Semester – IV

16MBU402 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 and host pathogen interaction

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 – Bacterial diseases

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 Diseases: *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, Clostridium difficle.*

Unit III – Viral diseases

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 – Fungal and Protozoan diseases

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 – Antimicrobial agents: General characteristics and mode of action

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. 4th edition. Elsevier.
7. Willey JM, Sherwood LM, and Woolverton CJ. (2013). Prescott, Harley and Klein's Microbiology. 9th edition. McGraw Hill Higher Education.

2017

II B. Sc Microbiology – Medical Microbiology

LECTURE PLAN - UNIT -I			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	History	
2	1	Normal micro flora of human body	
3	1	Host pathogen interactions	
4	1	Carriers and its types	
5	1	Opportunistic infections	
6	1	Nosocomial infections	
7	1	Transmission	
8	1	Pathophysiologic effects of LPS	
9	1	Collection and transport of samples	
10	1	Culturing and storage of samples	
11	1	Revision of Unit I	
12	1	Unit I test	
		Total hours	12
Textbooks :			
Reference books:			
Website:			
Journals:			

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

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II B. Sc Microbiology – Medical Microbiology

LECTURE PLAN - UNIT -II			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Various organ systems	
2	1	Details about symptoms, prophylaxis and control	
3	1	*Streptococcus pyogenes*	
4	1	*Haemophilus influenza*	
5	1	*Mycobacterium tuberculosis*	
6	1	*Escherichia coli*	
7	1	*Salmonella typhi*	
8	1	*Vibrio cholerae*	
9	1	*Helicobacter pylori*	
10	1	*Staphylococcus aureus*	
11	1	*Bacillus anthracis*	
12	1	*Clostridium*	
13	1	*Treponema pallidium*	
14	1	Revision of Unit II	
15	1	Unit II test	
		Total hours	15
Textbooks :			
Reference books:			
Website:			
Journals:			

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LECTURE PLAN - UNIT -III			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Polio	
2	1	Herpes	
3	1	Hepatitits	
4	1	Rabies	
5	1	Dengue and Chikungunya	
6	1	Swine flu and Ebola	
7	1	AIDS and Japanese encephalitis	
8	1	Revision of Unit III	
9	1	Unit III test	
		Total hours	09
Textbooks :			
Reference books:			
Website:			
Journals:			

II B. Sc Microbiology – Medical Microbiology

LECTURE PLAN - UNIT -IV			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**

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1	1	Mycoses	
2	1	Transmission, Symptoms and prevention	
3	1	Cutaneous mycoses	
4	1	Systemic mycoses	
5	1	Opportunistic mycoses	
6	1	Parasitic infections	
7	1	Amoebiasis	
8	1	Giardiasis	
9	1	Elephanttiasis	
10	1	Taeniasis	
11	1	Malaria and Kala-azar	
12	1	Revision of Unit IV	
13	1	Unit IV test	
		Total hours	13
Textbooks :			
Reference books:			
Website:			
Journals:			

II B. Sc Microbiology – Medical Microbiology

LECTURE PLAN - UNIT -V			
S. no	Lecture duration(Hr)	**Topics covered**	**Supporting materials**
1	1	Antimicrobial chemotheraphy	

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2	1	Antibacterial agents	
3	1	Inhibition of nucleic acid and cell wall synthesis	
4	1	Inhibition of protein and cell membrane synthesis	
5	1	Antibiotic resistance	
6	1	Resistance mechanisms	
7	1	Antifungal agents	
8	1	Antiviral agents	
9	1	Mechanism of the Antiparasitic agents	
10	1	Revision of Unit V	
11	1	Unit V test	
		Total hours	11
Textbooks :			
Reference books:			
Website:			
Journals:			

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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 environmental 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.

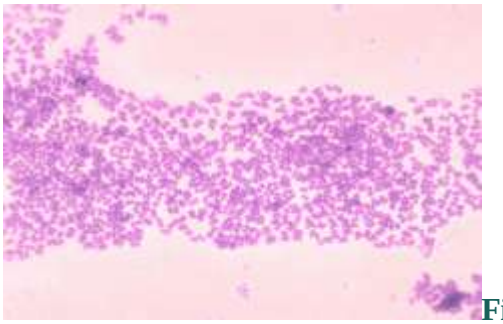


Figure 1. Gram stain of a species of *Micrococcus,* commonly isolated from the skin and nasal membranes of humans.

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.

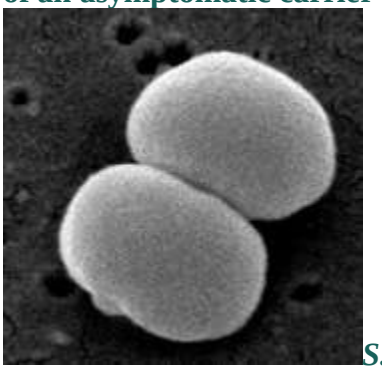
BACTERIUM	Skin	Con-junc-tiva	Nose	Pharynx	Mouth	Lower GI	Ant. ure-thra	Vagina
Staphylococcus epidermidis **(1)**	++	+	++	++	++	+	++	++
Staphylococcus aureus* **(2)**	+	+/-	+	+	+	++	+/-	+
Streptococcus mitis				+	++	+/-	+	+
Streptococcus salivarius				++	++			
Streptococcus mutans* **(3)**				+	++			
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

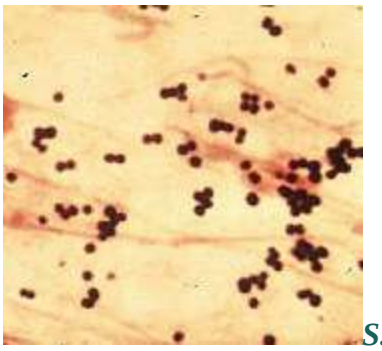
Table 1 Notes

(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.**

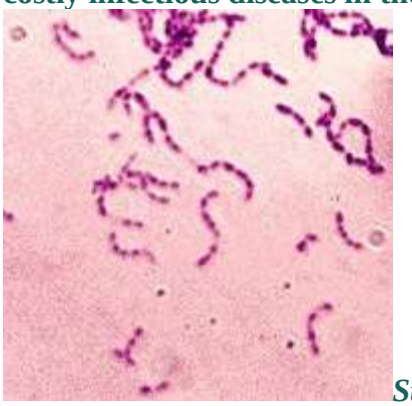
(2) Many of the normal flora are either pathogens or opportunistic pathogens, The asterisks indicate members of the normal flora a that may be considered major pathogens of humans.



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.

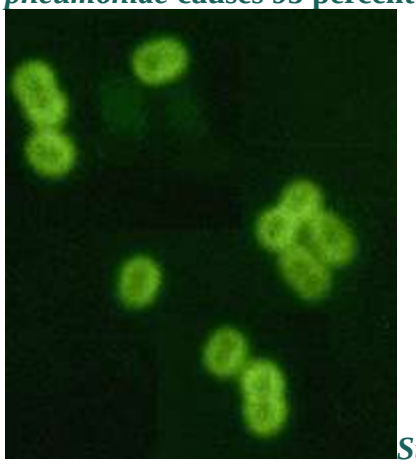


Streptococcus mutans. **Gram stain. CDC**

(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.**



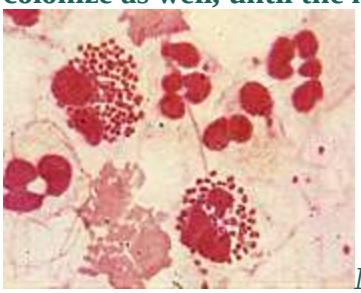
Streptococcus pneumoniae. **Direct fluorescent antibody stain. CDC.**

(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.**

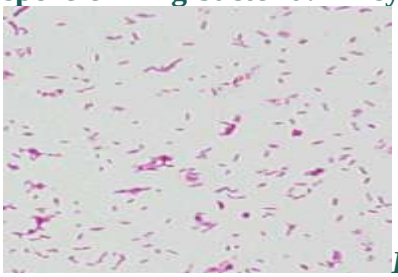
(9) *Pseudomonas aeruginosa* is the quintessential opportunistic pathogen of humans that can invade virtually any tissue. It is a leading cause of hospital-acquired (nosocomial) Gram-negative infections, but its source is often exogenous (from outside the host).

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.**

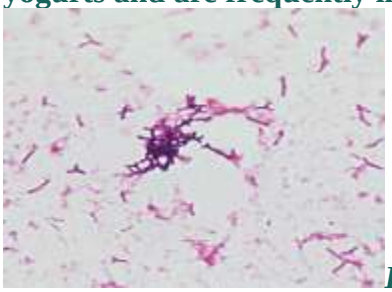
(11) The greatest number of bacteria are found in the lower intestinal tract, specifically the colon and the most prevalent bacteria are the *Bacteroides,* a group of Gram-negative, anaerobic, non-sporeforming bacteria. They have been implicated in the initiation colitis and colon cancer.



Bacteroides fragilis. **Gram stain.**

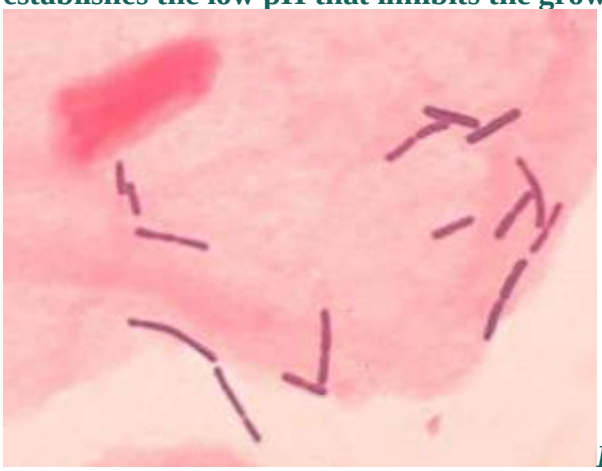
(12) Bifidobacteria are Gram-positive, non-sporeforming, lactic acid bacteria. They have been described as "friendly" bacteria in the intestine of humans.*Bifidobacterium bifidum* is the

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.



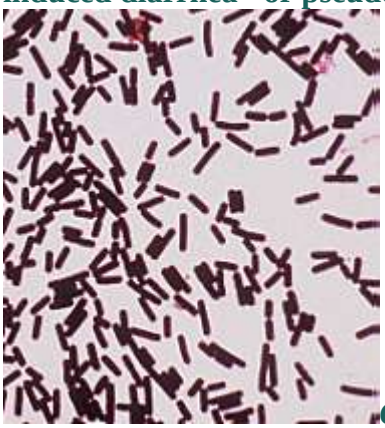
Bifidobacterium bifidum. **Gram stain**

(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 years and 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.**

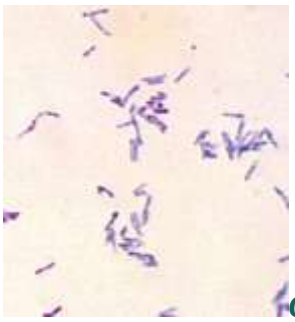


Clostridium perfringens**. Gram stain.**

(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 explanation for 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 that tissue 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.

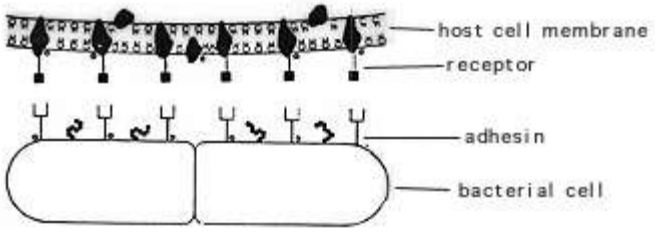


Figure 2. Specific adherence involves complementary chemical interactions between the host cell or tissue surface and the bacterial surface. In the language of medical microbiologist, a bacterial "adhesin" attaches covalently to a host "receptor" so that the bacterium "docks" itself on the host surface. The adhesins of bacterial cells are chemical components of capsules, cell walls, pili or fimbriae. The host receptors are usually glycoproteins located on the cell membrane or tissue surface.

Some examples of adhesins and attachment sites used for specific adherence to human tissues are described in the table below

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Table 2. Examples of bacterial specific adherence to host cells or tissue.

Bacterium	Bacterial adhesin	Attachment site
Streptococcus pyogenes	Cell-bound protein (M-protein)	Pharyngeal epithelium
Streptococcus mutans	Cell- bound protein (Glycosyl transferase)	Pellicle of tooth
Streptococcus salivarius	Lipoteichoic acid	Buccal epithelium of tongue
Streptococcus pneumoniae	Cell-bound protein (choline-binding protein)	Mucosal epithelium
Staphylococcus aureus	Cell-bound protein	Mucosal epithelium
Neisseria gonorrhoeae	N-methylphenyl- alanine pili	Urethral/cervical epithelium
Enterotoxigenic *E. coli*	Type-1 fimbriae	Intestinal epithelium

Uropathogenic *E. coli*	**P-pili (pap)**	**Upper urinary tract**
Bordetella pertussis	**Fimbriae ("filamentous hemagglutinin")**	**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.

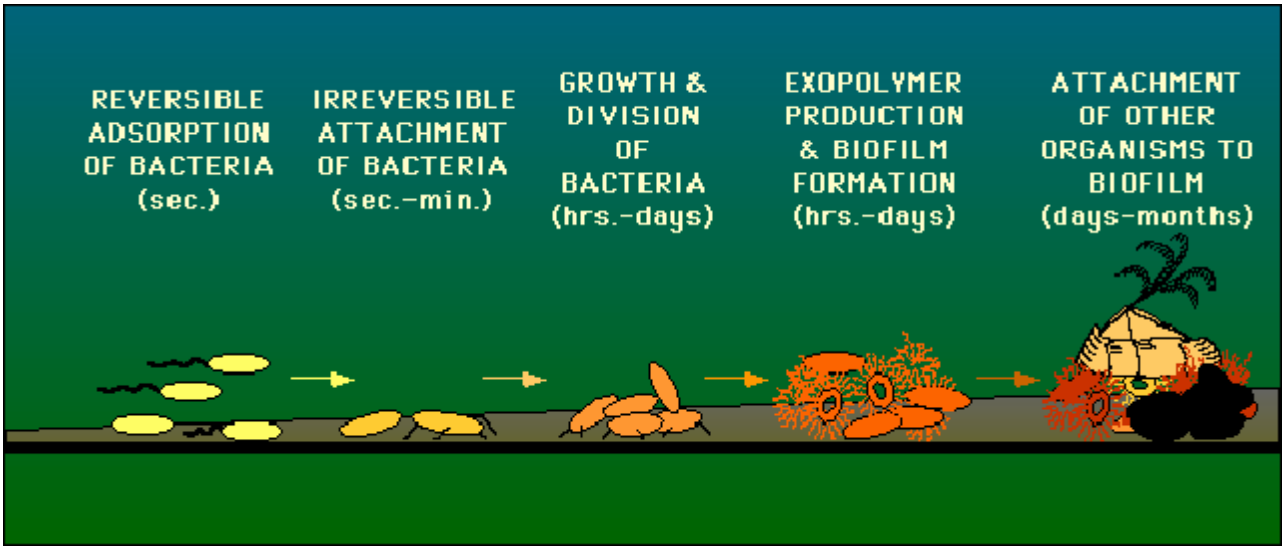


Figure 3. Cartoon depicting biofilm formation. Biofilms usually occur when one bacterial species attaches specifically or non specifically to a surface, and then secretes carbohydrate slime (exopolymer) that imbeds the bacteria and attracts other microbes to the biofilm for protection or nutritional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

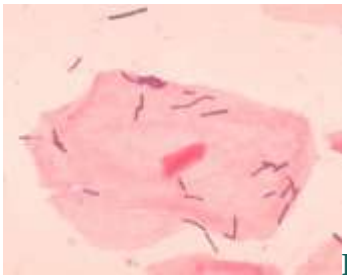


Figure 5. A *Lactobacillus* species, possibly Doderlein's bacillus, in association with a vaginal epithelial cell.

