animals or humans or by indirect contact with fomites.

9.4.2.3. Tinea cruris (L crura=leg): It is ringworm of the groin caused by *Epidermophyton floccosum, Trichophyton mentagrophytes* or *T. rubrum*. Factors predisposing are moisture, skin trauma, wet bathing suits, tight fitting clothes, obesity etc.

9.4.2.4. Tinea capitis (L capita=head): It is called ringworm of the scalp and is mainly caused by *Microsporum canis* or *Trichophyton tonsurans*. It is mainly a child hood disease and is characterized by circular bald patches with short stubs of hair or broken hair with hair follicles.

Microsporum infections occur in child hood and usually heals spontaneously by puberty. Untreated *Trichophyton* infections may persist into adult hood. In some patients, a pronounced inflammation may occur around the area of infection and may even resemble pyogenic infection. Such condition is described as Kerion.

9.4.2.5. Tinea barbae (L barba=beard): The infection of beard is called tinea barbae and is mainly caused by *Trichophyton verrucosum, T. rubrum* and *T. mentagrophytes*.

9.4.2.6. Tinea unguium (L unguis=nail): The infection of nail is called tinea unguium or onchomycosis. It is mainly caused by *T. rubrum, T. mentagrophytes* and *E. floccosum*. Nails become luster less, discolored and crumble distally. It usually follows prolonged tinea pedis.

9.4.3. SPREAD: Dermatophytes may be divided into two categories 1. Anthrophilic and 2. Zoophilic species.

Anthrophilic species are primarily parasites of humans and rarely of animals. Eg. *T. rubrum, M. audouni, E. floccosum* etc.

The zoophilic species are primarily parasites of animals and occasionally infect man. Eg. *M. canis* (dogs and cats), *T. mentagrophytes* (cat, dog, mouse, ox, horse) *T. verrucosum* (ox, horse).

The ring worm infections due to anthrophilic species spread from man to man. Eg. Tinea capitis, tinea pedis etc. The infections by zoophilic species spread from infected pets and form animals, and rarely from infected humans.

Apart from spread through contact, dermatophytic fungi also spread through fomites and air. The fungi are highly resistant to environmental conditions and may remain alive on contaminated objects for a long time. The fungi on clothes can survive through successive launderings. Tinea cruris spread through contamination of under wear clothes during laundries. Tinea capitis is often traced to shared barber shop clippers.

Airborne spread is through arthrospores released from active lesions.

M. gypseum has been found in soil and soil-borne infections may also occur.

9.4.4. DIAGNOSIS: Specimens for diagnosis consists of both skin and nails, hairs plucked from involved areas. Specimens are examined for the presence of hyphae and arthrospores.

Identification of the fungi is mainly based on cultures grown on agar media like sabourad's agar medium.

9.4.5. TREATMENT: No really effective control measures, except proper hygiene, are available. In severe cases, treatment consists of removal of infected and dead epithelial structures and application of antifungal chemicals.

For skin infections, use of imidazoles, salicylic acid 3% or benzoic acid 5% in cream are effective. Mixture of acetic acid and benzoic acid is available as white field ointment.

Imidazoles - miconazole, ketoconazole and clotrimazole - are broad spectrum agents available as creams and solutions for treatment of dermatophytic infections. They disrupt fungal membrane permeability and inhibit sterol synthesis.

Nystatin is an antibiotic derived from *Streptomyces noursei*, and it is effective against dermatophytes. It is used as a cream. It is not used for systemic use because it is not easily absorbed.

Griseofulvin, derived from *Penicillium griseofulvum*, is also very effective against dermatophytes. It interferes with nucleic acid synthesis.

For scalp infections, frequent head bath with soapnuts or shampoos and application of miconazole cream or other antifungal agents is suggested. The treatment should continue for many weeks or months.

9.5. SUBCUTANEOUS MYCOSES

The fungi that cause subcutaneous mycoses are normal saprophytic inhabitants of soil and decaying vegetation. They cannot penetrate the intact skin, and hence enter through puncture wounds, cracks etc. on the foot. Most infections involve bare footed agricultural workers. Once in the subcutaneous tissue, the fungus slowly develop over a period of months or years to produce a nodule that eventually ulcerates and the organism spread along the lymphatic channels producing more subcutaneous nodules. At times, such nodules drain to the surface of the skin.

Diagnosis is accomplished by culture of fungus from infected tissue, and therapy is usually by administration of oral 5-Flourocytosine, iodides, amphotericin-B, and surgical excision where necessary.

Important examples of subcutaneous mycoses are sporotrichosis and madura foot.

9.5.1. Sporotrichosis: It is caused by a dimorphic fungus *Sporothrix schenkii*. The fungus is found in soil and on decaying plant parts. Infections occur by puncture wounds. After an incubation period 1-12 weeks, a small red papule arises and begins to ulcerate. New lesions appear along the lymph channels and can remain localized or spread throughout the body producing extra-cutaneous sporotrichosis.

In the infected tissue and in cultures incubated at 37 °C the fungus occur as small, round to oval cells (yeast like), but on natural substrata and in cultures incubated at 25 °C septate mycelium develop with long, slender conidiophores bearing simple ovoid conidia in clusters at the tip.

The disease is an occupational hazard of those who work with wood, wood products or soil. The disease can be contracted by handling of sphagnum (peat) moss used to pack tree seedlings. The disease also spread by rose thorns, and often called rose-thorn disease.

Usually the disease is self-limiting. In severe cases, use of potassium iodide given orally or amphotericin-B given intravenously is useful. Treatment must continue for a number of weeks.

9.5.2. Mycetoma or maduromycosis or madura foot:

Mycetoma is a localized swollen lesion with granules that are compact colonies of causative agent. Oma means tumor and Mycetoma means tumor caused by fungi. A number of fungi and actinomycetes cause mycetoma in tropics. The disease was first reported from Madurai region of South India, where a fungus named *Madurella grisea* is mainly involved. Hence the disease is referred to as Madura foot or Maduromycosis. The disease is also reported from various other parts of the world, and a number of different fungi were reported to cause mycetoma. Other organisms involved in causing mycetoma are *Allescheria boydii, Acremonium* spp., *Cephalosporium* spp. and *Nocardia* spp.

Mycetoma granules are composed of broad hyphae which are often septate with chlamydospores. The actinomycete (*Nocardia*) granules contain thin gram positive filaments.

Fully developed mycetoma is a chronic, suppurative, granulomatous lesion with progressive destruction of contagious tissue and vascular dissemination. Skin and subcutaneous

tissues are involved originally, but as the disease progresses fascia and bones become infected. Fascia refers to a sheet or band of fibrous tissue that lies deep under the skin or invests muscles and various body organs. The foot is the most infected part. It becomes grossly deformed with multiple sinus formation and fistula tracts which communicate with each other, with deep abscesses including ulcerated areas of skin.

Actinomycotic mycetoma responds to antibiotics, but there is no established therapy for fungal mycetoma. Surgical excision of early lesions may prevent spread.

9.6. SYSTEMIC MYCOSES or DEEP MYCOSES

Inhalation of airborne inoculum liberated from some soilborne fungi initiate pulmonary infections which may later spread to other internal organs or skin through hematogenous spread. Such infections are termed as systemic mycoses or deep mycoses. Important examples for deep mycoses are Histoplasmosis, Blastomycosis and Cryptococcosis.

9.6.1. Histoplasmosis: Samuel Darling first described it in 1915. It is caused by a dimorphic fungus *Histoplasma capsulatum*, which grows abundantly in soils rich in excreta of birds and bats. Birds are not infected by the fungus but their excreta act as nutrient medium for the fungal growth, while bats are carriers of the fungus. The perfect stage of the fungus is *Emmonsiella capsulata* belonging to ascomycetes. The disease is endemic in central and eastern regions of USA.

The fungus forms oval, uninucleate budding cells measuring 2-4 μm in diameter in infected tissues, and in cultures incubated at 37 °C. In cultures incubated at room temperatures, the fungus produce cottony mycelial growth, with small (2-4 μm) microconidia and large (8-14 μm) thick walled spherical macroconidia (Fig. 9.2).

Figure 9.2. *Histoplasma capsulatum*. Macroconidia and microconidia

Infections occur through inhalation of airborne conidia. Inhaled conidia are engulfed by alveolar macrophages, and become intracellular, where they develop into budding cells. Most infections are asymptomatic but in a few highly susceptible persons, tuberculosis like disease symptoms develop. Tubercle like structures that form in the lung slowly heal up, but hypersensitivity develops in exposed individuals. In a few cases infection becomes progressive and widely disseminated with lesions in tissues and organs. Fever, wasting, enlargement of liver, spleen and lymph nodes also occur.

Diagnosis is by microscopy and culturing. For treatment, use of amphotericin -B and ketoconazole are suggested.

9.6.2. Blastomycosis: It was first described by Thomas Gilchrist in 1896 from USA. It is mainly prevalent in North American Continent - USA, Canada and Mexico, and is common in rural male in the age group of 30 -50. It is caused by a dimorphic soilborne fungus, *Blastomyces dermatidis*. The perfect stage of the fungus is *Ajellomyces dermatidis* belonging to plectomycetes of ascomycotina. The fungus occurs in dusty soil and bird droppings particularly near barns and sheds.

In the infected tissue fungus appears as a round, multinucleate, budding cell (8-15 μm) with a doubly refractile wall. Each cell usually has only one bud on a broad base. Colonies on blood agar medium at 37 °C also show yeast like growth. On Sabouraud's agar at 25 °C white or brownish mycelial colony develops with slender, terminal or later conidiophores bearing a conidium 2-10 μm in diameter (Fig. 9.3).

Figure 9.3. *Blastomyces dermatidis*. Hyphae with conidia and yeast like cells

The disease occurs in dogs and other animals in endemic areas. However, it is not communicable from animals or other humans. Infections arise by inhalation of airborne spores. The primary infection occurs in the lungs and then spread to other parts of the body including skin. The disease occurs in three clinical forms.

Pulmonary infection: Granulomatous pulmonary infection is the initial stage. Usually it is transitory, but may become chronic.

Cutaneous infection: The fungus has affinity to skin, and cutaneous infections are very prominent. They are chronic, granulomatous and often suppurative. Cutaneous infection may also occur directly through skin abrasions etc.

Disseminated infections: From the lung, the pathogen may spread to various body parts including bones and meninges, and cause abscesses. It occurs mainly in immune-suppressed individuals.

Diagnosis is by microscopy and culturing. Amphotericin-B and ketoconazole are suggested for therapy. Surgery may be necessary to remove large abscesses of the skin.

9.6.3. Cryptococcosis: It is caused by a basidiomycetous yeast, *Cryptococcus neoformans*. The perfect stage of the fungus is *Filobasidiella neoformans* belonging to Ustilaginales of Basidiomycotina. There are a number of species in the genus *Cryptococcus* but only *C. neoformans* is associated with human infections. It is found in soil throughout the world, especially in soils contaminated with bird droppings, particularly associated with pigeons.

C. neoformans occur as spherical cells of 5 - 20 μm in diameter, and surrounded by a very wide gelatinous capsule, which is visible in India ink preparations. Four serotypes, A B C D, are recognized basing on capsular polysaccharide. It reproduces by budding and does not give rise to mycelium or pseudomycelium. It is aerobic and culturable. Grow on a wide variety of common media. It shows rapid growth at 37°C and also at room temperatures. On Sabouraud's medium the growth appear as compact creamy glistening colonies.

Infections usually occur through inhalation of airborne yeast cells. Primary infections are pulmonary and in majority of individuals, they are symptom less and heal spontaneously. However in susceptible people, the infection spreads to various other parts especially to meninges and skin. Hence three types of infections are usually recognized.

Pulmonary infections: they are primary and often symptom less.

Cryptococcal meningitis: It is the most common result of fungal spread to central nervous system. Cryptococcal meningitis is usually chronic and often mistaken for a brain abscess or tumor. It is found in approximately 15% of AIDS patients.

Cutaneous cryptococcosis: When fungus spread to skin through blood, small ulcers to large granulomatous lesions are formed on the skin. Pus formation is common. It is often called European blastomycosis because the skin lesions resemble those caused by Blastomyces and the disease is common in European countries.

For diagnosis, the specimens include cerebrospinal fluid (CSF), sputum or pus from skin lesions. Identification is by microscopy and culture. Amphotericin-B and flucytosine are suggested for treatment.

9.7. OPPORTUNISTIC MYCOSES

Fungi that usually do not cause any disease in healthy persons, but may do so in persons who have altered host defence mechanisms are called opportunistic mycoses. The fungi that cause opportunistic infections may be endogenous i.e. members of normal flora, or exogenous, free living saprophytes. Candidiasis caused by *Candida albicans* is the best example for endogenous opportunistic infections, and Aspergillosis caused by *Aspergillus fumigatus* is the best example for exogenous opportunistic infections.

9.7.1. Candidiasis: *Candida albicans* is a yeast like fungus commonly present in the upper respiratory tract, alimentary canal, female genital tract and on the skin of healthy people and is an important fungal component of normal flora. It becomes pathogenic often as sequel to malnutrition, general debility, antibiotic suppression of bacterial flora which normally inhibit proliferation of these and other fungi. Other predisposing factors include use of oral contraceptives, steroid therapy and immunosuppressive therapy.

C. albicans possess two morphologically different types of cells (Fig. 9.4).

1. Spherical to oval, budding cells (3-5 x 5-10 μm) and
2. Elongated filamentous cells joined end to end (pseudohyphae).

Figure 9.4. *Candida albicans* showing two types of cells

It is aerobic and easily culturable. On agar medium, soft cream coloured colonies are formed. The surface growth consists of oval budding cells. The submerged growth consists of pseudomycelium. This is composed of psedohyphae that form blastospores at nodes and some times chlamydospores terminally.

The oval cells of *C. albicans* produce long germ tubes when placed in serum for three hours at 37 °C, and it is a distinguishing character.

Infections: *C. albicans* infections are usually endogenous. Cross infections may occasionally occur from mother to baby or from baby to baby in nurseries.

Candida infections occur in various parts of the body, and most infections involve skin or mucous membranes. This is because the fungus is a strict aerobe and finds such surfaces very suitable for growth. Candida infections are generally termed as candidiasis with a prefix basing on the organ infected or type of infection with prefix Candida.

1. Oral candidiasis or Thrush: Infection of mouth is called thrush or oral candidiasis. It occurs mainly in infants. Infection on the mucous membranes of buccal cavity appears as white adherent patches, consisting largely of pseudomycelium and epithelial cells.

2. Vaginal candidiasis: It resembles thrush. Produce irritation, intense itching and white discharge in females. Loss of acid pH in the vagina predisposes to Candida vulvovaginitis. Diabetes, pregnancy, antibiotic therapy etc. are the major predisposing factors.

3. Cutaneous candidiasis: Infection of skin occurs mainly in moist, warm parts of the body such as groin, intergluteal folds (gluteal = pertaining to buttocks). It is most common in obese and diabetic individuals. The infected areas become red and weepy, and vesicles may develop.

Candida infection of interdigital webs of the hands is seen most frequently following repeated, prolonged immersion in water. It is common in house wives, cooks, vegetable and fish handlers etc.

4. Pulmonary candidiasis: The fungus invades the bronchial walls and lung lobes, and may produce a generalized infection. It occurs mainly when a preexisting disease occurs (eg. TB). This must be clearly distinguished from the multiplication of C. albicans in bronchi, which often follows vigorous antibiotic treatment of bacterial infections. It ceases spontaneously when treatment is stopped.

5. Candida enteritis: Inflammation of small intestine is called enteritis. It is a consequence of suppression of normal flora by broad spectrum antibiotics.

6. Candida endocarditis: Inflammation of heart muscles is called carditis. It is mainly a disease of drug addicts and diabetics.

7. Candida paronychia: Painful, reddened swelling of nail fold, resembling a pyogenic paronychia, may lead to thickening and transverse grooving of the nails and eventual loss of the nail.

8. Candida septicaemia: It occurs with abscess formation in kidneys and other organs. Common predisposing factors are immunosuppression, malignant disease and broad spectrum antibacterial treatment.

Diagnosis of Candida infections is by microscopy and culturing of suitable specimens. Germ tube development from the cells kept in serum is diagnostic.

Treatment involves use of amphotericin-B for deep seated infections.For skin infections use of ketoconazole, miconazole and nystatin creams is effective. Use of 1% gentian violet applied topically reduce thrush.

9.7.2 ASPERGILLOSIS: Species of *Aspergillus* are ubiquitous saprophytes and a few species cause opportunistic infections in immune-compromised patients. The infections caused by species of *Aspergillus* are collectively called aspergilloses. Rippon (1974) and Emmons et al. (1977) have studied *Aspergillus* infections in detail and authenticated that atleast 8 species are involved in causing various types of infections, and *Aspergillus fumigatus* accounts for almost all infections. Other species involved include *A, terreus, A. flavus, A. niger* etc.

Species of *Aspergillus* grow on various substrata naturally, and are easily cultivable. Mycelium is well developed and comprise profusely branched well developed septate hyphae. They sporulate heavily. The conidiophores are long, erect filaments terminating in a bulbous vescicle. On the vesicle a large number of phialides are formed and they produce conidia at their tips one below the other, thus forming long chains (Fig. 9.5). The conidia are typically globose and unicellular measuring 4-6 μm in diameter. The conidia are aerially dispersed.

Figure 9.5. *Aspergillus fumigatus*

Infections start with inhalation of airborne spores of the pathogen. Inhalation of the spores leads to several types of infections.

Allergic aspergillosis: Infected individuals develop an immediate allergic response and suffer typical asthmatic attacks, when exposed to fungal antigens on the conidia.

Bronchopulmonary aspergillosis: The fungus grows in the lung, though tissue invasion is not apparent. It results in chronic bronchitis, resulting in type-I and type-III hypersensitivities.

Aspergilloma or colonizing aspergillosis: The most common manifestation of pulmonary involvement is the occurrence of colonizing aspergillosis, in which *Aspergillus* forms colonies with in the lungs, that develop into fungus like balls called 'aspergllomas'. These consist of tangled mass of mycelia.

Fulminating aspergillosis: It is a progressive, disseminating infection involving lungs, meninges, liver, bones etc. It occurs in immunosupressed patients in the final stages.

Otomycosis: It is a chronic condition in which fungal growth fills external ear.

Diagnosis of aspergillosis depends on identification of the pathogen microscopically in the specimens and culturing the fungus.

Treatment: The fungus is not susceptible to amphotericin-B or imidazoles, the drugs commonly used for treatment of deep seated mycoses.

Surgical operation may be required to remove aspergillomas.

9.8. SUMMARY:

Of the estimated 1,00,000 species of fungi, less than 100 species cause infection of humans and animals. Morphologically, the medically important fungi are four types viz. yeasts, yeast like fungi, filamentous fungi and dimorphic fungi. Infection of humans by a fungus is called mycosis and depending on the area of infection and nature of infection, the mycoses are grouped into five types viz. superficial mycoses, dermatomycoses, subcutaneous mycoses, systemic mycoses and opportunistic mycoses. Two types of piedras namely white piedra caused by *Trichosporon cutaeneum* and black piedra caused by *Piedraia hortai* are important superficial mycoses, and they are generally called dandruff. Fungal infections that spread through keratinized tissues of the skin, hair and nails are called dermatomycoses, and they are caused by three filamentous fungi viz. *Trichophyton, Microsporum* and *Epidermophyton*. The infections caused by these fungi are called tineas or ring worm infections. Important tineas are tinea pedis (infection of foot), tinea corporis (infection of non-hairy surface of skin), tinea cruris (infection of groin), tinea capitis (infection of hair on the head), tinea barbae (infection of beard) and tinea unguium (infection of nails). They mainly spread by contact with infected persons or animals. Imidazoles, nystatin and griseofulvin are effective drugs against tinea infections.

A number of soil fungi cause subcutaneous infections and among them sporotrichosis caused by *Sporothrix schenkii* and madura foot caused by *Madurella mycetoma* and other fungi are important. Most infections involve bare footed agricultural workers. Once in the subcutaneous tissue, the fungus slowly develop over a period of months or years to produce a nodule that eventually ulcerates and the organism spread along the lymphatic channels producing more subcutaneous nodules. At times, such nodules drain to the surface of the skin. Diagnosis is

accomplished by culture of fungus from infected tissue, and therapy is usually by administration of oral 5-Flourocytosine, iodides and amphotericin-B.

Inhalation of airborne inoculum liberated from some soil-borne fungi initiate pulmonary infections which may later spread to other internal organs or skin through hematogenous spread. Such infections are termed as systemic mycoses or deep mycoses. Important examples for deep mycoses are Histoplasmosis caused by *Histoplasma capsulatum*, Blastomycosis caused by *Blastomyces dermatidis* and Cryptococcosis caused by *Cryptococcus neoformans*. Of these *Histoplasma* and *Blastomyces* are dimorphic fungi which show filamentous hyphal growth in soil and show yeast like growth in the infected tissue. *Cryptococcus* is a true yeast. *Histoplasma* infections are mainly confined to lungs and cause tuberculosis like disease. *Blastomyces* has affinity to skin spread from lung to skin causing severe dermatidis. *Cryptococcus* cause severe meningitis on secondary spread from lungs. The diagnosis of systemic mycoses involve isolation and culturing of the fungi from infected tissues. For treatment amphotericin – B is used.

Fungi that usually do not cause any disease in healthy persons, but may do so in persons who have altered host defence mechanisms are called opportunistic mycoses. The fungi that cause opportunistic infections may be endogenous i.e. members of normal flora, or exogenous, free living saprophytes. Candidiasis caused by *Candida albicans* is the best example for endogenous opportunistic infection, and Aspergillosis caused by *Aspergillus fumigatus* is the best example for exogenous opportunistic infection.

9.9. MODEL QUESTIONS

Essay type questions

Give a general account of fungal infections of humans

Give an account of dermatophytic fungi and infections caused by them

Give an account of systemic mycoses and their control

Give an account of opportunistic infections caused by candida albicans and

Aspergillus species

Short answer type questions

Piedras
Tineas
Dermatophytic fungi

Subcutaneous mycoses
Histoplasmosis
Blastomycosis
Cryptococcosis
Opportunistic mycoses
Candidiasis
Aspergillosis

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LESSON–10: VIRAL DISEASES OF HUMANS (PART-1)

Objective: To study four diseases caused RNA viruses viz. poliomyelitis, Influenza, mumps and measles

Contents

10.1. INTRODUCTION

Among the human viruses RNA viruses are more common than DNA viruses. Polio, influenza, mumps and measles are very common human diseases and all of them are viral diseases caused by single stranded RNA viruses. Among these, polio virus is a very small naked virus belonging to the family picornaviridae and the virus mainly infects central nervous system and seriously effect the muscular function of the body parts enervated by the infected nerve tissue. The viruses that cause influenza, mumps and measles are relatively large enveloped ssRNA viruses having an affinity to mucous membranes, and hence called myxoviruses. Influenza virus contain segmented genome and is placed in the family orthomyxoviridae, while mumps and measles viruses have nonsegmented genome and are placed in the family paramyxoviridae. The details of these viruses and infections caused by them are explained in this lesson.

10.2. POLIOMYELITIS

Polio is probably of ancient origin. Various Egyptian heirographics (stone carvings) dated approximately 2000 B.C depict individuals with wasting whithered legs and arms, resembling present day polio patients. In 1840, the German orthopedist Jacob Von Heine described the clinical features of poliomyelitis and identified the spinal cord as the problem area. In 1908 Karl Landsteiner and William popper successfully transmitted the disease to monkeys and established the contagious nature of the disease. In 1949 John Enders, Thomas Weller and Frederick Robbins discovered that the poliovirus could be propagated in cultures of human embryonic tissues of non-neural origin. This is the first case of isolation of poliovirus. This led to the development of

vaccines, and Enders, Weller and Robins received Nobel Prize in 1954 for their contribution. In 1952 David Bodian recognized that there were three distinct serotypes of the poliovirus. In 1953 Jonas Salk successfully immunized humans with formalin killed poliovirus. This vaccine (IPV=intravenous polio vaccine) was licensed in 1955. In 1962 Albert Sabin and others developed live attenuated polio virus vaccine (OPV=Oral polio vaccine). Both Salk's and Sabin's vaccines (IPV & OPV) led to a dramatic decline of paralytic poliomyelitis in developed countries. At present, WHO has taken up polio eradication programme using the vaccine with an aim to achieve complete eradication by 2000.

10.2.1. Virus: Polomyelitis is caused by poliovirus belonging to the family picorna viridae and is a member of the genus *Enterovirus*. Natural infection of the virus occurs only in man but experimentally monkeys and chimpanzees may be infected.

The virion is a naked spherical particle of 26 to 27 nm diameter showing icosahedral symmetry with 32 capsomeres. Genome consists of a linear single stranded RNA molecule of (+) sense. The genome comprises of about 7440 bases, the 5' end of the virus RNA is joined covalently by a short polypeptide called VPg protein and 3' end is free. There is only one reading frame, 6627 bases in length, that codes for a polyprotein. This protein can be cleaved at multiple sites by proteolytic enzymes to yield 11 or 12 different peptides with various functions. Virus replication occurs in cytoplasm. Viral RNA which is (+) sense transcripts to synthesize one (-) sense RNA strand from which (+) strands are synthesized. Initially all + strands act as mRNAs to synthesize viral specific proteins. Once sufficient proteins have accumulated, newly formed RNA strands join with proteins to form new virions. The virions are released after cell lysis and hence no envelope is acquired.

10.2.2. Properties: The virus is very stable and can remain viable for relatively long periods in food and water - its main roots of transmission. It is resistant to ether, chloroform, proteolytic enzymes of the intestinal contents and detergents. It is stable at pH 3, can survive for years at 20^0C and for months at 40^0C.

It is readily inactivated by heat at 55^0C for 30 minutes. Formaldehyde and oxidizing disinfectants destroy the virus. Chlorination destroys the virus in water but organic matter delays inactivation. Phenolic disinfectants are not effective.

10.2.3. Strains: By neutralization test, poliovirus strains have been classified into three types 1, 2 and 3. Type-1 is the most common strain. Basing on the place at which the strains were first isolated, the type 1strain is called Brunhilde strain, type 2 strain is called Lansing strain and type 3 as Leon strain.

10.2.4. Antigens: Two antigens viz 'C' (colourless or capsid) and 'D' (dense) are recognized. The D antigen is also called 'N' antigen or native antigen, and is associated with the whole virion and type specific. The C-antigen, also called H antigen or coat antigen, is associated with 'empty' non infections virus coat and is less specific in serological tests. Anti-D antibody is protective and the potency of injectable polio vaccine is measured in terms of D antigen units.

10.2.5. Culture: The virus readily grows in a variety of tissue cultures of primate origin. Primary monkey kidney cell cultures are used for diagnostic purppose and vaccine production.

10.2.6. Pathogenesis: The virus enters the body mainly through ingestion of contaminated food and water. Inhalation of droplets can also be a mode of entry in close contacts of patients in the early stage of disease. Pathogenesis occurs in three phases.

10.2.6.1. Enteric Phase: Once ingested, or inhaled, the virions adsorb on to the specific cell receptors on the mucosa of the throat, enter the host cells and multiply. Sometimes the virions may pass down the small intestine and there adsorb on to the mucoepithelium, enter and multiply. From the throat, the virus invades the tonsils and lymph nodes of the neck.

10.2.6.2. Viremic Phase: After multiplication in the regional lymph nodes, the virus enter blood stream and is carried to spinal cord and brain. In most cases viremic phase is transient and clinical disease does not develop. However, in a small number of cases viremia persists and virus spreads to central nervous system (CNS). Direct neural transmission to CNS from throat (tonsils) may also occur under special circumstances as in polimyelitis following tonsillectomy.

10.2.6.3. Neural Phase: In CNS the virus multiply selectively in the neurons (nerve cells) and destroy them. The virus has a high affinity to anterior horn motor nerve cells of the spinal cord. The early changes due to infection is the degeneration of Nissl bodies. (Nissl bodies large granular basophilic bodies found in the cytoplasm of neurons, composed of rough endoplasmic reticulum and free polyribosomes). Nissl body degeneration is often described as chromatolysis. Nuclear changes follow. When degeneration becomes irreversible, the necrotic cell lyses or is phagocytosed by leukocytes or macrophages.

Though the most commonly affected nerve cells are motor nerve cells, other parts of CNS may also be infected and in severe cases, respiratory failure may occur due to degeneration of neurons enervating the muscles of respiratory tract.

10.2. 6.4. Path of pathogenesis:

Ingested virus

Oropharyngeal mucosa → Virus in throat → Intestinal mucosa

virus in faeces

Tonsils → Peyer's patches

Cervical lymph nodes → Mesenteric lymph nodes

Blood

CNS → Other susceptible Extra neural tissue

Regional Nerve ganglion

10.2.7. Clinical features: The incubation period is about 10 days but may range from 4 days to 4 weeks. The clinical manifestations vary very widely.

10.2.7.1.Inapparent infections: Most infections are symptomless and occurs in 95% of infected cases.

10.2.7.2. Abortive poliomyelitis: Occurs in approximately 4-5% of infected cases. It is characterized by are fever, headache, sore throat, vomiting, loss of appetite and general malaise lasting 1-5 days. These symptoms are associated with viremic phase. This is called minor illness and in many cases may be the only illness. In most cases (more than 99%) the infection will not proceed further, and the viremia phase is only transient.

10.2.7.3.Non-paralytic poliomyelitis: It has features of abortive poliomyelitis as well as signs of meningeal irritation which is most prominent in the neck and lumbar region. It occurs in less than 1% of cases, and symptoms are prominent during the stage of viral invasion of CNS. Sometimes the disease does not progress beyond this stage of aseptic meningitis (non paralytic poliomyelitis). The symptoms persist for a few days and recovery is complete, because the virus is eliminated before the nerve cells are affected.

10.2.7.4. Paralytic poliomyelitis: It occurs in approximately 0.1% of all infected cases, and is due to damage to the nerve cells. Flaccid paralysis occurs due to loss or impairment of motor function in affected parts of the body. Depending on the distribution of paralysis, cases are classified as different types. Mortality ranges from 5-10% and is mainly due to respiratory failure. In survivors, recovery of the paralysed muscles takes place in the next 4-8 weeks and is usually complete after 6 months, leaving behind varying degrees of residual paralysis.

The characteristic feature of paralytic polio is selective destruction of motor nerve cells resulting in permanent paralysis of one particular group of muscles such as those of an arm or leg. The nerves of sensation are not affected. Depending on the nature of damage, poliomyelitis may be

Paraplegia : Paralysis of lower part of the body including legs.

Paraparesis : Partial paralysis of the lower extremities

Tetraplegia or quadriplegia: Paralysis of both legs and arms

Tetra paresis : Muscular weakness affecting all four extremities

Following infection, muscles shrink and normal bone development fails in the affected area. In most severe cases, muscles of the other organs and respiratory tract may be affected leading to fatality.

10.2.8. Epidemiology: Poliomyelitis is an exclusively human disease and world wide in distribution. It mainly occurs during summer. Clinically severe poliomyelitis is not very prevalent (0.1%) but the visibility of the crippled survivors cause even small epidemics very terrifying.

Polio is essentially a child hood disease and over 80% of all paralytic cases occur before the age of three years, and occasionally occur in children above the age of 10. The severity of the disease increase with age and crippling affect is more severe in adults.

The only natural source of virus is man, the patient or much more commonly the symptomless carrier. Patients shed the virus in faeces for varying periods, about 50% for three weeks and a small proportion for 3-4 months. No permanent carriers occur. Faecal virus is the major source for infections in the community. The virus shed in the throat secretions during early part of the disease is an important source of infection for the contacts of patients. Infection is in general asymptomatic. The ratio of subclinical infections to active cases is stated to be 100-1000:1. Pregnancy and tonsillectomy during incubation period predispose the persons to paralysis.

Poliovirus type 1 is responsible for most epidemics of paralytic poliomyelitis. Type 3 also causes epidemics to a lesser extent. Type 2 usually causes inapparent infections in the western countries but in India paralysis due to type 2 is also quite common. There is cross protection between types l and 2, and 2 and 3 but little or none between 1 and 3.

Spread of polio occurs in poor living conditions with little sanitation. As such it is more prevalent in underdeveloped countries than in developed countries. In India and other tropical counties about 90% of children acquire antibodies to all three types in the early childhood, occasionally does a case occur after the age of 10 years. The incidence of paralytic poliomyelitis in India is 20-40 per 1,00,000 population per year. An estimated 20,000 children develop paralytic poliomyelitis annually in India, more than in all the rest of the world put together. The disease is equally prevalent in rural and urban areas.

The epidemiology of poliomyelitis has undergone marked changes during the last three decades with the use of polio vaccines. Herd immunity develop for polio, when 75% of the population is immune, the entire population is almost immune.

10.2.9.Diagnosis: Cultural and serological methods are used in diagnosis.

10.2.9.1. Culture: The virus can be isolated from throat in the early stages of the disease, and from faeces throughout the course of the disease and during convalescence. After appropriate processing to destroy bacteria, specimens are inoculated into tissue cultures. Primary monkey kidney cell cultures are usually employed. The virus growth is indicated by typical cytopathic effects in 2-3 days. Identification is made by neutralization tests with pooled and specific antisera. Mere isolation of poliovirus from faeces does not constitute a diagnosis of paralytic poliomyelitis as symptomless carriers are very common.

10.2.9.2. Serology: Serodiagnosis is less often employed. The antibodies can be demonstrated by neutralization or compliment fixation tests. They are useful only to identify exposure to poliovirus but not active disease.

10.2.10. Treatment: No specific drugs are available for therapy. Bed rest and respiratory support are suggested.

10.2.11. Immunization:

10.2.11.1. Passive immunization: It is by administration of human gamma globulin. It is of limited value and is restricted to special cases at high risk as in pregnant women exposed to infection.

10.2.11.2. Active Immunization: Salk's killed polio vaccine given as injection (IPV), live vaccine given orally (OPV) are available.

Killed Vaccine: Salk's killed polio vaccine is a formalin inactivated preparation of three types of poliovirus grown in monkey kidney tissue cultures. It is given by injection. Three doses given 4-6 weeks apart constitute the primary vaccination, to be followed by a booster to be given 6 months later. The first dose should be given to babies at the age of 6 months. Immunity can be sustained by booster doses every 3-5 years there after. Killed vaccine induces only systemic antibody response. There is no intestinal immunity. So, even in the vaccinated, infection with a wild strain may lead to intestinal multiplication and dissemination of virus. The individual is protected by circulating antibodies. Immunity needs to be protected by booster doses periodically.

Live vaccine: Sabin's attenuated vaccine (obtained by plaque selection in monkey kidney tissue culture) is administered orally in pleasantly flavoured syrup or candy. Both monovalent and trivalent vaccines are available. A single dose is sufficient but in practice threes doses are given at 6-8 week intervals, to ensure that virus multiply in intestine. Live vaccine induces local immunity in the gut so that wild viruses are unable to multiply in the intestine. Hence it protects the individual. Immunity resembles natural immunity in being life long. However there is a minor risk of attenuated virus acquiring virulence during enteric multiplication. The WHO at its 15^{th} planning meeting on 13^{th} May 1988, has planed global eradication of poliomyelitis by the year 2000 through global immunization using O.P.V. The programme is still going on.

10.3. INFLUENZA

Influenza is one of the great epidemic diseases. From time to time, influenza becomes pandemic and sweeps throughout the world. The name '**influenza**' is said to have been given by Italians during the epidemic of 1358, which they ascribed to the malevolent influence of the heavenly bodies or of inclement weather. Influenza virus belongs to orthomyxoviridae.

10.3.1. Virus Morphology: The virions are roughly spherical with an envelope, and measure 80-110 nm in diameter. Pleomorphism is common and filamentous forms are frequent in freshly isolated strains. The inner electron dense core is nucleocapsid of 70 nm diameter. The capsid shows helical symmetry. Viral genome is single stranded RNA of negative sense which is segmented and exists as 8 separate pieces (Fig. 10.1). Each segment is a gene and codes for a different protein. Because of the segmented nature, genetic reassortment occurs during replication forming stable hybrids.

Figure 10.1. Structure of influenza virus. C) Core containing eight strands of RNA.
M). Protein membrane H) Haemagglutinin spike N) Neuraminidase spike

The envelope has an inner membrane protein layer and an outer lipid layer. The membrane protein is also known as matrix protein or M- protein. The protein part of the envelope is virus coded but the lipid layer is derived from the modified host cell membrane, during the process of release by budding. Attached to the lipid layer of the envelope are two types of spikes or peplomers of about 10 nm size

1. hemagglutinin spikes which are triangular in shape and.
2. Neuraminidase spikes which are mushroom shaped and less numerous than H- spikes.

10.3.2. Function of the spikes:

10.3.2.1. N-Sikes: The spike neuraminidase is so called because it breaks down a mucopolysaccharide component of the plasma membrane called sialic acid, a derivative of neuraminic acid. When the virions deposit on mucous film of the respiratory tract, the neuraminidase by its activity lowers the viscosity of mucous layer, thus exposing the cellular surface receptors and promotes the spread of virus containing fluid to lower parts of the respiratory tract.

10.3.2.2. H-Spikes: The spike hemagglutinin is so called because it causes agglutination of red blood cells in vitro. The red blood cell is not the type of host cell which the virus normally infect but contains on its surface the same type of membrane component, chemically characterized as sialic acid, which the mucous membrane of the respiratory tract cells contain. Thus red blood cells are merely a convenient cell type for measurement of agglutination activity.

When the epithelial cells of the respiratory tract are exposed to virion, it attaches to the specific receptors on them, which are composed of sialic acid. Thus H-Spikes help in attachment of virion to the host cell membrane.

10.3.3. Antigenic Structures: Influenza viruses have three main antigens.

'S' or soluble antigen: It is the protein with ribonucleoprotein core of the virus particle. All influenza A viruses share a common S antigen, which is different from that shared by all influenza B viruses.

Hemagglutinin: It is contained in the radially projecting H-spikes in the virus envelope. It is type specific and considered as the major virulence factor because, antibodies against the hemagglutinin gives immunity against infections by blocking viral attachment.

Neuraminidase: It is also antigenic and contained in the N-spikes of virus envelope. Antibodies to N-spikes block the release of virions from the infected cells.

10.3.3.1. Antigenic variation: Influenza viruses uniquely able to undergo frequent antigenic change. Epidemics caused by Type A are due to the emergence of a new virus strain that contains

a haemagglutinin or a neuraminidase, different from those of previously circulating viruses, so that population has no herd immunity.

A minor antigenic change is called **antigenic drift**, and a major antigenic change is called **antigenic shift**.

Antigenic drift is due to spontaneous mutations in the hemagglutinin gene, causing minor changes in the amino acid sequence of the hemagglutinin protein. The drifted strain of virus then cause disease in the population by its ability to infect partially immune hosts. The drift occurs progressively from season to season.

Antigenic shift involves the replacement by genetic reassortment of the RNA segment which codes for the hemagglutinin by one from a different, possibly animal, virus strain. This can take place when two different influenza viruses infect and replicate in the same cell. As a result, new virus strain emerges with a new hemagglutinin gene, to which human populations have no preexisting antibody and can therefore spread pandemically. The same process may occur to new neuraminidase also.

The 1918 pandemic is deduced to be due to antigenic shift that occurred in preexisting strain from mixed infections with swine influenza virus and antigen combination termed H_1N_1. In 1957 a major shift occurred and H_2N_2 strain emerged and caused 'Asian flu'. In 1968 H_3N_3 appeared and caused 'Hong Kong Flu'. In 1977 H_1N_1 reappeared and caused epidemic in young people because older people had antibody from exposure to the virus before 1957. At present H_3N_3 and H_1N_1 strains are in circulation throughout the world.

Influenza virus type B also exhibits antigenic variation but the changes are not very marked. Type C virus has not undergone any antigenic variation.

10.3.4. O-D Variation : Barnet and Bull (1943) observed that Type A underwent certain changes, especially in hemagglutination activity – when serially passed in eggs. They called this O-D variation. The fresh isolate was said to be 'O' (original) phase and the virus cultivated in eggs is derived (D) phase. It was considered to be due to mutation. In 'O' Phase virions are predominantly filamentous while in 'D'-Phase they are predominantly spherical.

10.3.5. Pathogenesis: The virus enters the respiratory tract in airborne droplets emanated from nasopharyngeal secretions of infected persons. Even though influenza occurs in animals and birds in nature, they do not normally play an important role in human infections. In the respiratory tract,

neuraminidase facilitates infection by reducing the viscosity of the mucus film lining the respiratory tract and exposes the cell surface receptors for virus adsorption. The virion particle fuse with cellular membrane and it is mediated by hemagglutinin.

The virus particle enters the cell by the process of endocytosis. The endocytosed vesicle fuses with the lysosome. When fusion occurs between viral envelope and lysosomal membrane, the nucleocapsid is released into the cell cytoplasm and uncoating occurs in cytoplasm. Then viral genome migrates to the nucleus. Viral RNA pieces are synthesized individually in the nucleus and viral proteins are synthesized in the cytoplasm. The nucleocapsid assemblage occur in cytoplasm and bud through the host cell membrane, acquiring the envelope.

The ciliated cells of the respiratory tract are the main sites of virus infection. These cells are damaged and shed, laying bare the basal cells in trachea and bronchi, rendering the respiratory tract highly vulnerable to bacterial invasion.

Virus infection is ordinarily confined to respiratory tract and viremic phase is rare. Very rarely the virus had been isolated from spleen, liver, kidneys and other organs, during 1957 pandemic.

10.3.6. Clinical features: The incubation period is 1-4 days. Most infections are subclinical. In the typical clinical disease, the symptoms start with fever. The onset of influenza is abrupt and body temperature raises to 104^0F, nausea headache, generalized aches, sometimes with nasal discharge and sneezing. A non productive hacking cough is common and there may be sore throat and hoarseness. The symptoms usually last for about 4 days, but tiredness and weakness often persists for a long period.

People often confuse common cold and influenza (flu). Common cold is characterized by abundant nasal discharge with little or no fever, but influenza is often accompanied by high fever with little nasal discharge. The differences between common cold and flu are as follows

Symptom	**Common cold**	**Flue**
Fever	Rare	Common and high
Headache	Rare	Common
General weakness	Slight	Common and severe
Nasal discharge	Common, abundant	Less common

Sore throat	Common	Less common
Vomiting and diarrhoea	Rare	Common

10.3.7. Secondary infections: In a small proportion of cases the acute infection progresses to pneumonia. Two types of Pneumonia may follow influenza.

10.3.7.1. Primary influenzal pneumonia : It is very rare. It is characterized by on set of severe respiratory distress and symptoms of hypoxia (=reduction of 0xygen supply to tissue below physiological levels despite adequate perfusion of the tissue by blood), dyspnoea (= difficulty in breathing) and cyanosis (= a bluish discoloration of skin and mucous membranes due to excessive concentration of reduced haemoglobin in blood), leading to circulatory collapse, leading to the fatal final stage. This is due to congestion of the lungs with desquamated of ciliated epithelium and hyperaemia (=an excess of blood in a part) of tracheal and broncheal mucosa. No significant bacteria are present.

10.3.7.2. Secondary bacterial pneumonia: It is more common, especially in the elderly or in patients with preexisting cardiac or pulmonary disease. It is due to secondary invasion of lungs by bacteria such as *Staphylococcus aureus, Streptococcus pneumoniae, Haemophilous influenzae* etc. Though serious it is less lethal and can be cured.

10.3.8. Epidemiology: Influenza is the most typical example of epidemic disease, that spreads more rapidly than any another infectious disease. Influenza virus possesses an inherent capacity for rapid spread causing epidemics and pandemics. It is an airborne disease with a short incubation period of 3-4 days. Natural reservoir is present in populations in the form of inapparent infections. The virus infects both domestic animals and birds, which maintain the reservoir, though not directly spread infection to humans. It was widely believed that the virus spread from swine to man at the time of 1918 pandemic.

