18MBU402 MEDICAL MICROBIOLOGY (4H – 4C)

Instruction Hours / week: L: 4 T: 0 P: 0 **Marks:** Internal: **40 External**: **60** Total: **100**

End Semester Exam: 3 Hours

SCOPE

It provides the ability to characterize, isolate and identify different microbes. It includes a detailed study of characterization, etiology, pathogenicity, clinical systems, and laboratory diagnosis of disease causing Microorganisms.

OBJECTIVES

- To introduce the knowledge of the medically important microorganisms, microbial morphology with the main focuses being the characterization, isolation and identification of different microorganism.

Unit I

Normal micro flora of the human body: Importance of normal microflora, normal microflora of skin, throat, gastrointestinal tract, urogenital tract. Host pathogen interaction: Definitions – Infection, Invasion, Pathogen, Pathogenicity, Virulence, Toxigenicity, Carriers and their types, Opportunistic infections, Nosocomial infections. Transmission of infection, Pathophysiologic effects of LPS. Collection, transport and culturing of clinical samples – Sputum, Stool and Urine.

Unit II

List of diseases of various organ systems and their causative agents. The following diseases in detail with symptoms, mode of transmission, prophylaxis and control. Respiratory pathogens: *Streptococcus pyogenes, Haemophilus influenzae, Mycobacterium tuberculosis*. Gastrointestinal Diseases: *Escherichia coli, Salmonella typhi, Vibrio cholerae, Helicobacter pylori.* Others: *Staphylococcus aureus, Bacillus anthracis, Clostridium tetani, Treponema pallidum.*

Unit III

The detailed study of following diseases – Causative agents, Mode of transmissions, Pathogenicity, Symptoms and prophylaxis of Polio, Herpes, Hepatitis, Rabies, Dengue, AIDS, Influenza with brief description of swine flu, Ebola, Chikungunya, Japanese Encephalitis

Unit IV

Brief description of each of the following types of mycoses and one representative disease to be studied with respect to transmission, symptoms and prevention. Cutaneous mycoses: Tinea pedis (Athlete's foot). Systemic mycoses: Histoplasmosis. Opportunistic mycoses: Candidiasis. The detailed study of following diseases – Causative agents, Mode of transmissions, Pathogenicity, Symptoms and prophylaxis of Amoebiasis, Giardiasis, Elephanttiasis, Taeniasis, Malaria, Kala-azar.

Unit V

Antibacterial agents: Five modes of action with one example each: Inhibitor of nucleic acid synthesis; Inhibitor of cell wall synthesis; Inhibitor of cell membrane function; Inhibitor of protein synthesis; Antibiotic resistance - MDR, XDR, MRSA, NDM-1 – resistance mechanisms. Antifungal agents:

Mechanism of action of Amphotericin B, Griseofulvin. Antiviral agents: Mechanism of action of Amantadine, Acyclovir, Azidothymidine.

SUGGESTED READINGS

1. Brooks G.F., Carroll K.C., Butel J.S., Morse S.A. and Mietzner, T.A. (2013). Jawetz, Melnick and Adelberg's Medical Microbiology. 26th edition. McGraw Hill Publication.
2. Greenwood D, Slack R, Barer M, and Irving W. (2012). Medical Microbiology, 18th Edition. Churchill Livingstone.
3. Ryan KJ and Ray CG. (2014). Sherris Medical Microbiology, 6th Edition. McGraw-Hill Professional.
4. Ananthanarayan R. and Paniker C.K.J. (2009) Textbook of Microbiology. 8th edition, University Press Publication.
5. Madigan MT, Martinko JM, Dunlap PV and Clark DP. (2014). Brock Biology of Microorganisms. 14th edition. Pearson International Edition.
6. Goering R., Dockrell H., Zuckerman M. and Wakelin D. (2007). Mims' Medical Microbiology. 4 th edition. Elsevier.
7. Willey JM, Sherwood LM, and Woolverton CJ. (2013). Prescott, Harley and Klein's Microbiology. 9th edition. McGraw Hill Higher Education.

LECTURE PLAN-UNIT-1			
S.NO	Lecture duration hour	Topics	Supporting materials
1	1	Introduction	T1-451
2	1	Normal microflora of human body	T1451-453
3	1	Importance of microflora and normal flora of various organ	T1 255-256
4	1	Definition of infection,invasion,pathogen and pathogenicity,Virulence, Toxigenicity	T 257
5	1	Carriers and their types, Opportunistic infections, Nosocomial infections.	T1 258-260
6	1	Transmission of infection, Pathophysiologic effects of LPS.	T1 247
7	1	Collection, transport and culturing of clinical samples – Sputum, Stool and Urine.	T1 454-457
8	1	Unit Revision	
		TOTAL HOURS	8 h
Textbooks:		T1-Text book of microbiology-R.Vasanthakumari	
Journals:		-	
		-	
Website:			
Reference books:			

LECTURE PLAN-UNIT-2			
S.NO	Lecture duration hour	Topics	Supporting materials
1	1	List of diseases of various organ systems and their causative agents.	T1-190
2	1	Respiratory pathogens: Streptococcus pyogenes	T1-190-196
3	1	Haemophilus influenzae, Mycobacterium tuberculosis	T1283-284 231-242
4	1	Gastrointestinal Diseases: Escherichia coli, Salmonella typhi,	T1-254-256,258-263
5	1	Vibrio cholerae, Helicobacter pylori	T1 271-274,329-330
6	1	Staphylococcus aureus, Bacillus anthracis,	T1 185-188,222-225
7	1	Clostridium tetani, Treponema pallidum.	T1-216-217
8	1	Unit Revision-1	
		TOTAL HOURS	8 h
Textbooks:		T1-Text book of microbiology-R.Vasanthakumari	
Journals:		-	
		-	
Website:			
Reference books:			

LECTURE PLAN-UNIT-3			
S.NO	Lecture duration hour	Topics	Supporting materials
1	1	Diseases-polio	T2 355-359
2	1	Herpes virus	T1363-369
3	1	Hepatatis virus	T1 399-406
4	1	Rabies and dengue	T1 394-398
5	1	Aids and influenza	T2-381-385,334-339
6	1	Swine flu and Ebola	T3-900-903
7	1	Chickengunya and japanese encephalitis	T3-909
8	1	Unit Revision	
		TOTAL HOURS	8 h
Textbooks:		T1-Text book of microbiology-R.Vasanthakumari,T2-Medical microbiology--S.Rajan,T3-Microbiology-Prescott	
Journals:		-	
		-	
Website:			
Reference books:			

LECTURE PLAN-UNIT-4			
S.NO	Lecture duration hour	Topics	Supporting materials
1	1	Introduction to types of mycoses	T2-415-416
2	1	Cutaneous mycoses	T1 243-254
3	1	Systemic mycoses	T1 255-256
4	1	Oppurtunistic mycoses	T 2-467-470
5	1	Amoebiasis,Giardisis	T1 480-485
6	1	Elephantiasis,Taeniasis	T1 247
7	1	Malaria,Kala azar	T1-498-501
		TOTAL HOURS	7 h
Textbooks:		T1-Text book of microbiology-R.Vasanthakumari, T2-Medical microbiology-S.Rajan	
Journals:		-	
		-	
Website:			
Reference books:			

LECTURE PLAN-UNIT-5			
S.NO	Lecture duration hour	Topics	Supporting materials
1	1	Antibacterial agents:	T2 121-122,T11-4
2	1	Inhibitor of nucleic acid synthesis	T1243-254
3	1	Inhibitor of cell wall synthesis	T1 255-256
4	1	Inhibitor of cell membrane function; Inhibitor of protein synthesis;	T 257
5	1	Antibiotic resistance - MDR, XDR	T1 258-260
6	1	MRSA, NDM-1 – resistance mechanisms.	T2-844-845
7	1	Mechanism of action of Amphotericin B, Griseofulvin.	T3-847
8	1	Antiviral agents-Amantadine	T3-848
9	1	Acyclovir, Azidothymidine	T3-849
		TOTAL HOURS	9 h
Textbooks:		T1-Text book of microbiology-R.Vasanthakumari, T2-Medical microbiology-S.Rajan,T3-Microbiology-Prescott	
Journals:		-	
		-	
Website:			
Reference books:			

Unit I

Normal micro flora of the human body: Importance of normal microflora, normal microflora of skin, throat, gastrointestinal tract, urogenital tract. Host pathogen interaction: Definitions – Infection, Invasion, Pathogen, Pathogenicity, Virulence, Toxigenicity, Carriers and their types, Opportunistic infections, Nosocomial infections. Transmission of infection, Pathophysiologic effects of LPS. Collection, transport and culturing of clinical samples – Sputum, Stool and Urine.

Normal Bacterial Flora of Humans

The Normal Flora

In a healthy animal, the internal tissues, e.g. blood, brain, muscle, etc., are normally free of microorganisms. However, the surface tissues, i.e., skin and mucous membranes, are constantly in contact with MEDICAL organisms and become readily colonized by various microbial species. The mixture of organisms regularly found at any anatomical site is referred to as the **normal flora**, except by researchers in the field who prefer the term "**indigenous microbiota**". The normal flora of humans consists of a few eucaryotic fungi and protists, but bacteria are the most numerous and obvious microbial components of the normal flora.

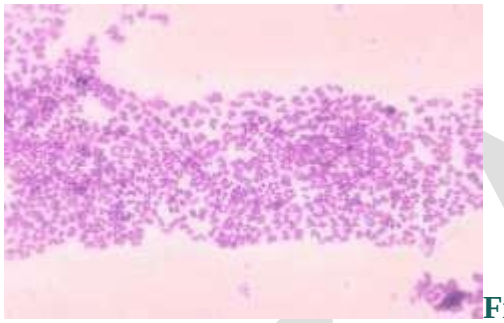


Figure 1. Gram stain of a species of *Micrococcus,* commonly isolated from the skin and nasal membranes of humans.

The predominant bacterial flora of humans are shown in Table 1. This table lists only a fraction of the total bacterial species that occur as normal flora of humans. A recent experiment that used 16S RNA probes to survey the diversity of bacteria in dental plaque revealed that only one percent of the total species found have ever been cultivated. Similar observations have been made with the intestinal flora. Also, this table does not indicate the relative number or concentration of bacteria at a particular site. If you are reading online, you can skip this table and use it as an ongoing reference. To continue this article, scroll to the bottom of the Table notes to**Associations Between Humans and the Normal Flora**

Table 1. Bacteria commonly found on the surfaces of the human body.

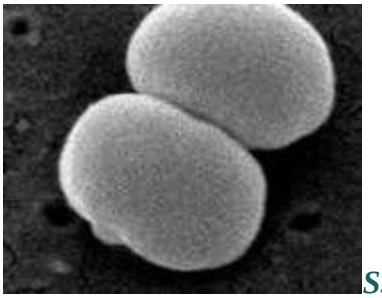
BACTERIUM	Skin	Con-junc-tiva	Nose	Pharynx	Mouth	Lower GI	Ant. ure-thra	Vagina

Staphylococcus epidermidis **(1)**	++	+	++	++	++	+	++	++
Staphylococcus aureus* **(2)**	+	+/-	+	+	+	++	+/-	+
Streptococcus mitis				+	++	+/-	+	+
Streptococcus salivarius				++	++			
Streptococcus mutans* **(3)**				+	++			
Enterococcus faecalis* **(4)**				+/-	+	++	+	+
Streptococcus pneumoniae* **(5)**		+/-	+/-	+	+			+/-
Streptococcus pyogenes* **(6)**	+/-	+/-		+	+	+/-		+/-
Neisseria sp. **(7)**		+	+	++	+		+	+
Neisseria meningitidis* **(8)**			+	++	+			+
Enterobacteriaceae*(Escherichia coli) **(9)**		+/-	+/-	+/-	+	++	+	+
Proteus sp.	+/-	+	+	+	+	+	+	
Pseudomonas aeruginosa* **(10)**	+/-	+/-	+	+/-				
Haemophilus influenzae* **(11)**		+/-	+	+	+			
Bacteroides sp.*						++	+	+/-
Bifidobacterium bifidum **(12)**						++		
Lactobacillus sp. **(13)**				+	++	++		++
Clostridium sp.* **(14)**					+/-	++		
Clostridium tetani **(15)**						+/-		
Corynebacteria (16)	++	+	++	+	+	+	+	+
Mycobacteria	+		+/-	+/-		+	+	
Actinomycetes				+	+			
Spirochetes				+	++	++		
Mycoplasmas				+	+	+	+/-	+

++ = nearly 100 percent + = common (about 25 percent) +/- = rare (less than 5%) * = potential pathogen

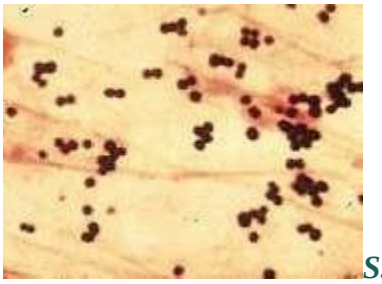
Table 1 Notes

(1) The staphylococci and corynebacteria occur at every site listed.*Staphylococcus epidermidis* is highly adapted to the diverse environments of its human host. *S. aureus* is a potential pathogen. It is a leading cause of bacterial disease in humans. It can be transmitted from the nasal membranes of an asymptomatic carrier to a susceptible host.



S. epidermidis. **Scanning EM. CDC.**

(2) Many of the normal flora are either pathogens or opportunistic pathogens, The asterisks indicate members of the normal flora a that may be considered major pathogens of humans.



S. aureus. **Gram stain.**

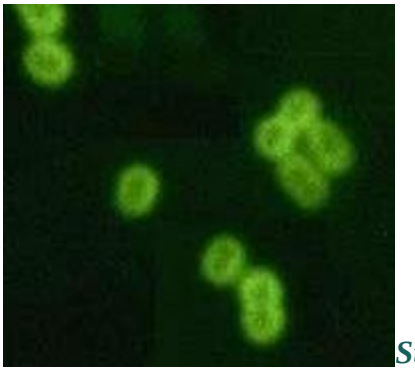
(3) ***Streptococcus mutans*** **is the primary bacterium involved in plaque formation and initiation of dental caries. Viewed as an opportunistic infection, dental disease is one of the most prevalent and costly infectious diseases in the United States.**

Streptococcus mutans. **Gram stain. CDC**

(4) ***Enterococcus faecalis*** **was formerly classified as** ***Streptococcus faecalis.*****The bacterium is such a regular a component of the intestinal flora, that many European countries use it as the standard indicator of fecal pollution, in the same way we use** ***E. coli*** **in the U.S. In recent years,** ***Enterococcus faecalis*** **has emerged as a significant, antibiotic-resistant, nosocomial pathogen.**

Vancomycin Resistant *Enterococcus faecalis.* Scanning E.M. CDC

(5) *Streptococcus pneumoniae* is present in the upper respiratory tract of about half the population. If it invades the lower respiratory tract it can cause pneumonia. *Streptococcus pneumoniae* causes 95 percent of all bacterial pneumonia.



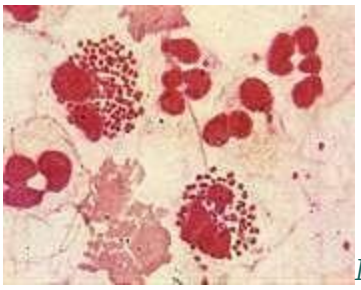
***Streptococcus pneumoniae.* Direct fluorescent antibody stain. CDC.**

(6) *Streptococcus pyogenes* refers to the Group A, Beta-hemolytic streptococci. Streptococci cause tonsillitis (strep throat), pneumonia, endocarditis. Some streptococcal diseases can lead to rheumatic fever or nephritis which can damage the heart and kidney.

***Streptococcus pyogenes.* Gram stain.**

(7) *Neisseria* and other Gram-negative cocci are frequent inhabitants of the upper respiratory tract, mainly the pharynx. *Neisseria meningitidis,* an important cause of bacterial meningitis, can

colonize as well, until the host can develop active immunity against the pathogen.



Neisseria meningitidis. **Gram stain.**

(8) While *E. coli* is a consistent resident of the small intestine, many other enteric bacteria may reside here as well, including *Klebsiella, Enterobacter* and*Citrobacter.* Some strains of *E. coli* are pathogens that cause intestinal infections, urinary tract infections and neonatal meningitis.

E. coli. **Scanning E.M. Shirley Owens. Center for Electron Optics. Michigan State University.**

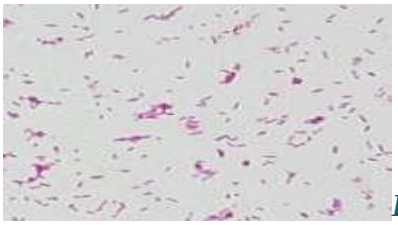
(9) *Pseudomonas aeruginosa* is the quintessential opportunistic pathogen of humans that can invade virtually any tissue. It is a leading cause of hospital-acquired (nosocomial) Gram-negative infections, but its source is often exogenous (from outside the host).

Colonies of *Pseudomonas aeruginosa* growing on an agar plate. The most virulent Pseudomonas species produce mucoid colonies and green pigments such as this isolate.

(10) *Haemophilus influenzae* is a frequent secondary invader to viral influenza, and was named accordingly. The bacterium was the leading cause of meningitis in infants and children until the recent development of the Hflu type B vaccine.

Haemophilus influenzae. **Gram stain.**

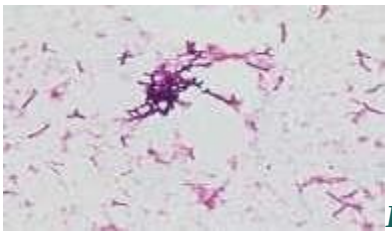
(11) The greatest number of bacteria are found in the lower intestinal tract, specifically the colon and the most prevalent bacteria are the *Bacteroides,* a group of Gram-negative, anaerobic, non-sporeforming bacteria. They have been implicated in the initiation colitis and colon cancer.



Bacteroides fragilis. **Gram stain.**

(12) Bifidobacteria are Gram-positive, non-sporeforming, lactic acid bacteria. They have been described as "friendly" bacteria in the intestine of humans.*Bifidobacterium bifidum* is the

predominant bacterial species in the intestine of breast-fed infants, where it presumably prevents colonization by potential pathogens. These bacteria are sometimes used in the manufacture of yogurts and are frequently incorporated into probiotics.



Bifidobacterium bifidum. **Gram stain**

(13) Lactobacilli in the oral cavity probably contribute to acid formation that leads to dental caries. *Lactobacillus acidophilus* colonizes the vaginal epithelium during child-bearing yearsand establishes the low pH that inhibits the growth of pathogens.

Lactobacillus **species and a vaginal squaemous epithelial cell. CDC**

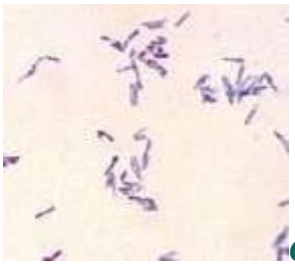
(14) There are numerous species of *Clostridium* that colonize the bowel.*Clostridium perfringens* is commonly isolated from feces. *Clostridium difficile*may colonize the bowel and cause "antibiotic-induced diarrhea" or pseudomembranous colitis.

***Clostridium perfringens*. Gram stain.**

(15) *Clostridium tetani* is included in the table as an example of a bacterium that is "transiently associated" with humans as a component of the normal flora. The bacterium can be isolated from feces in 0 - 25 percent of the population. The endospores are probably ingested with food and water, and the bacterium does not colonize the intestine.

Clostridium tetani. **Gram stain.**

(16) The corynebacteria, and certain related propionic acid bacteria, are consistent skin flora. Some have been implicated as a cause of acne.*Corynebacterium diphtheriae, the* agent of diphtheria, was considered a member of the normal flora before the widespread use of the diphtheria toxoid, which is used to immunize against the disease.



Corynebacterium diphtheriae. **No longer a part of the normal flora.**

Associations Between Humans and the Normal Flora

E. coli is the best known bacterium that regularly associates itself with humans, being an invariable component of the human intestinal tract. Even though *E. coli*is the most studied of all bacteria, and we know the exact location and sequence of 4,288 genes on its chromosome, we do not fully understand its ecological relationship with humans.

In fact, not much is known about the nature of the associations between humans and their normal flora, but they are thought to be dynamic interactions rather than associations of mutual indifference. Both host and bacteria are thought to derive benefit from each other, and the associations are, for the most part,**mutualistic**. The normal flora derive from their host a steady supply of nutrients, a stable environment, and protection and transport. The host obtains from the normal flora certain nutritional and digestive benefits, stimulation of the development and activity of immune system, and protection against colonization and infection by pathogenic microbes.

While most of the activities of the normal flora benefit their host, some of the normal flora are **parasitic** (live at the expense of their host), and some are**pathogenic** (capable of producing disease). Diseases that are produced by the normal flora in their host may be called **endogenous diseases**. Most endogenous bacterial diseases are **opportunistic infections**, meaning that the the organism must be given a special opportunity of weakness or let-down in the host defenses in order to infect. An example of an opportunistic infection is chronic bronchitis in smokers wherein normal flora bacteria are able to invade the weakened lung.

Sometimes the relationship between a member of the normal flora an its host cannot be deciphered. Such a relationship where there is no apparent benefit or harm to either organism during their association is referred to as a **commensal relationship**. Many of the normal flora that are not predominant in their habitat, even though always present in low numbers, are thought of as commensal bacteria. However, if a presumed commensal relationship is studied in detail, parasitic or mutualistic characteristics often emerge.

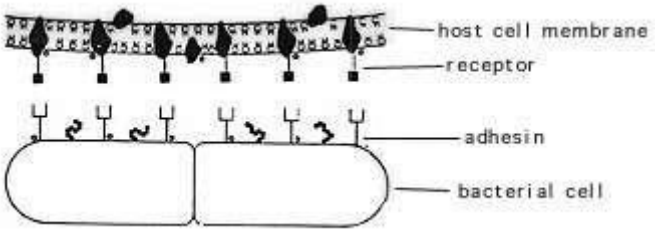
Tissue specificity

Most members of the normal bacterial flora prefer to colonize certain tissues and not others. This "tissue specificity" is usually due to properties of both the host and the bacterium. Usually, specific bacteria colonize specific tissues by one or another of these mechanisms.

1. **Tissue tropism** is the bacterial preference or predilection for certain tissues for growth. One explanationfor tissue tropism is that the host provides essential nutrients and growth factors for the bacterium, in addition to suitable oxygen, pH, and temperature for growth.

***Lactobacillus acidophilus,* informally known as "Doderlein's bacillus" colonizes the vagina because glycogen is produced which provides the bacteria with a source of sugar that they ferment to lactic acid.**

2. **Specific adherence** Most bacteria can colonize a specific tissue or site because they can adhere to thattissue or site in a specific manner that involves complementary chemical interactions between the two surfaces. Specific adherence involves biochemical interactions between bacterial surface components (**ligands** or **adhesins**) and host cell molecular **receptors**. The bacterial components that provide adhesins are molecular parts of their capsules, fimbriae, or cell walls. The receptors on human cells or tissues are usually glycoprotein molecules located on the host cell or tissue surface.



Streptococcus salivarius
Mucosal epithelium*Staphylococcus aureus*
Cell-bound proteinMucosal epithelium
***Neisseria gonorrhoeae* N-methylphenyl- alanine pili** **Urethral/cervical epithelium**

Enterotoxigenic *E. coli*

Respiratory epithelium		
Vibrio cholerae	**N-methylphenylalanine pili**	**Intestinal epithelium**
Treponema pallidum	**Peptide in outer membrane**	**Mucosal epithelium**
Mycoplasma	**Membrane protein**	**Respiratory epithelium**
Chlamydia	**Unknown**	**Conjunctival or urethral epithelium**

3. Biofilm formation

Some of the indigenous bacteria are able to construct **biofilms** on a tissue surface, or they are able to colonize a biofilm built by another bacterial species. Many biofilms are a mixture of microbes, although one member is responsible for maintaining the biofilm and may predominate.

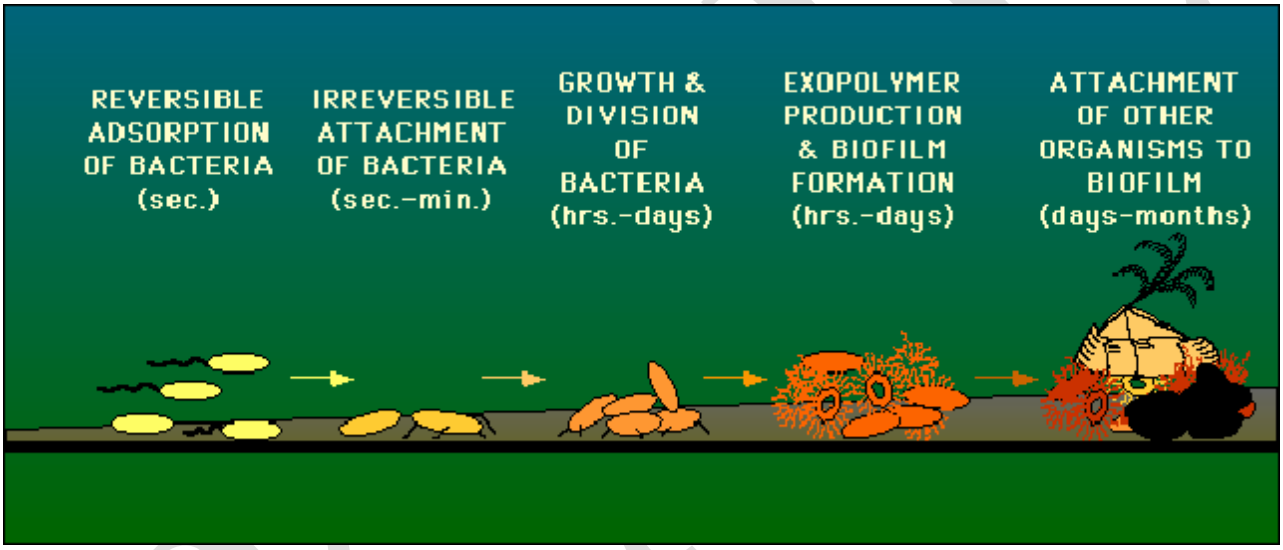


Figure 3. Cartoon depicting biofilm formation. Biofilms usually occur when one bacterial species attaches specifically or non specifically to a surface, and then secretes carbohydrate slime (exopolymer) that imbeds the bacteria and attracts other microbes to the biofilm for protection or nutritional advantages.

The classic biofilm that involves components of the normal flora of the oral cavity is the formation of dental plaque on the teeth. Plaque is a naturally-constructed biofilm, in which the consortia of bacteria may reach a thickness of 300-500 cells on the surfaces of the teeth. These accumulations subject the teeth and gingival tissues to high concentrations of bacterial metabolites, which result in dental disease.

The Composition of the Normal Flora

The normal flora of humans are exceedingly complex and consist of more than 200 species of bacteria. The makeup of the normal flora may be influenced by various factors, including genetics, age, sex, stress, nutrition

and diet of the individual.

Three developmental changes in humans, weaning, the eruption of the teeth, and the onset and cessation of ovarian functions, invariably affect the composition of the normal flora in the intestinal tract, the oral cavity, and the vagina, respectively. However, within the limits of these fluctuations, the bacterial flora of humans is sufficiently constant to a give general description of the situation.

A human first becomes colonized by a normal flora at the moment of birth and passage through the birth canal. In utero, the fetus is sterile, but when the mother's water breaks and the birth process begins, so does colonization of the body surfaces. Handling and feeding of the infant after birth leads to establishment of a stable normal flora on the skin, oral cavity and intestinal tract in about 48 hours.

It has been calculated that a human adult houses about 10^{12} bacteria on the skin, 10^{10} in the mouth, and 10^{14} in the gastrointestinal tract. The latter number is far in excess of the number of eucaryotic cells in all the tissues and organs which comprise a human. The predominant bacteria on the surfaces of the human body are listed in Table 3. Informal names identify the bacteria in this table. Formal taxonomic names of organisms are given in Table 1.

Table 3. Predominant bacteria at various anatomical locations in adults.

Anatomical Location	Predominant bacteria
Skin	staphylococci and corynebacteria
Conjunctiva	sparse, Gram-positive cocci and Gram-negative rods
Oral cavity	
teeth	streptococci, lactobacilli
mucous membranes	streptococci and lactic acid bacteria
Upper respiratory tract	
nares (nasal membranes)	staphylococci and corynebacteria
pharynx (throat)	streptococci, neisseria, Gram-negative rods and cocci
Lower respiratory tract	none
Gastrointestinal tract	
stomach	*Helicobacter pylori* (up to 50%)
small intestine	lactics, enterics, enterococci, bifidobacteria
colon	bacteroides, lactics, enterics, enterococci, clostridia, methanogens
Urogenital tract	

anterior urethra	**sparse, staphylococci, corynebacteria, enterics**
vagina	**lactic acid bacteria during child-bearing years; otherwise mixed**

Normal Flora of the Skin The adult human is covered with approximately 2 square meters of skin. The density and composition of the normal flora of the skin varies with anatomical locale. The high moisture content of the axilla, groin, and areas between the toes supports the activity and growth of relatively high densities of bacterial cells, but the density of bacterial populations at most other sites is fairly low, generally in 100s or 1000s per square cm. Most bacteria on the skin are sequestered in sweat glands.

The skin microbes found in the most superficial layers of the epidermis and the upper parts of the hair follicles are Gram-positive cocci (*Staphylococcus epidermidis* and *Micrococcus* sp.) and corynebacteria such as *Propionibacterium*sp. These are generally nonpathogenic and considered to be commensal, although mutualistic and parasitic roles have been assigned to them. For example, staphylococci and propionibacteria produce fatty acids that inhibit the growth of fungi and yeast on the skin. But, if *Propionibacterium acnes,* a normal inhabitant of the skin, becomes trapped in hair follicle, it may grow rapidly and cause inflammation and acne.

Sometimes potentially pathogenic *Staphylococcus aureus* is found on the face and hands in individuals who are nasal carriers. This is because the face and hands are likely to become inoculated with the bacteria on the nasal membranes. Such individuals may autoinoculate themselves with the pathogen or spread it to other individuals or foods.