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.

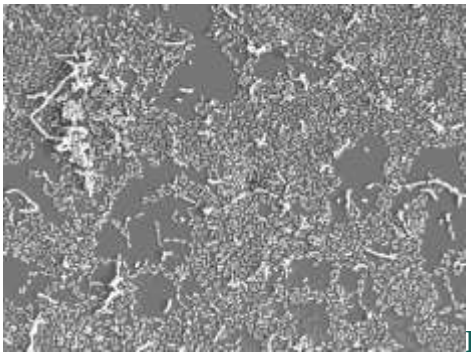


Figure 6. Various streptococci in a biofilm in the oral cavity.

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.

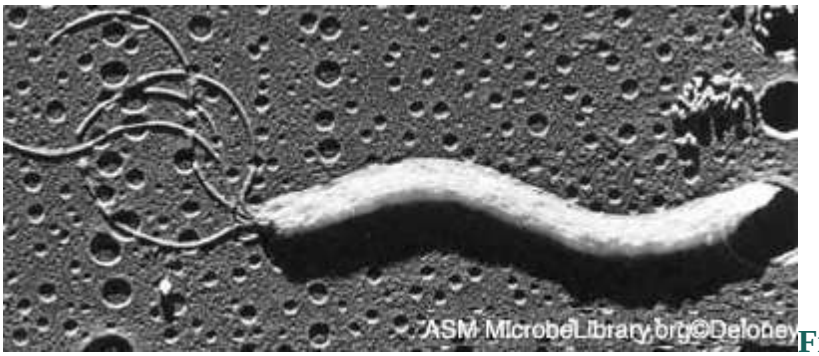


Figure 8. *Helicobacter pylori.* ASM

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.

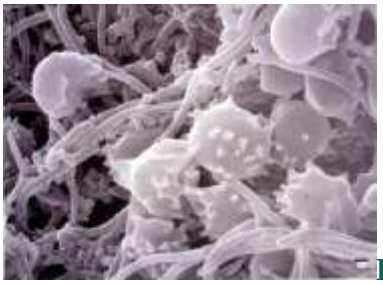
Dental Caries, Gingivitis and Periodontal Disease

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.

II BSc Microbiology (2016-2019 batch)						
MEDICAL MICROBIOLOGY - 16MBU402						
Unit I Question	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**		
The sputum specimen must be s	10C	5C	4C	8C		
the blood samples should be col	late	early	before dia	after symptom		
When a parasite is growing and	pathogen	infection	organism	diagnose		
For microbiological examinatio	good	sterile	normal	clean catch mid-stream		
Cary-Blair medium is used for t	blood	CSF	stool	urine		
a parasite organism or agent tha	pathagon	parasite	bacteria	virus		
the ability of an organism to cau	diagnosis	symptoms	pathogeni	disease		
The most important step in the c	Specimen	Organism	Symptoms	Prophylaxis		
In case of meningeal irritation o	blood	cerebrospi	urine	pus		
in blood specimens is se	haemoglol	serum	iron	protein		
The collected CSF should be sto	room temp	4C	2C	5C		
is a good transport	agar medi	peptone w	Cary-Blai	alkaline medium		
An alternate transport medium f	alkaline-p	peptone w	alkaline m	water		
A sterile is used for tran	container	plastic cor	screw-cap	metal container		
Specimens must be collected be	Vaccines	Antimicro	Symptoms	Culturing		
To eliminate normal flora skin s	Germicide	Soap	Vaseline	Cotton		
Pus swabs collected in transport	2	4	6	8		
During blood collection the skin	Isopropyl	Antiseptic	Soap	Antibiotic		
About _____ ml of blood sh	10	20	30	40		
the blood samples should be inc	25C	37C	4C	10C		
_____ ml of urine sample shoul	10	40	30	20		
In case of _____ only few m	Arthritis	Gas gangr	Renal failu	Paralysis		
If the urine sample is delayed fo	Amino aci	Boric acid	Sodium ch	Calcium chloride		
In case of suspected renal tuberc	1	2	3	4		
The CSF should be collected fro	Cerebrum	Cerebellu	Ventricle	Arachnoid space		
In case of infants the CSF shoul	Ventricle	Arachnoid	Cerebrum	Cerebellum		
About _____ ml of CSF sho	10	30	40	20		
For collecting nasopharyngeal a	Sterile cat	Syringe	Swab	Cotton		
_____ is collected in case of	Pus	Mucous	Conjuctiva	Conjunctival tissue		
Respiratory secretions should be	1	2	3	4		
The natural secretions of eye co	Antibiotic	Toxins	Antibacter	Amylase		
If not possible to obtain faeces a	Cotton wo	Syringe	Catheter	Cotton		
Salmonella, Shigella and Vibrio	24	48	32	76		
Campylobacter can survive in C	2	4	6	8		
If cholera is suspected the stool	1	3	5	8		
_____ is the transportation r	CB mediu	Boric acid	Alkaline p	Phosphate buffered saline		
For suspected viral enteritis the	Phosphate	Alkaline p	CB mediu	Boric acid		
About _____ ml of sterile pho	3	6	9	12		
_____ is the transportation m	Trisodium	Sodium ch	Boric acid	Calcium chloride		
About _____ ml of synovial,	2 to 3	5 to 6	3 to 5	4 to 6		
Synovial, pleural and ascitic flu	Antibiotic	Antiseptic	Anticoagu	Antibacterial		
In case of sputum sample the sa	morning	Evening	Mid night	Noon		
_____ bacilli requires entirely	Rod shape	Spindle sh	Club shap	Acid-fast		
In suspected anthrax the pus san	Lesions	Scrapings	Edema	Necrosis		

For darkfield examination pus fi	Salt soluti	Saline solu	Anticoagu	Antiseptic		
In case of tuberculosis ________	Stool	Urine	Blood	Sputum		
The sputum sample collected in	Saline solu	Anticoagu	Formalin s	Salt solution		
______ technique is used to stai	Ziehl Neel	Gram stai	Endospore	Quellung		
In bacilliary dysentry _______ y	Smears	Swabs	Rectal swa	Renal smears		
In case of salmonellosis ______	Urine	Stool	Sputum	Blood		
In case of thyphoid ______ sam	Sputum	Blood	Faeces	CSF		
In order to avoid drying of stool	Thioglyco	Sodium ci	Buffered g	Para amino benzoic acid		
A n alternative for Thioglycolla	Sodium ci	Buffered g	Para amin	Thioglycollate semisolid mediu		
_________ should be incorporate	Trisodium	Formalin s	Sodium ci	Para amino benzoic acid		
_________ is added to the blood	Sodium ci	Thioglyco	Antiseptic	Antibacterial		
Once the specimen is collected t	Labelled	Opened	Diluted	Defined		
Samples of blood and CSF for c	Labelled	Refrigerat	Processed	Incubated		
The sputum sample should be fr	Blood	Pus	Saliva	Tissue		
when the patient has symptoms	blood	Pus	Sputum	urine		
In case of suspected septicemia	blood	Pus	Sputum	urine		

Answer			
4C			
early			
infection			
clean catch mid-stream			
stool			
pathagon			
pathogenicity			
Specimen			
cerebrospinal fluid			
serum			
room temperature			
Cary-Blair medium			
alkaline-peptone water			
screw-cap container			
Antimicrobial drugs			
Germicide			
6			
Isopropyl alcohol			
20			
37C			
20			
Renal failure			
Boric acid			
3			
Arachnoid space			
Ventricle			
20			
Sterile catheter			
Conjuctival scrapings			
2			
Antibacterial			
Cotton woll swab			
48			
6			
8			
Alkaline peptone water			
Phosphate buffered saline			
9			
Trisodium citrate			
2 to 3			
Anticoagulant			
morning			
Acid-fast			
Lesions			

Saline solution			
Sputum			
Formalin solution			
Ziehl Neelsen			
Rectal swabs			
Blood			
Blood			
Thioglycollate semisolid medium			
Buffered glycerol water			
Para amino benzoic acid			
Sodium citrate			
Labelled			
Refrigerated			
Saliva			
urine			
blood			

2018

II B. Sc Microbiology –Medical Microbiology – 16MBU402
(2016-2019 Batch, IV semester)

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.

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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.

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• *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 of the 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 of effective 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 control review.

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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)

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Severe, sometimes life-threatening GAS disease may occur when these bacteria get into parts of the body where 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.

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.

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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 wound site. 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
23.1 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 or 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

INTEXT QUESTIONS 23.1

1. Salmonellae are gram ..... rods
2. Culturally salmonellae are facultative .....
3. ..... & ..... broth are commonly used as enrichment media for salmonellae
4. Flagella antigen is ..... & somatic antigen is .....
5. ..... antigen is unaffected by boiling, alcohol & weak acids

23.2 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 on this 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.

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Karen Brudney

Mycobacterium tuberculosis

1. Introduction
2. Epidemiology
3. Microbiology
4. Pathogenesis
a. Transmission
b. Host Reaction
5. Clinical Manifestations

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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 from infectious agent world wide among adults
3. HIV/TB relationship has exacerbated problem with TB increasing in areas with high AIDS incidence- Especially sub-Saharan Africa

4. Absolute numbers of cases of TB highest in Asia as population density highest 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 cavitary cases 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 high prevalence 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 greater than 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 from a 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

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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 infectious person 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 intermediate between 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

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*****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 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 people have 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
C. 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 produce organization 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

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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 successfully contained
4. 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 of organisms 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 apical cavitation soon after initial infection;
b. Data shows 23% of those infected between 15-19, 13% of those infected between ages 20-24, 4% of those 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

c. AIDS patients with no cellular immune function cannot mobilize CD4 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)

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c. Malnutrition
d. Old age: Hypersensitivity and cellular immunity wane with age
e. 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 in non-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

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 of incubation 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 of developing 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 serial passage
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

II BSc Microbiology (2016-2019 batch)						
MEDICAL MICROBIOLOGY - 16MBU402						
Unit II Question	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**		
An infectious disease is one that	transferred	caused by	spread fro	caused by all bacteria		
Epidemiologists are not interest	the causes	the frequ	the causa	diagnosis		
The science that studies the distribution, occurrence, and control of health and disease in a defined population is called	Bioinform	Epidemiol	Immunolo	Clinical laboratory science		
An infectious disease that can be transmitted from person to person is known as a	Systemic i	Communi	Transferal	Environmental disease		
The lodgement and multiplicati	disease	infection	immunity	parasitism		
Initial infection with a parasite i	primary	secondary	re-infectio	nosocomial		
Subsequent infection by the sam	Primary	Secondary	Re-infecti	Iatrogenic		
When a new parasite sets up an	Primary	Secondary	Re-infecti	Iatrogenic		
Infection or sepsis at localized s	Primary	Secondary	Focal	Iatrogenic		
In a patient already suffering fro	Cross	Focal	Re-infecti	Nosocomial		
Cross infection occurring in hos	Cross	Focal	Re-infection			
A person who arbours the patho	Patient	Carrier	Healthy pe	Immunodeficient person		
A person one who harbours the	Healthy ca	Convalesc	Contact ca	Paradoxical carrier		
A person who have recovered fr	Healthy	Convalesc	Contact	Paradoxical		
The _____ carrier state lasts	Acute	Temporary	Chronic	Healthy		
The term _____ carrier is ap	Acute	Temporary	Contact	Paradoxical		
The _____ carrier state lasts fo	Acute or c	Temporary	Contact	Paradoxical		
Physicians induced infections ar	Iatrogenic	Focal	Nosocomi	Re-infection		
When clinical effects are not ap	Atypical	Inapparent	Subclinica	Clinical		
_____ infection is the one in	Endogeno	Exogenou	Inapparent	Clinical		
Some parasites, following infect	Endogeno	Exogenou	Latent	Atypical		
The term _____ carrier refers t	Healthy	Convalesc	Contact	Paradoxical		
Infectious diseases transmitted f	Zoonosis	Anoosis	Xanthosis	Phytosis		
When the pathogen multiplies in	Mechanica	Biological	Healthy	Contact		
Some pathogens are able to cros	Congenita	Intracelair	Vertical	Horizontal		
_____ is generally employed	Infection	Immunity	Pathogeni	Virulence		
_____ is applied to the san	Infection	Immunity	Pathogeni	Virulence		
_____ is the science that e	Epidemiol	Oncology	Infection	Physiology		
An individual who practices epi	Epidemiol	Scientist	Investigat	Environmentalist		
A _____ is an impairement of t	Health	Disease	Infection	Immunity		
_____ is the condition in whic	Health	Disease	Infection	Immunity		
When a disease occurs occasion	Epidemic	Endemic	Sporadic	Pandemic		
When the disease occurs at a ste	Epidemic	Endemic	Sporadic	Pandemic		
_____ disease gradually i	hyperende	Epidemic	Endemic	Pandemic		
An_____is a sudden in	Epidemic	Endemic	sporadic	Pandemic		
The first case in an epidemic is	index case	infection	disease	outbreak		
A sudden unexpected occurrenc	index case	Infection	disease	outbreak		