Influenza occurs in successive waves of infection with peak during winter. Though all the three. Influenza virus types A, B and C occur in population, all known pandemics are caused by Type A and is considered as the most important Type. The period between epidemic waves of influenza A is 2-3 years. Epidemics may be started when the virus mutates to a new antigenic type (antigenic drift) that has survival advantage, when antibodies in the population are low to this new

types. Among the three influenza virus types only A type is found in animals notably birds, pigs and horses. When occasional mixed infections occur in human, major antigenic shift may occur leading to pandemic out breaks.

Type B virus tends to produce less extensive outbreaks at intervals of 3-6 years. Type C is much less important and has so far produced no large epidemics.

The most severe pandemic occurred during 1918-19, during which over 20 million people, especially young adults perished. The next pandemic occurred in 1957 when the Asian strain originated in china and spread throughout the world within in a short period. Though it caused wide spread morbidity, the mortality rate was low. It is called 'Asian Flu.' In 1968 a pandemic broke out due to a new strain originated in Hong Kong. It is called 'Hong Kong flu' epidemic. In 1977, epidemic influenza appeared in china and then in Russia (and hence called Red Flu). The disease was mainly confined to under 20 age group. All these pandemics were traced to appearance of new antigenic strains due to antigenic shift.

10.3.9. Diagnosis: serological and cultural techniques are employed in diagnosis. serology is most widely used.

10.3.9.1.Seological: Rapid diagnosis of influenza may be made by demonstration of the virus antigen on the surface of the nasopharyngeal cells by immunofluoresent technique. Smears of nasal swabs and nasopharyngeal secretions or centrifused deposit of throat garglings are prepared on slides and treated with fluorescent dyes conjugated influenza antiserum and examined under UV microscope. The cells will be found to fluoresce due to the presence of viral antigens on cell surface.

Compliment fixation test with 'S' or soluble antigen is also employed for diagnosis. Haemagglutination inhibition test is also used for diagnosis.

10.3.9.2. Cultural: Virus isolation is readily obtained from the patients during the first 2 or 3 days of illness but not usually in the later stages. Throat garglings are collected using broth saline or buffered salt solutions and incorporated into the amniotic cavity of 11-13 days old eggs, and fluids are harvested after 3 days of incubation at 35^0C. The fluids are tested for haemagglutination for presence of virus.

Tissue cultures of monkey kidney cells are also used for isolation of influenza virus.

10.3.10. Immunity: An attack of influenza confers protection effective for about one or two years. Circulating antibodies are formed against various viral antigens but the antibodies formed only in the respiratory tract (Ig A) provide protection. Influenza virus infection induces cell mediated immunity also but its role in protection has not been clarified.

10.3.11. Therapy: Amantadine inhibits RNA polymerase II and blocks viral production. It also inhibits uncoating. Amantadine hydrochloride has been found to be of some value in treatment of influenza, especially in high risk cases. Limited antibiotic therapy is suggested for prevention of secondary invasion of bacteria. Otherwise no drugs are suggested for treatment in general cases.

10.3.12. Proplylaxis: Influenza vaccines have been in use for over three decades but not in general use because of frequent appearance of new serological types.

Vaccination is currently recommended for the elderly and those suffering from cardiac or respiratory problems. This is to protect those at high risk of death or serious complications.

Both killed virus vaccines and live attenuated virus vaccines are available for influenza.

10.4. MUMPS

Mumps is an acute contagious disease usually affecting children of the age 5-15 years, characterized by non-suppurative inflammation and enlargement of parotid salivary glands (Fig. 10.2). Hippocrates in the 5^{th} century B.C. first described the condition. Johnson and Goodpasture (1934) first convincingly proved the association of a virus with the disease. Medically the disease is known as Myxovirus parotidis because parotid gland infection by a myxovirus is the main feature. The word mumps is probably derived from the 'mumbling' voice during acute phase of the disease, which is the result of the pain on moving the jaws.

Figure 10.2. Typical symptom of mumps due to swelling of parotid salivary gland

10.4.1. VIRUS: The mumps virus belongs to the virus family Paramyxoviridae and genus Paramyxovirus. It is an enveloped ssRNA virus. The virion varies in its size from 80-240 nm and is markedly pleomorphic, but roughly spherical.

The genome consists of a single stranded, unsegmented, negative sense RNA with a mol. Wt. of 5-8 x 10^6, contain 20,000 – 25,000 bases, and about 1000 nm in length. The core contains virus specific RNA dependent RNA polymerase, along with the genome. The capsid shows helical symmetry. The envelope contains two glycoproteins HN and F, that form spike like projections from the surface. The envelope has inner protein layer and outer lipid layer from which the spikes project out. HN spike is a large viral glycoprotein responsible for both hemagglutination and neuraminidase activity. F is a smaller spike involved in fusion of viral envelope with host cell membrane.

The virus is antigenically stable and there are no differences between various isolates. Two types of antigens are recognized in the virus. 1. S-antigen or soluble antigen associated with nucleoprotein core of the virus and 2. V-antigen or viral antigen found on the surface of the virus particle. The antibodies to S-antigen appear within 2-3 days after the onset of the disease and tend to diminish sooner, while antibodies to V-antigen appear 8^{th} or 9^{th} day of the disease and usually persist for years providing long standing immunity.

The virus can be cultured in yolk sac or amniotic cavity of chick embryos. It can also be cultivated in human or monkey kidney cell cultures. In tissue cultures cytopathic effect is weak, but syncytial formation is common.

10.4.2. Pathogenesis: Humans are the only natural sources for the virus, and infection is acquired through inhalation of droplet nuclei emitted from the patients. After inhalation the virus is deposited in the upper respiratory tract or nasopharyngeal region. Infection may also occur through conjunctiva. The virus may also be transmitted by fomites.

There are two views regarding the pathogenesis of mumps. 1. The virus travels from the mouth or nasopharynx to parotid salivary glands where it undergoes primary multiplication. This is followed by a generalized viremia, and hematogenous spread to testes, ovaries, pancreas, thyroid lands, brain etc. 2. Primary replication occurs in the superficial epithelium of the

respiratory tract, and it is followed by a generalized viremia and simultaneous localization in the parotid salivary glands and other organs.

10.4.3. Clinical features: About 30% of all mumps infections are symptomless. In clinical cases, the incubation period is long about 18-21 days, before manifestations of the main symptoms of parotidis. A prodromal period of malaise precedes the swelling of one or both the parotid glands. It coincides with the viremic phase of incubation period. The prodromal symptoms are nonspecific and include fever, malaise, headache and anorexia.

Parotidis is the characteristic symptom of mumps infections. Non-suppurative inflammation and enlargement of one or both parotid glands is called parotidis. Bilateral involvement is seen in 95% of cases but one side is involved one to five days before the other. The enlarged parotid glands obscure the angle of the mandible and may elevate the ear lobe. It results in difficulty in opening the mouth. Pain is common at this stage.

10.4.3.1. Complications: Parotidis is the main symptom of mumps and meningitis, orchitis and other organ infections are complications.

10.4.3.1.1.**Meningo-encephalitis:** It is an important complication of mumps and is the commonest extrasalivarygland manifestation of mumps. Clinical meningitis occurs in 5% of all infected patients and 30% of patients with CNS involvement have no evidence of parotid gland involvement. Most cases of mumps encephalitis resolve without sequale but rarely deafness may result.

10.4.3.1.2. Orchitis (infection of testes): In about 25 -33% cases of mumps in post pubertal or adult males, an acute interstitial orchitis occur, which is usually unilateral but occasionally bilateral. It occurs about one week after the swelling of the salivary glands. Only in rare cases orchitis occurs without parotidis. Mumps orchitis may lead to testicular atrophy, but rarely to sterility.

10.4.3.1.3. Other organs: Mumps virus has great affinity to glandular tissues. Inflammation of breast and ovary occur in females, and virus is excreted in breast milk. Lacryminal glands, pancreas and thyroid glands are also involved. In nephritis the virus is present in urine up to two weeks.

10.4.4. Epidemiology: Man is the only natural host for mumps virus, though experimentally the disease can be induced in monkeys. The disease is endemic throughout the world and has greatest

incidence in children of 5 to 15 years age. Though no age is exempt, it is uncommon below the age of two years and, occurs in adults who escape exposure to the virus in childhood.

Since the disease spread by droplet nuclei, it is more common in semiclosed communities, and epidemics usually recur every 3 years. A patient is infectious from about 7 days before and till about 9 days after the onset of symptoms. Close contact appear to be necessary for transmission. The virus is shed in the saliva and urine, though epidemiological significance of the latter is not clear.

The patient usually recovers 7-10 days after the onset, and one exposure confers solid immunity.

10.4.5. Diagnosis: Diagnosis is mainly based on clinical features. In doubtful cases, serological demonstration of a fourfold rise in antibodies, detected by indirect hemagglutination or neutralization tests, confirms the diagnosis. The virus can be isolated in monkey kidney cell cultures, using CSF or throat washings.

10.4.6. Treatment: Attention should be given to adequate nutrition and mouth care. Analgesics should be used to relieve pain. The role of steroids in the treatment of mumps orchitis is controversial.

10.4.7. Prophylaxis: Live attenuated mumps virus vaccine given as a single dose of 0.5 ml intramuscularly can prevent the disease in children over the age of one year. Mumps vaccine is now available as a triple vaccine MMR (mumps, measles and rubella) vaccine for general use. It is now included in universal immunization programme for children.

10.5. MEASLES

Measles is an acute and highly contagious disease of childhood, characterized by the appearance of maculo-papular rash. The disease is also called '**rubeola**' (rubus = red) because of the appearance of rash. The disease is probably of ancient origin, but the viral etiology of the disease was established only in 1911 by Goldberger and Anderson. Humans are the only natural hosts for the virus.

10.5.1. Virus: The virus belongs to the family Paramyxoviridae and the genus Morbillivirus. It is an enveloped virus, roughly spherical in shape and measures 120 – 250 nm in diameter. The envelope encloses a tightly coiled helical nucleocapsid (Fig. 10.3).

Figure 10.3. Measles virus. 1. Protein layer of the envelope 2. Tightly coiled nucleocapsid 3. Lipoprotein membrane of the envelope 4. Hemagglutinin spikes

The viral genome consists of single stranded, negative sense RNA. The viral envelope is a lipoprotein membrane, 10-20 nm in thickness, and two types of spikes H and F project from the surface. H-spikes posess haemagglutination activity, but has no neuraminidase activity. The F-spikes are composed of fusion protein and help in integration of viral envelope with host cell membrane.

The virus is antigenically uniform and no strain differences are present. The virus does not grow in chick embryos, but can be cultivated in human embryonic kidney cell cultures or in monkey kidney cell cultures.

10.5.2. Pathogenesis: The infection is acquired by inhalation of droplet nuclei. From the respiratory tract it reaches lymphoid tissue and multiply there. After sufficient multiplication and breakdown of infected cells, there is an overflow causing viremia. During next 2-3 days the virus is localized in the skin producing rash. The viremia disappears with the appearance of antibodies in the blood. In most cases, measles lasts a total of 7-10 days. Circulating antibodies to measles virus appear about 5 days after initiation of infection. The activities of both serum antibodies and cytotoxic t-lymphocytes combine to eliminate the virus from the system. However, in children with weak immune response the virus spread to various other organs leading to various complications.

10.5.3. Clinical features: The incubation period varies from 8 to 14 days, and rarely more. The prodromal symptoms, coinciding with viremic phase, are mainly nasal discharge and suffusion of

eyes. The main symptoms of measles are fever with maculopapular rash lasting for 2 to 5 days. In typical measles two distinct phases can be recognized. 1. infectious, preeruptive catarrhal stage and 2. noninfectious eruptive or exanthematous stage.

Preeruptive infectious phase is the stage of viremia and viral dissemination in the body. Malaise, fever, cough, rhinorhoea, conjunctival suffusion and the pathognomonic koplik's spots are present during this stage. Koplik's spots are small grayish, irregular lesions surrounded by an erythematous base, and are found in greatest numbers on the mucous membrane opposite the second molar tooth. They occur a day or two before the onset of rash, and are of diagnostic value.

The noninfectious eruptive stage is characterized by the presence of a maculopapular rash that initially appears on the face, chiefly on forehead, and then spreads rapidly to involve the rest of the body. At first the rash is discrete but later it may become confluent and patchy, especially on the face and neck. It fades in about a week and leaves behind a brownish discolouration which soon disappears with desquamation.

10.5.4. Complications: Although measles is a relatively mild disease in healthy child, it carries a high rate of mortality in malnourished and in those who have other diseases. Complications are common in such individuals and include bacterial pneumonia, bronchitis, otitis media and gastroenteritis. Giant cell pneumonia is a rare complication seen in children immunodeficient or with chronic debilitating disease. It is due to direct invasion of the lungs by measles virus and usually fatal. Numerous multinucleated giant cells form in the lungs. Latent tuberculosis infections may be activated following measles infection.

10.5.5. Epidemiology: Measles occur throughout the world, and epidemic outbreaks recur every 2-3 years. Epidemics are usually seen in late winter or early spring with a peak in April. The disease incidence is maximum in children 1-5 years of age. It is uncommon in the first 6 months of life due to the presence of maternal antibodies. One attack confers solid immunity.

Man is the only natural host. Patients are infectious from three days prior to the onset of symptoms until the rash desquamates. Spread is by direct contact or by aerial spread of nasal secretions as droplet nuclei in aerosols created by coughing and sneezing.

10.5.6. Diagnosis: The clinical features are distinctive and diagnosis can be made basing on symptoms. Hence, serological tests such as hemagglutination inhibition test are rarely used to confirm the diagnosis.

10.5.7. Treatment: Antibiotics are suggested only if secondary bacterial infections occur.

10.5.8. Prophylaxis: One attack of measles usually confers a high degree of immunity and second attack is uncommon. Hence, to prevent the outbreaks of the disease, vaccines are highly effective. Active immunization involves a single dose of 0.5 ml live attenuated measles vaccine given subcutaneously. A triple vaccine MMR (for mumps, measles and rubella) is adopted as a part of infant immunization programme since late 1980.

10.6. SUMMARY

Poliomyelitis is a viral disease caused by a member of the genus Enterovirus belonging to the family picornaviridae. The virus is a naked spherical particle of 27 nm diameter showing icosahedral symmetry. Genome consists of a linear single stranded RNA of + sense. Three serotypes were present and they are antigenically stable. It can be cultivated in primary monkey kidney cell cultures. Only humans are natural hosts. Infections are acquired by ingestion of contaminated water or food. Three stages of infections viz. enteric phase, viremic phase and neural phase are recognized. Primary multiplication occurs in the intestine and in 90 to 95% of cases infections do not progress further and they are called symptomless infections. In about 4-5% of cases infection progresses to viremic phase with generalized symptoms, but do not progress further and such infections are called abortive poliomyelitis. In about 1% of cases the virus enters central nervous system and cause meningeal infection and such infections are called nonparalytic poliomyelitis. In less than 0.1% of cases the virus infects the anterior horn cells of vertebrae leading to destruction of nerve cells that supply motor nerves to various parts of the body especially legs and hands, and it leads to paralysis of the affected parts. Recovery occurs very slowly leaving behind varying degrees of residual paralysis. Diagnosis mainly rests on clinical symptoms and confirmation made through culturing of the virus in primary monkey kidney cell cultures. Two types of vaccines viz. Salk's killed virus vaccine given intravenously and Sabin's live attenuated virus vaccine given orally, are available for prevention of polio outbreaks. Mass immunization programme was undertaken by World Health Organization for complete eradication of polio using oral polio vaccine.

Influenza is caused by an orthomyxovirus. It is an enveloped virus with segmented genome of single stranded RNA. The virus undergoes frequent antigenic changes. Minor variation caused by mutations is called antigenic drift and major changes caused by genetic reassortment is called

antigenic shift. Antigenic drift results in out break of frequent epidemics, while antigenic shift results in pandemics. The virus spread by droplet nuclei released from patients or carriers and infection is acquired by inhalation. The incubation period is very short, 1-4 days, and the typical clinical symptoms are headache, malaise and high fever. Symptoms subside in about 4 days but tiredness and weakness persists for a long period. Primary influenzal pneumonia and secondary bacterial pneumonia are general complications, while Reye's syndrome and Gullain-Barre syndrome are rare complications of Influenza. Diagnosis is by serological identification of virus in the throat washings and by compliment fixation test. Amantadine is a synthetic antiviral drug specific for treatment of influenza. Both killed virus vaccines and live attenuated vaccines are available, but not in general use because of frequent antigenic variations.

Mumps is an acute contagious disease usually affecting children, characterized by non-suppurative inflammation and enlargement of parotid salivary glands. The mumps virus belongs to the virus family Paramyxoviridae and genus paramyxovirus. It is an enveloped ssRNA virus. The genome consists of a single stranded, unsegmented, negative sense RNA. The virus is antigenically stable and recovery from one bout of infection usually provides long standing immunity. Humans are the only natural sources for the virus, and infection is acquired through inhalation of droplet nuclei emitted from the patients. After inhalation the virus is deposited in the upper respiratory tract or nasopharyngeal region. About 30% of all mumps infections are symptomless. In clinical cases, the incubation period is long about 18-21 days. It usually subsides in about a week. Meningo-encephalitis and mumps orchitis are complications. Diagnosis is mainly made by clinical symptoms. A live attenuated vaccine, as a part of triple vaccine MMR, is available for prevention of mumps infections.

Measles is an acute and highly contagious disease of childhood, characterized by the appearance of maculo-papular rash. The virus belongs to the family Paramyxoviridae and the genus Morbillivirus. It is an enveloped virus, The envelope encloses a tightly coiled helical nucleocapsid. The viral genome consists of single stranded, negative sense RNA. The virus is antigenically uniform. The infection is acquired by inhalation of droplet nuclei. From the respiratory tract it reaches lymphoid tissue and multiply there leading to massive viremia. During next 2-3 days the virus is localized in the skin producing rash. The viremia disappears with the appearance of antibodies in the blood. In most cases, measles lasts a total of 7-10 days. Diagnosis

is generally made by clinical symptoms. One bout of infection usually provides life long immunity. A live attenuated vaccine, as a part of triple vaccine MMR, is available for prevention of mumps infections.

10.7. MODEL QUESTIONS

Essay type questions

Discuss the pathogen, pathogenesis, clinical symptoms and epidemiology of Poliomyelitis
Discuss the pathogen, pathogenesis, clinical symptoms and epidemiology of Influenza
Give an account of the causal virus, symptoms and epidemiology of mumps and measles
Discuss the epidemiology and prophylactic measures to control outbreak of Polio, mumps and measles

Short answer type questions

Poliomyelitis
Clinical symptoms of polio
Influenza virus
Spread of flu
Mumps
Measles

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LESSON–11: VIRAL DISEASES OF HUMANS (PART – 2)

Objective: To study three important viral diseases of humans viz. rabies, chicken pox and hepatitis

Contents

11.1. INTRODUCTION

Rabies, chicken pox and hepatitis are very common and important diseases of humans caused by viruses. Rabies is a dreaded disease, an acute form of encephalitis, caused by a rigid bullet shaped, enveloped, ssRNA virus belonging to the family Rhabdoviridae. It is essentially a zoonotic disease, occasionally transmitted to humans by the bite of infected animals, and cause infections which are almost always fatal. Chicken pox is one of highly contagious diseases of humans caused by an enveloped, dsDNA virus described as Varicella-Zoster-Virus, belonging to the DNA virus family herpesviridae. Chicken pox is usually a mild, uneventful, self resolving paediatric disease, but the virus remains latent in the body for decades and cause more painful shingles in the later part of the life. Infection of liver is described as hepatitis, and it results in a clinical condition called jaundice characterized by yellowing of skin and sclera. A large number of viruses cause hepatitis, and so far six viruses are discovered to cause primary hepatitis. The details of the viruses causing rabies, chicken pox and hepatitis, their pathogenesis, clinical symptoms, spread, diagnosis and prevention are explained in this lesson.

11.2. RABIES

Rabies is a dreaded disease recognized since the time of ancient civilizations. References to the disease are found in Mesopotamian laws (1800 B.C). It is an acute infection of central nervous system that is almost always fatal. The name rabies came from the Latin word rabidus meaning madness and it refers to the behaviour of infected man and animals. The disease in

humans is also called **Hydrophobia** because the patient exhibits fear of water, being incapable of drinking, though subjected to intolerable thirst. This peculiar feature is not exhibited by infected animals. The studies of Louis pasture, during 1880's which led to the development of rabies vaccine, is regarded as an important milestone in the development of medicine.

11.2.1. Rabies virus: Rabies virus belongs to the virus family Rhabdoviridae and genus Lyssa virus. The family name is derived from the shape of the virus (Rhabdus ^{Gr} = rod) and the genus name is derived from the symptoms of the disease (Lyssa ^{Gr} = rage).

Virus is a bullet shaped rod (Fig. 11.1) with a diameter of 70-80 nm and length 170-180 nm, with one end round or conical and the other usually planar. The virus comprises of outer envelope which enclose nucleocapsid. The envelope is a two layered structure with outer lipid layer frame with numerous spikes of 10 nm project, except from planar surface. Below the lipid layer is protein membrane. Even though the virion is an enveloped one, it is not pleomorphic because it is firmly attached to inner core giving a characteristic rigid shape. However at the planar end, the envelope may either invaginate forming a concave layer or may project out wards from one end forming a blob.

Figure 11.1. Rabies virus. Bullet shaped virus showing tightly wound helix of Ribonucleoprotein in the core, and bilayered membrane envelope with glycoprotein spikes

Viral genome consists of negative sense SS RNA with a capsid showing helical symmetry, mot wt 3.5-4.6 x 10^6

The nucleocapsid core contains several enzymes like RNA polymerase etc. which are essential for infection and replication.

The virus replication occurs in cytoplasm of the infected cells. The nucleocapsid is formed by association of capsid proteins around viral RNA. It acquires envelope when it is released through the host cell by budding. Rabies virus produces specific cytoplasmic inclusion bodies called Negri bodies (named after their discoverer, Negri) in the infected nerve cells. Negri bodies are large spherical bodies of 2-10 μm in diam. and are clearly visible under light microscopic observation of stained preparation of infected cells.

11.2.2. Antigenic structures: A number of antigenic structures are identified.

1. The surface spikes are glycoproteins, which induce protective antibodies. Hence purified glycoprotein provide a safe and effective antigen.
2. The virus posses hemagglutinating activity. Hemagglutination inhibition (HI) antibodies develop following immunization. Hence, HI test provides a useful method for assessing immunity to rabies.
3. Nucleocapsid protein induce compliment fixing antibodies but they are not protective.
4. Two membrane proteins, glycolipid and RNA dependent RNA polymerase are also antigenic but antibodies to these antigens do not provide protection against infection.

11.2.3. Resistance/Sensitivity: Virus is sensitive to ethanol ether, acetone and ammonium compounds. It is inactivated by phenol, formalin, UV-irradiation and sunlight. Thermal inactivation occurs in one hour at 50^0C and in 5 minutes at 60^0C. The virus can be preserved at -70^0C or by lyophilization.

11.2.4. Virus culture: Rabies virus can grow in several primary and continuous cell cultures such as chick embryo, fibroblast, Human diploid cell lines etc.

Previously, the virus is maintained in experimental animals, especially rabbits. After several intracerebral passage in rabbits, the virus becomes more neurotropic but much less infective and is called **'fixed virus'**. Fixed virus is used for vaccine preparation by Pasteur. Fixed viruses do not produce negri bodies in infected cells.

Rabies virus isolated from natural hosts or animal infections are termed **'Street Virus'**. It cause fatal infection and Negri bodies are formed in brain cells.

11.2. 5. Host range: All warm blooded animals are susceptible to rabies infection. Cattle, cats, horses and foxes are highly susceptible, while skunks, fowls, and opossums are relatively resistant. Man and dogs occupy an intermediate position with respect to their susceptibility to rabies.

11.2.6. Pathogenesis: Rabies is a lethal form of encephalitis. It is transmitted to humans via the bite of an infected animal which is usually – but not always – a dog. In addition to mad dogs, other infected animals also transmit the disease. In South America and Trinidad, spread often occurs due to bite of vampire bats. Transmission by foxes and cats in Europe and by wolves in Russia is common. Involvement of other animals is occasional and incidental.

The virus once introduced into the subcutaneous tissue with saliva of rabid animals, first multiply in the muscles, connective tissue or nerve cells at the site of deposition. Then the virus penetrates the nerve endings either immediately or after varying periods of intervals and travels in the axoplasm towards the spinal cord and brain. The movement of virus in the axons is passive at a speed of about 3 mm per hour. The main replication occurs in brain and then the virus spreads outwards along the nerve trunks to various parts of the body, including salivary glands. It multiplies in salivary glands and shed in saliva. The virus ultimately reaches virtually every tissue in the body and fatality results from respiratory paralysis.

11.2.7. Clinical features: The incubation period is variable and may range from a few days to several years, but on average it is 1-3 months. In general, bites on the head, face and neck have a short incubation period (because of closeness of brain) than those on legs. The incubation period is shorter in children than in adults.

In humans, two distinct clinical variations of rabies are recognized.

1. Furious rabies – the classical variety.
2. Dumb rabies – the paralytic variety.

The prodromal syndrome, coincide with invasive phase when virus travels from site of bite to CNS, include low grade fever, headache, fatigue, anorexia, insomnia and restlessness. Pain and paresthesia around the wound is quite common. The initial symptoms are same in both furious and dumb rabies. When disease progresses to exciting hyperactivity it is called furious rabies, and if it progresses to paralytic symptoms it is called dumb rabies.

11.2.7.1. Furious rabies: Exciting stage, with hyper activity and hydrophobia characterize furious rabies. This stage occurs when virus after multiplication in brain move outward into salivary glands and other parts of the body. Tremors, muscular contractions and convulsions, hyper excitability precedes classical symptoms of hydrophobia. The patient experience intense thirst but exhibit fear of water because of difficulty in drinking water due to pharyngeal spasms. Patient may swallow dry solids but not liquids. This stage is followed by extreme irritability and madness. Aerophobia (fear of air) is also a distinctive symptom, **pathognomonic** of rabies.

The patient goes on to develop respiratory paralysis and death usually occurs in 10-14 days.

11.2.7.2. Dumb rabies: In some cases, hyperactivity or excitement stage may not be prominent and paralytic features dominate from the beginning. Hence, it is called dumb rabies. It is more common after bites from rabid bats in people of South America. It may also occur in persons who have received post exposure vaccination. In this case of rabies also death occurs due to respiratory paralysis.

11.2.8. Epidemiology: With the exception of Australia, New Zealand and Antarctic, human rabies has been reported from all continents. The virus is maintained in natural animal hosts and transmission by bite of mad dogs is the major route of infection, even though other animals also transmit occasionally. Human to human spread is rare.

Though the disease can occur at any time of the year, it is more common during late summer with the on set of first monsoon rains. Traditionally, the disease is associated with the appearance of dog star 'sirius' in the 'dog days' of summer when dogs were considered to be prone to spells of madness. It is unusual for the patients to survive an attack of rabies.

11.2.9. Diagnosis: Diagnosis of rabies is generally made clinically. Diagnosis in man and dogs can be confirmed by isolating the virus from saliva. After death, characteristic inclusion bodies (negri bodies) can be found in nerve cells. Recently, fluorescent antibody technique was developed to detect virus in the salivary secretions.

The suspected dog that bites should be kept in captivity and observation for 10 days. If it survives, it has not got rabies. If it dies, brain is examined for negri bodies for confirmation of virus infection.

11.2.10. Treatment: Persons bitten by rabid animals should be immediately given human antirabies immunoglobulin (or horse antirabies serum) injected partly into the wound and partly intramuscularly.

Five does of rabies vaccine on days 0, 3, 7, 14 and 28 are given as a course of post exposure prophylaxis The vaccine commonly used is HDCSV (Human diploid cell strain vaccine) .

Once the disease is established, therapy is symptomatic. The patient should be placed in quiet, darkened area. Nutrition, respiratory and cardiovascular supplements may be necessary.

Drugs such as morphine should be used liberally for patients who are excitable.

However, recovery is rare, once the symptoms develop.

11.2.11. Prophylaxis: Rabies vaccine was first developed by Pasteur in 1885. It consisted of virus attenuated by drying the spinal cords of infected rabbits for varying lengths of time over KOH. Later various types of vaccines are prepared.

Antirabies vaccines are mainly 2 types – neural vaccines and non-neural vaccines.

11.2.11.1. Neural vacciness: Pasteur's vaccine is the first prepared neural vaccine. Later by treating infected brain tissues, vaccines were prepared. However, because of serious risk of neurological complications, they are replaced by non-neural vaccines.

11.2.11.2. Non-neural vaccines: These are prepared by growing the virus in duck eggs and tissue cultures. Vaccines are prepared by culturing the virus in Human diploid cell lines. They are called HDCV (Human diploid cell vaccine).

11.2.11.3. Recombinant vaccines: These are in development stage. The glycoprotein subunits on the virus surface are protective antigens. Hence, the gene coding for the protein has been cloned to produce recombinant vaccines.

Because of long incubation period rabies is suitable for prophyplactic immunization after exposure.

Pasteur institute, Coonoor, is preparing rabies vaccines in India.

11.3. CHICKENPOX

Chicken pox (varicella) and Herpes zoster (shingles) are two diseases that differ greatly in their clinical manifestations but are caused by the same virus, Varicella-Zoster Virus (VZV). Chicken pox is the primary illness in children and zoster is a reactivation of infection in adults.

11.3.1. Virus: The virus belongs to DNA virus family Herpes viridae. Man is the only natural host for the virus. It is an enveloped dsDNA virus. The virion measures about 100 – 180 nm in diameter. There are four distinct morphological units in the virion viz. genome, capsid, tegument and envelope.

The genome consists of double stranded linear DNA molecule of mol. wt. 85 – 10 x 10^6. The capsid is composed of 162 capsomeres showing icosahedral symmetry. Tegument is a layer of electron dense amorphous material lying between nucleocapsid and envelope. It is unique to Herpes group of viruses. The envelope is the lipoprotein layer that surrounds the tegument. Many small spikes (peplomers) project from outer surface of the envelope.

11.3.2. Virus replication: When the virion attaches to the specific host cell receptors, envelope fuses with the cell membrane, and releases the nucleocapsid into the cell. It then moves towards the nucleus and uncoating occurs in the cytoplasm. The viral genome enters the nucleus of the host cell but do not fuse with host genome. Viral DNA is synthesized in the nucleus and viral proteins are synthesized in cytoplasm. Viral proteins move into the nucleus where nucleocapsids are assembled. Maturation occurs by budding of the nucleocapsids through altered nuclear membrane. The enveloped virions are then released from the cell through the tubular network of endoplasmic reticulum that is continuous with the outside of cell or from vacuoles that release their contents at the surface of the cell.

11.3.3. Primary infection (chicken pox):

Chicken pox is one of the most common and highly infectious diseases of childhood. Man is the only natural source of infection and the virus spread by droplet nuclei or by contact. Usually the portal of entry of the virus is the respiratory tract. After an incubation period of 2-3 weeks clinical symptoms of chicken pox develop with little prodromal illness. As the virus spread through out the body, mild fever and malaise is experienced before the appearance of vesicular rash. The rash occurs on the entire body but mainly on trunk, and very little or no rash on hands and feet. The evolution of rash is so rapid that the various stages – maculae (area of discoloration),

papulae (solid elevated region), vesicle (small sac containing fluid), pustule (pus containing lesion of skin) and scab (scarred or crusted lesion) – cannot be followed in individual lesions. The vesicles appear in successive waves in first 3 or 4 days so that lesions of different type are present together. The rash is very superficial without involvement of deeper layers of skin. The vesicles crust within 48 hours and desquamation leaves no scars.

Chicken pox is usually an uneventful disease and recovery is a rule rather than exception. However, in rare cases of adults and pregnant women varicella pneumonia occurs as a complication. Congenital varicella is very rare.

11.3.4. Immunity: In varicella immunity is usually life long and a second attack is very rare. However, it does not preclude the appearance of shingles in the later part of the life. The virus may remain latent for many years or life long in cranial or thoracic nerve ganglion.

11.3.5. Epidemiology: Chicken pox mainly occurs in tropical areas and is greatest among children. The highest incidence is in late winter and early spring. Since there are no animal reservoirs for the virus, the spread is rapid in semiclosed communities. Virus is transmitted in respiratory secretions, spread by droplet nuclei.

11.3.6. Diagnosis: Diagnosis is usually made basing on clinical symptoms. Diagnosis can also be made by demonstration of giant cells in smears prepared from vesicular contents. The virus can be isolated in human embryonic tissue cultures or HeLa cells. It does not grow in experimental animals or chick embryos.

11.3.7. Treatment: Since uncomplicated chicken pox is a self limiting disease, no treatment is usually needed. Drying solutions such as calamine or lotion may be applied for soothing. Use of antiseptic powders may help to limit secondary infections.

11.3.8. Prophylaxis: A live attenuated varicella vaccine has been developed by Japanese workers, and it is recommended for use in high risk children as those with leukaemia and immunodeficiencies. Varivax, a live attenuated vaccine, was licensed for use in USA during 1995, and it can be given to all persons of above one year of age.

11.3.9. Reactivation symptoms (shingles): Shingles is typically a disease of adults, usually over 50 years of age, and it occurs due to reactivation of virus latent in dorsal root or cranial nerve ganglion. Virus travels down the sensory nerves to produce painful vesicles in the area of skin

enervated from the affected ganglion. Thoracic nerve supplying dermatomes of the chest wall are most often affected. There is segmental rash which extends from the middle of the back in a horizontal strip around the side of the chest, often described as "belt of roses from hell", and appears as a chest girdle (zoster means girdle). The vesicles that develop are bigger than those of chicken pox and are painful. It slowly desquamates but leaves black depressed scars on the body.

The virus is not usually present in the vesicles and it is thought to be due to presence of residual antibody that developed during primary infection. However, the virus may some times present in the vesicles and in such cases contact with the patient cause primary infection in susceptible individuals. It leads to chicken pox only but not shingles. Hence, shingles as such is not a spreading disease.

The prodromal symptoms of pain and tingling sensation may precede the reemergence of virus into skin. It then produces characteristic vesicles, papules or bulbous lesion throughout the dermatome. The rash heals in about 2 weeks, but the pain and parasthecia of the affected area may persist for weeks or months.

As it is a reactivation disease, there is no pattern of incidence and seasonal distribution. One common feature is that it occurs in people over 50 years of age. It is attributed to a change in immune state and due to drop in antibody levels against the virus.

Since shingles is a painful disease, use of acyclovir is recommended in severe cases of the disease. Idoxuridine may be applied on dressings that are kept moist.

11.4. HEPATITIS

Inflammation of liver is described as Hepatitis and it results in a condition called Jaundice. Yellowing of skin and scelra (=tough white outer layer of eye balls) is the major symptom of jaundice, with other associated symptoms like passing of dark coloured urine and white stools. Theses symptoms occur mainly due to liver dysfunction. Hepatitis is caused due infection by various pathogens including a number of viruses. Voeghat in 1942 first reported the viral nature of hepatitis and now a number of hepatitis viruses are known. At least six viruses are recognized so far, and epidemiologic evidence points to the existence of a few more viruses that cause hepatitis.

11.4.1. HEPATITIS VIRUSES

Hepatitis A virus (HAV): It is a RNA virus, belongs to family Picorna viridae, spread by contaminated water and food, cause short incubation period hepatitis called infectious hepatitis.

Hepatitis B virus (HBA): It is a DNA virus, belongs to family Hepadna viridae, cause long incubation period hepatitis, spread by blood transfusion or contaminated blood and blood products, it is also known as serum hepatitis.

Hepatitis D virus (HDV): It is a defective RNA virus, which depends on HBV for its replication.

Hepatitis C virus (HCV): It is a RNA virus, belongs to Flavi viridae family, cause post transfusion hepatitis.

Hepatitis E virus (HEV): It is a RNA virus, belongs to Calciviridae family, cause waterborne hepatitis

Hepatitis G virus (HGV): It is a RNA virus, probably related to flaviviruses, cause post transfusion hepatitis.

11.4.2. HEPATITIS A VIRUS (HAV):

Infectious hepatitis caused by HAV is the most common type of viral hepatitis, world wide in distribution, and mainly affect children and young adults.

11.4.2.1. Pathogen: The virus is a naked, nonenveloped, spherical particle of 27 nm in diameter. It is a RNA virus belonging to the family Picornaviridae. It was originally described as Enterovirus–72, but because of its unique features, now treated as a member of a separate genus *Hepatovirus*. It is antigenically stable and only one serotype is known Genome is SS RNA + sense. Capsid shows icosahedral symmetry.

Man is the only natural host for the virus but infection can be transmitted to chimpanzees and other primates experimentally. It can be cultivated in human and simian cell cultures and is the only human hepatitis virus which can be cultivated in vitro. It retains viability for long periods in food and water. It is resistant to low pH and detergents. It can be destroyed by heat at 60 ^{0}C for 30 minutes, boiling water one minute. It is also destroyed by chlorination.

11.4.2.2. Transmission: There are two modes of spread. 1. case to case spread via faecal – oral route. It is the most common route of spread. Symptomless excretors are important source of infection. 2. General out breaks usually result from faecal contamination of a common source such as drinking water supplies, food or milk. Consumption of raw oysters from polluted waters also resulted in several out breaks.

11.4.2.3. Infection: Once the virus is ingested, the primary multiplication occurs in the intestine, and the virus then spread to liver where it multiplies in hepatocytes and destroy them. Viremic phase is brief and transient. Incubation period is usually 2-4 weeks. When compared to the incubation period of hepatitis caused by HBV, it is very short, and hence, this is called short incubation period hepatitis.

The virus is present in faeces and blood for 1-2 weeks before onset of the symptoms. With the onset of jaundice the patient ceases to be infectious. This stage coincides with the appearance of Ig M antibodies. They persist for several months. Ig G antibodies appear later than Ig M and persist for years. Chronic carrier state does not occur.

11.4.2.4. Clinical symptoms: Prodromal phase, characterized by low grade fever, nausea, vomiting and anorexia, lasts up to 2 weeks. As jaundice develops appetite returns, as jaundice deepens urine becomes dark and stools pale. The liver becomes moderately enlarged. The patient recovers slowly with in 3-6 weeks. In rare cases, the disease may become very severe with fulminant hepatitis, hepatic coma and final phase.

11.4.2.5. Epidemiology: It is world wide in distribution and endemic in most countries. It is a disease of poverty and unhygiene, and especially common in tropics where sewage treatment is negligible and protected water supplies are rare. The outbreaks are associated with contaminated food and water. It mainly effect children and young adults in the age group of 5 – 30 years, and account for 20 – 40% of all clinically apparent hepatitis.

11.4.2.6. Diagnosis: The experienced physicians diagnose the disease usually by symptoms. Serological tests confirm the disease. Serodiagnosis is by demonstration of Ig M or Ig G antibodies against HAV in the serum of the patient. The presence of Ig M antibodies in serum of the patient indicates current or recent infection. Presence of Ig G antibodies in the serum of the patient indicates recent or remote infection. Hence, serological tests for Ig M antibodies are more

specific. ELISA kits are commercially available for detection of Ig M and Ig G antibodies against HAV.

11.4.2.7. Treatment: No specific antiviral drug is available. Treatment is symptomatic. Bed rest and dietary control are suggested.

11.4.2.8. Prophylaxis: General prophylaxis consists of improved sanitary conditions and prevention of faecal contamination of food and water. A safe and effective formalin inactivated vaccine is available since 1992, and used for those at special risk.

11.4.3. HEPATITIS B VIRUS (HBV):

It is world wide in distribution and cause important type of viral hepatitis known as **serum hepatitis**. It is also called long incubation period hepatitis because it usually takes 1-6 months for the appearance of the clinical symptoms after exposure to infected blood or serum.

11.4.3.1. Virus: HBV is a DNA virus of 42 nm diameter, composed of a central core of 27 nm nucleocapsid core containing partially double stranded DNA and associated with DNA polymerase. It is placed in the genus Hepadna virus in the family Hepadnaviridae.

The full strand of DNA is composed of 3200 nucleotides and it is (-) strand. The incomplete strand is composed of 2500 nucleotides and DNA polymerase is attached to it at one end (Fig. 11.2).

Figure 11.2. Structure of Hepatitis B virus

In addition to virions, two other structures are also seen in the serum of patients.

1. Spherical particles of 22 nm diameter, more numerous and composed exclusively of surface antigen (HBsAg).

2. Large tubular or filamentous structures of 22 nm diameter and over 200 nm in length. They are also exclusively made up of HBsAg

Over production of surface component apparently results in particles and filaments of 22 nm diameter (Fig. 11.3).

Figure 11.3. Different types of particles seen in serum of patient with Type B Hepatitis: A. Spherical 22 nm particle B. Double shelled 42 nm particles and C. Tubular 22 nm particles.

11.4.3.2. Antigens: HBV possess two types of antigens 1. surface protein and 2. capsid protein. The surface antigen is called HBsAg and produced in large quantities. It is highly immunogenic and antibodies against it are protective in nature. The capsid protein is also antigenic and it is called HBcAg.

11.4.3.3. Transmission: HBV is normally transmitted through blood transfusions, contaminated equipment, drug user's unsterile needles etc. The virus can also pass from blood of an infected mother through placenta to infect foetus.

Natural infection occurs only in humans. There is no animal reservoir. The virus is maintained in the large pool of carriers whose blood contains circulating virus for long periods.

11.4.3.4. Pathogenesis: The virion pass through blood to hepatic cells where it multiplies. HBV enters the host cell through envelope fusion. Uncoating occurs in cytoplasm and genome enters the host nucleus. In the nucleus, viral mRNA is synthesized from (-) DNA strand utilizing host RNA

polymerase. Viral mRNA enters cytoplasm and synthesizes viral specific proteins including viral DNA polymerase, viral DNA synthesized through reverse transcription.

Virions are released by budding acquiring envelope. The cells do not lyse. The injury to liver cells and pathogenesis appears to be immune mediated. The hepatocytes carrying viral antigens (HBsAg and HBcAg) are recognized by T-cells (CD^+8) and activate cytotoxic T-cells and natural killer cells which destroy the cells having the virus antigens coated with antibodies.

Thus, prompt host immune reaction cause cellular injury but at the same time eliminate the virus. The extent of liver damage is dependent upon the degree of immune response. Inadequate immune response may lead to carrier state. Therefore infants and immunodeficient persons are more likely to become asymptomatic carriers.

11.4.3.5. Clinical features: The clinical signs of Hepatitis B vary widely. Most infections are asymptomatic and virus is eliminated without much liver damage. About 5-10% of infected persons become long term carriers. In clinical cases, symptoms appear after a long incubation period of 2-6 months. After prolonged prodromal stage with fever, loss of appetite, abdominal pain and vomiting, jaundice develops.

About 90-95% of adults with acute hepatitis B infections recovers within 1-2 months of onset, and eliminates the virus from the body within about 6 months and remains immune thereafter. Mortality rate is about 0.5 to 2%, but may be more in post- transfusion cases.

In some patients, extrahepatic complications like arthralgia (=pain in joints), urticaria (=allergic skin lesions) or glomerulonephritis (=inflammation of kidneys) may occur. These are attributed to deposition of circulating antigen in these parts.

Hepatocellular carcinoma (liver cancer) may occur in chronic patients of serum hepatitis. HBV is strongly implicated in causation of liver cancer, though there is no direct evidence.

11.4.3.6. Epidemiology: The disease occurs throughout the world, and there is no seasonal distribution. The infection is usually sporadic, though occasional outbreaks have occurred in hospitals, orphanages etc. The incidence is more in adults than in children, and in urban than in rural areas.

The prevalence of hepatitis carriers varies widely in different countries, and is directly related to their living standards.