Normal Flora of the Conjunctiva A variety of bacteria may be cultivated from the normal conjunctiva, but the number of organisms is usually small.*Staphylococcus epidermidis* and certain coryneforms (*Propionibacterium acnes*) are dominant. *Staphylococcus aureus, some* streptococci, *Haemophilus* sp. and*Neisseria* sp. are occasionally found. The conjunctiva is kept moist and healthy by the continuous secretions from the lachrymal glands. Blinking wipes the conjunctiva every few seconds mechanically washing away foreign objects including bacteria. Lachrymal secretions (tears) also contain bactericidal substances including lysozyme. There is little or no opportunity for microorganisms to colonize the conjunctiva without special mechanisms to attach to the epithelial surfaces and some ability to withstand attack by lysozyme.

Pathogens which do infect the conjunctiva (e.g. *Neisseria gonorrhoeae* and*Chlamydia trachomatis*) are thought to be able to specifically attach to the conjunctival epithelium. Newborn infants may be especially prone to bacterial attachment. Since *Chlamydia* and *Neisseria* might be present on the cervical and vaginal epithelium of an infected mother, silver nitrate or an antibiotic may be put into the newborn's eyes to avoid infection after passage through the birth canal.

Normal Flora of the Respiratory Tract A large number of bacterial species colonize the upper respiratory tract (nasopharynx). The nares (nostrils) are always heavily colonized, predominantly with *Staphylococcus*

epidermidis and corynebacteria, and often (in about 20% of the general population) with*Staphylococcus aureus*, this being the main carrier site of this important pathogen. The healthy sinuses, in contrast are sterile. The pharynx (throat) is normally colonized by streptococci and various Gram-negative cocci. Sometimes pathogens such as *Streptococcus pneumoniae, Streptococcus pyogenes, Haemophilus influenzae* and *Neisseria meningitidis* colonize the pharynx.

The lower respiratory tract (trachea, bronchi, and pulmonary tissues) is virtually free of microorganisms, mainly because of the efficient cleansing action of the ciliated epithelium which lines the tract. Any bacteria reaching the lower respiratory tract are swept upward by the action of the mucociliary blanket that lines the bronchi, to be removed subsequently by coughing, sneezing, swallowing, etc. If the respiratory tract epithelium becomes damaged, as in bronchitis or viral pneumonia, the individual may become susceptible to infection by pathogens such as *H. influenzae* or *S. pneumoniae* descending from the nasopharynx.

Normal Flora of the Urogenital Tract Urine is normally sterile, and since the urinary tract is flushed with urine every few hours, microorganisms have problems gaining access and becoming established. The flora of the anterior urethra, as indicated principally by urine cultures, suggests that the area my be inhabited by a relatively consistent normal flora consisting of S*taphylococcus epidermidis*, *Enterococcus faecalis* and some alpha-hemolytic streptococci. Their numbers are not plentiful, however. In addition, some enteric bacteria (e.g. *E. coli, Proteus*) and corynebacteria, which are probably contaminants from the skin, vulva or rectum, may occasionally be found at the anterior urethra.

The vagina becomes colonized soon after birth with corynebacteria, staphylococci, streptococci, *E. coli*, and a lactic acid bacterium historically named "Doderlein's bacillus" (*Lactobacillus acidophilus*). During reproductive life, from puberty to menopause, the vaginal epithelium contains glycogen due to the actions of circulating estrogens. Doderlein's bacillus predominates, being able to metabolize the glycogen to lactic acid. The lactic acid and other products of metabolism inhibit colonization by all except this lactobacillus and a select number of lactic acid bacteria. The resulting low pH of the vaginal epithelium prevents establishment by most other bacteria as well as the potentially-pathogenic yeast, *Candida albicans.* This is a striking example of the protective effect of the normal bacterial flora for their human host.

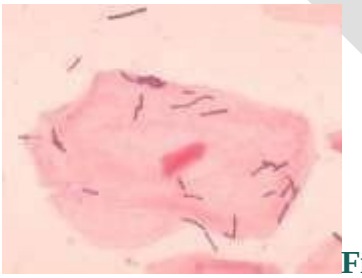


Figure 5. A *Lactobacillus* species, possibly Doderlein's bacillus, in association with a vaginal epithelial cell.

Normal Flora of the Oral Cavity The presence of nutrients, epithelial debris, and secretions makes the mouth a favorable habitat for a great variety of bacteria. Oral bacteria include streptococci, lactobacilli, staphylococci

and corynebacteria, with a great number of anaerobes, especially bacteroides.

The mouth presents a succession of different ecological situations with age, and this corresponds with changes in the composition of the normal flora. At birth, the oral cavity is composed solely of the soft tissues of the lips, cheeks, tongue and palate, which are kept moist by the secretions of the salivary glands. At birth the oral cavity is sterile but rapidly becomes colonized from the environment, particularly from the mother in the first feeding. *Streptococcus salivarius* is dominant and may make up 98% of the total oral flora until the appearance of the teeth (6 - 9 months in humans). The eruption of the teeth during the first year leads to colonization by *S. mutans* and *S. sanguis*. These bacteria require a nondesquamating (nonepithelial) surface in order to colonize. They will persist as long as teeth remain. Other strains of streptococci adhere strongly to the gums and cheeks but not to the teeth. The creation of the gingival crevice area (supporting structures of the teeth) increases the habitat for the variety of anaerobic species found. The complexity of the oral flora continues to increase with time, and bacteroides and spirochetes colonize around puberty.

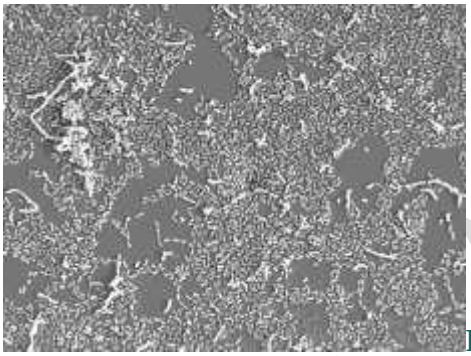


Figure 6. Various streptococci in a biofilm in the oral cavity.

The normal bacterial flora of the oral cavity clearly benefit from their host who provides nutrients and habitat. There may be benefits, as well, to the host. The normal flora occupy available colonization sites which makes it more difficult for other microorganisms (nonindigenous species) to become established. Also, the oral flora contribute to host nutrition through the synthesis of vitamins, and they contribute to immunity by inducing low levels of circulating and secretory antibodies that may cross react with pathogens. Finally, the oral bacteria exert microbial antagonism against nonindigenous species by production of inhibitory substances such as fatty acids, peroxides and bacteriocins.

On the other hand, the oral flora are the usual cause of various oral diseases in humans, including abscesses, dental caries, gingivitis, and periodontal disease. If oral bacteria can gain entrance into deeper tissues, they may cause abscesses of alveolar bone, lung, brain, or the extremities. Such infections usually contain mixtures of bacteria with *Bacteroides melaninogenicus* often playing a dominant role. If oral streptococci are introduced into wounds created by dental manipulation or treatment, they may adhere to heart valves and initiate subacute bacterial endocarditis.

Normal Flora of the Gastrointestinal Tract The bacterial flora of the gastrointestinal (GI) tract of animals has been studied more extensively than that of any other site. The composition differs between various animal species, and within an animal species. In humans, there are differences in the composition of the flora which are influenced by age, diet, cultural conditions, and the use of antibiotics. The latter greatly perturbs the composition of the intestinal flora.

In the upper GI tract of adult humans, the esophagus contains only the bacteria swallowed with saliva and food. Because of the high acidity of the gastric juice, very few bacteria (mainly acid-tolerant lactobacilli) can be cultured from the normal stomach. However, at least half the population in the United States is colonized by a pathogenic bacterium, *Helicobacter pylori.* Since the 1980s, this bacterium has been known to be the cause of gastric ulcers, and it is probably a cause of gastric and duodenal cancer as well. The Australian microbiologist, Barry Marshall, received the Nobel Prize in Physiology and Medicine in 2005, for demonstrating the relationship between *Helicobacter* and gastric ulcers.

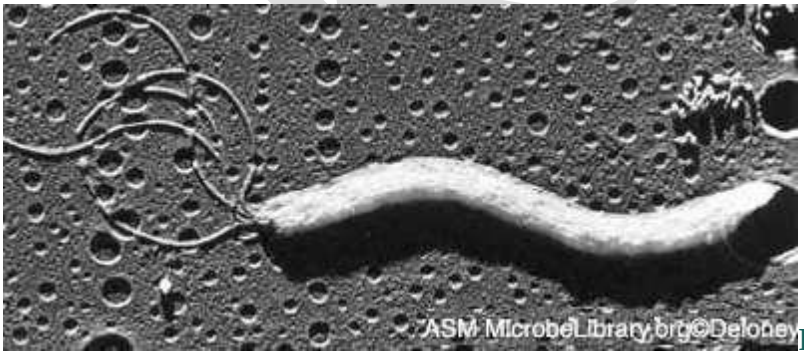


Figure 8. *Helicobacter pylori.* ASM

The proximal small intestine has a relatively sparse Gram-positive flora, consisting mainly of lactobacilli and *Enterococcus faecalis.* This region has about 10^5 - 10^7 bacteria per ml of fluid. The distal part of the small

intestine contains greater numbers of bacteria (10^8/ml) and additional species, including coliforms (*E. coli* and relatives) and *Bacteroides,* in addition to lactobacilli and enterococci.

The flora of the large intestine (colon) is qualitatively similar to that found in feces. Populations of bacteria in the colon reach levels of 10^{11}/ml feces. Coliforms become more prominent, and enterococci, clostridia and lactobacilli can be regularly found, but the predominant species are anaerobic *Bacteroides*and anaerobic lactic acid bacteria in the genus *Bifidobacterium* (*Bifidobacterium bifidum*). These organisms may outnumber *E. coli* by 1,000:1 to 10,000:1. Sometimes, significant numbers of anaerobic methanogens (up to 10^{10}/gm) may reside in the colon of humans. This is our only direct association with archaea as normal flora. The range of incidence of certain bacteria in the large intestine of humans is shown in Table 4 below.

Table 4. Bacteria found in the large intestine of humans.

BACTERIUM	RANGE OF INCIDENCE
Bacteroides fragilis	**100**
Bacteroides melaninogenicus	**100**
Bacteroides oralis	**100**
Lactobacillus	**20-60**
Clostridium perfringens	**25-35**
Clostridium septicum	**5-25**
Clostridium tetani	**1-35**
Bifidobacterium bifidum	**30-70**
Staphylococcus aureus	**30-50**
Enterococcus faecalis	**100**
Escherichia coli	**100**
Salmonella enteritidis	**3-7**
Klebsiella sp.	**40-80**
Enterobacter sp.	**40-80**
Proteus mirabilis	**5-55**
Pseudomonas aeruginosa	**3-11**
Peptostreptococcus sp.	**?common**
Peptococcus sp.	**?common**

At birth the entire intestinal tract is sterile, but bacteria enter with the first feed. The initial colonizing bacteria vary with the food source of the infant. In breast-fed infants, bifidobacteria account for more than 90% of the total intestinal bacteria. *Enterobacteriaceae* and enterococci are regularly present, but in low proportions, while bacteroides, staphylococci, lactobacilli and clostridia are practically absent. In bottle-fed infants, bifidobacteria

are not predominant. When breast-fed infants are switched to a diet of cow's milk or solid food, bifidobacteria are progressively joined by enterics, bacteroides, enterococci lactobacilli and clostridia. Apparently, human milk contains a growth factor that enriches for growth of bifidobacteria, and these bacteria play an important role in preventing colonization of the infant intestinal tract by non indigenous or pathogenic species.

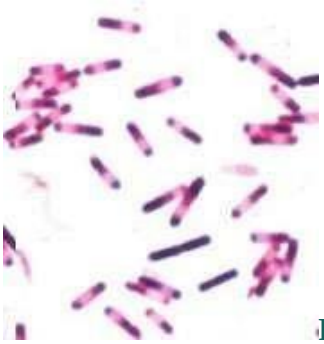


Figure 9. *Clostridium difficile*. Gram stain. The growth of "C. diff" in the intestinal tract is normally held in check by other members of the normal flora. When antibiotics given for other infections cause collateral damage to the normal intestinal flora, the clostridium may be able to "grow out" and produce a serious diarrheal syndrome called pseudomembranous colitis. This is an example of an "antibiotic induced diarrheal disease".

The composition of the flora of the gastrointestinal tract varies along the tract (at longitudinal levels) and across the tract (at horizontal levels) where certain bacteria attach to the gastrointestinal epithelium and others occur in the lumen. There is frequently a very close association between specific bacteria in the intestinal ecosystem and specific gut tissues or cells (evidence of tissue tropism and specific adherence). Gram-positive bacteria, such as the streptococci and lactobacilli, are thought to adhere to the gastrointestinal epithelium using polysaccharide capsules or cell wall teichoic acids to attach to specific receptors on the epithelial cells. Gram-negative bacteria such as the enterics may attach by means of specific fimbriae which bind to glycoproteins on the epithelial cell surface.

It is in the intestinal tract that we see the greatest effect of the bacterial flora on their host. This is due to their large mass and numbers. Bacteria in the human GI tract have been shown to produce vitamins and may otherwise contribute to nutrition and digestion. But their most important effects are in their ability to protect their host from establishment and infection by alien microbes and their ability to stimulate the development and the activity of the immunological tissues.

On the other hand, some of the bacteria in the colon (e.g. *Bacteroides*) have been shown to produce metabolites that are carcinogenic, and there may be an increased incidence of colon cancer associated with these bacteria. Alterations in the GI flora brought on by poor nutrition or perturbance with antibiotics can cause shifts in populations and colonization by nonresidents that leads to gastrointestinal disease.

Beneficial Effects of the Normal Flora

The effects of the normal flora are inferred by microbiologists from experimental comparisons between **"germ-**

free" animals (which are not colonized by any microbes) and conventional animals (which are colonized with a typical normal flora). Briefly, some of the characteristics of a germ-free animals that are thought to be due to lack of exposure to a normal flora are:

1. vitamin deficiencies, especially vitamin K and vitamin B12
2. increased susceptibility to infectious disease
3. poorly developed immune system, especially in the gastrointestinal tract
4. lack of "natural antibody" or natural immunity to bacterial infection

Because these conditions in germ-free mice and hamsters do not occur in conventional animals, or are alleviated by introduction of a bacterial flora (at the appropriate time of development), it is tempting to conclude that the human normal flora make similar contributions to human nutrition, health and development. The overall beneficial effects of microbes are summarized below.

1. The normal flora synthesize and excrete vitamins in excess of their own needs, which can be absorbed as nutrients by their host. For example, in humans, enteric bacteria secrete Vitamin K and Vitamin B12, and lactic acid bacteria produce certain B-vitamins. Germ-free animals may be deficient in Vitamin K to the extent that it is necessary to supplement their diets.

2. The normal flora prevent colonization by pathogens by competing for attachment sites or for essential nutrients. This is thought to be their most important beneficial effect, which has been demonstrated in the oral cavity, the intestine, the skin, and the vaginal epithelium. In some experiments, germ-free animals can be infected by 10 *Salmonella* bacteria, while the infectious dose for conventional animals is near 10^6 cells.

3. The normal flora may antagonize other bacteria through the production of substances which inhibit or kill nonindigenous species. The intestinal bacteria produce a variety of substances ranging from relatively nonspecific fatty acids and peroxides to highly specific bacteriocins, which inhibit or kill other bacteria.

4. The normal flora stimulate the development of certain tissues, i.e., the caecum and certain lymphatic tissues (Peyer's patches) in the GI tract. The caecum of germ-free animals is enlarged, thin-walled, and fluid-filled, compared to that organ in conventional animals. Also, based on the ability to undergo immunological stimulation, the intestinal lymphatic tissues of germ-free animals are poorly-developed compared to conventional animals.

5. The normal flora stimulate the production of natural antibodies. Since the normal flora behave as antigens in an animal, they induce an immunological response, in particular, an antibody-mediated immune (AMI) response. Low levels of antibodies produced against components of the normal flora are known to cross react with certain related pathogens, and thereby prevent infection or invasion. Antibodies produced against antigenic components of the normal flora are sometimes referred to as "natural" antibodies, and such antibodies are lacking in germ-free animals.

Harmful Effects of the Normal Flora

Harmful effects of the normal flora, some of which are observed in studies with germ-free animals, can be put in the following categories. All but the last two are fairly insignificant.

1. Bacterial synergism between a member of the normal flora and a potential pathogen. This means that one organism is helping another to grow or survive. There are examples of a member of the normal flora supplying a vitamin or some other growth factor that a pathogen needs in order to grow. This is called**cross-feeding** between microbes. Another example of synergism occurs during treatment of **"staph-protected infections"** when a penicillin-resistant staphylococcus that is a component of the normal flora shares its drug resistance with pathogens that are otherwise susceptible to the drug.

2. Competition for nutrients Bacteria in the gastrointestinal tract must absorb some of the host's nutrients for their own needs. However, in general, they transform them into other metabolisable compounds, but some nutrient(s) may be lost to the host. Germ-free animals are known to grow more rapidly and efficiently than conventional animals. One explanation for incorporating antibiotics into the food of swine, cows and poultry is that the animal grows faster and can therefore be marketed earlier. Unfortunately, this practice contributes to the development and spread of bacterial antibiotic resistance within the farm animals, as well as humans.

3. Induction of a low grade toxemia Minute amounts of bacterial toxins (e.g. endotoxin) may be found in the circulation. Of course, it is these small amounts of bacterial antigen that stimulate the formation of natural antibodies.

4. The normal flora may be agents of disease. Members of the normal flora may cause **endogenous disease** if they reach a site or tissue where they cannot be restricted or tolerated by the host defenses. Many of the normal flora are potential pathogens, and if they gain access to a compromised tissue from which they can invade, disease may result.

5. Transfer to susceptible hosts Some pathogens of humans that are members of the normal flora may also rely on their host for transfer to other individuals where they can produce disease. This includes the pathogens that colonize the upper respiratory tract such as *Neisseria meningitidis, Streptococcus pneumoniae, Haemophilus influenzae* and *Staphylococcus aureus,* and potential pathogens such as *E. coli, Salmonella* or *Clostridium* in the gastrointestinal tract.

Dental Caries, Gingivitis and Periodontal Disease

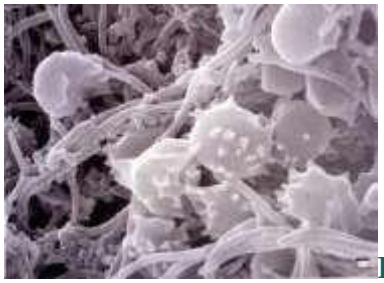
The most frequent and economically-important condition in humans resulting from interactions with our normal flora is probably dental caries. Dental plaque, dental caries, gingivitis and periodontal disease result from actions initiated and carried out by the normal bacterial flora.

Dental plaque, which is material adhering to the teeth, consists of bacterial cells (60-70% the volume of the plaque), salivary polymers, and bacterial extracellular products. Plaque is a naturally-constructed biofilm, in which the consortia of bacteria may reach a thickness of 300-500 cells on the surfaces of the teeth. These accumulations subject the teeth and gingival tissues to high concentrations of bacterial metabolites, which result

in dental disease.

The dominant bacterial species in dental plaque are *Streptococcus sanguis* and*Streptococcus mutans,* both of which are considered responsible for plaque.

Plaque formation is initiated by a weak attachment of the streptococcal cells to salivary glycoproteins forming a pellicle on the surface of the teeth. This is followed by a stronger attachment by means of extracellular sticky polymers of glucose (glucans) which are synthesized by the bacteria from dietary sugars (principally sucrose). An enzyme on the cell surface of *Streptococcus mutans,* glycosyl transferase, is involved in initial attachment of the bacterial cells to the tooth surface and in the conversion of sucrose to dextran polymers (glucans) which form plaque.



Dental plaque, scanning electron micrograph illustrating the diversity of microbes in plaque. Image courtesy of Rachel Sammons, University of Birmingham School of Dentistry (UK).

Dental Caries is the destruction of the enamel, dentin or cementum of teeth due to bacterial activities. Caries are initiated by direct demineralization of the enamel of teeth due to lactic acid and other organic acids which accumulate in dental plaque. Lactic acid bacteria in the plaque produce lactic acid from the fermentation of sugars and other carbohydrates in the diet of the host.*Streptococcus mutans* and *Streptococcus sanguis* are most consistently been associated with the initiation of dental caries, but other lactic acid bacteria are probably involved as well. These organisms normally colonize the occlusal fissures and contact points between the teeth, and this correlates with the incidence of decay on these surfaces.

Streptococcus mutans in particular has a number of physiological and biochemical properties which implicate it in the initiation of dental caries.

1. It is a regular component of the normal oral flora of humans which occurs in relatively large numbers. It readily colonizes tooth surfaces: salivary components (mucins, which are glycoproteins) form a thin film on the tooth called the enamel pellicle. The adsorbed mucins are thought to serve as molecular receptors for ligands on the bacterial cell surface.

2. It contains a cell-bound protein, glycosyl transferase, that serves an adhesin for attachment to the tooth, and as an enzyme that polymerizes dietary sugars into glucans that leads to the formation of plaque.

3. It produces lactic acid from the utilization of dietary carbohydrate which demineralizes tooth enamel. *S. mutans* produces more lactic acid and is more acid-tolerant than most other streptococci.

4. It stores polysaccharides made from dietary sugars which can be utilized as reserve carbon and energy sources for production of lactic acid. The extracellular glucans formed by *S. mutans* are, in fact, bacterial capsular polysaccharides that function as carbohydrate reserves. The organisms can also form intracellular polysaccharides from sugars which are stored in cells and then metabolized to lactic acid.

Streptococcus mutans appears to be important in the initiation of dental caries because its activities lead to colonization of the tooth surfaces, plaque formation, and localized demineralization of tooth enamel. It is not however, the only cause of dental decay. After initial weakening of the enamel, various oral bacteria gain access to interior regions of the tooth. Lactobacilli, *Actinomyces*, and various proteolytic bacteria are commonly found in human carious dentin and cementum, which suggests that they are secondary invaders that contribute to the progression of the lesions.

Actinomyces israelii

Periodontal Diseases are bacterial infections that affect the supporting structures of the teeth (gingiva, cementum, periodontal membrane and alveolar bone). The most common form, **gingivitis**, is an inflammatory condition of the gums. It is associated with accumulations of bacterial plaque in the area. Increased populations of *Actinomyces* have been found, and they have been suggested as the cause.

Diseases that are confined to the gum usually do not lead to loss of teeth, but there are other more serious forms of periodontal disease that affect periodontal membrane and alveolar bone resulting in tooth loss. Bacteria in these lesions are very complex populations consisting of Gram-positive organisms (including*Actinomyces* and streptococci) and Gram-negative organisms (including spirochetes and *Bacteroides*). The mechanisms of tissue destruction in periodontal disease are not clearly defined but hydrolytic enzymes, endotoxins, and other toxic bacterial metabolites seem to be involved.

Short Answer Questions 2 Marks

1. Define Infection.
2. Define Invasion
3. Define Pathogen
4. Define Pathogenicity
5. Define Virulence
6. Define Toxigenicity
7. Define Carriers and their types
8. Define Opportunistic infections
9. Define Nosocomial infections.
10. Write about Transmission of infection

Essay Answer Questions 8 Marks

11. Write about the normal flora present in skin.
12. Write the importance of normal flora present in gastrointestinal tract and urogenital tract.

Unit I Questions	Option 1	Option 2	Option 3	Option 4	Answer
The sputum specimen must be stored at	10C	5C	4C	8C	4C
the blood samples should be collected at the stage of the disease.	late	early	before diagnosis	after symptom	early
When a parasite is growing and multiplying within a host, it is said have	pathogen	infection	organism	diagnose	infection
For microbiological examination urine must be collected as a specimen	Good	sterile	normal	clean catch mid-stream	clean catch mid-stream
Cary-Blair medium is used for the transportation of specimen.	Blood	CSF	stool	urine	stool
a parasite organism or agent that produce such a disease is a	Pathagon	parasite	bacteria	virus	pathagon
the ability of an organism to cause disease is called	Diagnosis	symptoms	pathogenicity	disease	pathogenicity
The most important step in the diagnosis of an infection is the proper collection of _____.	Specimen	Organism	Symptoms	Prophylaxis	Specimen
In case of meningeal irritation or affected cerebrum specimen is collected.	Blood	cerebrospinal fluid	urine	pus	cerebrospinal fluid
in blood specimens is seperated and used for serological techniques.	haemoglobin	serum	iron	protein	serum
The collected CSF should be stored at	room temperature	4C	2C	5C	room temperature
is a good transport medium for stool sample.	agar medium	peptone water	Cary-Blair medium	alkaline medium	Cary-Blair medium
An alternate transport medium for stool sample is	alkaline-peptone water	peptone water	alkaline medium	water	alkaline-peptone water

A sterile is used for transportation of specimens to labs.	Container	plastic container	screw-cap container	metal container	screw-cap container
Specimens must be collected before administration of _____	Vaccines	Antimicrobial drugs	Symptoms	Culturing	Antimicrobial drugs
To eliminate normal flora skin surface is cleansed with _____.	Germicide	Soap	Vaseline	Cotton	Germicide
Pus swabs collected in transport medium should reach lab within ____ hours	2	4	6	8	6
During blood collection the skin should be vein punctured by cleansing with _____.	Isopropyl alcohol	Antiseptic	Soap	Antibiotic	Isopropyl alcohol
About _____ ml of blood should be collected.	10	20	30	40	20
the blood samples should be incubated at _____.	25C	37C	4C	10C	37C
_____ ml of urine sample should be collected.	10	40	30	20	20
In case of _____ only few millilitres of urine specimen can be collected.	Arthritis	Gas gangrene	Renal failure	Paralysis	Renal failure
If the urine sample is delayed for more than an hour _____ can be added.	Amino acid	Boric acid	Sodium chlloride	Calcium chloride	Boric acid
In case of suspected renal tuberculosis the urine should be collected for _____ successive mornings	1	2	3	4	3
The CSF should be collected from _____	Cerebrum	Cerebellum	Ventricle	Arachnoid space	Arachnoid space
In case of infants the CSF should be	Ventricle	Arachnoid space	Cerebrum	Cerebellum	Ventricle

collected by puncturing _____.					
About _____ ml of CSF should be collected in sterile tubes.	10	30	40	20	20
For collecting nasopharyngeal aspirates _____ is passed gently through one nostril.	Sterile catheter	Syringe	Swab	Cotton	Sterile catheter
_____ is collected in case of eye specimens.	Pus	Mucous	Conjuctival scrapings	Conjunctival tissue	Conjuctival scrapings
Respiratory secretions should be transported to laboratory within _____.hours	1	2	3	4	2
The natural secretions of eye contains _____ enzymes	Antibiotic	Toxins	Antibacterial	Amylase	Antibacterial
If not possible to obtain faeces a specimen is collected by inserting _____	Cotton woll swab	Syringe	Catheter	Cotton	Cotton woll swab
Salmonella, Shigella and Vibrio survive in Cary Blair medium for _____ hrs.	24	48	32	76	48
Campylobacter can survive in C-B medium for ___hes.	2	4	6	8	6
If cholera is suspected the stool sample should be sent to the lab within _____-	1	3	5	8	8
_____ is the transportation medium used in case of cholera.	CB medium	Boric acid	Alkaline peptone water	Phosphate buffered saline	Alkaline peptone water
For suspected viral enteritis the stool sample is transferred to 9ml of _____	Phosphate buffered saline	Alkaline peptone water	CB medium	Boric acid	Phosphate buffered saline
About _____ ml of sterile phosphate	3	6	9	12	9

buffered saline is needed for suspected viral enteritis					
_____ is the transportation medium used for the collection of synovial, pleural or asceptic fluid samples	Trisodium citrate	Sodium chlloride	Boric acid	Calcium chloride	Trisodium citrate
About _____ ml of synovial, pleural and ascitic samples were collected.	2 to 3	5 to 6	3 to 5	4 to 6	2 to 3
Synovial, pleural and ascitic fluids should be mixed with _____.	Antibiotic	Antiseptic	Anticoagulant	Antibacterial	Anticoagulant
In case of sputum sample the sample should be collected best in _____.	morning	Evening	Mid night	Noon	morning
_____ bacilli requires entirely different method of identification	Rod shaped	Spindle shaped	Club shaped	Acid-fast	Acid-fast
In suspected anthrax the pus sample should be collected from _____.	Lesions	Scrapings	Edema	Necrosis	Lesions
For darkfield examination pus from syphilitic ulcers are suspected in 0.2ml of _____.	Salt solution	Saline solution	Anticoagulant	Antiseptic	Saline solution
In case of tuberculosis _____ specimen is collected	Stool	Urine	Blood	Sputum	Sputum
The sputum sample collected in paper towel should be soaked in _____-.	Saline solution	Anticoagulant	Formalin solution	Salt solution	Formalin solution
_____ technique is used to stain acid fast bacilli	Ziehl Neelsen	Gram staining	Endospore staining	Quellung	Ziehl Neelsen
In bacilliary dysentry _____ yields more positive cultures than stools.	Smears	Swabs	Rectal swabs	Renal smears	Rectal swabs

In case of salmonellosis _____ sample is collected.	Urine	Stool	Sputum	Blood	Blood
In case of thyphoid _____ sample should be collected.	Sputum	Blood	Faeces	CSF	Blood
In order to avoid drying of stool sample the swabs should be placed in _____.	Thioglycollate semisolid medium	Sodium citrate	Buffered glycerol water	Para amino benzoic acid	Thioglycollate semisolid medium
A n alternative for Thioglycollate semisolid medium is _____.	Sodium citrate	Buffered glycerol water	Para amino benzoic acid	Thioglycollate semisolid medium	Buffered glycerol water
_____ should be incorporated in all blood culture media	Trisodium citrate	Formalin solution	Sodium citrate	Para amino benzoic acid	Para amino benzoic acid
_____ is added to the blood sample to prevent coagulation	Sodium citrate	Thioglycollate semisolid medium	Antiseptic	Antibacterial	Sodium citrate
Once the specimen is collected the container should be _____.	Labelled	Opened	Diluted	Defined	Labelled
Samples of blood and CSF for culture should not be _____	Labelled	Refrigerated	Processed	Incubated	Refrigerated
The sputum sample should be free from _____	Blood	Pus	Saliva	Tissue	Saliva
when the patient has symptoms of cystitis _____ should be collected.	blood	Pus	Sputum	urine	urine
In case of suspected septicemia _____ sample should be collected	blood	Pus	Sputum	urine	blood

Unit II

List of diseases of various organ systems and their causative agents. The following diseases in detail with symptoms, mode of transmission, prophylaxis and control. Respiratory pathogens: *Streptococcus pyogenes, Haemophilus influenzae, Mycobacterium tuberculosis*. Gastrointestinal Diseases: *Escherichia coli, Salmonella typhi, Vibrio cholerae*, *Helicobacter pylori*. Others: *Staphylococcus aureus, Bacillus anthracis, Clostridium tetani, Treponema pallidum.*

Streptococcus pyogenes (GAS) Invasive Disease 10/26/2015

Organism: *Streptococcus pyogenes* Group A beta-hemolytic (130 different serotypes associated with different clinical manifestations).