A___________is an increase in	epidemic	Endemic	Pandemic	Sporadic		
The factors that influence the fr	epizootiol	Zootiology	Epidemiol	Entamology		
Moderate prevalance of a diseas	epizootic	enzootic	panzootic	zoonoses		
A sudden outbreak of disease in	epizootic	enzootic	panzotic	zoonoses		
A wide dissemination of anioma	epizoolic	enzoolic	panzootic	zoonoses		
Animal disease that can be trans	epizootic	enzootic	panzootic	zoonoses		
A ________ period is the period	incubation	survival	lag	log		
The _________ is the period in	incubatiob	prodromal	survival p	death		
The _______ represents the eve	infectious	disease	infection	none		
_______ is an illness caused by	communic	epidemic	epidemic	none		
__________ controls the microb	Air pressu	a Aer	Atmosphe	oxygen		
The circulating air is filtered in	Pad	Candle	HEPA	Catridge		
The discard materials to be____	Dissuaded	Dispenced	Decontam	sterilized		
Quality control checks the quali	QA	QC	GTL	QB		
The proper disposal of biologica	Epidemic	Endemic	Pandemic	Randamic		
There is no single reliable test a	Antibiotic	Antiseptic	Disinfecta	Diluent		
In the Rideal walker test typhoic	Phenol	Boron	Acid	Alcohol		
_____________ is a product ket	HCL	BPL	TNT	SDS		
QA stands for _________	Quality as	Quality as	Quality ac	Quality abolision		
The ________ is the time during	incubation	the period	predormal	none		
cholerae caused by	V.cholerae	E.coli	V. paraher	E.histolytica		
The Goods purchased is stored a	Dispatch s	Materials	Quarentur	Stock section		
The water used in laboratory is	Catridge	Pad	Sinted gla	Earthern ware		
The monitoring of the lab envir	QA	QC	GTL	QB		
The microbial load of lab enviro	Settle plat	Pour plate	Spread pla	Streak plate		

Answer		
spread from person to person		
diagnosis		
Epidemiology		
Communicable disease		
infection		
primary		
Re-infection		
Secondary		
Focal		
Cross		
Nosocomial		
Carrier		
Healthy carrier		
Convalescent		
Temporary		
Contact		
Acute or chronic		
Iatrogenic		
Inapparent		
Inapparent		
Latent		
Paradoxical		
Zoonosis		
Biological vector		
Vertical		
Pathogenicity		
Virulence		
Epidemiology		
Epidemiologist		
Disease		
Infection		
Endemic		
Endemic		
hyperendemic		
Pandemic		
index case		
outbreak		

Pandemic		
epizootiology		
enzootic		
panzotic		
zoonoses		
zoonoses		
incubation		
prodromal stage		
infectious disease cycle		
communicable disease		
Air pressure		
HEPA		
Decontaminated		
QA		
Epidemic		
Disinfectants		
Phenol		
BPL		
Quality assurance		
predormal stage		
V.cholerae		
Quarentum section		
Catridge		
QC		
Settle plate		

II B. Sc Microbiology –Medical Microbiology – 16MBU402
(2016-2019 Batch, IV semester)

GENERAL PROPERTIES OF VIRUSES

INTRODUCTION

Viruses occupy the twilight zone that separates the 'living' from the 'non-living'. They do not have a cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both. The medical importance of viruses lies in their ability to cause a very large number of human diseases. Viral diseases range from minor ailments like common cold to terrifying diseases like rabies and AIDS.

In this chapter, we shall be discussing the morphology and general properties of viruses.

After reading this lesson you will be able to:

- explain the concept of viruses, in relation to other microorganisms
- describe the morphological features of viruses
- explain the multiplication of viruses (replication)
- describe the methods of cultivation of viruses
- explain the classification and naming (nomenclature) of viruses

Concept of Viruses in relation to other Organisms

Viruses occupy the twilight zone that separates the 'living' from the 'non-living'. They do not have a cellular organization and contain only one type of nucleic acid, either DNA or RNA but never both. Viruses are obligate intracellular parasites. They lack the enzymes necessary for protein and nucleic acid synthesis. They are dependent for replication on the synthetic machinery of host cells. They multiply by a complex process and not by binary fission. They are unaffected by antibacterial antibiotics. Viruses cause a wide range of human diseases. They cause infections like common cold, chicken pox, measles, viral encephalitis, rabies and AIDS.

Properties Bacteria Viruses

Cellular organization Present Absent Growth on inanimate media Yes No Binary fission Yes No DNA and RNA Both are present Either DNA or RNA Ribosomes Present Absent Sensitivity to antibacterial Yes No antibiotics

Morphology of Viruses

Size: The extracellular infectious virus particle is called virion. Viruses are much smaller than bacteria. They are too small to be seen under the light microscope. Some large viruses like the poxviruses can be seen under the light microscope when suitably stained. The viruses range in size from 20 nm to 300 nm. Poxviruses are one of the largest viruses and parvoviruses are one of the smallest viruses. The earliest method of estimating the size of virus particles was by passing them through collodion membrane filters of graded porosity. The average pore diameter of the finest filter that permitted passage of the virion gave an estimate of its size. With the development of the ultracentrifuge, a second method became available. From the rate of sedimentation of the virus in the ultracentrifuge, the particle size could be calculated using Stoke's law. The third and the most direct method of measuring virus size is electron microscopy. By this method, both the shape and size of virions can be studied.

Structure, shape and symmetry: The virion consists essentially of a nucleic acid surrounded by a protein coat, the **capsid**. The capsid with the enclosed nucleic acid is called the **nucleocapsid**. The capsid protects the nucleic acid from harmful agents in the environment. It is composed of a large number of capsomers which form its morphological units. The chemical units of the Capsid are polypeptide molecules which are arranged symmetrically. They form a shell around the nucleic acid. The capsid shows two kinds of symmetry – icosahedral (cubical) and helical. An icosahedron is a polygon with 12 vertices and 20 facets or sides. Each facet is in the shape of an equilateral triangle. Two types of capsomers are present in the icosahedral capsid. They are the pentagonal capsomers at the vertices (pentons) and the hexagonal capsomers making up the facets (hexons). There are always 12 pentons but the number of hexons varies with the virus group. Examples of viruses with icosahedral symmetry of the capsid are Adenovirus and Herpes Simplex Virus. In the nucleocapsids with helical symmetry, the capsomers and nucleic acid are wound together to form a helical or spiral tube, for example tobacco mosaic virus. All viruses do not show the typical icosahedral or helical symmetry. Some, like the poxviruses, show a complex symmetry. Virions may be enveloped or nonenveloped. The envelope of viruses is derived from the host cell membrane. This occurs when the virus is released from the host cell by budding. Protein subunits may be present as projecting spikes on the surface of the envelope. They are called **peplomers**. The influenza virus carries two kinds of peplomers: haemagglutinin and neuraminidase. Haemagglutinin is a triangular spike and neuraminidase is mushroom-shaped. Envelope is sensitive to the action of lipid solvents. Envelopes confer chemical, antigenic and biological properties on viruses.
The overall shape of the virus particle varies in different groups of viruses. Most animal viruses are roughly spherical. The rabies virus is bullet shaped. Poxviruses are brick-shaped.

Chemical properties: Viruses contain only one type of nucleic acid, either DNA or RNA. Viruses are unique because they carry genetic information on RNA. This property is not seen in any other organism in nature. Viruses also contain protein which makes up the capsid. Enveloped viruses contain lipids derived from the host cell membrane. Most viruses do not have enzymes for the synthesis of viral components or for energy production. Some viruses have enzymes, for example the influenza virus has neuraminidase.

Resistance: Viruses are destroyed by heat except a few. They are stable at low temperatures. For long term storage, they are kept at -70°C. A better method for prolonged storage is lyophilisation or freeze-drying. Viruses are inactivated by sunlight, UV rays and ionising radiation. They are, in general, more resistant than bacteria to chemical disinfectants. Phenolic disinfectants have a weak action on viruses.

Multiplication of Viruses
Multiplication of viruses is called viral **replication**. Viruses contain the genetic information for their replication but they lack the enzymes. They depend on host cell machinery for replication. The viral replication cycle can be divided into six phases – adsorption, penetration, uncoating, biosynthesis, maturation and release.
Adsorption: In this phase, the virus gets attached to the host cell. The host cell should have specific receptors on its surface. These receptors recognize viral surface components. This cell-virus interaction helps the virus to attach to the host cell surface.
Penetration: In this phase, the virus enters into the host cell. Bacteria have rigid cell wall. So, viruses which infect bacteria cannot penetrate into the bacterial cell. Only the nucleic acid of the virus enters the bacterial cell.

Animal and human cells do not have cell walls. Therefore, whole virus enters the cell. Virus particle may be engulfed by a process called **viropexis**. In case of enveloped viruses, the viral envelope may fuse with the cell membrane of the host cell. Then the nucleocapsid is released into the cytoplasm.

Uncoating: This is the process in which the outer layers and capsid of the virus are removed. This mostly occurs by the action of lysosomal enzymes of the host cell. This can also occur by a viral uncoating enzyme. Finally, the viral nucleic acid is released into the cell.

Biosynthesis: In this phase, the viral nucleic acid and capsid are synthesised. The enzymes necessary in the various stages of viral synthesis, assembly and release are also synthesised. Certain 'regulator proteins' are synthesised. They shut down the normal metabolism of the host cell. They direct the production of viral components. In general, most DNA viruses synthesise their nucleic acid in the host cell nucleus. Exceptions are the poxviruses. They are DNA viruses, but they synthesise all their components in the host cell cytoplasm. Most RNA

viruses synthesise all their components in the cytoplasm. Orthomyxoviruses and some paramyxoviruses are exceptions. They synthesise some components in the host cell nucleus. Biosynthesis consists essentially of the following steps:

1. Transcription of messenger RNA (mRNA) from the viral nucleic acid
2. Translation of mRNA into "early proteins" or "non-structural proteins".

They are enzymes responsible for the synthesis of viral components.

3. Replication of viral nucleic acid
4. Synthesis of "late proteins" or "structural proteins". They are the components

of daughter virion capsids.

Maturation: This is the assembly of daughter virions following the synthesis of viral nucleic acid and proteins. It can take place in the host cell nucleus or cytoplasm. Herpesviruses and adenoviruses are assembled in the nucleus.

Picornaviruses and poxviruses are assembled in the nucleus.

Release: Viruses which infect bacteria (bacteriophages) are released by lysis of the infected bacterium. Animal viruses are usually released without cell lysis. Myxoviruses are released by budding from the cell membrane. The host cell is unaffected. Daughter virions are released into the surrounding medium and may infect other cells. In some viruses (for eg. varicella), transmission occurs directly from cell to cell. In this case, there is very little free virus in the medium. The poliovirus causes cell damage and may be released by cell lysis. From the stage of penetration till the appearance of mature daughter virions, the virus cannot be demonstrated inside the host cell. During this period, the virus seems to disappear. This is called the "eclipse phase". The time taken for a single cycle of replication is about 15-30 minutes for bacteriophages. It is about 15- 30 hours for animal viruses. A single infected cell may release a large number of progeny virions.

PLANT DISEASES CAUSED BY VIRUSES

- Plant viruses consist of a nucleoprotein that multiplies only in the living cells of a host. The presence of viruses in host cells often results in disease.
- 400 or more viruses are known to attack plants (2000 viruses are described for plants, animals, bacteria, etc.). viruses are generally specific, what infects a plant does not cause disease in an animal, and vice versa.
- The first record of a disease that was later found to be caused by a plant virus was on tulips in the 17th century in the Netherlands.

- First experimental demonstration of the infectious nature of viral disease was recorded by Lawrence, who described the transmission of a disease of jasmine by grafting.
- Adolf Mayer (1886) described a disease of tobacco called mosaikkranheit (tobacco mosaic). Disease could be transmitted to healthy plants with sap from diseased plants.
- Dmitrii Iwanowski (1892) demonstrated that the agent in tobacco mosaic was filterable. He demonstrated that the causal agent of tobacco mosaic could pass through a filter that retains bacteria.
- 1898 Martinus Beijerinck - demonstrated that the causal agent was not a microorganism but a *contagium vivum fluidum* (contagious living fluid). He was the first to use the term *virus*, which is the Latin word for poison. He concluded that this was not a toxin, because repeated inoculations of diluted infected sap yielded similar amounts of disease as it was passed from one plant to another. If it had been a toxin, it would eventually be diluted away.
- Loefler and Frosch (1898) described the first filterable infectious agent in animals - the foot-and-mouth disease virus and Walter Reed (1900) - described the first human virus, yellow fever virus.
- In 1929, F. O. Holmes provided a tool by which the virus could be measured by showing that the amount of virus present in a plant sample preparation is proportional to the number of local lesions produced on appropriate host plant leaves rubbed with the contaminated sap.
- 1935 W. M. Stanley isolated and purified some tiny white crystals from leaves of mosaic-infected tobacco plants. He treated healthy plants with TMV, which had been precipitated out of infected tobacco juice with the help of ammonium sulfate and a technique he had developed. The healthy plants contracted tobacco mosaic disease. Due to the high protein content of the purified virus particles, he concluded that the virus was an autocatalytic protein that could multiply within living cells. Although his conclusions were later proved incorrect, Stanley's work merited him receiving the Nobel Prize. He won the Nobel Prize in chemistry in 1946 for this work.
- 1937 - Bawden and Pirie demonstrated that virus consists of protein and nucleic acid (RNA).
- 1939 - Kausche - saw virus particles for the first time with the electron microscope.
- 1955-1960's Much was learned by various workers, regarding the infectivity of viral (TMV) RNA and the structure and arrangement of viral (TMV) coat protein.
- 1971 - T. O. Diener discovered viroids, which only consist of nucleic acids. Smaller than viruses, caused potato spindle tuber disease (250-400 bases long of single-stranded circular molecule of infectious RNA). About a dozen other viroids that cause disease in a variety of plants have been isolated. No viroids have ever been found in animals.
- 1980- Cauliflower mosaic virus, whose genome is a circular double-stranded DNA chromosome, was the first plant virus for which the exact sequence of all its 8,000 base pairs was determined. In 1982, the complete sequence of the bases in the single-stranded tobacco mosaic virus RNA was determined, as were those of smaller viral RNA and of viroids.
- 1986 - Use of transgenic plants to obtain resistance against viruses (TMV).

VIRUS DISEASES OF PLANTS ARE USUALLY DESCRIPTIVE OF THE TYPE OF SYMPTOMS THAT THESE CAUSE IN THE HOST

- For example, the symptoms of specific plant diseases form the basis for the following disease names: tobacco mosaic, turnip crinkle, barley yellow dwarf, ring spot of watermelon, cucumber mosaic, spotted wilt of tomato.
- Some viruses have a broader host range than the name of disease or virus may imply. For example, tobacco mosaic virus (TMV) infects tomato, eggplant, peppers, in addition to tobacco.

PROPERTIES AND MORPHOLOGY OF PLANT VIRUSES

- noncellular, ultramicroscopic particles, that multiply only in living cells. very, very small! (size measured in nanometers).

- most plant viruses consist of protein shells surrounded by a core of positive-stranded nucleic acid (normally ssRNA - nucleotides (guanine, uracil, cytosine, adenine) + 5 carbon sugar called ribose + a phosphate group), but sometimes these viruses contain dsRNA or dsDNA (2 strands of nucleotides with thymine substituted for uracil and deoxyribose instead of ribose).
- 5-40% of virus is nucleic acid 60-95% is protein
- Protein coats or shells can be different shapes, but are normally rod, filamentous, isometric, quasi-isometric/bacilliform or variants of these structures. For example, Tobacco Mosaic and Barley Stripe Mosaic viruses are rods, while broad bean wilt and maize chlorotic dwarf viruses are isometric or more spherical in shape.