High prevalence areas (10-20%):	East and South East Asia, Pacific islands, Tropical Africa.
Medium prevalence areas (2-10%):	Russia, Indian subcontinent, parts of Africa, parts of Latin America, South-East Europe
Low prevalence areas (< 1%):	USA, Canada, Australia, Europe

Certain groups and occupations carry a high risk of infection. These include medical and paramedical personnel, staff of blood banks, dialysis units, medical laboratories, mental health institutions, barbers and sex workers.

11.4.3.7. Diagnosis: Detection of HBsAg in the blood is the common diagnostic test for HBV infections. It appears in blood even before the clinical symptoms develop. ELISA kits are available for detection of HBsAg in the blood samples.

Occasionally when the level of HBsAg is too low to be detected, serological for Ig M antibodies against HBcAg is carried out for detecting recent infection. Presence of Ig G antibodies against HBcAg indicates recent infection.

11.4.3.8. Treatment: No specific drug is available. Use of α-interferon in combination with antiviral agents like lamcivudine, flamciclovir is beneficial in some cases.

11.4.3.9. Prophylaxis: Passive prophylaxis is with intramuscular injection of Hepatitis B immunoglobulin within 7 days of exposure.

For active prophylaxis, two recombinant vaccines Energix-B abd Rcombivax-HB are available. Recombinant vaccines are prepared by cloning the gene which produce HBsAg in *Saccharomyces cerevisiae*.

11.4.4. HEPATITIS C VIRUS (HCV)

11.4.4.1. VIRUS: HCV is a ssRNA virus designated as Hepacivirus. It was first recognized in 1989 and is responsible for over 80% cases of Non-A Non B post transfusion hepatitis.

The virus is a spherical particle of 50-60 nm in diameter. ssRNA is enclosed in a capsid showing icosahedral symmetry and the nucleocapsid is surrounded by an envelope with glycoprotein spikes.

The virus is poorly antigenic and antibodies against HCV cannot be detected for several months after acute infection. Further, the antibodies do not neutralize the virus because antibody containing blood can transmit the virus. The virus frequently mutates shows considerable antigenic variability. The virus is mainly transmitted through blood transfusion, and also prevalent in intravenous drug abusers and male homosexuals, indicating other routes of transmission like contaminated needles and contact.

11.4.4.2. Clinical manifestations: The incubation period is very long, 15 to 160 days with an average of 50 days. The clinical manifestations are similar in many respects to those caused by HBV but mild. Overt jaundice is seen in about 5% of patients only. The important character of HCV infection is that it becomes chronic, and 50-80% of patients progress to chronic hepatitis with some developing hepatocellular carcinoma,

11.4.4.3. Diagnosis: ELISA kits are available for identification of antibodies in the serum

11.4.4.4. Prophylaxis: No specific active or passive immunizing vaccine available. Screening of blood and blood products is important.

11.4.4.5. Treatment: Prolonged treatment with α-interferon, alone or in combination with antiviral agents like Ribavirin has been reported to be useful.

11.4.5. HEPATITIS D (DELTA) VIRUS (HDV):

In 1977 Italian workers, Mario Rizzetto *et al*. have identified a cytopathic hepatitis virus in patients infected with HBV and termed the virus Hepatitis Delta virus (HDV). HDV is a defective ssRNA virus that requires HBV for its replication. It replicates only in liver cells in which HBV is also replicating. Hence it is a satellite virus.

HDV is a spherical, 36 nm particle with an outer coat composed of HBsAg surrounding circular single stranded RNA genome.

Its mode of transmission is the same as that of HBV. Two types of infections are recognized. 1. Coinfection – HDV and HBV are transmitted together at the same time and 2. Super infection – delta virus infection occurs in a person already harbouring HBV.

Coinfection clinically presents as acute hepatitis B ranging from mild to fulminating disease. Superinfection usually leads to more serious and chronic illness However, HDV is not implicated in hepatocellular carcinoma.

HDV is distributed world wide but is more common in endemic areas like Mediterranean countries. In endemic areas, infection spread by non percutaneous routes, especially close contact.

In non-endemic areas, such as Northern Europe and North America, infection is more often through blood and blood products and is commonly seen in drug addicts and hemophiliacs.

Epidemiology of HDV is similar to that of HBV, because simultaneous occurrence is necessary and mode of transmission is also same for both. In 1990, it was reported that 25-35% of all HBsAg positive intravenous drug users were infected with HDV.

No specific prophylaxis exists, but immunization with HBV vaccine is effective as HDV cannot infect persons immune to HBV.

Screening of blood and blood donors for HBsAg automatically limits blood borne HDV infections.

11.4.6. HEPATITIS E VIRUS (HEV):

11.4.6.1. Virus: It is enterically transmitted non-A non-B virus that often causes epidemics. It was particularly reported from Indian subcontinent, Central and South Asia, North Africa and South America. HEV is characterized as a calcivirus. Genus Hepevirus, Family Calciviridae. Viral genome is ssRNA (-) of mol. Wt. 2.7 x 10^6. Capsid shows icosahedral symmetry and characteristically possess 32 calix like (cup shaped) depressions. There is no envelope. Virion size is 27 – 34 nm.

11.4.6.2. Transmission: Transmission occurs through contaminated waters like HAV. Incubation period is 2-9 weeks. After initial multiplication in the intestine, it reaches liver where it causes short incubation period hepatitis. It does not develop to chronic liver disease.

11.4.6.3. Clinical manifestations: Clinical manifestations are generally mild and disease is self limiting, with low case mortality of about 1%. A unique feature is the clinical severity and high case fatality of 20 -40% in pregnant woman, especially in the last trimester of pregnancy.

11.4.6.4. Epidemiology: HEV infections often occur in epidemic form mainly due to faecal contamination of drinking water supplies. Hence, it is also referred to as epidemic NANB. The

largest epidemic of HEV infections occurred in Delhi during the winter of 1955-56, affecting over 30,000 people within six weeks. HEV infections are common in young to middle aged adults in the age group of 15-40 years. No carrier state develops in humans. It has been reported to be prevalent in animals such as pigs, which may act as reservoirs.

11.4.6.5. Diagnosis: ELISA kits are available for detection of Ig M and Ig G antibodies against HEV in the serum of patients.

11.4.6.6. Treatment: No vaccine is available against HEV. Treatment is symptomatic and no special drugs are available.

11.4.7. HEPATITIS G VIRUS (HGV):

Two flavivirus like isolates were obtained in 1995 from Tamarin monkeys inoculated with blood from a young surgeon (named GB) with acute hepatitis. A similar virus was isolated from another human specimen in the same year. These isolates were called GB viruses A, B and C.

In 1966, an isolate closely resembling GBV-C was isolated from a patient with chronic hepatitis. This was designated as Hepatitis G virus (HGV). The virus has not yet been fully characterized, but it has RNA genome and resembles flaviviruses.

HGV RNA has been found in patients with chronic and fulminating hepatitis, hemophiliacs, patients with multiple transfusions, intravenous drug addicts etc.

HGV appears to be a blood borne virus resembling HCV. Its role in hepatitis is yet to be clarified.

11.5. SUMMARY

Rabies is an acute infection of central nervous system that is almost always fatal. Rabies virus belongs to the virus family Rhabdoviridae and genus Lyssa virus. Virus is a bullet shaped rod with a diameter of 70-80 nm and length 170-180 nm. The virus comprises of outer envelope which enclose nucleocapsid. Viral genome consists of negative sense SS RNA with a capsid showing helical symmetry. The virus replication occurs in cytoplasm of the infected cells. It produces specific cytoplasmic inclusion bodies called Negri bodies which are large spherical bodies and are clearly visible under light microscope, and are of diagnostic value. It is transmitted to humans via the bite of an infected animal which is usually – but not always – a dog. The

incubation period is about 1-3 months. The disease occurs in two forms viz. Furious rabies – the classical variety, and Dumb rabies – the paralytic variety. Exciting stage, with hyper activity and hydrophobia characterize furious rabies. This stage occurs when virus after multiplication in brain move outwards into salivary glands and other parts of the body. In dumb rabies, hyperactivity or excitement stage may not be prominent and paralytic features dominate from the beginning. Diagnosis of rabies is generally made clinically. Persons bitten by rabid animals should be immediately given human antirabies immunoglobulin (or horse antirabies serum) injected partly into the wound and partly intramuscularly.

Chicken pox (varicella) and Herpes zoster (shingles) are two diseases that differ greatly in their clinical manifestations but are caused by the same virus, Varicella-Zoster Virus (VZV). Chicken pox is the primary illness in children and zoster is a reactivation of infection in adults. The virus belongs to DNA virus family Herpes viridae. There are four distinct morphological units in the virion viz. genome, capsid, tegument and envelope. The genome consists of double stranded linear DNA molecule, capsid shows icosahedral symmetry, tegument is a layer of electron dense amorphous material lying between nucleocapsid and envelope. The envelope is the lipoprotein layer that surrounds the tegument. The virus spread by droplet nuclei or by contact. After an incubation period of 2-3 weeks clinical symptoms of chicken pox develop with little prodromal illness. The rash occurs on the entire body but mainly on trunk, and very little or no rash on hands and feet. The rash is very superficial without involvement of deeper layers of skin. The vesicles crust within 48 hours and desquamation leaves no scars. In varicella immunity is usually life long and a second attack is very rare. But the virus may remain latent for many years or life long in cranial or thoracic nerve ganglion and cause reactivation symptoms in the later part of the life. Shingles is characterized by large vesicles. The rash heals in about 2 weeks, but leaves black depressed scars on the body. Diagnosis is generally made by clinical symptoms. In case of painful shingles, idoxuridine is suggested for treatment.

Inflammation of liver is described as Hepatitis and it results in a condition called Jaundice. Yellowing of skin and scelra (=tough white outer layer of eye balls) is the major symptom of jaundice, with other associated symptoms like passing of dark coloured urine and white stools. The term viral hepatitis refers mainly to primary infection of liver by viruses. At least six viruses are recognized so far, and epidemiologic evidence points to the existence of a few more viruses

that cause hepatitis. Hepatitis A Virus is a naked single stranded RNA virus belonging to Picornaviridae family. It spreads by contaminated food and water and causes short incubation hepatitis. Damage to the liver cells occurs due to lysis of the infected cells. Hepatitis B virus is an enveloped DNA virus in which the genome is partially double stranded. It spreads mainly by contaminated blood and blood products and is called serum hepatitis. The incubation period is relatively long, 1-3 months, and is also called long incubation hepatitis. The damage to liver cells by HBV is mainly due to immune reaction of the host. Hepatitis C virus is an enveloped, single stranded RNA virus designated as Hepacivirus. It spreads mainly by blood transfusion. The virus is poorly antigenic and it is strongly implicated in hepatocellular carcinoma. Hepatitis D virus is a defective virus always associated with HBV infections, and is dependent on HBV for its replication. It also spreads mainly by contaminated blood. Hepatitis E virus is a calcivirus designated as Hepevirus, and it spreads by contaminated water and cause epidemic outbreaks. The infections caused by HEV are similar to those of HAV. HGV virus is an enveloped, single stranded RNA virus belonging to Flaviviridae, and it spread mainly by blood transfusions and cause chronic fulminating hepatitis in haemophiliacs, drug addicts and persons with multiple blood transfusions.

11.6. MODEL QUESTIONS

Essay type questions

Discuss the causal virus, transmission, pathogenesis and clinical symptoms of rabies

Discuss the causal virus and clinical symptoms of chicken pox and shingles

Give an account of hepatitis viruses and their spread

Discuss the pathogenesis and clinical symptoms of infectious hepatitis and Serum hepatitis

Short answer type questions

Rhabdovirus

Hydrophobia

Furious rabies

Dumb rabies

Chicken pox

Shingles

Varicella zoster virus

Symptoms of hepatitis

Serum hepatitis

Infectious hepatitis

NANB hepatitis viruses

HbsAg

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LESSON–12: AIDS AND ONCOVIRUSES

Objective: To study about AIDS and viruses implicated in oncogenesis

Contents

12.1. INTRODUCTION

AIDS is the latest pandemic disease that is still increasing in populations, and no effective control measures were developed against this disease. It mainly infects the T – cells with $CD4^+$ receptors and slowly decreases their population over the years resulting in loss of immunity in the affected persons. Cancer is another killer disease that slowly affects the diseased individuals because of uncontrolled growth of some cells resulting in malignant tumors. Various physical and chemical factors are mainly in causation of cancer, and some viruses are also implicated in causation of cancer. The evidence for involvement of viruses in causation of cancer comes mainly from studies on animals, but in case of humans the involvement of viruses in causation of cancer is strongly circumstantial, and only a few viruses are suspected in causing cancer in humans. The cancer causing viruses are called oncoviruses. The details of AIDS and oncoviruses are explained in this lesson.

12.2. AIDS

Acquired Immunodeficiency Syndrome (AIDS) is a profound immunoregulatory disorder that is often fatal. Normal immune system of the patient is severely impaired due to the depletion of Helper T cells of CD_4^+ subset due to infection by a retrovirus named Human immunodeficiency virus (HIV).

12.2.1. Discovery: In the summer of 1981, the Centres for Disease Control (CDC) in Atlanta reported an unusual prevalence of *Pneumocystis carinii* pneumonia (PCP) in a group of young, healthy male homosexuals. Before this, PCP had been associated with disease only in patients whose immune system had been seriously impaired as a result of immune therapy or congenital cellular deficiency.

Almost at the same time the incidence of a rare cancer called 'Kaposi's sarcoma' was reported in young homosexual males in New York and Sanfrancisco. It is also known to be associated earlier with only patients whose immune system is greatly impaired.

The disease, though at first described under various names like 'gay-disease', slim disease etc, came to be known as AIDS, the acronym for acquired immunodeficiency syndrome.

12.2.3. Pathogen: In 1983, Luc Montagnier and Barre Sinoussi of Pasteur Institute, Paris, isolated the virus from a patient suffering from persistent lymphoadenopathy syndrome and named it as Lymphoadenopathy associated virus (LAV).

In 1984, Robert Gallo of National Cancer Institute of USA, isolated a virus from lymphocytes of AIDS patients and named it as HTLV-III (Human Lymphotrophic Virus-III).

In 1986 International committee on Taxonomy of viruses (ICTV) decided to name it as HIV; and it was placed in the family Retroviridae, sub family Lentivirinae.

12.2.4. Characters of the Virus: The virus is an enveloped particle of 100-120 nm diameter in size. Capsid shows icosahedral symmetry (some authors have described it as helical symmetry) and inner core shaped like a cone. The diagrammatic structure of HIV is given in the figure 12.1.

Figure 12.1. Structure of HIV (diagrammatic representation) 1. Envelope glycoprotein spike (gp 120), 2. Transmembrane pedicle glycoprotein (gp41), 3. Outer icosahedral shell of nucleocapsid (p18), 4. Cone shaped core of nucleo capsid (p24), 5. Inner core, 6. Viral proteins associated with RNA (p7 p9), 7. Viral RNA, 8. Reverse transcriptase, 9. Envelope lipid bilayer

The genome comprises two molecules of ss RNA (+sense) of approximately 10,000 nucleotides in total. The nucleocapsid core contains several enzymes, the most important being reverse transcriptase which is an RNA dependent DNA polymerase and transfer genetic in formation from RNA to DNA which is integrated into the chromosomal DNA of host cells. The core also contains two other proteins p^9 which is the major core protein and p^7 which is directly attached to the genome.

The capsid is made of protein p^{24} and inner protein layer of envelope which is viral specific is made up of p^{17}. There are 72 spikes allover the surface of virion. The spikes of the envelope contain two glycoproteins gp^{41} and gp^{120}. gp^{41} is the stalk and gp^{120} is the cap protein.

The genome contains three structural genes gag, pol, env and at least 6 regulatory genes. The genomic structure of HIV can be diagrammatically represented as in the figure 12.2.

Figure 12.2. HIV genome diagrammatic representation

Structural genes:

gag --- code for core proteins P^7 P^9 P^{17} and p^{24}.

pol --- code for reverse transcriptase and other enzymes

env --- code for protein P^{160} which cleaves to form gp^{120} and gp^{41}.

Regulatory genes:

rev --- regulation of structural gene expression

tat --- potent transcription activator

vpr --- weak transcription activator.

vif --- Promotes of infectivity

vpu --- required for virus budding.

nef --- function not known.

12.2.4. Resistance: HIV is thermolabile being inactivated in 10 minuets at 60 ^{0}C and 10 seconds at 100 ^{0}C. But it can withstands lyophilization.

It is inactived in 10 minuets by treatment with 50% etnanol, 35% isopropanol, 0.5% lysol, 0.5% formaldehyde 0.3% hydrogenperoxide, 10% household bleaching powder.

12.2.5. Antigenic nature: HIV induces both humoral and cell mediated response to viral antigens. However viral antigens are weak antigens and antibodies develop very slowly. gp^{120} and gp^{41} are surface antigens. p^{24} (the capsid protein) and reverse transcriptase are internal antigens. Antibodies against gp^{41} and p^{24} appear first but decrease markedly as symptoms appear. Antibodies to gp^{120} remain more constant but gp^{120} is highly variable and contains at least 5 hyper variable regions and hence antigenic shift occurs frequently every 6 months.

12.2.6. Transmission: Infected cells, rather than free virions, are important in transmission. The infected CD_4^+ T lymphocytes are important vectors for HIV transmission from infected person to healthy person. HIV containing CD_4^+ T-lymphocytes occur in sufficient concentration, to make a biological fluid infectious are found in (1) blood (2) sexual secretions of both males and female and in (3) breast milk, especially if a mother is in early or very late stages of infection when virus multiplies most rapidly. Consequently the important modes of transmission are

1) Sexual : Homosexual, heterosexual

2) Parenteral : Intravenous drug users, who share common contaminated needles
blood transfusion
Invasive medical dental procedures (rare)

3) Maternal to child ` : Perinatal, breast feeding.

4) Cutaneous(very rare) : mucous membrane, skin.

All humans are equivocally susceptible to HIV, but those persons who are more frequently exposed to major modes of transmission are considered as high risk groups. The groups of people who constitute the high risk groups are as follows in the descending order

Homosexual / bisexual males
Intravenous drug users (drug addicts)
Heterosexuals
Transfusion patients or haemophiliacs
Children born of infected mothers

The factors which predispose to infection are

1) Life style	:	Large number of sexual partners commercial sex drug abuse within needle sharing
2) Traumatic sexual Practices.	:	Abnormal sexual practices that cause genital abrasions.
3) Preexisting disease	:	ulcerative lesions and exudative genital infections.

Normal social and domestic contact does not transmit the disease. Shaking hands, hugging, putting cheeks together or dry kissing is safe. There is no evidence that mosquitoes, bed bugs or other blood sucking insects transmit the disease. Infection is not transmitted through air, food, water or fomites. Sharing of bathrooms, cooking and eating facilities are not considered dangerous. Though wet kissing is a form of intimate contact, no documented cases of transmission have been traced to it. Epidemiologists at CDC are convinced that if any of these modes of transmission existed, they would have become evident by now.

12.2.7. Replication cycle: Once the virus enters the blood, it selectively infects the T-helper cells which possess CD_4 protein, which is the receptor for the virus (CD stands for clusters of differentiation). The events in replication cycle are shown in figure 12.3.

12.3. Replication of HIV

1.The virion attaches to the CD_4 protein receptor on T-lymphocytes and then the envelope of the virus fuses with the host cell membrane releasing the nucleocapsid into the host cell cytoplasm, where uncoating occurs releasing the genome.

2. The enzyme reverse transcriptase, which is released into host cytoplasm along with rival genome, uses the viral RNA as a template to make a single stranded DNA and then using the DNA strand as a template completes a DNA double helix. As viral DNA is formed, the original viral RNA is degraded. The DNA then enters the nucleus and integrates into the chromosomal DNA of the host, becoming a provirus.

3. On activation, proviral DNA is transcribed into RNA, which enters the cytoplasm and translated into proteins.

4. Viral specific proteins arrange themselves into new capsids that surround viral RNA and attached reverse transcriptase molecules.

5. The nucleocapsid is budded through host cell membrane and acquires the envelope during the process.

12.2.8. Pathogenesis: Infected CD_4^+ T cells are destroyed by cytotoxic T-cells and Natural Killer (NK) cells or eliminated by syncytial formation.

When Virus enters the cell, the envelope fuse with host cell membrane, and thus the host cell membrane expresses gp^{120} on its surface. Cytotoxic T cells and NK cells recognize gp^{120} and destroy the infected cells.

Expression of gp^{120} on the surface of the infected CD_4 cells allows other non infected cells to bind to them via CD_4 receptors producing a group of bound cells know as syncytium. The cells of the syncytium become non functional and promptly eliminated by degeneration and immune mechanisms.

When the virus is replicating in the infected CD_4 cells, it produces viral specific gp^{120} which attach to cell membrane and may become detached and circulate freely in the blood. These circulating gp^{120} bind to CD_4 receptors of new CD_4 cells. Even though these CD_4 cells are not infected, because they have attached gp^{120}, they are attacked by cytotoxic T cells and NK cells and are promptly eliminated.

Healthy individual have about 1000 CD_4^+ T cells per ml of blood. At first only a few cells are infected. For a period of time, body can replace the T-lymphocytes destroyed but eventually the infected individual suffers a gradual decline of helper T-lymphocytes. The rate of decrease is 40 to 80 cells per ml per year. When CD_4^+ cell count falls below 400 per ml, the first opportunistic infections develop. When CD_4^+ cells fall below 200 per ml, it is considered as crisis phase or a case of full blown AIDS.

CD_4 lymphocytes act as an important central communicating lymphocytes. They secrete soluble factors that result in the replication of other T-lymphocytes as well as B-lymphocytes. As these cells decrease, immune system involving both antibody mediated and cell mediated systems gradually fail and the infected individual is exposed to the risk of attack of opportunistic pathogens and certain types of neoplasms.

Apart from T_4 lymphocytes, the virus also infects, to a lesser extent, B-lymphocytes, macrophages, dendritic cells of brain etc. These too may have a few CD_4 receptors. Clinically significant dementia, characterized by impaired ability to concentrate and increased forgetfulness, develop in 60% of patients with HIV infections, and it is thought to be due to the direct effect of

the virus on central nervous system. The infection may spread to brain when the virus infected macrophages cross the blood brain barrier.

12.2.9. Clinical features of HIV infection: Natural evolution of HIV infection can be considered in the following stages.

12.2.9.1. Acute HIV infection: It is the first stage of primary infection. Within a few weeks of infection with HIV, about 10-15% persons experience low grade fever, malaise, headache and other generalized symptoms of viral infection. Spontaneous resolution occurs within a few weeks. Tests for HIV antibodies are negative at the onset of illness but become positive during the course. Hence, this syndrome has been termed sero-conversion illness. During this stage antibodies remove the virus in the blood and CD_4 -lymphocytes minimize further production.

12.2.9.2. Asymptomatic infection: This is the latency phase. All persons infected with HIV, whether they experience initial symptoms of seroconversion illness or not, pass through a phase of symptomless infection lasting for several months or years. In some cases infection may not proceed further, while in others it may lead to full blown AIDS.

12.2.9.3. Persistent Generalized Lymphoadenopathy (PGL): This is the preeruptive stage. This has been defined as the presence of enlarged lymph nodes, at least 1.0 cm in diameter, in two or more non-contiguous, extra-inguinal sites, that persist for at least three months in the absence of any apparent other illness. This by it self benign but a proportion of cases may progress further.

12.2.9.4. AIDS- Related complex (ARC): Typical symptoms of ARC are fatigue, unexplained fever, persistent diarrhoea, marked weight loss (more than 10% of body weight). The common opportunistic infections are oral candidiasis, herpeszoster, TB, typhoid etc., ARC patients are usually severely ill, and many of them progress to AIDS in a few months.

12.2.9.5. AIDS: This is the end stage. World Health Organization (WHO) has defined AIDS as irreversible breakdown of immune system when CD_4 lymphocyte count falls below 200 per ml. The patient is prone to the attack of opportunistic infections and development of malignancies leading to death.

12.2.10. Opportunistic pathogens: The common opportunistic pathogens that attack AIDS patients and the diseases are

Protozoa	---	*Pneumocystis carinii*	---	Pneumonia
		Toxoplasma gondii	---	Encephalitis, diarrhoea
		Cryptosporidium sp	---	severe diarrhoea

Fungi	---	*Candida albicans*	---	Oral candidiasis and other symptoms
		Cryptococcus neoformans	---	Meningitis
		Histoplasma capsulatum	---	Pulmonary infections
Viruses	---	Cytomegaloviruse	---	Disseminative disease
		Epstein-Barr virus	---	Whitish patches on torgue Oral Hairy leukoplakia
		Herpes simplex virus	---	Cutaneous lesions.
		Herpes zoster virus	---	Shingles
Bacteria	---	*Mycobacterium intracellulare*	---	Lymphoadenopathy
		M. tuberculosis	---	TB
		Listeria monocytogenes	---	Meningitis
		Nocardia spp.	---	Lung infections
		Salmonella spp.	---	Fever

12.2.11. Malignancies in AIDS: Malignancies or cancers also commonly develop in AIDS patients, and they include Kaposi's sarcoma, lymphomas and intra epithelial dysplasia.

12.2.11.1. Kaposi's sarcoma: It is seen in about 30% of AIDS patients, mainly in homosexuals. It comprises skin lesions and multiple lymph node involvement. Skin lesions progress slowly. They are small raised reddish-purple nodules (Fig. 12.4). Nearly all organs of the body can be affected. It is rare in infected women and children.

Figure 12.4. Kaposis's sarcoma lesions as they appear on distal leg and ankle

12.2.11.2. Lymphomas: They are seen mostly in patients with haemophilia. It is seen as a late manifestation where $_{CD4}^{+}$ count is 200 or below. Three types of lymphomas viz. Immunoblastic lymphoma commonly in elderly patients, Burkitt's lymphoma in children, and Primary CNS lymphoma, develop in AIDS patients.

12.2.11.3. Intra epithelial dysplasia: It is increasingly seen in women but relationship between HIV and this is not established. Dysplasia means abnormal development cells or alterations in size, shape and organization of adult cells.

12.2.12. AIDS Dementia Complex (ADC): It is characterized by an impaired ability to concentrate, increased forgetfulness and difficulty in performing complicated tasks. Clinically significant ADC occurs in up to 60% of patients with HIV infection. It is thought to be due to the direct effect of virus on central nervous system. HIV dementia usually progresses quickly to severe deterioration and death.

12.2.13. Epidemiology:

AIDS is the latest pandemic disease, which officially started in the summer of 1981 and still going on with increased number of new cases every year. It is a wold wide pandemic and reported from all the continents.

In September 1985, WHO reported about 14,000 cases in USA, 1300 cases in Europe, 725 in Latin America and 100 in Australia. By 1988 WHO estimated that HIV infections occurred in 10 million people in 142 countries and 1,50,000 people developed clinical AIDS.

In 1994 WHO estimated that about 18 million adults and 1.5 million children were infected with HIV and reported cases of AIDS are 1,025,073. Vast majority of them are from America and Africa, while Asia accounted for less than 2% of the total.

A present estimate of HIV infections in the world put figure at 30-40 million in men, women and children put together.

AIDS is an unusual disease because carriers of HIV virus typically show no symptoms of the specific illness that define them as having AIDS for 8 to 10 years after they are infected. Hence there is a great difference between HIV infections and AIDS cases. Hence WHO is regularly making reports on HIV infection and AIDS separately.

12.2.13.1. AIDS in India:

The first case of HIV infection in India was detected in 1986 through a random blood test of a commercial sex worker in Madras. From 1986 to January 1995, about 24,43,141 individiduds have been tested for HIV through the nation wide surveillance, of whom 17,283 tested positive. HIV infection was present in almost all parts of the country but was highest in the states of Maharastra, Tamilnadu and North eastern states of Manipur, Mizoram and Nagaland. In NE states which border Myanmar, the main mode of HIV transmission is through needle sharing by drug users, where as in the rest of the country most infections are transmitted through heterosexual activity. National AIDS control organization, New Delhi, estimated that about 35 lakhs people in India are infected with HIV by 2000 AD.

India's first AIDS case was reported from Bombay in 1986. Since then AIDS cases are reported from almost all the states. Though the exact figure is not known, the mortality due to AIDS is increasing.

In India, commercial sex is considered as the major cause of spread because of two reasons (1) commercial sex workers have higher than normal rate of infection and (2) The large number of sexual partners often increased opportunities for the virus to spread from client to sex worker and vice-versa.

12.2.13.2. AIDS in Asia: In early 80's it was thought that Asia might avoid a major HIV/AIDS epidemic, because no cases were reported from ASIA when it is constantly increasing in Europe, America and Africa. However, by early 90's it became clear that most of the Asian countries are also having HIV problem.

The major HIV affected countries in Asia are Thailand, Japan, India, Myanmar, Philippines, Malaysia, Hong Kong, Vietnam, Singapore etc., Thailand is considered as a 'hot spot' in Asia because of high degree of commercial sex. In Japan, Hong Kong and Malaysia casual sex is also considered as a major cause of HIV spread, apart from commercial sex. Casual sex is relatively high among adolescents and adults who travel frequently.

12.2.13.3. Changing trends: Majority of AIDS case and HIV infections prior to 1988 came from North America, and Europe, and male homosexuals and intravenous drug users are considered as high risk groups. Even now 90% of HIV infections in America and Europe are in male homosexuals and bisexuals and it was attributed to the prevalence of HIV-1 type B, which has

high affinity for blood borne transmission via homo sexual contact or contaminated needles and had a low efficiency of transmission by vaginal route.

Unlike in the West, HIV infection in Asia and Africa are equally distributed among males and females, because of the prevalence of HIV-1 strains which mainly spread by heterosexual activity. There is an increasing incidence in children mainly through maternal transmission in Asia.

12.2.14. Diagnosis: Diagnosis includes 2 types of tests.

1. Non specific immunologic tests and 2. Specific tests for HIV.

12.2.14.1. Non specific tests include:

a. total leucocyte count (below 2000 per ml)
b. T_4 cell count (below 200 per ml)
c. Platelet count showing severe thrombopenia (deficieny of platelets)
d. Raised Ig G and Ig M levels
e. diminished CMI as indicated by skin tests

12.2.14.2. Specific tests for HIV:

Once the host is infected by HIV, a detectable antibody response occurs in most cases within 6 to 8 weeks. Antibodies against p^{24} (capsid protein) and gp^{120} (spike glycoprotein) are detected by ELISA tests. ELISA kits for detection of antibodies against p^{24} and gp^{120} are commercially available. A positive ELISA result should be repeated and if again positive, should be confirmed by more specific western blot test.

If HIV is suspected, but ELISA and western blot tests are negative gene probe test should be performed to detect viral fragments or HIV genetic material RNA or DNA.

Virus isolation is not a routine test for diagnosis and is done only in specialized laboratories.

12.2.15. Treatment:

Azidothymidine (AZT) or zidovudine, marketed under trade name retrovir is licensed for use in treatment of AIDS. It is a structural analogue of deoxythymidine but lacks a proper or

correct attachment point for next nucleotide and hence serves as a DNA chain terminator. AZT do not completely eliminate the virus but increase the survival period of patients with symptomatic HIV infection, because it temporarily inhibits viral replication, thus decreasing the viral load in patients, but unfortunately it is toxic, and when used continuously severe side effects develop.

Other anti-HIV agents that have been approved in recent years include dideoxyinosine (DDI) and dideoxycytidine (DDC) which are similar to AZT but have fewer side effects.

Protease inhibitors are the drugs that interfere with the final processing steps of the protein used in HIV capsid. They inhibit enzyme protease, which is responsible for sectioning of molecules of large protein for final use in capsid formation. By 1996 three protease inhibitors are approved as drugs. These are Saquinavir, Indinavir and Ritonavir. Soluble CD_4 is under trial for use as a drug.

No effective vaccine has been released for use against HIV so far, but trials are going on.

12.2.16. AIDS Awareness:

Since, AIDS is a killer disease with no effective drug or vaccine, individuals must be aware of it and take necessary steps or avoid risk factors to contact the disease.

HIV infection by it self do not produce any symptoms but result in development of a variety of illnesses because of diminished immunity. Hence, people must be aware of suspected symptoms of HIV infection. In HIV infections some major and minor symptoms develop.

Major Signs of AIDS:

a) kaposis's sarcoma
b) Cryptococcal meningitis
c) Fever for more than one month
d) Weight loss more than 10% of body weight.

Minor signs of AIDS:

a) Cough for more than are one month.
b) generalized pruritic dermatitis
c) recurrent herpes zoster or shingles.
d) chronic or aggressive ulcerative herpes simplex
e) oropharyngeal candidiasis or Thrush
f) Persistent generalized lymphoadenopathy

The people who suffer from any two major signs or one major sign and 2 minor signs or 2 or 3 minor signs should under go medical checkup.

People must consciously avoid to major mode of transmission of the virus. The major modes of transmission of HIV virus and methods to avoid them are as follows

1. Needle transmission	---	needle and syringes must not be shared
2. Sexual transmission		
absolutely safe	---	mutually monogamous relationship
Safe	---	Normal heterosexual activity with non risk group of individuals using condoms
Risky	---	anything else.
3. Perinatal transmission:	---	Persons exposed to HIV should have antibody test. Antibody positive women should not become pregnant

Sex is a natural urge and it cannot be avoided. Hence, mutually monogamous sexual relation, which is not only morally, ethically, legally and socially acceptable, but also scientifically correct, and one who cares for himself/herself and is socially responsible must strictly adhere to it.

12.3. ONCOVIRUSES

12.3.1. General account of cancer: The tissues of the human body are formed by the regulated growth of their component cells. When a cell or cells escape normal regulatory constraints and divide in an uncontrolled manner to form a mass of tissue, the health of the individual is impaired. Such masses formed by uncontrolled growth are called neoplasms or tumors. Tumors are mainly two types basing on the pattern of growth. 1. **Benign tumors**: those that do not invade surrounding tissue but grow by displacing adjacent cells, and 2. **Malignant tumors**: those that invade and destroy surrounding tissue, as they grow. The malignant tumors are also called **CANCERS**.

Cancers have three main characteristic features 1. Hyperplasia, 2. Anaplasia, and 3. Metastasis.

Uncontrolled proliferation of cells is called hyperplasia. For most types of cancers, the evidence points to a single cell origin. The cell that escapes normal regulating constraints is called transformed cell, and it undergoes rapid divisions to form a clone which constitute the cancerous tissue. The cells fail to exhibit contact inhibition i.e. they do not adhere to one another as normal cells do. Thus they over grow on one another and form a tumor.

Anaplasia refers to the structural abnormality of cells. The anaplastic cells are markedly pleomorphic in size and shape. Nuclei are extremely large and hyperchormatic. Nucleus:cytoplasm ratio is about 1:1 (in normal cells it is 1:4 or 1:6). Anarchic multiple spindles are formed during cell division. Abberations occur in 1,5,7,8,9,17 and 21 (in leukemias) chromosomes. Neoplastic cells produce quantitatively more organic acids and mucopolysaccharides. The cumulative effect of structural abnormality is loss of normal function.

Metastasis is the ability of a malignant cell to detach itself from a tumor and establish a new tumor at another site in the host. The property of metastasis unequivocally identify a neoplasm as a malignant one than any other character.

More than 100 clinically distinct types of cancers have been recognized. Most of them can be grouped into 4 major categories viz. carcinomas, sarcomas, lymphomas and leukemias.

Malignant neoplasms of epithelial cell origin are called carcinomas. Epithelial cells include ectoderm (skin) and endoderm (cells lining intestinal, respitatory, circulatory and urogenital tracts and ducts of various glands).

Malignant neoplasms arising in mesenchymal tissue or its derivatives are called sarcomas.

Cancerous growth of lymphocytes or lymph nodes is described as lymphoma. Abnormal increase in number of T-lymphocytes due to HTLV-I infection is similar to leukemia and is often described as T - cell leukemia.

Leukemia is commonly called blood cancer. It is characterized by the uncontrolled proliferation of leucocytes most of which do not mature into functional cells.

Of the different types of cancers that occur in humans Carcinomas account for 85% of all cancers Carcinomas of lung, intestine and breasts account for about half of all human cancers.

Sarcomas account for about 2% of all cancers; Leukemias account for about 3%, Lymphomas account for about 5%. The remaining cancers are of mixed origin.

Among males, lung cancer is the most prevalent (about 18%), followed by stomach cancer (12%), and cancer of large intestine (9%).

Among females, breast cancer (19%), uterine cervical cancer (12%) and that of large intestine (9%) are the most prevalent types of cancers

Cancerous growths do not produce toxins or otherwise kill host cells directly but create a condition of malnutrition by utilizing the nutrients that are meant for tissues of the host. Hence, many cancer patients suffer from progressive loss of body fat, become lean and experience profound weakness, anorexia and anemia. This wasting syndrome is referred to as **cachexia.** Severity of cachexia is correlated with size and extent of spread of cancer.

12.3.2. CARCINOGENS: Agents that cause cancer are called carcinogens. Various chemical (both inorganic and organic), physical factors like radiation, and viruses are considered as important carcinogens.

12.3.3. CARCINOGENESIS: Fundamentally, cancers develop because a cell or cells undergo some genetic change that transforms the cells to neoplastic state. The genes responsible for transformation of a normal cell into a cancerous cell are called oncogenes. Normal cells also possess genes similar to oncogenes but in repressed state and are called protooncogenes. The protooncogene may become oncogenic by 1. mutation by chemical or physical mutagens, 2. chromosomal translocations or rearrangement which may derepress oncogenes and 3. integration of viral genome with host cell genome contributing oncogenes or activating protooncogenes

By whatever means oncogenes are activated or expressed, cells derived from the cancerous cell under go anaplastic changes. All the cells in a tumor do not exhibit same type of change. Hence various types of abnormal cells are formed. Competition among the abnormal cell types usually result in selection of one that proliferate successfully.

12.3.4. ONCOGENIC VIRUSES

The viruses that transform the infected cells from normal to malignant cells, either in vivo or in vitro in tissue cultures are termed oncogenic viruses.

In 1908 V. Ellerman and O. Bang demonstrated that a type of leukemia that affects fowls could be transmitted to healthy fowls by injecting them with a cell free filtrate of the blood of leukemia Fowl.

In l911 Francis Peyton Rous demonstrated that a chicken sarcoma could similarly be transmitted, and established that active agent in the filtrate was a virus. That virus is now named Rous Sarcoma virus (RSV), a member of retro virus family.

In 1932 Richard Shoppe showed that rabbit papilloma was also caused by a virus. In 1938 John Bittner demonstrated that a virus of mice termed mammary tumor virus (MTV) is transmitted in milk from a female mouse to her off spring.

It is very difficult to prove the viral causation of cancer in humans, since indirect method of study must be used.

At present five viruses have been strongly implicated in the genesis human cancers, and the viruses are EBV, HBV, HPV and HTLV-1 & HTLV- II.

Details of important Human oncoviruses

Virus	Group	Genome	Capsid	Enve-lope	Size (nm)	Disease
Epstein - Barr Virus (EBV)	Herpes Viridae	DNA	Icosa-hedral	+	100-180	l. Burkitt's lymphoma, 2. Nasopharyngeal carcinoma, 3. Infectious mononucleosis
HBV	Hepadna Viridae	DNA	Icosa-hedral	+	42	Hepatocellular carcinoma
HPV	Papova Viridae	DNA	Icosa-hedral	-	50	Uterine cervical cancer and skin cancer
HTLV-I	Retro-Viridae	RNA	Icosa-hedral	+	100-120	Adult T cell Leukemia
HTLV-II	Retro-Viridae	RNA	Icosa-hedral	+	100-120	Hairy cell Leukemia

12.3.4.1. Epstein Barr Virus (EBV): EBV belongs to Herpes viridae group. It is associated with 3 types of cancers.

In 1958, Dennis Burkitt, a British missionary surgeon working in Uganda reported that a large number of African children between the ages of 4 and 16 suffered from tumors in the connective tissue of Jaw. In 1964 M.A. Epstein and Y.M. Barr demonstrated the virus particles in the tumor cells using electron microscope and the virus is known as Epstein Barr virus. Later it was also implicated in Nasopharyngeal carcinoma and self limiting infectious mononucleosis.

12.3.4.1.1. Burkitt's lymphoma: It is a tumor of B-lymphocytes in jaw (Fig. 12.5), and is endemic in certain parts of Africa and sporadic else where. In endemic areas virtually all patients carry EBV genome. Further, it was observed that chromosomal translocation between 8 and 2 in 5% of patients and between 8 and 22 in the other 5% patients. The chromosomal translocation relocate C-myc gene which is considered important in oncogenesis. In non endemic areas EBV is not always associated with lymphoma but chromosomal translocations do occur in all cases.

Figure 12.5. Burkitt's lymphoma, a form of cancer of the jaw that results from infection with EBV, usually seen only in African children

12.3.4.1.2. Nasopharyngeal carcinoma: EBV also causes nasopharyngeal carcinoma in Hong Kong and surroundings in adults. Both the virus particles and EBV genome have been found within the tumor cells. The disease was also reported from Southern China, where it is endemic.

12.3.4.1.3. Infectious Mononucleosis (IM): It is considered as a self limiting leukemia. In 1968 Werner and Gertrude reported that IM is also caused by a virus similar to EBV. Further, IM occur

only in persons who do not have antibodies against EBV, the disease spread by mouth to mouth contact and hence called kissing disease. The virus is present in oropharyngeal secretions. It also spread by shared drinking bottles and glasses. The virus infects B-lymphocytes and the infected B cells proliferate. The disease is manifested by enlargement of lymph nodes and spleen accompanied by sore throat, tiredness and mild fever. It lasts for one to six weeks and then slowly resolve. The peak incidence of the disease occurs in young adults of age group 15-25 years.

12.3.4.2. HEPATITIS B VIRUS: HBV belongs to Hepadna Viridae. It is associated Hepatoma or hepatocellular carcinoma.

Although epidemiologic evidence linking chronic HBV infection with liver cancer is strong, the role of virus in tumor production is not very clear, HBV genome does not encode for any transforming proteins and there is no consistent pattern of integration in liver cells. The oncogenic effect of HBV appears to be multifactorial.

1. By causing chronic liver cell injury accompanied by regeneration, HBV predisposes hepatic cells to mutation caused possibility by dietary toxins. Mutational inactivation of P53 gene, a tumor supressor gene on chromosome 17, has been observed in liver cancers that occur in areas of the world where HBV and exposure to aflatoxin is endemic.
2. HBV encodes for protein called x-protein which disrupts normal growth control of infected liver cells.
3. In some patients viral integration seems to cause secondary rearrangement of chromosomes and possibly homozygous inactivation of p53 gene

12.3.4.3. HUMAN PAPILLOMA VIRUS(HPV): There are about 50 distinct human papilloma viruses. Some cause benign tumors or warts. HPV types 6 and 11 are implicated in uterine cervix carcinoma, and HPV types 16 and 18 in causing squamous cell carcinoma. The oncogenic potential of HPV can be related to products of two viral genes E6 and E7. The proteins encoded by these genes bind to and neutralize the products of Rb and p53 genes, which are tumor suppressor genes.

12.3.4.4. HUMAN T-CELL LYMPHOTROPIC VIRUS-1 (HTLV-I): It is a typical retrovirus and causes adult T cell leukemia (ATL). The disease is endemic in certain parts of Japan and Caribbean but sporadic else where. Like HIV, HTLV-1 has an affinity for CD_4^+ T-cells and hence

this subset of T cells is the major target for neoplastic transformation. Human infection occur by transmission of infected T-cells via sexual intercourse, blood products or breast feeding. Leukemia develops in only about 1% of infected individuals after a very long latent period of 20-30 years.