- Most common is NON-invasive disease, e.g., impetigo or pharyngitis
- Only INVASIVE presentations, i.e., necrotizing fasciitis (NF), streptococcal toxic shock syndrome (STSS), bacteremia, are reportable
- Can also see nonsuppurative sequelae, e.g., rheumatic fever, post-streptococcal glomerulonephritis
- Rheumatic fever is reportable as a separate condition

Incubation period: Short, usually 1-3 days, rarely longer. Non-invasive infections may have variable incubation periods: pharyngitis – 2-5 days and impetigo – 7-10 days. Can also have asymptomatic carriage.

Infectious period: With adequate penicillin treatment, transmissibility generally ends within 24 hours.

- In untreated, uncomplicated cases, 10-21 days.
- In untreated cases with purulent discharges, weeks to months.
- In untreated cases with pharyngitis, contagiousness sharply reduced after 2-3 weeks.

Transmission route:

Person to person by contact with infectious secretions from the nose or throat of infections persons or by contact with infected skin lesions. Asymptomatic pharyngeal carriage occurs among all age groups but is most common among children. Persons with acute upper respiratory tract symptoms are particularly likely to transmit infections. Carriers have been responsible for nosocomial outbreaks, particularly following surgical procedures. Explosive outbreaks of sore throat may follow ingestion of contaminated food. Spread of GAS from humans to cattle has been responsible for outbreaks associated with raw milk.

Treatment:

- Standard therapy is Penicillin G, IM for 10 days. While antibiotics might shorten clinical illness somewhat, pharyngitis cases would likely improve in 3-4 days without treatment. Antibiotics are given to guard against suppurative complications and development of rheumatic fever.
- Erythromycin can be used for pen-sensitive patients, but resistance has been documented.
- Clindamycin or a cephalosporin may also be used. Sulfonamides and tetracyclines should not be used.
- For NF, aggressive surgical debridement of necrotic tissue is recommended in addition to antibiotic therapy.
- Immune Globulin Intravenous (IVIG) may also be used in the treatment of STSS in addition to antibiotic therapy.

10/26/2015

Information Needed for the Investigation Verify the Diagnosis

- Clinical description: NF causes severe local pain and tissue destruction. It is often characterized by rapid progression, rash, blistering, tachycardia, high fever and hypotension. STSS is a severe illness characterized by

hypotension, coagulation disorder, and multiorgan failure. Incidence of invasive GAS infection highest among infants, the elderly, immune-suppressed, etc.

• *Confirmed:* isolation of GAS from a normally sterile site (CSF, blood, joint fluid, etc.) ABCs case definition (http://www.cdc.gov/abcs/methodology/case-def-ascertain.html GAS isolated from a wound culture and accompanied by NF or STSS.

Determine the Extent of Illness

Currently additional epidemiologic and laboratory data for individual cases are gathered by CDC-AIP. For cluster detection, SOE reviews case counts and geographic distribution monthly, and CDC-AIP reviews molecular data quarterly. If GAS epidemiology appears to be changing, SOE will coordinate with CDC-AIP for additional follow up, including confirming/determining if a cluster exists and discussing with partners about the need to launch a larger investigation. See CDC's website calculator for direction on launching an investigation: http://www2a.cdc.gov/ncidod/dbmd/abcs/calc/calc_new/index.htm . See also below for CDC guidance on when public health action may be indicated.

Settings requiring urgent public health action

• Postpartum and post-surgical GAS infections (see Prevention of Invasive Group A Streptococcal Disease among Household Contacts of Case Patients and among Postpartum and Postsurgical Patients: Recommendations from the Centers for Disease Control and Prevention. *Clin Infect Dis* 2002;35:950-59.)
• Outbreaks of rheumatic fever
• Clusters in military institutions
• Clusters in hospitals or long-term care facilities
• Outbreaks of invasive disease in child care centers and other school settings
• Outbreaks of invasive disease among young children following varicella (chicken pox) infections

Laboratory Specimens

• In suspected cases of invasive GAS, cultures of blood and focal sites of infection are indicated.
• Request that any isolate be sent to CDC/AIP directly.

Contact and Control Measures

• If multiple cases occur, notify parents, healthcare providers and emergency rooms in the area ofthe occurrence of GAS.
• People with skin lesions should not handle food.

10/26/2015

• Culture symptomatic contacts in outbreak settings, e.g., families with cases of NF, or healthcare outbreaks.

Hospital Considerations

• Minor skin, wound or burn use Standard Precautions.
• Endometriosis (puerperal sepsis) use Standard Precautions.
• Major skin, wound or burns (draining wounds) use Contact and Droplet Precautions for the first 24 hours after initiation of effective therapy.
• Pharyngitis in infants and children use Droplet Precautions for the first 24 hours after initiation ofeffective therapy.

• Pneumonia, scarlet fever in infants and young children, and serious invasive disease (concern for secondary transmission to patients or health care workers) use Droplet Precautions for the first 24 hours after initiation of effective therapy.
• Post-partum and post-surgical cases should prompt a facility internal infection controlreview.

Reporting Requirements
• FTR: write up cluster investigations only
• AK-STARS Database o Enter all *confirmed* cases
o Indicate "NF" as a Secondary Condition if that is present
o Indicate "STSS" as a Secondary Condition if that is present
o Ensure especially for NF that the "Death" Y/N/U field is completed
o AIP will fax all Case Report Forms to SOE for NF and deaths
o Rheumatic Fever cases are entered as RF and not GAS cases, although sometimes a person might have two entries if they meet both case definitions

• Fax notification of any suspected GAS (not RF) cases to CDC/AIP (729-3473) upon receipt (within one working day.)
• May use ABCs (CDC) Case Report Form and Definition to define *confirmed* cases
• NOTE: GAS no longer reportable to CDC via NETSS since 2009. Case definition not updated since 1995. Included in chapter for historical reference only.

References
• CDC annual ABCs surveillance reports are available at: http://www.cdc.gov/abcs/reports-findings/surv-reports.html
• Red Book 2012 Report of the Committee on Infectious Diseases, 29th Edition
• Control of Communicable Diseases Manual 20th Edition
• Siegel JD, Rhinehart E, Jackson M, Chiarello L, and the Healthcare Infection Control Practices Advisory Committee. 2007 Guideline for Isolation Precautions: Preventing Transmission of Infectious Agents in Healthcare Settings. Available at http://www.cdc.gov/hicpac/pdf/isolation/isolation2007.pdf

Page 1
Streptococcus pyogenes or Group A Strep (GAS) What is group A streptococcus (GAS)?
Group A streptococcus (GAS) is a bacterium often found in the throat and on the skin. People may carry group A streptococci in the throat or on the skin and have no symptoms of illness. Most GAS infections are relatively mild illnesses such as "strep throat," or impetigo (a skin infection). Occasionally, these bacteria can cause other severe and even life-threatening diseases.
How are group A streptococci spread?
These bacteria are spread through direct contact with mucus from the nose or throat of people who are sick with a GAS infection or through contact with infected wounds or sores on the skin. The bacteria may also be spread through contact with persons without symptoms but who carry the bacteria in their throat or on their skin. Ill persons, such as those who have strep throat or skin infections, are most likely to spread the infection. Persons who carry the bacteria but have no symptoms are much less contagious. Treating an infected person with an antibiotic for 24 hours or longer generally prevents the spread of the bacteria to others. However, it is important to complete the entire course of antibiotics as prescribed. It is not likely that household items like toys spread

these bacteria. However, it is possible to spread these bacteria by drinking from the same glass or eating from the same plate as someone who is ill with a GAS infection like strep throat.

What kinds of illnesses are caused by group A streptococcal infection? Infection with GAS can result in a range of illnesses:

- Mild illness such as strep throat or impetigo
- Severe illness (necrotizing fasciitis, streptococcal toxic shock syndrome)

Severe, sometimes life-threatening GAS disease may occur when these bacteria get into parts of the bodywhere bacteria usually are not found, such as the blood, muscle, or the lungs. These infections are called "invasive GAS disease." Two of the most severe, but least common, forms of invasive GAS disease are necrotizing fasciitis and streptococcal toxic shock syndrome (STSS). Necrotizing fasciitis (occasionally described by the press as "the flesh-eating bacteria") rapidly destroys muscles, fat, and skin tissue. STSS causes blood pressure to drop rapidly and organs (e.g., kidney, liver, lungs) to fail. STSS is not the same as the staphylococcal toxic shock syndrome that has been associated with tampon usage. Less severe invasive illnesses caused by GAS include cellulitis and pneumonia. In the U.S, about 25% of patients with necrotizing fasciitis due to GAS and approximately 40% with STSS die. About 10%-15% of patients with any form of invasive group A streptococcal disease die.

How common is invasive group A streptococcal disease?

About 9,000-11,500 cases of invasive GAS disease occur each year in the United States, resulting in 1,000-1,800 deaths annually. STSS and necrotizing fasciitis each comprise an average of about 6%-7% of these invasive cases. In contrast, there are several million cases of strep throat and impetigo each year.

Section of Epidemiology *Streptococcus pyogenes* (GAS) Invasive Disease Page 2 3601 C Street, Suite 540
Updated May 2015 Anchorage, AK 99503 907-269-8000 (f) 907-562-7802

What is reportable in Alaska?

In Alaska, health care providers and laboratories are required to report *only* invasive GAS cases. Annual counts are published in Epidemiology *Bulletins* and have averaged between 40-70 cases each year.

Who is most at risk of getting invasive group A streptococcal disease?

Few people who come in contact with GAS will develop invasive GAS disease. Most people will have a throat or skin infection, and some may have no symptoms at all. Although healthy people can get invasive GAS disease, people with chronic illnesses like cancer, diabetes, and chronic heart or lung disease, and those who use medications such as steroids have a higher risk. People with skin lesions (such as cuts, chickenpox, or surgical wounds), the elderly, and adults with a history of alcohol abuse or injection drug use also have a higher risk for disease.

What are the early signs and symptoms of necrotizing fasciitis and STSS? Early signs and symptoms of necrotizing fasciitis:

- Severe pain and swelling, often rapidly increasing
- Fever
- Redness at the wound site

Early signs and symptoms of STSS:

- Sudden onset of generalized or localized severe pain, often in an arm or leg
- Dizziness
- Flu-like symptoms such as fever, chills, muscle aches, nausea, vomiting

• Confusion
• A flat red rash over large areas of the body (only occurs in 1 in 10 cases)

How is invasive group A streptococcal disease treated?
GAS infections can be treated with many different antibiotics. For STSS and necrotizing fasciitis, high dose penicillin and clindamycin are recommended. For those with very severe illness, supportive care in an intensive care unit may also be needed. With necrotizing fasciitis, early and aggressive surgery is often needed to remove damaged tissue and stop disease spread. Early treatment may reduce the risk of death from invasive GAS; however, even the best medical care does not prevent death in every case.

What can be done to help prevent group A streptococcal infections?
The spread of all types of GAS infection can be reduced by good hand washing, especially after coughing and sneezing and before preparing foods or eating. People with sore throats should be seen by a doctor who can perform tests to find out whether the illness is strep throat. If the test result shows strep throat, the person should stay home from work, school, or day care until 24 hours after taking an antibiotic. All wounds should be kept clean and watched for possible signs of infection such as redness, swelling, drainage, and pain at the woundsite. A person with signs of an infected wound, especially if fever occurs, should immediately seek medical care. It is not necessary for all persons exposed to someone with an invasive GAS infection (i.e., necrotizing fasciitis or STSS) to receive antibiotics to prevent infection. However, in some situations, antibiotics may be recommended. That decision should be made after talking with your doctor.
Invasive, Group A (GAS) *Streptococcus pyogenes* Case Definition Clinical Description
Invasive group A streptococcal infections may manifest as any of several clinical syndromes, including pneumonia, bacteremia in association with cutaneous infection (e.g., cellulitis, erysipelas, or infection of a surgical or nonsurgical wound), deep soft-tissue infection (e.g., myositis or necrotizing fasciitis), meningitis, peritonitis, osteomyelitis, septic arthritis, postpartum sepsis (i.e., puerperal fever), neonatal sepsis, and nonfocal bacteremia.
Laboratory Criteria for Diagnosis
Isolation of group A *Streptococcus* (*Streptococcus pyogenes*) by culture from a normally sterile site (e.g., blood or cerebrospinal fluid, or, less commonly, joint, pleural, or pericardial fluid)
Case Classification Confirmed
A case that is laboratory confirmed Comments
The 1995 case definition appearing on this page was later re-published in the 1997 *MMWR* Recommendations and Reports titled *Case Definitions for Infectious Conditions Under Public Health Surveillance*.[1] Thus, the 1995 and 1997 versions of this case definition are identical. As of 2009, GAS was no longer considered nationally notifiable.

SALMONELLA
INTRODUCTION
Salmonella consists of bacilli leading to Enteric fever, Gastroenteritis, Speticemia etc. The important member of the genus is Salmonella typhi, which causes Typhoid fever.
OBJECTIVES
After reading this lesson, you will be able to:
describe the morphology of Salmonella
discuss the cultural characteristics of Salmonella

explain the biochemical reactions of Salmonella
demonstrate the Widal reaction. Salmonella are of two groups;
(i) Enteric fever group consisting of typhoid & Paratyphoid bacilli exclusively primary human parasites
(ii) Food poisoning group, which are animal parasite but may infect humans causing Gastrointestinal infections

Morphology

Salmonellae are gram negative rods. They are motile with peritrichate flagella except for S. gallinarum pullorum

Cultural Characteristics

Salmonellae are aerobic and facultatively anaerobic bacteria growing readily on simple media over a range of pH 6-8 & temperature 15-41°C with optimum temperature of 37°C. Colonies are large, circular and smooth on MacConkey and Deoxycholate citrate media, colonies are colourless due to absence of lactose fermentation. Selenite F and Tetrathionate broth are commonly employed as enrichment media.

Biochemical reaction

Salmonellae ferment glucose, mannitol and maltose forming acid and gas. Whereas S. typhi is an aerogenic i.e. it does not form fermentation of sugars like glucose etc. Lactose, Sucrose and Salicin are not fermented. Indole is not produced. They are MR positive, VP negative and citrate positive.

Resistance

The bacilli are killed at 55oC in one hour or at 60oC in 15 minutes. Boiling or chlorination of water and pasteurization of milk destroy the bacilli. In polluted water it may survive for weeks and in ice for months.

Antigenic Structure

Salmonellae possess the antigens and based on which they are classified as

(i) flagella antigen H,
(ii) Somatic antigen O and
(iii) surface antigen Vi

H antigen

This antigen present on flagella is heat labile protein. It is destroyed by boiling or by treatment with alcohols but not by formaldehyde.

O antigen

O antigen is a Phospholipid-protein-polysaccharide complex which forms an integral part of the cell wall. It is identical with endotoxin. This is unaffected by boiling, alcohol or weak acids

CLASSIFICATION AND NOMENCLATURE

Classification within the genus is on antigenic characterisation based on Kauffman-White scheme and this depends on identification by agglutination of the O and H antigens of the strains. Salmonellae are classified into serological groups based on the presence of distinctive O antigen factors and designated as 1, 2, 3 etc

Biochemically Kauffman proposed Salmonellae classification as

Subgenus I: Largest and medically most important group causing human and animal infections

Subgenus II: Species isolated from reptiles.

Subgenus III: Species isolated from reptiles and human beings Subgenus IV: These are rarely encountered.

Pathogenecity

Salmonellae cause the following clinical syndrome in human beings

1. Enteric fever

2. Gastroenteritis or food poisoning
3. Septicemia with or without local suppurative lesions

I. Enteric Fever

This includes typhoid fever caused by S.typhi and paratyphoid fever caused by S. Paratyphi A,B,C. the infection is acquired by ingestion of contaminated food, on reaching the gut the bacilli attach to microvilli of the ileal mucosa and penetrate submucosa. They are phagocytosed by polymorphs and macrophages. Their ability to resist intercellular killing and to multiply within the cells is a measure of their virulence. They enter the mesenteric lymph nodes, where they multiply and via thoracic duct, enter the blood stream causing bacteremia.

Fig. 23.2

As bile is a good culture medium for the bacillus it multiplies abundantly in the gall bladder and is discharged continuously into the intestine where it involves the Peyer's Patches and lymphoid follicles of the ileum, which ulcerate and may lead to intestinal perforation & haemorrhage as complication. The incubation period is usually 7-14 days but may range from 3-56 days.

Laboratory Diagnosis

Bacteriological diagnosis of enteric fever consists of isolation of the bacilli and demonstration of antibodies in serum. A positive blood culture is diagnostic; demonstration of antibodies is not conclusive of current infection. A third method is the demonstration of typhoid bacilli in blood or urine.

Blood culture

Bacteremia occurs early in the disease and blood cultures are positive mostly in the first week of fever. About 5-10ml of blood is collected and inoculated into culture bottle containing 50-100ml of 0.5 percent bile broth. After incubation

overnight at 37oC, the bile broth is subcultured on MacConkey agar, pale nonlactose fermenting colonies that may appear on this medium are picked up for

biochemical tests and motility. Salmonellae are motile, indole and urease negative and ferment glucose, mannitol and maltose but not lactose or sucrose. The typhoid bacillus will be anaerogenic, while the paratyphoid bacilli will form and gas from sugars. Identification of the isolate is by slide agglutination. A loopful of the growth from an agar slope is emulsified in two drops of saline on a slide. One emulsion acts as a control to show that the strain is not auto agglutinable.

If Salmonellae are not obtained from first subculture from bile broth, subcultures should be repeated every other day till growth is obtained. Cultures should be declared negative only after incubation for ten days.

Feces culture

Salmonellae are shed in feces throughout the course of disease and even in

convalescence, with varying frequency. A positive fecal culture, however may occur in carriers as well as in patients. The use of enrichment and selective media and repeated sampling increase the rate of isolation. Fecal samples are plated directly on MacConkey, DCA and Wilson-Blair media. On MacConkey and DCA it appears as pale colonies. On Wilson-Blair medium S typhi forms large black colonies. S paratyphi A produces green colonies onthis medium.

Urine culture

Salmonellae are shed in urine irregularly and infrequently. Hence urine culture is less useful than culture of blood or feces. Cultures are generally positive in second and third weeks.

Widal reaction

This is a test for measurement of H and O agglutinins for typhoid and paratyphoid bacilli in the patient's sera. Two types are generally used for the testa narrow tube with a conical bottom (Dreyer's agglutination tube) for H

agglutination and short round-bottomed tube (Felix tube) for O agglutination.
Equal volumes (0.4 ml) of serial dilutions of the serum and the H and O antigens are mixed in Dreyer's and Felix agglutination tubes and incubated in a water bath at 37°C overnight. Control tubes containing the antigen and normal saline are set to check for autoagglutination. The agglutination titres of the serum are read. 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.

Salmonellae gastroenteritis

Salmonellae gastroenteritis or food poisoning is zoonotic disease, the source of infection being animal products and may be caused by any Salmonellae except S typhi.
Human infection results from ingestion of contaminated food and most common source of food poisoning are poultry, meat, milk products. Salmonellae can enter through the shell if eggs are left on contaminated chicken feed or feces and grow inside.
Laboratory diagnosis is made by isolating the Salmonellae from feces and from the article of food which confirms the diagnosis.

Salmonellae Septicemia

S choleraesuis in particular, may cause septicemic disease with focal suppurative lesions such as osteomyelitis, deep abscesses, endocarditis, pneumonia and meningitis.

MID 24

Karen Brudney

Mycobacterium tuberculosis

1. Introduction
2. Epidemiology
3. Microbiology
4. Pathogenesis
a. Transmission
b. Host Reaction
5. Clinical Manifestations
6. Diagnosis
7. Treatment
8. Latent Infection
9. Prevention

I. Introduction

A. History
1. Evidence for spinal TB in Egyptian mummies and pre-Columbian remains
2. Not a significant problem until the 17th and 18th centuries as urbanization and crowding in unventilated living conditions increased
3. By the 19th century with industrialization, TB caused one quarter adult deaths in Europe
4. Germ theory of diseases and discovery of TB bacillus by Koch

B. Pre-antibiotic era
1. Sanatorium regimens and rest
2. Recognition of importance of cavitary disease- collapse therapy
3. Fresh air and sunshine-rooftops and solaria

C. Antibiotics
1. 1946 –Streptomycin

2. Rapid development of failure with monotherapy-PAS
3. INH- the magic bullet-1952
4. Rifampin and short course treatment era-1970

D. Rising incidence in world:
Failure of public health=failure of political will since Rx to cure costs $12 per person; all drugs off patent

II. Epidemiology

A. World wide: WHO Maps: Estimated incidence vs. case notifications
1. *M. tuberculosis* infects one third world's population Causes 8 million new cases active disease annually
2. Causes 2 million deaths= 2nd only to HIV as cause of death frominfectious agent world wide among adults
3. HIV/TB relationship has exacerbated problem with TB increasing in areaswith high AIDS incidence-Especially sub-Saharan Africa

MID 24

4. Absolute numbers of cases of TB highest in Asia as population densityhighest there but case rates highest in sub-Saharan Africa: 300 per 100,000 estimated incidence rates in sub-Saharan Africa vs. 100-299 per 100,000 in Asia

B. Developed World- Europe
1. Downward trend in incidence even before advent of antibiotics
2. 10% infected people develop active disease and mainly cavitarycases infectious; only 50% cases are cavitary
3. Each cavitary case needs to infect 20 to maintain constant rate of cases
4. Data from Pre-WW2 Holland shows 1 infectious case produced 13 new infections
5. Annual decrease in mortality and morbidity of 4%-6% in developed countries between 1900 and WW2:
a. Progressively higher natural residual resistance in those who had survived infection
b. Better living conditions less conducive to airborne spread.
6. Advent of antibiotics late 1940s (Streptomycin) and INH in 1952: Tuberculosis is curable

C. United States
1. Decline steady until 1984 when slowly increasing incidence
2. Causes- Neglect of TB control programs; Increase in urban homelessness and resultant crowding into homeless shelters; Advent of AIDS epidemic among this population
3. Currently, restored TB control program funding and decreasing crowding of homeless leaves background rates high among immigrants from highprevalence countries
4. One half cases in US are now among foreign born; Dramatic change between 1993 and 2003; New York, New England, west coast states all have greaterthan 50% cases foreign born in 2003; 300 per 100,000 estimated incidence rates (maps)

III. Microbiology

A. *M.tuberculosis* complex includes several species, all probably derived froma soil bacterium:
1. *Mycobacterium tuberculosis*
2. *Mycobacterium bovis*- unpasteurized milk; recent rash of cases in US among immigrants who have favorite cheeses made from unpasteurized milk sent them from home, especially Mexico and Dominican Republic
3. *Mycobacterium bovis*-BCG= used to treat bladder cancer
4. *Mycobacterium africanum* and *Mycobacterium canetti*= rare causes of tuberculosis in Africa
5. *Mycobacterium microti*= pathogen for rodents

B. Organism characteristics

1. Aerobic, non-motile, non-spore forming bacillus
2. High cell wall content of high molecular weight lipids- mycolic acid
3. SLOW GROWTH RATE

a. generation time of 20 hours vs *E.coli* generation time of 20 minutes

MID 24

b. 3-8 weeks before growth on solid media;
c. implications for length of treatment for complete sterilization compared with most bacterial pathogens

IV. Pathogenesis

A. Transmission: Lungs are the portal of entry except *M. bovis* in unpasteurized dairy products from other countries

1. Inhalation of droplet nuclei (bacillus 5 microns): from infectiousperson with active pulmonary tuberculosis, NOT just positive PPD

a. cough: most efficient at 3000 infectious droplet nuclei per cough
b. talking: similar quantity over 5 minutes
c. sneezing more efficient than coughing; singing intermediatebetween talking and coughing.
d. Inoculum size relevant: Autopsy suite transmissions; cutting through

lung tissue aerosolized millions of bacilli; PPD conversion and progression to active tuberculosis astonishingly high

e. Virulence of strain: Kentucky outbreak after minimal contact with index patient
f. Bacillus remains alive and infectious in air for long period; ventilation key in preventing transmission; isolation of patient and mandated number of air exchanges in hospital rooms

2. Primary infection: BEFORE IMMUNE RESPONSE:

a. Bacillus reaches alveoli
b. replicates extracellularly in alveolar space and intracellularly in alveolar macrophage
c. lack of immediate host immune response:

alveolar macrophage ingests TB bacillus; bacillus sits in phagosome; phagosome normally incorporates proton-ATPase into membrane leading to decreased pH and acidification within phagosome; acidified phagosome then normally fuses with cell lysosome, exposing organism to lysosome's toxic enzymes

BUT MTB prevents insertion of proton-ATPase into phagosome so phagosome never gets acidified and never merges with lysosome

d. MTB multiplies for weeks, both in initial focus in alveolar macrophages and in cells transported lymphohematogenously throughout body:
e. metastatic foci well established in regional nodes (hilar, mediastinal) and then to tissues which retain bacilli and favor multiplication:

apical posterior areas of lungs lymph nodes in neck kidneys epiphyses of long bones vertebral bodies juxtaependymal meningeal areas adjacent to subarachnoid space

*****These will be areas of reactivation disease in future as organisms seeded remain alive but dormant once immune response occurs

MID 24

*****Reactivation can occur in any one of these areas of the body with or without reactivation in others i.e. TB meningitis or "scrofula" with no pulmonary TB

B. Development of immune response: **MUST HAVE INTACT CELLULAR**
C. IMMUNE SYSTEM, INCLUDING CD4 CELLS

1. 6-12 weeks after initial infection

2. alveolar macrophage infected with *M. tuberculosis* releases interleukins 12 and 18
3. These attract and stimulate T lymphocytes (mainly CD4): all peoplehave native population of CD4 cells which can recognize mycobacterial antigens which have been processed and presented by macrophages
4. CD4 cell meets mycobacterial antigen presented by macrophage and becomes activated= transformed -- Transformed CD4 cell proliferates and produces clone of similarly reactive T lymphocytes
5. When population of activated lymphocytes is large enough, get cutaneous delayed reaction to tuberculin=tissue hypersensitivity: Positive PPD (Implications in AIDS patients with low CD4 cells: Cannot perform this process so no positive PPD response to tuberculin)
6. Meanwhile, CD4 cells release interferon gamma -- Interferon gamma stimulates additional macrophage phagocytosis AND interferon gamma stimulates macrophage to secrete regulatory factors including tumor necrosis factor alpha (TNF alpha).
7. TNF alpha increases macrophages ability to kill *M. tuberculosis* and is required for granuloma formation

Lack of TNF alpha results in inability to control initial TB infection as well as reactivation of latent organisms:

a.Murine experiments:
-Blockade of TNF alpha resulted in reactivation, high bacillary burden, persistent tuberculosis and death
-TNF alpha knock-out mice infected with *M. tuberculosis* followed similar course
b. Anti-TNF alpha agents for rheumatoid arthritis and auto-immune disorders cause reactivation of tuberculosis
D. Pathology: Tissue response depends on activation of macrophages with secretion of lytic enzymes which cause tissue necrosis
-Epithelioid cells= highly stimulated macrophages
-Langhans giant cell=fused macrophages oriented around tuberculosis antigen with multiple nuclei lined up peripherally
1. Small antigen load and high tissue hypersensitivity produceorganization of lymphocytes, macrophages, Langhans giant cells, fibroblasts and result in granuloma
a. Granuloma=successful tissue reaction resulting in containment of infection, healing with fibrosis, encapsulation and scar formation **MID 24**
2. Large antigen load and high tissue hypersensitivity produce few or no epithelioid clls or Langhans cells, disorganized lymphocytes, macrophages and polys and result in necrosis and caseation
a.-caseous material is acellular and inhibits multiplication of organisms due to its pH and oxygen tension but is inherently unstable, liquefies and discharges through the bronchial tree
b.this discharge produces a cavity in which TB bacillus multiplies to make population 5-6 logs greater than in noncavitary lesions
3. Large or small antigen load with no tissue hypersensitivity produces few cells (polys and mononuclear cells) and huge numbers of bacilli=seen in AIDS patients with low CD4 counts -implications for post treatment appearance of lung and chest x-ray in AIDS patients- lack of fibrosis or granuloma

V. Clinical Syndromes

A. Primary Infection with Resolution: 85% of cases
1. Patient asymptomatic or mild viral syndrome
2. Enlargement of hilar and peribronchial nodes if chest x-ray taken at time
3. Calcified granuloma on Chest x-ray=evidence that tuberculosis infection
4. successfully contained
5. Development of positive PPD 6-12 weeks later