VIRUS GENOME

Minimum number of genes in a plant RNA virus could be two: a coat protein and an RNA replicase gene (as is the case with RNA phages). Evidence indicates there are usually 3-5 gene products.

Plant positive-stranded RNA viruses frequently possess divided genomes. In addition, viral genomes are separately encapsulated. Viral genomes consisting of two or three different nucleic acid components, all required for infection are called bipartite, tripartite, or multipartite viruses. More than a single species of genomic RNA.

Multipartite viruses are potentially at an evolutionary disadvantage. Infectivity dilution curve for Alfalfa mosaic virus (requiring B, M, Tb particles for infectivity) is steeper than for tobacco necrosis virus (single particle). Partition of genome could potentially hinder transmission or infection by a virus.

SATELLITE VIRUSES AND RNAs

Kasinis in 1962, described the first satellite viruses. These viruses are serologically unrelated to their helpers and the two genomes exhibit little if any sequence similarity. Satellite viruses are dependent for its replication on the presence of a second, independently replicating virus.

Satellite RNAs have no coat protein of their own and are encapsulated with the help of other viral RNAs.

TRANSMISSION

- Mechanical transmission through sap by plants touching one another, through root grafts, and manhandling.
- Vegetative propagation and grafting.
- Seed, pollen, mites, nematodes, dodder, fungi (carried by zoospores and mycelium) and insects (aphids, leafhoppers, scale insects, thrips, grasshoppers, beetles, whiteflies). For example, cucumber mosaic virus and barley yellow dwarf virus moved by aphids.

DETECTION OF PLANT VIRUSES

Due to the inability to observe plant viruses visually by observing them directly through the light microscope, virologists must resort to the following methods of detecting their presence and in diagnoses.

1. Ability to transmit disease via plant sap by rubbing plant, grafting, dodder or insect transmission.
2. Indexing - indicator plants - sensitive to specific virus and will react a certain way if exposed..
3. Visual inspection with EM.
4. By eliminating possibility that symptoms are not due to other sources (e.g., herbicide, nutritional deficiencies.
5. Serological Tests (ELISA - enzyme-linked immuno sorbent assay).

   Indirect (virus + Ab virus + Enzyme conjugated Ab) and direct (double-antibody sandwich technique) (Ab virus + virus + Enzyme-conjugated Ab).

   1. Virus or Ab virus added to well and these become attached to walls.
   2. Antibody or virus added to well and these attach to their counterpart (i.e., antigen to antibody).
   3. Second antibody with enzyme conjugate attaches to first antibody/virus complex.
   4. Substrate is catalyzed by enzyme and this causes a color change.

ELISA tests are extremely sensitive (small amounts of antisera are needed) results are quantitative, large samples can be run at same time (96 well plates), results can be gathered in a few hours instead of days. ELISAs along with serial dilutions of plant sap and applications of this to the leaves of susceptible hosts (by counting the number of lesions) can be used to quantify the amount of virus present.

MANAGEMENT

- Milk inactivates many viruses - use milk to wash tools/hands. "Milk does a plant body good!" Soap and water work well too!
- Removing diseased plants, killing and removing potential virus vectors (primarily weeds and insects).
- disease-resistant cultivars.
- disease or virus free seed, roots or tubers.
- cross protection (inoculation with a less-virulent strain of a virus protects the plant from a more virulent strain later when exposed to it).
- heat (some viruses are killed at temperatures that will not kill host). For example, dormant propagative organs dipped in hot water (35 C) for few minutes or hours, or by growing plants in greenhouse at 35-40 C for several days, weeks or months may inactivate virus.

TOBACCO MOSAIC

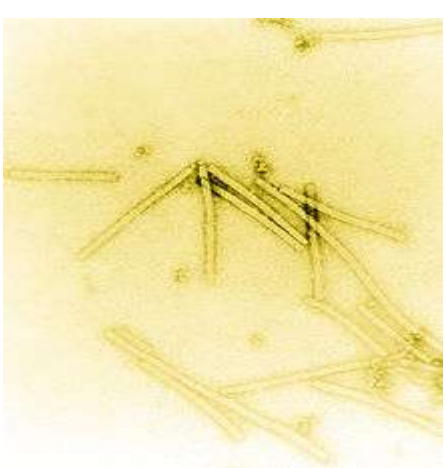
- Caused by Tobacco Mosaic Virus (TMV) worldwide distribution primarily infects tobacco and tomato, but more than 350 species are susceptible.
- tobacco leaves become mottled with light and dark green areas; leave become distorted, puckering or blistering, especially areas of new growth.
- stunting of plant growth. in tomato, mottling of leaves occurs and leaflets become long and pointed.
- TMV is a rod-shaped particle which are 300 nm long by 15-18 nm in diameter. It possesses ssRNA and a protein coat.
- difficult to inactivate, and can survive for 5 years in dead, dried tissues and many months in living plant tissues.
- many strains, that vary in virulence from severe to mild symptoms. virus is spread from plant to plant through injuries caused by crop worker, contaminated equipment and chewing insects.
- virus overwinters in dead plant tissues and debris, on contaminated equipment, in contaminated soil, greenhouse containers, bedding, tools, and in living hosts, including weeds like horsenettle, *Solanum carolinense*, and other crop plants (tomato, pepper, and eggplant).

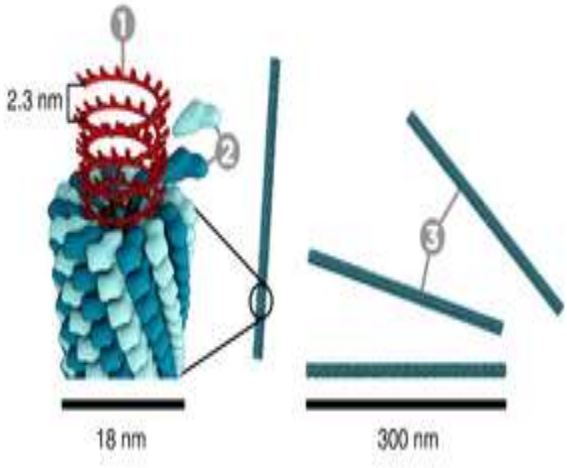
Management of Tobacco Mosaic Disease

- use virus-free seed (tomato seed can by treated with acid or bleach)
- transplant in noninfested soil
- fumigatation with methyl bromide or heated.
- no chewing of tobacco or smoking around seedbeds or in greenhouses.
- to eliminate spreading of virus wash hand with soap and water or milk.
- spraying plants with milk (whole or skim) seems to help reduce
- infections. crop rotation with nonhost crops (corn, rice, other cereal grains).
- resistant cultivars

Tobacco Mosaic Virus: The Prototype Plant Virus

The stability of the TMV virus particle accounts for its having been the first virus to be identified, purified to homogeneity, and then biochemically and biologically characterized.





Small coat protein subunits (capsomeres) aggregate to form a helical protein coat or). The virus particle contains an axial channel that is 4 nm wide and the viral RNA lies within a groove in the surrounding protein helix. The nucleic acid core is not in the axial channel, but passes about halfway between the interior channel and the exterior surface of the rod. The overall particle is rod-shaped, narrow, and rigid. The pitch of the helix is 2.3 nm, and each turn contains 16 1/3 coat protein molecules. A full-length virion contains 130 helical turns. TMV particle is resistant to nucleases and proteolytic enzymes. TMV particles will fall apart in both alkaline and acid solutions. Denaturation is often reversible, as long as temperature and pH are not too extreme. Removal of the denaturant allows the native structure of the viral protein to re-form and near its isoelectric point (pH 4 to 6), the TMV coat protein aggregates to form rod-shaped particles that look exactly like TMV virions. When virus is subjected to neutral pH with either detergents (e.g., SDS) or 6 M urea or by extraction with phenol then RNA can be extracted in an intact form. When isolated TMV RNA are added to native TMV protein, these form stable "reconstituted" virus, which is more stable (stable from pH 3 to 9) then protein alone (unstable below pH of 4 and above pH 6).

Protein and RNA are more infectious than naked RNA alone (nearly 1000 times the amount of naked RNA is required to cause infection).

Proof that the viral RNA was the sole determinant of tobacco mosaic disease was obtained by a mixed reconstitution of RNA from Holmes ribgrass mosaic virus (RMV) with the protein subunits from TMV. Reconstituted virus caused localized lesions on plants instead of a systemic infection and formed new RMV virus (RMV RNA + protein coat containing histidine and methionine - not found in TMV). Refer to Figure 2.13 on page 50 in handout #2

Assembly of Helical Viruses

Aggregates of 33 protein molecules form the double disk. This combines with viral RNA. Attachment of the nucleic acid to the protein aggregate begins at the origin of assembly site (OAS) about 800 nucleotides from the 3' terminus of TMV common strain RNA. Rod growth toward the 5' terminus of the viral RNA is rapid, involving addition of double disks; encapsidation of the 3' terminus proceeds more slowly, through the addition of A protein monomers or small aggregates. Refer to Figure 6.6 in the textbook or to Figure 2.14 on page 50 in handout #2). Cotranslation disassembly - the protein coat is displaced at the 5' end by ribosomes in host cell.

TMV RNA 3' TERMINUS

3' end of TMV RNA ends with the sequence -C-C-C-A and can be charged with an amino acid (histidine). This region is non-coding and be folded into a tRNA-like structure preceded by a series of four pseudoknots. Why? Four possibilities exist.

1. Donating an amino acid during some stage of protein synthesis.
2. Facilitating translation by disrupting base pairing between the 3' and 5' - terminal regions of the viral RNA

3. Acting as a recognition site for the viral replicase to initiate negative-strand synthesis
4. A molecular fossil from the original RNA world where tRNA-like structures tagged RNAs for replication and prevented the uncontrolled loss of nucleotides from third 3' terminus.

Subgenomic mRNAs and translational read-through in TMV replication.
Five open reading frames or ORFs are found in the genome of TMV. Subgenomic mRNAs and translational read-through are two strategies employed by TMV to regulate gene expression.
Plant positive-sense RNA viruses have developed several other mechanisms to facilitate and/or regulate the expression of individual genes. 5 strategies of regulating gene expression.

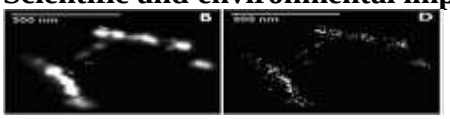
Environment

TMV is known as one of the most stable viruses. It has a very wide survival range. As long as the surrounding temperature remains below approximately 40 degrees Celsius, TMV can sustain its stable form. All it needs is a host to infect. If necessary, greenhouses and botanical gardens would provide the most favorable condition for TMV to spread out, due to the high population density of possible hosts and the constant temperature throughout the year.

Treatment and management

One of the common control methods for TMV is sanitation, which includes removing infected plants, and washing hands in between each planting. Crop rotation should also be employed to avoid infected soil/seed beds for at least two years. As for any plant disease, looking for resistant strains against TMV may also be advised. Furthermore, the cross protection method can be administered, where the stronger strain of TMV infection is inhibited by infecting the host plant with mild strain of TMV, similar to the effect of a vaccine.
In the past ten years, the application of genetic engineering on a host plant genome has been developed to allow the host plant to produce the TMV coat protein within their cells. It was hypothesized that the TMV genome will be re-coated rapidly upon entering the host cell, thus it prevents the initiation of TMV replication. Later it was found that the mechanism that protects the host from viral genome insertion is through gene silencing.[20]

Scientific and environmental impact



TMV virus: super resolution light microscopy

The large amount of literature about TMV and its choice for many pioneering investigations in structural biology (including X-ray diffraction), virus assembly and disassembly, and so on, are fundamentally due to the large quantities that can be obtained, plus the fact that it does not infect animals. After growing a few infected tobacco plants in a greenhouse and a few simple laboratory procedures, a scientist can easily produce several grams of the virus. As a result of this, TMV can be treated almost as an organic chemical, rather than an infective agent.
James D. Watson, in his memoir *The Double Helix*, cites his x-ray investigation of TMV's helical structure as an important step in deducing the nature of the DNA molecule.[21]

Investigational uses

Due to its cylindrical shape, high aspect-ratio, self-assembling nature, and ability to incorporate metal coatings (nickel and cobalt) into its shell, TMV is an ideal candidate to be incorporated into battery electrodes. Addition of TMV to a battery electrode increases the reactive surface area by an order of magnitude, resulting in an increase in the battery's capacity by up to six times compared to a planar electrode geometry.[

Cauliflower mosaic virus

Cauliflower mosaic virus (**CaMV**) is a member of the genus *Caulimovirus*, one of the six genera in the *Caulimoviridae* family, which are pararetroviruses that infect plants.[1] Pararetroviruses replicate through reverse transcription just like retroviruses, but the viral particles contain DNA instead of RNA.[2]

Definition

Aphid species *Myzus persicae*

Cauliflower mosaic virus (CaMV) is the type species of the family *Caulimoviridae*. This family is grouped together with *Hepadnaviruses* into the *Pararetrovirus* group due to its mode of replication via reverse transcription of a pre-genomic RNA intermediate.

CaMV infects mostly plants of the *Brassicaceae* family (such as cauliflower and turnip) but some CaMV strains (D4 and W260) are also able to infect *Solanaceae* species of the genera *Datura* and *Nicotiana*. CaMV induces a variety of systemic symptoms such as mosaic, necrotic lesions on leaf surfaces, stunted growth, and deformation of the overall plant structure. The symptoms exhibited vary depending on the viral strain, host ecotype, and environmental conditions.[3]

CaMV is transmitted in a non-circulatory manner by aphid species such as *Myzus persicae*.[4] Once introduced within a plant host cell, virions migrate to the nuclear envelope of the plant cell.

Structure

The CaMV particle is an icosahedron with a diameter of 52 nm built from 420 capsid protein (CP) subunits arranged with a triangulation T = 7, which surrounds a solvent-filled central cavity.[5][6]

CaMV contains a circular double-stranded DNA molecule of about 8.0 kilobases, interrupted by nicks that result from the actions of RNAse H during reverse transcription. These nicks come from the Met-tRNA, and two RNA primers used in reverse transcription. After entering the host cell, these single stranded "nicks" in the viral DNA are repaired, forming a supercoiled molecule that binds to histones. This DNA is transcribed into a full length, terminally redundant[*clarification needed*], 35S RNA and a subgenomic 19S RNA.