The genome of HTLV-1 comprises special gene called Tax gene along with pol, gag and env genes. The tax gene activates several host genes which encode for T cell growth factor. Hence HTLV-1 infection stimulates proliferation of secondary transformations (mutations) which ultimately lead to the out growth of monoclonal neoplastic T-cell population. The transformed T-cell populations are functionally inactive, and the infected person shows immunodeficiency like in AIDS, and fatality result from opportunistic infections

12.3.4.5. HUMAN T-CELL LYMPHOTROPIC VIRUS-II (HTLV-II): This virus was isolated in 1982 from patients suffering from hairy cell leukemia. The virus shares the same trans-activating mechanism of HTLV-I. Hairy cell leukemia gets its name from many membrane derived protrusions that give white blood cells the appearance of being hairy. The malignancy is believed to originate in a stage of B cell development. The disease is chronic and progressive.

The result of this leukemia is severe loss of immune competence, and patient is prone to secondary infections by opportunistic pathogens.

12.3.4.6. Other viruses:

A part from the above 5 types of viruses, a number of other viruses, especially those belonging to DNA virus family Herpesviridae, are also implicated in human cancers.

Among the Herpes viruses, Herpes simplex type 2 is associated with cancer of uterine cervix (neck of uterus) in females. There is a high correlation with HSV-2 infection and incidence of cervical cancer. A higher incidence of antibody to the virus has been demonstrated in women with cervical cancer and the virus has been more frequently isolated from genital tract of cancer patients.

HSV-1 has been associated with cancer of lip.

Cytomegalovirus infection is associated with cancer of prostrate glands and kaposi's sarcoma.

Among the Adenovirus group, many serotypes (12, 18, 21) of human adeno virus produce sarcoma in new borne rodents but no evidence of any human cancer. Among the Pox viridae, molluscum contagiosum virus has been reported to be associated with skin tumors or warts.

12.3.5. CANCER-VIRUS HYPOTHESES:

Three hypotheses were proposed to explain cancer production by viruses. They are 1) provirus hypothesis 2) oncogenic hypothesis and 3)protovirus hypothesis.

12.3.5.1. Provirus hypothesis: H.M. Temin proposed this hypothesis in 1960. According to this hypothesis, after infection of a cell by an RNA tumor virus, the cell makes a DNA copy of the viral RNA and incorporates this genetic information into its own DNA. This gives the cell the capacity to produce ontogenic viruses and transform it from a normal cell to a neoplastic cell.

12.3.5.2. Oncogenic hypothesis: It was proposed by Huebner and Tudor in 1969. According to this concept, every cell is assumed to contain an oncogene, a region of DNA, that is normally repressed (prevented from functioning). When the oncogene is derepressed, possibly by a virus or by any other carcinogen, it expresses itself by bringing about the formation of a transforming protein. This protein could change a normal cell into a malignant one, even though no virus could be recovered from the malignant cell.

12.3.5.3. Protovirus hypothesis: Temin proposed it in 1970. It holds that cancer viruses arise from segments of genetic information randomly brought together by a variety of cellular and genetic events. These segments form the protovirus. It differs from the oncogenic concept, in that, the cells do not possess genetic information as such for cancer production, but have potential for assembling such information.

Probably no single hypothesis holds good to explain all types of cancers, but each may account for different types of cancers.

12.3.6. VIRAL ONCOGENESIS:

Much of the information on viral oncogenesis comes from the studies on animal viruses. Two important steps in viral oncogenesis are

1. integration of viral genome with host cell genome.
2. expression of viral oncogenes.

12.3.6.1. Integration: Integration of the viral genome with host genome is the essential step in transformation of the normal cells into cancerous cells, though all cases of integration do not result in carcinogenesis.

Among the RNA viruses, only retroviruses show integration of viral genome with host genome. For retroviruses, it is an essential step in multiplication process. But, in some retroviruses, integration may lead to carcinogenesis and such viruses are placed in subfamily on oncovirinae of retroviridae eg: HTLV-I, HTLV-II etc.

With DNA viruses, integration is not an essential event for their replication, and integration might occur only when an infection is abortive. Since integration has no survival value for DNA viruses, some loss of genetic information may occur. Hence, in the cases where DNA viruses are implicated in cancer, the evidence for their association is only indirect.

In the study of virus induced transformation system, it has been demonstrated in monkey cell line using SV40 that a transformed cell contains integrated viral DNA.

It has been observed that SV 40 does not have a fixed site of integration. It can integrate at many different sites in cellular DNA. Further, integration may occur not with one specific chromosome but with different chromosomes.

Since the process of integration is random, transformation may occur in different ways.

1. integration can occur within a host gene, there by inactivating it.
2. integration may separate a gene from its promoter there by preventing its expression.
3. integration can put a host gene under the influence of viral promoter and cause over production of host gene product
4. integration may lead to chromosome break up and rearrangement which can alter gene expression.
5. viral integration may lead to chromosomal translocations that may activate oncogenes.

12.3.6.2. Expression of viral oncogenes: In chicken sarcoma, caused by Rous Sarcoma Virus (RSV), the transformation is brought about by the activity of a single gene called Src (for sarcoma producing). The product of Src-gene is a single protein called PP60-V-Src (60 refers to MW which is 60,000, and V for virus). The protein is an enzyme that is able to transfer a phosphate group from ATP to amino acid tyrosine in cellular proteins. An enzyme that phosphorylates a protein is called protein kinase. Most protein kinases act on amino acid serine but Src enzyme is unusual in its preference for Tyrosine and is called tyrosine kinase. This enzyme is located mainly in the

plasma membrane and its activity alters cell growth and behaviour. Later it was found that normal cells also have Src-gene called C-Src (c for normal cell) and protein product of C-Src is also a tyrosine kinase but its enzymatic activity is less than 10% of that of V-Src kinase.

The oncogene of Maloney Murine sarcoma virus, denoted V-mos, also encodes for a kinase which phosphorylates serine residues in cellular proteins.

The oncogene of simian sarcoma virus, denoted V-Sis, encodes a protein (M.W. 28,000) that closely resembles platelet-derived growth factor, a protein released from platelets that stimulates cells to divide during normal process of wound healing. The product of V-Sis cause uncontrolled stimulation of cell division.

More than 20 oncogenic proteins have discovered so far, some of which are kinases, some are nuclear proteins and some are growth factor proteins.

12.3.6.3. Deregulation of cellular oncogenes: Some oncogenic viruses promote the activity of cellular oncogenes. If these viruses integrate themselves next to a cellular oncogene, the viral gene stimulate it leading to cancer. Oncogene might be necessary for normal cell growth and causes cancer only when it functions too rapidly or at the wrong time. For eg. some chicken retroviruses induce lymphomas when they are integrated next to the c-myc cellular oncogene which codes for protein that appears to be involved in the induction of DNA or RNA synthesis.

12.4. SUMMARY

Acquired immunodeficiency syndrome (AIDS) is caused by a retrovirus named Human immunodeficiency virus (HIV). It came to light in 1981 in USA, but is now reported from all parts of the world. The virus is an enveloped particle of 100-120nm diameter in size. Capsid shows icosahedral symmetry and inner core shaped like a cone. The genome comprises two molecules of ssRNA (+sense) of approximately 10,000 nucleotides in total. The nucleocapsid core contains several enzymes, the most important being reverse transcriptase which is an RNA dependent DNA polymerase and transfer genetic in formation from RNA to DNA which is integrated into the chromosomal DNA of host cells. Viral antigens are weak antigens and antibodies develop very slowly. Antibodies to gp^{120} remain more constant but gp^{120} is highly variable and contains at least 5 hyper variable regions and hence antigenic shift occurs frequently every 6 months. The important modes of transmission are sexual contact, contaminated needles, blood transfusion and

breast feeding. All humans are equivocally susceptible to HIV, but those persons who are more frequently exposed to major modes of transmission are considered as high risk groups. Once the virus enters the blood, it selectively infects the T-helper cells which possess CD_4 protein, which is the receptor for the virus. Infected CD_4^+ T cells are destroyed by cytotoxic T-cells and Natural Killer (NK) cells or eliminated by syncytial formation. Healthy individual have about 1000 CD_4^+ T cells per ml of blood. The rate of decrease is of 40 to 80 cells per ml per year. When CD_4^+ cell count falls below 400 per ml, the first opportunistic infections develop. When CD_4^+ cells fall below 200 per ml, it is considered as crisis phase or a case of full blown AIDS. HIV infections pass through 5 stages viz. primary infection, latent stage, persistent lymphoadenopathy stage, AIDS related complex and full blown AIDS. Opportunistic infections appear in the fourth stage. Apart from opportunistic infections malignancies like kaposi's sarcoma and lymphomas may also develop. AIDS dementia develops due to direct infection of brains cells. For treatment of AIDS very few drugs are available. Azidothymidine (AZT) is the first licensed drug against HIV. It can prolong the life but cannot completely cure the disease. No vaccine has yet been developed against the disease. For diagnosis ELISA test and western blot technique are commonly used. Since, AIDS is a killer disease with no effective drug or vaccine, individuals must be aware of it and take necessary steps or avoid risk factors to contact the disease.

The viruses that transform the infected cells from normal to malignant cells, either in vivo or in vitro in tissue cultures are termed oncogenic viruses. It is very difficult to prove the viral causation of cancer in humans, since indirect method of study must be used. At present viruses have been implicated in the genesis of at least six human cancers and the viruses are EBV, HBV, HPV and HTLV-1 & HTLV- II.

Epstein Barr Virus (EBV) is a double stranded DNA virus belonging to Herpes viridae group. It is associated with 3 types of cancers viz. Burkitt's lymphoma, Nasopharyngeal carcinoma and self limiting infectious mononucleosis. HEPATITIS B VIRUS (HBV) is a partially double stranded DNA belonging to Hepadna Viridae. It is associated Hepatoma or hepatocellular carcinoma. Although epidemiologic evidence linking chronic HBV infection with liver cancer is strong, the role of virus in tumor production is not very clear. Human papilloma virus (HPV) comprise about 50 distinct human papilloma viruses, and of these HPV types 6 and 11 are implicated in uterine cervix carcinoma, and HPV types 16 and 18 in causing squammous cell carcinoma. The oncogenic

potential of HPV can be related to products of two viral genes E6 and E7. The proteins encoded by these genes bind to and neutralize the products of Rb and p53 genes, which are tumor suppressor genes. Human T-cell lymphotropic virus-1 (HTLV-I) is a typical retrovirus and causes adult T cell leukemia (ATL). The genome HTLV-1 comprises special gene called Tax gene and it activates several host genes which encode for T cell growth factor which cause proliferation of infection T-cell population. The transformed T-cell populations are functionally inactive, and the infected person shows immunodeficiency like in AIDS, and fatality result from opportunistic infections. HTLV-II causes hairy cell leukemia. The virus shares the same trans-activating mechanism of HTLV-I. The infected cells possess many membrane derived protrusions that give white blood cells the appearance of being hairy. The malignancy is believed to originate in a stage of B cell development and is chronic, progressive disease. A part from the above 5 types of viruses, a number of other viruses, especially those belonging to DNA virus family Herpesviridae, are also implicated in human cancers. These are Herpes simplex type 2 is associated with cancer of uterine cervix (neck of uterus) in females, HSV-1 associated with cancer of lip, Cytomegalovirus infection is associated with cancer of prostrate glands and kaposi's sarcoma. Among the Pox viridae, molluscum contagiosum virus has been reported to be associated with skin tumors or warts.

Three hypotheses were proposed to explain cancer production by viruses. They are 1) provirus hypothesis 2) oncogenic hypothesis and 3) protovirus hypothesis. Much of the information on viral oncogenesis comes from the studies on animal viruses. Two important steps in viral oncogenesis are 1. integration of viral genome with host cell genome and 2. expression of viral oncogenes.

12.5. MODEL QUESTIONS

Essay type questions

Describe the structure of HIV virus, its method of transmission, replication cycle and pathogenesis

Give an account of opportunistic infections and malignancies associated with AIDS

Discuss the etiology and epidemiology of AIDS

Give an account of oncoviruses and viral oncogenesis

Short answer type questions

Structure of HIV
Transmission of HIV
Genomic structure of HIV
Replication cycle of HIV
Oncoviruses
Epstein – Barr virus
Hepatocellular carcinoma
Cancer – virus hypotheses
Integration of viral genome with host genome
Expression of viral oncogenes

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LESSON–13: PROTOZOAN PARASITES OF MAN

Objective: To study the diseases caused by two important protozoan parasites viz. *Entamoeba histolytica* and *Plasmodium* species

Contents

13.1. INTRODUCTION

Of the several thousand (more than 30,000) species described in protozoa, only about 20 species are medically important pathogens. Of the different protozoan diseases, amoebic dysentery caused by *Entamoeba histolytica* and malaria caused by species of *Plasmodium* are important.

13.2. AMOEBIC DYSENTERY

Amoebic dysentery is caused by *Entamoeba histolytica*. It is world wide in distribution. About 500 million people are infected and as many as 1,00,000 die of disease every year. In India, amoebiasis affects about 15% of the population.

13.2.1. PATHOGEN: *E. histolytica* is a typical unicellular protozoan. The cells are spherical to oval, 20 - 40 μm in diameter. They possess a central nucleus and cytoplasm is clearly seen as outer more dense ectoplasm and inner endoplasm (Fig. 13.1). Cell membrane is thin and elastic. They show amoeboid movements with elongated pseudopodia which may be suddenly protruded and retracted. They feed on intestinal bacteria, mucins, RBCs etc. The cells transform into cysts under unfavourable conditions. Cysts are thick walled, spherical resting structures of 10 - 20 μ in diameter. Immature cyst is uninucleate. As it matures nucleus divides twice to become quadrinucleate.

Figure 13.1 *Entamoeba histolytica*. Trophozoite, precyst and mature cyst

E. histolytica is differentiated into 18 zymodemes. Zymodemes are recognized basing on electrophoretic mobility of one or more enzymes. Of the 18 types, only 7 are potentially pathogenic and 11 are non-pathogenic.

13.2.2. LIFE CYCLE AND PATHOGENESIS: Infection occurs by ingestion of mature cysts. Cysts are very resistant and can survive outside the body for long periods and are transmitted through contaminated food, particularly raw vegetables, fruits and water. Ingested quadrinucleate mature cysts survive passage through stomach and reach small intestine. Excystation occur in the lower region of small intestine. The emerging protoplast, called metacyst or primary trophozoite, divides rapidly to produce 8 uninucleate trophozoites. These trophozoites begin feeding on mucous and intestinal bacteria, and move to the large intestine or colon, where they can invade the host tissue causing the disease or live as commensals in the lumen of the intestine or undergo encystations (Fig. 13.2). The behaviour of trophozoites in the colon depend upon the strains and host resistance.

Figure 13.2. Entamoeba histolytica. Excystation and trophozoite formation occurs in small intestine. Trophozoites that enter large intestine may 1. invade the host tissue, or 2. live in the lumen of large intestine without invasion, or 3. undergo encystations and pass out of the host

The pathogenic strains invade the intestinal tissues, multiply rapidly and spread to deeper tissues of intestinal wall, by producing cytotoxins and proteolytic enzymes. Necrotic lesions or amoebic abscesses are formed on the intestinal wall due to invasion of the pathogen. Some times amoeboma, a tumor like mass develop in the wall of large intestine. The irritating effect of the amoeba on the cells lining the intestine cause intestinal cramps and diarrhoea. Due to intestinal lesions or ulcers, the diarrhoeal fluid is often bloody and the condition is referred to as dysentery. The severity of the disease is directly related to the extent of invasion and ulceration of intestinal wall.

The pathogen may also invade and produce lesions in extra-intestinal foci, especially liver to cause hepatic amoebiasis (Fig. 13.3). It occurs through hematogenous spread of the pathogen. The pathogen may also spread to lungs. However, all extra-intestinal amoebic lesions are secondary to ones established in the large intestine.

Figure 13.3. The course of amoebiasis

13.2.3. CLINICAL FEATURES: The incubation period is highly variable and may be short (a few days) or very long (several months or even a year). The symptoms also vary from asymptomatic infection to fulminating dysentery.

Onset of the disease is gradual with mild intermittent diarrhoea and abdominal discomfort, usually progressing to bloody diarrhoea with mucous. In most cases because of the deep ulcers formed the patients experience appendicitis like sharp pain, but relatively little diarrhoea or dysentery because the ulcers are separated and do not drastically effect water absorption. Hence, the older term amoebic dysentery has been replaced by amoebiasis. Other systemic manifestations of the disease are headache, nausea and anorexia. The disease is chronic and slowly subside.

Complications are unusual but may occur such as severe haemorrhage due to extensive damage to the intestinal wall or due to spread of the parasite to extra-intestinal parts through hematogenous spread and cause liver damage.

13.2.4. DIAGNOSIS: Laboratory diagnosis is mainly based on finding trophozoites in fresh warm stools and cysts in ordinary stools. *E. histolytica* trophozoites must be distinguished from nonpathogenic amoebae and cysts from PMNLs with which they are some times confused.

13.2.5. THERAPY: Metronidazole is the drug of choice for treating *Entamoeba* infections. The suggested course of drug is three times daily for 5 days in case of dysentery and a more prolonged course, for 10 - 14 days, in case of liver abscess or other extra-intestinal spread.

Asymptomatic cyst passers should always be treated because they represent the most important reservoir of the parasite in the population. Carriers discharge 1.5 to 10^7 cysts daily. Amoeboquin (diiodohydroxyquinine) is the drug of choice for carriers.

13.2.6. CONTROL AND PREVENTION: The disease is very difficult to eradicate because of substantial human reservoir of asymptomatic cases. Further, epidemic out breaks are associated with sewage seepage into water supplies. Hence, proper sanitation and protected water supplies are essential for prevention of the disease. The cysts of the pathogen are destroyed by boiling water for atleast 10 minutes, but chlorination does not destroy the cysts.

13.3. MALARIA

Malaria is one of the most important and wide spread febrile diseases of humans, and is known since antiquity. Hippocrates (460 - 377 BC) utilized earlier records of malaria and described fever cycles that repeat every 24, 48 and 72 hours. In 17^{th} Century, Italians named the

disease as malaria (=bad air) because of its association with foul smelling vapours from swamps near Rome.

French army surgeon Charles Louis Alphonse Laveran in 1880 first observed gametocytes in the blood of infected patients. In 1885, Camillo Golgi, an Italian histologist, observed the multiplication of asexual blood forms. In late 1890s Patrick Manson postulated that malaria was transmitted by mosquitoes. Ronald Ross, a British army surgeon, while working in Secunderabad, India, observed developing plasmodia in the intestine of mosquitoes. Using birds as experimental models, Ross (1895) established the major features of life cycle of *Plasmodium vivax*, and he was honoured with Nobel prize for his work in 1902.

13.3.1. MALARIAL PARASITE: Four species of ***Plasmodium*** cause malaria.

***P. vivax* :** Most widely distributed in both tropical and temperate regions. Fever occurs every 48 hours. Affect mainly young RBCs. 70% of malarial infections are due to this species.

***P. falciparum*:** cause most severe form of malaria, common in tropics; fever recurs every 48 hours, infect all RBCs (both young and old); 25 to 30% of infections are due to this species.
***P. malariae*:** mainly found in subtropics and temperate zones fever recurs every 72 hours, affect senescent or old RBCs, cause less than 1% of all infections

***P. ovale*:** predominant in West Africa, rare in other areas, cause fever every 48 hours, affect young RBCs only, its incidence is very rare

13.3.2. LIFE CYCLE OF *PLASMODIUM*: *Plasmodium* completes its life cycle in two hosts, the asexual stage in humans and sexual stage in female anopheles mosquitoes (Fig. 13.4).

Figure 13.4. Life cycle of Malarial parasite. Stages in man and mosquitoes

13.3.2.1. Growth stages in Humans: The pathogen enters the human blood stream in the form of small spindle shaped sporozoites, when infected female anopheles mosquitoes bite human host. The growth of *Plasmodium* in humans occurs in the following four stages or cycles.

Pre - erythrocytic cycle: The sporozoites do not infect RBCs directly but invade liver cells, increase in size and develop into a large multinucleate structure called schizont. The schizont breaks up into a large number of small structures called **micromerozoites.** Hepatic cell breaks and release the micromerozoites, which once again infect the fresh liver cells. This hepatic cycle

continues for 10 - 14 days. During this stage clinical symptoms are absent. Eventually the micromerozoites enter the blood stream.

Erythrocytic cycle: This is the phase when RBCs become infected by micromerozoites. In RBCs, they develop into trophozoite, schizont and finally breaks up into merozoites. The merozoites are released into the blood by the rupture of RBCs. They reinfect fresh RBCs. Each cycle in the RBCs takes 48 hours in *P. vivax, P. falciparum* and *P. ovale*, but takes 72 hours in *P. malariae*. The erythrocytic cycle continues for a considerable period.

Gametogony: As the percent of RBCs infected increase, some merozoites which infect RBCs develop into gametocytes instead of forming schizonts and merozoites. Gametocytes are specialized sexual forms, and do not develop further in human host. The gametocytes are recognized into male and female basing on their structure, size and nature of nucleus.

Exoerythrocytic cycle: As natural erythrocytic cycle subsides, some merozoites reenter liver cells and develop into trophozoite, schizont and spores called hypnozoites or resting spores. They usually are not released quickly and may remain latent. It is believed to be responsible for relapses. This stage is absent in *P. falciparum*.

13.3.2.2. Growth stages in mosquitoes:

When female *Anopheles* mosquitoes suck the blood of infected humans, gametocytes enter the intestine of the mosquitoes along with human blood. Stimulated by drop in temperature, the male gametocytes produce tiny whip like bodies that unite with female gametocytes. The resulting zygote burrows into the wall of the midgut of the mosquito and forms a cyst, called oocyst. It enlarges as the zygote undergoes meiosis, dividing asexually into large number of spores. The cyst then ruptures in the body cavity of the mosquito and release structures called sporozoites, which are spindle shaped. These sporozoites find their way to the mosquito's salivary glands and saliva, from which they may be injected into a new human host.

13.3.3. PATHOGENESIS:

The incubation period varies from 10 - 14 days in P. vivax, P. ovale and P. falciparum infection while it is 3 to 6 weeks in P. malariae, which cause mild disease.

During pre-erythrocytic cycle, no symptoms develop and this period represents the incubation period.

The RBC infections and release of merozoites occur in a remarkably synchronous manner. Rupture of all infected cells at a time, not only release large number of merozoites, but also massive amounts of pyrogens into blood, which results in fever. Fever usually lasts for 4 to 6 hours, and with disappearance of pyrogens, fever also subsides.

As the malarial parasites selectively infect RBCs and disrupt them anemia and generalized weakness results. When more than 1% of total RBCs are infected, the clinical condition is considered very severe.

The symptoms of infection last as long as erythrocytic cycle goes on. exoerythrocytic cycle represents convalescing stage. Relapses of malarial fever result when hypnozoites in liver cells are activated and released into blood stream.

13.3.4. CLINICAL FEATURES:

The regular recurrence of fever at every 24 or 48 hours is considered as the classical symptom, and the regular recurrence of fever is due to synchronized release of merozoites and erythrocytic debris into the blood stream. It results in malarial paroxysms - shaking, chill, high fever followed by profuse sweating. Cold stage, hot stage and sweating stage are thus the three major clinical features.

Cold stage: It lasts for 30 minutes to 1 hour. Patient feels intensely cold and uncomfortable. There is marked shrivering. Temperature rapidly rises up to 41 °C (104 °F).

Hot stage: It lasts for 2 - 6 hours. Patient feels intensely hot and uncomfortable. Delirium may be present. Fever is due to schizont rupture and release of pyrogens.

Sweating stage: At the end of hot stage, there is profuse sweating. Bed clothes are drenched. Patient feels fatigued and exhausted but other wise comfortable and often sleeps well.

Anemia is a general symptom of malarial infection and is largely a result of hemolysis.

The nature and severity of clinical symptoms vary with species. Fever caused by *P. vivax* and *P. ovale* recurs every other day (48 hr cycles). These species of *Plasmodium* give rise to a clinically mild infection. The presence of exoerythrocytic stage is responsible for relapses and makes eradication of the organisms difficult. The fever caused by *P. malariae* is mild recurs after every

72 hours. It tends to run a more chronic course. *P. falciparum* causes the most severe form of malaria with high levels of parasitemia. Infected RBCs develop peculiar knob like surface projections that facilitate adhesion of these RBCs to the endothelium of blood vessels. The consequent vascular occlusion causes severe organ damage chiefly in brain, kidneys, liver and gastrointestinal tract. Characteristic cold, hot and sweating stages are not prominent. Depending up on the organ most severely affected, falciparum malaria is described differently.

Cerebral malaria: characterized by marked elevation of body temperature, rapid deterioration of consciousness, convulsions, coma and death. It is mainly due to loss of blood supply to brain. It is also called brain-fever malaria.

Algid malaria: It occurs when intestinal tract is affected and is characterized by severe vomiting and diarrhoea.

Septicaemic malaria: High levels of parasitemia results in high, continuous fever, vomiting and symptoms resembling those of typhoid fever.

Black water malaria: It is characterized by fever with passage of black urine. It occurs due to intravascular haemolysis.

Spleenomegaly: Enlargement of spleen is called spleenomegaly. It responds to anti-malarial therapy. But malarial parasites are not detected in spleen.

13.3.5. EPIDEMIOLOGY: The disease is world wide in distribution and is one of the most serious infectious diseases of humans. It is estimated that 100 million people are infected every year with one million deaths.

The disease incidence is very high in endemic areas especially in India, far east, parts of Africa, parts of South and Central America. Some hyper-endemic areas are also recognized.

People of all ages are susceptible to the disease. Even though both male and female are equally susceptible, the disease is more in males than in females because they are more frequently exposed to the risk than females. Among the females, pregnancy increases the risk of the disease. Immunity to malaria is acquired only after repeated exposures over several years. In endemic areas immunity becomes established slowly. The native people of West Africa are naturally resistant to *P. vivax* infections as they lack specific receptor sites for merozoites on their red blood cells.

There are about 45 species in the Genus Anopheles, and of these *A. culicifacies* is common in rural areas and *A. stephani* is common in urban areas. Other species commonly occurring are *A. minimus, A. sundaccus, A. maculatus* etc. Since the mosquitoes occur every where the disease is also wide spread.

The factors affecting the incidence of the mosquito vectors also influence the disease incidence. Malaria is a seasonal disease because the mosquitoes mainly occur during rainy season from July to November. The stagnant water pools are the breeding places for the mosquitoes. A minimum RH of 60% is essential for the survival of mosquitoes. Optimum temperature for the vector and the parasite is 20 - 30 °C. The parasite ceases to undergo development in mosquitoes if mean temperature is below 16 °C, and temperatures higher than 30 °C are lethal to the parasite. Anopheles mosquitoes are not found at altitudes above 2000 - 2500 meters due to unfavourable conditions, and the disease also is rare at these altitudes.

13.3.6. DIAGNOSIS: It is made mainly by demonstrating the presence of the parasite within the erythrocytes by using giemsa stain or wright stain. When blood smears are negative, serological testing can establish the presence of infection. Serological tests include immunofluorescence, gel diffusion and indirect haemagglutination techniques, but they are not widely used.

13.3.7. THERAPY: Treatment of severe malaria (more than 1% RBCs infected) or any other pernicious forms of falciparum malaria constitutes medical emergency.

---- Administration of chloroquine 600 mg is the main stay of treatment.

---- Chloroquin resistant malaria is treated with quinine sulphate 650 mg.

---- Mefloquine and Halofantrine are promising but expensive drugs.

---- Chloroquin and Mefloquine eradicate erythrocytic stage of the parasite.

---- Primaquine satisfactorily eradicate exoerythrocytic stage of the parasite.

13.3.8. PROPHYLAXIS: Mosquito eradication and personal protection against exposure to mosquitoes by maintaining clean dry hygienic conditions, using mosquito repellents and mosquito-nets are important prophylactic measures.

Recognizing the importance of malaria on world scale, World Health Organization (WHO) in 1955 began a world wide malaria eradication programme by using DDT to eradicate mosquitoes

and chloroquine to treat existing cases. The progrmme was implemented in 52 countries by 1960. But it lost its impetus in early 1970s and collapsed by 1976. The major reason for its failure is development of DDT resistance in mosquitoes and chloroquine resistance in *Plasmodium*.

Chemoprophylaxis is essential for those visiting endemic areas. Generally chloroquine 400 once a week is suggested. Prophylaxis should be continued for 6 weeks after leaving high risk areas.

Trials are on for developing a vaccine for malaria, but so far are not successful.

In 1967 Ruth Nussenzweig and her coworkers at New York State University started trials on vaccine preparation. They isolated large amount of antibodies that react with sporozoites. Then using the antibodies, located the immune stimulating antigens on the sporozoite surface by 1984. Then they located the gene that codes for the antigen, it was cloned, permitting antigen to be mass produced. This is used in vaccine trials.

In 1980s Manuel Elkin Patarroyo *et al*. at Columbia developed a vaccine containing 4 different synthetic protein antigens (one sporozoite antigen and 3 merozoite antigens). In 1993 they announced that in trials using the vaccine 39 to 68% reduction in disease incidence.

In 1995, Ruth Nussenzweig announced a peptide vaccine which is highly effective in mice.

In India also the trials on producing vaccine for malaria are on.

13.4. SUMMARY

Entamoeba histolytica, a typical unicellular protozoan, causes amoebic dysentery. The cells of *E. histolytica* are spherical to oval 20-40 μm in diameter. It shows amoeboid movements with elongated pseudopodia. Thick walled cysts are formed under unfavourable conditions. Immature cysts are uninucleate and mature cysts are quadrinucleate. Infection occurs by ingestion of mature cysts along with contaminated water or food. Cysts germinate in the lower region of small intestine and primary trophozoite divide rapidly produce 8 uninucleate trophozoites. They rapidly multiply and enter large intestine. The pathogenic strains invade intestinal epithelium and cause haemorrhagic lesions and ulcers. The severity of the disease is directly related to the extent of invasion and ulceration. The pathogen may spread to other areas also and cause extraintestinal lesions in liver and lungs. The diagnosis of the disease is based on finding the cysts or trophozoites in fresh warm stools. Metronidazole is the drug of choice for treatment of infections.

Malaria is caused by four species of *Plasmodium*, a sporozoan parasite. The plasmodium species that cause malaria are *P. vivax, P. falciparum, P. malariae* and *P. ovale.* Of these *P. vivax* is the most widespread pathogen causing 70% of all malarial infections, and *P. falciparum* accounts for about 25 to 30% of infections and it causes most severe type of disease. The pathogen completes its life cycle in humans and female Anopheles mosquitoes. In mosquitoes the pathogen completes its sexual reproduction and produce sporozoites on reduction division of mature zygote or oocyst. The sporozoites enter the blood stream of humans when infected female Anopheles mosquitoes bite the humans. From the blood stream the sporozoites enter liver and multiply there for about 2 weeks, and produce merozoites which enter the blood stream and infected RBCs. The replication cycle of the pathogen in RBCs takes 48 or 72 hours and merozoites are released by simultaneous rupture of infected RBCs. Fever occurs coinciding with the rupture of RBCs and last for about 6 hours. Cold stage, hot stage and sweating stage characterize the malarial fever. *P. falciparum* cause cerebral malaria or brain fever malaria which is killer disease. The diagnosis of the disease is made by observing the merozoites in the RBCs. Chloroquin is the drug of choice for treatment of malaria. Mefloquine and halofantrine are promising but expensive drugs. No vaccine has yet been released for malarial infections but trials are in advanced stage.

13.5. MODEL QUESTIONS

Essay type questions

Describe the life history and infections caused by Entamoeba histolytica

Describe the life history and infections caused by Plasmodium species

Discuss the causal organisms, epidemiology and control of malaria

Short answer type questions

Entamoeba histolytica

Amoebiasis

Plasmodium vivax

Plasmodium falciparum

Growth stages of malarial parasite in humans

Growth stages of malarial parasite in mosquitoes

Symptoms of malaria

Control of malaria

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Medical Microbiology	14	Protozoan Parasites

LESSON–14: EMERGING AND RESURGENT INFECTIOUS DISEASES

Objective: To study the factors effecting resurgent and emerging infectious diseases

Contents:

14.1 INTRODUCTION

Infectious diseases are global health problems, and the scope and focus of these problems are constantly changing. Before the antibiotic era the microbial diseases are the major cause of morbidity and mortality in both developed and developing countries. With the advent of antibiotics and improved public health system, the incidence of infectious diseases drastically came down in developed countries, but they still are major health concern in developing countries. A recent estimate of World Health Organization (WHO) revealed that in developed countries, of approximately 11.5 million deaths per year, about 500,000 (less than 4%) are attributed to infectious disease, with nearly all deaths in this category due to pneumonia, but in developing countries, of approximately 38.5 million deaths per year, about 17.5 million (nearly 50%) are attributed to infectious disease.

The worldwide distribution of diseases can change dramatically and rapidly. Alterations in the pathogen, the environment, or the host population can contribute to the rapid spread of new diseases, with potential for high morbidity and mortality among infected individuals. The diseases that suddenly become prevalent are described as emerging diseases. Emerging infections are not limited to "new" diseases but also include resurgence of diseases thought to be controlled, especially as antibiotics become less effective and public health systems fail. Some of the most recent, dramatic examples of emerging and resurgent disease on a global scale are given in the table 14.1. and figure 14.1.

Table 14.1. Recent out break of infectious diseases in various countries

Disease	Year	Country

AIDS	1981	USA
Lyme disease	1982	USA
Cholera	1991	Peru
	1993	India
Lassa fever	1992	South America
Dengue fever	1992	Australia
	1995	Mexico
Hanta virus	1993	USA
Pertussis	1993	USA
Anthrax	1993	Pacific Islands
Diphtheria	1993	Russia
Rift valley fever	1993	Egypt
Yellow fever	1993	East Africa
SARS	2003	China

Figure 14.1. Recent outbreaks of emerging and resurgent infectious diseases on a global scale

The phenomenon of sudden emergence of diseases in epidemic proportions is not new. Some of the diseases that suddenly emerged into prominence in the past were syphilis (caused by *Treponema pallidum*) and plague (caused by *Yersinia pestis*). In the Middle Ages, up to one third of all living humans were killed by the plague epidemics that swept Europe, Asia, and Africa. More recently, influenza became a major public health threat in the early part of the twentieth century. In the 1980s, legionellosis (caused by *Legionella pneumophila*), acquired immunodeficiency syndrome (AIDS), and lyme disease became major epidemic diseases. In 2003, SARS (severe acute respiratory syndrome) disease caused by a corona virus, appeared in China and spread to a large number of countries within a short period.

14.2. EMERGENCE FACTORS

Some factors responsible for the emergence of new pathogens are

1) human demographics and behaviour
2) technology and industry
3) economic development and land use
4) international travel and commerce
5) microbial adaptation and change
6) breakdown of public health measures
7) abnormal natural occurrences that upset the usual host-pathogen balance
8) increased recognition and prompt diagnosis

14.2.1. Human demographics and behaviour: The demographics of human populations have changed dramatically in the last two centuries. In 1800, less than 2% of the world's population lived in urban areas. By contrast, today nearly one-half of the world's population lives in cities. The numbers, sizes, and population densities of modern urban centers make disease transmission much easier. For example, dengue fever is now recognized as a serious hemorrhagic disease in tropical cities, largely because of the spread of dengue virus by the mosquito *Aedes aegypti*. The

disease now spreads as an epidemic in tropical urban areas. Prior to 1950, dengue fever was rare, presumably because the virus was not easily spread among a more dispersed, smaller population.

Human behavior, especially in large population centers, also contributes to disease spread. For example, sexual promiscuity and the use of injectable drugs, centered mainly in large urban areas, have been a major contributing factor to the spread of AIDS and hepatitis.

14.2.2. Technology and industry: Although technological advances and industrial development have had a generally positive impact on living standards worldwide, in some cases these advances have contributed to the spread of diseases. For example, one of the chief technological advances of the twentieth century has been in the health care area. However, the health care environment, especially in hospitals, has resulted in an explosive increase in nosocomial infections. For example, during the 1980s there was a threefold rise in hospital-associated bacteremias in the United States. Antibiotic resistance in microorganisms is another negative outcome of modern health care practices; vancomycin-resistant enterococci and multiple-drug-resistant *Streptococcus pneumoniae* have become important emerging diseases, especially in developed countries.

Transportation, bulk processing, and central distribution methods have become an important factor for quality assurance and economy in the food industry. However, these same factors can increase the potential for common-source epidemics when sanitation measures fail. For example, a single meat processing plant spread *Escherichia coli* O157:H7 to at least 500 individuals in four states in the United States. Finally, the food source, ground beef, was recalled and the epidemic was curtailed, but not before several people died.

14.2.3. Economic development and land use: Economic development and changes in land use also have potential implications for promoting disease spread. For example, Rift Valley fever, a mosquito-borne viral infection, has been on the increase since completion of the Aswan High Dam in Egypt in 1970. The dam created 2 million acres of flooded land, which dramatically increased mosquito breeding grounds at the edge of the new reservoir. The first major epidemic of Rift Valley fever occurred in Egypt in 1977 when an estimated 200,000 people became ill and 598 died. Several epidemic outbreaks have occurred since then including a major outbreak in 1993, and the disease has become endemic near the reservoir.

Lyme disease, the most common vector-borne disease in the United States, is probably on the rise because of changes in land use. Reforestation and the concomitant increase in the numbers of deer (the natural host for the disease-producing *Borrelia burgdorferi*) have resulted in greater numbers of infected ticks, the arthropod vector. In addition, increasing numbers of people are building homes and pursuing recreational activities in and near forests, resulting in increased contact between the infected ticks and humans and, consequently, increased disease.

14.2.4. International travel and commerce:

International travel and commerce can also affect the spread of pathogens. For example, filoviruses (Filoviridae), a group of ribonucleic acid (RNA) viruses, cause fevers culminating in hemorrhagic disease in infected hosts. These diseases, because of their viral origin, are not treatable. They generally have a mortality rate higher than 20%. Most outbreaks of these diseases have been restricted to equatorial central Africa, where the still-unidentified natural hosts and vectors undoubtedly live. Travel of potential hosts to or from endemic areas is usually implicated in disease transmission. For example, one of these viruses was imported into Marburg, Germany, with a shipment of African green monkeys, a species used for laboratory work. The virus quickly spread from the primate vector to some of the human handlers. Twenty-five people were initially infected, and six more developed disease as a result of contact with the human cases. Seven people died in this outbreak of what came to be known as the Marburg virus. Another shipment of laboratory monkeys brought a filovirus to the United States. At least four individuals who worked with the imported monkeys were infected with what is now called the Reston virus (named for Reston, Virginia, the site of the outbreak). The Reston virus was highly contagious and spread through the monkeys, presumably by a respiratory route. However, only four humans were infected and none developed clinical disease. Fortunately, this virus did not cause significant human disease. These two filoviruses are closely related to the Ebola virus. Recent Ebola outbreaks in central Africa, characterized by mortality rates of greater than 50%, have again underscored the existence of highly virulent human pathogens for which there is little or no immunity. These pathogens could potentially be disseminated via air travel throughout the world in a matter of days. A single agent that combines the highly contagious respiratory transmission

route of the Reston virus and the high mortality rate of the Ebola virus could start a major pandemic that could devastate population centers worldwide in a matter of weeks.

14.2.5. Microbial adaptation and change:

Microbial adaptation and change also contribute to pathogen emergence. For example, nearly all RNA viruses, including influenza and human immunodeficiency virus (HIV), undergo genetic mutations. Hepatitis B virus, a deoxyribonucleic acid (DNA) virus known for rapidly mutating, also uses reverse transcriptase to replicate. These viruses lack correction mechanisms for replication steps, and so they incorporate genomic mutations at an extremely high rate compared to most DNA viruses. RNA viruses are considered to be major epidemiological problems because of their constantly changing genomes.

Bacteria also have genetic mechanisms that enhance virulence and promote emergence of new epidemics. One group of virulence enhancing mechanisms are the mobile genetic elements: bacteriophages, plasmids, and transposons. Some representative virulence factors that are carried on these mobile genetic elements and contribute to pathogen emergence are shown in the following table 14.2.

Table-14.2. Virulence factors encoded by bacteriophages, plasmids and transposons

Genetic element	Organism	Virulence factors
Bacteriophage	*Streptococcus pyogenes*	Erythrogenic toxin
	Escherichia coli	Shiga like toxin
	Corynebacterium diphtheriae	Diphtheria toxin
	Clostridium botulinum	Neurotoxin
	Staphylococcus aureus	TSS – toxin, enterotoxin
Plasmid	*Escherichia coli*	Enterotoxins
	Bacillus anthracis	Edema factor
	Yersinia pestis	Coagulase, murine toxins
Transposon	*Vibrio cholerae*	Cholera toxin
	Shigella dysenteriae	Shiga toxin

Antibiotic resistance is also a major factor in bacterial pathogen resurgence. Drug resistance is also a factor for virus emergence. Although several drugs are effective against certain viral diseases, resistance to these drugs is very common, especially among the RNA viruses. For example, most strains of HIV develop resistance to azidothymidine very rapidly unless it is used in combination with other drugs.

14.2.6. Breakdown of public health measures:

A breakdown of public health measures is sometimes responsible for the emergence or resurgence of diseases. For instance, cholera (caused by *Vibrio cholerae*) can be adequately controlled, even in endemic areas, by providing proper sanitation, especially for water sources. However, contaminated municipal water supplies in Peru led to a major cholera pandemic, involving nearly 400,000 people by 1991, with almost 4000 deaths. In another case, the municipal water supply of Milwaukee, Wisconsin, was contaminated with the chlorine-resistant protozoan *Cryptosporidium* in 1993. The contamination resulted in 370,000 cases of intestinal disease, 4000 of which required hospitalization. More effective treatment procedures including enhanced filtration systems were required to rid the water supply of the pathogen.

Inadequate public vaccination programs are an important potential reason for the resurgence of some previously controlled diseases. For example, recent outbreaks of diphtheria (caused by *Corynebacterium diphtheriae*) in the former Soviet Union are the result of inadequate immunization of susceptible children resulting from the breakdown of the formerly centralized public health infrastructure. Pertussis, another vaccine-preventable childhood respiratory disease (caused by *Bordetella pertussis*), has increased recently in the United States because of inadequate immunization and record keeping. The incidence of measles was also on the rise in the United States owing to a lack of effective, timely vaccination programs.

14.2.7. Abnormal natural occurrences that upset the usual host-pathogen balance:

Abnormal natural occurrences such as rapid environmental changes sometimes upset the usual host-pathogen balance. For example, Hantavirus is a well-known human pathogen that occurs in many rodent populations, even in laboratory animals. Over the last decade, several

isolated cases of Hantavirus infection have occurred in laboratory animal handlers. However, a number of lethal cases of Hantavirus infection were reported in 1993 in the American Southwest and were linked to exposure to wild animal droppings. Abundant rainfall and a long growing season, coupled with a mild winter, caused a tremendous increase in the number of mice in 1993. Virtually everyone who acquired the Hantavirus infection had been exposed to rodents or their droppings. Thus, increased human contact with the larger-than-normal mouse population resulted in propagation and transfer of a deadly virus to a large number of human hosts, all because of abnormally mild weather conditions.

14.2.8. Increased recognition and prompt diagnosis:

Some of the infectious diseases when occur only sporadically are usually not recognized, and when they breakout in unusually large number of individuals they are recognized as clinically significant and attempts are made to identify the causal organisms. The most important recent pandemic is that of AIDS, recognized for the first time during 1981. It might have been present earlier also, but when some of the unusual diseases like pneumocystis pneumonia caused by *Pneumocystis carini* and Kaposi's sarcoma occurred in male homosexuals, it was recognized as due to immunosuppression in these people and cause is promptly diagnosed as infection of CD4 subset of T-lymphocytes by a retrovirus.

Legionellosis caused by *Legionella pneumophila* was recognized because of its sudden out break in people attending a convention. From July 21 to July 24, 1976, Bellevue-Statford Hotel in Philadelphia was the site of 58^{th} Annual convention of Pennysylvania chapter of the American Legion. Towards the end of the convention, 140 conventioners and 72 other people in or near the hotel became ill with fever, cough and pneumonia. Eventually 34 individuals died of the disease. The disease is came to be known as Legionnaires disease. The scientists at CDC began investigating and by January 1977 identified the pathogen as a gram negative rod and named it as *Legionella pneumophila*. The bacterium occurs where water collects. Industrial air conditioning units, lakes, stagnant pools and puddles of water have been identified as sources of bacteria. Humans breathe the contaminated droplets into respiratory tract and disease develops a few days later. Thus this disease was recognized because of its sudden out break in a group of important people.