B. Primary Infection with progression
1. Progressive Primary Disease
a. very young children (below age 5): unable to resolve infection;
local progression with mid or lower lung field pneumonitis initially, then dissemination, miliary pattern in lungs and frequent CNS involvement -almost always in developing world countries where tuberculosis remains endemic
b. tuberculous pleurisy: hypersensitivity reaction to small number oforganisms which reach pleura in primary infection; exudative pleural effusion,
culture negative as very few organisms present; 90% resolve spontaneously but WW II studies of soldiers showed 65% relapse to active TB (pre-antibiotic era); TB pleurisy should be treated
2. Primary infection in adolescence and young adulthood results in "adult type" upper lobe cavitary disease
a. epidemiologic data shows puberty influences tendency to apicalcavitation soon after initial infection;
b. Data shows 23% of those infected between 15-19, 13% of those infected between ages 20-24, 4% ofthose infected from 25-29 develop cavitary disease
c. Only 2% of those infected after 30 do so.
3. AIDS nosocomial outbreaks of tuberculosis: AIDS wards, homeless shelters and prisons
a. undiagnosed patient with active pulmonary tuberculosis hospitalized in AIDS ward or shelter for AIDS patients; all patients have CD4 <50
b. index patient coughs and infects other AIDS patients
MID 24
c. AIDS patients with no cellular immune function cannot mobilizeCD4 and macrophages to contain or kill bacillus
d. rapid dissemination and death if untreated; blood cultures positive for *M.tuberculosis*
e. MDR (multi-drug resistance) outbreaks killed many as resistance not monitored
C. Reactivation: 10-15% of those infected
1. Persistence of viable organisms following containment of initial infection
2. Disease occurs years after infection when cellular immune response no longer able to contain MTB:
a. Iatrogenic (transplant patients, immunosuppressive Rx for connective tissue disorders)
b. Immunocompromising diseases (AIDS, malignancies, end stage renal disease, cirrhosis)

Malnutrition
c. Old age: Hypersensitivity and cellular immunity wane with age
d. Unknown- possibly hormonal, stress (immigrants)
3. Pulmonary location most frequent site of reactivation: 85%
a. Posterior aspect of upper lobe is focus where reactivation begins
-location attributed to increased oxygen in apices and MTB's aerophilia
-other possibility= deficient lymphatic flow at apices resulting in retention of bacillary antigen; with hypersensitivity get necrosis
b. localized pneumonitis, inflammatory response produces fibrin rich exudates into alveoli, caseating necrosis, liquefaction
c. drainage into bronchial tree with cavity formation
d. cavity favors bacillary multiplication to huge amounts: 5-6 logs greater than # of organisms innon-cavitary lesions: $10^9 - 10^{10}$ organisms/gram tissue
e. cavitary disease is most contagious as cough aerosolizes hundreds of thousands of organisms

f. implications for development of drug resistance

4. Viable organisms remain alive, dormant for years in all sites to which disseminated during primary infection: extrapulmonary tuberculosis

a. lymph nodes: scrofula; most frequent form of extra pulmonary TB

-usually cervical or supraclavicular chain

-biopsy and culture essential (fine needle aspirate usually smear and culture neg.)

b. meninges: rupture of subependymal tubercle into subarachnoid space (distinct from meningitis in young children following dissemination as discussed above)

-meningitis most severe at base of brain causing thick gelatinous exudate; affects cranial nerves as they exit

-CSF exam essential to make diagnosis: low glucose; elevated protein; lymphocytic pleocytosis

c. bones: one third involve spine=Pott's disease; hematogenous spread, contiguous disease, lymphatic spread from pleural disease

-early focus is anterior part of vertebral body; spreads to disk and then to adjacent vertebra; X-ray shows anterior wedging of 2 adjacent vertebral bodies and

MID 24

destruction of disk; tender spine prominence on exam=gibbus

VI Diagnosis

A. Symptoms

1. Systemic symptoms non-specific: fever, fatigue, night sweats, weight loss

2. Pulmonary symptoms: cough, productive or dry-most patients have cough but may be ignored by patient for weeks

3. Hemoptysis

a. mild-moderate, chronic blood streaking: results from caseous sloughing or endobronchial erosion; seen in advanced disease

b. sudden massive hemoptysis= erosion of pulmonary artery=only TB emergency (Rasmussen's aneurysm)

B. Diagnostic procedures: **SPUTUM**: staining, cultures and molecular diagnostics

1. Acid fast stain: Acid fast implies mycobacterial species although nocardia is weakly acid fast; many other species besides *M. tuberculosis* complex will all be

AFB positive (*Mycobacterium avium, kansasii, abscessus, chelonae*)

a. Ziehl-Neelsen stain=fixed smear covered with carbol-fuchsin, heated, rinsed, decolorized with acid alcohol; Kinyoun stain is similar but heating unnecessary

b. Fluorochrome stain with phenol-auramine or auramine-rhodamine; modified acid alcohol step and potassium permanganate counterstaining; fluorescent mycobacteria visible with 20 or 40X magnification

SMEAR POSITIVITY MEANS AT LEAST 10,000 ORGANISMS/mL SPUTUM

2. Culture: Gold Standard. Now available in most of world via WHO reference labs

a. Solid media= Lowenstein Jensen (egg based) or Middlebrook 7H11 (agar based): can detect colony morphology, mixed infections; can detect 10-100 organisms/mL; 3-8 weeks incubation to detect organisms

b. Liquid broth= Middlebrook 7H12, BACTEC systems; 1-3 weeks ofincubation to detect organisms

CULTURE NECESSARY TO DETERMINE DRUG SUSCEPTIBILITIES

3. Nucleic acid amplification- can detect *M. tuberculosis* complex in fresh sputum: developed world technology: too costly for resource poor countries

a. sensitivity intermediate between acid fast smear and culture

b. AFB smear is negative, nucleic acid amplification is 40-77% sensitive

c. AFB smear is positive, nucleic acid amplification is 95% sensitive and nearly 100% specific

4. DNA fingerprinting: Molecular epidemiologic tool: RFLP (Restriction fragment length polymorphism); also developed world technology

a. Restriction endonuclease produces DNA fragments; separate fragments by electrophoresis; probe to repetitive DNA sequence=Insertion sequence (IS)6110 numerous copies of IS6110 present in *M.tuberculosis* chromosome at highly variable locations

b. Utility in identifying transmission from person to person; distinguishing endogenous reactivation from exogenous reinfection in recurrent TB; laboratory cross-contamination

C. Chest X-ray: Luxury of developed world technology

1. Upper lobe infiltrate with or without cavity: apical or sub-apical

a. most common in reactivation disease in intact immune system

MID 24

b. radiologic extent of disease reflects tissue damage

c. tissue damage reflects host's ability to have hypersensitivity reaction

2. Hilar adenopathy with or without infiltrates

a. most common in AIDS patients

b. reflects minimal cellular immune response

3. Pleural effusion; always exudative

a. seen in post-primary infection as above

-scant organisms=hypersensitivity tissue reaction;

-smear never positive; culture may be positive 25%

b. seen as complication of reactivation pulmonary tuberculosis

-more likely to have organisms

-smear positive 50%; culture positive 60%

4. Miliary- from description of pathologic lesions as "millet seeds"; chest x-ray shows 0.5-1.0 mm nodules

a. following childhood infection and progression as discussed above

b. immunocompromised: alcoholism, cirrhosis, rheumatologic diseases, treatment with immunosuppressive agents;

-diagnosis difficult; may have multiple organ involvement with millet seeds granulomas in tissues

-transbronchial bx=highest yield for diagnosis

5. Atypical infiltrates

VII Treatment

A. General Principles

1. Always use at least 2 drugs; usually begin with 3 or 4 pending sensitivities

a. natural incidence of spontaneous drug resistance: 1 in 10^5 organisms resistant to each drug;

b. bacilli resistant to 1 drug will be killed by other drug

c. natural resistance to 2 drugs spontaneously 1 in 10^{10} or 1 in 10^{11}

2. Prolonged length necessary: 6-9 months if organism pan-sensitive

3. Directly Observed Therapy for all patients

a. No one is 100% compliant regardless of age, sex, race, education

b. Daily treatment for first 2 months; intermittent with adjusted doses for continuation phase of 4-7 months depending on regimen

B. Drugs: **ALL GIVEN ONCE DAILY TOGETHER: NEVER DIVIDE DOSES**

1. Isoniazid=INH; bactericidal against dividing organisms

-hepatitis: Chemical (20%) vs clinical (age related:<35=0.3%; >65=4%)

2. Rifampin=RMP=bactericidal; Enables short course treatment (6-9 months vs 18-24 months with non-RMP regimens

-drug-drug interactions:RMP is potent inducer of hepatic microsomal enzymes: cytochrome p450

3. Pyrazinamide=PZA; Enables shortening of regimen from 9 months to 6 months

4. Ethambutol=EMB: Used in drug resistance and situations where INH or RMP cannot be used(INH hepatotoxicity; RMP drug-drug interactions)

MID 24

VIII Prophylaxis: Latent tuberculosis infection (LTBI)

A. Targeted testing: PPD is NOT a general screen; Only use PPD for patients at high risk ofdeveloping active tuberculosis

1. Immunocompromised: HIV infected, chemotherapy patients, patients undergoing organ transplant, patients on immunosuppressive Rx for autoimmune diseases, rheumatoid arthritis

2. Close contacts of infectious case (household or close working quarters)

3. Previously untreated patients with Chest X-ray evidence of old fibrotic changes

-not just calcified granuloma

4. Recent immigrants (in US < 5 years) from endemic areas (see map at beginning)

5. People who work in institutions where TB exposure likely: hospitals, nursing homes, homeless shelters, prisons

B. Positive PPD: Purified Protein Derivative= protein extract derived from=skin test *M.tuberculosis*; contains multiple antigens

1. Definition of positive PPD

a. 5 mm: HIV infected, close contacts of infectious case, chest x-ray evidence of old disease (fibrotic scarring, not just calcified granuloma)

b. 10mm: patients from endemic areas of tuberculosis

2. Booster phenomenon: 2 step testing essential for all those >55 whose exposure/infection in distant past and for those with BCG

C. Treatment: Only for those at high risk of reactivation (see above): INH for 9 months

D. New tests: Need to know if reactivity represents tuberculosis infection, not BCG: Enzyme-linked immunospot (ELISPOT): T-cell based assay from blood;

- M.*tuberculosis* genes not present in *M.bovis* BCG produce antigen to which T-cell reacts

- 1 tube of blood needed: not feasible in resource poor settings

- Useful in outbreaks for contact investigations- UK school outbreak showed greater sensitivity and specificity than PPD

IX Prevention: BCG

Most widely used and most controversial vaccine in the world

A. What is it?

1. *M. bovis* strain attenuated through serialpassage

2. No standardized strain or procedure to make one

B. Does it work?

1. Largest study: India= no protection from TB infection

2. Other studies: England= protection from TB infection

3. Prevalence of non tuberculous mycobacteria in given region may interfere

4. Background prevalence of tuberculosis determines utility

C. Who uses it?
-All agree that it is highly effective for infants and small children in preventing dissemination and meningitis when infected by *M. tuberculosis*
-Newborns vaccinated in all high prevalence areas of world shown on first map

Unit II possible questions

Part B

Define *Streptococcus pyogenes, Haemophilus influenzae, Mycobacterium tuberculosis*. Gastrointestinal Diseases: *Escherichia coli, Salmonella typhi, Vibrio cholerae, Helicobacter pylori.* Others: *Staphylococcus aureus, Bacillus anthracis, Clostridium tetani, Treponema pallidum.*

1. Define hemolysis in Blood agar.
2. Say about Acid fast Staining
3. What is clinical specimen of tuberculosis?
4. What is the identification test for *Staphylococcus aureus*?
5. Write the morphology of *Haemophilus influenzae*?
6. Write the morphology of *Clostridium tetani?*

Part C

1. Give the detailed information about *Mycobacterium tuberculosis* transmission and prophylaxis measures.
2. State the pathogenic role and diagnosis of *Staphylococcus aureus*.
3. Discuss the pathogenicity of *Clostridium tetani*.
4. Explain in detail about *Treponema pallidum*.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS : II B.Sc MB **COURSE NAME: MEDICAL MICROBIOLOGY**
COURSE CODE: 18MBU402 UNIT 2 **BATCH-2018-2021**

Unit II Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
An infectious disease is one that is _____	transferred by mosquitoes	caused by a pathogen or its products	spread from person to person	caused by all bacteria	spread from person to person
Epidemiologists are not interested in learning about	the causes of diseases and how to cure or control them	the frequency and geographic distribution of diseases	the causal relationships between diseases	diagnosis	diagnosis
The science that studies the distribution, occurrence, and control of health and disease in a defined population is called	Bioinformatics	Epidemiology	Immunology	Clinical laboratory science	Epidemiology
An infectious disease that can be transmitted from person to person is known as a	Systemic infection	Communicable disease	Transferable disease	Environmental disease	Communicable disease
The lodgement and multiplication of a parasite in or on the loscies of a host constitute _____	disease	infection	immunity	parasitism	infection
Initial infection with a parasite in a host is termed _____	primary	secondary	re-infection	nosocomial	primary
Subsequent infection by the same parasite in the host is termed _____ infection	Primary	Secondary	Re-infection	Iatrogenic	Re-infection
When a new parasite sets up an infection in a host whose resistance is lowered by a preexisting infectious disease, this is termed _____	Primary	Secondary	Re-infection	Iatrogenic	Secondary

infection					
Infection or sepsis at localized sites is called _____ infection	Primary	Secondary	Focal	Iatrogenic	Focal
In a patient already suffering from a disease, a new infection is set up from another host or external source it is termed _____ infection	Cross	Focal	Re-infection	Nosocomial	Cross
Cross infection occurring in hospitals are called ___ infection	Cross	Focal	Re-infection		Nosocomial
A person who arbours the pathogenic microorganism without suffering from any ill effects because of it is called _____	Patient	Carrier	Healthy person	Immunodeficient person	Carrier
A person one who harbours the pathogen but has never suffered from the disease caused by the pathogen is called _____	Healthy carrier	Convalescent carrier	Contact carrier	Paradoxical carrier	Healthy carrier
A person who have recovered from the disease and continous to harbour the pathogen inside the body is called _____ carrier	Healthy	Convalescent	Contact	Paradoxical	Convalescent
The _____ carrier state lasts than six months	Acute	Temporary	Chronic	Healthy	Temporary

The term _____ carrier is applied to a person who acquires the pathogen from a patient	Acute	Temporary	Contact	Paradoxical	Contact
The _____ carrier state lasts for several years	Acute or chronic	Temporary	Contact	Paradoxical	Acute or chronic
Physicians induced infections are termed _____ infection	Iatrogenic	Focal	Nosocomial	Re-infection	Iatrogenic
When clinical effects are not appaent it is called _____ infection	Atypical	Inapparent	Subclinical	Clinical	Inapparent
_____ infection is the one in which the typical or characteristic clinical manifestation of the particular infectious disease are not present	Endogenous	Exogenous	Inapparent	Clinical	Inapparent
Some parasites, following infection may remain in the tissues in a hidden form proliferating and producing clinical disease termed _____ infection	Endogenous	Exogenous	Latent	Atypical	Latent
The term _____ carrier refers to a person who acquires the pathogen from another carrier	Healthy	Convalescent	Contact	Paradoxical	Paradoxical
Infectious diseases transmitted from animals to human being are called _____	Zoonosis	Anoosis	Xanthosis	Phytosis	Zoonosis

When the pathogen multiplies in the body of the vector and then transmits the infection to humans are called _____ vector	Mechanical	Biological vector	Healthy	Contact	Biological vector
Some pathogens are able to cross the placental carrier and infect the fetus in uterus This is known as _____ transmission	Congenital	Intracelaine	Vertical	Horizontal	Vertical
_____ is generally employed to refer to the ability of a microbial species to produce diseases	Infection	Immunity	Pathogenicity	Virulence	Pathogenicity
_____ is applied to the same property in a strain of microorganism to produce disease	Infection	Immunity	Pathogenicity	Virulence	Virulence
_____ is the science that evaluates the occurrence, determinants, distribution and control of health and disease in a defined human population	Epidemiology	Oncology	Infection	Physiology	Epidemiology
An individual who practices epidemiology is an _____	Epidemiologist	Scientist	Investigator	Environmentalist	Epidemiologist
A _____ is an impairement of the normal state of an organism or any of its components that hinders the performance of vital	Health	Disease	Infection	Immunity	Disease

components					
_____ is the condition in which the organism performs its vital functions normally	Health	Disease	Infection	Immunity	Infection
When a disease occurs occasionally and at irregular intervals in a human population it is a _____ disease	Epidemic	Endemic	Sporadic	Pandemic	Endemic
When the disease occurs at a steady low-level frequency at a moderately regular interval it is an ____ disease	Epidemic	Endemic	Sporadic	Pandemic	Endemic
_____ disease gradually increase in the occurrence frequency beyond the endemic level but do not to the epidemic level	hyperendemic	Epidemic	Endemic	Pandemic	hyperendemic
An_____is a sudden increase in the occurrence of a disease above the expected level	Epidemic	Endemic	sporadic	Pandemic	Pandemic
The first case in an epidemic is called_____	index case	infection	disease	outbreak	index case
A sudden unexpected occurrence of a disease in a limited segment of population is called_____	index case	Infection	disease	outbreak	outbreak
A_____is an increase in disease occurrence within a	epidemic	Endemic	Pandemic	Sporadic	Pandemic

large population over a very wide region					
The factors that influence the frequency of a disease in an animal population is known as	epizootiology	Zootiology	Epidemiology	Entamology	epizootiology
Moderate prevalance of a disease in animals is termed _____	epizootic	enzootic	panzootic	zoonoses	enzootic
A sudden outbreak of disease in animals is called _____	epizootic	enzootic	panzotic	zoonoses	panzotic
A wide dissemination of aniomals is callled _____	epizoolic	enzoolic	panzootic	zoonoses	zoonoses
Animal disease that can be transmitted to humans are termed _____	epizootic	enzootic	panzootic	zoonoses	zoonoses
A _____ period is the period between pathogen entry and the expression of signs and symptoms	incubation	survival	lag	log	incubation
The _____ is the period in which ther is an onset of sign and symptoms	incubatiob period	prodromal stage	survival period	death	prodromal stage
The _____ represents the events in the form of an intriguing epidemiological mystery story	infectious disease cycle	disease	infection	none	infectious disease cycle
_____ is an illness caused by a pathogen or its products that has been transmitted from an infected	communicable disease	epidemic	epidemic	none	communicable disease

person or a reservoir					
_____ controls the microbes in air	Air pressure	a Aerosol	Atmosphere	oxygen	Air pressure
The circulating air is filtered in lab by _____ filter	Pad	Candle	HEPA	Catridge	HEPA
The discard materials to be_____ before disposal	Dissuaded	Dispenced	Decontaminated	sterilized	Decontaminated
Quality control checks the quality of the product based on the norms of_____	QA	QC	GTL	QB	QA
The proper disposal of biologicals helps to prevent _____	Epidemic	Endemic	Pandemic	Randamic	Epidemic
There is no single reliable test available to determine efficiency of a_____	Antibiotics	Antiseptics	Disinfectants	Diluent	Disinfectants
In the Rideal walker test typhoid bacilli is tested with _____	Phenol	Boron	Acid	Alcohol	Phenol
_____ is a product ketane and formaldehyde	HCL	BPL	TNT	SDS	BPL
QA stands for _____	Quality assurance	Quality association	Quality acceptance	Quality abolision	Quality assurance
The _____ is the time during which the source is infectious or is disseminating the pathogen	incubation period	the period of infectivities	predormal stage	none	predormal stage
cholerae caused by	V.cholerae	E.coli	V. parahemolyticus	E.histolytica	V.cholerae
The Goods purchased	Dispatch	Materials	Quarentum	Stock section	Quarentum

is stored at_____	section	section	section		section
The water used in laboratory is purified by _____ filter	Catridge	Pad	Sinted glass	Earthern ware	Catridge
The monitoring of the lab environment is done by_____	QA	QC	GTL	QB	QC
The microbial load of lab environment is tested by_____method	Settle plate	Pour plate	Spread plate	Streak plate	Settle plate

UNIT 3

Poliovirus:

Poliovirus is the etiologic agent of the paralytic disease known as poliomyelitis. It's also the virus I've worked on for most of my career. The World Health Organization is in the midst of a massive effort to eradicate the disease, an undertaking that has encountered a number of obstacles. In coming posts I'd like to discuss the polio eradication effort, but first we need some background on poliovirus.

Poliovirus is a member of a family of viruses called the *Picornaviridae*. Viruses are classified into families, and then genera, based on many criteria, including physical and biological properties. The *Picornaviridae* includes many other human pathogens, such as Coxsackieviruses, echoviruses, enteroviruses, hepatitis A virus, and rhinoviruses (which cause the common cold). The International Committee for the Taxonomy of Viruses is responsible for virus classification and maintains the Universal Virus Database, where you can find information about most known viruses. Poliovirus is a rather small and simple virus. It is composed of a shell, or capsid, made of protein, as shown. The poliovirus capsid is about 30 nanometers in diameter. Within the capsid is the information to make new virus particles – a single molecule of ribonucleic acid, or RNA. In the image, part of the capsid has been cut away to reveal the viral RNA. When the virus infects a cell, the RNA genome enters the cell and programs it to make new virus particles. These virus particles are released from the cell and go on to infect new cells.

In humans, poliovirus is ingested, and replicates in cells of the gastrointestinal tract. Newly synthesized virus particles are released into the intestine and shed in the feces. Transmission of poliovirus to another human occurs through contact with virus-containing feces or contaminated water. After multiplying in the gastrointestinal tract, poliovirus may enter the spinal cord and brain. Destruction of motor neurons by the virus leads to limb paralysis.

Poliomyelitis became a common disease at the turn of the 20th century. Two vaccines were developed in the 1950s that can effectively prevent the disease – inactivated poliovaccine (IPV, developed by Jonas Salk) and live, oral poliovaccine (OPV, developed by Albert Sabin). In 1988 the World Health Organization announced that it would eradicate poliomyelitis from the globe by the year 2000. We'll discuss that goal, and obstacles that might prevent it from being attained, in subsequent posts.

INTRODUCTION TO HERPES SIMPLEX VIRUS (HSV)

Herpes Simplex Virus causes the most common viral infection in human and worldwide in distribution.
The herpes simplex virus is of two types –

- HSV type 1 (human herpesvirus type 1 or HHV type 1)
- HSV type 2 (human herpesvirus type 2 or HHV type 2)

Herpes Simplex Virus type 1 is usually associated with oral and ocular lesions, while type 2 is responsible for the majority of genital infection.

DISEASES CAUSED BY HERPES SIMPLEX VIRUS (HSV)

⇒ HSV type 1 causes

- Acute Gingivostomatitis
- Herpes labialis
- Keratoconjunctivitis
- Encephalitis

⇒ HSV type 2 causes

- Genital herpes
- Neonatal herpes
- Aseptic meningitis.

PATHOGENESIS OF HERPES SIMPLEX VIRUS (HSV)

⇒ The sources of infection are Saliva, Skin lesions or Respiratory Secretions. Primary infection is usually acquired in early childhood, between 2-5 years of age.
Transmission occurs by close contact and maybe
venereal in genital herpes.
⇓
Virus enters through defects in skin or
mucous membranes & multiply locally.
⇓
Virus enters Cutaneous nerve fibers & is
transported to ganglia where it replicates.
⇓
Migration of virus can take place from the ganglia
to the Skin and Mucosa causing lesions.
⇓
Virus remains latent in ganglia, particularly of
Trigeminal (HSV type 1) & Sacral (HSV type 2) nerves.
Hepatitis refers to an inflammatory condition of the liver. It's commonly caused by a viral infection, but there are other possible causes of hepatitis. These include autoimmune hepatitis and hepatitis that occurs as a secondary result of medications, drugs, toxins, and alcohol. Autoimmune hepatitis is a disease that occurs when your body makes antibodies against your liver tissue.

Your liver is located in the right upper area of your abdomen. It performs many critical functions that affect metabolism throughout your body, including:

bile production, which is essential to digestion
filtering of toxins from your body
excretion of bilirubin (a product of broken-down red blood cells), cholesterol, hormones, and drugs breakdown of carbohydrates, fats, and proteins
activation of enzymes, which are specialized proteins essential to body functions
storage of glycogen (a form of sugar), minerals, and vitamins (A, D, E, and K)
synthesis of blood proteins, such as albumin
synthesis of clotting factors

According to the Centers for Disease Control and Prevention (CDC)Trusted Source, approximately 4.4 million Americans are currently living with chronic hepatitis B and C. Many more people don't even know that they have hepatitis.

Treatment options vary depending on which type of hepatitis you have. You can prevent some forms of hepatitis through immunizations and lifestyle precautions.

The 5 types of viral hepatitis

Viral infections of the liver that are classified as hepatitis include hepatitis A, B, C, D, and E. A different virus is responsible for each type of virally transmitted hepatitis.

Hepatitis A is always an acute, short-term disease, while hepatitis B, C, and D are most likely to become ongoing and chronic. Hepatitis E is usually acute but can be particularly dangerous in pregnant women.

Hepatitis A

Hepatitis A is caused by an infection with the hepatitis A virus (HAV). This type of hepatitis is most commonly transmitted by consuming food or water contaminated by feces from a person infected with hepatitis A.

Hepatitis B

Hepatitis B is transmitted through contact with infectious body fluids, such as blood, vaginal secretions, or semen, containing the hepatitis B virus (HBV). Injection drug use, having sex with an infected partner, or sharing razors with an infected person increase your risk of getting hepatitis B.

It's estimated by the CDCTrusted Source that 1.2 million people in the United States and 350 million people worldwide live with this chronic disease.

Hepatitis C

Hepatitis C comes from the hepatitis C virus (HCV). Hepatitis C is transmitted through direct contact with infected body fluids, typically through injection drug use and sexual contact. HCV is among the most common bloodborne viral infections in the United States. Approximately 2.7 to 3.9 million AmericansTrusted Source are currently living with a chronic form of this infection.

Hepatitis D

Also called delta hepatitis, hepatitis D is a serious liver disease caused by the hepatitis D virus (HDV). HDV is contracted through direct contact with infected blood. Hepatitis D is a rare form

of hepatitis that only occurs in conjunction with hepatitis B infection. The hepatitis D virus can't multiply without the presence of hepatitis B. It's very uncommon in the United States.

Hepatitis E

Hepatitis E is a waterborne disease caused by the hepatitis E virus (HEV). Hepatitis E is mainly found in areas with poor sanitation and typically results from ingesting fecal matter that contaminates the water supply. This disease is uncommon in the United States. However, cases

of hepatitis E have been reported in the Middle East, Asia, Central America, and Africa, according to the CDCTrusted Source.

Causes of noninfectious hepatitis

Alcohol and other toxins

Excessive alcohol consumption can cause liver damage and inflammation. This is sometimes referred to as alcoholic hepatitis. The alcohol directly injures the cells of your liver. Over time, it can cause permanent damage and lead to liver failure and cirrhosis, a thickening and scarring of the liver.

Other toxic causes of hepatitis include overuse or overdose of medications and exposure to poisons.

Autoimmune system response

In some cases, the immune system mistakes the liver as a harmful object and begins to attack it. It causes ongoing inflammation that can range from mild to severe, often hindering liver function. It's three times more common in women than in men.

Common symptoms of hepatitis

If you have infectious forms of hepatitis that are chronic, like hepatitis B and C, you may not have symptoms in the beginning. Symptoms may not occur until the damage affects liver function.

Signs and symptoms of acute hepatitis appear quickly. They include:

- fatigue
- flu-like symptoms
- dark urine
- pale stool
- abdominal pain
- loss of appetite
- unexplained weight loss
- yellow skin and eyes, which may be signs of jaundice

Chronic hepatitis develops slowly, so these signs and symptoms may be too subtle to notice.

How hepatitis is diagnosed

Hepatitis A

Hepatitis A usually doesn't require treatment because it's a short-term illness. Bed rest may be recommended if symptoms cause a great deal of discomfort. If you experience vomiting or diarrhea, follow your doctor's orders for hydration and nutrition.

The hepatitis A vaccine is available to prevent this infection. Most children begin vaccination between ages 12 and 18 months. It's a series of two vaccines. Vaccination for hepatitis A is also available for adults and can be combined with the hepatitis B vaccine.

Hepatitis B

Acute hepatitis B doesn't require specific treatment.

Chronic hepatitis B is treated with antiviral medications. This form of treatment can be costly because it must be continued for several months or years. Treatment for chronic hepatitis B also requires regular medical evaluations and monitoring to determine if the virus is responding to treatment.

Hepatitis B can be prevented with vaccination. The CDCTrusted Source recommends hepatitis B vaccinations for all newborns. The series of three vaccines is typically completed over the first six months of childhood. The vaccine is also recommended for all healthcare and medical personnel.

Hepatitis C

Antiviral medications are used to treat both acute and chronic forms of hepatitis C. People who develop chronic hepatitis C are typically treated with a combination of antiviral drug therapies. They may also need further testing to determine the best form of treatment.

People who develop cirrhosis (scarring of the liver) or liver disease as a result of chronic hepatitis C may be candidates for a liver transplant.

Currently, there is no vaccination for hepatitis C.

Hepatitis D

No antiviral medications exist for the treatment of hepatitis D at this time. According to a 2013 studyTrusted Source, a drug called alpha interferon can be used to treat hepatitis D, but it only shows improvement in about 25 to 30 percent of people.

Hepatitis D can be prevented by getting the vaccination for hepatitis B, as infection with hepatitis B is necessary for hepatitis D to develop.

Hepatitis E

Currently, no specific medical therapies are available to treat hepatitis E. Because the infection is often acute, it typically resolves on its own. People with this type of infection are often advised to get adequate rest, drink plenty of fluids, get enough nutrients, and avoid alcohol. However, pregnant women who develop this infection require close monitoring and care.

Autoimmune hepatitis

Corticosteroids, like prednisone or budesonide, are extremely important in the early treatment of autoimmune hepatitis. They're effective in about 80 percent of people with this condition.

Azothioprine (Imuran), a drug that suppresses the immune system, is often included in treatment. It can be used with or without steroids.

Other immune suppressing drugs like mycophenolate (CellCept), tacrolimus (Prograf) and cyclosporine (Neoral) can also be used as alternatives to azathioprine for treatment.