Genome

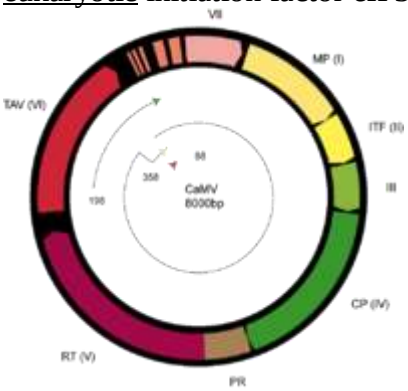
The promoter of the 35S RNA is a very strong constitutive promoter responsible for the transcription of the whole CaMV genome. It is well known for its use in plant transformation. It causes high levels of gene expression in dicot plants. However, it is less effective in monocots, especially in cereals. The differences in behavior are probably due to differences in quality and/or quantity of regulatory factors. Interestingly, recent study has indicated that the CaMV 35S promoter is also functional in some animal cells, although the promoter elements used are different from those in plants. While this promoter had low activity compared to canonical animal promoters, levels of reporter products were significant. This observation suggests that the 35S promoter may have potential for use in animals.[7]

The promoter was named CaMV 35S promoter ("35S promoter") because the coefficient of sedimentation of the viral transcript, whose expression is naturally driven by this promoter, is 35S. It is one of the most widely used, general-purpose constitutive promoters. It was discovered at the beginning of the 1980s, by Chua and collaborators at The Rockefeller University.

The 35S RNA is particularly complex, containing a highly structured 600 nucleotide long leader sequence with six to eight short open reading frames (ORFs).[8][9][10]

This leader is followed by seven tightly arranged, longer ORFs that encode all the viral proteins. The mechanism of expression of these proteins is unique, in that the ORF VI protein (encoded by the 19S RNA) controls translation reinitiation of major open reading frames on the polycistronic 35S RNA, a process that

normally only happens on bacterial mRNAs. TAV function depends on its association with polysomes and eukaryotic initiation factor eIF3.[11]



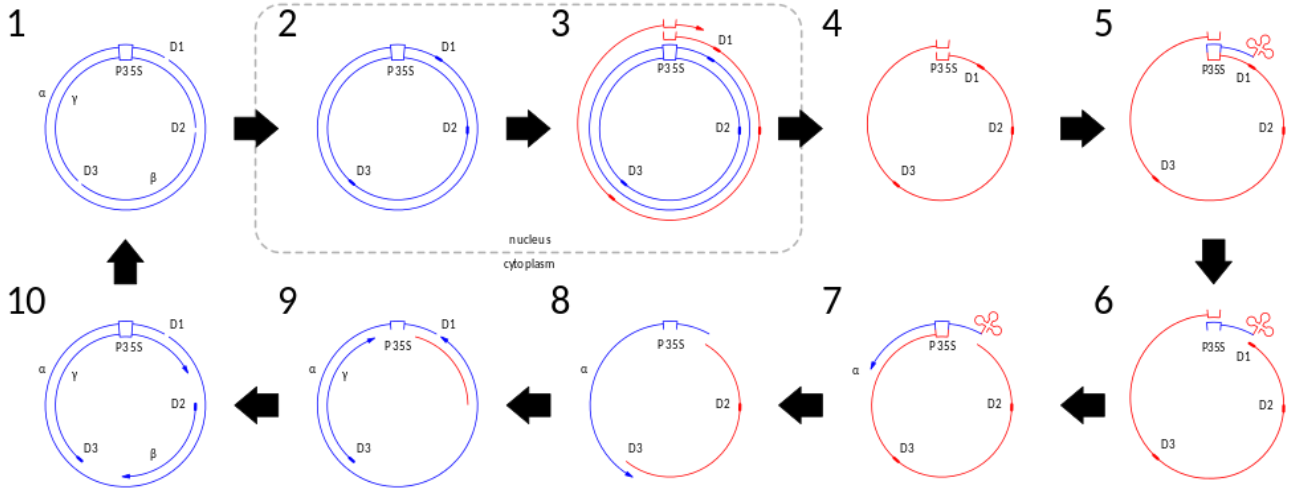
Genomic map of CaMV

- ORF I - Movement Protein
- ORF II - Insect Transmission Factor
- ORF III - Structural Protein, DNA-Binding Capabilities
- ORF IV - Capsid Protein
- ORF V - Protease, Reverse Transcriptase and RNaseH
- ORF VI - Translational Activator, Inclusion Body Formation/Trafficking; Possibly more functions (See Below)
- ORF VII - Unknown (Appears to not be required for infection)

In addition to its functions regarding translational activation and formation of inclusion bodies, P6 has been shown to interact with a number of other CaMV proteins, such as P2 and P3, suggesting that it may also contribute in some degree to viral assembly and aphid-mediated transmission. In addition, P6 has been shown to bind to P7; investigating interactions between the two may help to elucidate the as yet unknown function of P7.[12]

Another interesting function of P6 involves modification of host NONEXPRESSOROFPATHOGENESISRELATED1 (NPR1) during the course of infection. NPR1 is an important regulator of salicylic acid (SA) and jasmonic acid (JA)-dependent signaling, and is most closely associated with crosstalk between the two. Modification of NPR1 serves to inhibit plant cells' defensive responses by preventing SA-dependent signaling; modified NPR1 can properly traffic to the nucleus and bind the PR-1 promoter, but is unable to initiate transcription. Because active NPR1 is required for accumulation of SA, this leads to a further depletion of SA. Whereas regulation of SA-dependent signaling by P6-modified NPR1 is localized to the nucleus, regulation of JA-dependent signaling is cytoplasmic in nature and involves the COI1 pathway. In contrast to that of SA, JA-dependent signaling is increased in the presence of modified NPR1.[13]

Replication



CaMV replicates by reverse transcription:

1. Viral particles enter a plant cell and are unencapsidated. At this stage the viral DNA consists of three fragments, one on the – strand (α) and two on the + strand (β and γ) which are imperfectly assembled into a circular genome with three gaps or discontinuities (D1, D2, and D3).
2. The viral DNA enters the nucleus where the discontinuities are filled in. At this point the viral DNA also associates with host histones, forming a minichromosome (not shown).
3. The host DNA-dependent RNA polymerase transcribes from the 35S promoter all the way around the viral genome, surpassing the 35S promoter. (This creates two copies of the 35S promoter in the resulting RNA.) Transcription also initiates at the 19S promoter (not shown).
4. The viral RNAs pass into the host cytoplasm where they are transcribed.
5. The 3′ end of a $tRNA^{fMet}$ anneals to a site corresponding to discontinuity 1 (D1) near the 5′ end of the 35S RNA.
6. The $tRNA^{fMet}$ primes synthesis, by the viral reverse transcriptase (encoded by ORF V), of a new α strand.
7. RNase H removes the RNA from the DNA–RNA duplex, leaving behind the DNA.

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8. This new DNA binds the 35S promoter at the 3′ end of the RNA template and synthesis of the α strand of DNA continues and RNase H continues to degrade RNA complexed to DNA.
9. Synthesis of the α strand completes. RNase H activity exposes purine-rich regions at the position of discontinuity 3 (D3), which primes the synthesis of the γ DNA strand.
10. RNase H activity exposes purine-rich regions at the position of discontinuity 2 (D2), which primes the synthesis of the β DNA strand. When the new γ strand of DNA reaches the 5′ end of the new α strand it switches to the 5′ end of the new α strand, recreating discontinuity 1 (D1). When the new γ strand of DNA reaches the 5′ end of the new β strand, it displaces the primer and some of the newly synthesized β strand, resulting in the recreation of discontinuity 2 (D2). When the new β strand of DNA reaches the 5′ end of the new γ strand, it displaces the primer and some of the newly synthesized γ strand, resulting in the recreation of discontinuity 3 (D3).

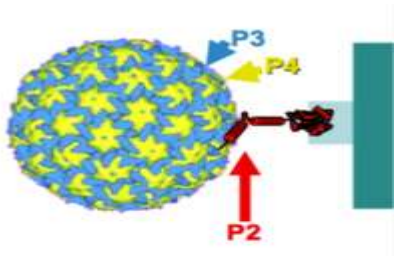
At this point the new viral genome can either be packaged into capsids and released from the cell or they can be transported by movement proteins into an adjacent, uninfected cell.[14]

The Cauliflower mosaic virus promoter (CaMV 35S) is used in most transgenic crops to activate foreign genes which have been artificially inserted into the host plant. It is inserted into transgenic plants in a form which is different from that found when it is present in its natural *Brassica* plant hosts. This enables it to operate in a wide range of host-organism environments which would otherwise not be possible.

CaMV contains about 8 kb double-strand DNA genome and produces spherical particles. CaMV infections are systemic, and even its DNA is infectious when inoculated on abraded plant surfaces. The CaMV genome has 8 tightly packed genes, of which only two small genes, genes II and VII, are nonessential; as a result, only these two genes can be replaced/deleted without a loss of infectivity. In addition, modified CaMV genomes exceeding the natural genome size (8024 bp) by even a few hundred bp are not packaged into virions. These two factors seriously limit the size of DNA insert clonable in CaMV. The bacterial dihydrofolate reductase DHFR gene has been successfully cloned into the CaMV genome, in place of gene II, and has been successfully expressed in plants.

Molecular Mechanisms of Vector-Mediated CaMV Transmission

The virus is acquired from an infected host during feeding by the aphid vector. To occur, a transmissible complex is composed of virions and protein P2 located in the vector's stylets. The P2 N-terminal domain recognizes a protein receptor located at the tip of the stylet and the P2 C-terminal domain binds to the P3-decorated virions.[15]



Transmissible complex of CaMV

The mode of acquisition by the vector is controlled by the tissue and intracellular-specific localization of P2. This protein is only found in epidermis and parenchyma cells. Moreover, in these cells, P2 is localized in single viral electron-lucent inclusion bodies (ELIB).[16] In host cells, viral protein P2 and P3 are first produced in numerous viral factories (electron-dense inclusion bodies), and are later exported and co-localize with microtubules, before concentrating in ELIB. CaMV specifically uses the microtubules to form the transmissible body and thus enable vector transmission.[17] The complete molecular characterization and study of this virus was not carried further.

Evasion of Plant Defenses

Cauliflower mosaic virus possesses a number of mechanisms that allow it to counteract host plant cell defenses. While the pregenomic 35S RNA is responsible for genome replication by reverse transcriptase, it also contains a non-coding 600 base pair leader sequence that serves as an important mRNA for the production of factors involved in viral counter-defense. A number of hosts of CaMV possess small RNA-based viral silencing mechanisms that serve to limit viral infection. The products of the aforementioned 600-bp sequence are viral small RNAs (vsRNA) of 21, 22, and 24 nucleotides in length that serve as decoys, binding and inactivating effectors of host silencing machinery, such as Argonaute 1 (AGO1). As proof-of-principle, experimental overexpression of these vsRNAs allows for increased viral accumulation in infected plants.[18]

Concerns About Use of CaMV 35S Promoter in Transgenic Plants

Recently, some concerns have been raised about using the CaMV 35S promoter for expression in transgenic plants because sequence overlap exists between this promoter and the coding sequences of P6. Fifty four transgenic events certified for release in the USA contain up to 528 bp of ORF VI (encoding C-terminal domains of P6).[19] As P6 is a multifunctional protein whose full range of functions is unknown, there is some concern that expression of one or more of its domains may have unforeseen consequences in the transgenic organisms. Recent studies have attempted to determine what length of CaMV 35S promoter has the least chance of inadvertently producing P6 domains, while still retaining full promoter activity. As one might expect, using shorter promoter lengths decreases the number of P6 domains included and also decreases the likelihood of unwanted effects.[19]

Geminiviridae

Geminiviridae is a family of plant viruses. There are currently 325 species in this family, divided among 7 genera. Diseases associated with this family include: bright yellow mosaic, yellow mosaic, yellow mottle, leaf curling, stunting, streaks, reduced yields.[1][2] They have single-stranded circular DNA genomes encoding genes that diverge in both directions from a virion strand origin of replication (i.e. geminivirus genomes are ambisense). According to the Baltimore classification they are considered class II viruses. It is the largest known family of single stranded DNA viruses.

Mastrevirus transmission is via various leafhopper species (e.g. maize streak virus and other African streak viruses are transmitted by *Cicadulina mbila*), curtoviruses and the only known topocuvirus species, *Tomato pseudo-curly top virus*, are transmitted by treehopper species (e.g.*Tomato pseudo-curly top virus* is transmitted by the treehopper *Micrutalis malleifera*), and begomoviruses are transmitted by the whitefly species, *Bemisia tabaci*.

These viruses are responsible for a significant amount of crop damage worldwide. Epidemics of geminivirus diseases have arisen due to a number of factors, including the recombination of different geminiviruses coinfecting a plant, which enables novel, possibly virulent viruses to be developed. Other contributing factors include the transport of infected plant material to new locations, expansion of agriculture into new growing areas, and the expansion and migration of vectors that can spread the virus from one plant to another.[3]

Virology

The genome can either be a single component between 2500–3100 nucleotides, or, in the case of some begomoviruses, two similar-sized components each between 2600 and 2800 nucleotides. They have elongated, geminate capsids with two incomplete T=1 icosahedra joined at the missing vertex. The capsids range in size from 18–20 nm in diameter with a length of about 30 nm. Begomoviruses with two component (i.e. bipartite) genomes have these components separated into two different particles both of which must usually be transmitted together to initiate a new infection within a suitable host cell.