Some of the diseases that are commonly occurring, and often attributed to nonmicrobial cause, are identified as caused by microorganisms due to intensive research. One of the more remarkable discoveries of modern era is that many cases of peptic ulcers are caused by bacteria. Traditionally ulcers are thought to occur due to excess acid production in the stomach due to factors such as nervous stress, smoking, alcohol consumption, diet and physiological dysfunction. However, in 1982, the work of Barry Marshall and Robin Warren of Australia identified that bacteria living in the stomach lining of ulcer patients cause the disease. They identified the organism as *Campylobacter pyloris*, a gram negative curved rod. Its name was later changed to *Helicobacter pylori*. But it received widespread skepticism. By intensive work, they proved the cause of ulcers by bacteria and reported that 48 of 52 peptic ulcer patients could be cured with two antibiotics over a 12 day period. Thus, intensive research provided conclusive proof of ulcer causation by bacteria.

14.3. EMERGING DISEASES AND THE CAUSES:

Some of the important emerging and resurgent infectious diseases, the causal organisms and cause of their resurgence are shown in the following table 14.3.

Table 14.3. Some important emerging and resurgent infectious diseases

Pathogen	Disease	Cause of emergence
BACTERIA:		
Borrelia burgdorferi	Lyme disease	Increase in deer population and human populations in forest areas
Campylobacter jejuni	Diarrhoea	Increased recognition, Consumption of under cooked poultry products
Chlamydia trachomatis	Trachoma, LGV	Increased sexual activity

Escherichia coli O-157 H7	Haemorrhagic colitis	Development of a new strain
Helicobacter pylori	Peptic ulcers	Increased recognition
Legionella pneumophila	Respitatory illness	Recognition in an epidemic situation
Streptoccus pyogenes	Scarlet fever Rheumatic fever Toxic shock	Change in virulence possibly by mutation
Vibrio cholerae	Severe diarrhea	Poor sanitation
Mycobacterium tuberculosis	Tuberculosis	Immunosuppression
VIRUSES:		
Dengue Virus	Haemorrhagic fever	Poor mosquito control Increased urbanization Increased air travel
Hepatitis – B	Jaundice	Intravenous drug abuse Blood transfusions
Hepatitis – C	Jaundice	Increased recognition
HIV – 1 and 2	AIDS	Life styles, Intravenous drug abuse International travel Blood transfusions
Influenza virus	Flu	Antigenic shift Animal-human virus reassortment
Rubeola virus	Measles	Failure of public health system
Rhabdovirus	Rabies	Introduction of infected host reservoir to new areas
Rota virus	Infantile grastroenteritis	Increased recognition
Encephlitis Viruses	Encephalitis	Movement of mosquitoes and horses

Yellow fever Virus	Yellow fever	Lack of effective mosquito control
Varicella Zoster virus	Shingles	Immune suppression
Hanta virus	Intestinal disorders	Human intrusion into virus ecological niche
PROTOZOA:		
Plasmodium spp.	Malaria	Changing parasite biology Drug resistance, air travel
Pneumocystis carini	Pneumonia	Immunosuppression
Toxoplasma gondii	Toxoplasmosis	Immunosuppression
Giardia lamblia	Diarrhoea	Inadequate control in some water supply systems Increased recognition
Cryptosporidium	Cryptosporidiosis	Develoment near water shed areas
FUNGI:		
Candida albicans	Candidiasis	Antibiotic abuse Immunosuppression
Cryptococcus neoformans	Cryptococcal meningitis	Immunosuppression

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14.4. ADDRESSING EMERGING DISEASES:

The key features for addressing emerging diseases are recognition of the disease and intervention to prevent spread of the disease.

The first step in disease recognition is surveillance. Epidemic diseases that exhibit particular clinical syndromes warrant intensive public health surveillance. These syndromes are (1) acute respiratory diseases, (2) encephalitis and aseptic meningitis, (3) hemorrhagic fever, (4) acute diarrhoea, (5) clusterings of high fever cases, (6) unusual clusterings of any disease or deaths, and (7) resistance to common drugs or treatment. Thus, new diseases are recognized because of their epidemic incidence, clusterings, and syndromes. As the prevalence and pathology

of an emerging disease are recognized, it is added to the notifiable disease list. For example, AIDS was recognized as a disease in 1981 and was added to the notifiable disease list in 1984. Lyme disease was first recognized as a separate clinical disease in the 1980s and added to the notifiable disease list in 1991. Likewise, outbreaks of gastrointestinal disease due to enteropathogenic *Escherichia coli* O157:H7 have been increasing in recent years, and the strain was added to the notifiable disease list in 1995.

Intervention to prevent spread of emerging infections must be a public health response involving a variety of methods. General strategies such as strengthening the public health system and supporting research and training are useful, but disease-specific intervention is the key to controlling individual outbreaks. In addition, intervention must include drug and vaccine development to prevent and treat specific diseases. Finally, a number of the emerging diseases are propagated in nonhuman hosts, or vectors. We must identify the alternate hosts and vectors and develop means to intervene in the life cycle of the pathogen to prevent disease propagation.

14.5. SUMMARY

Infectious diseases are global health problems, and the scope and focus of these problems are constantly changing. The diseases that suddenly become prevalent are described as emerging diseases. The emerging infections are not limited to new diseases but also include resurgence of diseases that are thought to have been controlled. The factors responsible for emergence of new pathogens and resurgence of old diseases are many, and some of the important factors are 1. changes in human demographics and behaviour 2. changes brought about by technology and industry 3. economic development and land use 4. international travel and commerce 5. microbial adaptation and change 6. break down of public health measures 7. abnormal natural occurrences that upset the usual host-pathogen balance 8. increased recognition and prompt diagnosis etc. The effect of these factors on emergence and resurgence of infectious diseases are explained. The important examples of emerging diseases and the cause for their emergence are tabulated. For addressing the problem of emerging diseases, epidemiological surveillance and public health measures for control are very important.

14.6. MODEL QUESTOINS

Essay type questions

Discuss the factors responsible for emergence and resurgence of infectious diseases

Give an account of important diseases that emerged or reappeared in recent times, and discuss the factors responsible for it.

"Population explosion and technological advance are responsible for emergence of resurgence of infectious diseases" - Discuss

Short answer type questions

Resurging diseases

Emerging diseases

Human factors responsible for resurgence of diseases

Pathogen factors responsible for resurgence of diseases

Weather factors responsible for resurgence of diseases

Addressing the emerging diseases

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LESSON–15: EPIDEMIOLOGY (PART – 1)

OBJECTIVE: To study the pattern of disease out break in populations, types of epidemics, host and pathogen factors effecting epidemic outbreaks, and disease reservoirs.

CONTENTS:

15.1. INTRODUCTION:

The study of infectious disease in population is known as epidemiology (Gr. Epi = upon, demos=population, logos = study). The science of epidemiology originated and evolved in response to the great epidemic diseases such as cholera, typhoid, small pox and yellow fever.

Heironymous Fracastorius of Italy was the early epidemiologist, and he published a book '**De Contagione**' in 1546 basing on his studies on epidemic diseases of that time, especially syphilis. He presented the fundamental concept that epidemic disease is due to the transmission of an agent from one individual to another by direct contact between the individuals or by the agency of inanimate objects such as clothing and personal possessions (which he called fomites) or air. He suggested that the spread involved passage of small infective particles, which he called "seminaria" from an affected person to others. But he could not demonstrate the existence of these particles and his theory received little attention.

John Snow, a British Physician, systemically studied the cause of cholera epidemics in London during 1849 - 55, and established that contaminated water supply was the main cause of cholera epidemics. He is considered as "**Father of Epidemiology**".

15.2. NATURE OF DISEASE INCIDENCE IN POPULATIONS:

A disease is said to be **epidemic** when it occurs in an unusually large number of individuals in a community at the same time. A world wide epidemic is designated as **pandemic**.

When a disease is constantly present in a population, usually at a low incidence, it is described as **endemic** disease. When a disease occurs occasionally and at irregular intervals it is termed as **sporadic** disease.

The **incidence** of a disease refers to the number of diseased individuals in a population. The **prevalence** of a disease refers to the proportion or percentage of diseased individuals in a population at any one time. The incidence and prevalence are determined by obtaining statistics of illness and death. The severity of disease is expressed in terms of mortality rate and morbidity rate.

Morta1ity rate expresses the incidence of death in a population due to a given disease

$$\text{Mortality rate} = \frac{\text{Number of deaths due to a given disease}}{\text{Size of the population with the disease}}$$

Morbidity refers to the incidence of disease in population and includes both fatal and nonfatal cases

$$\text{Morbidity rate} = \frac{\text{No. of new cases of a disease during a specific period}}{\text{No. of individuals in the population}}$$

Morbidity statistics define the health of the population more precisely than mortality rates because many diseases have low mortality rates.

15.3. EPIDEMIOLOGICAL SURVEILLANCE

An epidemiologist studying an infectious disease is concerned with the causative agent, the sources or reservoir of the pathogen, how it was transmitted, and host and environmental factors that could have contributed to the outbreak of disease with in a defined population. The relevant data is obtained through surveillance methods. Surveillance is a dynamic activity that includes gathering information on the development and occurrence of a disease, collating and analyzing the data, summarizing the findings and using the information to select control methods.

Surveillance methods are commonly used for the following aspects

1. Generation of morbidity data from case reports
2. Collection of mortality data from death certificates
3. Investigation of actual cases
4. Collection of data from reported epidemics
5. Field investigation of epidemics
6. Review of laboratory results; survey of a population for antibodies against the pathogens and specific microbial serotypes; skin tests; cultures; stool analyses etc.
7. Population surveys using valid statistical sampling to determine who has the disease
8. Use of animal and vector disease data
9. Collection of information on the usage of specific aspects such as effective antibiotics, antitoxins, vaccines, other prophylactic measures etc.
10. Use of demographic data on population characteristics such as human movements during a specific time of the year.

Developing statistical models to predict the course of epidemics and testing their validity is also an important activity of epidemiologist. In the field of medicine, statistics are used extensively only in epidemiological studies.

15.4. IMPORTANCE OF EPIDEMIOLOGICAL STUDIES:

Epidemiological studies are very important because analysis of the data collected on the occurrence and distribution of infectious diseases provide important information on the nature of epidemic buildup, its structure and other factors. Further, reliable data on disease situation helps to formulate sound public health policies.

The number of cases of a particular disease may be plotted against time, geographic region, age, sex, race, occupation or other parameters to bring out the correlation with these factors.

The finding of a seasonal incidence may provide important clues as to the mode of transmission of pathogen. For example, an epidemic disease that occurs mainly during cooler months (winter) suggests an airborne mode of transmission as in case of pneumonia, influenza or

chicken pox. This is because during the cooler months people are more likely to transmit microorganisms via aerosols generated through coughing and sneezing. On the other hand, the agent of a disease that occurs mainly in the warmer months would possibly be transmitted by other means such as vectors and contaminated food and water. For example, an arthropod borne disease like Rocky mountain spotted fever is prevalent in summer, as vectors are more common at that time. Salmonella food poisoning also mainly occurs during summer.

Geographical correlations may help to determine the mode of transmission. An epidemic occurring in a particular town or city suggests a common source such as a particular water source or food source, as in typhoid fever and bacillary dysentery.

Correlation of a disease with age groups of affected persons may indicate factors of epidemiological significance. A disease that occurs mainly in the age group of over 65 may be suggestive of a breakdown of immunity. Eg. New active cases of TB occur in elderly people. Diseases that effect mainly children also suggest that lack of active immunity may be a major factor. Eg. whooping cough caused by *Bordetella pertussis* is most common in infants.

A relatively high rate of incidence in drug addicts suggests the transmission through use of contaminated needles Eg. AIDS.

A correlation with occupation or life style can be made with some diseases. For example, a disease that occurs in veterinarians and slaughter house workers suggest that direct contact with the tissues of infected animals may be responsible for infection eg. brucellosis. Physicians, hospital staff, nurses, laboratory workers dealing with pathogenic cultures are also potential patients for the pathogens with which they are dealing. The classic and tragic example is that of Howard Taylor Ricketts, who died due to infection while working on rickettsial disease.

15.5. TYPES OF EPIDEMICS:

Basing on the nature of spread and rapidity of disease outbreaks, epidemics are described as 1. common source epidemics 2. propagated epidemics 3. rapid epidemics and 4. slow epidemics.

15.5.1. Common source epidemic: A common source epidemic arises as a result of infection (or intoxication) of a large number of people from a single contaminated source such as food or water. Usually such contamination occurs because of malfunction in some aspect of distribution system

providing food or water to the population. Food and waterborne diseases are primarily intestinal diseases, the pathogens leaving the body in faecal material contaminate food or water due to improper sanitary procedures and then enter the intestinal tract of recipient during ingestion. The common source epidemic is characterized by a sharp rise to a peak since a large number of individuals succumb with in a relatively brief period of time. These epidemics usually decline rapidly, although the decline is less abrupt than rise. Cases continue to be reported approximately equal to the duration of one incubation period of the disease.

15.5.2. Propagated epidemic: It is also described as "**person to person**" epidemic. In this type of epidemic, the outbreak and progress occur slowly and decline is also gradual. Cases continue to be reported over a period of time equivalent to several incubation periods of the disease. The epidemic may have been initiated by the introduction of a single infected individual into susceptible population, and this individual infects one or a few people in the population, who in turn spread the disease to others. An example is the increase of mumps, measles or chicken pox cases that coincide with new population of sensitive children, who arrive in class rooms each year. Only one infected child is sufficient to propagate the epidemic.

15.5.3. Rapid epidemics: The disease that spread through air or water, and has short incubation period breakout very rapidly and are often described as explosive epidemics. The best example for a rapid epidemic disease is influenza. It has a very short incubation period of 2-4 days and spreads through inoculum that becomes airborne when released from patients. Cholera, which spread through contaminated water, is another example of a rapid epidemic. It is also having a short incubation period of 1-4 days.

15.5.4. Slow epidemics: When the incidence of disease steadily increase over a period of time running into years or decades, the epidemic is described as a slow epidemic. Such epidemics usually result from spread of the disease from person to person by intimate contact and incubation period is unusually long. The best example for such a type of epidemic is AIDS, which spread mainly from person to person by sexual contact and has a long incubation period of 2-10 years before final crisis phase develops. But because of lack of any effective control measures the epidemic is progressing unabatedly.

15.6. FACTORS EFFECTING EPIDEMIC OUTBREAKS

An infectious disease breaks out in an epidemic form when a highly virulent pathogen having an extensive natural reservoir and efficient mode of transmission, attack a highly susceptible host population which is not exposed previously to the pathogen. Hence, four important aspects that determine the pattern and extent of epidemic out-break are 1. host factors 2. pathogen factors 3. disease reservoirs and 4. modes of transmission.

15.7. HOST FACTORS EFFECTING EPIDEMIC OUT BREAKS:

15.7.1. Nature of host community:

Acquired immunity, herd immunity, life styles, living environment etc are important host factors that influence the epidemic out breaks.

15.7.2. Acquired immunity: The colonization of a susceptible, non-immunized host by a pathogen may first lead to an explosive infection and an epidemic. As the host population develops resistance, the spread of the pathogen is checked and eventually a balance is reached in which host and parasite are in equilibrium. A subsequent genetic change in the pathogen could lead to the formation of a more virulent form which would then initiate another explosive epidemic until the host again responds and another balance was reached. An important example is influenza virus A and B. Influenza epidemics caused by strain A recur in 2-3 year cycles and those caused by strain B in 4-6 year cycles. These are traced to genetic changes in the strains, especially in H and N antigens.

15.7.3. Herd immunity: Herd immunity is a concept used to explain resistance of a group to invasion and spread of an infectious agent due to immunity of a high proportion of the members of the group. If the proportion of immune individuals is sufficiently high, then the whole population will be protected. The percentage of resistant individuals necessary to prevent an epidemic is higher for a highly virulent pathogen or one with a long period of infectivity, and lower for a mildly virulent agent or one with a short period of infectivity. From epidemiological studies on the incidence of poliomyelitis in large populations, it appears that if a population is 70% immune, the disease will be essentially absent in the population. For highly infectious disease such as small pox, the proportion of immunes necessary to confer herd immunity has been estimated to be 90 -

95%. A value of 70% has been estimated for Diphtheria. However, presence of chronic carriers is an additional complication and higher proportion may have to be immunized to prevent the spread.

15.7.4. Life styles: Male homosexuality and drug addiction are considered as most important human practices responsible for spread of AIDS in USA and West where 90% of AIDS patients are males, while in Asia and Africa heterosexuality is main mode of spread of HIV and incidence of AIDS is almost equal in males and females. In Asia, Thailand is considered as hot spot of AIDS because of high incidence of commercial sex in that country.

15.7.5. Food habits: Humans are generally very rigid in their food habits and foodborne infections are associated with the habit. People in some European countries drink relatively little water, and hence largely escape waterborne diseases like typhoid. Some Europeans savor dishes prepared from raw meat or fish, and become subject to high attack rates of foodborne diseases. Consumption of raw shell fish growing in contaminated waters is one of the reasons for spread of Hepatitis A virus. Eating of raw marine products spread *Vibrio parahaemolyticus* which cause intestinal disorders.

15.7.6. Living conditions: Slum dwelling, over crowding, unhygienic living conditions associated with poverty are main reasons for spread of a number of diseases in underdeveloped countries. Lack of proper water supplies and sewage disposal results in outbreak of waterborne infectious intestinal diseases in epidemic proportions. Accumulation of garbage which is breeding place for disease carrying animals and insect vectors result in outbreak of diseases like malaria, plague etc.

15.8. PATHOGEN FACTORS EFFECTING DISEASE OUT BREAKS:

Virulence, antigenic variability, inoculum density, incubation period, latency etc are important pathogen factors that influence the epidemic out break of infectious diseases.

15.8.1. Virulence: That the pathogen strains differ widely in their virulence comes from the study of a number of pathogens like *Escherichia coli, Neisseria meningitidis* etc. The isolates of *E. coli* vary from completely avirulent type to highly pathogenic ones. The spread of *N. meningitidis* in a population will produce low disease rates at one time whereas at another time it produces high rates and significant mortality. Such differences are attributed to the virulence of the strains. The coagulase positive strains of *Staphylococcus aureus* are virulent while coagulase negative strains

are avirulent. Haemolytic streptococci are pathogenic while nonhaemolytic strains are non pathogenic.

15.8.2. Antigenic variability: The pathogen that undergo frequent antigenic change cause epidemics at regular intervals. The best example is influenza virus type A. In this virus haemagglutinin and neuraminidase proteins associated with viral envelope are major antigens, and antibodies against them are protective in nature. When minor variations occur in the structure of these antigens due to spontaneous mutations it is called antigenic drift, and drift increases progressively from season to season and within a 2-3 year period sufficiently new antigenic type emerge to initiate a new epidemic. Hence influenza epidemics occur in 3 year cycles.

When major change occurs in antigens due to genetic reassortment, it is called antigenic shift and is responsible for major pandemics. The pandemic of influenza in 1918 is due to H_1N_1 strain, that in 1957 is due to H_2N_2 strain, and that in 1968 is due to H_3N_2 strain.

15.8.3. Intensity of exposure: The probability of developing infection and disease may be low or absent if the environmental concentration of microbial pathogen (i.e. inoculum density) is low. On the otter hand, there is probably no human infection for which immunity is absolute. Heavy exposure to a microbial pathogen may produce serious or even fatal disease in a person who has specific immunity to ordinary doses of pathogens. Even immunized persons should therefore take precautions to minimize exposure to epidemic agents.

The inoculum density required for pathogenesis varies with virulence of the strain. Highly virulent ones can cause infection at low densities while others require fairly high doses of inoculum. For example, species of *Shigella* are highly communicable and minimum infective dose is as few as 10 - 100 cells, and under normal conditions minimum infective dose is 10^3. The infective dose for Salmonellae is 10^5 and that for Vibrios is 10^8.

15.8.4. Incubation period: Different pathogens differ widely in their incubation period i.e. number of days taken for appearance of clinical symptoms after entry of the pathogen. The incubation period for influenza is 2-4 days, for measles it is 10 - 14 days, for mumps 18-21 days, for rabies 1-3 months, for AIDS 2-10 years. In general, the pathogens with short incubation cause explosive epidemics (eg. influenza), while those with long incubation period cause slow epidemics (eg. AIDS).

15.8.5. Latency: Tuberculosis pathogen after primary infection remains dormant in the host and cause post primary tuberculosis in elderly patients. Viruses belonging to Herpesviridae family show long periods of latency to cause reactivation symptoms later in life. Though the reactivation diseases may occur widely in elderly people over 50 years of age, there is no particular pattern of epidemic outbreak of such diseases.

15.9. DISEASE RESERVOIRS

All living organisms, for their continued existence, prefer certain natural sites or locations. In epidemiological terminology, a site or a natural environmental location in which pathogen populations are normally found living and from which infection can occur is described as a "**disease reservoir**". Reservoirs can be animate (eg. Humans, animals, birds, insects etc.) or inanimate (eg. soil, water etc.). Generally it is also called a source of pathogenic microorganisms. However, a fine distinction is maintained between a source and a reservoir in epidemiological studies. A **source** is defined as a location from which the pathogen is immediately transmitted to the host either directly or indirectly whereas **reservoir** is referred to as the natural source of existence of the pathogens. Like reservoirs, the sources can also be animate or inanimate. In certain cases the source and reservoir may be the same. Identifying the source and/or reservoir is an important aspect of epidemiology.

For many pathogenic microorganisms humans are victims as well as reservoirs. A large number of pathogens are maintained in animals including birds, insects etc. and are occasionally transmitted to humans. Some pathogenic microorganisms are essentially free living in soil or water and their pathogenesis is only incidental. Hence, important disease reservoirs are 1. Humans 2. Animals 3. Soil and 4. Water.

15.9.1. HUMAN SOURCES

A large number of pathogens, especially those that affect respiratory tract and urogenital tract, occur only in humans, with no nonhuman host or any other site of natural occurrence. Important diseases that affect only humans are

Bacterial diseases : Diphtheria, whooping cough, relapsing fever, typhoid, tuberculosis, leprosy, streptococcal infections, staphylococcal infections, gonorrhoea, syphilis etc

Viral diseases : AIDS, small pox, mumps, measles, poliomyelitis, Herpes simplex, Hepatitis A & B etc.

Protozoan diseases : Amoebic dysentery, Trichomoniasis, Giardiasis etc.

Continued infection of humans is essential for the maintenance of the pathogens causing the above diseases. Small pox is now considered as completely eliminated because immunity against the pathogen is complete.

Human sources of pathogenic microorganisms are called carriers. A carrier is defined as an infected individual with or without clinical symptoms, who is a potential source of infection to others. Carriers play an important role in the epidemiology of the concerned disease. Four types of carriers are recognized.

Active carrier : an individual who has overt disease, usually called a patient.

Incubatory carrier : an individual who is incubating the pathogen but has not yet developed symptoms.

Convalescent carrier : an individual who has recovered from infection, but continues to harbour large numbers of the pathogen .

Healthy carrier : an individual who harbours the pathogen but donot show any disease symptoms

Incubatory, convalescent and healthy carriers may harbour the pathogen for only a short period, and then they are called casual or transient carriers. If they harbour the pathogen for long periods (months or years) they are called chronic carriers.

The occurrence of healthy carriers indicate that mere acquiring of a pathogen may not automatically ensure the disease. For example, 20 to 40% of population harbours virulent *Streptococcus pneumoniae* in the upper respiratory tract, and disease develop only when resistance is

lowered. The pathogen may be carried in the nose or in the throat. The nasal carriers release the pathogen as droplet nuclei more efficiently than throat carriers and hence are epidemiologically more important.

Carrier state may develop during convalescence. For example, about 3% of persons recovering from typhoid fever become chronic carriers. In these carriers, typhoid bacilli establish a persistent harmless infection of gall bladder or bile ducts from which they pass to the intestine and are excreted in faeces. Some typhoid carriers harbor the pathogen in urinary bladder, but their epidemiological significance is not understood. One important case history of chronic carriage of typhoid is that of Mary Mallon, better known as 'Tyhoid Mary', who was a cook in several homes and restaurants in New York during the early years of 20th Century, and is responsible for atleast 10 outbreaks of typhoid fever involving 51 cases and 3 deaths.

Trichomonas vaginalis, a protozoan flagellate, Infect urinary tract of both males and females, but symptoms develop only in females, and males are symptomless carriers but can transmit the disease to their sexual partner.

Many of the human viruses infect without clinical symptoms. Among such viruses, those belonging to Herpesviridae group are important. The herpes viruses set up latent infections in nerve cells and reactivated at irregular intervals. Chicken pox is a childhood disease and latency of the virus and its reactivation in persons over 50 years of age result in shingles. Infection with poliomyelitis virus is usually asymptomatic in more than 90% of cases.

Carriers can be identified by routine surveys of populations using cultural, immunologic or radiological techniques. Testing positive for a pathogen in serological tests do not indicate active infection but only establish that the person is previously exposed to the infection. Whether the person is active carrier or not should be confirmed by cultural methods and other collateral data.

15.9.2. ANIMAL SOURCES:

A large number of diseases are known to occur both in animals and humans. Diseases which occur primarily in animals but are occasionally transmitted to humans are called zoonoses, and for them the infected animals are reservoirs. Basing on the mode of disease spread from animals to humans, animal reservoirs are three types 1. that transmit the disease directly without

vector involvement 2. that transmit the disease through vectors and 3. which are primary hosts essential for completion of life cycle.

15.9.2.1. Type-I Reservoirs: Some animal reservoirs act as sources i.e. the animals transmit the disease to humans either directly or indirectly without any vector involvement. Eg. Anthrax, Brucellosis, Leptospirosis, Tuberculosis etc.

Brucellosis or undulant fever caused by *Brucella melitensis* is essentially a disease of cattle and spread to humans either by drinking of milk from infected animals or by handling infected meat or milk.

Anthrax caused by *Bacillus anthracis* is primarily a disease of herbivorous animals like cattle, sheep, goats etc. The disease spread to humans either by contact with infected animals or by inhalation of infected material (wool sorter's disease) or by ingesting meat from infected sources.

Leptospirosis is jaundice like disease caused by *Leptospira icterohaemorrhagiae*. Rats are natural reservoirs. Leptospires are discharged in the urine of rats and transmitted to humans by contaminated food or water.

Among the fungi, some ring worm fungi like *Microsporum canis* (dogs and cats), *Trichophyton mentagrophytes* (cat,dots,mouse, horse etc.), *T. verrucosum* (ox, horse) are zoophilic and spread to humans by contact with infected animals.

15.9.2.2. Type-II Reservoirs: Animal reservoirs from which disease is transmitted through insect vectors (eg. plague, encephalitis, yellow fever etc.) or through bite of intermediate animal hosts (eg. rabies).

Birds are natural reservoirs for a number of encephalitis viruses like Eastern Equine Encephalitis Virus, Western Equine Encephalitis Virus, Japanese B encephalitis Virus etc. and mosquitoes belonging to the genera Aedes, Culex etc are vectors. Equine encephalitis Virus is first transmitted to horses, and from horses to humans, and horses are considered not as reservoirs but as amplifier hosts. Like wise, for Japanese B encephalitis Virus pigs are the amplifier hosts.

For yellow fever virus, reservoir is tree dwelling monkeys in the African jungles and forest mosquitoes are the vectors.

Plague is an important epidemic disease for which rats are reservoirs and it is transmitted by rat flea *Xenopsylla cheopis*.

Rabies is an important zoonotic disease for which wild carnivorous animals are reservoirs and is transmitted to humans mainly through bite of infected dogs or bats. Dogs do not develop carrier state and invariably die of infection and hence cannot be considered as reservoirs but essentially they are transmitters .

Marburg and Ebola virus diseases which appeared in epidemic form during 1970's and 1980's are transmitted to humans who handled the monkeys which are not considered as reservoirs but are collateral hosts.

15.9.2.3. Type-III Reservoirs: For some diseases insects are primary hosts and man is secondary host essential for completion of life cycle. Eg. Malaria.

Malarial parasite (*Plasmodium* spp.) completes its life cycle in two hosts 1. Female anopheles mosquitoes - in which sexual reproduction occur and 2. Humans - in which asexual multiplication takes place. Since the host in which sexual stage occurs is considered as primary host, technically humans are reservoirs for malarial parasite.

Some important animal reservoirs and diseases they harbour are given in the table15.1.

15.1. Animal reservoirs some of the important human diseases

Reservoir / source	important diseases
Cattle	Brucellosis
sheep	Anthrax
Cats	Toxoplasmosis
Dogs	Rabies, Ring worm fungi
Rats	Bubonic plague, Leptospirosis
Rabbits	Tularemia
Pigs	Japanese B Encephalitis
Monkeys	Yellow fever, Marburg and Ebola diseases
Horses	Equine Encephalitis, Glanders
Birds	A no. of encephalitis viruses
Female Anapheles mosquitos	Malaria

15.9.3. SOIL AS RESERVOIR OF PATHOGENS:

Some pathogens are primarily saprophytic free living organisms in soil and their pathogenesis is only accidental and not essential for their survival. *Clostridium tetani* which cause tetanus is the best example. *C. tetani* is wide spread in soil, especially as resistant resting spores, and when accidentally it enters the human body during accidents, scratches, pricks etc. it cause disease. Actinomycetes are essentially freeliving in dry soils and *Nocardia* species cause subcutaneous infections (Madura foot) when accidentally enters the foot of humans, especially barefooted rural agricultural workers with cracks or lesions in the foot.

A number of pathogenic fungi, especially those causing subcutaneous mycoses and deep mycoses are free living soilborne fungi, and pathogenesis is only incidental. Eg. *Sporothrix, Histoplasma, Blastomyces, Cryptococcus* etc.

Table 15.2. Important examples of soilborne pathogens

Group	Organism	Mode of spread	Disease
Bacteria	*Clostridium tetani*	contact	Tetanus
	Nocardia spp.	contact	Madura foot
Fungi	*Microsporum gypseum*	contact	Ring worm
	Sporothrix schenckii	contact	Sporotrichosis
	Histoplasma capsulatum	airborne	Histoplasmosis
	Blastomyces dermatidis	airborne	Blastomycosis
	Cryptococcus neoformans	airbone	Cryptococcosis

15.9.4. WATER AS RESERVOIR OF PATHOGENS:

Water bodies are not only vehicles for transmission of certain human diseases but also are reservoirs for some important pathogens belonging to bacteria and protozoa. Some waterborne pathogenic microorganisms in surface waters are essentially free living and can be maintained in

the environment independent of humans. For eg. Vibrios are abundant in surface waters. *Pseudomonas aeruginosa* is a freeliving organism thriving in moist environments in hospitals, laboratories and other dwelling places and spread to susceptible people.

Water based microbial pathogens that can be maintained in the environment independent of humans are given in the table -15.3.

Table – 15.3. Some important examples of human pathogens for which water is the reservoir.

Group	Organism	Reservoir	Disease
Bacteria	*Vibrio cholerae*	free living in surface waters	Cholera
	V.parahaemolyticus	freeling in coastal waters	
	Pseudomonas aeruginosa	freeliving in moist places	Skin in fections
Protozoa	*Naegleria fowleri*	swimming pools	amoebicmeningo-encephalitis

15.10. SUMMARY

The study of infectious disease in population is known as epidemiology. The science of epidemiology originated and evolved in response to the great epidemic diseases such as cholera, typhoid, small pox and yellow fever. John Snow worked extensively on the spread of cholera in England during 1849-55, and he is considered as "**Father of Epidemiology**". The severity of disease is expressed in terms of mortality rate and morbidity rate. Morbidity statistics define the health of the population more precisely than mortality rates because many diseases have low mortality rates. Epidemiological studies are very important because analysis of the data collected on the occurrence and distribution of infectious diseases provide important information on the nature of epidemic buildup, its structure and other factors. Further, reliable data on disease situation helps to formulate sound public health policies. Basing on the nature of spread and rapidity of disease outbreaks, epidemics are described as 1. common source epidemics 2. propagated epidemics 3. rapid epidemics and 4. slow epidemics. An infectious disease breaks out in an epidemic form when a highly virulent pathogen having an extensive natural reservoir and efficient mode of transmission, attack a highly susceptible host population which is not exposed

previously to the pathogen. Hence, four important aspects that determine the pattern and extent of epidemic out-break are 1. host factors 2. pathogen factors 3. disease reservoirs and 4. modes of transmission. All living organisms, for their continued existence, prefer certain natural sites or locations. In epidemiological terminology, a site or a natural environmental location in which pathogen populations are normally found living and from which infection can occur is described as a "**disease reservoir**". A **source** is defined as a location from which the pathogen is immediately transmitted to the host either directly or indirectly whereas **reservoir** is referred to as the natural source of existence of the pathogens. For many pathogenic microorganisms humans are victims as well as reservoirs. A large number of pathogens are maintained in animals including birds, insects etc. and are occasionally transmitted to humans. Some pathogenic microorganisms are essentially free living in soil or water and their pathogenesis is only incidental. Hence, important disease reservoirs are 1. Humans 2. Animals 3. Soil and 4. Water.

15.11. MODEL QUESTIONS

Essay type questions

Give a general account of epidemics and significance of epidemiological studies

Discuss the host and pathogen factors responsible for out break of epidemics

Discuss the importance of disease reservoirs and sources on outbreak of infectious diseases

Short answer types questions

Nature of disease incidence in populations

Epidemiological surveillance

Common source epidemics

Propagated epidemics

Human carriers

Animal sources

Water as reservoir of pathogens

Soil as reservoir of pathogens

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LESSON–16: EPIDEMIOLOGY (PART – 2)

OBJECTIVE: To study the pattern of transmission of the pathogens causing epidemic out breaks, and strategies adopted for control of epidemic out break of diseases

CONTENTS

16.1. INTRODUCTION

In the first part of the lesson (lesson-15) various aspects of epidemics like the nature of disease incidence in populations, epidemiological surveillance, significance of epidemiological studies, types of epidemics, host and pathogen factors effecting disease out breaks and survival of the pathogens out side the host are explained. In this lesson, the methods of transmission of the infectious diseases and strategies employed for preventing the epidemic out break of infectious diseases are explained.

16.2. MODES OF SPREAD

Different pathogens have adopted different modes of spread or transmission, which are usually related to the habitats of the organisms in the body. In general, respiratory tract pathogens spread through air, intestinal tract pathogens spread through contaminated food and water, urogenital tract pathogens and skin pathogens spread by contact, and blood borne pathogens spread by insect vectors or other parenteral modes. Transmission of pathogens from person to person either by contact or by airborne droplet nuclei is described as direct host to host transmission. Spread by other means is described as indirect transmission. Biological agents involved in indirect spread are called vectors while inanimate agents like fomites, food or water are called vehicles. From epidemiological stand point four main routes of transmission are

recognized 1. airborne transmission 2. contact transmission 3. vehicle transmission and 4. vector transmission.

16.3. AIRBORNE TRANSMISSION

Many microbial pathogens have an airborne mode of transmission and cause infections of respiratory tract. Aerial spread of such pathogens occurs in the form of drop let nuclei, contaminated dust or spores in case of fungi.

16.3.1. Transmission of droplet nuclei: A number of bacterial and viral pathogens spread through air as droplet nuclei. The infections caused by such pathogens tend to occur in epidemic form, appearing explosively, attacking a large number of people within a short time. Their incidence usually increases during winter when people are more likely to occupy crowded quarters. The airborne spread of droplet nuclei is effective mainly in the indoor environment, not significant in outdoor because inoculum gets diluted quickly in the outdoor air. The important diseases that spread by droplet nuclei are streptococcal pneumonia, Diphtheria, whooping cough, tuberculosis, influenza, common cold, measles, mumps, and chicken pox.

The secretions containing bacterial cells or virus particles from site of infection in the upper respiratory tract, either nose or throat, are liberated with much force as a cloud of mucous droplets during sneezing, coughing or even loud talking. The mucous droplets in aerosol generated during sneezing travel at high speed of about 100 m/sec. (over 200 mph) and those liberated in cough or loud talk travel at a speed of 16 to 48 m/sec. The number of bacteria in a single sneeze from active patient may range from 10,000 to 100,000. The larger droplets (10μm or more) in the aerosol tend to settle out quickly after traveling a few feet, while smaller droplets (1 to 4 μm) tend to remain in air for much longer time during which the moisture evaporates leaving out the core of the droplet containing organic matter and mucous to which bacterial cells or virus particles are attached. These are called droplet nuclei. These airborne droplet nuclei can remain suspended in the air for a long period in the indoor environment and serve as effective source of inoculum, acquired by inhalation.

16.3.2. Dustborne transmission: Large aerosol droplets settle out rapidly and eventually become part of dust on various surfaces. Mechanical disturbances of such surfaces liberate dust particles and microorganisms into air and inhalation of such contaminated dust particles may also cause infection. A pathogen that can survive for relatively long periods in or on dust creates an

epidemiological problem, particularly in hospitals where dust can be the source of hospital acquired infections. Tubercle bacilli have been isolated from dust of sanitoria. Diphtheria bacilli and haemolytic streptococci have been found in floor dust near patients or carriers harbouring these organisms.

16.3.3. Airborne fungal spores: Among the fungal pathogens of humans, dimorphic fungi causing deep mycoses are soilborne, and the microconidia or arthrospores come into air during mechanical disturbances and cause lung infections on inhalation. Eg. *Histoplasma, Blastomyces, Coccidioides, Paracoccidioides* etc.

Some important examples human pathogens that spread through air are given the table 16.1.

Table – 16.1.Important human pathogens adopted to aerial spread

Group	Pathogen	Disease
Bacteria	*Streptococcus pneumoniae*	Pneumonia
	Corynebacterium diphtheriae	Diphtheria
	Bordetella pertussis	Whooping cough
	Mycobacterium tuberculosis	Tuberculosis
Viruses	Rhinoviruses	Common cold
	Influenza virus	Viral flu
	Morbilli virus	Measles
	Myxovirus parotidis	Mumps
	Varicella Zoster virus	Chicken pox
Fungi	*Histoplasma capsulatum*	Histoplasmosis
	Blastomyces dermatidis	Blastomycosis
	Coccidioides immitis	Coccidioidomycosis
	Cryptococcus neoformans	Cryptococcosis

16.4. CONTACT TRANSMISSION

Spread of the pathogens from infected host to healthy one by contact is the most common type of spread. Contact can be direct or indirect. Direct contact implies actual physical interaction with infectious source, while indirect contact refers to spread of droplet nuclei between closely associated persons. Spread of droplet nuclei technically comes under airborne transmission but

often described as spread by indirect contact because they always spread between persons closely associated, though intimate physical contact is not necessary.

Direct contact transmission from person to person is involved mainly in skin infections and sexually transmitted diseases. Some pathogens spread by contact with tissues of infected animals.

16.4.1. Skin pathogens: Direct contact is involved in the transmission of skin pathogens such as Staphylococci causing pyogenic skin lesions such as boils, furuncles, carbuncles etc., Streptococci causing skin infections, and fungi causing ring worm infections. These pathogens are relatively resistant to environmental influences such as drying, and intimate person to person contact is not the only means of transmission for them.

16.4.2. Sexually transmitted diseases: Some of the diseases of urogenital tract caused by bacteria, virus and protozoa spread mainly through intimate sexual contact. Such diseases are called sexually transmitted diseases or venereal diseases. Important examples are gonorrhoea, syphilis, lymphogranuloma venereum, AIDS, genital herpes, trichomoniasis etc. (Table – 16.2.

Table – 16.2. Important sexually transmitted diseases

Group	Pathogen	Disease
Bacteria	*Neisseria gonorrhoeae*	Gonorrhoea
	Treponema pallidum	Syphilis
	Chlamydia trachomatis	LGV, NGU
Viruses	Human immunodeficiency virus	AIDS
	Human Herpes Virus – 2	Genital herpes
	Epstein – Barr virus	Infectious mononucleosis
Protozoa	*Trichomonas vaginalis*	Trichomoniasis

Unlike respiratory infections where large number of infectious particles may be expelled by an individual, sexually transmitted pathogens are generally not shed in large numbers other than during sexual activity. Consequently transmission will be limited to physical contact, generally during sexual intercourse. In addition, many sexually transmitted pathogens are very sensitive to drying. Their habitat, the human urogenital tract, is generally a moist environment. Thus, these organisms colonize moist niches and have apparently lost the ability to survive outside the host. The effect of drying is most pronounced in *Neisseria gonorrhoeae* and *Treponema*

pallidum. Both these pathogens are killed easily when dried. The possibility of contacting gonorrhoea and syphilis from activities other than sexual intercourse is therefore very rare.

16.4.3. Contact with animal tissues: Some pathogens can be transmitted via contact with tissues of infected animals. Diseases caused by such pathogens often have an occupational incidence, being contracted mainly by Hunters, Veterinarians, Butchers, Slaughter house workers etc. Important examples are brucellosis, tularemia, anthrax etc. Zoophilic dermatophytic fungi spread from pet animals to humans by contact.

16.5. VEHICLE TRANSMISSION

Inanimate materials or objects involved in pathogen transmission are called vehicles. Surgical instruments, fomites, food and water are important examples of vehicles that transmit the pathogens. Surgical instruments and fomites are of only minor importance, but food and water are important causes of major epidemics originating from a single source, since these are actively consumed in large quantities.

16.5.1. Surgical instruments: Contaminated surgical instruments are major cause of sepsis of surgical wounds before introduction of antiseptic surgery by Lord Joseph Lister. At present, contaminated needles used by drug addicts are considered as major cause of spread of AIDS among drug abusers. Serum hepatitis (HBV, HCV) also spread mainly by contaminated needles among the drug addicts.

16.5.2. Fomites: Substances such as clothing, bedding, eating utensils etc. which are used by patients, carry the pathogenic microorganisms and transmit them to healthy susceptibles. Such common articles that transmit the pathogens are called fomites and spread is described as fomite transmission. Important examples are hospital staphylococci, dermatophytic fungi etc.

Microorganisms that cause respiratory tract infections can also be transmitted indirectly by fomites such as drinking glasses, eating utensils, and hand kerchiefs that have been recently used by infected persons.

The pathogens transmitted by fomites are resistant to drying or washing and can retain viability for long periods.

16.5.3. Waterborne transmission: Waterborne pathogens usually cause intestinal infections such as typhoid fever, shigellosis, cholera, amoebiasis etc. Such infections are usually acquired by the

consumption of polluted water containing human faecal matter from patients or healthy carriers. When human faeces pollute a municipal water supply or other common source of drinking water, the outbreaks of intestinal diseases tend to occur in epidemic form.

Pathogenic bacteria such as *Salmonella, Shigella* etc. cannot survive in water for long periods but vibrios can occur in surface waters for relatively long periods. Water is not a natural habitat for *Entamoeba* and it occur mainly as cysts in water. Most of the waterborne pathogens are destroyed by chlorination effectively, but some escape the effect of chlorination, being associated and protected by organic matter in polluted water. Some important examples of waterborne diseases are given in the table- 16.3.

Table – 16.3. Important waterborne diseases

Group	Pathogen	Disease
Bacteria	*Vibrio cholerae*	Cholera
	Salmonella typhi	Typhoid
	Shigella dysenteriae	Bacterial dysentery
Viruses	Polio virus	Poliomyelitis
	Hepatitis A virus	Infectious hepatitis
Protozoa	*Entamoeba histolytica*	Amoebic dysentery

16.5.3.1. Contact with polluted waters: Drinking of water is not always required for transmission of some waterborne infections. For eg. Leptospirosis - a non-intestinal disease characterized by bacteremia and kidney damage, caused by spirochete *Leptospira* - can be acquired merely by coming in contact with water contaminated with urine from infected domestic or wild animals. Such activities as swimming in a farm pond frequented by infected cattle or by working in rat infested sewer, can result in transmission. The leptospires can penetrate the conjunctiva of the eye, abrasions in skin or mucous membranes of nose and mouth. Similarly, *Naegleria fowleri*, a common inhabitant of swimming pools, can cause direct infection.