Tips to prevent hepatitis

Hygiene

Practicing good hygiene is one key way to avoid contracting hepatitis A and E. If you're traveling to a developing country, you should avoid:

- local water
- ice
- raw or undercooked shellfish and oysters
- raw fruit and vegetables

Hepatitis B, C, and D contracted through contaminated blood can be prevented by:

- not sharing drug needles
- not sharing razors
- not using someone else's toothbrush
- not touching spilled blood

Hepatitis B and C can also be contracted through sexual intercourse and intimate sexual contact. Practicing safe sex by using condoms and dental dams can help decrease the risk of infection. You can find many options available for purchase online.

Vaccines

The use of vaccines is an important key to preventing hepatitis. Vaccinations are available to prevent the development of hepatitis A and B. Experts are currently developing vaccines against hepatitis C. A vaccination for hepatitis E exists in China, but it isn't available in the United States.

Complications of hepatitis

Chronic hepatitis B or C can often lead to more serious health problems. Because the virus affects the liver, people with chronic hepatitis B or C are at risk for:

- chronic liver disease
- cirrhosis

When your liver stops functioning normally, liver failure can occur. Complications of liver failure include:

- bleeding disorders
- a buildup of fluid in your abdomen, known as ascites
- increased blood pressure in portal veins that enter your liver, known as portal hypertension
- kidney failure
- hepatic encephalopathy, which can involve fatigue, memory loss, and diminished mental abilities due to the buildup of toxins, like ammonia, that affect brain function
- hepatocellular carcinoma, which is a form of liver cancer
- death

People with chronic hepatitis B and C are encouraged to avoid alcohol because it can accelerate liver disease and failure. Certain supplements and medications can also affect liver function. If you have chronic hepatitis B or C, check with your doctor before taking any new medications.

Hepatitis B

Hepatitis B is transmitted through contact with infectious body fluids, such as blood, vaginal secretions, or semen, containing the hepatitis B virus (HBV). Injection drug use, having sex with an infected partner, or sharing razors with an infected person increase your risk of getting hepatitis B.

It's estimated by the CDC Trusted Source that 1.2 million people in the United States and 350 million people worldwide live with this chronic disease.

Hepatitis C

Hepatitis C comes from the hepatitis C virus (HCV). Hepatitis C is transmitted through direct contact with infected body fluids, typically through injection drug use and sexual contact. HCV is among the most common bloodborne viral infections in the United States. Approximately 2.7 to 3.9 million AmericansTrusted Source are currently living with a chronic form of this infection.

Hepatitis D

Also called delta hepatitis, hepatitis D is a serious liver disease caused by the hepatitis D virus (HDV). HDV is contracted through direct contact with infected blood. Hepatitis D is a rare form of hepatitis that only occurs in conjunction with hepatitis B infection. The hepatitis D virus can't multiply without the presence of hepatitis B. It's very uncommon in the United States.

Hepatitis E

Hepatitis E is a waterborne disease caused by the hepatitis E virus (HEV). Hepatitis E is mainly found in areas with poor sanitation and typically results from ingesting fecal matter that contaminates the water supply. This disease is uncommon in the United States. However, cases of hepatitis E have been reported in the Middle East, Asia, Central America, and Africa, according to the CDCTrusted Source.

Causes of noninfectious hepatitis

Alcohol and other toxins

Excessive alcohol consumption can cause liver damage and inflammation. This is sometimes referred to as alcoholic hepatitis. The alcohol directly injures the cells of your liver. Over time, it can cause permanent damage and lead to liver failure and cirrhosis, a thickening and scarring of the liver.

Other toxic causes of hepatitis include overuse or overdose of medications and exposure to poisons.

Autoimmune system response

In some cases, the immune system mistakes the liver as a harmful object and begins to attack it. It causes ongoing inflammation that can range from mild to severe, often hindering liver function. It's three times more common in women than in men.

Rabies:

Rabies is an infectious viral disease that is almost always fatal following the onset of clinical symptoms. In up to 99% of cases, domestic dogs are responsible for rabies virus transmission to

humans. Yet, rabies can affect both domestic and wild animals. It is spread to people through bites or scratches, usually via saliva.

Rabies is present on all continents, except Antarctica, with over 95% of human deaths occurring in the Asia and Africa regions.

Rabies is one of the neglected tropical diseases that predominantly affects poor and vulnerable populations who live in remote rural locations. Although effective human vaccines and immunoglobulins exist for rabies, they are not readily available or accessible to those in need. Globally, rabies deaths are rarely reported and children between the ages of 5–14 years are frequent victims. Treating a rabies exposure, where the average cost of rabies post-exposure prophylaxis (PEP) is US$ 40 in Africa, and US$ 49 in Asia, can be a catastrophic financial burden on affected families whose average daily income is around US$ 1–2 per person.

Every year, more than 29 million people worldwide receive a post-bite vaccination. This is estimated to prevent hundreds of thousands of rabies deaths annually.

Prevention

Eliminating rabies in dogs

Rabies is a vaccine-preventable disease. Vaccinating dogs is the most cost-effective strategy for preventing rabies in people. Dog vaccination reduces deaths attributable to rabies and the need for PEP as a part of dog bite patient care.

Awareness on rabies and preventing dog bites

Education on dog behaviour and bite prevention for both children and adults is an essential extension of a rabies vaccination programme and can decrease both the incidence of human rabies and the financial burden of treating dog bites. Increasing awareness of rabies prevention and control in communities includes education and information on responsible pet ownership, how to prevent dog bites, and immediate care measures after a bite. Engagement and ownership of the programme at the community level increases reach and uptake of key messages.

Preventive immunization in people

Human rabies vaccines exist for pre-exposure immunization. These are recommended for people in certain high-risk occupations such as laboratory workers handling live rabies and rabies-related (lyssavirus) viruses; and people (such as animal disease control staff and wildlife rangers) whose professional or personal activities might bring them into direct contact with bats, carnivores, or other mammals that may be infected.

Pre-exposure immunization is also recommended for travellers to rabies-affected, remote areas who plan to spend a lot of time outdoors involved in activities such as caving or mountain-climbing. Expatriates and long term travellers to areas with a high rabies exposure risk should be immunized if local access to rabies biologics is limited. Finally, immunization should also be considered for children living in, or visiting, remote, highrisk areas. As they play with animals, they may receive more severe bites, or may not report bites.

Symptoms

The incubation period for rabies is typically 2–3 months but may vary from 1 week to 1 year, dependent upon factors such as the location of virus entry and viral load. Initial symptoms of rabies include a fever with pain and unusual or unexplained tingling, pricking, or burning sensation (paraesthesia) at the wound site. As the virus spreads to the central nervous system, progressive and fatal inflammation of the brain and spinal cord develops.

There are two forms of the disease:

- People with furious rabies exhibit signs of hyperactivity, excitable behaviour, hydrophobia (fear of water) and sometimes aerophobia (fear of drafts or of fresh air). Death occurs after a few days due to cardio-respiratory arrest.
- Paralytic rabies accounts for about 20% of the total number of human cases. This form of rabies runs a less dramatic and usually longer course than the furious form. Muscles gradually become paralyzed, starting at the site of the bite or scratch. A coma slowly develops, and eventually death occurs. The paralytic form of rabies is often misdiagnosed, contributing to the under-reporting of the disease.

Diagnosis

Current diagnostic tools are not suitable for detecting rabies infection before the onset of clinical disease, and unless the rabies-specific signs of hydrophobia or aerophobia are present, clinical diagnosis may be difficult. Human rabies can be confirmed intra-vitam and post mortem by various diagnostic techniques that detect whole viruses, viral antigens, or nucleic acids in infected tissues (brain, skin, urine, or saliva).

Transmission

People are usually infected following a deep bite or scratch from an animal with rabies, and transmission to humans by rabid dogs accounts for 99% of cases. Africa and Asia have the highest rabies burden in humans and account for 95% of rabies deaths, worldwide.

In the Americas, bats are now the major source of human rabies deaths as dog-mediated transmission has mostly been broken in this region. Bat rabies is also an emerging public health threat in Australia and Western Europe. Human deaths following exposure to foxes, raccoons, skunks, jackals, mongooses and other wild carnivore host species are very rare, and bites from rodents are not known to transmit rabies.

Transmission can also occur when infectious material – usually saliva – comes into direct contact with human mucosa or fresh skin wounds. Human-to-human transmission through bites is theoretically possible but has never been confirmed.

Contraction of rabies through inhalation of virus-containing aerosols or through transplantation of infected organs is rare. Contracting rabies through consumption of raw meat or animal-derived tissue has never been confirmed in humans.

Post-exposure prophylaxis (PEP)

Post-exposure prophylaxis (PEP) is the immediate treatment of a bite victim after rabies exposure. This prevents virus entry into the central nervous system, which results in imminent death. PEP consists of:

- extensive washing and local treatment of the wound as soon as possible after exposure;
- a course of potent and effective rabies vaccine that meets WHO standards; and
- the administration of rabies immunoglobulin (RIG), if indicated.

Effective treatment soon after exposure to rabies can prevent the onset of symptoms and death.

Extensive wound washing

This involves first-aid of the wound that includes immediate and thorough flushing and washing of the wound for a minimum of 15 minutes with soap and water, detergent, povidone iodine or other substances that kill the rabies virus.

Dengue (pronounced DENgee) fever is a painful, debilitating mosquito-borne disease caused by any one of four closely related dengue viruses. These viruses are related to the viruses that cause West Nile infection and yellow fever.

Transmission;

Dengue viruses are spread to people through the bites of infected *Aedes* species mosquitoes (*Ae. aegypti* or *Ae. albopictus*). These are the same types of mosquitoes that spread Zika and chikungunya viruses.

- These mosquitoes typically lay eggs near standing water in containers that hold water, like buckets, bowls, animal dishes, flower pots, and vases.
- These mosquitoes prefer to bite people, and live both indoors and outdoors near people.
- Mosquitoes that spread dengue, chikungunya, and Zika bite during the day and night.
- Mosquitoes become infected when they bite a person infected with the virus. Infected mosquitoes can then spread the virus to other people through bites.

Aedes albopictus mosquito.

From mother to child

- A pregnant woman already infected with dengue can pass the virus to her fetus during pregnancy or around the time of birth.
- To date, there has been one documented report of dengue spread through breast milk. Because of the benefits of breastfeeding, mothers are encouraged to breastfeed even in areas with risk of dengue.
    - Dengue in pregnancy

Through infected blood, laboratory, or healthcare setting exposures

Rarely, dengue can be spread through blood transfusion, organ transplant, or through a needle stick injury.

Prevention:

- Prevent dengue by avoiding mosquito bites.
- All four dengue viruses are spread primarily through the bite of an infected *Aedes* species (*Ae. aegypti* and *Ae. albopictus*) mosquito. These mosquitoes also spread chikungunya and Zika viruses.
- The mosquitoes that spread dengue are found in most tropical and subtropical regions of the world, including many parts of the United States.
- *Ae. aegypti* and *Ae. albopictus* bite during the day and night.

- A dengue vaccine is available for use in some parts of the world, including United States territories.
- Swine flu is a type of influenza that originates in pigs. Swine flu is caused by a specific strain of the H1N1 virus and the newest swine flu virus, H3N2v. There are many strains of the virus that only infect pigs. Only certain strains also affect humans. Swine flu is contagious and is spread from person-to-person.
- Symptoms of both H1N1 and H3N2v strains of the swine flu include a respiratory infection. Signs of respiratory infection include fever, cough, and sore throat. Other symptoms of swine flu include fatigue, chills, headache, body aches, nausea, vomiting, or diarrhea. Symptoms in very young children may include listlessness or shortness of breath.
- Rarely, swine flu can cause severe symptoms including pneumonia or respiratory failure leading to death.

HIV-AIDS virus working of immunne system in the presence of HIV- Replication in tarrget cell

Introduction

Human immunodeficiency virus (HIV) is a virus that causes the condition acquired immunodeficiency syndrome(AIDS). The virus attacks a specific type of immune system cell in the body, known as CD4 helper lymphocyte cells. HIV destroys these cells, making it harder for your body to fight off other infections. When you have HIV,even a minor infection (like a cold) can be much more severe because your body has difficulty healing.

HIV is transmitted through contact with the following bodily fluids:

- blood
- breast milk
- semen
- vaginal fluid

Sexual contact and sharing contaminated needles — even tattoo or piercing needles — cann result in thetransmission of HIV.

Not only does HIV attack CD4 cells, it allso uses the cells to make more of the virus. When the virus has destroyed a certain number of CD4 cells, doctors will call this stage AIDS. A person with AIDS is very vulnerable to infections,such as pneumonia. People with lowereed immune systems can also get cancers, such as ly mphoma.

HIV doesn't always multiply rapidly. It can take years for a person's immune system to be affected enough to have symptoms. A person with HIV will often progress through several

phases beffore their condition is considered AIDS. Taking medications caarefully can help to slow the disease's progression.

Stages of HIV

Doctors have classified three HIV stages: acute HIV infection, chronic HIV infection, and AIDS.

Acute HIV infection

Once a person becomes infected with HIV, an acute infection will take place two to four weeks later. At this time, the virus is multiplying in the body, attacking CD4 cells. This initial infection can result in flu-like symptoms. Examples of these symptoms include:

- fever
- headache
- rash

However, not all people with HIV experience initial flu-like symptoms. The flu symptoms are due to the increase of HIV viruses in the body. During thiis time, the amount of CD4 cells starts to fall very quickly. The immune system then kicks in, which causes CD4 levels to once again rise. However, the CD4 levels may not return to their preinfection height.

In addition to causing symptoms, the acute stage is when HIV is at its greatest risk forr transmission to other people. This is because HIV levels are very high at this time. The acute stage typically lasts between several weeks and months.

Clinical latency

The chronic HIV infection stage is known as the latent or asymptomatic stage. During this stage, you usually won't have as many symptoms as you did duuring the acute phase. The virus multiplies less quickly during the chronic stage. However, you can still transmit the HIV infection.

Without any treatment, the chronic HIV infection stage lasts anywhere from 10 to 12 yeaars before advancing to AIDS. If a person is taking treatments for HIV, the chronic HIV infection stage may last several decades. According to AIDS.gov, if you take treatments for HIV and your HIV levels are low, you can live a normmal to nearly normal life span. It's also possible that the infection will never progress to the AIDS phase.

AIDS

A doctor diagnoses a person with AIDS when they have a CD4 count of less than 200 cells/^{3}(a measurement ofthe cells in the blood) and they've had an opportunistic infection, such as tuberculosis, caancer, or pneumonia. A
normal CD4 count ranges from 500–1,600 cells/mm^3 in healthy adults.

Unfortunately, when a person's HIV proogresses to AIDS, the survival rate is usually about thhree years.

What factors affect disease progression?

While HIV does progress in phases, some people go through the phases more quickly than others. Taking medicatijons, known as antiretroviral therapy (ART), can slow this progression for morre people. Factors that affect HIV progression can include:

- Your age when your symptoms started: Being older can result in faster progression of HIV.
- Your health before treatment: If you had other diseases, such as tuberculosis, hepatitis C, or other
  sexually transmitted diseases, it can affect your overall health.
- How soon you were diagnosed after you were infected: The longer betweeen your diagnosis and
  treatment, the faster the disease can progress.
- Your lifestyle: Maintaining an unhealthy lifestyle, such as having a poor diet and experiencing severe
  stress, can aid HIV progression .
- Whether you take your medications as prescribed
- Your genetic history: Some people just seem to progress more quickly through their disease.

Some factors can delay or slow the progression of HIV. These include:

- taking your ART medications as your doctor prescribes
- seeing your doctor as recommeended for HIV treatments
- eating a healthy diet

- taking care of yourself, includinng having protected sex, trying to minimize stress inn your life, and sleeping regularly

Living a healthy lifestyle and seeing your doctor regularly can make a big difference in your overall health.

Treatments for HIV typically involve ART. This isn't a specific regimen, but instead a combination of several drugs. There are currently 25 different FDA approved medicines to treat HIV. ART works to prevent the virus from copying itself. This maintains your immunity levels while slowing the progression of HIV.

Your doctor will take into consideration your health history, the levels of the virus in yoour blood, possible sideeffects, costs, and any allergies you may have before prescribing medications. There are six classes of HIV drugs. Most doctors will start you on a combination of three medications from at least two differrent drug classes. These classes are:

- CCR5 antagonists (CCR5s)
- fusion inhibitors
- integrase stand transfer inhibitors (INSTIs)
- non-nucleoside reverse transcriptase inhibitors (NNRTIs)
- nucleoside reverse transcriptase inhibitors (NRTIs)
- protease inhibitors

Your doctor may prescribe several different medication types before you find the best regimen for you.

How is HIV prevented?

HIV is an especially dangerous virus beecause it doesn't cause a lot of outward or noticeable symptoms until the disease has progressed. For this reason, it's important to understand how HIV is transmiitted and ways you can work to prevent transmission.

HIV can be transmitted by:

- having sex, including oral, vaginnal, and anal sex
- sharing needles, including tattooo needles, needles used for body piercing, and needles used for injecting drugs
- coming in contact with body fluuids, such as blood, semen, vaginal fluid, and breast milk

HIV is not transmitted by:

- breathing the same air as an inffected person
- getting bitten by a mosquito or other biting insect
- hugging, holding hands with, kissing, or touching an infected person touching a door handle or toilet seat an infected person has used

Keeping this in mind, some of the ways you can prevent HIV include:

- refraining from oral, anal, or vaginal sex (known as the abstinence method)
- always using a latex barrier, succh as a condom, when you have oral, anal, or vaginaal sex
- never sharing needles with others

If you've had unprotected sex or shhared needles with anyone in the past, doctors usually recommend gettingan HIV test at least once a year. Symptoms can take years to appear, which is why it's so important to get tested regularly.

Conclusion

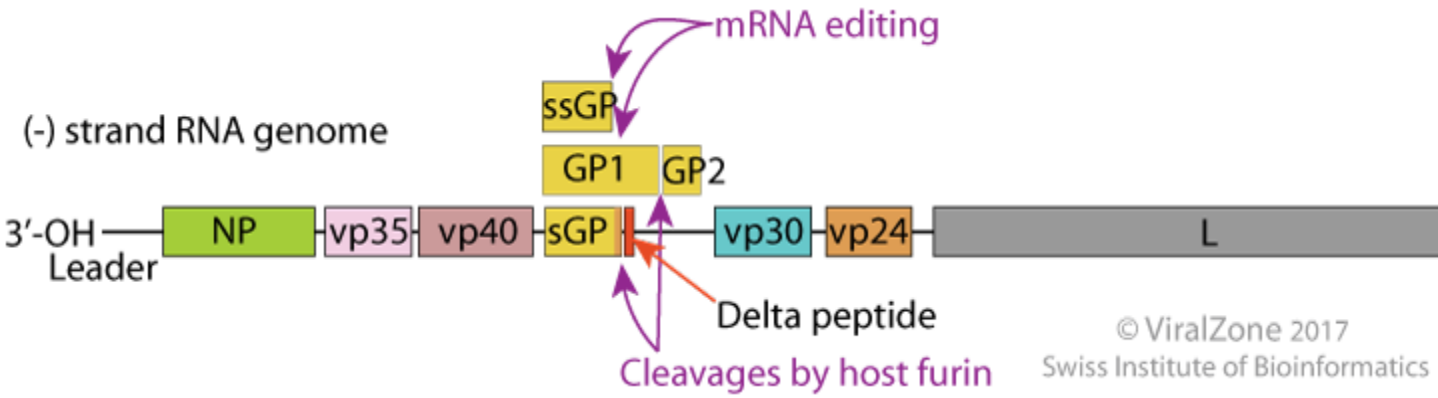
Advances in HIV treatments mean that people can live longer with the condition. Getting tested regularly and taking good care of yourself is vital to keeping your disease from progressing to the AIDS

Ebola Virus:

- It falls under the Filoviridae family.
- The virion is filamentous, enveloped measuring 800 nm length and 80 nm in diameter.
- It comprises of negative sense, single stranded RNA genome.
- Structural proteins associated with the nucleocapsid are the nucleoprotein (NP), VP30, VP35, and the polymerase (L) protein

Genome of Ebola Virus

- It comprises of linear, negative-stranded RNA genome, about 18-19 kb in size.



Introduction to Chikungunya

Chikungunya is a viral disease transmitted to humans by the bite of infected Aedes Aegypti mosquitoes. Chikungunya virus (CHIKV) is a member of the genus Alphavirus, in the family Togaviridae. CHIKV was first isolated from the blood of a febrile patient in Tanzania in 1953, and has since been identified repeatedly in west, central and southern Africa and many areas of Asia, and has been cited as the cause of numerous human epidemics in those areas since that time. The virus circulates throughout much of Africa, with transmission thought to occur mainly between mosquitoes and monkeys.

Symptoms of Chikungunya

Symptoms of Chikungunya includes debilitating arthralgia (joint pain), swelling of joints, stiffness of joints, myalgia (muscular pain), headache, fatigue (weakness), nausea, vomiting and rash and fever.

The incubation period (time from infection to illness) can be 2-12 days, but is usually 3-7 days. "Silent" CHIKV infections (infections without illness) do occur; but how commonly this happens is not yet known.

Acute chikungunya fever typically lasts a few days to a couple of weeks, but some patients have prolonged fatigue lasting several weeks. Additionally, some patients have reported incapacitating joint pain, or arthritis which may last for weeks or months. No deaths, neuro-invasive cases, or hemorrhagic cases related to CHIKV infection have been conclusively documented in the scientific literature.

CHIKV infection (whether clinical or silent) is thought to confer life-long immunity.

Spread of Chikungunya

CHIKV is spread by the bite of an infected mosquito. Mosquitoes become infected when they feed on a person infected with CHIKV. Monkeys, and possibly other wild animals, may also serve as reservoirs of the virus. Infected mosquitoes can then spread the virus to other humans when they bite. Aedes aegypti (the yellow fever mosquito), a household container breeder and aggressive daytime biter which is attracted to humans, is the primary vector of CHIKV to humans. Aedes albopictus (the Asian tiger mosquito) may also play a role in human transmission in Asia, and various forest-dwelling mosquito species in Africa have been found to be infected with the virus.

Treatment of Chikungunya

Effective treatment for Chikungunya is available in homeopathy.

Homeopathy has very effective treatment for Chikungunya. After diagnosis of Chikungunya, homeopathic treatment should be started as early as possible. Homeopathic treatment can also be given along with conventional treatment if desired. Conventional treatment consists of only symptomatic treatment of fever, joint stiffness, joint pain of this viral illness, whereas homeopathic treatment gives better and long lasting relief from the disease. Effective remedy can be chosen by a homeopathic doctor according to clinical picture of that particular case. Homeopathy works effectively in all viral diseases. Homeopathic pills are simply chewed (dissolved) on tongue and are sweet in taste. Homeopathic medicines do not cause gastric irritation, are safe on kidneys and liver even when taken for long duration. Individual case study, preparing and shipping the Chikungunya treatment to your address worldwide is available from our clinic. Click here to order.

In epidemics, prevention of Chikungunya can be achieved by administering Homeopathic "Genus Epidemicus". Genus Epidemicus is a homeopathic remedy that is chosen as a preventive remedy for that particular epidemic in that particular locality. Homeopathic medicines are equally effective for post-chikungunya complaints like weakness, stiffness in joints, muscles pain etc. Infected persons should be protected from further mosquito exposure (staying indoors and/or under a mosquito net during the first few days of illness) so that they can't contribute to the transmission cycle.

Know more about Chikungunya Homeopathy Treatment

Know more about Curative and Preventive Homeopathy in Chikungunya.

Prevention of Chikungunya

The best way to avoid CHIKV infection is to prevent mosquito bites. In addition we strongly recommend to have "Homeopathic Genus Epidemicus" for double protection.

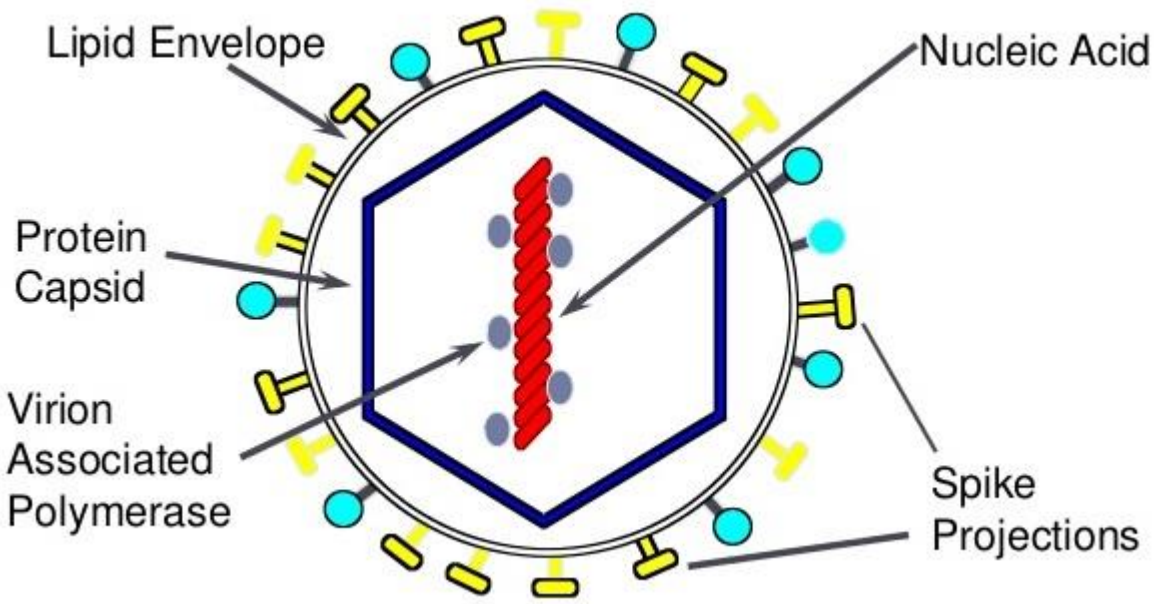
Prevention tips:

- Use mosquito repellent on exposed skin.
- Wear long sleeves shirts and pants.
- Have secure screens on windows and doors to keep mosquitoes out.
- Get rid of mosquito breeding sites by emptying standing water from flower pots, buckets and barrels. Change the water in pet dishes and replace the water in bird baths weekly. Drill holes in tire swings so water drains out.
- Additionally, a person with chikungunya fever should limit their exposure to mosquito bites in order to avoid further spreading the infection. The person should stay indoors or under a mosquito net.

Japanese Encephalitis (JE) Virus

Structure of Japanese Encephalitis (JE) Virus

- Japanese encephalitis (JE) virus falls under the family Flaviviridae.

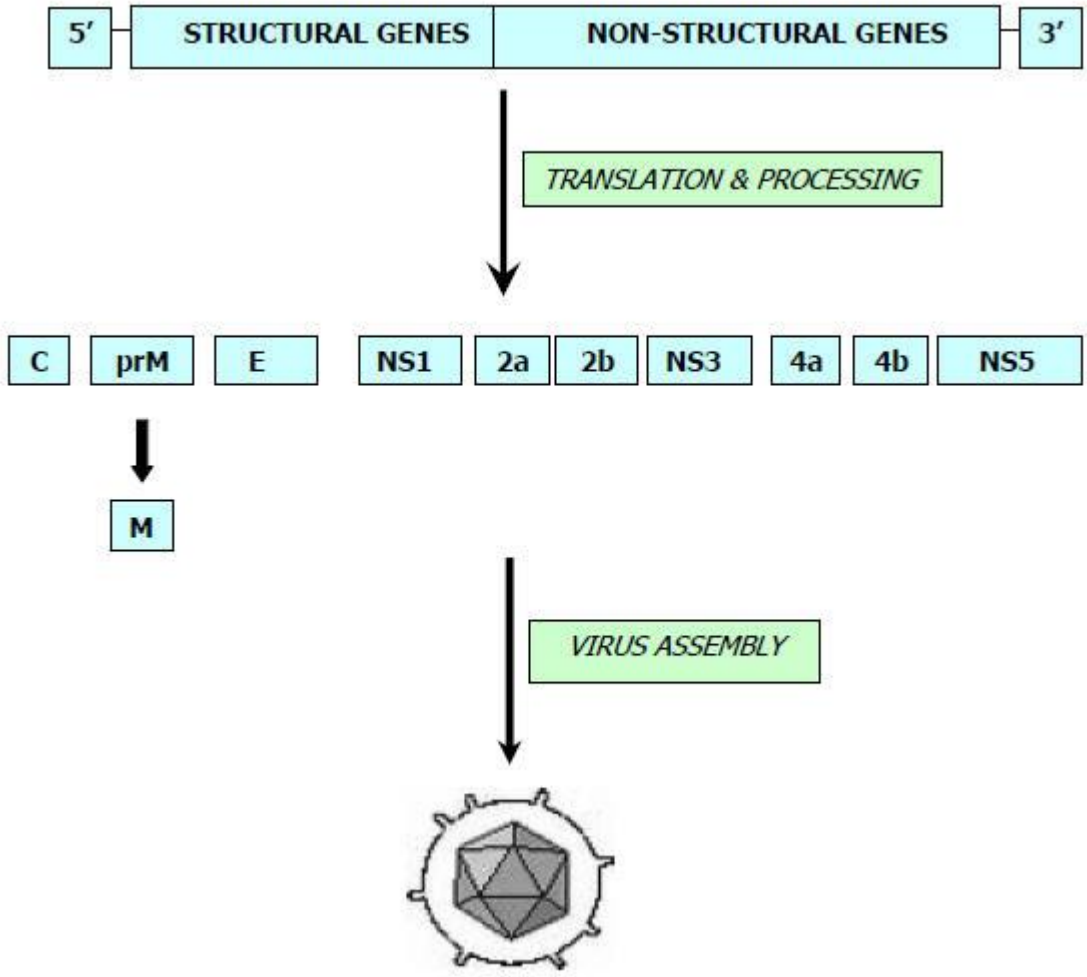


The particles appear to be spherical, 50 nm in diameter, containing an electron dense core (about 30 nm diameter) surrounded by a lipid bilayer.

- Mature virions sediment between 170 and 210S, have a buoyant density of 1.19 to 1.23 g/mL.
- It has a small lipoprotein envelope surrounding a nucleocapsid comprising of the core protein.
- The nucleocapsid also encloses the single stranded RNA genome with positive polarity which is 11kb in length.