Genus	Structure	Symmetry	Capsid	Genomic Arrangement	Genomic Segmentation

Genus	Structure	Symmetry	Capsid	Genomic Arrangement	Genomic Segmentation
Eragrovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Curtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Begomovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Segmented
Becurtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Topocuvirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Turncurtovirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite
Mastrevirus	Twinned Icosahedral	Incomplete T=1	Non-Enveloped	Circular	Monopartite

Taxonomy

Group: ssDNA

Order: Unassigned

Several additional genera have been proposed: Baminivirus, Nimivirus and Niminivirus.[4]

Some viruses have yet to be assigned a genus: one of these is the Grapevine Cabernet Franc-associated virus/Grapevine red blotch-associated virus/Grapevine redleaf-associated virus.[5]

Replication

Drawing of geminiviruses

Geminivirus genomes encode only a few proteins; thus, they are dependent on host cell factors for replication: these include factors such as DNA polymerase—and probably repair polymerases—in order to amplify their genomes, as well as transcription factors. Geminiviruses replicate via a rolling circle mechanism like bacteriophages such as M13, and many plasmids. Replication occurs within the nucleus of an infected plant cell. First the single-stranded circular DNA is converted to a double-stranded circular intermediate. This step involves the use of cellular DNA repair enzymes to produce a complementary negative-sense strand, using the viral genomic or plus-sense DNA strand as a template. The next step is the rolling circle phase, where the viral strand is cleaved at a specific site situated within the origin of replication by the viral Rep protein in order to initiate replication.[6] This process in a eukaryotic nucleus can give rise to concatemeric double-stranded forms of replicative intermediate genomes, although double-stranded unit circles can be isolated from infected plants and cells. New single-stranded DNA forms of the virus genome (plus-sense) are probably formed by interaction of the coat protein with replicating DNA intermediates, as genomes lacking a CP gene do not form ssDNA. The ssDNA is packaged into germinate particles in the nucleus. It is not clear if these particles can then leave the nucleus and be transmitted to surrounding cells as virions, or whether ssDNA associated with coat protein and a movement protein is the form of the genome that gets trafficked from cell to cell via the plasmodesmata.[7]

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These viruses tend to be introduced into and initially infect differentiated plant cells, via the piercing mouthparts of the vector insect: however, these cells generally lack the host enzymes necessary for DNA replication, making it difficult for the virus to replicate. To overcome this block geminiviruses can induce plant cells to reenter the cell cycle from a quiescent state so that viral replication can occur.[8]

Genus	Host Details	Tissue Tropism	Entry Details	Release Details	Replication Site	Assembly Site	Transmission
Eragrovirus	Plants	None	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Treehopper; leafhopper
Curtovirus	Dicotyledonous plants	Phloem-limited	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Beet leefhopper
Begomovirus	Dicotyledonous plants	Phloem; sieve; phloem-limited	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Bemisia tabaci whiteflies
Becurtovirus	Spinach	Phloem; sieve; phloem-limited	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Viral movement; contact
Topocuvirus	Dicotyledonous plants	None	Cell receptor endocytosis	Budding	Nucleus	Nucleus	Leafhopper
Turncurtovirus	Turnip	None	Cell receptor endocytosis	Budding	Nucleus	Nucleus	Leafhopper
Mastrevirus	Monocots[9]	None	Viral movement; mechanical inoculation	Budding	Nucleus	Nucleus	Leafhopper

Evolution

These viruses may have evolved from a phytoplasma plasmid.[10] Geminiviruses are capable of horizontal gene transfer of genetic information to the plant host.[11]

HIV

"AIDS virus" redirects here. For the computer virus, see AIDS (computer virus).

The **human immunodeficiency virus** (**HIV**) is a lentivirus (a subgroup of retrovirus) that causes HIV infection and over time acquired immunodeficiency syndrome (AIDS).[1][2] AIDS is a condition in humans in which progressive failure of the immune system allows life-threatening opportunistic infections and cancers to thrive. Without treatment, average survival time after infection with HIV is estimated to be 9 to 11 years, depending on the HIV subtype.[3] Infection with HIV occurs by the transfer of blood, semen, vaginal fluid, pre-ejaculate, or breast milk. Within these bodily fluids, HIV is present as both free virus particles and virus within infected immune cells.

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HIV infects vital cells in the human immune system such as helper T cells (specifically $CD4^+$ T cells), macrophages, and dendritic cells.[4] HIV infection leads to low levels of $CD4^+$ T cells through a number of mechanisms, including pyroptosis of abortively infected T cells,[5] apoptosis of uninfected bystander cells,[6] direct viral killing of infected cells, and killing of infected $CD4^+$ T cells by CD8 cytotoxic lymphocytes that recognize infected cells.[7] When $CD4^+$ T cell numbers decline below a critical level, cell-mediated immunity is lost, and the body becomes progressively more susceptible to opportunistic infections.

Virology

Classification

Comparison of HIV species

Species	Virulence	Infectivity	Prevalence	Inferred origin
HIV-1	High	High	Global	Common chimpanzee
HIV-2	Lower	Low	West Africa	Sooty mangabey

HIV is a member of the genus *Lentivirus*,[8] part of the family *Retroviridae*.[9] Lentiviruses have many morphologies and biological properties in common. Many species are infected by lentiviruses, which are characteristically responsible for long-duration illnesses with a long incubation period.[10] Lentiviruses are transmitted as single-stranded, positive-sense, enveloped RNA viruses. Upon entry into the target cell, the viral RNA genome is converted (reverse transcribed) into double-stranded DNA by a virally encoded reverse transcriptase that is transported along with the viral genome in the virus particle. The resulting viral DNA is then imported into the cell nucleus and integrated into the cellular DNA by a virally encoded integrase and host co-factors.[11] Once integrated, the virus may become latent, allowing the virus and its host cell to avoid detection by the immune system. Alternatively, the virus may be transcribed, producing new RNA genomes and viral proteins that are packaged and released from the cell as new virus particles that begin the replication cycle anew.

Two types of HIV have been characterized: HIV-1 and HIV-2. HIV-1 is the virus that was initially discovered and termed both LAV and HTLV-III. It is more virulent, more infective,[12] and is the cause of the majority of HIV infections globally. The lower infectivity of HIV-2 compared to HIV-1 implies that fewer of those exposed to HIV-2 will be infected per exposure. Because of its relatively poor capacity for transmission, HIV-2 is largely confined to West Africa.[13]

Structure and genome

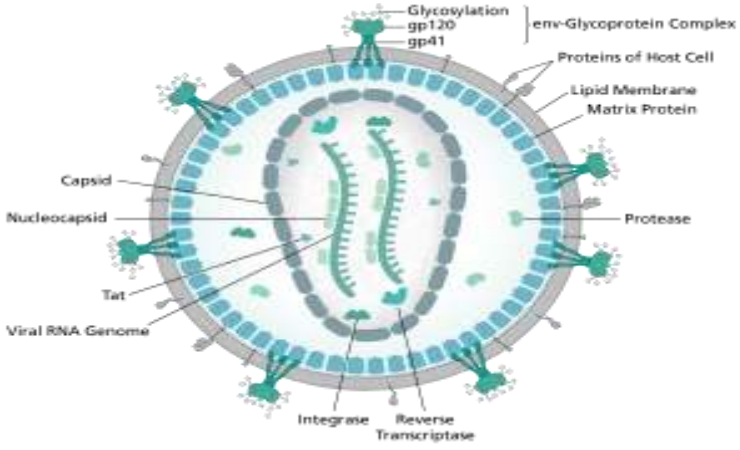

Diagram of HIV virion

HIV is different in structure from other retroviruses. It is roughly spherical[14] with a diameter of about 120 nm, around 60 times smaller than a red blood cell.[15] It is composed of two copies of positive single-stranded RNA that codes for the virus's nine genes enclosed by a conical capsid composed of 2,000 copies of the viral protein

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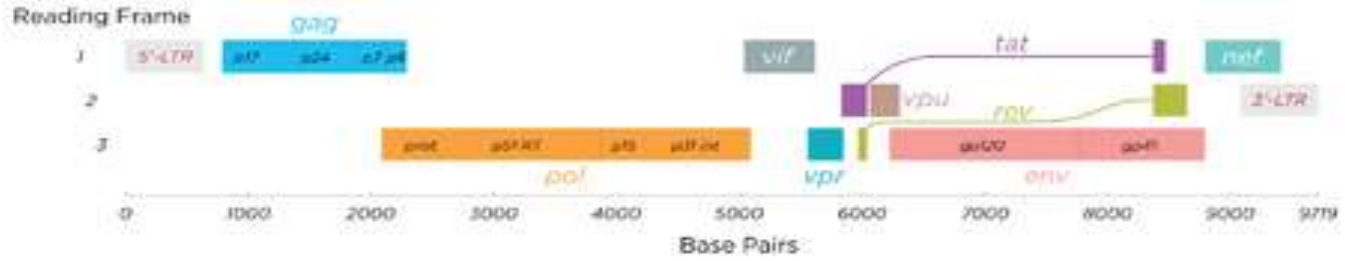
p24.[16] The single-stranded RNA is tightly bound to nucleocapsid proteins, p7, and enzymes needed for the development of the virion such as reverse transcriptase, proteases, ribonuclease and integrase. A matrix composed of the viral protein p17 surrounds the capsid ensuring the integrity of the virion particle.[16]
This is, in turn, surrounded by the viral envelope, that is composed of the lipid bilayer taken from the membrane of a human cell when the newly formed virus particle buds from the cell. The viral envelope contains proteins from the host cell and relatively few copies of the HIV Envelope protein,[16] which consists of a cap made of three molecules known as glycoprotein (gp) 120, and a stem consisting of three gp41 molecules which anchor the structure into the viral envelope.[17][18] The Envelope protein, encoded by the HIV *env* gene, allows the virus to attach to target cells and fuse the viral envelope with the target cell membrane releasing the viral contents into the cell and initiating the infectious cycle.[17] As the sole viral protein on the surface of the virus, the Envelope protein is a major target for HIV vaccine efforts.[19] Over half of the mass of the trimeric envelope spike is N-linked glycans. The density is high as the glycans shield the underlying viral protein from neutralisation by antibodies. This is one of the most densely glycosylated molecules known and the density is sufficiently high to prevent the normal maturation process of glycans during biogenesis in the endoplasmic and Golgi apparatus.[20][21] The majority of the glycans are therefore stalled as immature 'high-mannose' glycans not normally present on secreted or cell surface human glycoproteins.[22] The unusual processing and high density means that almost all broadly neutralising antibodies that have so far been identified (from a subset of patients that have been infected for many months to years) bind to or, are adapted to cope with, these envelope glycans.[23]
The molecular structure of the viral spike has now been determined by X-ray crystallography[24] and cryo-electron microscopy.[25] These advances in structural biology were made possible due to the development of stable recombinant forms of the viral spike by the introduction of an intersubunit disulphide bond and an isoleucine to proline mutation in gp41.[26] The so-called SOSIP trimers not only reproduce the antigenic properties of the native viral spike but also display the same degree of immature glycans as presented on the native virus.[27] Recombinant trimeric viral spikes are promising vaccine candidates as they display less non-neutralising epitopes than recombinant monomeric gp120 which act to suppress the immune response to target epitopes.[28]



Structure of the RNA genome of HIV-1
The RNA genome consists of at least seven structural landmarks (LTR, TAR, RRE, PE, SLIP, CRS, and INS), and nine genes (*gag*, *pol*, and *env*, *tat*, *rev*, *nef*, *vif*, *vpr*, *vpu*, and sometimes a tenth *tev*, which is a fusion of tat env and rev), encoding 19 proteins. Three of these genes, *gag*, *pol*, and *env*, contain information needed to make the structural proteins for new virus particles.[16] For example, *env* codes for a protein called gp160 that is cut in two by a cellular protease to form gp120 and gp41. The six remaining genes, *tat*, *rev*, *nef*, *vif*, *vpr*, and *vpu* (or *vpx* in the case of HIV-2), are regulatory genes for proteins that control the ability of HIV to infect cells, produce new copies of virus (replicate), or cause disease.[16]
The two Tat proteins (p16 and p14) are transcriptional transactivators for the LTR promoter acting by binding the TAR RNA element. The TAR may also be processed into microRNAs that regulate the apoptosis genes ERCC1 and IER3.[29][30] The Rev protein (p19) is involved in shuttling RNAs from the nucleus and the

cytoplasm by binding to the RRE RNA element. The Vif protein (p23) prevents the action of APOBEC3G (a cellular protein that deaminates Cytidine to Uridine in the single stranded viral DNA and/or interferes with reverse transcription[31]). The Vpr protein (p14) arrests cell division at G2/M. The Nef protein (p27) down-regulates CD4 (the major viral receptor), as well as the MHC class I and class II molecules.[32][33][34]
Nef also interacts with SH3 domains. The Vpu protein (p16) influences the release of new virus particles from infected cells.[16] The ends of each strand of HIV RNA contain an RNA sequence called the long terminal repeat (LTR). Regions in the LTR act as switches to control production of new viruses and can be triggered by proteins from either HIV or the host cell. The Psi element is involved in viral genome packaging and recognized by Gag and Rev proteins. The SLIP element (TTTTTT) is involved in the frameshift in the Gag-Pol reading frame required to make functional Pol.[16]

Tropism

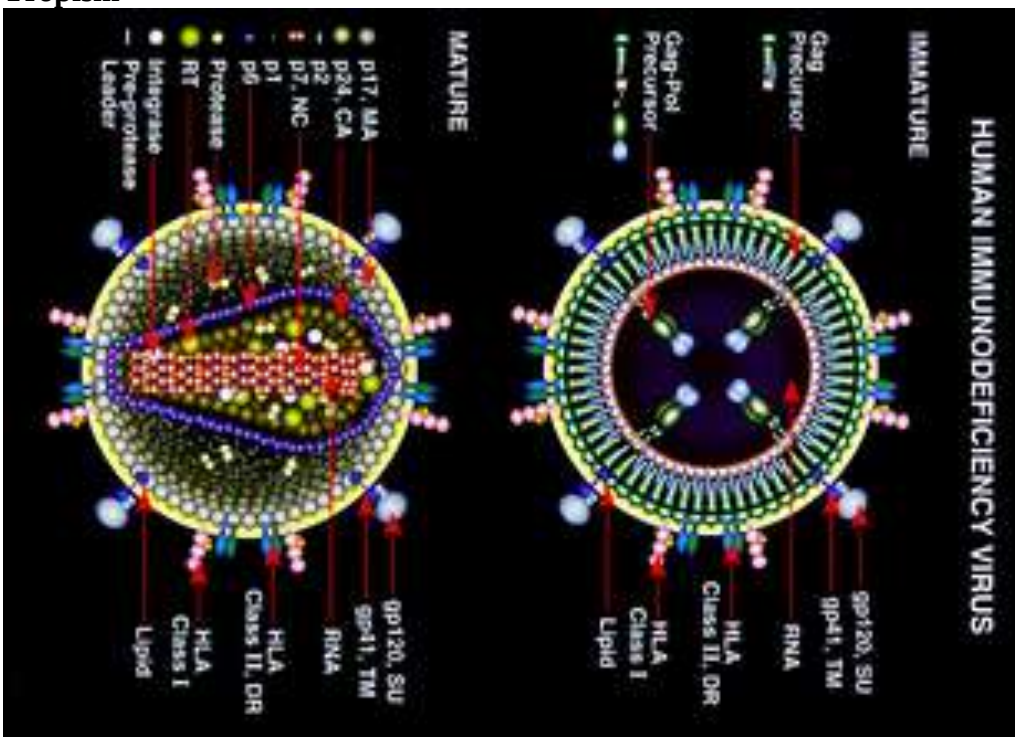


Diagram of the immature and mature forms of HIV

The term viral tropism refers to the cell types a virus infects. HIV can infect a variety of immune cells such as $CD4^+$ T cells, macrophages, and microglial cells. HIV-1 entry to macrophages and $CD4^+$ T cells is mediated through interaction of the virion envelope glycoproteins (gp120) with the CD4 molecule on the target cells and also with chemokine coreceptors.[17][35]
Macrophage (M-tropic) strains of HIV-1, or non-syncytia-inducing strains (NSI; now called R5 viruses[36]) use the β-chemokine receptor CCR5 for entry and are, thus, able to replicate in macrophages and $CD4^+$ T cells.[37] This CCR5 coreceptor is used by almost all primary HIV-1 isolates regardless of viral genetic subtype. Indeed, macrophages play a key role in several critical aspects of HIV infection. They appear to be the first cells infected by HIV and perhaps the source of HIV production when $CD4^+$ cells become depleted in the patient. Macrophages and microglial cells are the cells infected by HIV in the central nervous system. In tonsils and adenoids of HIV-infected patients, macrophages fuse into multinucleated giant cells that produce huge amounts of virus.
T-tropic isolates, or syncytia-inducing (SI; now called X4 viruses[36]) strains replicate in primary $CD4^+$ T cells as well as in macrophages and use the α-chemokine receptor, CXCR4, for entry.[37][38][39] Dual-tropic HIV-1

strains are thought to be transitional strains of HIV-1 and thus are able to use both CCR5 and CXCR4 as co-receptors for viral entry.