16.5.4. Foodborne transmission:

Foodborne diseases are mainly intestinal and are characterized by diarrhoea or vomiting. There are mainly two types of foodborne diseases

16.5.4.1. Foodborne intoxication: Eg. Botulism, Staphylococcal food poisoning. The microorganisms produce exotoxin in food and disorders are mainly due to exotoxin, which is usually thermos table.

16.5.4.2. Foodborne infections: Eg. *Salmonella, Shigella* etc. The microorganisms in the contaminated food, grow in the body and cause disease.

Food such as meat, milk and eggs which come from infected animal become contaminated. For example, infection of chickens, turkeys, swine and cattle by certain serotypes of *Salmonella* is common. If such food is stored at a warm temperature the salmonellae may multiply sufficiently to cause infection of persons who consume the food. Major food borne infections are given the table – 16.4.

Table – 16.4. Major food borne infections

Disease	Pathogen	Major foods involved
Salmonellosis	*Salmonella typhimurium* *S. enteritidis*	Meats, Poultry, Fish eggs, diary products
Campylobacteriosis	*Campylobacter jejuni*	Milk, pork, poultry Products
Listeriosis	*Listeria monocytogenes*	Meat products especially pork, and milk.
Diarrhoea and Colitis	*Escherichia coli*	Undercooked groundbeef, raw milk
Shigellosis	*Shigella sonnei* *S.flexneri*	Egg products, puddings
Yersiniosis	*Yersinia enterocolitica*	Milk, meat products
Gastroenteritis	*Vibrio parahaemolyticus*	Sea food, Shellfish

Food may become contaminated by means of humans or animal carriers who have access to the food during its preparation or storage. Cooks, who are chronic carriers, may be the main source and the case of "**Typhoid Mary**" is a classic example.

16.6. VECTOR TRANSMISSION:

Live transmitters of a pathogen are called vectors. Most vectors are arthropods (insects, ticks, mites, fleas) or vertebrates (cats, dogs, bats etc.).

The vertebrate vectors are always diseases animals, which transmit the disease by biting or scratching the humans. Such diseases are called zooneses, and the most important zoonotic disease is Rabies, which is usually transmitted to humans by bite of rabid dogs.

The arthropod vectors may or may not be hosts for the pathogens they transmit from one person to the other. Basing on the vector - pathogen relationship vector transmission can be divided into two categories 1. mechanical transmission and 2. biologic transmission.

16.6.1. Mechanical transmission:

Mechanical transmission implies that the vector merely carries the pathogen either externally or internally, but the pathogens do not increase in number or change its morphology. In external mechanical transmission, the pathogen is carried on the body surface of a vector. Carriage is passive with no growth of the pathogen during transmission. The common house fly, *Musca domestica*, is a classic example of mechanical vector. They carry pathogens from faecal matter to exposed food material. Such a faecal-oral route of transmission is considered important in spread of some bacterial diseases like bacterial dysentery caused by *Shigella dysenteriae*.

Large numbers of arthropods obtain nourishment by biting, and if the pathogen is present in the blood, the arthropod vector will receive some of the pathogenic cells and may transmit them when it bites another individual.

16.6.2. Biologic transmission:

A vector in which the pathogen undergoes a period of incubation, resulting in increase of inoculum, is called a biologic vector. In such cases the vectors are also considered as hosts for the pathogen. If the growth of the pathogen in vector is merely an increase in population, it is called a collateral host, and if the pathogen undergoes some changes in its life history, the vector is called an alternative host. In either case the transmission is called biologic transmission.

A number of bacterial, viral and protozoan pathogens are transmitted by insects that act as biologic vectors. The classic example for biologic transmission is malarial parasite (*Plasmodium* species) for which female *Anopheles* mosquitoes are both primary hosts (in which sexual stage occurs) and vectors.

Plague caused by *Yersinia pestis* is an important example of bacterial pathogen transmitted by vectors. Rat fleas (*Xenopsylla cheopis*) acquire the pathogen by feeding on infected rats, the natural reservoirs. Multiplication of the pathogen occurs in the gut of the flea before it is transmitted to humans or other rats.

Rickettsias are obligate intracellular parasites that are transmitted by lice, ticks, mites or fleas.

Among the viral diseases, yellow fever, dengue fever, encephalitis caused by various equine viruses, and others are transmitted by mosquitoes. Some encephalitis viruses are transmitted by ticks.

16.7. CONTROL OF EPIDEMICS

The control measures are usually directed towards that part of the disease cycle which is most susceptible to control. Hence, finding this weakest link in the chain of events that result in explosive out break of disease is the most important aspect in control of epidemics. The nature of control measures depend upon the type of epidemics. The important measures directed against the out break of epidemics are

1. imposing legal restrictions on entry of diseased individuals in to a disease free area i.e. quarantine
2. maintaining public hygiene and eliminating the sources of pathogens already established i.e. sanitation
3. when elimination is not possible, measures are taken to prevent transmission
4. increasing the disease resistance in the host populations through immunization procedures.

All these measures are essentially the work of public health authorities, and individual participation and cooperation is important in control of epidemic out breaks of infectious diseases.

16.7.1. Quarantine:

Quarantine is a legal restriction on the movement of individuals with active infections to prevent the spread of the disease to other members of the population. The time limit of quarantine or forced isolation is the longest period of communicability of a given disease.

By international agreement six diseases are considered as quarantinable. They are

Disease	**Pathogen**

Small pox	Variola virus
Yellow fever	Flavivirus of arbovirus group
Cholera	*Vibrio cholerae*
Plague	*Yersinia pestis*
Typhoid	*Salmonella typhi*
Relapsing fever	*Borrelia recurrentis*

Smallpox has since been completely eradicated, and quarantine for other diseases is still mandated. Each of these diseases is considered as a highly serious disease and highly communicable.

Quarantine departments are set up at all major international ports (sea, air and land ports) and their clearance is essential for international travel. Quarantine restrictions are absolute in case of persons with active infection of mandated diseases. Not only diseased persons but also healthy persons going to going through the areas considered as hot spots or endemic areas for a particular disease, have to produce the immunization certificates.

Quarantine regulations also include strict regulation of movement of diseased animals. Unwanted and undocumented animal immigrants can be a serious problem. Hence, ships in ports have shields which prevent 'hitch-hiking' by plague carrying rats. Aeroplanes which pass through yellow fever zones are sprayed with insecticides incase they pick up infected mosquitoes.

16.7.2. Control measures directed against source/ reservoir: The type of control depends upon the nature of source/reservoir. If the disease occurs primarily in domestic animals, immunization procedure or slaughter of infected animals may be used to wipe out the disease in animals, thus removing the source. In many European countries, slaughtering of domestic animals to eradicate the disease is followed, and it was quite effective in case of brucellosis (slaughtering of infected cattle), anthrax (sheep) and glanders (horses).

When the reservoir is a wild animal (Tularemia, Plague etc.) the eradication is much more difficult. Rabies is a disease that occurs both in domestic animals and wild animals. The control of rabies can be achieved to some extent by immunization of domestic animals and slaughtering of rabid dogs, though this may not completely eradicate the disease.

Many of the serious diseases are transmitted to man by mosquitoes. Stagnant water pools near dwelling places are the main source of mosquito breeding. Municipal health workers regularly spray DDT or other insecticides to control the mosquito menace.

When humans are the reservoir (*Streptococcus, Staphylococcus, Neisseria* etc.) the eradication is not possible, and therapy that reduce or eliminated infectivity of the individual is practiced. The elimination of infectivity is difficult, especially if there are asymptomatic carriers.

16.7.3. Control measures directed against transmission: These are designed to break the connection between the source of infection and susceptible individuals.

Treatment of drinking water supplies using various methods like flocculation, filtration and chlorination makes the water almost biologically pure. It eliminates the spread of such waterborne pathogens like *Vibrio cholerae, Salmonella typhi, Shigella dysenteriae* etc.

Pasteurization of milk prevents the diseases from cattle like bovine tuberculosis and brucellosis.

Supervision and strict inspection of food preparation and storage, and people who handle food, eliminates the incidence of food poisoning such as staphylococcal food poisoning, botulism, Salmonellosis etc. Hygienic conditions and personal hygiene of food makers and handlers is essential precaution in controlling foodborne diseases.

Destroying of vectors by spraying with insecticides is important control measure in mosquito transmitted diseases like Malaria, yellow fever, Encephalitis etc. Though the complete elimination of any species of insects is almost impossible, the reduction in numbers can be achieved by eliminating or destroying the insect vectors.

Isolation of infected individuals or highly susceptible healthy individuals is also practiced to prevent spread of infections. The purpose of isolating a patient is to contain an infectious disease agent with in a prescribed area, thus preventing the spread of infection. For treating highly contagious diseases, infectious disease hospitals (ID hospitals) are specially set up. Sanitoria are built far away from the cities for treatment of tuberculosis.

Reverse isolation is employed to shield highly susceptible persons from pathogens in the hospital environment. Such persons include premature infants, organo-transplant patients, leukemia patients, individuals with severe burn injuries, patients receiving radiation therapy etc. The person in reverse isolation is placed in a room that has been thoroughly cleaned and

disinfected prior to the patient's admission. Every one entering the room wears a grown to prevent pathogens from being carried into the rooms on cloths. No one with a known infection is allowed to enter the room.

16.7.4. Immunization procedures: Boosting of individual's immune response against a specific disease is called immunization. It is also commonly known as vaccination. Immunization reduces the number of susceptible individuals in a population and raises the general level of herd immunity. By global immunization programme World Health Organization (WHO) could achieve complete eradication of small pox in 1970s, and at present polio eradication using oral vaccine is going on with an air to eradicate the disease by early 2000 AD. 100% immunization is not necessary in order to prevent the disease incidence in a population. Herd immunity varies with the disease but it operates at a certain minimum proportion of immune individuals in a population.

Apart from small pox and polio, some of the important diseases that can be prevented by vaccination are diphtheria, pertussis, tetanus, mumps, measles, rubella etc. and these are commonly referred to as VPDs (i.e. Vaccine Preventable Diseases). For all these diseases effective vaccines are available.

For preventing the out break of diseases (VPDs) in children, universal immunization programmes are implemented in each country, depending on their priority. The schedule of child immunization generally followed in India is as follows

National Immunization schedule (India)

Age of the child	**Vaccine**
At birth[1]	BCG-1, OPV-0
6 weeks	BCG-2 (when BCG-1 is not given) DPT-1, OPV-1
10 weeks	DPT-2 OPV-2
14 weeks	DPT-3 OPV-3

9 months	Measles
16-24 months	DPT OPV
5-6 years (school entry)	DT-3
10 years	TT-4
16 years	TT-4
For pregnant women[2]	TT-1 or Booster
One month after TT-1	TT-2)

1 immunization at birth is only for institutional births
2 for prevention of tetanus in the neonate primarily but also for mother

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16.8. Summary:

Different pathogens have different modes of spread or transmission. From epidemiological stand point four main routes of transmission are recognized 1. airborne transmission 2. contact transmission 3. vehicle transmission and 4. vector transmission.

The secretions containing bacterial cells or virus particles from site of infection in the upper respiratory tract, either nose or throat, are liberated with much force as a cloud of mucous droplets during sneezing, coughing etc. and on evaporation of mucous the core of the droplet containing bacterial cells or virus particles remain suspended in the air and these are called droplet nuclei. These serve as effective source of inoculum in the indoor environments. The important diseases that spread by droplet nuclei are streptococcal pneumonia, Diphtheria, whooping cough, tuberculosis, influenza, common cold, measles, mumps, and chicken pox. Large aerosol droplets settle out rapidly and eventually become part of dust on various surfaces. Mechanical disturbances of such surfaces liberate dust particles and microorganisms into air and inhalation of such contaminated dust particles may also cause infection. Among the fungal pathogens of humans, dimorphic fungi causing deep mycoses are soilborne, and the microconidia or arthrospores come into air during mechanical disturbances and cause lung infections on inhalation.

Spread of the pathogens from infected host to healthy one by contact is the most common type of spread. Contact can be direct or indirect. Direct contact transmission from person to person is involved mainly in skin infections and sexually transmitted diseases. Skin pathogens such as staphylococci and streptococci causing pyogenic skin lesions and fungi causing ring worm infections mainly spread by direct contact. Some of the diseases of urogenital tract caused by bacteria, virus and protozoa such as gonorrhoea, syphilis, lymphogranuloma venereum, AIDS, genital herpes, trichomoniasis etc. spread mainly through intimate sexual contact.

Inanimate materials or objects involved in pathogen transmission are called vehicles. Surgical instruments, fomiites, food and water are important examples of vehicles that transmit the pathogens. Live transmitters of a pathogen are called vectors. Most vectors are arthropods (insects, ticks, mites, fleas) or vertebrates (cats, dogs, bats etc.). Vector transmission can be divided into two categories 1. mechanical transmission and 2. biologic transmission. Mechanical transmission implies that the vector merely carries the pathogen either externally or internally, but the pathogens do not increase in number or change its morphology. A vector in which the pathogen undergoes a period of incubation, resulting in increase of inoculum, is called a biologic vector.

The important measures directed against the out break of epidemics are 1. imposing legal restrictions on entry of diseased individuals in to a disease free area i.e. quarantine 2. by maintaining public hygiene and eliminating the sources of pathogens already established i.e. sanitation 3. when elimination is not possible, measures are taken to prevent transmission 4. by increasing the disease resistance in the host populations through immunization procedures.

16.9. MODEL QUESTIONS

Essay type questions

Discuss the methods of transmission of infectious diseases

Discuss the strategies employed for control of epidemic out break of infectious diseases

Short answer type questions

Airborne transmission

Vehicle transmission

Water transmission

Vector transmission

Quarantine

Immunization

Immunization schedules

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LESSON–17: CHEMOTHERAPY (PART – I)

Objective: To study the development of chemotherapy, properties of antimicrobial drugs, and synthetic antibacterial drugs

Contents:

17.1. INTRODUCTION

The treatment of a disease with a chemical substance is known as chemotherapy and the chemical substance is called chemo-therapeutic agent or drug. The essential requirement of such a drug is that it should have selective toxicity i.e. under the conditions of use, it must be active against the pathogen than it is to the host. Disinfectants and antiseptics will kill bacteria but they are highly toxic to host tissues also, and hence unsuitable as chemotherapeutic agents.

17.2. DEVELOPMENT OF CHEMOTHERAPY

Treatment of human diseases using powders or extracts prepared from plant parts is in practice from times immemorial. Ayurveda, based on therapeutic extracts from plants, is a highly developed form of medicine in ancient India. Europeans used natural quinine from the bark of cinchona tree to treat malaria as early as 1630. It was used even earlier by South Americans who relieved symptoms of malarial fever by chewing the bark of cinchona tree. However, chemotherapy involving the use of chemo-therapeutic drugs of known composition, developed only in 20th century, and Paul Ehrlich, a German chemist, who first carried out a systematic search for chemicals showing selective toxicity against pathogens and synthesized first chemical drug Salvarsan, is considered as founder of chemotherapy as a branch of medicine.

At the beginning of 20th century, Paul Ehrlich started a systematic screening of a number of chemicals, especially dyes, for their selective toxicity against microbial pathogens, and by 1904

he found that dye "trypan red" was active against trypanosomes that cause African sleeping sickness. Subsequently Ehrlich along with a young Japanese scientist Sahachiro Hata, tested a number of arsenical compounds on syphilis infected rabbits and found that compound number 606, arsphenamine, was active against syphilis spirochete, Treponema pallidum. It was commercially made available in 1910 under the trade name Salvarsan, and it was hailed as "Magic bullet' of Ehrlich. Ehrlich's contributions were especially important because his was the first systematic and deliberate search for a chemotherapeutic drug having selective toxicity. For his valuable contributions he received Nobel Prize in 1908, along with Eli Metchnikoff. Though Salvarsan proved to be effective against syphilis, its low solubility necessitated intravenous injection of large doses of the drug and there are side effects. Hence it lost its appeal..

In 1927, the German chemical industry giant, I.G. Farben Industrie, began a long term search for chemotherapeutic agents under the direction of Gerhard Domgk. In 1935, Domagk reported successful treatment of streptococcal infections with a red dye Prontosil (sulphonamidochrysoidin). French scientists Jacques and Trefouel, at Pasteur Institute, showed that prontosil has no antibacterial action in vitro but its antibacterial activity in infected animals is due to a colourless breakdown product, sulphanilamide. Later it was reported by Woods that inhibition of bacterial growth by sulphanilamide can be reversed by a structural analogue Para aminobenzoic acid (PABA). PA8A is a precursor of a coenzyme folic acid and sulphonilamide blocks its conversion to the end product by competitive metabolic inhibition. In succeeding years, thousands of structural analogues of aminoacids, purines, pyrimidines and vitamins were synthesized and tested for their antimicrobial activity and some of them were successfully used as drugs. For his contribution to chemotherapy Domagk was awarded Nobel Prize in 1939.

In 1929, a Scottish Physician Alexander Fleming discovered that Penicillium notatum, a laboratory contaminant growing in petri plates seeded with staphylococci, produced an antibacterial chemical that diffused into the medium and inhibited the growth of the bacteria. He named the antimicrobial principle as penicillin and found that it is strongly active against a wide range of gram positive pathogenic bacteria, but nontoxic to mammalian cells. However, he could not isolate penicillin in stable pure form. In 1940, Howard Florey and Ernst Chain of Oxford University were able to isolate Penicillin in pure and stable form for use as a chemotherapeutic

drug. For the discovery and production of penicillin, Fleming, Florey and Chain were awarded Nobel Prize in 1945.

In 1944, Selman Waksman announced that he had found an antimicrobial drug Streptomycin produced by an actinomycete *Streptomyces griseus*, after a patient screening of about 10,000 strains of soil bacteria and fungi. He coined the term 'antibiotic' and defined it as "a substance produced by microorganisms which can inhibit the growth or destroy other microorganisms in very low concentrations". Waksman received Nobel Prize for his discovery in 1952.

Waksman's success led to a world wide search for other antibiotic producing soil microorganisms and Chloramphenicol, neomycin, tetracycline and other antibiotics were isolated by 1953.

Later, a large number of chemicals were synthesized which are similar or identical with those produced by microorganisms. By chemical alteration of microbial products a large number of semisynthetic drugs are produced. Now all chemotherapeutic drugs are commonly called antibiotics irrespective of their origin i.e. microbial,semisynthetic or purely synthetic.

17.3. PROPERTIES OF ANTIMICROBIAL DRUGS

A large number of antimicrobial drugs are now available, and they show wide variation with respect to various properties like selective toxicity, range of effectiveness, type of activity and other properties.

17.3.1. SELECTIVE TOXICITY

A chemotherapeutic agent must have selective toxicity i.e. it must kill or inhibit the microbial pathogen while damage to the host is as little as possible. The degree of selective toxicity may be expressed in terms of therapeutic dose and toxic dose. The dose or concentration of the drug required for clinical treatment of a particular infection is called therapeutic dose. The dose or concentration of the drug at which it becomes too toxic for the host is called toxic dose. The ratio of the toxic dose to the therapeutic dose is known as the therapeutic index. Larger the therapeutic index better the chemotherapeutic agent.

A drug that disrupts a microbial function or structure not found in eukaryotic animal cells often has a greater selective toxicity and a higher therapeutic index. For eg. Penicillin inhibits the

synthesis of bacterial cell wall, but has little effect on host cells because they lack cell walls. Therefore, Penicillin has high therapeutic index.

A drug may have low therapeutic index because it inhibits the same process in host cells or damages the host in other ways. The drugs that inhibit nucleic acid synthesis have low therapeutic index because nucleic acids have essentially similar structure either in procaryotes or eucaryotes. Even highly antimicrobial drugs with selective action may also have low therapeutic index because they have undesirable side effects on the host. For eg. Chloramphenicol is a highly effective antibiotic but it cause severe bone marrow depression.

17.3.2. RANGE OF EFFECTIVENESS

Drugs vary considerably in their range of effectiveness. Many are narrow spectrum drugs i.e. they are effective against a limited variety of pathogens. Some have broad spectrum activity and attack many different kinds of pathogens.

Antibiotics, which are effective against bacteria, fall into three main categories basing on their range of action.

i). Active against gram positive organisms : Eg.: Penicillin, Cephalosporins etc.

ii). Active against gram negative organisms: Eg.: Streptomycin etc.

iii). Active against both gram positive and gram negative organisms : Eg. : Tetracyclines etc.

These are generalizations only and there are many exceptions. For example, Neisseriae are gram negative but are highly sensitive to penicillins, while gram positive mycobacteria are highly susceptible to streptomycin.

Chemotherapeutic drugs in general show specific action against one particular group of microbial pathogens only. Thus, chemotherapeutic drugs are often described as antibacterial, antifungal, antiprotozoan, antiviral etc. Some drugs can be used against more than one group. For example, Sulpha drugs are active against bacteria and some protozoa.

17.3.3. TYPE OF ACTION

Basing on the type of action, chemotherapeutic drugs are two types viz. cidal and static. The drug that kills the pathogen is called cidal drug, while the drug that only inhibits the growth of the pathogen is called static drug.

Penicillins, Cephalosporins , aminoglycosides etc. show rapid lethal action against bacteria, and are called bactericidal drugs.

Sulphonamides , Tetracyclines, Chloramphenicol etc. merely inhibit the growth, and if the drug is removed the microorganism will recover and grow again. Such drugs are described as bacteristatic drugs.

The differences between cidal and static action are not clear cut and most drugs are, to varying extents, both bactericidal and bacteristatic . A cidal agent kills the target pathogen but its activity is dependent on concentration, and the same drug may be static at low concentration and cidal at normal dose. The effect of a drug may also vary with target species i.e. a drug may be cidal for one pathogen and static for another.

Because static agents do not directly destroy the pathogen, elimination of infection depends on the host's own resistance mechanisms. A static agent may not be effective if the host's resistance is low.

The effectiveness of a chemotherapeutic agent against a pathogen can be obtained from minimal inhibitory concentration (MIC). MIC is the lowest concentration of a drug that prevents growth of a particular pathogen. The minimum lethal concentration (MLC) is the lowest concentration of a drug that kills the pathogen. A cidal drug kills pathogens at levels only 2 to 4 times the MIC where as a static drug may kill the pathogens at very higher concentrations only.

17.3.4. ACTIVITY IN COMBINATION

When two or more chemotherapeutic drugs are used together, they may show i) Synergism ii) antagonism or iii) indifference.

Synergism: It is usually seen when both the drugs used are bactericidal Eg. Penicillin + Gentamycin. The drugs that are effective on the same metabolic pathway of the pathogens also show synergism. For example, sulpha drugs and trimethoprim both act on folic acid synthesis at two different points in the metabolic pathway, and hence show synergistic action.

Antagonism: In general, antagonism is liable to occur when a bactericidal agent is used with a bacteristatic agent eg. Penicillin + Tetracyline. Bactericidal drug (Penicillin) is active against rapidly multiplying cells only whereas static drug (tetracycline) inhibits the growth. Hence, when penicillin and tetracycline are used simultaneously, penicillin cannot act because tetracycline inhibits the growth.

However, for treatment of tuberculosis, a cidal drug like Rifampcin and a bacteistatic drug like ethambutol are used in combination so as to prevent development of drug resistance.

Indifference: When two or more drugs are used, mostly their interaction is one of indifference.

Sulphonamides donot antagonize penicillin because their activity is very slow. Bacteristatic drugs do not antagonise polymyxin because polymyxin disrupts cell membranes of both resting spores and multiplying cells.

17.4. MECHANISM OF ACTION

Chemotherapeutic drugs differ in their mechanism of action (Fig. 17.1), and the major modes of action are

i). Inhibition of cell wall synthesis: Eg. Penicillin, Bacitracin

ii). Damage to cell membranes : Eg. Polymyxin

iii). Inhibition of protein synthesis: Eg. Streptomycin, Tetracycline, Chloramphenicol,

iv). Inhibition of Nucleic acid synthesis: Eg. Rifampicin Ciprofloxacin

v). Metabolic antagonism : Eg. Sulphonamides, Trimethoprim

Figure 17.1. Major modes of action of chemotherapeutic drugs on bacterial cells

The most effective drugs are those that interfere with the synthesis of bacterial cell wall. They have a high therapeutic index because bacterial cell walls have a unique structure not found in eukaryotic cells.

The drugs that inhibit protein synthesis by binding with procaryotic ribosomes like streptomycin, gentamicin etc. also have high therapeutic index because these drugs discriminate between procaryotic and eucaryotic ribosomes.

The drugs that inhibit nucleic acid synthesis or damage cell membranes are not as selectively toxic as other drugs because procaryotes and eucaryotes do not differ very greatly with respect to nucleic and synthetic mechanisms or cell membrane structures. Hence quinolones and polymyxins have less therapeutic index.

Antimetabolites block the functioning of metabolic pathways by competetively inhibiting the use of metabolites by key enzymes. Sulphonamides and other similar drugs inhibit folic acid synthesis and have high therapeutic index because humans cannot synthesize folic acid and must obtain it in their diet, whereas most bacterial pathogens synthesize their own folic acid, and hence are susceptible to inhibitors of folic acid metabolism. Antimetabolic drugs can also inhibit other pathways. For example, isoniazid interferes with either pyridoxol or NAD metabolism.

17.5. FACTORS INFLUENCING THE EFFECTIVENESS OF DRUGS

Various factors that influence the effectiveness of drugs include methods of administration, dosage, growth stage of the pathogen, site of infection etc.

17.5.1. Method of administration: Antibiotics are prepared mainly in three forms viz. tablets for oral administration, injections for intravenous or intramuscular use, and drops and ointments for surface use.

The drug must actually be able to reach the site of infection and mode of administration plays an important role in this aspect. A drug such as penicillin G is not suitable for oral administration because it is relatively unstable in acidic stomach juices.

Some antibiotics like gentamicin and other aminoglycosides are not well absorbed from intestinal tract and must be injected intramuscularly or intravenously.

For skin infections antibiotics like neomycin, soframycin, bacitracin are to be applied topically to skin.

For eye and ear infections antibiotic preparations available as drops are commonly used.

17.5.2. Dosage of the drug: The dose of chemotherapeutic drug must exceed the pathogen's minimum inhibitory concentration (MIC) value, if it is to be effective. The concentration reached the site of infection will depend upon the amount of drug administered, route of administration, speed of uptake and the rate at which the drug is cleared or eliminated from the body. Ideally, a drug that is absorbed over a long period and excreted slowly will remain at high concentrations for longer time.

17.5.3. Growth stage of the pathogen: The pathogen must be in susceptible stage for the drug to be effective. Bacteria in abscesses may be dormant and therefore resistant to many antibiotics. Penicillins and many other antibiotics affect pathogens only if they are acively growing and dividing.

17.5.4. Site of infection: The site of infection and severity is often very important because some sites in the body may not get the antibiotic properly. For example, blood clots or necrotic tissue can protect bacteria from a drug either because body fluids containing the drug may not easily reach the pathogens or because the drug is absorbed by materials surrounding it. Intracellular pathogens are also not easily destroyed by the drugs because they are safely present in the host cells.

17.5.5. Misuse and drug resistance: Wide spread, indiscriminate use of broad spectrum antibiotics in large quantities even for minor infections is main reason for developement of resistance in pathogens. Chemotherapy has been rendered less effective and much more complex by spread of drug resistance plasmids.

17.6. SYNTHETIC ANTIBACTERIAL DRUGS

There is an extensive array of chemotherapeutic drugs for treatment of bacterial diseases. These drugs mainly fall under two categories 1). Synthetic drugs of inorganic origin and 2). Antibiotics - which may be purely of microbial origin or chemically modified semisynthetic antibiotics or purely synthetic compounds resembling microbial products.

The group of synthetic antibacterial drugs include Sulphonamides, Trimethoprim, Cotrimoxazole, Quinolones etc.

17.6.1. SULPHA DRUGS:

Sulphonamides are important therapeutic drugs of nonmicrobial origin and their antibacterial activity was reported by Gerhard Domagk in 1930s. Compounds containing a sulphonamide (SO_2 NH_2) group are called sulphonamides or sulpha drugs. All Sulphonamides have a basic structure and different sulphonamides differ primarily by the virtue of different constitutents attached to the basic structure. Sulphanilamide is the simplest sulpha drug. and in this 'H" is represented at 'R' position (Fig.17.2). It is a structural analogue of paraaminobenzoic acid (PABA).

Figure 17.2. Structures of PABA and sulfa drugs

Sulphanilamide, Sulphamethoxazole, sulphapyridiazine, Sulphathiozole, Sulphadiazine, Sulphadimidine, Sulphafurazole, Sulphamethiozole etc. are some important sulpha drugs.

The sulpha drugs are white crystalline powders, mildly acidic in character and relatively insoluble in water. They form salts with bases and their sodium salts are water soluble.

17.6.1.1. Mode of administration: Sulpha drugs are normally given orally. Some are available as eye drops and eye ointments.

17.6.1.2. Spectrum of activity: Sulpha drugs are effective against a wide range of bacteria such as Streptococcus pyogenes, S. pneumoniae, Neisseria meningitidis, Escherichia coli and other coliforms etc. They are mainly bacteristatic and donot antagonize bactericidal effects of penicillin and can be used in combination with it for treating susceptible infections.

17.6.1.3. Duration of activity: Sulphonamides intended for systemic use are rapidly absorbed from the gastrointestinal tract, and 70 to 90 percent of the oral dose reaches the blood stream. The main site of absorption is the small intestine. After a single oral dose of a short acting suphonamide, the peak plasma concentration is usually reached within 2 to 4 hours. The free and the acetylated sulphonamides are mainly eliminated in urine, mostly by glomeru-lar filtration.

Basing on their duration of action the sulphadrugs used systemically may be divided into 3 groups

a. short acting sulphonamides - Sulphadiazine, sulphafurazone, sulphamethizole etc.

b. intermediate acting sulpha drugs - Sulphamethoxazole

c. Long acting suphonamides - Sulphamethoxypyridazine.

17.6.1.4. Clinical use: Sulphadimidine, Sulphafurazole and Sulphamethiozole are used to treat urinary tract infections. Sulpha drugs are rapidly absorbed when given orally but renal excretion is slow, to give good blood levels. There is little protein binding, consequently it diffuses into tissues and also into cerebrospinal fluid. This makes them best for treating meningitis due to meningococci.

17.6.1.5. Mechanism of action: Sulpha drugs are best examples of competitive metabolic inhibition by structural analogues. Sulphanilamide is structurally similar to PABA, which is a constituent of folic acid. When Sulphanilamide or other sulpha drug enters a bacterial cell it competes for the active sites in the enzyme folic acid synthetase, thus inhibiting folic acid synthesis. Folic acid is an essential coenzyme in the synthesis of purines, pyrimidines and other important cell constituents. Hence, inhibition of folic acid synthesis results in cessation of bacterial growth and its eventual death and elimination by phagocytization.

Folic acid is an essential growth factor for both host and bacterial pathogen. Many bacteria synthesize it from PABA while mammalian host cell cannot synthesize it but depend on external supply in diet. This accounts for the selective antibacterial action of sulphonamides.

17.6.1.6. Side effects: Use of sulphonamides some times causes serum sickness like illness or other side effects.

17.6.1.7.Resistance: Bacteria develop resistance easily against supha drugs. Their medical use is slowly declining partly because of wide spread resistance and also because more effective

antibiotics with similar spectra of activity are available. But still they are preferred in treatment of urinary tract infections.

17.6.2. TRIMETHOPRIM:

It is a broad spectrum drug, and is similar to sulpha drugs in mode of activity as well as spectrum of activity. It is marketed under the trade name septran

17.6.2.1. Structure: It is a pyrimidine analogue of dihydrofolate derivative

17.6.2.2. Administration: Normally given by oral route, some times intravenously .

17.6.2.3. Spectrum: It is effective against many common pathogenic bacteria such as staphylococci, Corynebacterium diphtheriae, E. coli. Salmonella, Shigella, Haemophilous influenzae, Vibrio cholerae etc. However, it is only weakly active against Neisseriae and ineffective against Pseudomonas aeruginosa.

17.6.2.4. Mode of action: It acts by inhibiting the synthesis of folic acid like sulpha drugs. While sulpha drugs inhibit the first step in folic acid synthesis i.e. conversion of PABA to dihydrofolic acid, Trimethoprim inhibits the conversion of dihydrofolic acid to tetra hydrofolic acid. Since trimethoprim is a structural analogue of dihydrofolate, it inhibits the activity of enzyme dihydrofolate reductase by binding to it (Fig. 17.3).

Figure 17.3. Structure and mode of action of trimethoprim

Since sulphadrugs and trimethoprim inhibit folic acid synthesis by inhibiting two sequential steps, they show synergism in action.

17.6.2.5. Side effects: It is relatively free from side effects. But a few patients may experience rashes, nausea and vomiting.

17.6.2.6. Resistance: Resistance to trimethoprim develop due to production of resistant enzymes. The gene for resistance is often present on a transposon.

17.6.3. COTRIMOXAZOLE:

Since sulpha drugs and trimethoprim act sequentially to inhibit folic acid synthesis, a drug is developed with a combination of the two. It is called cotrimoxazole and commercially available under the trade name Bactrim.

Cotrimoxazole contain trimethoprim and sulphamethoxazole in 1 : 5 ratio. This ratio was chosen because it becomes 1 : 20 ratio in the blood, and invitro studies had shown that this was the optimum ratio of the drug for effectiveness against many sensitive bacteria.

17.6.3.1. Administration: Oral, intramuscular, intravenous

17.6.3.2. Mode of action: It is a bacteristatic drug. Sulphamethoxazole and trimethoprim components of the drug block sequential steps in folic acid synthesis.

17.6.3.3. Spectrum: It is having broad spectrum activity against both gram positive and gram negative bacteria.

17.6.3.4. Clinical use: Widely used for urinary and respiratory tract infections, invasive salmonellosis, Pnemocystis pneumonia etc.

17.6.3.5. Side effects: Nausea, vomiting, rashes, mouth ulceration, folate deficiency etc.

17.6.4. QUINOLONES:

Quinolones are broad spectrum synthetic drugs that contain quinolone ring structure. Nalidixic acid is the first quinolone synthesized in 1962, and quinolone drugs are the derivatives of nalidicxic acid.

Ciprofloxacin, Norflocacin and Enofloxacin are the derivatives of nalidixic acid by addition of flourine and are called fluoroquinolones.

17.6.4.1. CIPROFLOXACIN: It is the first fluoroquinalone developed from nalidicxic acid and widely used. The structure of ciprofloxacin is shown in the figure 17.4.

Figure 17.4. The structure of ciprofloxacin

17.6.4.1.1. Administration: Mainly oral, also intravenous

17.6.4.1.2. Spectrum: It is highly effective against enteric bacteria such as E. coli and Klebsiella pneumoniae. It is also effective against Haemophilous, Neisseria, Psuedomonas, staphylococci, streptococci and mycobacteria.

17.6.4.1.3. Clinical use: Fluoroquinalones are currently used in treating urinary tract infections, sexually transmitted diseases caused by Neisseria and Chlamydia; gastro-intestinal infections, respiratory tract infections, skin infections etc.

17.6.4.1.4.Mechanism of action: Fluoroquinalones selectively block the bacterial DNA gyrase activity but not mammalian DNA gyrase. The enzyme DNA gyrase allows for breaking and rejoining of double stranded DNA as it coils or uncoils. Hence this enzyme is important in chromosomal replication, regulation of transcription and DNA repair. The mechanism of action is diagramatically represented below

17.6.4.1.5. Side effects: In general, these drugs are well tolerated. However in a few patients they may cause nausea, vomiting, abdominal discomfort and diarrhoea.

17.7. SUMMARY

The treatment of a disease with a chemical substance is known as chemotherapy, and chemotherapy developed due to the pioneering contributions of Paul Ehrlich and Gerhard Domagk of Germany, Alexander Fleming of UK, Selman Waksman of USA and others. A large number of

antimicrobial drugs are now available, and they show wide variation with respect to various properties like selective toxicity, range of effectiveness, type of activity and other properties.

A chemotherapeutic agent must have selective toxicity i.e. it must kill or inhibit the microbial pathogen while damage to the host is as little as possible. A drug that disrupts a microbial function or structure not found in eukaryotic animal cells often has a greater selective toxicity. Drugs vary considerably in their range of effectiveness. Many are narrow spectrum drugs i.e. they are effective against a limited variety of pathogens. Some have broad spectrum activity and attack many different kinds of pathogens. Basing on the type of action, chemotherapeutic drugs are two types viz. cidal and static. The drug that kills the pathogen is called cidal drug, while the drug that only inhibits the growth of the pathogen is called static drug. When two or more chemotherapeutic drugs are used together, they may show i) Synergism ii) antagonism or iii) indifference. Synergism is usually seen when both the drugs used are bactericidal or act on the same metabolic pathway of the pathogens. Antagonism is liable to occur when a bactericidal agent is used with a bacteristatic agent. However in most cases when two or more drugs are used, their interaction is one of indifference. Chemotherapeutic drugs differ in their mechanism of action, and the major modes of action are inhibition of cell wall synthesis (Eg. Penicillin), damage to cell membranes (Eg. Polymyxin), inhibition of protein synthesis (Eg. Streptomycin), inhibition of nucleic acid synthesis (eg. Rifampicin) and metabolic antagonism (eg. Sulphonamides). Various factors that influence the effectiveness of drugs include methods of administration, dosage, growth stage of the pathogen, site of infection etc. Among the synthetic antibacterial drugs, sulpha drugs are very important. Compounds containing a sulphonamoide (SO_2 NH_2) group are called sulpha drugs. Paraaminobenzoic acid (PABA), an important precursor of folic acid, is a structural analogue of sulpha drugs. When the drugs are used, they compete with PABA and inhibit folic acid synthesis in bacteria. Sulpha drugs are effective against a number of bacterial pathogens, and are the drugs of choice for treatment of urinary tract infections. Trimethoprim is a pyrimidine derivative and a structural analogue of dihydrofolic acid and inhibit folic acid synthesis. Its range of activity is similar to that of sulpha drugs, since both act on same metabolic pathway to inhibit folic acid synthesis. Since sulpha drugs and trimethoprim act sequentially to inhibit folic acid synthesis, a drug is developed with a combination of the two. It is called cotrimoxazole and contain trimethoprim and sulphamethoxazole in 1: 5 ratio. Ciprofloxacin is a drug developed from

nalidixic acid. It is highly effective against a number of bacterial pathogens, and it inhibits the bacterial growth by selectively blocking the bacterial DNA gyrase activity, thus inhibiting DNA synthesis.

17.8. MOEL QUESTIONS

Essay type questions

Give an account of development of chemotherapy

Give an account of properties of antimicrobial drugs

Discuss the mechanisms action of antimicrobial drugs and factors influencing their effectiveness

Discuss the structure, mode of action and effectiveness of synthetic antibacterial drugs

Short answer type questions

Selective toxicity

Mechanisms of action of antimicrobial drugs

Sulpha drugs

Trimethoprim

Cotrimoxozole

Ciprofloxacin

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LESSON–18: CHEMOTHERAPY (PART – II)

Objective: To study the details of antibiotics, antiviral drugs, antifungal drugs, antiprotozoan drugs, and drug resistance

Contents:

18.1 INTRODUCTION

In the first part of this lesson (lesson – 17) development of chemotherapy, properties of antimicrobial drugs and details of some of the important synthetic antibacterial drugs are explained. In this lesson, the structure, mode of action and clinical use of various important antibiotics, antiviral drugs and antifungal drugs are explained. The development of drug resistance in pathogens against the commonly used drugs is an important problem in chemotherapy, and the mechanisms of development of drug resistance in pathogens are also explained.

18.2. ANTIBIOTICS

Antibiotics are chemical compounds produced by living microorganisms which in small concentrations inhibit the growth of other microorganisms.

Some species of fungi, actinomycetes and bacteria produce antibiotics. Pasteur and Joubert (1877) first reported that the growth of *Bacillus anthracis* was inhibited by the presence of other bacteria that contaminated the cultures and termed the phenomenon as antibiosis. Emmerich and Low (1899) discovered that Pyocyanase from the growth of *Pseudomonas aeruginosa* and other chemical now known as pyocyanin, a blue green soluble pigment, having antibiotic properties. Alexander Fleming (1929) discovered Penicillin from the cultures of a fungus *Penicillium notatum*. Dubos (1939) discovered tyrothricin from the cultures of *Bacillus brevis*. Tyrothricin is a mixture of tyrocidin and gramicidin, two polypeptides. Waksman and his colleagues in 1940's discovered that streptomycin is produced by an actinomycete *Streptomyces griseus*. In 1942

Waksman coined the term antibiotic and defined it as a substance produced by microorganism which can inhibit the growth or destroy other microorganisms. Since then a large number of antibiotics were discovered. However, only a limited number are commercially successful. Commercially important antibiotics include Penicillins, Cephalosporins, Aminoglycosides, Tetracyclines, Chloramphenicol, Erythromycin, Polymixin etc.

18.2.1. PENICILLINS:

One of the most important groups of antibiotics, both historically and medicinally are penicillins. Penicillin was first extracted from the culture filtrates of the fungus *Penicillium notatum*. Subsequently, a related mould, *P. chrysogenum* was found to give highest yield of penicillin and is now employed for commercial production .

A number of natural penicillins are produced by the fungus and the type of natural penicillin produced mainly depends on the substratum used for growing the fungus. For example, when the fungus is grown on corn steep liquor it mainly yields Penicillin G. Other natural penicillins are Penicillin- V, Penicillin-0. Penicillin- F, K and X.

All the natural penicillins are structurally related in having a basic struture called 6-aminopenicillanic acid (6-APA). APA is a two ring structure having a thiazolidine ring and a condensed β-lactam ring. The 6-APA carries a variable side chain in position 6, and different penicillins mainly differ in the chemical nature of the side chain.

Figure 18.1. General structure of 6-amino penicillanic acid

By using Penicillin - G as base a number of semisynthetic penicillins were prepared by chemically changing the side chain of APA at position 6. Important semisynthetic penicillins are Ampicillin, Carbenicillin, Amoxycillin, Methicillin, Oxacillin, Ticaricillin etc.

18.2.1.1. Spectrum: All pecinicillins are basically effective against gram positive bacteria including staphylococci and streptococci. Some semisynthetic penicillins have extended spectrum and are effective against some gram negative bacteria also.

18.2.1.2. Clinical use: Pneumococci are extremely susceptible to benzylpenicillin and hence, it continues to be the drug of choice for the treatment of pneumococcal infections.

Infections such as streptococcal pharyngitis, meningitis, otitis media, mastoiditis and acute bacterial endocarditis respond satisfactorily to penicillin.

Penicillin is the drug of choice in the treatment of venereal diseases like gonorrhoea and syphilis. Diphtheria, tetanus and gasgangrene respond well to penicillin treatment.

On oral administration, benzyl penicillin is inactivated to a significant extent by the gastric acids. Because of this and irregular absorption, the oral dose of benzylpenicillin required to achieve an effective therapeutic plasma level is 4 to 5 times larger than the equivalent muscular dose. As food interferes with the absorption, benzylpenicillin should be given orally at least 30 minutes before or 2 or 3 hours after a meal.

Benzylpenicillin in aqueous solution is rapidly absorbed after subcutaneous or muscular administration. Peak plasma level of 8 to 10 units per ml is reached within 15 to 30 minutes, and the drug disappears from the plasma within 3 to 6 hours.

Penicillin is widely distributed in the body after absorption. Nearly 30 percent of a single parenteral dose is metabolised within the body; small amounts appear in bile, milk and saliva but the major portion is eliminated by the kidneys.