Genome of Japanese Encephalitis (JE) Virus

- The genome is monopartite, linear single stranded RNA genome with positive polarity.
- The ssRNA genome is 11kb in length.
- This RNA contains a 5′ cap (m7G5’ppp5’A) at the 5′ end and lacks a polyadenylate tail.
- Genomic RNA is the messenger RNA for translation of a single long open reading frame (ORF) as a large polyprotein.



<img

Surrounding the ORF are 5′ and 3′ noncoding regions (NCRs) of around 100 nucleotides (nt) and 400 to 700 nt, respectively.

- The genome is translated as a large polyprotein that is processed co- and posttranslationally by cellular proteases and a virally encoded serine protease into at least 10 discrete products.
- The N-terminal one quarter of the polyprotein encodes the structural proteins, and the remainder contains the nonstructural (NS) proteins, in the following order: C-prM-E-NS1-NS2A-NS3-NS4A-NS4B-NS5.
- Three viral proteins are associated with virions: the E (envelope), M (membrane), and C (capsid) proteins.
- The E protein (50kd) is the major surface protein of the viral particle, probably interacts with viral receptors, and mediates virus–cell membrane fusion.
- M protein is a small proteolytic fragment of prM protein (26kd), which is important for maturation of the virus into an infectious form.

- C protein (about 11 kd) is highly basic, consistent with its proposed role in forming a ribonucleoprotein complex with packaged genomic RNA.

Epidemiology and Transmission of Japanese Encephalitis (JE) Virus

- JE virus is transmitted by the bite of Culex mosquitoes and transmitted naturally between wild and domestic birds and pigs.
- The virus is maintained in an enzootic cycle between mosquitoes and amplifying vertebrate hosts (mainly pigs).
- Most important one for human infection is *Culex tritaeniorrhynchus*, which breeds in pools of stagnant water.
- The virus occurs in countries across Eastern, Southern Asia and Pacific.
- Humans become infected coincidentally when living or travelling in close proximity to animals and birds infected with JE.
- Human are considered as dead end host from which transmission doesn't not normally occur.

Unit III possible questions

Part B

1. Define endemic ad epidemic
2. Say about viral infections
3. What are clinical specimens for viral diagnosis?
4. What are Infection and immunity?
5. What is IFT?

Part C

1. Give the laboratory diagnosis of pox virus
2. State the pathogenic role and diagnosis of GI tract pathogenesis.
3. Discuss the properties of viruses.
4. Explain in detail about hepatitis.
5. Comment on viral diagnosiss

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS : II B.Sc MB **COURSE NAME: MEDICAL MICROBIOLOGY**
COURSE CODE: 18MBU402 UNIT 3 **BATCH-2018-2021**

Unit III Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
The genera that doesnot belongs to the family Micrococcaceae is	*Staphylococcus*	*Micrococcus*	*Planococcus*	*diplococcus*	*diplococcus*
Identify the bacteria which is oxidase-negative and catalase-positive?	*Staphylococcus*	*Streptococcus*	*Neisseria*	*Pseudomonas*	*Staphylococcus*
The coagulase test is used to differentiate Staphylococcus aureus from	*other staphylococci*	*streptococci*	*micrococci*	*enterococci*	*other staphylococci*
Scalded skin syndrome is caused by exotoxin (exfoliatins) produced by:	*Streptococcus pyogens*	*Staphylococcus aureus*	*Propionibacterium acne*	*Pseudomonas aeruginosa*	*Staphylococcus aureus*
The ability to clot blood plasma is one of the most reliable laboratory tests available for the identification of which of the following organisms?	*Escherichia coli*	*Streptococcus pyogenes*	*Staphylococcus aureus*	*Haemophilus influenzae*	*Staphylococcus aureus*
Streptococcus pyogenes are more sensitive to than other species	amoxycillin	penicillin	erythromycin	bacitracin	bacitracin
the disease common in dockworkers carrying loads of skin and animals was known as	hide porter's disease	gas gangrene	edeme	toxemia	hide porter's disease
_____ strains usually secrete both coagulase and clumping factor	*micrococcus*	*streptococcus*	*staphylococcus aureus*	*E coli*	*Staphylococcus aureus*
Vegetative cells of Bacilli are destroyed at C in 30 min	60C	40C	20C	10C	60C
The spores of Bacillus anthracis	20	30	40	60	60

were isolated from the infected soil after _____ years					
Lepra bacilli have been found to remain viable in moist soil for days	100	80	46	20	46
The Lepra bacilli seen in large numbers as golbi inside Lepra cells is known as _____	Common disease	Multibacilir y disease	Gas gangrene	Symptomatic disease	Multibacilir y disease
was the first effective chemotherapeutic agent against leprosy	rifampicin	clofazimine	ethionamide	dapsone	dapsone
_____ vaccine was used to prevent leprosy	BCG	Polio vaccine	Leprosy vaccine	Rabies vaccine	BCG
The Lepra bacillus was first observed by _____	Jenner	Behring	Pasteur	Hansen	Hansen
The diphtheria bacillus was first observed by _____	Pasteur	Hansen	Klebs	Loeffler	Klebs
Typing in Pneumococcus may be carried out by reaction	serum typing	Quellung	Bamboo-stick	stickland	Quellung
Corynebacterium exists in a _____ morphology	Rod	Cocci	Varied	Spindle shaped	Varied
On repeated subculture Pneumococci undergo a variation	smooth-rough	rough-smooth	smooth	rough	smooth-rough
is always a secondary infection caused by any serotype of Pneumococcus	meningitis	hemorrhage	paralysis	Broncho-pneumonia	Broncho-pneumonia
The strain used to produce the diphtheria toxin is _____	Bacillus Calmette	Park Williams 8 Strain	Park Williams 5 Strain	Bacillus Calmette 8 strain	Park Williams 8 Strain

The diagnostically important constant property of Pneumococcus is	Bile solubility	serum solubility	symptoms	pathogeicity	Bile solubility
The incubation period of Lepra bacilli ranges from years	5 to 6	2 to 5	4 to 9	1 to 3	2 to 5
The BCG vaccine used to prevent leprosy was suggested by	Fernandez	Pasteur	Jenner	Hansen	Fernandez
Preventation of anthrax in animals is aided by	prophlaxix	treatment	active immunization	antibiotics	active immunizatio n
reaction is useful for the primary diagnosis of anthrax in animals	M'Fadyean's	Quellung	Nagler	immunization	M'Fadyean's
Staphylococci are lysed under the influence of the drug	Penicillin	streptomyci n	amoxycillin	dapsone	Penicillin
is a typical of Staphycoccal infection	pus	edema	focal suppuration	impetigo	focal suppuration
Streptococcal sore throat is commonly called	sore throat	Strep throat	infection	disease	sore throat
named the strains *Staphylococcus aureus* and *S albus*	Jenner	Behring	Rosenbach	Klebs	Rosenbach
Typical Staphylococci are seen in the stained smears of	pus	blood	stool	urine	pus
is the selective medium used for the isolation of Bacillus anthracis	PLET	blood agar	Mac-conkey	PDA	PLET
In cultures Bacillus were arranged ene to end in a long chain gives a appearance	chain	Bamboo-stick	cluster	thread-like	Bamboo-stick
The of	exotoxin	antitoxin	size	shape	antitoxin

Corynebacterium was described by Von Behring					
type of complications are most common in gravis type of Corynebacterium	fever	headache	paralytic	nausea	paralytic
Food poisoningby Clostridium perfringens is usually caused by cold or warmed up dish	fish	egg	soup	meat	meat
Pneumococci were first noticed by	Nagler	Robert Hooke	Pasteur and Sternberg	Kleb and Loeffler	Pasteur and Sternberg
The toxin produced by virulent strain of diphtheria is known as	exotoxin	endotoxin	antitoxin	toxin	exotoxin
The drug of choice for treating gas gangrene is	Penicillin	streptomycin	metranidazole	Amoxycillin	metranidazole
The irregularly stained boat or leaf shaped Clostridium septicum is known as	citron bodies	pleomorphs	irregular bodies	boat bodies	pleomorphs
The arrangement of diphtheria bacilli at various angles to each other resembling the letters V or L has been called as _____	V shaped	L shaped	Chinese	Bamboo-stick	Chinese
The causative agent of gas gangrene is ____	*streptococcus*	*micrococcus*	*Clostridium perfringens*	*Corynebacterium*	*Clostridium perfringens*
Clostridium species can grow well in _____ broth	PLET	Robertson's cooked meat	PDA	Mac-conkey	Robertson's cooked meat
_____ dyes are used to stain Pneumococci	Acidic dyes	Basic dyes	Neutral dyes	Aniline dyes	Aniline dyes
The most important antigen of Pneumococcus is _____	Type specific capsular polysaccharide	Type specific Nucleotide	Type specific antigen	Type specific antibody	Type specific capsular polysaccharide
When sputum is not	blood	serum	serum coated	egg	serum

available may be used for culturing Pneumococcus			laryngeal swabs		coated laryngeal swabs
is an antigeniccomponent of the cell wall facilitates adhesion of the cocci to the host cell surface	lipopolysaccharide	teichoic acid	lactate	iron	teichoic acid
Staphylococcus cause_____ __	Tumour	Boils	Lesion	Fever	Lesion
Staphylococcus were first observed by_____	Vonreckling hausen	Ogsten	Passet	Pasteur	Vonreckling hausen
Staphylococcus citreus produce_____ ____ colour	Red	Blue	Brown	Yellow	Yellow
Non virulent Staphylococcus is found in _____	Water	Hospital	Skin	Hair	Skin
Mannitol is fermented by _____ Staphylococcus	Virulant	Avirulant	Commensal	Normal flora	Virulant
Oil paint appearance is seen in_____ medium	Nutrient broth	Nutrient agar	Blood agar	Blood broth	Nutrient agar
In intoxication the disease is caused by _____	Toxoid	Tetroid	Enzyme	Toxin	Toxin
Alpha haemolysis means _____	Complete	Irregular	Partial	Nolysis	Partial
Beeta haemolysis means _____	Complete	Irregular	Partial	Nolysis	Complete
Selective media for Staphylococcus _____	Manittol salt agar	Mac conkey agar	Blood agar	LJ agar	Manittol salt agar
Streptococcus shows _____ type of haemolysis	Alpha	Beta	Gamma	delta	Beta
BCG expansion	bacillus calmette guarine	bdello vibrio guarine		bacillo vibrio guarine	none of the above
AIDS Expansion	acquired	acquired	active immuno	all of the	acquired

	immuno deficiency syndrome	infected deficiency symptom	deficiency syndrome	above	immuno deficiency syndrome

Unit IV

Brief description of each of the following types of mycoses and one representative disease to be studied with respect to transmission, symptoms and prevention. Cutaneous mycoses: Tinea pedis (Athlete's foot). Systemic mycoses: Histoplasmosis. Opportunistic mycoses: Candidiasis. The detailed study of following diseases – Causative agents, Mode of transmissions, Pathogenicity, Symptoms and prophylaxis of Amoebiasis, Giardiasis, Elephanttiasis, Taeniasis, Malaria, Kala-azar.

1. Use universal precautions for collecting and handling all specimens.

2. Whenever possible, collect all culture specimens prior to administration of any antimicrobial agents.

3. Avoid contamination with indigenous flora.

4. Swabs are convenient but inferior to tissue and fluid. Tissue and fluid are essential for fungaland mycobacterial culture.

5. All specimens must be appropriately labeled with two patient identifiers. Identifiers used at University of Iowa Hospitals and Clinics (UI Hospitals and Clinics) include patient name, birthdate and/or hospital number. The requisition will include the patient name, hospital number, hospital service, date and time of collection, specimen type and tests requested. A requisition needs to accompany each different specimen type.

6. Deliver all specimens to the laboratory as soon as possible after collection. Specimens for bacterial culture should be transported at room temperature. If transport is delayed the following specimens should be refrigerated: urines (within 30 min), stool (within 1 h), respiratory specimens. Specimens for viral culture must be transported to the laboratory immediately on ice. See specific specimen and culture type for detailed collection and transport guidelines.

7. Specimens may be hand delivered to the laboratory or transported via the runners from Technical Services if the specimens are not indicated as deliver immediately. Specimens may be transported through the pneumatic tube system if approved by Pneumatic Tube Administration. This includes blood culture bottles (if placed in plastic carrier), Vacutainer® tubes and swabs.

8. Specimens should be in tightly sealed, leak proof containers and transported in sealable, leak-proof plastic bags. Specimens for TB should be double bagged. Specimens should not be externally contaminated. Specimens grossly contaminated or compromised may be rejected.

9. If anaerobic culture is requested, make certain to use proper anaerobic collection containers (fluid: 59546, tissue: 59547, or ESwab, 74541).

10. Further questions may be referred to the Microbiology laboratory (356-2591) or pathology resident (pager 4903 weekdays; pager 3404 evenings and weekends).

Bacterial Cultures: Transport at room temperature unless otherwise specified.

A. Abscess – Tissue or aspirates are always superior to swab specimens. Remove surface exudate by wiping with sterile saline or 70% alcohol. Aspirate with needle and syringe. Cleanse rubber stopper of anaerobic transport vial with alcohol; allow to dry 1 min before inoculating; push needle through septum and inject all abscess material on top of agar. If a swab must be used, pass the swab deep into the base of the lesion to firmly sample the fresh border. Transport time ≤ 2 hours.

B. Anaerobic cultures - Aspirates are preferred rather than swabs. Fluid collections should be aspirated through disinfected tissue or skin. For superficial ulcers, collect material from below the surface (after surface debridement or use a needle and syringe). Submit specimens using anaerobic transport media:

a. Anaerobic transport vial :Cleanse rubber stopper with alcohol; allow to dry 1 min before inoculation; push needle through septum and inject specimen on top of agar
b. Anaerobic jar . Place sample on top of agar. Keep jar upright to maintain atmosphere in jar.
c. A sterile container may be used for tissue if transported to the microbiology lab immediately (add drops of sterile saline to keep small pieces of tissue moist).
d. Copan Liquid Amies Elution Swab – swab specimens are suboptimal, but will be accepted if no other sample can be obtained.
e. Deliver all specimens to the laboratory immediately after collection.
f. Anaerobic flora is prevalent on mucosal surfaces of the oral cavity, upper respiratory, gastrointestinal, and genital tracts; specimens collected from these sites should not ordinarily be cultured for anaerobic bacteria. The following is a list of specimens that are likely to be contaminated with anaerobic normal flora and are NOT routinely accepted for anaerobic culture.
    1. Throat or nasopharyngeal swabs
    2. Gingival or other intraoral surface swabs
    3. Expectorated sputum
    4. Sputum obtained by nasotracheal or endotracheal suction
    5. Bronchial washings
    6. Voided or catheterized urine
    7. Vaginal or cervical swabs
    8. Gastric and small bowel contents (except for "blind loop" or bacterial overgrowth syndrome)
    9. Feces (except for specific etiologic agents such as *C. difficile* and *C. botulinum*)
    10. Rectal swabs - Surface swabs from ulcers and wounds (collect material from below the surface)
    11. Material adjacent to a mucous membrane that has not been adequately decontaminated

C. Blood

a. Adult – Cleanse skin with disinfectant:

1. Holding the applicator sponge downward, pinch wings on applicator to break ampuleand release the antiseptic.
2. Use a side-to-side motion to scrub the site with the friction pad for a full 30 sec; allow site to dry completely (at least 30 sec) before venipuncture. Do not touch site after prep.
3. Remove overcaps from bottles (1 aerobic and 1 anaerobic) and cleanse each rubber septum with separate 70% alcohol swabs. Allow septum to dry for 1 min before inoculating.
4. Draw 20 mL of blood and inoculate each bottle with 10 mL of blood. Do not vent or overfill bottles. Adding low (<8 mL) or high (>10 mL) volumes may adversely affect the recovery of organisms. Transport time <2 h.
5. For adults with a suspected bloodstream infection (BSI), collect two initial sets of blood cultures sequentially from separate phlebotomy procedures followed by a third and a fourth set at 4-6 hour intervals (will detect >99% of BSIs). Three sets of blood cultures collected within a 24 hour period will detect 96.9 - 98.3% of BSIs. A single set of blood cultures to detect BSIs in adults is inadequate (only 73% sensitivity); two sets of blood cultures will allow detection of 87.7-89.7% of BSI episodes. If patient is allergic to chlorhexidine, prep site with a povidone iodine swab stick applied in concentric circles (start at center). Allow to dry at least 1 min before venipuncture. If patient is allergic to iodine, cleanse site with 70% alcohol for 60 sec.

b. Pediatric – Apart from NICU patients, the minimum volume drawn should be 1 mL per year of age per blood culture set. This volume should be split between an aerobic and anaerobic bottle. See pediatric blood culture order for more detail.

D. Bone marrow aspirate – Prepare puncture site as for surgical incision. Inoculate yellow top tube (104184). Transport time <2 hours.

E. Burn – Clean and debride burn. Place tissue in sterile screw-cap container. Transfer aspirates to a sterile container. These are processed for aerobic culture only. Quantitative culture may or may not be valuable. A 3 to 4 mm punch biopsy specimen is optimum when quantitative cultures are ordered. Cultures of surface samples can be misleading.

F. Catheter Tips – Catheter tips are not routinely accepted for culture. Consult Microbiology laboratory for approval. Foley catheters are not accepted for culture since growth represents distal urethral flora.

G. Cerebrospinal Fluid (CSF) – Obtain CSF for gram stain, cell count, protein, glucose and aerobic culture where able. The kit should contain 4 pre-numbered tubes to be filled in chronological order. Avoid covering tube numbers with stickers to ensure appropriate routing of samples.

- With low volume, one-tube specimens not all testing may be possible and the clinician must determine which tests should be prioritized. If cultures are desired, Microbiology must receive the specimen first to ensure the culturing of a sterile specimen.
- Transport time <15 minutes. Do not refrigerate CSF for bacterial culture.

If adequate volume is obtained, orders are placed per tube as follows:

Tube #	Orders

#1 – Chemistry/Immunology	Protein and Glucose
#2 – Microbiology	Aerobic culture with gram stain. Select "CSF lumbar puncture", "CSF shunt", or "CSF ventricular trap" as source to ensure appropriate culturing. If cryptococcal meningitis is suspected, fungal culture and cryptococcal antigen should be ordered as well. The Meningitis/Encephalitis PCR Panel should be ordered on all lumbar punctures where infections is being considered and cannot be ordered on non-lumbar puncture samples (order individual tests instead).
#3 – Hematology	CSF Cell Count and Differential. If a manual differential is needed, a pathologist review can be obtained by completing the "Staff Pathologist Slide Review (Information Required)" area of the A-1a Pathology Doctor's Order Form.
#4 – Specimen Control	Specimen storage. Select "Spinal fluid" as source and request to hold for 30 days at -80°C unless 4°C or -20°C storage is needed for a specific test.

. Inner ear – Tympanocentesis should be reserved for complicated, recurrent, or chronic persistent otitis media. For intact eardrum, clean ear canal with soap solution and collect fluid via syringe aspiration. Submit in sterile container. For ruptured eardrum, collect fluid on flexible shaft swab via an auditory speculum. Transport time <2 hours.

a. Outer ear – Use moistened swab to remove any debris or crust from ear canal. Obtainsample by firmly rotating swab in outer canal. For otitis externa, vigorous swabbing is required – surface swabbing may miss streptococcal cellulitis.

Eye

. Conjunctiva – Sample each eye with separate swabs (premoistened with sterile saline) byrolling over conjunctiva. When only one eye is infected, sampling both can help distinguish indigenous microflora from true pathogens.

a. Corneal scrapings – Collected by ophthalmologist. Using sterile spatula, scrape ulcers and lesions; inoculate scraping directly onto media (BHI with 10% sheep blood, chocolate, and inhibitory mold agar). Prepare 2 smears by rubbing material onto 1-2 cm area of slide. Transport time ≤15 min.

b. Vitreous fluid – Prepare eye for needle aspiration of fluid. Transfer fluid to sterile tube. Transport time ≤15 min.

Feces - see stool.

Fistula - see abscess.

Fluids - see sterile body fluids.

Genital –Cultures for *Neisseria gonorrhoeae* should be collected using an Copan Liquid Amies Elution Swab (ESwab). Transport to the laboratory immediately.

. Endocervical - Remove cervical mucus with swab and discard. Insert a second swab into endocervical canal and rotate against walls. Allow time for organisms to absorb onto the swab

surface.

a. Urethral - Collect urethral specimens at least 1 h after patient has urinated. Insert small swab 2-4 cm into urethral lumen, rotate, leave for 2s to facilitate absorption.

Pilonidal cyst – see abscess.

Respiratory, lower – Transport time ≤2 hours.

. Bronchoalveolar lavage or brush, endotracheal aspirate – Collect fluid in a sputum trap ;transfer to leak-proof container for transport to microbiology labortory; place brush in sterile container with 1 mL sterile saline.

a. Sputum, expectorated - Patient should rinse mouth and gargle with sterile water prior to collection; instruct patient to cough deeply. Collect specimen in sterile transport containers

b. Sputum, induced – Have patient brush gums and teeth, then rinse mouth thoroughly with sterile water. Using a nebulizer, have the patient inhale 20-30 mL of 3 to 10% sterile saline. Collect sputum in sterile container.

c. If Nocardia is suspected, culture for Nocardia should be requested as an add-on test as standard culture is inadequate for its recovery.

Respiratory, upper – Transport time ≤2 hours.

. Oral – remove oral secretions and debris from surface of lesion with a swab. Use a second swab to vigorously sample lesion, avoiding normal tissue. Superficial swab specimens should not be submitted. Tissue or needle aspirates are preferred.

a. Nasal swabs– Insert a sterile swab (use Copan dual swab) into the nose until resistance is met at the level of the turbinates (approximately 1-2 cm into one nostril). Rotate the swab against the nasal mucosa for 3 sec. Apply slight pressure with a finger on the outside of the nose to ensure good contact between swab and inside of nose. Using the same swab, repeat for the other nostril.

b. Sinus aspirates – Aspirate with needle and syringe. Cleanse rubber stopper of anaerobic transport vial with alcohol; push needle through septum and inject specimen on top of agar.

c. Throat - Routine throat cultures will be processed only for growth of ß-hemolytic *Streptococcus* species. Do not obtain throat samples if epiglottis is inflamed, as sampling may cause serious respiratory obstruction. Sample the posterior pharynx, tonsils, and inflamed areas using a Copan Liquid Amies Elution Swab(ESwab).

Sterile body fluids (other than CSF)

. Transport fluid to laboratory in sterile, leak-proof container (BD Vacutainer®, no additive, yellow top,) or anaerobic transport vial (Vial,).

a. Cleanse rubber septum of container with 70% alcohol. Allow septum to dry for 1 min before inoculating.

b. Disinfect overlying skin with iodine or chlorhexidine preparation. Obtain specimen with needle and syringe. Push needle through septum of transport container and inject fluid.

c. Amniotic and culdocentesis fluids should always be transported in an anaerobic transport vial .

Agar in anaerobic vial should be clear before inoculation; inject fluid on top of agar.

d. Submit as much fluid as possible. NEVER submit a swab dipped in fluid. NEVER inject fluid into swab container.

e. One aerobic blood culture bottle inoculated at bedside (up to 10 mL) is highly recommended provided adequate sample is available. If blood culture bottle is inoculated, submit separate aliquot inanaerobic transport vial or sterile container for preparation of cytocentrifuged Gram stain and inoculation of solid media (allows quantitation, aids in culture interpretation).

f. Transport time ≤15 min, room temperature.

Stool – Stools submitted on patients admitted for >3 days will be rejected without prior preapproval (pager 4903 weekdays, pager 3404 evenings and weekends).

. Please use Fecal Swabs. 1) Obtain a stool specimen in a clean pan or container. Stool specimens should not contain urine or water. 2) Holding FecalSwab shaft above the red breakpoint mark, insert the entire tip of the FecalSwab into the stool sample and rotate. Do not use FecalSwab as a spoon; rather, coat swab with a visible layer. 3) If visible stool is not coating the FecalSwab tip, reinsert until swab is coated. 4) Using swab and aiming tube away, mash and mix the stool sample against the side of the tube to suspend the sample. 5) Invert the tube several times to homogenize the sample and expose the sample to Cary Blair preservative fluid.

a. The FilmArray Gastrointestinal Panel is a multiplex PCR test capable of qualitatively detecting DNA or RNA of 22 pathogens (bacteria, parasites, and viruses). It requires a FecalSwab. The panel is **used to diagnose infection caused by *Campylobacter* species, *Plesiomonas shigelloides*, *Salmonella* species, *Vibrio* species, *V. cholerae*, *Yersinia* species, enteroaggregative *E. coli*, enteropathogenic *E. coli*, enterotoxigenic *E. coli*, Shiga toxin producing *E. coli*, *E. coli* O157, *Shigella*/Enteroinvasive *E. coli*, *Cryptosporidium* species, *Cyclospora cayetanensis*, *Entamoeba histolytica*, *Giardia lamblia*, Adenovirus F 40/41, Astrovirus, Norovirus, Rotavirus and Sapovirus.**

b. Stools for *C. difficile* toxin detection must be transported to the laboratory immediately or refrigerated if transport is delayed. This test requires raw stool, not a FecalSwab.

c. Surveillance cultures may be ordered on Bone Marrow transplant and other immunocompromised patients to detect overgrowth of normal flora by *Staph aureus*, yeast or a gram negative bacillus.

d. Test of Cure Stool Culture (Salmonella, Shigella, EHEC) is only for the listed organisms. For organisms other than these please contact the Microbiology laboratory for approval.

e. Aeromonas Culture – Should be collected in Fecal Swabs. This test may be added onto the FilmArray Enteric Panel.

Tissue – Submit in anaerobic collection jar or sterile screw-cap container ; add drops of sterile saline to keep small pieces of tissue moist. Transport time ≤15 min.

Urine – Collect 4 mL of urine in a sterile specimen container . Transfer urine to a gray top C&S urine container. Tubes must be filled to 3 mL do prevent inhibition of bacterial growth. Transport to the microbiology laboratory. If unable to collect 3 mL of urine, collect in sterile specimen container or yellow top tube and transport urine specimens to the Microbiology Laboratory or refrigerate **within 30 minutes**. Refrigerated specimens should be delivered to the lab as soon as possible, and may be rejected if not received within 24 hours of collection.

Gray top C&S urine containers are not acceptable for urinalysis and urine chemistries because the preservative interferes with testing.

Cultures can not be performed as an add-on test to urinalysis. Send separate sample for urinalysis (random urine yellow top, round bottom tube (no additive)) and culture (as above).

. Midstream clean catch method: Patients should be instructed to wash hands prior to collection and offered exam gloves.

1. **Female** patients should be instructed to sit on toilet with legs apart and spread labia with one hand. First void in toilet and then, continuing to void, hold specimen container in "midstream" to collect sample.
2. **Male** patients should be instructed to retract foreskin if uncircumcised. First void in toilet and then, continuing to void, hold specimen container in "midstream" to collect sample.

a. Straight catheter: Clamp catheter below port and allow urine to collect in tubing. Disinfect the catheter collection port with 70% alcohol. Use needle and syringe to aseptically collect 4 mL freshly voided urine though catheter port. Transfer to gray top C&S urine container. Do not collect urine from collection bag.

b. Indwelling catheter: Clamp catheter below port and allow urine to collect in tubing. Disinfect the catheter collection port with 70% alcohol. Use needle and syringe to aseptically collect 20 mL freshly voided urine though catheter port. Transfer to gray top C&S urine container. Do not collect urine from collection bag.

c. Ileal conduit: Remove the external device and discard urine within device. Gently cleanse the stoma with 70% alcohol followed by povidone-iodine swab stick . Using sterile technique, insert a double catheter into the cleansed stoma, to a depth beyond the fascial level, and collect the urine into a sterile container. Transfer to gray top C&S urine container. Use of a double catheter helps to minimize contamination of the specimen with skin flora.

Wound – See abscess.

Fungal Culture

1. Deliver all specimens to the laboratory as soon as possible after collection.
   A. Blood: Cleanse skin with disinfectant. Collect 8-10 mL of blood for adult (1.5 mL for child) and inoculate into an Isolator tube (Adult; Pediatric). Collect in addition to bacterial blood culture bottles. Isolator tubes are for molds, Histoplasma, Blastomyces, and Malassezia spp.; for bloodstream infection by Candida spp., inoculate aerobic blood culture bottles instead.

   B. Skin: Using a scalpel blade, scrape the periphery of the lesion border and transport in asterile container.

   C. See Bacterial Culture for collection and transport of all other specimen types.

Mycobacterial Culture (AFB Culture)

1. Deliver all specimens to the laboratory as soon as possible after collection. Specimens for mycobacteria should be double bagged and sent sealed in leak-proof containers.
   A. Blood: Media and instructions available upon request from the Microbiology Lab. Test available for limited patient populations only.

   B. Sputum: Collect an early morning specimen on three consecutive days. Collect 5-15 mL in a sterile container.

   C. See Bacterial culture for collection and transport of all other specimen types.

   D. Swabs are suboptimal for recovery of mycobacteria due to limited material and the hydrophobicity of the mycobacterial cell envelope (often compromises a transfer from swabs onto media). Dry swabs are unacceptable. The lab only accepts Copan Liquid Amies Elution Swab (ESwab) for AFB culture when the ordering physician confirms that the swab is the only possible way to obtain the specimen.

Viral/Molecular Infectious Disease PCR Testing

Collect specimens for PCR testing early in illness when viral shedding is maximal. Place swabs in viral transport medium. Collect bronchoalveolar lavage, tracheal aspirate, or nasopharyngeal wash/aspirate and specimens from normally sterile sites in a sterile, leak-proof container . Transport the specimen to the Microbiology laboratory immediately.

Respiratory Virus PCR
PCR assay includes the detection of respiratory viruses (influenza A including H1N1; influenza B; RSV; parainfluenza 1, 2, 3; adenovirus; human metapneumovirus).