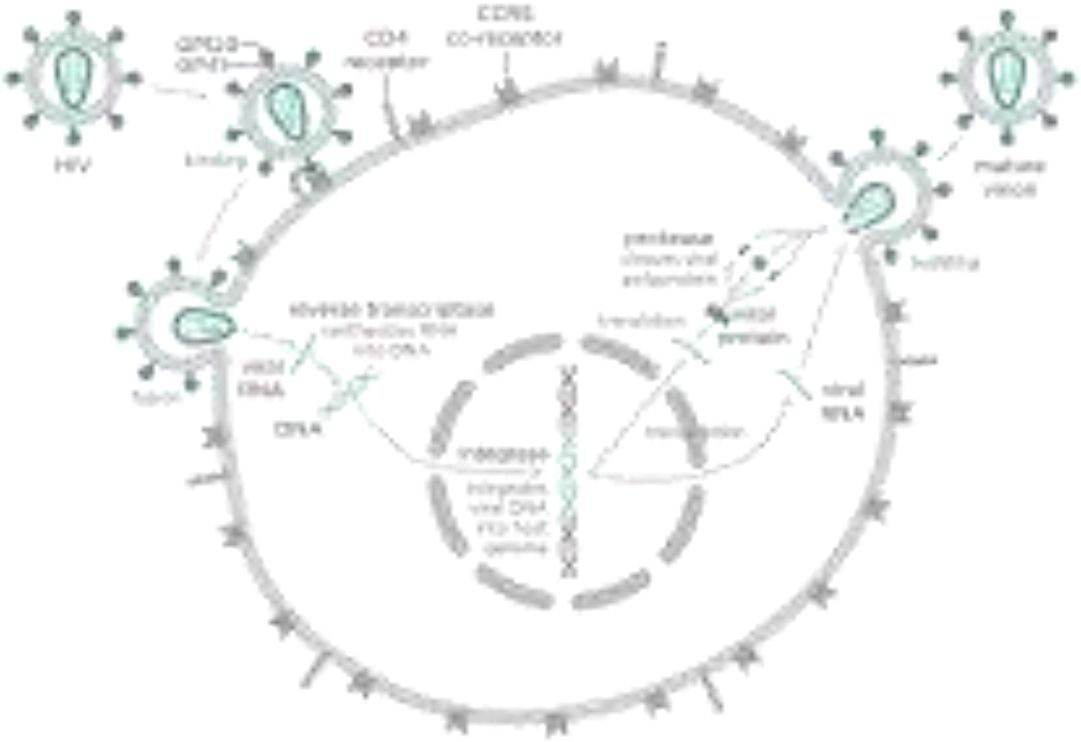
The α-chemokine SDF-1, a ligand for CXCR4, suppresses replication of T-tropic HIV-1 isolates. It does this by down-regulating the expression of CXCR4 on the surface of these cells. HIV that use only the CCR5 receptor are termed R5; those that use only CXCR4 are termed X4, and those that use both, X4R5. However, the use of coreceptor alone does not explain viral tropism, as not all R5 viruses are able to use CCR5 on macrophages for a productive infection[37] and HIV can also infect a subtype of myeloid dendritic cells,[40] which probably constitute a reservoir that maintains infection when $CD4^+$ T cell numbers have declined to extremely low levels. Some people are resistant to certain strains of HIV.[41] For example, people with the CCR5-Δ32 mutation are resistant to infection with R5 virus, as the mutation stops HIV from binding to this coreceptor, reducing its ability to infect target cells.

Sexual intercourse is the major mode of HIV transmission. Both X4 and R5 HIV are present in the seminal fluid, which is passed from a male to his sexual partner. The virions can then infect numerous cellular targets and disseminate into the whole organism. However, a selection process leads to a predominant transmission of the R5 virus through this pathway.[42][43][44] How this selective process works is still under investigation, but one model is that spermatozoa may selectively carry R5 HIV as they possess both CCR3 and CCR5 but not CXCR4 on their surface[45] and that genital epithelial cells preferentially sequester X4 virus.[46] In patients infected with subtype B HIV-1, there is often a co-receptor switch in late-stage disease and T-tropic variants appear that can infect a variety of T cells through CXCR4.[47] These variants then replicate more aggressively with heightened virulence that causes rapid T cell depletion, immune system collapse, and opportunistic infections that mark the advent of AIDS.[48] Thus, during the course of infection, viral adaptation to the use of CXCR4 instead of CCR5 may be a key step in the progression to AIDS. A number of studies with subtype B-infected individuals have determined that between 40 and 50 percent of AIDS patients can harbour viruses of the SI and, it is presumed, the X4 phenotypes.[49][50]

HIV-2 is much less pathogenic than HIV-1 and is restricted in its worldwide distribution. The adoption of "accessory genes" by HIV-2 and its more promiscuous pattern of coreceptor usage (including CD4-independence) may assist the virus in its adaptation to avoid innate restriction factors present in host cells. Adaptation to use normal cellular machinery to enable transmission and productive infection has also aided the establishment of HIV-2 replication in humans. A survival strategy for any infectious agent is not to kill its host but ultimately become a commensal organism. Having achieved a low pathogenicity, over time, variants more successful at transmission will be selected.[51]

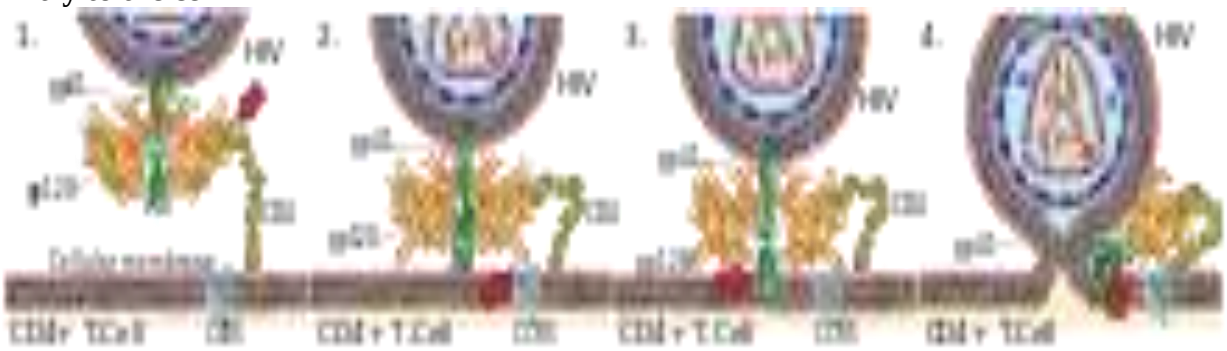
Replication cycle

2017



The HIV replication cycle

Entry to the cell



Mechanism of viral entry

1. Initial interaction between gp120 and CD4. **2.** Conformational change in gp120 allows for secondary interaction with CCR5. **3.** The distal tips of gp41 are inserted into the cellular membrane. **4.** gp41 undergoes significant conformational change; folding in half and forming coiled-coils. This process pulls the viral and cellular membranes together, fusing them.

The HIV virion enters macrophages and $CD4^+$ T cells by the adsorption of glycoproteins on its surface to receptors on the target cell followed by fusion of the viral envelope with the cell membrane and the release of the HIV capsid into the cell.[52][53]

Entry to the cell begins through interaction of the trimeric envelope complex (gp160 spike) and both CD4 and a chemokine receptor (generally either CCR5 or CXCR4, but others are known to interact) on the cell surface.[52][53] gp120 binds to integrin $\alpha_4\beta_7$ activating LFA-1 the central integrin involved in the establishment of virological synapses, which facilitate efficient cell-to-cell spreading of HIV-1.[54] The gp160 spike contains binding domains for both CD4 and chemokine receptors.[52][53]

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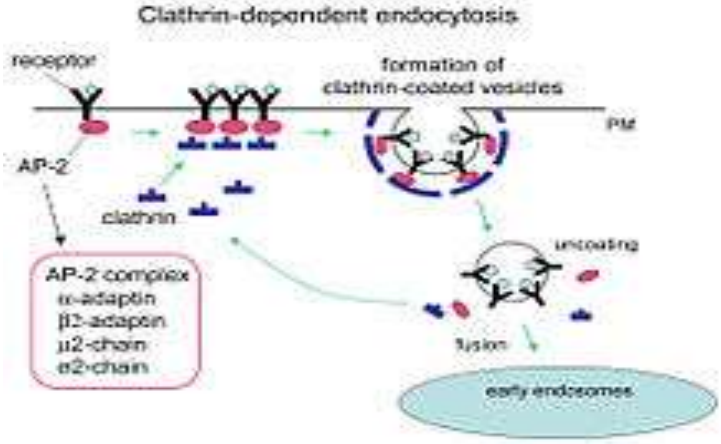
The first step in fusion involves the high-affinity attachment of the CD4 binding domains of gp120 to CD4. Once gp120 is bound with the CD4 protein, the envelope complex undergoes a structural change, exposing the chemokine binding domains of gp120 and allowing them to interact with the target chemokine receptor.[52][53] This allows for a more stable two-pronged attachment, which allows the N-terminal fusion peptide gp41 to penetrate the cell membrane.[52][53] Repeat sequences in gp41, HR1, and HR2 then interact, causing the collapse of the extracellular portion of gp41 into a hairpin. This loop structure brings the virus and cell membranes close together, allowing fusion of the membranes and subsequent entry of the viral capsid.[52][53]

After HIV has bound to the target cell, the HIV RNA and various enzymes, including reverse transcriptase, integrase, ribonuclease, and protease, are injected into the cell.[52][*not in citation given*] During the microtubule-based transport to the nucleus, the viral single-strand RNA genome is transcribed into double-strand DNA, which is then integrated into a host chromosome.

HIV can infect dendritic cells (DCs) by this CD4-CCR5 route, but another route using mannose-specific C-type lectin receptors such as DC-SIGN can also be used.[55] DCs are one of the first cells encountered by the virus during sexual transmission. They are currently thought to play an important role by transmitting HIV to T-cells when the virus is captured in the mucosa by DCs.[55] The presence of FEZ-1, which occurs naturally in neurons, is believed to prevent the infection of cells by HIV.[56]

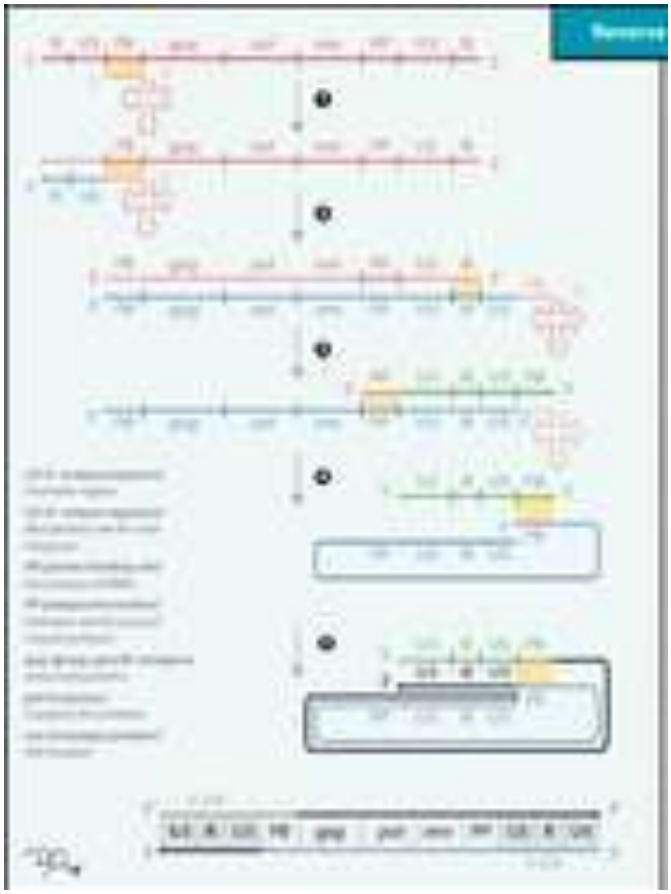


Clathrin-dependent endocytosis

HIV-1 entry, as well as entry of many other retroviruses, has long been believed to occur exclusively at the plasma membrane. More recently, however, productive infection by pH-independent, clathrin-dependent endocytosis of HIV-1 has also been reported and was recently suggested to constitute the only route of productive entry.[57][58][59][60][61]

Replication and transcription

Shortly after the viral capsid enters the cell, an enzyme called *reverse transcriptase* liberates the single-stranded (+)RNA genome from the attached viral proteins and copies it into a complementary DNA (cDNA) molecule.[62] The process of reverse transcription is extremely error-prone, and the resulting mutations may cause drug resistance or allow the virus to evade the body's immune system. The reverse transcriptase also has ribonuclease activity that degrades the viral RNA during the synthesis of cDNA, as well as DNA-dependent DNA polymerase activity that creates a sense DNA from the *antisense* cDNA.[63] Together, the cDNA and its complement form a double-stranded viral DNA that is then transported into the cell nucleus. The integration of the viral DNA into the host cell's genome is carried out by another viral enzyme called *integrase*.[62]



Reverse transcription of the HIV genome into double strand DNA

This integrated viral DNA may then lie dormant, in the latent stage of HIV infection.[62] To actively produce the virus, certain cellular transcription factors need to be present, the most important of which is NF-κB (NF kappa B), which is upregulated when T-cells become activated.[64] This means that those cells most likely to be killed by HIV are those currently fighting infection.