18.2.1.3. Mode of action:

All penicillins inhibit cell wall synthesis by blocking transpeptidation. Penicillin does not enter the cell but binds to bacterial enzymes (peptidyl transferases) which are responsible for cross linkage in peptidoglycan layer in the cell wall.

Peptidoglycan is a microfibrillar structure and each filament is composed of N-acetyl glucosamine (NAG) and N-acetyl muramic acid (NAM) in a strictly alternating arrangement. Each NAM molecule has a pentapeptide side chain. The peptide chain of NAM molecule in a filament is connected to peptide chain of NAM molecule on the adjacent filament by formation of a pentapeptide bridge. This peptide bridge formation is called transpeptidation. The peptide bridge

in *Staphylococcus* is composed of 5 glycine molecules and is called pentaglycine bridge. The aminoacid composition of the peptide bridge may vary in other bacterial genera.

As the transpeptidation occurs outside the cytoplasmic membrane where energy is not available, it is mediated by an enzyme called peptidyl transferase. Penicillin inhibits the activity of peptidyl transferase by binding to it.

Thus, penicillin neither kills nor inhibits the cell growth directly but interrupt the completion of cell wall synthesis. Such cells then become increasingly vulnerable to osmotic shock and under normal circumstances eventually die.

Penicillin mainly effect gram positive bacteria but not gram negative bacteria, because peptidoglycan layer is thick and composed of many layers in gram positive bacteria while it is very thin and composed of only one or two layers in gram negative bacteria.

Penicillin inhibits only actively growing cells because peptidoglycan synthesis occurs only in actively growing cells but not in resting cells. Further, peptidoglycan synthesis occurs only to add new material to the existing peptidoglycan layer. For new peptidoglycan to be added to the existing peptodoglycan, the peptide bonds in the preexisting peptidoglycan are lysed by autolysins. In actively growing cells autolysins continue to act, peptodoglycan filaments are added, but since cross bridges do not occur, the cell wall becomes progressively weaker and osmotic lysis occur.

It is also reported that penicillins bind to several penicillin binding proteins and may destroy bacteria by activating their own autolytic enzymes (autolysins) even when there is no active synthesis of peptodoglycan.

Penicillin is not a structural analogue of peptidyl transferase enzyme but the shape of the molecule appears to be important to its activity because β-lactam ring is essential for the activity of the molecules.

18.2.1.4. Side effects: Penicillins are remarkably safe drugs. However, some persons may show nausea and vomiting. Some exhibit allergy to penicillin. Anaphylaxis is the most serious reaction of penicillin usage. It occurs in 1-5% of patients. A positive skin reaction is relatively reliable indicator of potentially serious penicillin allergy.

18.2.1.5. Resistance to penicillin: Resistance to penicillin is quite common in hospital staphylococci and other bacteria. The resistant strains produce β-lactamase enzyme, which disrupts the β-lactam ring structure, which is important for the activity of the drug.

18.2.1.6. DIFFERENT MEDICALLY IMPORTANT PENICILLINS:

18.2.1.6.1. Natural penicillins : Among the natural penicillins, penicillin-G and penicillin - V are important medically.

Penicillin - G : Chemically it is Benzyl penicillin. It is the most fungus side chain is commonly used natural penicillin produced by growing the on corn steep liquor as substrate. It is effective against gonococci, meningococci and several gram positive pathogens such as streptococci and staphylococci.

It is highly acid labile and is easily inactivated by acidic stomach fluids, if used orally. Hence, it must be administered parenterally.

The main disadvantages are inactivation by stomach fluids, short duration of activity and rapid development of resistant staphylococci. Allergy may occur on continuous use.

Penicillin-V: Chemically it is phenoxymethyl penicilin. It is acid resistant and can be given orally. Other properties are similar to those of Penicillin-G.

18.2.1.6.2. SEMISYNTHETIC PENICILLINS:

The semisynthetic penicillins may be grouped into two categories 1. Penicillinase resistant penicillins and 2. extended spectrum penicillins. Penicillinase resistant penicillins: Ampicillin, Amoxycillin and Cloxacillin are important semisynthetic penicillins that are resistant to penicillinase activity. Carbenicillin is an extended spectrum semisynthetic penicillin.

Ampicillin: It is the most commonly used semisynthetic penicillin. It is not only resistant to penicillinase activity but also acid resistant and possess extended spectrum of activity.

The antibacterial activity of ampicillin is generally the same as that of benzylpenicillin.It is more effective than benzylpanicillin against a variety of gram negative bacteria. The drug is effective against *Haemophilous influenzae, Streptococcus viridans, Proteus mirabilis, Neisseria gonorrhoea, Salmonella typhi,* many strains of *E. coli* and several strains of *Shigella*.

It is mainly used for treatment of urinary tract infections, respiratory tract infections, meningitis and intestinal infections due to *E.coli*, enterococci, *Salmonella* and *Shigella*.

Amoxycillin: It has a broad spectrum of activity similar to that of ampicillin. It is acid resistant and effective on oral administration, and the blood levels are twice as high as those after similar dose of ampicillin. Its absorption is not influenced by food.

Cloxacillin : It is also acid resistant and after a single oral dose, peak plasma levels are attained within an hour and persist for 4 to 6 hours.

Carbenicillin: It is acid labile and ineffective orally. It shows extended spectrum being effective against a wide variety of gram negative bacteria including Pseudomonas aeruginosa. However it is much less active against gram positive bacteria.

18.2.2. CEPHALOSPORINS:

In 1945 Professor G. Brotzu of Sardinia isolated a fungus *Cephalosporium acremonium* from sea water at a sewage fall and found that it produces antimicrobial principle. He sent the culture to Howard Florey (of penicillin fame) in 1948. In 1955 Florey reported that the fungus produce not one but a group of 7 antibiotics and called them cephalosporins because they are produced by *Cephalosporium acremonium*.

Important members of cephalosporin group of antibiotics are Cephalothin, Cefoxitin, Cefoperazone, Ceftriaxone etc.

18.2.2.1. Structure: Cephalosporins have 7 - aminocephalosporanic acid nucleus which bears close resemblance to the 6- aminopenicillanic acid necleus of Penicillins. Because of structural similarity both penicillins and cephalosporins are called β-lactam antibiotics and have similar spectrum of activity. The basic structure of cephalosporins is shown in the figure 18.2.

Figure 18.2. The basic structure of cephalosporins

18.2.2.2. Mode of action: Cephalosporins resemble penicillin in their mechanism of action because of structural similarity. They inhibit cell wall synthesis by blocking transpeptidation during peptidoglycan synthesis.

18.2.2.3. Spectrum: Originally cephalosporins were similar to penicillin in their spectrum of activity- being effective against gram positive bacteria. However, new generation cephalosporins have extended spectrum of activity.

First generation cephalosporins, Eg.: Cephalothin, are effective against gram positive bacteria like pneumococci, Streptococci, corynebacteria.

The second generation cephalosporins, Eg.: Cefoxitin, are effective against many gram negative bacteria as well as gram positive pathogens. Eg.: *E.coli, Klebsiella pneumoniae, Neisseria gonorrhoeae, Salmonella, Shigella, Staphylococcus, Streptococcus* etc.

The third generation cephalosporins, Eg.: Cefoperazone, Ceftrizxone, are particularly effective against gram negative bacteria including *Pseudomonas aeruginosa* and often reach central nervous system also.

18.2.2.4. Administration: Cephalosporins are administered either orally or intravenously; intramuscular administration is painful. Cephalosporins are eliminated mainly by renal excretion and high concentrations are achieved in the urinary tract.

18.2.2.5. Clinical use: They are effective against a large group of diseases like Pneumonia, Dysentery, Typhoid, Gonorrhoea, pyogenic infections, urinary tract infections etc. They are preferred in treatment of patients who are allergic to penicillins.

18.2.2.6. Side effects: In general, cephalosporins are well tolerated. However, a few persons may experience skin rash, fever, serum sickness, eosinophilia etc. Large doses can cause kidney damage.

18.2.3. AMINOGLYCOSIDES:

This is a large group of antibiotics which include Streptomycin, Gentamicin, Neomycin, Kanamycin, Framycetin etc. Chemically all the members of the group are aminoglycosides. All contain cyclohexane ring and amino sugars joined by glycosidic bonds.

18.2.3.1. Streptomycin:

18.2.3.1.1. Structure : Streptomycin, the first discovered antibiotic in this group, comprise 3 components in its structure. 1. N - methyl-L-glucosamine 2. Streptose sugar and 3. Streptidine. The three components are linked by glycosidic bonds (Fig. 18.3).

Figure 18.3. The structure of streptomycin

18.2.3.1.2. Spectrum : Aminoglycosides are mainly active against gram negative bacteria such as *E.coli, Klebsiella, Vibrio cholerae, Yersinia pestis, Salmonella, Shigella, Neisseria* and *Brucella*. Streptomycin act variously and is effective against some gram positive bacteria, especially *Mycobacterium tuberculosis*.

18.2.3.1.3. Mode of action: All aminoglycosides mainly inhibit protein synthesis. The mechanism of action was mainly studied in case of Streptomycin.

The drug is more effective at alkaline (7-8) than acidic pH. Streptomycin is bacteristatic in low concentrations and bactericidal in high concentrations.

On intramuscular administration peak plasma level is reached within 30 to 60 minutes and antibacterial activity persists in the plasma for 6 to 8 hours.

The uptake of streptomycin by the susceptible bacteria is triphasic

a). An immediate absorption to the exterior of the cell wall. The uptake is irreversible because of extensive binding to anionic surfaces within the cell

b). Following absorption, there is a lag period during which there is very little entry.

c). Rapid entry phase, following damage to the cell membrane.

Following absorption, only a few molecules of the antibiotic enter the cell, perhaps through transient imperfections in the growing membrane. These molecules bind to polysomal ribosomes (which predominate in the cell) yielding misread proteins. Some of these proteins enter the cell membrane, where their misfolding creates aqueous channels. Further entry of the antibiotic then

increase autocatalytically as more misreading leads to more membrane damage. Finally, when enough antibiotic has entered to block all ribosomes, it rreversibly halts protein synthesis.

At relatively higher concentrations, streptomycin completely inhibits protein synthesis and cause cell lysis. However, at sublethal concentrations, antibiotic cause misreading in protein synthesis. The irreversible binding of the antibiotic molecule to 30 S subunit of mRNA distorts the ribosomes in a way that increase errors. It explains streptomycin dependent mutations.

The bacterial type ribosomes (70S) are present in eukaryotic cellular organelles such as mitochondria. This may account, in part, for the side effects often observed in patients under going treatment with high doses of streptomycin.

18.2.3.1.4. Side effects : Aminoglycosides, especially streptomycin, are quite toxic and produce various side effects.

Local irritation : Streptomycin may occasionally cause nausea and vomiting.

Intolerance : The manifestations include various skin rashes accompanied by eosinophilia, drug fever and lymphoadenopathy.

Central nervous system: The most serious adverse effect of streptomycin is the damage to the 8th nerve.

Super infections : Superinfection with *Staphylococcus* and *Candida* has been reported on either local or systemic administration of streptomycin.

Because of these side effects streptomycin is used very rarely now a days and is replaced by other aminoglycosides.

18.2.3.2. GENTAMICIN: At present it is the most commonly used aminoglycoside. It is extracted from the cultures of *Micromonospora purpura*. It shows wide spectrum of activity against a number of gram negative bacteria including *Pseudomonas aerugnosa, E. coli, Proteus* and also against gram positive streptococci and staphylococci.

The drug is administered as intramuscular injection. After giving a single dose, peak plasma levels are reached within 60 to 90 minutes and therapeutically effective concentration persists for 6-8 hours.

It is the drug of choice for treatment of a wide variety of skin infections. It is combined with carbenicillin for *Pseudomonas* infections, with ampicillin for streptococcal infections, and

with cephalosporins for staphylococcal infections. A 0.1% cream, ointment or eye drops is used for topical applications.

18.2.3.3. NEOMYCIN: It is obtained from *Streptomyces fradiae*. It is stable but poorly absorbed from the intestinal tract. Hence used to disinfect intestinal tract prior to operations. Because of poor absorption and serious toxic side effects, it is now used mainly for topical applications to treat skin infections and bacterial conjunctivitis.

18.2.3.3. KANANYCIN: It is derived from *Streptomyces kanamyceticus*, and is closely related to neomycin. It is a drug of choice to treat urinary tract infections with *Proteus* species, but if the organism is not eradicated within a few days it is liable to become resistant. It has now been replaced by other aminoglycosides because of toxicity.

18.2.3.4. FRAMYCETIN: It is derived from *Streptomyces decaris*. The antimicrobial spectrum and toxicity of framycetin are similar to those of neomycin. The ointment is commercially available under the trade name soframycin and is widely used for skin infections.

18.2.4. TETRACYCLINES

Tetracyclines are a group of antibiotics with a common four ring structure, hence the name tetracylines. They were discovered as a result of systematic search by pharmaceutical industry by screening a multitude of soil microorganisms for potential antibiotic activity. Chlortetracycline was the first in the group isolated from *Streptomyces aureofaciens* in 1948. Oxytetracycline was the second in the group isolated from *Streptomyces rimosus* in 1950. Tetracycline was the first semisynthetic drug of the group prepared by catalytic hydrogenation of Chlortetracycline in 1953. Later a number of semisynthetic tetracyclines were synthesized by partial modification of natural tetracyclines.

Natural tetracyclines : Chlortetracycline, Oxytetracycline

Semisynthetic tetracyclines : 1. Tetracycline
2. Dimethyl Chlortetracycline (Ledermycin)
3. Doxycycline (vibramycin)
4. Minocycline (Minocin)

18.2.4.1. Structure : Chemically the tetracyclines are naphthacene derivatives. The naphthacene nucleus is made up of fusion of four partially unsaturated cyclohexane radicals, and hence the name tetracyclines.

Figutre18.4. Structure of tetracyclines

The various tetracyclines differ only slightly in structure. The crystalline bases of these compounds are pale yellow, slightly bitter and sparingly soluble in water. However, they form water soluble sodium salts. The acid salts are more stable in the dry powdered state and are usually preferred in therapy.

18.2.4.2. Spectrum: Tetracyclines are called broad spectrum antibiotics as they are effective against both gram positive bacteria, gram negative bacteria and also inhibit growth of certain actinomycetes, rickettsiae and chlamydiae.

Tetracyclines are highly effective against mycoplasmal pneumonia, ricketssial fevers, chlamydial conjunctivitis and lymphogranuloma venereum. They are also used to treat cholera, plague, brucellosis, acute bronchitis and other diseases.

18.2.4.3. Mechanism of action: They are essentially bacteriostatic, and they interfere with protein synthesis by blocking the attachment of aminoacyl transfer RNA to the acceptor site on the messenger RNA - ribosome complex by binding to 30 S sub unit of ribosome.

18.2.4.4. Administration : The absorption of tetracyclines on oral administration is variable but adequate. After oral administration peak plasma level is reached within 3 to 4 hours. Oxytetracycline and tetracycline given intramuscularly produce peak plasma levels within one hour and adequate levels are maintained for 12 hours, the injection is, however, painful.

The drug is also available in the form of opthalmic ointments and eye drops for topical application.

Chlortetracycline, oxytetracycline and tetracycline are the original members of the group and are still in use. Their irregular absorption when given orally necessitates high dosage. New generation semisynthetic tetracyclines like doxycycline and minocycline are better absorbed and slowly excreted, and hence given as a daily dose, as against 6-hourly doses for the older tetracyclines.

18.2.4.5. Resistance: Fairly common, generally plasmid mediated

18.2.4.6. Side effects: Generally safe when used in recommended doses. Larger doses can cause severe liver damage, particularly in pregnancy. In young children, it may deposit on teeth causing yellow staining and also interfere with bone development. Superinfection with drug resistant *Staphylococcus aureus* is a serious complication.

18.2.4.7. Semisynthetic tetracyclines:

18.2.4.7.1. Dimethylchlortetracycline (Ledermycin): It is more stable to temperature and pH changes than the older analogues. The drug is slowly excreted by kidneys and therapeutic blood levels are claimed to be maintained for at least 24 hours after cessation of treatment.

18.2.4.7.2. Doxycycline (Vibramycin): This antibiotic, administered orally, is better absorbed and more slowly excreted than ledermycin and others.

18.2.4.7.3. Minocycline (Minocin): This tetracycline is absorbed completely from the gastrointestinal tract and exhibits greater antibacterial activity than older tetracyclines. It is also long acting.

18.2.5. CHLORAMPHENICOL

It is a broad spectrum antibiotic originally derived from *Streptomyces venezuelae* in 1947, and now produced synthetically.

8.2.5.1. Structure : Chemically chloramphenicol is a derivative of dichloroacetic acid and contains a nitrobenzene ring with nonionic chlorine (Fig.18.5). It is stable over the pH range of 2 to 9.

Figure 18.5. The structure of chloramphenicol

8.2.5.2. Spectrum : The antibacterial spectrum of chloramphenicol resemble that of tetracyclines but with little or no cross resistance with tetracyclines. The drug is active against gram positive and gram negative bacteria. *Rickettsia, Chlamydia* and *Mycoplasma pneuoniae. Salmonella typhi* and *Haemophilous influenzae* are more susceptible to chloramphenicol than to any other antibiotic.

8.2.5.3. Mode of action: It is a bacteriostatic drug. It inhibits protein synthesis by combining with 50 S subunit of bacterial ribosomes.

8.2.5.4. Administration: It is well absorbed when given orally and is better diffusible into the tissues. It is also used as intravenous injections, and from blood stream it passes into cerebrospinal fluid more readily than any other antibiotic. However, intramuscular injection is not suggested because absorption is slow and gives relatively poor blood levels.

8.2.5.5. Clinical use: It is the drug of choice in treatment of typhoid, and meningitis caused by *Haemophilous influenzae*. Eye drops and ointments are used for treating eye infections.

8.2.5.6. Side effects: There are several reports of fatal bone marrow depression following its use. The drug also causes severe shock and depression in premature infants (grey baby syndrome). Because of these side effects the drug is in disgrace, and less toxic drugs replaced its use wherever possible. It is regarded as a professional malpratice to give synthetic chloramphenicol for minor infections.

However, severe respiratory tract and other infections which have shown little response to other antibiotics, often respond dramatically to chloramphenicol probably through its action on certain gram negative bacteria such as *Haemophilous influenzae, Bordetella pertussis* and coliforms. Most strains of staphylococci and other trouble some bacteria are still sensitive to it. In such cases, restricted use of chloramphenicol in short duration treatment is still useful. A fall in the

reticulocyte count is the earliest evidence of toxicity and blood counts should be performed daily when choramphenicol is used for treatment of a disease.

18.2.6. POLYMYXINS

Polymyxins are a group of polypeptide antibiotics produced by *Bacillus polymyxa*. Five polymyxins viz. polymyxin A, B, C, D and E are known. Of these, polymyxin B is therapeutically important.

18.2.6.1. Structure: Polymyxin B is a cyclic polypeptide made up of the aminoacid residues leucine, phenyl alanine, threonine and diaminobutyric acid (Fig. 18.6).

Figure 18.6. The structure of polymyxin - B

18.2.6.2. Spectrum : Polymyxin B is effective against a number of gram negative bacteria including *Pseudomonas aeruginosa*.

18.2.6.3. Mode of administration : Spray, cream or powder for local applications. It is not suggested for systemic use because of its nephrotoxicity.

18.2.6.4. Clinical use: For wound infections, especially those caused by *Pseudomonas aeruginosa*.

18.2.6.5. Resistance : Development of bacterial resistance to polymyxin is rare.

18.2.6.6. Mechanism of action: It shows bactericidal activity. The drug binds to phospholipid component of procaryotic cell membrane and disrupts its permeability, resulting in leakage of essential cytoplasmic constituents. Since its activity is disruption of cell membrane, it is bactericidal to non-growing cells also. Since the membrane structure is essentially similar in all organisms, the drug is cytotoxic even to the host cells to some extent. However, it has much lower

affinity for phosphatidyl choline, an important constituent of animal cell membranes that is not present in bacteria, and this accounts for lower toxicity to host cells than to bacterial cells

Because of its nephrotoxicity, polymtxin B is rarely used systemically except against *Pseudomonas aeruginosa*.

18.3. ANTIVIRAL DRUGS

For many years, the possibility of treating viral infections with drugs appeared remote because viral nucleic acid is pathogenic agent and it uses host metabolism for its replication. However, with the discovery of viral specific enzymes and some viral specific life processes, chemotherapeutic drugs were synthesized for a few important diseases like herpetic infections, influenza, AIDS etc. All the antiviral drugs synthesized so far are mainly structural analogues of nucleosides, and competitively inhibit viral replication or attachment.

Important antiviral drugs include Acyclovir, Zidovudine (AZT), Amantadine etc. The structures of the important antiviral drugs are shown in the figure 18.7.

Figure 18.7. The structures of acyclovir, zidovudine and amantadine

18.3.1. ACYCLOVIR: A break through in antiviral therapy was achieved with the introduction of Acyclovir in 1982. It is nontoxic to host cells but strongly inhibits herpes simplex virus type I and type II, and to a lesser extent Varicella - Zoster virus.

18.3.1.1. Structure: It is an analogue of Guanine-nucleoside.

18.3.1.2. Mode of action : On phosphorylation it resembles deoxy-GTP and inhibit Virus DNA polymerase activity. The drug acts specifically in two ways in inhibiting viral replication.

First, the chemical is phosphorylated to monophosphate only in cells infected with herpes simplex virus since this step requires herpes thymidine kinase (TK) and cannot be achieved by normal cellular TK. The viral TK is less precise than the corresponding cellular TK, and so unlike the latter it will accept fradulent substrate like acyclovir. Phosphorylation to di- and tri- phosphate is achieved by cellular enzymes.

The second specific feature of the drug is that the triphosphate, the active moiety, binds to and specifically inhibits the functions of the herpes virus DNA polymerase. It has little effect on normal cell DNA polymerase.

18.3.1.3. Administration: Oral, topical (as cream) and intravenous (for severe infections).

18.3.1.4. Clinical use: It is mainly used in severe cases of herpes encephalitis. It is also useful in treating other infections of herpes simplex virus type I & II. It is effective against Varicella-Zoster virus which causes chicken pox and shingles. Generally chicken pox is a self limiting milder disease, but shingles is a painful infection, and hence the drug is suggested for severe cases of shingles.

18.3.1.5. Side effects : Minor side effects like rash and gastrointestinal disturbances.

18.3.1.6. Draw back : Latent virus is not eradicated by this drug. Hence reactivations are not prevented. The herpes viruses on which the drug is effective are well known for their latency for long periods. In addition to this draw back, resistant strains of viruses also appeared.

18. 3.2. ZIDOVUDINE : It is also called Azidothymidine and its trade name is retrovir. It is a thymine - nucleoside analogue effective against HIV.

18.3.2.1. Mode of action: As with acyclovir, the molecule has to be phosphorylated intracellularly to produce the active antiviral drug. The triphosphate is a very potent inhibitor of viral reverse transcriptase (RT) enzyme, and prevents nucleotide chain elongation. The 3' positioning of the

azido group blocks the essential phosphodiester linkage which would normally enable the next nucleotide to be added to the growing DNA chain. Azidothymidine triphosphate binds to the viral reverse transcriptase rather than to the cellular DNA polymerase, giving some specificity of action.

The cell enzymes can phosphorylate the molecule and hence intracellular concentrations of the active drug increase in normal cells also. This partly explains its toxic effects.

18.3.2.2. Clinical use: It is a specific drug for treatment of AIDS. If used before the symptoms develop it can prevent or delay the onset of symptomatic AIDS. It can delay the death but does not eradicate infection or effect cure. It is the only drug licensed for use against AIDS so far.

18.3.2.3. Side effects: It is toxic when used on continuing basis. Side effects include anemia, bone marrow depression, hepatic and renal impairment etc.

18.3.3. AMANTADINE: It is a synthetic chemical effective against Influenza - A virus. It could be useful in an influenza A pandemic for patients of high risk group.

18.3.3.1. Mode of action: The antiviral action of amantadine is mediated by its ability to increase the pH of intracellular vacuoles. Influenza-A virus normally infects cells by catalysing the fusion of its viral membrane with a cellular membrane in intracellular vacuoles at low pH. If the vacuolar pH is raised by amantadine virus induced fusion is prevented and subsequent release of viral nucleic acid and hence, viral infection is blocked. One of the structural proteins of the virus, M2 protein, normally acts as an ion channel, allowing passage of hydrogen ions to the interior of the virus. Amantadine binds to this protein and blocks the channel, and acidification cannot occur.

Amantadine also inhibit the action of RNA polymerase- II and blocks viral replication.

18.3.3.2. Administration: Oral

18.3.3.3. Clinical use: It is a specific drug against Influenza.

18.3.3.4. Side effects: Side effects mainly occur in elderly and include insomnia, nervousness and dizziness.

18.4. ANTIFUNGAL DRUGS

The treatment of fungal infections has been less successful than that of bacterial infections largely because eucaryotic fungal cells are much more similar to human cells than are bacteria.

Further, most fungi have a detoxification system that modifies many antibiotics probably by hydroxylation. Hence, relatively few effective antifungal drugs are available.

Imidazoles, Flucytosine, Nystatin, Griseofulvin and Amphotericin-B are important antifungal drugs. Of these imidazoles and flucytosine are synthetic drugs, while the other three are antibiotics of microbial origin.

The antifungal drugs mainly act on sterols in the fungal cell wall. They either extract membrane sterols or prevent their synthesis. Since host cells do not have cell walls, it accounts for specificity of their action.

Fungal infections are mainly two types viz. a).superficial mycoses that effect skin and b). deep mycoses or systemic infections that cause lung infections. Treatment of these two types of diseases is different. Drugs used to treat superficial mycoses are imidazoles, nystatin and griseofulvin. The drugs used to treat deep mycoses are amphotericin-B and Flucytosine. The structures of important antifungal drugs are shown in the figure 18.8.

Figure 18.8. Structures of antifungal drugs

18.4.1. IMIDAZOLES: Three drugs containing imidazole ring structure - Miconazole, Ketonazole and Clotrimazole - are broad spectrum agents available as creams and solutions for the treatment of dermatophytic infections such as athlete's foot, tinea corporis, tinea cruris, oral and vaginal candidiasis etc. They disrupt fungal membrane permeability and inhibit sterol synthesis.

18.4.2. FLUCYTOSINE: It is a synthetic, oral antimycotic agent. It is a structural analogue of cytosine, synthesized by adding Flourine at position 5 and hence called 5-Flucytosine. It is effective against most systemic fungi, although drug resistance often develop rapidly.

The drug is converted to 5-Fluorouracil by the fungi, incorporated into RNA in place of uracil, and disrupts RNA function.

Side effects include skin rash, diarrhoea, nause, liver damage etc.

18.4.3. AMPHOTERICIN – B : It is a polyene antibiotic obtained from *Streptomyces nodosus*, and has a wide antifungal activity. It inhibits the growth of *Histoplasma capsulatum*, *Cryptococcus neoformans Sporothrix schenkii, Coccidioides immitis* and *Blastomyces dermatidis* in low concentrations. All these fungi cause systemic lung infections. The drug is given intravenously in treatment of systemic infections. Topically it is useful in the treatment of *Candida* infections.

Amphotericin B is a highly toxic antibiotic and a variety of reactions may develop after its intravenous use. However, this is the only antibiotic available for serious systemic fungal infections, and hence, though toxic could be life saving in some cases.

18.4.4. NYSTATIN: This antibiotic. obtained from *Streptomyces noursei*, contains many double bonds in its chemical structure, and hence called a polyene antibiotic. It shows inhibitory activity against *Histoplasma, Blastomyces, Trichophyton, Microsporum* and *Candida*. It has no antibacterial activity. On parenteral administration it produces a variety of toxic effects. Further, it is poorly absorbed by the tissues. Hence, its use is restricted to the treatment of localized skin infections, mainly those caused by *Candida*.

18.4.5. GRISEOFULVIN: It is isolated from *Penicillium griseofulvum*, and the first antibiotic to cure effectively the dermatophytic infections. It is administered orally. It acts mainly on the growing fungal cells, probably acts as purine analogue and interfere with nucleic acid synthesis.

It is quite effective against species of *Trichophyton, Microsporum* and *Epidermophyton*, which cause dermatophytic infections, but ineffective against *Candida* or any of systemic mycoses. Side effects are usually mild and include headache, nausea, vomiting and diarrhoea.

18.5 SUMMARY

Among the antibiotics, Pencillins, Cephalosporins, Aminoglycosides, Tetracyclines, Chloramphenicol, and Polymyxins are very important and commonly used against a number of bacterial diseases. Natural penicillin mainly acts against gram positive bacteria by inhibiting the synthesis of their cell wall, and a drug of choice for treatment of staphylococcal and streptococcal skin infections, pneumococcal pneumonia, diphtheria, tetanus, gasgangrene and also against venereal diseases like gonorrhoea and syphilis. Semisynthetic penicillins like ampicillin, amoxicillin, cloxacillin, carbenicillin etc. have extended spectrum of activity and are effective against gram negative bacteria also. Cephalosporins are a group of antibiotics derived from a fungus *Cephalosporium acremonium*, and have similar mode and spectrum of activity as penicillins. They are used as alternate drugs to patients who are allergic to penicillin. Aminoglycosides are a large group of antibiotics derived from species of *Streptomyces* and *Micromonospora* and include streptomycin, neomycin, kanamycin, framycetin, gentamicin etc. All of them contain cyclohexane ring and aminosugars joined by glycosidic bonds. They inhibit protein synthesis, and are mainly effective against gram negative bacteria. Tetracyclines are a group of antibiotics with a common four ring structure. Chlortetracycline and oxytetracycline are natural tetracyclines derived from *Streptomyces* species, and tetracycline, ledermycin, doxycycline and minocycline are semisynthetic tetracyclines. Tetracyclines are broad spectrum antibiotics effective against both gram positive and gram negative bacteria and also against certain actinomycetes, mycoplasms, ricekettsiae and chlamydiae. They interfere with protein synthesis by blocking the attachment of aminoacyl transfer RNA to the acceptor site on the messenger RNA by binding to 30 S subunit of ribosome. Chloramphenicol is a broad spectrum antibiotic derived from *Streptomyces venezulae* and now produced synthetically. It inhibits protein synthesis by combining with 50 S subunit of bacterial ribosome. Its spectrum of activity is similar to that of tetracyclines. It is a drug of choice for treatment of typhoid and meningitis. However, it has serious side effects, and hence its use is now restricted. Polymyxins are a group of polypeptide

antibiotics produced by *Bacillus polymyxa*, and polymyxin-B is therapeutically important. It selectively disrupts the cell membrane of bacteria, and is a drug of choice against infections caused by *Pseudomonas aeruginosa*. However, because of its nephrotoxicity, it is rarely used systemically, but used as ointment for topical application.

There are very few antiviral drugs, and all the antiviral drugs synthesized so far are mainly structural analogues of nucleosides. Acyclovir is a structural analogue of guanine nucleoside and specific against infections caused by herpes viruses. Azidothymidine is a structural analogue of thymine nucleoside and effective against HIV infections. Amantadine is a synthetic chemical drug effective against influenza infections.

Imidazoles, Flucytosine, Nystatin, Griseofulvin and Ampho-tericin - B are important antifungal drugs. Of these imidazoles and flucytocine are synthetic drugs, while the other three are antibiotics of microbial origin. The antifungal drugs mainly act on sterols in the fungal cell wall. They either extract membrane sterols or prevent their synthesis. Imidazoles, nystatin and griseofulvin are used against dermatomycoses, while flucytosine and amphotericin – B are used against deep seated systemic infections.

18.6. MODEL QUESTIONS

Essay type questions

Give an account of structure and mode of action of penicillin, and a note on semisynthetic penicillins

Give an account of aminoglycosides, their mode action and uses

Give an account of structure, mode of action and uses of tetracyclines and chloramphenicol

Give an account of antiviral and antifungal drugs

Discuss the development of drug resistance in pathogenic microorganisms

Short answer type questions

Mode of action of penicillin

Semisynthetic penicillins

Cephalosporins

Mode of action of streptomycin

Tetracyclines

Chloramphenicol

Polymyxin – B

Anti viral drugs

Antifungal drugs

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LESSON–19: DIAGNOSTIC MICROBIOLOGY (PART – 1)

Objective : To study about the methods of diagnosis of infectious diseases

Contents

19.1. INTRODUCTION

The most important activity of the Microbiologist in medicine is to isolate and identify the causal organisms of infectious diseases. This major area of Microbiology is called *Clinical Microbiology or Diagnostic Microbiology*. For proper diagnosis, Collection of specimens, preliminary processing and transport of the specimens to the laboratory, microscopic observation of specimens, culturing of the pathogenic microbes, biochemical tests and serological tests for identification, are very important.

19.2. COLLECTION OF SPECIMENS:

In diagnosis of aetiological agent of infectious disease, collection of suitable specimens in good condition is as important as laboratory techniques for identification of the pathogen. The specimens collected should be representative of the disease process. They should be collected at proper time or course of disease because recovery of organisms is greatest at that time. Specimens should be collected without risk of contamination from actual site of infection. Sterile containers should always be used for collection. Sufficient material should be collected to ensure a complete and accurate examination.

The clinical specimens are collected in a number of ways, and the methods vary with the type of disease and organ involved.

19.2.1. Direct collection: Material such as saliva, sputum, faeces, urine and freely discharging pus can be collected directly into sterile containers. Sterilized screw capped glass bottles disposable waxed card-board or plastic cartons are used as specimen containers.

Urine is usually collected by clean catch method. After the patient has cleansed the urethral meatus (= opening) a small container is used to collect the urine. In clean catch midstream method, the first urine is voided and is not collected because it will be contaminated with microbes in the lower portion of urethra. Only the midstream portion is collected, since it will most likely to contain those microorganisms found in the urinary bladder.

Sputum is the mucous secretion expectorated from the lungs, bronchi and trachea through the mouth. Sputum is collected in specifically designed sputum cups.

For culturing of intestinal pathogens, specimens of faecal matter are collected. For the purpose, stools passed into a clean container and a small sample is obtained with a disposable spoon.

19.2.2. Swabs: For collection of material from skin and mucous surfaces and also exudates and discharges which are too small in amount for direct collection a swab can be used. Swab usually consists of a wooden or wire rod about 6 inches long with a small quantity of cotton wool tightly twisted around at one end and the other end is inserted into the cork or stopper of the container in which it is supplied. For use, the swab is withdrawn from the container, applied to the patient, and then replaced into the container for transmission to the lab or else placed in a suitable transport medium.

Collection of organisms from dry skin is more efficient if the swab is moistened with sterile broth immediately before use. Use of serum or albumin coated wool swab may increase the chances that relatively delicate bacteria such as *Streptococcus pyogenes* will reach the laboratory alive.

A throat swab is taken by depressing the tongue with a spatula, and then rubbing the swab firmly on the exposed part of the throat lesion.

The efficiency of transfer of organisms from the patient to culture media by means of a swab is low, particularly if the amount of material on swab is small, because some of it becomes entangled in the cotton wool. Alginate wool which is soluble in sodium hexametaphosphate solution is used in some cases, as it releases all the microbes.

Swab is not a satisfactory substitute for direct collection. It should be used only when inadequacy of materials or other factors make direct collection impossible.

19.2.3. Washings: Throat washings are preferred to throat swabs for virus investigations. The patient is asked to gargle with saline and expectorate it.

19.2.4. Aspiration: Aspiration using a needle and syringe is used to collect materials confined with in the patient's body such as blood, cerebrospinal fluid, effusions into body cavities, joints etc. aspirated materials are either placed in a sterile container or added to a suitable medium directly from syringe.

19.2.5. Intubation: Intubation is the insertion of a tube into a body canal or hollow organ. For example, intubation can be used to collect specimens from stomach. In this procedure a long sterile tube attached to a syringe, and the tube is passed through the nostril into the person's stomach. Specimens are then withdrawn periodically into the sterile syringe. The most common intubation tube is Levine tube.

19.2.6. Collection for anaerobic culturing: The material for anaerobic culture is best obtained by tissue autopsy or by using a needle and syringe. Use of swabs for anaerobic culturing is a poor alternative because of excessive exposure of the specimen to the deleterious effects of oxygen and drying.

19.3. PROCESSING OF THE SPECIMENS

The collected specimens should be taken to laboratory without delay so that the pathogens remain viable and physiological changes do not occur in the specimens. Inoculation of media at the bed side, though ideal, is unsuitable for general use because of the risk of contamination.

19.3.1. Preservation: If delay is inevitable, all the specimens, other than for blood cultures, are better kept in a refrigerator than at room temperature. It is still better to use transport media, which are designed to keep the pathogens alive. Proper labeling of the specimens is very essential.

19.3.2. Transport of specimens: Special precautions must be taken when transport of specimens from place of collection to diagnostic laboratories become necessary. A number of transport media are available for this purpose. These transport media usually contain only minimal nutrients to prevent over growth of fast growing bacterial contaminants. Reducing agents like sodium thioglycolate may be added to the media to protect anaerobes from oxygen. Charcoal may be added to the media to neutralize components liberated from the specimens that might be toxic to bacteria.

Stuart's transport medium (commercially available) is used to transport swab specimens. It is particularly useful to maintain the viability of sensitive bacteria like gonococci during their transport to the laboratory.

Cary-Blair transport medium is used for transport of faecal specimens and it is particularly useful for the specimens that may contain species of *Salmonella, Shigella, Vibro, Campylobacter* etc. The medium consists of only mineral salts like NaCl, CaCl etc.

Glycerol-Saline transport medium is mainly used for the enteric bacilli.

Bile-peptone transport medium is useful in field work in hot climates where cholera may occur.

For transport of specimens suspected to contain anaerobic pathogens, a plastic collection device with its own anaerobic transport medium are available.

19.3.3. Long distance transport: Some times it may become necessary to submit specimens to a reference laboratory in a distant city, thus requiring transportation by mail or other modes. In such cases, the specimens should be refrigerated immediately and packed in a styrofoam box with commercial refrigerant packs (eg. 3 M cryogel) and mailed.

For mailing of faecal specimens containing salmonellae over long distances, filter paper method may be employed. In this method, fresh faecal matter is spread fairly thin over a strip of filter paper and allowed to dry at room temperature and folded inwards using forceps. The folded specimens are then inserted in a plastic container and then mailed with all postal precautions.

19.3.4. Handling in lab: Since it is not always possible for many specimens to be processed as soon as they arrive in the laboratory, refrigeration at 4 to 6°C offers a safe and dependable method for storing many clinical samples, until they can be conveniently handled. However, some may require immediate plating (eg. specimens containing gonococci or *Bordetella pertussis*, which are very delicate pathogens), where as others must be immediately frozen (eg. Serum for subsequent antimicrobial assay). The length of the time for storage varies with individual pathogens.

19.4. DIAGNOSIS OF THE PATHOGENS

Microscopy, culturing for isolation and biochemical characterization, and serological tests are mainly employed for the diagnosis various types of pathogenic microorganisms. Microscopy is mainly used for identifying protozoan pathogens. For fungal pathogens, microscopic observation and cultural characters are used for identification. For identifying bacterial pathogens, microscopic

observations, biochemical characterization of the pathogen brought into pure culture and in some cases serological tests are used. For viral pathogens, serological tests are mainly used in diagnosis.

19.5. MICROSCOPIC METHODS OF DIAGNOSIS:

Microscope is the most important tool in observation and identification of microorganisms. Many infectious agents can readily be identified with only a few simple stains and a basic microscope under high power or oil immersion. Phase contrast microscope is routinely used in some laboratories for observation of unstained bacterial cultures. Electron microscope, though is of greatest importance in observing viral pathogens, since it is a major capital investment, few laboratories use it on routine basis.

There are various methods of preparing the specimens for microscopic examination.

19.5.1. Direct examination of wet mounts: Many clinical specimens may be examined directly under bright field or phase contrast microscope, preferably as soon as they are collected from the patient. Specimens that can be applied directly to the surface of a slide for this purpose include sputum, exudates from lesions, aspirated fluids, stool, vaginal discharge and urine sediment. If the material is too thick for observation, it can be diluted with equal parts of sterile saline. This type of preparation is known as direct wet mount.

Wet preparations are often used to detect motile trophozoites of faecal parasites such as *Giardia lamblia* and *Entamoeba histolytica*. The eggs and cysts of other parasites, larvae and adult worms are also often seen in wet mounts made from freshly passed stool. *Trichomonas vaginalis* can be seen moving in wet mounts prepared from vaginal discharge material or from fresh urine specimens. Parasites can also be found in direct wet mounts made from material aspirated from duodenum, lung or abscess contents.

Stool may be examined directly by phase contrast microscopy for certain bacterial pathogens also. Eg. *Campylobacter jejuni* causing diarrhoea, and *Vibrio cholerae* causing cholera.

Examination of blood can establish the diagnosis of relapsing fever or leptospirosis. Trypanosomes and other flagellates may also be seen in direct preparations of blood.

19.5.2. Slightly modified direct preparations:

19.5.2.1. KOH Prepartion: The use of 10% Potassium hydroxide (KOH) will help to distinguish fungal elements in a direct wet preparation of clinical material. Proteinaceous components such as host cells are partially digested by the alkali, leaving intact the polysaccharide containing fungal

cell walls. The material to be examined whether fluid or skin or nail scrapings, is added to a drop of 10% aqueous KOH on a glass slide and may be examined after some time for the presence of fungal elements. Gentle healing may speed up the activity of KOH. A small amount of lactophenol cotton blue can be added to 10% KOH for enhanced visibility of fungal elements.

19.5.2.2. Negative staining: The presence of encapsulated yeast *Cryptococcus neoformans*, particularly in CSF specimens, can be determined by adding equal parts of India ink or Nigrosin stain to the sediment of the spinal fluid. The polysaccharide capsules will exclude the particles in ink and the capsule will appear as a clear halo around the organisms.

19.5.2.3. Iodine preparations: Lugol's Iodine is often added to the direct wet mounts of faecal material to differentiate parasitic cysts from host white blood cells. Many cysts will take up the iodine appearing light brown color, other objects remain clear.

19.5.2.4. Methylene blue preparations: Loeffler's methylene blue may be added to wet mounts of faeces (equal amounts of stain and faeces) for determination of the presence of leucocytes. The presence of many polymorphonuclear leucocytes is indicative of invasive disease such as bacterial dysentery, as opposed to the non-inflammatory nature of the diarrhoea of most parasitic diseases or certain food poisonings.

19.5.2.5. Antiserum preparations: By adding specific antiserum to a wet preparation of selected clinical material, certain organisms may be identified by a visible antigen-antibody reaction, called quellung reaction. Organisms with capsules such as *Haemophilous influenzae* and *Streptococcus pneumoniae*, exhibit apparent capsular swelling in the presence of homologous antibody. Pathogens in CSF and sputum can be identified rapidly by this method.

19.5.2.6. Dark field microscopy: Certain bacteria are very thin for direct microscopic examination. These bacteria, primarily spirochetes such as *Borrelia* and *Treponema* are best visualized under dark field microscopy, a method of allowing light to be reflected off the surface of the object, which appears brightly lit against black back ground. This method is used most often for the demonstration of motile treponemes in the exudates from a primary chancre of syphilis. Motile *Campylobacter* in stools may also be seen under dark field microscopy.

19.5.3. Fixed and stained preparations:

Examination of stained material, either direct clinical specimens or samples of growth from cultures, is the most useful method for presumptive

identification of bacteria and presence of certain viruses, and for definitive identification of many fungi and most parasites.

Simple staining with a single dye, differential staining using more than one dye, fluorescent stains, antibody-conjugated stains, enzyme conjugated stains etc are used for identification of different pathogens in clinical specimens.