1. Mycoplasma PCR: Collect throat swab in ESwab.

2. Biopsy or tissue: Keep moist with sterile saline or viral transport media [do not use viral transport media (VTM) if bacterial, AFB, or fungal cultures are also requested; VTM is available in kits from Hospital Stores

3. CSF: Collect 0.5 mL in a sterile container. Transport immediately to laboratory.

4. Nasopharyngeal swab: Collect specimen using the flexible minitip flocked swab (Hospital Stores #33595). Measure the distance from the patient's nostril to the nasopharynx (half the distance from nostril to base of the ear) and hold the swab at that location. Do not advance the swab beyond that point. Gently insert the swab along the base of one nostril (straight back, not upwards) and continue along the floor of the nasal passage until reaching the nasopharynx. Rotate swab 2-3 times and hold in place for 5 seconds. Place swab in tube containing viral transport medium. Break off the excess length of swab at the score mark to permit capping of the tube.
5. Nasopharyngeal wash/aspirate:
   A. Assemble equipment:

- Sterile specimen trap
- Personal Protective Equipment (gloves, surgical mask, eye protection)
- Appropriate size suction catheter (8 fr for infants/children, 10/12 fr for adults)
- Normal saline vial
- Wall suction
- Bag or cup of ice for specimen transport to laboratory

B. Place patient with the head tilted slightly back.

C. With sterile gloved hand, insert suction catheter into the patient's nose to the depth of the nasopharyngeal area (beyond the turbinates). Do not remove catheter until end of procedure (see picture below).

D. With the non-sterile gloved hand, instill approximately 1-2 mL normal saline outside the catheter.

E. Apply suction to aspirate nasopharyngeal secretions.

F. Above steps may need to be repeated to obtain 1 mL sample in specimen trap.

G. Remove catheter from patient. With specimen trap still in-line, rinse catheter withremaining saline to clear secretions.

H. Specimens transported by tube system must be transferred from trap to a leak-proofsterile container (be sure the lid is tightly secured).

6. Respiratory secretions: Collect specimens in a sterile, leak-proof container. Sputum is unacceptable for viral cultures.

PCR Assays are available for the following viruses: HSV1&2, VZV, enterovirus (CSF), EBV (Blood and CSF) and CMV (blood, CSF, or BAL). PCR testing requires a dedicated collection tube and cannot be added onto a previously opened Vacutainer® tube. For CSF from lumbar puncture, most patients should have an order placed for the Meningitis/Encephalitis Panel (LAB8514).

1. HSV 1,2 or VZV PCR: submit CSF in sterile container. Submit vesicle fluid, surface swab, or BAL (sputum and tracheal aspirates are unacceptable) in UTM media. Transport to laboratory immediately.

2. Enterovirus PCR: submit CSF in sterile container. Keep on ice and deliver to laboratoryimmediately.

3. EBV PCR: Collect one 5 mL pink (EDTA) top tube. For CSF collect a minimum of 1.0 mL in a sterile container. Deliver to laboratory immediately after collection. **EBV PCR is useful only for diagnosis and monitoring of posttransplant lymphoproliferative disorder and similar disorders and is not appropriate for the diagnosis of mononucleosis or meningitis/encephalitis in immunocompetent patients.**

4. CMV Quantitative PCR: Collect one 5 mL pink (EDTA) top tube. For CSF collect a minimum of 0.5

mL in a sterile container. Deliver to laboratory immediately.

5. CMV Qualitative PCR: Submit a minimum of 2.0 mL BAL or 1.0 mL of amniotic fluid in a sterile container. Transport to laboratory immediately.

6. HIV Viral Load by PCR, Hepatitis C Virus RNA by PCR and Hepatitis B Virus DNA byPCR: Foreach test collect at least 6 mL whole bold in one pink (EDTA) top tube. Deliver immediately to laboratory. Each test requires a dedicated collection tube and cannot be added onto a previously opened Vacutainer® tube. All collection tubes need to be processed within 6 hours ofcollection.

7. Neisseria gonorrhoeae & Chlamydia trachomatis Detection by PCR: Amplified DNA (PCR) testing is recommended for urine, endocervical, urethral, oral or pharyngeal and rectal swab. Culture is recommended for suspected failure of therapy.
   A. Endocervical, urethral, oral or pharyngeal, rectal swab: Use multicollect specimen kit available from hospital stores (46161). Specimens must be aseptically collected with the orange shaft swab provided with the kit. After collection, specimens may be stored and transported at 2 to 30 ºC for up to 14 days. Do not freeze.

   B. Urine: The patient should not have urinated for at least one hour prior to sample collection. Collect urine in a typical collection cup (not provided in multi-collect kit). Using plastic transfer pipette provided in multi-collect specimen kit available from Hospital stores (46161), transfer urine from collection cup into the transport tube until the liquid level in the tube falls within the clear fill window of the transport tube label. Do not overfill. Slightly more than one full squeeze of the transfer pipette bulb may be required to transfer the necessary volume of urine specimen. After collection, specimens may be stored and transported at 2 to 30 ºC for up to 14 days. Do not freeze.

Parasitology

1. Enteric Panel: The Enteric Panel can only be performed from Fecal Swab samples. If test of cure is needed for Salmonella, Shigella or EHEC, Test of Cure Stool Culture instead.
   Please use Fecal Swabs. 1) Obtain a stool specimen in a clean pan or container. Stool specimens should not contain urine or water. 2) Holding Fecal Swab shaft above the red breakpoint mark, insert the entire tip of the Fecal Swab into the stool sample and rotate. Do not use Fecal Swab as a spoon; rather, coat swab with a visible layer. 3) If visible stool is not coating the Fecal Swab tip, reinsert until swab is coated. 4) Using swab and aiming tube away, mash and mix the stool sample against the side of the tube to suspend the sample. 5) Invert the tube several times to homogenize the sample and expose the sample to Cary Blair preservative fluid.

2. Ova and parasite exam: Within 1 hour of collection, transfer a few grams of stool to each vial of SHL collection kit. Order on EPIC as "SHL, Routine O+P with trichrome stain", complete the SHL requisition that is in the box, and deliver to Specimen Control for transport to SHL. A minimum ofthree stool specimens collected on alternate days is recommended. Onset of diarrhea in patients hospitalized for >3 days is usually not attributed to a parasitic infection. Requests to include Microsporidia or Cyclospora detection must be specified on the SHL requisition.

3. Pinworm exam: Submit scotch tape prep. Touch the perianal folds with clear scotch tape, then attach the tape to a clean glass slide and transport to the laboratory sealed in a ziplock bag. Clear tape must be used, not invisible tape.

4. Parasite exam: For direct examination of parasites (worms), arthropods (insects, spiders), and suspect material passed in stool. This is not a stool ova and parasite (O&P) exam. An O&P can be ordered in EPIC as "SHL, Routine O+P with trichrome stain" and uses SHL collection kit

Instructions:

A. Submit whole worms, worm segments or other objects in 70% alcohol or 10% formalin.

B. Submit arthropods in a clean, dry container.

5. Scabies exam: Sterile mineral oil is available from Pharmacy (item 991565, 10 mL container). Collect skin scrapings as follows:

A. Place a drop of mineral oil on a sterile scalpel blade.

B. Allow some of the oil to flow onto the papule. Scrape vigorously six or seven times to remove the top of the papule. (Tiny flecks of blood should be seen in the oil.)

C. Transfer the oil and scrapings onto a glass slide (an applicator stick can be used).

D. Add 1-2 extra drops of mineral oil to the slide and mix well. Clumps can be crushed to expose hidden mites.

E. Place a coverslip onto the slide and transport to the Microbiology Lab immediately.

6. Blood Parasite EXAM (R/O Malaria/Blood Parasites): Collect venous blood in EDTA collection tube and deliver immediately to lab. Malaria antigen testing is available 24 hrs/day, 7 days a week. Antigen results will be available within one hour of specimen arrival. Preliminary slide results will be available within 90 minutes if specimen received between 0700-1900 or by 0930 if after 1900. If clinical suspicion for malaria remains after one set of negative smears, additional specimens should be submitted at 12 hour intervals for the subsequent 36 hour period. Note on request if parasite infection other than malaria is suspected.

7. Vaginosis/Vaginitis Panel (Trichomonas, Yeast and Gardnerella): Collect vaginal specimen in Affirm VPIII Collection and Transport System. Deliver to laboratory within 24 hours of collection.

Unit IV possible questions

Part B

1. Define Cutaneous mycoses
2. Say about Systemic mycoses
3. What are clinical specimens for Candidiasis?
4. What are Infection and immunity?

5. What is Amoebiasis?

Part C

1. Give the laboratory diagnosis of Histoplasmosis.
2. State the pathogenic role and diagnosis of Elephanttiasis.
3. Discuss about Malaria.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS : II B.Sc MB **COURSE NAME: MEDICAL MICROBIOLOGY**

COURSE CODE: 18MBU402 UNIT 4 **BATCH-2018-2021**

Unit IV Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
Which of the following is the best bacteriological indicator of water contamination?	E coli	Streptococci	Clostridium perfringens	Salmonella	E coli
_____is a common cause of traveler's diarrhea and diarrhea in infants	Salmonella sp	Enterotoxigenic E coli	Staphylococcus aureus	Klebsiella	Enterotoxigenic E coli
With reference to infections with Escherichia coli the following are true except ?	Enteroaggregative E coli is associated with Persistent diarrhoea	Enterohemorrhagic E coli can cause haemolytic uraemic syndrome	Enteroinvasive E coli produces a disease similar to salmonellosis	Enterotoxigenic E coli is a common cause of traveler's diarrhoea	Enteroinvasive E coli produces a disease similar to salmonellosis
E coli produces _____ during fermentation of carbohydrates	acid alone	acid and gas	gas alone	No acid No gas	acid and gas
Transmission of intestinal disease is commonly by _____	airborne	blood contamination	fecal-oral route	animals	fecal-oral route
E coli is an _____ in humans	Pathogen	predator	Parasite	commensal	Parasite
*E coli exhibits IMViC*_____	++--	--++	+-+-	++++	++--
The K antigen in E coli is composed of _____	Protein	lipid		carbohydrate	Polysaccharide
_____is known as travelers diarrhea	EPEC	ETEC	EHEC	EAEC	ETEC
EHEC is also known as_____	EPEC	EAEC	VTEC	EXEC	VTEC
SIDS is seen in case of _____	*E coli*	*Klebsiella*	*Proteus*	*Pseudomonas*	*E coli*
Klebsiella is_____	Non motile and non capsulated	motile and capsulated	Non motile and capsulated	motile and non capsulated	Non motile and capsulated
Klebsiella exhibits_____ colonies	Dry	mucoid	Pale	diffuse	mucoid

K pneumoniae is also known as _____	Jansen's bacilli	Koch's bacilli	Friedlander's bacilli	Escherich's bacilli	Friedlander's bacilli
Klebsiella exhibits IMViC_____ __	++--	--++	+++	----	--++
The tribe proteae are_____	Fermentors	non fermentors	Late fermentors	early fermentors	non fermentors
The proteae is classified into_____genera	One	two	three	Four	three
Proteus exhibits _____ motility	Swarming	no	Fish in stream	Darting	Swarming
The predominant aerobic bacterial flora of the large intestines of human beings and animals is composed of_____	Non sporing bacilli	non acid fast bacilli	viruses	Gram negative bacilli	Gram negative bacilli
The clinical picture of dysentery is exhibited by_____	*Mycobacteria*	*Pseudomonas*	*Klebsiella*	*Shigella*	*Shigella*
Shigella is _____	Flagellated	sporing	capsulated	Non motile	Non motile
The selective medium used for *Shigella* is_____	Deoxycholate citrate agar	EMB	MSA	Martin Thayer	Deoxycholate citrate agar
The Shigella culture filtrates demonstrates_____ ___ type toxicity	Hypersensitive	Lytic	Chemotoxicity	Neurotoxicity	Neurotoxicity
The minimum infective dose for outcome of Shigellosis is_____	10-50 bacilli	10-100 bacilli	100-1000 bacilli	1-10 bacilli	10-100 bacilli
Bacillary dysentery has an incubation period of _____	6 hours	1 day	1-7 days	more than 7 days	1-7 days
The main features of bacillary dysentery are_____	Rice watery diarrhoea	presence of parasite	Abdominal discomfort	Loose scanty feces	Loose scanty feces
The infection with *Salmonella* is	Malaise	gastric ulcer	Septicemia	Enteric fever	Enteric fever

characterized by_____					
Salmonella is known as_____bacilli	Eberth gaffky	Shiga	Friedlander's	Escherich	Eberth gaffky
Salmonella typhi is the causative agent of _____	Typhoid fever	paratyphoid fever	Enteric fever	Malaise	Typhoid fever
The incubation period of *Salmonella typhi* is_____	6 hours	1 day	1-7 days	7-14 days	7-14 days
The infective dose for *Salmonella typhi* is_____	1-10 bacilli	100000 bacilli	100000000 bacilli	10000 bacilli	100000000 bacilli
Vibrio is _____rods	Helical	Elongated	Twisted	Curved	Curved
Vibrio cholerae was first isolated by_____	Pasteur	Koch	Paccini	Boyd	Koch
Vibrio is_____	Motile	Non motile	Slime	Capsulated	Motile
Vibrio cholerae are_____rods	Gram positive	Non motile	Spore forming	curved, Cylindrical	curved, Cylindrical
_____Is used as transport medium for Vibrio	Alcohol medium	Acid	sea water	V R Medium	V R Medium
Vibrio colonies may be easily identified by_____	Biochemical test	String test	Cultural characteristics	Coombs test	String test
Heiberg classified Vibrios into_____groups based on sugar fermentation	2	4	6	8	6
The route of infection with Vibrio is by_____ route	Oral	respiratory tract	Ingestion	inhalation	Oral
Pseudomonas is motile by_____flagella	polar	bipolar	peritrichous	Atrichous	polar
Vibrio is motile by_____ flagella	Atrichous	peritrichous	polar	Lopotrichous	polar
Glycocalyx is composed of _____	Protein	lipid	Polysaccharide	carbohydrate	Polysaccharide

*Pseudomonas produces*_____ __ *pigments*	Ruby	lucosin	Pyocynanin	Verdin	Pyocynanin
Pyocyanin is a _____ colored pigment	Blue	green	Red	yellow	Blue
Pyocynanin is soluble in_____	Acid	Base	chloroform , Water	ether	chloroform , Water
Fluorescin is_____ colored pigment	Greenish yellow	Green	red	blue	Greenish yellow
Pyorubin is_____colored pigment	yellow	Green	red	blue	red
Pyomelanin is_____ colored pigment	brown	red	Green	yellow	brown
Pseudomonas aeruginosa produces_____pigment	Pyocyanin	melanin	rubin	verdin	Pyocyanin
The term 'blue pus' is associated with_____	*Proteus*	*Pseudomonas*	*E coli*	*Vibrio*	*Pseudomonas*
_____is used as selective media for Pseudomonas	Citrimide agar	EMB	DCA	MSA	Citrimide agar
enteric fever is caused by _____	Salmonella typhii	S paratyphi D	S enterica	Proteus	Salmonella typhii
EHEC strains are able to secrete _____	verotoxin	exotoxin	endotoxin	exfoliate toxin	verotoxin
_____isolated influenza bacillus	Andrews and Laidlaw	Koch	Boyd	Pasteur	Andrews and Laidlaw
The accessory factors required by H influenza is called as_____	X and Y	X and V	X and Z	Y and Z	X and V
_____ enzyme aids the invasion of Pseudomonas into tissues	pectinase	elastase	protease	amylase	elastase
_____is the drug of choice for Pseudomonas	Chloramiphenicol	Bacitracin	ceftazidime, Cefatoxime	Streptomycin	Chloramiphenicol

selective media for Pseudomonas aerugonisa is _____	Mac conkey agar	blood agar	PLET	Dettol agar	Dettol agar
*T pallidum causes*_____ __	Syphilis	typhoid	Tuberculosis	pertusis	Syphilis
T pallidum are highly sensitive to _____	Acid	Antiseptics	drying	antibiotic	drying
Pseudomonas mainly causes_____	Primary infection	secondary infection	re- infection	nosocomial infection	nosocomial infection

Unit V

Antibacterial agents: Five modes of action with one example each: Inhibitor of nucleic acid synthesis; Inhibitor of cell wall synthesis; Inhibitor of cell membrane function; Inhibitor of protein synthesis; Antibiotic resistance - MDR, XDR, MRSA, NDM-1 – resistance mechanisms. Antifungal agents: Mechanism of action of Amphotericin B, Griseofulvin. Antiviral agents: Mechanism of action of Amantadine, Acyclovir, Azidothymidine.

Antimicrobial Susceptibility Testing

Introduction

Resistance to antimicrobial agents (AMR) has resulted in morbidity and mortality from treatment failures and increased health care costs. Although defining the precise public health risk and estimating the increase in costs is not a simple undertaking, there is little doubt that emergent antibiotic resistance is a serious global problem.

Appropriate antimicrobial drug use has unquestionable benefit, but physicians and the public frequently use these agents inappropriately. Inappropriate use results from physicians providing antimicrobial drugs to treat viral infections, using inadequate criteria for diagnosis of infections that potentially have a bacterial aetiology, unnecessarily prescribing expensive, broad-spectrum agents, and not following established recommendations for using chemo prophylaxis. The availability of antibiotics over the counter, despite regulations to the contrary, also fuel inappropriate usage of antimicrobial drugs in India. The easy availability of antimicrobial drugs leads to their incorporation into herbal or "folk" remedies, which also increases inappropriate use of these agents.

Widespread antibiotic usage exerts a selective pressure that acts as a driving force in the development of antibiotic resistance. The association between increased rates of antimicrobial use and resistance has been documented for nosocomial infections as well as for resistant community acquired infections. As resistance develops to "first-line" antibiotics, therapy with new, broader spectrum, more expensive antibiotics increases, but is followed by development of resistance to the new class of drugs.

Resistance factors, particularly those carried on mobile elements, can spread rapidly within human and animal populations. Multidrug-resistant pathogens travel not only locally but also globally, with newly introduced pathogens spreading rapidly in susceptible hosts. Antibiotic resistance patterns may vary locally and regionally, so surveillance data needs to be collected from selected sentinel sources. Patterns can change rapidly and they need to be monitored closely because of their implications for public health and as an indicator of appropriate or inappropriate antibiotic usage by physicians in that area.

The results of in-vitro antibiotic susceptibility testing, guide clinicians in the appropriate selection of initial empiric regimens and, drugs used for individual patients in specific situations. The selection of an antibiotic

panel for susceptibility testing is based on the commonly observed susceptibility patterns, and is revised periodically.

Principle

The principles of determining the effectivity of a noxious agent to a bacterium were well enumerated by Rideal ,Walker and others at the turn of the century, the discovery of antibiotics made these tests(or their modification)too cumbersome for the large numbers of tests necessary to be put up as a routine. The ditch plate method of agar diffusion used by Alexander Fleming was the forerunner of a variety of agar diffusion methods devised by workers in this field .The Oxford group used these methods initially to assay the antibiotic contained in blood by allowing the antibiotics to diffuse out of reservoirs in the medium in containers placed on the surface.

With the introduction of a variety of antimicrobials it became necessary to perform the antimicrobial susceptibility test as a routine. For this, the antimicrobial contained in a reservoir was allowed to diffuse out into the medium and interact in a plate freshly seeded with the test organisms. Even now a variety of antimicrobial containing reservoirs are used but the antimicrobial impregnated absorbent paper disc is by far the commonest type used. The disc diffusion method of AST is the most practical method and is still the method of choice for the average laboratory. Automation may force the method out of the diagnostic laboratory but in this country as well as in the smaller laboratories of even advanced countries, it will certainly be the most commonly carried out microbiological test for many years to come. It is, therefore, imperative that microbiologists understand the principles of the test well and keep updating the information as and when necessary. All techniques involve either diffusion of antimicrobial agent in agar or dilution of antibiotic in agar or broth. Even automated techniques are variations of the above methods.

3. Factors Influencing Antimicrobial Susceptibility Testing

pH

The pH of each batch of Müeller-Hinton agar should be checked when the medium is prepared. The exact method used will depend largely on the type of equipment available in the laboratory. The agar medium should have a pH between 7.2 and 7.4 at room temperature after gelling. If the pH is too low, certain drugs will appear to lose potency (e.g., aminoglycosides, quinolones, and macrolides), while other agents may appear to have excessive

activity (e.g., tetracyclines). If the pH is too high, the opposite effects can be expected. The pH can be checked by one of the following means:

* Macerate a sufficient amount of agar to submerge the tip of a pH electrode
* Allow a small amount of agar to solidify around the tip of a pH electrode in a beaker or cup.
* Use a properly calibrated surface electrode.

Moisture

If, just before use, excess surface moisture is present, the plates should be placed in an incubator (35°C) or a laminar flow hood at room temperature with lids ajar until excess surface moisture is lost by evaporation (usually 10 to 30 minutes). The surface should be moist, but no droplets of moisture should be apparent on the surface of the medium or on the petri dish covers when the plates are inoculated.

Effects of Thymidine or Thymine

Media containing excessive amounts of thymidine or thymine can reverse the inhibitory effect of sulfonamides and trimethoprim, thus yielding smaller and less distinct zones, or even no zone at all, which may result in false-resistance reports. Müeller-Hinton agar that is as low in thymidine content as possible should be used. To evaluate a new lot of Müeller-Hinton agar, *Enterococcus faecalis* ATCC 29212, or alternatively, *E. faecalis* ATCC 33186, should be tested with trimethoprim/sulfamethoxazole disks. Satisfactory media will provide essentially clear, distinct zones of inhibition 20 mm or greater in diameter. Unsatisfactory media will produce no zone of inhibition, growth within the zone, or a zone of less than 20 mm.

Effects of Variation in Divalent Cations

Variation in divalent cations, principally magnesium and calcium, will affect results of aminoglycoside and tetracycline tests with *P. aeruginosa* strains. Excessive cation content will reduce zone sizes, whereas low cation content may result in unacceptably large zones of inhibition. Excess zinc ions may reduce zone sizes of carbapenems. Performance tests with each lot of Müeller-Hinton agar must conform to the control limits.

Testing strains that fail to grow satisfactorily

Only aerobic or facultative bacteria that grow well on unsupplemented Müeller-Hinton agar should be tested on that medium. Certain fastidious bacteria such as *Haemophilus* spp.,

N. gonorrhoeae, *S. pneumoniae*, and viridans and ß-haemolytic streptococci do not grow sufficiently on

unsupplemented Müeller-Hinton agar. These organisms require supplements or different media to grow, and they should be tested on the media described in separate sections

4. Methods of Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing methods are divided into types based on the principle applied in each system. They include:

Diffusion	Dilution	Diffusion&Dilution
Stokes method	Minimum Inhibitory Concentration	E-Test method
Kirby-Bauer method	i) Broth dilution	
	ii)Agar	Dilution

Disk Diffusion

Reagents for the Disk Diffusion Test

1. Müeller-Hinton Agar Medium

Of the many media available, Müeller-Hinton agar is considered to be the best for routine susceptibility testing of nonfastidious bacteria for the following reasons:

* It shows acceptable batch-to-batch reproducibility for susceptibilitytesting.
* It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.
* It gives satisfactory growth of most nonfastidiouspathogens.
* A large body of data and experience has been collected concerning susceptibility tests performed with this medium.

Although Müeller-Hinton agar is reliable generally for susceptibility testing, results obtained with some batches may, on occasion, vary significantly. If a batch of medium does not support adequate growth of a test organism, zones obtained in a disk diffusion test will usually be larger than expected and may exceed the acceptable quality control limits. Only Müeller-Hinton medium formulations that have been tested according to, and that meet the acceptance limits described in, NCCLS document M62-A7- Protocols for Evaluating Dehydrated Müeller-Hinton Agar should be used.

Preparation of Müeller-Hinton Agar

Müeller-Hinton agar preparation includes the following steps.

1. Müeller-Hinton agar should be prepared from a commercially available dehydrated base according to the manufacturer's instructions.
2. Immediately after autoclaving, allow it to cool in a 45 to 50°C water bath.

3. Pour the freshly prepared and cooled medium into glass or plastic, flat-bottomed petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm. This corresponds to 60 to 70 ml of medium for plates with diameters of 150 mm and 25 to 30 ml for plates with a diameter of 100 mm.
4. The agar medium should be allowed to cool to room temperature and, unless the plate is used the same day, stored in a refrigerator (2 to 8°C).
5. Plates should be used within seven days after preparation unless adequate precautions, such as wrapping in plastic, have been taken to minimize drying of the agar.
6. A representative sample of each batch of plates should be examined for sterility by incubating at 30 to 35°C for 24 hours or longer.

2. Preparation of antibiotic stock solutions

Antibitiotics may be received as powders or tablets. It is recommended to obtain pure antibiotics from commercial sources, and not use injectable solutions. Powders must be accurately weighed and dissolved in the appropriate diluents (Annexure III) to yield the required concentration, using sterile glassware. Standard strains of stock cultures should be used to evaluate the antibiotic stock solution. If satisfactory, the stock can be aliquoted in 5 ml volumes and frozen at -20ºC or -60ºC.

Stock solutions are prepared using the formula (1000/P) X V X C=W, where P+potency of the anitbiotic base, V=volume in ml required, C=final concentration of solution and W=weight of the antimicrobial to be dissolved in V.

Preparation of dried filter paper discs

Whatman filter paper no. 1 is used to prepare discs approximately 6 mm in diameter, which are placed in a Petri dish and sterilized in a hot air oven.

The loop used for delivering the antibiotics is made of 20 gauge wire and has a diameter of 2 mm. This delivers ml of antibiotics to eachdisc.

Storage of commercial antimicrobial discs

Cartridges containing commercially prepared paper disks specifically for susceptibility testing are generally packaged to ensure appropriate anhydrous conditions. Discs should be stored as follows:

* Refrigerate the containers at 8°C or below, or freeze at -14°C or below, in a nonfrost-free freezer until needed. Sealed packages of disks that contain drugs from the ß-lactam class should be stored frozen, except for a small working supply, which may be refrigerated for at most one week. Some labile agents (e.g., imipenem, cefaclor, and clavulanic acid combinations) may retain greater stability if stored frozen until the

day of use.

* The unopened disc containers should be removed from the refrigerator or freezer one to two hours before use, so they may equilibrate to room temperature before opening. This procedure minimizes the amount of condensation that occurs when warm air contacts colddisks.
* Once a cartridge of discs has been removed from its sealed package, it should be placed in a tightly sealed, desiccated container. When using a disc-dispensing apparatus, it should be fitted with a tight cover and supplied with an adequate desiccant. The dispenser should be allowed to warm to room temperature before opening. Excessive moisture should be avoided by replacing the desiccant when the indicator changes color.
* When not in use, the dispensing apparatus containing the discs should always be refrigerated.
* Only those discs that have not reached the manufacturer's expiration date stated on the label may be used. Discs should be discarded on the expiration date.

Turbidity standard for inoculum preparation

To standardize the inoculum density for a susceptibility test, a $BaSO_4$ turbidity standard, equivalent to a 0.5 McFarland standard or its optical equivalent (e.g., latex particle suspension), should be used. A $BaSO_4$ 0.5 McFarland standard may be prepared as follows:

1. A 0.5-ml aliquot of 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 . 2H_2O$) is added to 99.5 ml of 0.18 mol/L H_2SO_4 (1% v/v) with constant stirring to maintain a suspension.
2. The correct density of the turbidity standard should be verified by using a spectrophotometer with a 1-cm light path and matched cuvette to determine the absorbance. The absorbance at 625 nm should be 0.008 to 0.10 for the 0.5 McFarland standard.
3. The Barium Sulfate suspension should be transferred in 4 to 6 ml aliquots into screw-cap tubes of the same size as those used in growing or diluting the bacterialinoculum.
4. These tubes should be tightly sealed and stored in the dark at room temperature.
5. The barium sulfate turbidity standard should be vigorously agitated on a mechanical vortex mixer before each use and inspected for a uniformly turbid appearance. If large particles appear, the standard should be replaced. Latex particle suspensions should be mixed by inverting gently, not on a vortex mixer
6. The barium sulfate standards should be replaced or their densities verified monthly.

Disc diffusion methods

The Kirby-Bauer and Stokes' methods are usually used for antimicrobial susceptibility testing, with the Kirby-

Bauer method being recommended by the NCCLS. The accuracy and reproducibility of this test are dependent on maintaining a standard set of procedures as describedhere.

NCCLS is an international, interdisciplinary, non-profit, non-governmental organization composed of medical professionals, government, industry, healthcare providers, educators etc. It promotes accurate antimicrobial susceptibility testing (AST) and appropriate reporting by developing standard reference methods, interpretative criteria for the results of standard AST methods, establishing quality control parameters for standard test methods, provides testing and reporting strategies that are clinically relevant and cost-effective

Interpretative criteria of NCCLS are developed based on international collaborative studies and well correlated with MIC's and the results have corroborated with clinical data. Based on study results NCCLS interpretative criteria are revised frequently. NCCLS is approved by FDA-USA and recommended by WHO.

Procedure for Performing the Disc Diffusion Test

Inoculum Preparation

Growth Method

The growth method is performed as follows

1. At least three to five well-isolated colonies of the same morphological type are selected from an agar plate culture. The top of each colony is touched with a loop, and the growth is transferred into a tube containing 4 to 5 ml of a suitable broth medium, such as tryptic soy broth.
2. The broth culture is incubated at 35°C until it achieves or exceeds the turbidity of the 0.5 McFarland standard (usually 2 to 6 hours)
3. The turbidity of the actively growing broth culture is adjusted with sterile saline or broth to obtain a turbidity optically comparable to that of the 0.5 McFarland standard. This results in a suspension containing approximately 1 to 2 x 10^8 CFU/ml for *E.coli* ATCC 25922. To perform this step properly, either a photometric device can be used or, if done visually, adequate light is needed to visually compare the inoculum tube and the 0.5 McFarland standard against a card with a white background and contrasting black lines.

Direct Colony Suspension Method

1. As a convenient alternative to the growth method, the inoculum can be prepared by making a direct broth or saline suspension of isolated colonies selected from a 18- to 24-hour agar plate (a nonselective medium, such as blood agar, should be used). The suspension is adjusted to match the 0.5 McFarland turbidity

standard, using saline and a vortex mixer.

2. This approach is the recommended method for testing the fastidious organisms, *Haemophilus* spp., *N. gonorrhoeae*, and streptococci, and for testing staphylococci for potential methicillin or oxacillin resistance.