19.5.3.1. Gram staining: It is the most popular differential staining technique for identification of bacteria. In this procedure crystal violet is the first stain and saffranin is the second stain. Those that retain the first stain and appear violet are called gram positive bacteria and those that lose the primary stain after cleaning with an organic solvent and take up the saffranin and appear red are called gram negative bacteria. By the gram reaction and morphology of the isolated bacteria a tentative identification may be possible.

Gram staining can also be used to examine clinical material directly for assessing its suitability for cultures. For example, by staining sputum, the number of bacterial cells present can be assessed. High bacterial count indicate contamination of the sputum with normal flora, and unsuitable for culture. Urine samples can also be gram stained to determine the presence of significant bacteria.

19.5.3.2. Ziehl-Neelsen acid fast stain: It is a specific differential staining for *Mycobacterium* and used for observation of samples suspected of tuberculosis. Carbolfuschin is the primary stain and malachite green is the alternate stain. The smears are first stained with carbolfuschin and washed with acid alcohol mixture and stained with the alternate stain. If the carbolfuschin is retained even after washing with acid-alcohol mixture the cells appear red and those that lose the primary stain and take up the alternate stain appear green. Whether it is made routinely or not for sputum and other specimens sent for general bacteriological investigation depends on the local prevalence of tuberculosis. It is also used to identify *Nocardia* and coccidean parasites such as *Cryptosporidium* species.

19.5.3.3. Methylene blue staining: For microscopic examination diphtheria pathogen, *Corynebacterium diphtheriae*, methylene blue stain is used. It is a simple stain, and by the metachromatic granules of diphtheria bacilli.

The stain is also used to observe fusiform bacteria and spirochetes in oral specimens.

19.5.3.4. Giemsa staining: The giemsa stain (containing eosin, glycerin and methanol) is used for staining protozoan parasites such as *Trypanosoma, Leishmania* etc., bacteria such as *Leptospira*, *Borrelia, Rickettsia* etc., and viral inclusion bodies such negri bodies.

To identify the blood-borne parasites, thin films of blood samples are fixed with methanol (to preserve the red cell morphology) and stained, which reveal nuclei and cytoplasmic features of parasites.

Giemsa stain is also used to visualize inclusion bodies in cells infected by viruses or bacteria either directly from clinical material such as the base of a suspected herpetic vesicle or a corneal scrapings from a suspected case of *Chlamydia trachomatis* conjunctivitis or for staining a monolayer of infected cell cultures.

19.5.4. Fluorescent microscopy:

Certain dyes called fluorochromes or fluors have the property of becoming excited after absorbing UV light. As the excited molecules return to their normal state, they release excess energy in the form of visible light of longer wave length. This property of becoming self luminous is called fluorescence. Fluorescent dyes commonly used in diagnostic labs are 1. Acridine orange and 2. Rhodamine-auramine stain.

19.5.4.1. Acridine orange: The fluorochrome acridine orange binds to nucleic acid and is commonly used to observe bacteria in blood culture media. The stain is also used for detection of cell wall deficient bacteria such as mycoplasmas in broth cultures and from suspected colonies on the agar plates.

19.5.4.2. Rhodamine-auramine stain: The mycolic acid in the cell walls of *Mycobacterium* has an affinity for the fluorochromes auramine and rhodamine. The dyes will bind to mycobacteria which appear bright yellow orange against a greenish back ground. The counter stain, potassium permanganate, helps to prevent non specific fluorescence.

19.5.5. Antibody conjugated stains:

The most specific detectors of microbial pathogens (or antigens) are antibodies that bind tightly to antigens against which they are directed. If antibodies are conjugated to a dye or chromogenic substrate that allows their reactive sites to interact with homologous antigens, they

serve as visible flags for the presence of that antigen. Antibodies bound to the fluorochrome fluorescein isothiocyanate (FITC) are used to visualize bacteria. FITC fluoresce an intense apple green when excited. Fluorescein conjugated antibodies are used to detect *Bordetella pertussis* in nasopharyngeal smears from children suspected of having whooping cough, and *Legionella* species in respiratory specimens or tissue of patients with Legionnaires disease.

Monoclonal antibodies have been successfully conjugated to fluoresceine for detection of Chlamydiae, herpes, respiratory syncytia, rabies and other viruses, treponemes and other pathogens in directly stained clinical material.

Fluorescence conjugated antibodies are also used to identify pure cultures of organisms such as *Actinomyes* spp., *Legionella* spp., *Streptococcus pyogenes* and *Nessieria gonorrhoeae*.

19.5.6. Enzyme conjugated stains:

For those laboratories that do not have access to a fluorescent microscope, enzymes that catalyze the production of a coloured precipitin product are an excellent alternative as specific antibody detector reagents.

Horseradish peroxidase is a small enzyme that produces an orange brown precipitate as its easily visible end product. Conjugated to antibodies, it is known as immunoperoxidase stain, and it is used to detect cytomegalovirus and other virus proteins or nucleic acids in cells. The most commonly used substrates for the action of horseradish peroxidase are α-naphthol, 3,3,-diaminobenzidine etc.

Alkaline phosphotase is another commonly used enzyme to conjugate with the antibodies. When alkaline phosphatase enzyme is conjugated to antibodies the substrate commonly used is nitrophenylphosphate. The enzyme reacts with the substrate to produce a yellow precipitate as its end product. It is used as a detector for viral antigens as well as inclusions of chlamydiae.

19.5.7. Electron microscopy:

The greatest use of electron microscope has been for detection of virus particles in the infected tissue or clinical specimens. All enteric viruses can be identified in either direct electron microscopic preparation of faecal material or by immuno-electron microscopy in which faecal samples are mixed with specific antiviral antibody before staining.

Since electron microscope is a major capital investment, few laboratories have capability to use these techniques on routine basis.

19.6. CULTURAL METHODS OF DIAGNOSIS

Culturing of the pathogen from the specimens is the most important aspect of diagnosis, and is mainly carried out for bacteria, the most important group of human pathogens. A pure culture of the pathogen is essential for identification, and for performing drug sensitivity tests and biochemical tests.

19.6.1. MEDIA: A number of culture media are used for isolation of bacteria from the specimens. They include general purpose media, differential media, selective media, characterization media etc.

19.6.1.1. Blood agar medium: Most specimens received in a clinical Microbiology laboratory are plated on blood agar, since it supports all but the most fastidious, clinically significant pathogens and also because most microbiologists have become adept at making decisions about the identification of bacteria from colony morphology on blood agar. Blood agar medium usually consists of all the ingredients of Nutrient agar medium and in addition 5% blood. The source of blood may be sheep (USA) or horse (Europe).

Composition:		
	Beef extract	3.0 g
	Peptone	5.0 g
	Blood	50 ml (5%)
	Agar	20 g
	Distilled water	1000 ml

Culturing on blood agar make possible various types of haemolysis, which permits differentiation of some species of bacteria. Three haemolytic patterns generally observed on blood agar are

Alpha(α) haemolysis : greenish to brownish halo around colony due to partial lysis of red blood cells
Eg. *Streptococcus pneumoniae*

Beta (β) haemolysis : a clear zone around the colony due to complete lysis of red blood cells.
Eg. *Streptococcus pyogenes Staphylococcus aureus*

Gama (γ) haemolysis : No change in the medium
Eg. *Staphylococcus epidermidis*

19.6.1.2. Chocolate agar: This medium uses the same base as blood agar. Originally, blood was added to the molten base and temperature raised enough to lyse partially the red blood cells (about 85 °C) causing the medium to turn to a chocolate brown colour. Now, haemoglobin and other nutrients present in the lysed red blood cells, hemin (also known a x-factor) and coenzyme nicotine adenine dinucleotide (called v-factor) are added as supplements to nutritionally rich agar base. *Neisseria gonorrhoeae* and *Haemophilous* sp. and other fastidious organisms will grow best in the medium.

19.6.1.3. Chopped meat broth: Cooked meat medium (boiled minced meat in peptone water) supports the growth of most aerobic and anaerobic bacteria, and is commonly used in addition to the solid media for primary culture of pus and swabs from many sites.

Where there is a delay in transport of the specimens or when the patient has been on antibiotic treatment, growth may occur in cooked meat medium, even when there is none on the primary culture plates. The colonies on the meat medium are then sub-cultured on blood agar for further growth and other studies.

19.6.1.4. MacConkey agar: It is the most frequently used primary selective and differential agar. It consist bile salts and crystal violet to inhibit growth of gram positive cocci and pH indicator, neutral red, to impart differential characteristics. Lactose is the sole carbon source and lactose fermenting bacteria produce colonies that are various shades of red where as non-lactose fermentors produce colourless colonies.

Composition:		
	Peptone	17.0 g
	Lactose	10.0 g
	Bile salts	1.5 g
	Proteose peptone	3.0 g
	NaCl	5.0 g
	Neutral red	0.03 g
	Crystal violet	0. 001 g
	Agar	20.0 g
	Distilled water	1000 ml
	pH	7.1

This medium is mainly used for isolation of bacteria from faeces, urine, pus samples, wound swabs and many ulcerative skin lesions, especially those on the lower half of the body.

19.6.1.5. Eosine methylene blue (EMB) agar: It differentiates between lactose fermentors and non-lactose fermentors. EMB contain lactose, salts and two dyes namely Eosin and Methylene blue. *Escherichia coli,* a lactose fermentor, produce dark colonies with or without metallic sheen, while *Salmonella typhi,* a non-lactose fermentor appear colourless.

Composition:		
	Lactose	5.0 g
	Sucrose	5.0 g
	Peptone	10.0 g
	K_2HPO_4	12.0 g
	Eosin	0.4 g
	Methylene blue	0.065 g
	Agar	15.0 g
	Distilled water	1000 ml
	pH	7.2

19.6.1.6. Lowenstein-Jensen medium: It is a selective medium for isolation of *Mycobacterium tuberculosis* from sputum and other specimens. The medium contain glycerol, egg yolk, asparagine and mineral salts. It is supplemented with malachite green, an antibiotic dye and other antibiotics like cycloserine to inhibit the fast growing bacteria in the samples

Composition:		
	KH_2PO_4	2. 4 g
	$MgSO_4$	0.24 g
	Magnesium citrate	0.6 g
	Asparagine	3.6 g
	Glycerol	12 ml
	Malachite green	20 ml
	Homogenised whole egg	
	Or Egg yolk	1000 ml
	Distilled water	600 ml
	Cycloserine	400 µm

19.6.1.7. Loeffler's medium: It is a specific medium for isolation of *Corynebacterium diphtheriae*, the causative agent of Diphtheria. It mainly consists of serum (25%) and infusion broth (75%). It enhances production of metachromatic granules in the cells of the pathogen.

Composition:		
	Serum	750 ml
	Infusion broth	250 ml
	Dextrose	2.5 g
	pH	7.6

19.6.1.8. Other media: Apart from the above media, a number of selective or differential media are also used routinely. They include

Tripple Sugar Iron (TSI) medium: It is used to differentiate bacteria by their ability to utilize dextrose, lactose and sucrose to liberate H_2S, when growing in the medium containing Ferrous ammonium sulphate or Sodium thiosulphate as sulphur source.

Mannitol-salt agar (MSA): It is a highly selective medium for isolation of coagulase positive *Staphylococcus aureus*.

***Salmonella* - *Shigella* agar (SSA):** It is a selective medium for recovery and isolation of *Salmonella* and *Shigella* from stools and food materials.

Bismuth sulphate agar (BSA): It is a selective medium for cultivation of *Salmonella typhi*.

Trypticase Soy broth: It is also used for testing antibiotic sensitivity by Kirby- Bauer method.

Cystine trypticase Agar: It is used for determination of fermentation reactions of *Neisseria* species.

Bordet-Gengou medium: It is used for isolation of *Bordetella pertussis*

Tellurite blood agar: It is used for isolation of *Corynebacterium diphtheriae*.

Levinthal agar: It used for isolation of *Haemophilous* species.

Thayer's Martin agar: It is a selective medium for isolation of *Neisseria gonorrhoeae* and *N. meningitidis*.

Deoxycholate citrate agar (DCA): It is a differential medium used for isolation of lactose fermenting and non-lactose fermenting organisms of enterobacteriaceae.

Urea broth/agar: It is used to detect the ability of an organism to hydrolyze urea. The positive *Proteus* can be differentiated from *Salmonella* and *Shigella*.

Simmon's citrate agar: It is a medium used in IMVC tests to determine the ability to utilize citrate as a sole source of carbon.

MR-VP broth: It is used to detect the production of acids or neutral compounds in IMVC tests

Muller-Hinton agar: It is used for antibiotic sensitivity testing by Kirby- Bauer method. It does not contain any fermentable sugars which on utilization produce acids and change pH.

19.6.2. CULTURING OF THE SPECIMENS:

Different types of specimens are sent to the diagnostic laboratories and methods used for culturing of the specimens differ.

19.6.2.1. Blood culturing:

Since bacteremia frequently portends life-threatening illness, its early detection is essential. Blood culture is the single most important procedure to detect systemic infection due to bacteria. Two important examples with early bacteremic stage are typhoid caused by *Salmonella typhi*, and meningitis caused by *Neisseria meningitides*.

The standard blood culture procedure is to remove sufficient amount of blood aseptically from a vein and inject it into a blood culture bottle containing an anticoagulant and an all purpose culture medium.

Several factors determine whether blood cultures will yield positive results: the volume of blood cultured, the dilution of blood in the culture medium, the duration of incubation. For adults, a 20 ml blood sample is usually taken and half is placed in an aerobic blood culture bottle and half in an anaerobic one. However, different volumes of blood may be required for the many different blood cultures systems that exist. An optimum dilution of blood in a liquid culture medium is 1:150 to 1:300, this minimizes the effects of the antibody, complement and white blood cell antibacterial systems that are usually present in the blood. Since such large dilutions are impractical in blood cultures, most such media contain 0.05% sodium polyaetholesulfonate (SPS) which inhibits the antibacterial systems. However, SPS also inhibits growth of some neisseriae, anaerobic gram positive cocci etc. and in such cases, alternative blood culture systems should be used.

Two culture systems are set up, with one bottle being incubated aerobically and the other anaerobically. Blood culture bottles are incubated at 35 °C and examined daily for up to 7 days. Clinically significant bacteria are generally recovered with in this period.

19.6.2.2. Urine culture: Urine culture is used to detect urinary tract pathogens. Urinary tract infections are usually caused by *E. coli, Neisseria gonorrhoeae*, species of *Proteus*, *Klebsiella* etc.

Urine is normally sterile but samples are often contaminated during collection. Bacterial counts of more than 100 million per litre indicate urine infection while counts less than 10 million

per litre indicate contamination. Intermediate counts are equivocal. Hence, culturing methods to quantify the count are used. The specimens are used with or without centrifugation.

Strip method: Using standard strips of sterile filter paper bent at one end to form 6 x 12 mm part, dipped in urine and then pressed on surface of MacConkey agar medium.

Loop method: Using a standard inoculation loop (5 mm), loopful of urine is taken from the sample and plated out on medium.

Dip-slide method: In this method, slides coated with nutrient medium are dipped in urine, drained and incubated.

By referring to the standard curves, bacterial counts can be estimated from the number of colonies developed.

When infections are strongly suspected, the urine samples are centrifused and deposit is resuspended in saline and used for isolation of the pathogens.

Since bacteria grow rapidly in urine, the samples should be used for culture within three hours, or alternately refrigerated not more than for 24 hours.

19.6.2.3. Faecal cultures: Faecal specimens, either collected directly or swabs, are used to isolate enteric pathogens. The important pathogens that are commonly isolated from faecal cultures are *Vibrio cholerae, Salmonella typhi* and *Shigella dysenteriae.*

The faeces specimens diluted with sterile saline are plated out on a variety of selective media for the isolation of specific bacteria or characterization of intestinal parasites. The common media used are MacConkey agar, Salt nutrient agar, citrate agar etc.

The plates are incubated aerobically at 37°C and examined daily. The first characterization of cultures is into lactose fermentors and non-lactose fermentors. Other biochemical tests are later used to characterize the bacteria isolated.

19.6.2.4. Culturing of sputum: Sputum is plated out on two blood agar plates, incubated aerobically and anaerobically. Sputum may be diluted with sterile saline if it is very thick. A plate of chocolate agar can be inoculated to improve the chances of isolating *Haemophilus influenzae*.

For isolating *Mycobacterium tuberculosis*, sputum is treated to destroy contaminants, inoculated on to Lowenstein-Jensen medium, and incubated for at least 2 weeks because the pathogen is a slow grower.

19.6.2.5. Throat swabs: The swabs are plated out on blood agar medium and incubated aerobicallv and anaerobically. If there is any reason to suspect diphtheria, swab is used to inoculate on blood tellurite agar and Loeffler's media. The plates are examined after 24 hours.

19.6.2.6. Cerebrospinal fluid (CSF): It is streaked out on a plate of blood agar or chocolate agar, which is incubated aerobically with 2-10% CO_2. Anaerobic plate culture is not necessary as a routine, but may be desirable in special circumstances. CSF is cultured mainly to isolate *Neisseria meningitidis* which cause meningitis, and also for other pathogens causing meningo-encephalitis.

19.6.2.7. Culturing of specimens from wound, burns, abscesses, pus etc: The specimens are routinely plated out on Blood agar medium and MacConkey agar medium, and incubated aerobically. The blood agar plates are also incubated anaerobically.

Additional media can be inoculated for special purposes. The plates are inspected after incubation at 37 °C for 24 hours.

19.7. SUMMARY

The most important activity of the Microbiologist in medicine is the isolation and identification of the causal organisms of infectious diseases. For diagnosis of aetiological agent, collection of specimens is carried out in a number of ways. Specimens such as saliva, sputum, faeces, urine and freely discharging pus are collected directly into sterile containers. When the specimens are too small in amount for direct collection sterile swabs are used. Throat washings are collected for virus investigations. Aspiration using syringe is used to collect materials in the patient’s body such as blood, cerebrospinal fluid etc. Intubation tubes are used for collection of stomach contents. The collected specimens are properly labeled and immediately taken to the laboratory. If delay is inevitable, the specimens are immediately refrigerated and stored in transport media with minimal nutrients. Microscopy is the most important tool in observation and identification of microorganisms. Direct wet mounts are prepared from specimens like sputum, exudates from lesions, aspirated fluids, stool, vaginal discharge, urine sediment etc and observed for protozoan parasites and bacteria like *Campylobacter jejuni*. Negative staining is used for observations of specimens with conspicuous capsules. Dark field microscopy is used for spirochete pathogens. Gram staining is the most common differential staining used for tentative identification of bacteria. Acid fast staining or fluorescent staining is used for identification of mycobacteria in specimens. Antibody conjugated stains and enzyme conjugated stains are used for

specific pathogens. For isolation of pathogens a number of bacteriological media are used which include blood agar medium, chocolate agar medium, chopped meat broth, MacConkey agar medium, EMB medium, Lowenstein-Jensen medium, Loffler's medium, tellurite agar agar medium etc. In addition to these a number of specific media are used for culturing of bacteria for biochemical tests.

Blood culture is the single most important procedure to detect systemic infection due to bacteria like *Salmonella typhi*, and *Neisseria meningitides*. The standard blood culture procedure is to remove sufficient amount of blood aseptically from a vein and inject it into a blood culture bottle containing an anticoagulant and an all purpose culture medium.

Urine culture is used to detect urinary tract pathogens. The specimens are used to isolate bacteria on media like MacConkey agar medium to estimate the total bacterial counts. When infections are strongly suspected, the urine samples are centrifused and deposit is resuspended in saline and used for isolation of the pathogens.

Faecal specimens, either collected directly or swabs, are used to isolate enteric pathogens like V*ibrio cholerae*, *Salmonella typhi* and *Shigella dysenteriae*. The faeces specimens diluted with sterile saline are plated out on a variety of selective media for the isolation of specific bacteria or characterization of intestinal parasites.

Sputum is used for isolation of throat and respiratory tract pathogens like *Mycobacterium tuberculosis.* For isolation of *M. tuberculosis*, sputum is treated to destroy contaminants, inoculated on to Lowenstein-Jensen medium, and incubated for at least 2 weeks because the pathogen is a slow grower.

Throat swabs are used for isolation of pathogens like *Corynebacterium diphtheriae*. Swab is used to inoculate on blood tellurite agar and Loeffler's media. The plates are examined after 24 hours.

Cerebrospinal fluid is cultured mainly to isolate *Neisseria meningitidis* which cause meningitis, and also for other pathogens causing meningo-encephalitis.

For culturing of specimens from wound, burns, abscesses. pus etc. the specimens are routinely plated out on Blood agar medium and MacConkey agar medium, and incubated at 37 °C for 24 hours.

19.8. MODEL QUESTIONS

Essay type questions

Discuss the methods of collection and processing of specimens for diagnosis

Discuss the microscopic methods used for diagnosis of pathogens

Discuss the methods of culturing of blood, urine and faecal specimens

Short answer type questions

Collection of clinical specimens
Transport of specimens
Wet mounts
Differential staining
Antibody conjugated stains
Blood agar medium
Differential media
Blood culturing
Urine culture
Faecal culture

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LESSON–20: DIAGNOSTIC MICROBIOLOGY (PART – 2)

Objective: To study the biochemical and serological methods of diagnosis of bacterial infections.

Contents:

20.1. INTRODUCTION

In the first part of this lesson (lesson – 19) the collection of specimens and their processing, microscopic methods of diagnosis of the pathogens, culture media commonly used in diagnostic laboratories to isolate the bacterial pathogens and culturing of various types of specimens are explained. In this lesson, the biochemical tests commonly employed for identification of pure cultures of pathogenic bacteria, and serological tests used for diagnosis of bacterial diseases are explained.

20.2. BIOCHEMICAL TESTS USED FOR DIAGNOSIS:

Maintenance of pure cultures and growing them on specialized media are very important for biochemical characterization and identification of pathogenic bacteria. A number of biochemical tests are performed on pure cultures, and the type of tests used depends up on the pathogen involved.

20.2.1. IMVC TESTS:

Four biochemical tests widely employed in the characterization of enteric bacteria are 1. Test for indole production 2. Fermentation of glucose producing acids 3. Production of neutral compounds in fermentation of glucose and 4. The ability to utilize citrate as sole carbon source. These tests are called Indole production test, Methyl red reduction test, Voges-Proscauer test and citrate utilization test, and are commonly called IMVC tests.

20.2.1.1. INDOLE TEST:

The ability of an organism to degrade the aminoacid tryptophan can be detected by testing for Indole, the product of tryptophanase enzyme. The enzyme degrades tryptophan in peptone and other components of the medium to indole, skatole and indole acetic acid. When combined with certain aldehydes, indole yields a red coloured product.

The test organism is grown in a medium rich in tryptophan and the medium is then tested for the presence of indole end product, using aldehyde indicator, either Kovac's reagent or Ehrlich reagent. For testing, 2 ml of the medium is taken in another test tube after 24 hour incubation and 0.5 ml (5 drops) of Kovac's reagent is added to the broth, tube is shaken gently and observed for pink colour in a ring around the interface between the broth and the reagent which rises to the surface.

20.2.1.2. MR TEST &VP TEST:

Organisms that utilize glucose may do so by producing abundant acidic end products such as formate and acetate from the intermediate pyruvate metabolites or they may metabolize the carbon compounds to acetoin and butanediol which are more neutral in pH. The methyl red reduction and Voges-Proskauer tests detect the presence of the end products of these two divergent metabolic pathways. The same substrate broth is used for both the tests. MR/VP broth contains peptone, glucose and buffers. The medium is commercially available.

The tubes with 5 ml of MR/VP broth are inoculated with the test organism and incubated at $37^{O}C$ for at least 48 hours. Then the broth is divided into two equal aliquots of 2.5 ml each in two test tubes, and one is tested for acidic end products and another for neutral end products.

20.2.1.2.1. MR test: It is a test for acid fermentation. To the 2.5 ml of substrate 0.5 ml (5 drops) of MR reagent is added and observed for colour. Methyl red is a pH indicator. It is red at pH 4.0 or below, and yellow at pH 6.0 or above. If the indicator remains red, the test is positive, if it turns to yellow, it indicates that the pH of the medium is greater than 6 and is considered negative. If the reagent remains orange the test must be repeated after a longer incubation period.

20.2.1.2.2. VP test: It is a test for production of neutral compounds. To 2.5 ml of broth suspension 0.6 ml (6 drops) of VP reagent - A (α-naphthol) is added and then 0.2 ml (2 drops) of reagent -B (40% KOH) is added. The tube is gently shaken and allowed to stand for 15 minutes, and observed

for colour change. Pink or red colour in the medium is indicative of positive test. It shows the presence of acetoin (acetyl methyl carbinol) in the broth. In the negative test, the broth appears colourless or yellow.

20.2.1.3. CITRATE UTILIZATION TEST:

Certain organisms are able to utilize a single substrate as a sole carbon source. The ability to use citrate can help to differentiate among the members of Enterobacteriaceae. Simmon's Citrate agar medium is used for the purpose. It consists of sodium citrate as sole source of carbon, ammonium phosphate as nitrogen source, salts and bromothymol blue as pH indicator. The medium is available commercially. The test organism is inoculated to the surface of an agar slant of the medium, incubated for 1 to 4 days at 37°C. Growth of the organism on the slant with change of colour from green to blue is evidence of positive test, indicating that the organism was able to grow citrate as sole carbon source. During the growth, ammonium phosphate is used as nitrogen source with production of ammonia which results in shift of pH to alkaline side. Hence medium turns from green to blue. Luxuriant growth on the slant without a blue colour may indicate a positive test but the test should be repeated.

IMVC reactions of some common enteric bacteria are as follows

Organism	**I**	**M**	**V**	**C**	
Escherichia coli	+	+	-	-	
Salmonella typhi	+	-	+	-	
Shigella dysenteriae	v	+	-	-	(v=variable)
Aerobacter aerogenes	-	-	+	+	

20.2.2. CATALASE TEST:

Catalase is an enzyme that splits hydrogen peroxide into water and oxygen. It is produced by most of the aerobes and facultative anaerobes. The ability of the test organism to produce the enzyme catalase is determined by the test. When hydrogen peroxide is added to colony growth of the test organism on agar medium, bubbles of oxygen are formed rapidly giving frothy appearance to the colony.

This test can be performed to distinguish

Staphylococcus (+) and *Streptococcus* (-)

Bacillus (+) and *Clostridium* (-)

20.2.3. COAGULASE TEST:

Coagulase is an enzyme which clot human or rabbit plasma. The enzyme along with the coagulase reacting factor (CRF) present in plasma converts fibrinogen into fibrin. Coagulase production is one of the important virulence factors in *Staphylococcus*. This test is commonly used to distinguish pathogenic *Staphylococcus aureus* from non-pathogenic *Staphylococcus epidermidis* and *Micrococcus* spp.

The coagulase production can be detected either by slide test or tube test.

20.2.3.1. Slide test: It is commonly used to screen quickly a large number of *Staphylococcus* isolates. The presence of a cell surface associated substance that binds fibrinogen and thus allows aggregation of organisms in plasma containing fibrinogen is detected by observation of clumps of cells. For this test, a drop of plasma is placed on a slide and a loopful of fresh bacterial culture is added and mixed. Prompt clumping of the cells or formation of a precipitate within 20 to 30 seconds indicates positive test.

20.2.3.2. Tube test: This test detects free coagulase in the medium which forms a clot. In the test, about 0.5 ml of rabbit plasma is added to an equal volume of broth culture of the test isolate in a test tube and incubated overnight. The formation of a clot can be detected by tilting the tubes and clot formation is positive for the test. It is more sensitive than the slide test, but takes more time to perform.

20.2.4. OXIDASE TEST: It is performed to identify *Neisseria* species presumptively, and to characterize gram-negative bacilli initially. Oxidase test indicates the presence of the enzyme Cytochrome oxidase. This iron containing porphyrin enzyme participates in the electron transport mechanism and in the nitrate metabolic pathways of some bacteria. The test can be performed by flooding agar surface of a petri plate on which a number colonies developed, with the reagent (1% solution of tetramethyl para phenylene diamine). The oxidase positive colonies turn purple within 10 seconds.

20.2.5. BILE SOLUBILITY TEST: *Streptococcus pneumoniae* possess an active autocatalytic enzyme that lyses the organism's own cell wall during cell division. Under the influence of bile salts (Sodium deoxycholate) the organism rapidly autolyse. Other α- streptococci do not possess such an active enzyme and will not dissolve in bile. Hence the test is routinely used to distinguish *Streptococcus pneumoniae* from other streptococci. In the test, a few drops of bile salt solution is added to a fresh culture of *Streptococcus pneumoniae* and incubated at 37°C. Complete clearance of the broth in about 30 minutes is the positive result.

20.2.6. OPTOCHIN SENSITIVITY TEST:

Optochin (Methyl hydroxy cupriene hydrochloride) is a water soluble quinine derivative which selectively inhibits the growth of *Streptococcus pneumoniae*. It has no effect on other streptococci. Hence, optochin is impregnated into filter paper discs and sensitivity to it studied following the procedure of Kirby-Bauer method of antibiotic sensitivity testing.

The growth of *S. pneumoniae* near the disc is inhibited. The zone of inhibition of about 14 mm should be considered a positive test.

S. pneumoniae	—	positive
S. faecalis	—	negative

20.2.7. RAPID UREASE TEST:

Species of *Proteus, Klebsiella, Citrobacter* and some *Haemophilus* spp., yeast *Cryptococcus neoformans*, several other bacteria and fungi produce enzyme urease, which hydrolyzes urea into ammonia, water and CO_2. The alkaline end products cause the indicator phenol red to change from yellow to red. This test can be used as a component of screening methods for lactose negative colonies on differential media plated with material from stool specimens, helping to differentiate *Salmonella* and *Shigella* which are urease negative from urease positive non-pathogens. For this test urea broth is used as medium.

20.2.8. H_2S PRODUCTION TEST:

Some members of Enterobacteriaceae produce hydrogen sulphide when cultured on substrate containing sugars and sulphur containing aminoacids. H_2S production can be detected by culturing the test organisms on Triple Sugar Iron (TSI) medium which contain ferrous sulphate.

In the test, the test organism is grown on TSI medium at 37°C for 24 hours. The development of a black insoluble precipitate indicates positive test.

Escherichia coli — Negative

Salmonella paratyphi — Positive

20.2.9. LITMUS MILK TEST:

Litmus milk consists of skim milk with sufficient litmus solution added to give a pale mauve colour (litmus solution is added in 10 ml amounts to one litre of skim milk). Litmus milk indicates both saccharolytic and proteolytic properties of bacteria by detecting whether they ferment lactose or degrade casein. Fermentation of lactose in the medium produces sufficient amounts of acids and milk turns pink. The production of large amounts of acids results in clotting of casein and gas production. This process is referred to as stormy fermentation. Proteolytic bacteria may degrade casein to transparent solution of soluble products.

20.2.10. OTHER TESTS:

A number of other tests are also performed for biochemical characterization of various pathogenic bacteria and include Niacin formation and Nitrate reduction tests for *Mycobacterium tuberculosis*, amidase test for atypical mycobacteria, Phosphatase test for *Staphylococcus aureus*.

Other general biochemical characteization tests include gelatin liquifaction test, Phenyl alanine deaminase test, test for decarboxylases, gluconate test, Aryl sulphatase test, Tween hydrolysis test etc.

20.3. SEROLOGICAL TESTS USED FOR DIAGNOSIS:

The culturing of certain viruses, bacteria, fungi and parasites from clinical specimens may not be possible because the methodology remains undeveloped (eg. *Treponema pallidum*, hepatitis A,B,C; EB virus etc) or is unsafe (Rickettsiae). In such and other cases serological diagnostic techniques are employed.

Although humans produce several different classes of antibodies routine diagnostic serological methods are usually used to measure only two antibody classes Ig M and Ig G. Normally humans produce both Ig M and Ig G in response to most pathogens. In most cases Ig M

is produced by a patient only after the first interaction with a given pathogen and afterwards the cells that were producing Ig M switch to producing Ig G, often more specific for the antigen. A second encounter with the same pathogen will usually induce only Ig G response, because B-lymphocytes retain memory of this pathogen.

Antibodies can be detected in many different ways. Serological tests for agglutination, precipitation, enzyme linked antibody detection are commonly used in diagnostic laboratories.

20.3.1. AGGLUTINATION TESTS:

The most basic tests for antibody detection are those that measure the antibody produced by a host to determinants on the surface of bacterial agent in response to the infection with that agent. The specific antibodies bind to surface antigens of bacteria and cause the bacteria to clump together to form visible aggregates. The reaction is called agglutination. Bacterial agglutination tests can be performed on the surface of slides or in tubes. Tube agglutination test are more sensitive, since longer incubation period allow more antigen and antibody to interact. Slide agglutination tests are more rapid. The tests can be performed with homologous antigens on pathogen surface, or heterophile antigens on nonpathogenic bacteria or with antigens coated on special particles.

20.3.1.1.Tests with homologous antigens: Diseases commonly diagnosed by this method include typhoid, tetanus, leptospirosis, brucellosis etc.

20.3.1.1.1. Widal test:

It is a routinely used serological test for detecting the antibodies against *Salmonella typhi* and *Salmonella paratyphi* which cause typhoid and paratyphoid respectively. These pathogens contain both H (flagellar) antigens and O (somatic) antigens. If a patient with fever is suspected to be infected with *S. typhi* or *S. paratyphi*, widal test is performed to detect the antibodies against O and H antigens in the serum of the patient's blood.

In the test, the patient's serum (in different concentrations) is mixed with commercially available H and O antigens of *S. typhi* and *S. paratyphi* separately in special test tubes and incubated in a water bath at 37°C over night. H- agglutination leads to the formation of loose

cotton woolly clumps, while O-agglutination is seen as a disc like pattern at the bottom of the tube. In both the supernatant is clear.

20.3.1.2. Tests with heterophile antigens:

Antibodies formed against the surface antigens of a pathogenic bacterium may some times react with surface antigens of nonpathogenic bacteria. Such antibodies are called heterophile antibodies. For example, *Rickettsia rickettesii* which cause Rocky mountain spotted fever stimulate the production of antibodies in the patient's blood which can react with *Proteus vulgaris*, a common normal flora member of intestinal tract. In such cases, nonpathogenic bacteria can be used in agglutination tests instead of pathogenic bacteria, to detect the specific antibodies against the pathogen. An important test with such heterophile antigens is Weil-Felix test for diagnosis of Rickettsial fever.

20.3.1.2.1. Weil-Felix test:

Weil and Felix (1915) isolated a strain of *Proteus* from the urine of a typhus patient, which was agglutinated with patients serum. They thought that *Proteus* is the pathogen. Later studies showed that *Proteus* is not the pathogen but possess antigens which are similar to the somatic antigens of pathogenic Rickettsiae. The test came to be known as Weil-Felix test. Even though least specific, it is most commonly used serological test for diagnosis of Rickettsial fever, because the use of Rickettsial cultures is unsafe.

In the test, patient's serum is mixed with commercially available antigens and incubated overnight. Clumping indicates positive reaction.

20.3.1.3. Tests with antigens coated on particles (or carriers):

Numerous serological procedures have been developed for detection of antibodies via the agglutination of an artificial carrier particle with antigen bound to its surface. Treated latex beads or treated erythrocytes are commonly used. The size of the carrier enhances the visibility of agglutination reaction and the artificial nature of the system allows the antigen bound to the surface to be extremely specific.

Latex bead tests are commonly used for Staphylococci and also for detection of antibodies to Cytomegalovirus, Rubella virus, Varicella-Zoster virus, infectious mononucleosis virus etc. In the test, a few drops of patient's serum are placed on a slide and latex beads coated with the suspected antigen are added and mixed. If clumping of the latex beads occurs it indicates that the

test is positive. The agglutination of latex beads give milky white colour to the mixture and agglutination can be easily recognized.

Treated animal red blood cells have also been used as carriers of antigen for agglutination tests called indirect hemagglutination or passive hemagglutination tests, since it is not the antigens on the blood cells themselves but passively attached antigens that are being bound by antibody. It is commonly used for detection of antibodies against *Treponema pallidum*, some Clostridia, *Psuedomonas, Corynebacterium diphtheriae, Leptospira* and several agents of viral and parasitic diseases.

20.3.1.4. Cold agglutination test:

It is the most commonly used serological test for diagnosis of *Mycoplasma pneumoniae* which cause primary atypical pneumonia. In the test, the patient's serum is mixed with equal volume of Human O-group erythrocytes and incubated overnight at 4 °C. Clumping of RBCs is positive, if the serum contains Ig M antibodies. It is called cold agglutination because the reaction can occur at low temperatures. This test is positive in 80% of the cases.

20.3.2. PRECIPITATION TESTS:

In precipitation reaction, soluble antigen combines with its specific antibody to form a visible precipitate. The precipitate usually sediments at the bottom of the tube. However, if the precipitate is found suspended in the medium, the reaction is described as flocculation.

Precipitation reactions are used to detect antigens in specimens, filtrates and cultures, and also in identification and quantification of antibodies. The precipitation tests can be carried out on slides, tubes and gels. VDRL test is a commonly used slide flocculation test for rapid screening of serum samples of suspected syphilis patients. Kahn test is a tube flocculation test for syphilis. Gel diffusion tests are used for diagnosis of bacterial pathogens like *Corynebacteriunm diphtheriae*, and fungal pathogens like *Histoplasma, Blastomyces* etc.

20.3.2.1. VDRL test:

It is the simplest and the most commonly used serological test for syphilis. It is a slide flocculation test developed by Venereal Disease Research Laboratory (VDRL). In this test a few drops of antigen preparation (cardiolipin) and serum from patient are mixed on a slide. For thorough mixing, a VDRL shaker is used. The slides are observed for flocculation which may be visible with naked eye or under microscope. The test is positive if flocculation is observed.

20.3.2.2. KAHN test:

It is a tube precipitation test for syphilis developed by Kahn. In this test, to a fixed volume of diluted serum in test tubes, equal amount of serially diluted antigen preparation is added and the tubes are incubated over night. Formation of floccules in the tubes indicates positive result.

20.3.3. Immunodiffusion tests:

Precipitation in gels is often referred to as immunodiffusion. There are various techniques that use diffusion principle, and double diffusion technique is commonly used in diagnosis

In **Double diffusion** method, commonly described as Ouchterlony's double diffusion technique, antiserum is placed in a central well and different antigens in the surrounding wells cut in an agar gel plate. Following incubation a line of precipitate occurs when an antigen and antibody meet in optimal concentration.

If the adjacent antigens are identical the line of precipitate formed by them will fuse. If they are unrelated the lines of precipitate will cross each other. If spur formation occurs it indicates cross-reacting or partial antigens (Fig.20.1).

Figure 20.1. Ouchterlony's double diffusion technique. A. Line of confluence formed by adjacent antigens (identical antigens); B. spur formation (partial identity of antigens); C. crossing over lines (non identical antigens)

Among the bacterial pathogens, diagnosed by this method, are toxigenic strains of *Corynebacterium diphtheriae* are important. The technique is also routinely used to detect antibodies against *Histoplasma, Blastomyces, Coccidioides* and other fungal pathogens.

20.3.4. OTHER TESTS

20.3.4.1. COMPLEMENT FIXATION TEST:

Wasserman (1906) first developed this test for diagnosis of syphilis. In the test, patient's serum is heated to 56°C to inactivate complement in the serum (but antibodies retain their activity) and mixed with antigen and external source of compliment is added. After 24 hours of incubation, sheep red blood cells and antibodies to them are added and incubated for the second time. If the complement is not fixed in the first set up, it will be available for lysing the sheep red blood cells. If the complement is fixed in the original set up, the sheep red blood cells do not lyse because complement is not available.

Set up 1: antigen + patient serum (Heated to 56°C) + external complement (from sheep) — incubated for 24 hrs

Set up 2: First set up + Sheep RBCs + antibodies to Sheep RBCs — incubated for 24 hrs

Observation:

1. Lysis of sheep RBCs — Patient's serum do not Contain syphilis antibodies.

Patient is negative for syphilis

2. No lysis of RBCs — Patient's serum contain antibodies to syphilis pathogen.

Patient is positive for syphilis

Complement fixation test is not now used for syphilis because more rapid serological tests were available. However, it is still useful in serodiagnosis of various bacterial and viral pathogens.

20.3.4.2. ENZYME LINKED IMMUNOSORBENT ASSAY (ELISA):

Elisa technique is used extensively nowadays because it is sensitive and requires only small amounts of reagents. It is based on the ability of antigen or antibody

1. to adsorb to a solid phase support surface and
2. to be linked to an enzyme forming a complex showing detectable immunological and enzyme activity.

Two methods of ELISA are commonly used in diagnosis. They are

1. Direct ELISA method for detection of antigen
2. Indirect ELISA for detection of antibodies.

DIRECT ELISA: It is used to detect antigens using specific antibodies (Fig. 20.2).

1. for detection of antigen, the specific antibody is first coated on the plastic lining of the wells of ELISA plate
2. then the specimen (usually from culture solution) is added, and incubated for antigen-antibody reactions to take place
3. the well is then washed to remove the unbound antigens
4. then antibodies conjugated with an enzyme system (such as alkaline phosphatase) is added to the well and incubated
5. the well is again washed to remove unbound enzyme linked antibodies
6. then a chromogenic substrate (nitrophenyl phosphate for alkaline phosphatase) is added
7. the enzyme acts on the substrate to give yellow colour
8. the colour change can be visually observed, and it indicates the presence of antigen specific to the antibody used
9. the colour can be quantitatively estimated using spectrophometer.

Figure 20.2. ELISA for detection of antigen

INDIRECT ELISA: This technique is used to detect antibodies in the serum using specific antigens (Fig. 20.3).

1. antigen is coated on linings ELISA plate
2. serum with antibodies is added and incubated
3. wells washed to remove unbound antibodies
4. enzyme linked antibodies against the antibodies is added and incubated
5. wells again washed to remove unbound enzyme labeled antibodies
6. chromogen substrate is added
7. colour change indicate positive reaction and no change in colour is negative reaction.

Figure 20.3. ELISA test for detection of antibody

20.4. SUMMARY

A number of biochemical tests are performed on pure cultures, and the type of tests used depends up on the pathogen involved. IMVC tests are four biochemical tests widely employed in the characterization of enteric bacteria. Catalase test used to differentiate strong aerobes from facultative or obligate anaerobes. Coagulase test is used to differentiate pathogenic strains of *Staphylococcus aureus* from nonpathogenic staphylococci. Oxidase test used for tentative identification of *Neisseria* species in a plate of mixed cultures. Bile solubility test and optochin sensitivity test are used for identification of *Streptococcus pneumoniae*. Urease test, H_2S production test, litmus milk test and a number of other biochemical tests are used for identification of bacterial pathogens.

A number of serological tests are performed for rapid identification of bacterial pathogens. Serological tests for agglutination, precipitation, and ELISA are commonly used in diagnostic laboratories. Widal test is an agglutination test widely used for diagnosis of typhoid. Weil-Felix test is used for diagnosis of rickettsial fevers, cold agglutination test is used for diagnosis of mycoplasmal pneumonia. Latex bead test is used for identification of a number of bacterial pathogens like staphylococci. Passive hemagglutination test commonly used for detection of antibodies against *Treponema pallidum, Corynebacterium diphtheriae, Pseudomonas, Leptospira,* Clostridia etc. Among the precipitation tests, VDRL test is a slide precipitation test widely used for diagnosis of syphilis. ELISA tests are commonly performed serological tests in the diagnostic laboratories. Direct ELISA tests are used for detection of antigen in the serum, and indirect ELISA tests are used for detection of antibodies in the serum. Compliment fixation test is a laborious time taking serological test first developed for diagnosis of syphilis, and it is now used but rarely for serodiagnosis of various bacterial and viral pathogens.

20.5. MODEL QUESTIONS:

Essay type questions

Discuss the important biochemical tests employed for diagnosis of bacterial Pathogens

Discuss the serological tests employed for diagnosis of bacterial diaseses

Short answer type questions

IMVC tests
Coagulase test
Bile solubility test
Widal test
Weil – Felix test
VDRL test
Complement fixation test
ELISA

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