Inoculation of Test Plates

1. Optimally, within 15 minutes after adjusting the turbidity of the inoculum suspension, a sterile cotton swab is dipped into the adjusted suspension. The swab should be rotated several times and pressed firmly on the inside wall of the tube above the fluid level. This will remove excess inoculum from the swab.

The dried surface of a Müeller-Hinton agar plate is inoculated by streaking the swab over the entire sterile agar surface. This procedure is repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar is swabbed.

3. The lid may be left ajar for 3 to 5 minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug impregnated disks.

NOTE: Extremes in inoculum density must be avoided. Never use undiluted overnight broth cultures or other unstandardized inocula for streaking plates.

Application of Discs to Inoculated Agar Plates

1. The predetermined battery of antimicrobial discs is dispensed onto the surface of the inoculated agar plate. Each disc must be pressed down to ensure complete contact with the agar surface. Whether the discs are placed individually or with a dispensing apparatus, they must be distributed evenly so that they are no closer than 24 mm from center to center. Ordinarily, no more than 12 discs should be placed on one 150 mm plate or more than 5 discs on a 100 mm plate. Because some of the drug diffuses almost instantaneously, a disc should not be relocated once it has come into contact with the agar surface. Instead, place a new disc in another location on the agar.

2. The plates are inverted and placed in an incubator set to 35°C within 15 minutes after the discs are applied. With the exception of *Haemophilus* spp., streptococci and *N. gonorrhoeae*, the plates should not be incubated in an increased CO_2 atmosphere, because the interpretive standards were developed by using ambient air incubation, and CO_2 will significantly alter the size of the inhibitory zones of some agents.

Reading Plates and Interpreting Results

1. After 16 to 18 hours of incubation, each plate is examined. If the plate was satisfactorily streaked, and the inoculum was correct, the resulting zones of inhibition will be uniformly circular and there will be a confluent lawn of growth. If individual colonies are apparent, the inoculum was too light and the test must

be repeated. The diameters of the zones of complete inhibition (as judged by the unaided eye) are measured, including the diameter of the disc. Zones are measured to the nearest whole millimeter, using sliding calipers or a ruler, which is held on the back of the inverted petri plate. The petri plate is held a few inches above a black, nonreflecting background and illuminated with reflected light. If blood was added to the agar base (as with streptococci), the zones are measured from the upper surface of the agar illuminated with reflected light, with the cover removed. If the test organism is a *Staphylococcus* or *Enterococcus* spp., 24 hours of incubation are required for vancomycin and oxacillin, but other agents can be read at 16 to 18 hours. Transmitted light (plate held up to light) is used to examine the oxacillin and vancomycin zones for light growth of methicillin- or vancomycin- resistant colonies, respectively, within apparent zones of inhibition. Any discernable growth within zone of inhibition is indicative of methicillin or vancomycin resistance.

2. The zone margin should be taken as the area showing no obvious, visible growth that can be detected with the unaided eye. Faint growth of tiny colonies, which can be detected only with a magnifying lens at the edge of the zone of inhibited growth, is ignored. However, discrete colonies growing within a clear zone of inhibition should be subcultured, re-identified, and retested. Strains of *Proteus* spp. may swarm into areas of inhibited growth around certain antimicrobial agents. With *Proteus* spp., the thin veil of swarming growth in an otherwise obvious zone of inhibition should be ignored. When using blood-supplemented medium for testing streptococci, the zone of growth inhibition should be measured, not the zone of inhibition of hemolysis. With trimethoprim and the sulfonamides, antagonists in the medium may allow some slight growth; therefore, disregard slight growth (20% or less of the lawn of growth), and measure the more obvious margin to determine the zonediameter.

3. The sizes of the zones of inhibition are interpreted by referring to Tables 2A through 2I (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement, and the organisms are reported as either susceptible, intermediate, or resistant to the agents that have been tested. Some agents may only be reported as susceptible, since only susceptible breakpoints are given.

Dilution Methods

Dilution susceptibility testing methods are used to determine the minimal concentration of antimicrobial to inhibit or kill the microorganism. This can be achieved by dilution of antimicrobial in either agar or broth

media. Antimicrobials are tested in log_2 serial dilutions (two fold).

Minimum Inhibitory Concentration (MIC)

Diffusion tests widely used to determine the susceptibility of organisms isolated from clinical specimens have their limitations; when equivocal results are obtained or in prolonged serious infection e.g. bacterial endocarditis, the quantitation of antibiotic action vis-a-vis the pathogen needs to be more precise. Also the terms 'Susceptible' and 'Resistant' can have a realistic interpretation. Thus when in doubt, the way to a precise assessment is to determine the MIC of the antibiotic to the organisms concerned.

There are two methods of testing for MIC:

(a) Broth dilution method

(b) Agar dilution method.

Broth Dilution Method

The Broth Dilution method is a simple procedure for testing a small number of isolates, even single isolate. It has the added advantage that the same tubes can be taken for MBC tests also:

Materials

Sterile graduated pipettes of 10ml, 5ml, 2ml and 1ml Sterile capped 7.5 x 1.3 cm tubes / small screw-capped bottles, Pasteur pipettes, overnight broth culture of test and control organisms (same as for disc diffusion tests), required antibiotic in powder form (either from the manufacturer or standard laboratory accompanied by a statement of its activity in mg/unit or per ml. Clinical preparations should not be used for reference technique.), required solvent for the antibiotic, sterile Distilled Water - 500ml and suitable nutrient broth medium.

Trimethoprim and sulphonamide testing requires thymidine free media or addition of 4% lysed horse blood to the media

A suitable rack to hold 22 tubes in two rows i-e 11 tubes in each row.

Stock solution

Stock solution can be prepared using the formula

$$\frac{1000}{P} \times V \times C = W$$

Where P=Potency given by the manufacturer in relation to the base

V= Volume in ml required

C=Final concentration of solution (multiples of 1000)

W= Weight of the antimicrobial to be dissolved in the volume V

Example: For making 10 ml solution of the strength 10,000mg/l from powder base whose potency is 980 mg per gram,the quantities of the antimicrobials required is

$$W = \frac{1000}{980} \times 10 \times 10 = 102.04 \text{mg}$$

Note:the stock solutions are made in higher concentrations to maintain their keeping qualities and stored in suitable aliquots at -20ºC .Once taken out,they should not be refrozen or reused.

Suggested dilution ranges of some antimicrobials are shown in Annexure II.

Method

Prepare stock dilutions of the antibiotic of concentrations 1000 and 100 µg/L as required from original stock solution (10,000mg/L). Arrange two rows of 12 sterile 7.5 x1.3 cm capped tubes in the rack. In a sterile 30ml (universal) screw capped bottle, prepare 8ml of broth containing the concentration of antibiotic required for the first tube in each row from the appropriate stock solution already made. Mix the contents of the universal bottle using a pipette and transfer 2ml to the first tube in each row. Using a fresh pipette ,add 4 ml of broth to the remaining 4 ml in the universal bottle mix and transfer 2ml to the second tube in each row. Continue preparing dilutions in this way but where as many as 10 or more are required the series should be started again half the way down. Place 2ml of antibiotic free broth to the last tube in each row. Inoculate one row with one drop of an overnight broth culture of the test organism diluted approximately to 1 in 1000 in a suitable broth and the second row with the control organism of known sensitivity similarly diluted. The result of the test is significantly affected by the size of the inoculum.The test mixture should contain 10^6 organism/ml.If the broth culture used has grown poorly,it may be necessary to use this undiluted. Incubate tubes for 18 hours at 37ºC. Inoculate a tube containing 2ml broth with the organism and keep at +4ºC in a refrigerator overnight to be used as standard for the determination of complete inhibition.

Calculations for the preparation of the original dilution.

This often presents problems to those unaccustomed to performing these tests. The following method advocated by Pamela M Waterworth is presented. Calculate the total volume required for the first dilution. Two sets of dilution are being prepared (one for the test and one for the control), each in 2ml volumes i-e a total of 4 ml for each concentration as 4ml is required to make the second dilution, the total requirement is 8ml. Now calculate the total amount of the antibiotic required for 8ml. For 64 g/l concentration, 8x64mg/l =512µg in 8 ml. Place a

decimal point after the first figure (5.12) and take this volume in ml (i.e 5.12 ml) of the dilution below 512mg/l and make upto 8ml with broth. In this example given above, the series has to be started again mid way at 2 mg/l which would be obtained in the same way:

8ml of 2mg/l=16µg in 8ml.

1.6 ml of 10 mg/ l + 6.4 ml of broth

Reading of result

MIC is expressed as the lowest dilution, which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself, the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition.

Standard strain of known MIC value run with the test is used as the control to check the reagents and conditions.

Minimum Bactericidal Concentrations(MBC)

The main advantage of the 'Broth dilution' method for the MIC determination lies in the fact that it can readily be converted to determine the MBC as well.

Method

Dilutions and inoculations are prepared in the same manner as described for the determination of MIC. The control tube containing no antibiotic is immediately subcultured (Before incubation) by spreading a loopful evenly over a quarter of the plate on a medium suitable for the growth of the test organism and incubated at 37ºC overnight. The tubes are also incubated overnight at 37ºC. Read the MIC of the control organism to check that the drug concentrations are correct. Note the lowest concentration inhibiting growth of the organisms and record this as the MIC. Subculture all tubes not showing visible growth in the same manner as the control tube described above and incubate at 37ºC overnight. Compare the amount of growth from the control tube before incubation,which represents the original inoculum. The test must include a second set of the same dilutions inoculated with an organism of known sensitivity .These tubes are not subcultured; the purpose of the control is to confirm by its MIC that the drug level is correct,whether or not this organism is killed is immaterial.

Reading of result

These subcultures may show

- Similar number of colonies- indicating bacteriostasis only
- A reduced number of colonies-indicating a partial orslow bactericidal activity.
- No growth- if the whole inoculum has beenkilled

- The highest dilution showing at least 99% inhibition is taken as MBC

Micro-broth dilution test

This test uses double-strength Müeller-Hinton broth, 4X strength antibiotic solutions prepared as serial two-fold dilutions and the test organism at a concentration of $2x10^6$/ml. In a 96 well plate, 100 µl of double-strength MHB, 50 µl each of the antibiotic dilutions and the organism suspension are mixed and incubated at 35°C for 18-24 hours. The lowest concentration showing inhibition of growth will be considered the MIC of the organism.

Reading of result

MIC is expressed as the highest dilution which inhibited growth judged by lack of turbidity in the tube. Because very faint turbidity may be given by the inoculum itself,the inoculated tube kept in the refrigerator overnight may be used as the standard for the determination of complete inhibition. Standard strain of known MIC, run with the test is used as the control to check the reagents and conditions.

The Agar dilution Method

Agar dilutions are most often prepared in petri dishes and have advantage that it is possible to test several organisms on each plate .If only one organism is to be tested e.g *M.tuberculosis*,the dilutions can be prepared in agar slopes but it will then be necessary to prepare a second identical set to be inoculated with the control organism.The dilutions are made in a small volume of water and added to agar which has been melted and cooled to not more than 60°C.Blood may be added and if 'chocolate agar' is required,the medium must be heated before the antibiotic is added.

It would be convenient to use 90 mm diameter petri dishes and add one ml of desired drug dilutions to 19 ml of broth.The factor of agar dilution must be allowed for in the first calculation as follows.

final volume of medium in plate = 20 ml

Top antibiotic concentrations = 64mg/l

Total amount of drug = 1280µg to be added to 1 ml of water

2ml of 1280 µg /ml will be required to startthe dilution = 2560µg in 2 ml

= 1.28ml of 2000µg /ml

± 0.72 ml of water.

1 ml of this will be added to 19 ml agar.

(Note stock dilution of 2000µg /ml is required for this range of MIC)

The quickest way to prepare a range of dilutions in agar is as follows:

Label a sterile petri dish on the base for each concentration required. Prepare the dilutions in water placing 1 ml of each in the appropriate dish. One ml water is added to a control plate. Pipette 19 ml melted agar, cooled to 55^{o}C to each plate and mix thoroughly. Adequate mixing is essential and if sufficient technical expertise is not available for the skilled manipulation, it is strongly recommended that the agar is first measured into stoppered tubes or universal containers and the drug dilution added to these and mixed by inversion before pouring into petri dishes. After the plates have set they should be well dried at 37^{o}C with their lids tipped for 20 to 30 minutes in an incubator. They are then inoculated either with a multiple inoculator as spots or with a wire loop or a platinum loop calibrated to deliver 0.001ml spread over a small area. In either case the culture should be diluted to contain 10^5 to 10^6 organisms per ml. With ordinary fast growing organisms, this can be obtained approximately by adding 5 µl of an overnight broth culture to 5ml broth or peptone water.

It is possible to test spreading organism such as *P.mirabilis* by this method either by cutting ditches in the agar between the inocula, or by confining each with small glass or porcelain cylinders pressed into the agar. Although swarming of *P.mirabilis* can be prevented by the use of higher concentration of agar in the medium, this is not recommended for determination of MIC because of the difficulty of ensuring adequate mixing of the drug with this very viscous medium. Selective media should not be used and electrolyte deficient media will give false results because of the effect of variation in the salt content on the action of many antibiotics.

Reading of results

The antibiotic concentration of the first plate showing ≥ 99% inhibition is taken as the MIC for the organism.

Dilution and Diffusion

E test also known as the epsilometer test is an ‘exponential gradient’ testing methodology where ‘E’ in E test refers to the Greek symbol epsilon (ε).The E test(AB Biodisk) which is a quantitative method for antimicrobial susceptibility testing applies both the dilution of antibiotic and diffusion of antibiotic into the medium.. A predefined stable antimicrobial gradient is present on a thin inert carrier strip. When this E test strip is applied onto an inoculated agar plate, there is an immediate release of the drug. Following incubation , a symmetrical inhibition ellipse is produced. The intersection of the inhibitory zone edge and the calibrated carrier strip indicates the MIC value over a wide concentration range (>10 dilutions) with inherent precision and accuracy . E test can be used to determine MIC for fastidious organisms like *S. pneumoniae*, ß-hemolytic streptococci, *N.gonorrhoeae*, *Haemophilus* sp. and anaerobes. It can also be used for Nonfermenting Gram Negative bacilli (NFGNB) for eg-*Pseudomonas* sp. and *Burkholderia pseudomallei*.

Resistance of major consequence may be detected for e.g., the test is very useful in detecting glycopeptide resistant Enterococci (GRE) and glycopeptide intermediate *S.aureus* (GISA) and slow growing pathogens such as *Mycobacterium tuberculosis.* Further it can be used for detection of extended spectrum beta lactamases (ESBL). In conclusion E test is a simple, accurate and reliable method to determine the MIC for a wide spectrum of infectious agents.

5. Susceptibility of Fastidious Bacteria

DISC DIFFUSION FOR FASTIDIOUS ORGANISMS

Antibiotic susceptibility testing of *S.pneumoniae*

Media for disc diffusionMüeller -Hinton Sheep blood agar

Standardization of inoculum.

The inocula for seeding the susceptibility media with *S.pneumoniae* is prepared from fresh pure cultures (grown overnight on Chocolate agar). Cell suspensions of the bacteria to be tested are prepared in sterile saline or Müeller-Hinton broth. The cell suspension is prepared by transferring a portion of the fresh growth with a swab or inoculating loop to the suspending medium, using caution when mixing the cells with the suspending medium so as not to form bubbles. The suspension is then compared to the McFarland standard by holding the suspension and McFarland standard in front of a light against a white background with contrasting black lines and comparing the turbidity. If the turbidity is too heavy, the suspension should be diluted with additional suspending medium. If the turbidity is too light additional cells should be added to the suspension.

For *S.pneumoniae* – Direct colony suspension is made in normal saline and turbidity adjusted to 0.5 McFarland standard. Within 15 minutes after adjusting the turbidity of the suspension the plate should be inoculated.

Inoculation of the susceptibility test media

After proper turbidity is achieved, a new sterile swab (cotton or dacron) is submerged in the suspension, lifted out of the broth, and the excess fluid is removed by pressing and rotating the swab against the wall of the tube. The swab is then used to inoculate the entire surface of the supplemented Müeller Hinton agar plate three times, rotating the plate 60 degrees between each inoculation. The inoculum is allowed to dry (usually taking only a few minutes but no longer than 15 minutes) before the discs are placed on the plates. The discs should be placed on the agar with sterile forceps and tapped gently to ensure the adherence to the agar. The plates containing the disks are incubated at 35^{o}C for 16 to 18 h in an inverted position in a 5% CO_2 incubator. A candle extinction jar may be used if a CO_2 incubator is not available.

Estimating the susceptibility of the strains

After overnight incubation, the diameter of each zone of inhibition is measured with a ruler or calipers. The zones of inhibition on the media containing blood are measured from the top surface of the plate with the top removed. It is convenient to use a ruler with a handle attached for these measurements, holding the ruler over the surface of the disk when measuring the inhibition zone. Care should be taken not to touch the disk or surface of the agar. Sterilize the ruler occasionally to prevent transmission of bacteria. In all measurements, the zones of inhibition are measured from the edges of the last visible colony-forming growth. The ruler should be positioned across the center of the disc to make these measurements. The results are recorded in millimeters (mm) and interpretation of susceptibility is obtained by comparing the results to the standard zone sizes. For *S.pneumoniae* the zone measurement is from top of plate with the lid removed. Faint growth of tiny colonies that may appear to fade from the more obvious zone should be ignored in the measurement.

Interpretation

Each zone size is interpreted by reference to the Table 2G (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints for *S.pneumoniae*) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and resistant.

Antibiotic susceptibility of *Haemophilus* species

The medium of choice for disc diffusion testing of *Haemophilus* sp. is Haemophilus Test Medium (HTM). Müeller-Hinton chocolate agar is not recommended for routine testing of *Haemophilus* spp.

In its agar form, Haemophilus Test medium consists of the following ingredients.

* Müeller-Hinton agar,
* 15 µg/ml ß-NAD,
* 15 µg/ml bovine hematin, and
* 5-mg/ml yeast extract.

To make HTM, first a fresh hematin stock solution is prepared by dissolving 50 mg of bovine hematin powder in 100 ml of 0.01 mol/L NaOH with heat and stirring until the powder is thoroughly dissolved. Thirty ml of the hematin stock solution are added to 1 L of MHA with 5 g of yeast extract. After autoclaving and cooling to 45 to 50°C, 3 ml of an NAD stock solution (50 mg of NAD dissolved in 10 ml of distilled water and filter sterilized) are also aseptically added. The pH should be 7.2 to 7.4.

Test Procedure

The direct colony suspension procedure should be used when testing *Haemophilus* sp. Using colonies taken directly from an overnight (preferably 20 to 24 hour) chocolate agar culture plate, a suspension of the test organism is prepared in Müeller-Hinton broth or 0.9% saline. The suspension should be adjusted to a turbidity equivalent to a 0.5 McFarland standard using a photometric device. This suspension will contain approximately 1 to 4 x 10^8 CFU/ml. Care must be exercised in preparing this suspension, because higher inoculum concentrations may lead to false-resistant results with some ß-lactam antibiotics, particularly when ß-lactamase producing strains of *H. influenzae* are tested. Within 15 minutes after adjusting the turbidity of the inoculum suspension, it should be used for plate inoculation.

2. The procedure for the disc test should be followed as described for nonfastidious bacteria, except that, in general, no more than 9 discs should be applied to the surface of a 150-mm plate or no more than 4 discson a 100-mm plate.
3. Plates are incubated at 35°C in an atmosphere of 5% CO_2 for 16 to 18 hours before measuring the zones of inhibition.

The zone margin should be considered as the area showing no obvious growth visible with the unaided eye. Faint growth of tiny colonies that may appear to fade from the more obvious zone should be ignored in the measurement.

Zone Diameter Interpretive Criteria

The antimicrobial agents suggested for routine testing of *Haemophilus* sp. are indicated in Annexure I. Each zone size is interpreted by reference to the Table 2E (Zone Diameter Interpretative Standards and equivalent Minimum Inhibitory Concentration Breakpoints for *Haemophilus* sp.) of the NCCLS M100-S12: Performance Standards for Antimicrobial Susceptibility Testing: Twelfth Informational Supplement as susceptible, intermediate and diffusion testing of *Haemophilus* spp. with other agents is not recommended.

Unit III possible questions

Part B

1. Define Antibacterial agents
2. Say about Inhibitor of nucleic acid synthesis
3. What are Inhibitor of cell membrane function?
4. What is Antibiotic resistance?
5. Write about MDR?

Part C

1. Give short notes on MDR, XDR, MRSA, NDM-1 – resistance mechanisms.
2. State the Mechanism of action of Amphotericin B and Griseofulvin.
3. Discuss the properties of Acyclovir and Azidothymidine.

4. Explain about the antiviral agents: Mechanism of action of Amantadine.

KARPAGAM ACADEMY OF HIGHER EDUCATION

CLASS : II B.Sc MB **COURSE NAME: MEDICAL MICROBIOLOGY**

COURSE CODE: 18MBU402 UNIT 5 **BATCH-2018-2021**

Unit V Question	Opt 1	Opt 2	Opt 3	Opt 4	Answer
which of the following is non – pathogenic	*Entamoeba coli*	*Entamoeba histolytica*	*Balantidium coli*	*Trichomonas vaginalis*	*Entamoeba coli*
Amphotericin B is effective treatment for all the following except	Leishmaniasis	Amoebiasis	Naegleria infection	Acanthamoeba infection	Amoebiasis
Which of the following is not an antiprotozoal drug:	Albendazole	Tetracycline	Trimethoprin+sulfamethoxazole	Pyrimethamine	Albendazole
Which of the following is not a flagellate :	Naegleria	Giardia	Leishmania	Dientamoeba	Naegleria
Montenegro skin test used to be done for the diagnosis of:	Kala-azar	Hydatid disease	Toxocariasis	Cysticercosis	Kala-azar
Amoebiasis can mimic in clinical presentation with :	Appendicitis	Schistosomiasis	Toxocariasis	Cysticercosis	Appendicitis
Entamoeba histolytica gains access to the liver via:	Lymphatics	Portal system	Direct invasion	Through perineural space	Portal system
In case of hepatic amoebiasis ESR is:	Normal	Almost always elevated	Below normal	Variable	Almost always elevated
Meningoencephalitis due to Naegleria fowleri best respond to:	Amphotericin B	Chloroquine	Metronidazole	Tetracycline	Amphotericin B
Malabsorption seen in giardiasis is most likely due to :	Mechanical barrier to absorption	Poor enzymatic digestion	Insolublising the proteins and fats	Direct invasion	Mechanical barrier to absorption
Which of the following use pseudopodia for locomotion :	Ciliates	Amoebae	Flagellates	Microsporidia	Amoebae
A host that harbours the larval or asexual stage of parasite is known as :	Definite host	Intermediate host	Reservoir host	None of the abo	Intermediate host
Ribbon like helminth parasites are known	Tapeworms	Flukes	Round worms	Amoeba	Tapeworms

as:					
The test for an accurate faecal examination for ova and cysts is:	Direct wet mount for motility	Permanent stained side	PCR	Serological test	Permanent stained side
The best test to distinguish amoebic liver abscess from a bacterial liver abscess is:	Ultra sound examination	Good physical examination by an expert	CT scan	Serological evidence of E. histolytica	Serological evidence of E. histolytica
There is 100 percent infection in Giardia lamblia when the number of ingested cysts is:	100 or more	1,000 or more	1,000 or more	100,000 or more	100 or more
A 'pear shaped' trophozoite is normally seen in :	Entamoeba histolytica	Giardia lamblia	B.coli	Dientamoeba fragilis	Giardia lamblia
A 30-years –old male who is anaemic, cachexic and has hepatosplenomegaly accompanied with fever, shows on a bone marrow smear many organisms within macrophages. These organisms have a nucleus and a smaller but distinct kinetoplast. The likely diagnosis is:	Leishmania donovani	Histoplasma capsulatum	Toxoplasma gondi	Toxocara canis	Leishmania donovani
Blood smears for suspected malaria should first be taken:	Just after the fever	Before the chills	When the diagnosis is first suspected	At night	When the diagnosis is first suspected
After initiation of specific treatment in malaria, how soon the patient, s symptoms abate:	Within 6 hour	Within 24-48 hours	within a week	After one more febrile attack	Within 24-48 hours
Which of the following is not a component of Lugol's	Potassium iodide	Iodine crystals	Ethyl alcohal	Water	Ethyl alcohal

iodine:					
All the following are advantages of using formalin in preservation of stool except:	Good overall fixative	Easy to prepare	Long shelf- life	Preserves trophozoites	Preserves trophozoites
A series of 3 stool specimens for diagnosis of parasitic infections be examined within no more than:	2 days	4 days	7days	10 days	10days
The aldehyde test is based on the principle of:	Altered WBC: RBC ratio	Altered albumin: globulim ratio	Decreased cell mediated immunity	. Antigen antibody complement complex formation	Altered albumin: globulim ratio
"Eating at the same table" would be an appropriate description of :	Parasitism	Symbiosis	Commenalism	Mutualism	Commenalism
A chest X-ray in cases of pneumonia due to pneumocystis carinii can reveal all the following except:	Cavitation	Nodular densities	Ground glass appearance	Lobar infiltration	Cavitation
Treatment of choice for vaginitis due to trichomonas vaginalis is :	Metronidazole	Thiabendazole	Piperazine	Chloroquine	Metronidazole
The partner of a Patient who suffers from trichomonal vaginitis be treated:	Only if symptomatic	Only if examination is positive	Always be treated	No need of treatment	Always be treated
What percentage of males infected with Trichomonas vaginalis are symptomatic?	Nil	5-10%	10-15%	50-60%	10-15%
Chagas' disease is usually treated wih:	Nifurtimox	Niclosamide	Spraziquantel	Ivermectin	Nifurtimox
Stains used for parasite observation include	Gomori's trichrome stain	Iron hematoxylin stain	Both (a) & (b)	Giemsa Stain	Both (a) & (b)

……					
Using blood films which of the following infections can be identified?	Trichomonas	Entameoba	Plasmodium	Ascaris	Plasmodium
. ……. Film allows rapid confirmation of presence of parasite in the blood.	Thick	Thin	Hot	Cold	Thick
…….. film is used to identify the species of parasite.	Thick	Thin	Hot	Cold	Thin
Cerebrospinal fluid is collected by …… puncture	Spinal	Bone marrow	Liver	Spleen	Spinal
The adult worm of *Chlonorchisa sinensis* is most frequently found in:	Intrahepatic bile duct	Lung parenchyma	Urinary bladder submucosa	Lumen of Intestine	Intrahepatic bile duct
The drug of choice for treating cryptosporidiosis is:	Pyrimethamine	Metronidazole	Tetracycline	There is no effective treatment	There is no effective treatment
The drug of choice for balantidiasis is:	Metronidazole	Oxytetracycline	Chloroquine	Albendazole	Oxytetracycline
Which of the following drug used to treat infection with Loa loa and kills worms by causing flaccid paralysis:	Invermectin	Metronidazole	suramin	mefloquine	Invermectin
Nucleus of Entamoeba coli has	Central karyosome	Peripheral or eccentric karyosome	No karyosome	Many karyosomes	Peripheral or eccentric karyosome
Parasites causing human infections can be divided into ……. groups.	Two	Three	Four	Five	Two
Protozoan parasites consists of ……. Cell	Multiple	Single	Row	Double	Single
Protozoan parasites that infect humans	Apicomplexa	Microspora	Kinetoplastida	Retortamonadida	Apicomplexa

include ……					
A parasite that lines inside the body of a host is ……..	Endoparasite	Ectoparasite	Obligate parasite	Facultative parasit	Endoparasite
……. Technique is used to separate parasitic ova and cysts from most of the fecal matter	Floatation	Sedimentation	Evaporation	Concentration	Sedimentation
Which of the following causes amoebic meningo encephalitis ?	Entamoeba histolytica	Entamoeba Coli	Entamoeba gingivalis	Naeglaria fowleri	Naeglaria fowleri
Which of the following causative agent is not transmitted by mosquito:	Plasmodium vivax	Brugia malayi	Wuchereria bancrofti	Loa loa	Loa loa
Ticks are the vectors in which of the following:	Malaria	Babesiosis	Loiasis	Chagas' disease	Babesiosis
All the following parasites can be present in spleen except:	Leishmania donovani	Plasmodium falciparum	Plasmodium vivax	Balantidium coli	Balantidium coli
Which of the following requires two intermediate host for completion of its life cycle:	Taeniasolium	Diphyllobothrium latum	Hymenolepis nana	Taenia saginata	Diphyllobothrium latum
The invasion of tissue by parasites elicits production of antibodies that include	IgM	Protein	Aminoacid	Peptidoglycan	IgM
Carbolic acid is the name commonly referred for:	Phenol	Methyl alcohol	Ethyl alcohol	Potassium dichromate	Phenol
Parasite F test is used for the diagnosis of:	Filariasis	Falciparum malaria	Amoebiasis	Cysticercosis	bFalciparum malaria
Entero-test capsule is used for sampling	Duodenal conte	CSF	Urogenital specimens	Rectal smear	Duodenal conte
Modified acid fast staining is used for	Leishmania	Isospora belli	Pneumoystis carini	Entamoeba fragilis	Pneumoystis carini

demonstration of:	donovani				
The lenth of adult worm of Diphyllobothrium latum could be up to:	2 metres	5 metres	10 metres	20 metres	10 metres
Chronic infecton with which of the followingmay be associated with megaesphagus or megacolon:	Trypanosoma cruzi	Trypanosoma b gambiense	*Leishmania tropica*	Leishmania donovani	Trypanosma cruzi
Adult worms of which of the following are found in subcutaneous nodules:	Loa loa	Dracunculus medinensis	Onchocerca volvulus	Brugia malayi	Onchocerca volvulus
Amastigotes of leishmania are found in:	Liver parenchymal cell	In- vitro culture	Salivary gland of sandfly	Human mononuclear phagoecytes	Human mononuclear phagoecytes
Cysts of all the following intestinal protozoa have 4 or more nuclei except:	Entamoeba histolytica	Entamoeba coli	Iodamoeba butschlii	Entamoeba hartm	Iodamoeba butschlii