During viral replication, the integrated DNA provirus is transcribed into RNA, some of which then undergo RNA splicing to produce mature mRNAs. These mRNAs are exported from the nucleus into the cytoplasm, where they are translated into the regulatory proteins Tat (which encourages new virus production) and Rev. As the newly produced Rev protein accumulates in the nucleus, it binds to full-length, unspliced copies of virus RNAs and allows them to leave the nucleus.[65] Some of these full-length RNAs function as new copies of the virus genome, while others function as mRNAs that are translated to produce the structural proteins Gag and Env. Gag proteins bind to copies of the virus RNA genome to package them into new virus particles.[66]

HIV-1 and HIV-2 appear to package their RNA differently[*citation needed*]. HIV-1 will bind to any appropriate RNA[*citation needed*]. HIV-2 will preferentially bind to the mRNA that was used to create the Gag protein itself.[67]

Recombination

Two RNA genomes are encapsidated in each HIV-1 particle (see Structure and genome of HIV). Upon infection and replication catalyzed by reverse transcriptase, recombination between the two genomes can occur.[68][69] Recombination occurs as the single-strand (+)RNA genomes are reverse transcribed to form DNA. During reverse transcription the nascent DNA can switch multiple times between the two copies of the viral RNA. This form of recombination is known as copy-choice. Recombination events may occur throughout the genome. From 2 to 20 events per genome may occur at each replication cycle, and these events can rapidly shuffle the genetic information that is transmitted from parental to progeny genomes.[69]

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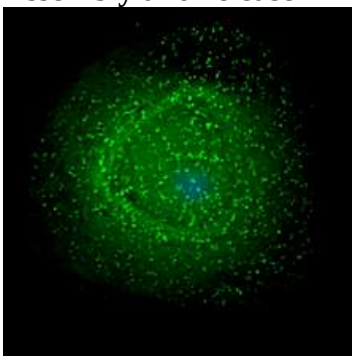
Viral recombination produces genetic variation that likely contributes to the evolution of resistance to anti-retroviral therapy.[70] Recombination may also contribute, in principle, to overcoming the immune defenses of the host. Yet, for the adaptive advantages of genetic variation to be realized, the two viral genomes packaged in individual infecting virus particles need to have arisen from separate progenitor parental viruses of differing genetic constitution. It is unknown how often such mixed packaging occurs under natural conditions.[71] Bonhoeffer et al.[72] suggested that template switching by the reverse transcriptase acts as a repair process to deal with breaks in the ssRNA genome. In addition, Hu and Temin[68] suggested that recombination is an adaptation for repair of damage in the RNA genomes. Strand switching (copy-choice recombination) by reverse transcriptase could generate an undamaged copy of genomic DNA from two damaged ssRNA genome copies. This view of the adaptive benefit of recombination in HIV could explain why each HIV particle contains two complete genomes, rather than one. Furthermore, the view that recombination is a repair process implies that the benefit of repair can occur at each replication cycle, and that this benefit can be realized whether or not the two genomes differ genetically. On the view that that recombination in HIV is a repair process, the generation of recombinational variation would be a consequence, but not the cause of, the evolution of template switching.[72]

HIV-1 infection causes chronic ongoing inflammation and production of reactive oxygen species.[73] Thus, the HIV genome may be vulnerable to oxidative damages, including breaks in the single-stranded RNA. For HIV, as well as for viruses generally, successful infection depends on overcoming host defensive strategies that often include production of genome-damaging reactive oxygen. Thus, Michod et al.[74] suggested that recombination by viruses is an adaptation for repair of genome damages, and that recombinational variation is a byproduct that may provide a separate benefit.

Assembly and release



HIV assembling on the surface of an infected macrophage.

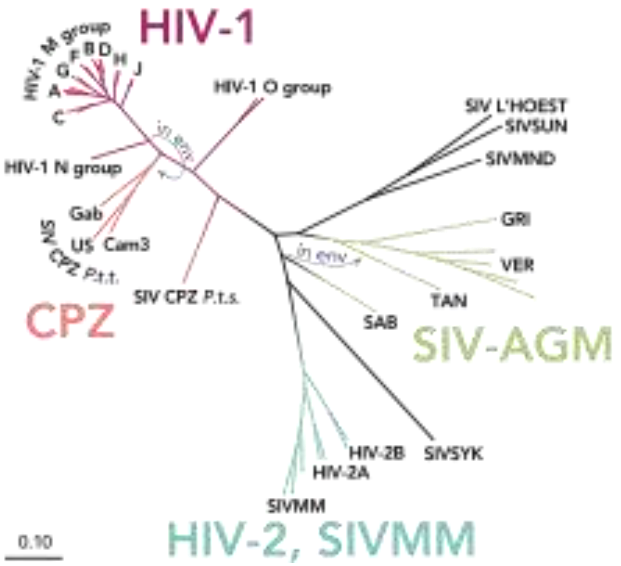
The final step of the viral cycle, assembly of new HIV-1 virions, begins at the plasma membrane of the host cell. The Env polyprotein (gp160) goes through the endoplasmic reticulum and is transported to the Golgi complex where it is cleaved by furin resulting in the two HIV envelope glycoproteins, gp41 and gp120.[75] These are transported to the plasma membrane of the host cell where gp41 anchors gp120 to the membrane of the infected cell. The Gag (p55) and Gag-Pol (p160) polyproteins also associate with the inner surface of the plasma membrane along with the HIV genomic RNA as the forming virion begins to bud from the host cell. The budded virion is still immature as the gag polyproteins still need to be cleaved into the actual matrix, capsid and nucleocapsid proteins. This cleavage is mediated by the also packaged viral protease and can be inhibited by antiretroviral drugs of the protease inhibitor class. The various structural components then assemble to produce a mature HIV virion.[76] Only mature virions are then able to infect another cell.

Spread within the body

HIV is now known to spread between CD4+ T cells by two parallel routes: cell-free spread and cell-to-cell spread, i.e. it employs hybrid spreading mechanisms.[77] In the cell-free spread, virus particles bud from an

infected T cell, enter the blood/extracellular fluid and then infect another T cell following a chance encounter.[77] HIV can also disseminate by direct transmission from one cell to another by a process of cell-to-cell spread. Two pathways of cell-to-cell transmission have been reported. Firstly, an infected T cell can transmit virus directly to a target T cell via a virological synapse.[54][78] Secondly, an antigen presenting cell (APC) can also transmit HIV to T cells by a process that either involves productive infection (in the case of macrophages) or capture and transfer of virions *in trans* (in the case of dendritic cells).[79] Whichever pathway is used, infection by cell-to-cell transfer is reported to be much more efficient than cell-free virus spread.[80] A number of factors contribute to this increased efficiency, including polarised virus budding towards the site of cell-to-cell contact, close apposition of cells which minimizes fluid-phase diffusion of virions, and clustering of HIV entry receptors on the target cell to the contact zone.[78] Cell-to-cell spread is thought to be particularly important in lymphoid tissues where CD4+ T lymphocytes are densely packed and likely to frequently interact.[77] Intravital imaging studies have supported the concept of the HIV virological synapse *in vivo*.[81] The hybrid spreading mechanisms of HIV contribute to the virus's ongoing replication against antiretroviral therapies.[77][82]

Genetic variability



The phylogenetic tree of the SIV and HIV

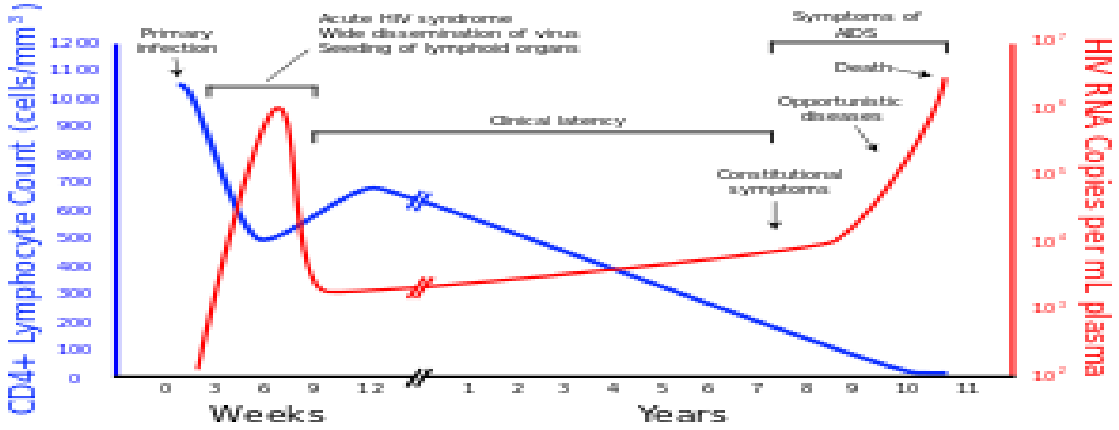
HIV differs from many viruses in that it has very high genetic variability. This diversity is a result of its fast replication cycle, with the generation of about 10^{10} virions every day, coupled with a high mutation rate of approximately 3×10^{-5} per nucleotide base per cycle of replication and recombinogenic properties of reverse transcriptase.[83][84][85]

This complex scenario leads to the generation of many variants of HIV in a single infected patient in the course of one day.[83] This variability is compounded when a single cell is simultaneously infected by two or more different strains of HIV. When simultaneous infection occurs, the genome of progeny virions may be composed of RNA strands from two different strains. This hybrid virion then infects a new cell where it undergoes replication. As this happens, the reverse transcriptase, by jumping back and forth between the two different RNA templates, will generate a newly synthesized retroviral DNA sequence that is a recombinant between the two parental genomes.[83] This recombination is most obvious when it occurs between subtypes.[83]

The closely related simian immunodeficiency virus (SIV) has evolved into many strains, classified by the natural host species. SIV strains of the African green monkey (SIVagm) and sooty mangabey (SIVsmm) are thought to have a long evolutionary history with their hosts. These hosts have adapted to the presence of the

virus,[86] which is present at high levels in the host's blood but evokes only a mild immune response,[87] does not cause the development of simian AIDS,[88] and does not undergo the extensive mutation and recombination typical of HIV infection in humans.[89]
In contrast, when these strains infect species that have not adapted to SIV ("heterologous" hosts such as rhesus or cynomologus macaques), the animals develop AIDS and the virus generates genetic diversity similar to what is seen in human HIV infection.[90] Chimpanzee SIV (SIVcpz), the closest genetic relative of HIV-1, is associated with increased mortality and AIDS-like symptoms in its natural host.[91] SIVcpz appears to have been transmitted relatively recently to chimpanzee and human populations, so their hosts have not yet adapted to the virus.[86] This virus has also lost a function of the Nef gene that is present in most SIVs. For non-pathogenic SIV variants, Nef suppresses T-cell activation through the CD3 marker. Nef's function in non-pathogenic forms of SIV is to downregulate expression of inflammatory cytokines, MHC-1, and signals that affect T cell trafficking. In HIV-1 and SIVcpz, Nef does not inhibit T-cell activation and it has lost this function. Without this function, T cell depletion is more likely, leading to immunodeficiency.[91][92]
Three groups of HIV-1 have been identified on the basis of differences in the envelope (*env*) region: M, N, and O.[93] Group M is the most prevalent and is subdivided into eight subtypes (or clades), based on the whole genome, which are geographically distinct.[94] The most prevalent are subtypes B (found mainly in North America and Europe), A and D (found mainly in Africa), and C (found mainly in Africa and Asia); these subtypes form branches in the phylogenetic tree representing the lineage of the M group of HIV-1. Coinfection with distinct subtypes gives rise to circulating recombinant forms (CRFs). In 2000, the last year in which an analysis of global subtype prevalence was made, 47.2% of infections worldwide were of subtype C, 26.7% were of subtype A/CRF02_AG, 12.3% were of subtype B, 5.3% were of subtype D, 3.2% were of CRF_AE, and the remaining 5.3% were composed of other subtypes and CRFs.[95] Most HIV-1 research is focused on subtype B; few laboratories focus on the other subtypes.[96] The existence of a fourth group, "P", has been hypothesised based on a virus isolated in 2009.[97] The strain is apparently derived from gorilla SIV (SIVgor), first isolated from western lowland gorillas in 2006.[97]
HIV-2's closest relative is SIVsm, a strain of SIV found in sooty mangabees. Since HIV-1 is derived from SIVcpz, and HIV-2 from SIVsm, the genetic sequence of HIV-2 is only partially homologous to HIV-1 and more closely resembles that of SIVsm.[*citation needed*][98]

Diagnosis



A generalized graph of the relationship between HIV copies (viral load) and CD4 counts over the average course of untreated HIV infection; any particular individual's disease course may vary considerably.
$CD4^+$ T cell count (cells per µL)
HIV RNA copies per mL of plasma

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Many HIV-positive people are unaware that they are infected with the virus.[99] For example, in 2001 less than 1% of the sexually active urban population in Africa had been tested, and this proportion is even lower in rural populations.[99] Furthermore, in 2001 only 0.5% of pregnant women attending urban health facilities were counselled, tested or receive their test results.[99] Again, this proportion is even lower in rural health facilities.[99] Since donors may therefore be unaware of their infection, donor blood and blood products used in medicine and medical research are routinely screened for HIV.[100]

HIV-1 testing is initially by an enzyme-linked immunosorbent assay (ELISA) to detect antibodies to HIV-1. Specimens with a nonreactive result from the initial ELISA are considered HIV-negative unless new exposure to an infected partner or partner of unknown HIV status has occurred. Specimens with a reactive ELISA result are retested in duplicate.[101] If the result of either duplicate test is reactive, the specimen is reported as repeatedly reactive and undergoes confirmatory testing with a more specific supplemental test (e.g., western blot or, less commonly, an immunofluorescence assay (IFA)). Only specimens that are repeatedly reactive by ELISA and positive by IFA or reactive by western blot are considered HIV-positive and indicative of HIV infection. Specimens that are repeatedly ELISA-reactive occasionally provide an indeterminate western blot result, which may be either an incomplete antibody response to HIV in an infected person or nonspecific reactions in an uninfected person.[102]

II BSc Microbiology (2016-2019 batch)						
MEDICAL MICROBIOLOGY - 16MBU402						
Unit III Question	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**		
The genus/genera that doesnot b	Staphyloc	Micrococ	Planococc	diplococcus		
Identify the bacteria which is ox	Staphyloc	Streptococ	Neisseria	Pseudomonas		
The coagulase test is used to dif	other stapl	streptococ	micrococc	enterococci		
Scalded skin syndrome is caused	Streptococ	Staphyloc	Propioniba	Pseudomonas aeruginosa		
The ability to clot blood plasma is one of the most reliable laboratory tests available for the identification of which of the following organisms?	Escherichi	Streptococ	Staphyloc	Haemophilus influenzae		
Streptococcus pyogenes are mor	amoxycilli	penicillin	erythromy	bacitracin		
the disease common in dockwor	hide porte	gas gangre	edeme	toxemia		
_____ strains usually secret	micrococc	streptococ	staphyloc	E coli		
Vegetative cells of Bacilli are d	60C	40C	20C	10C		
The spores of Bacillus anthracis	20	30	40	60		
Lepra bacilli have been found to	100	80	46	20		
The Lepra bacilli seen in large n	Common	Multibacil	Gas gangr	Symptomatic disease		
was the first effective che	rifampicin	clofazimin	ethionami	dapsone		
_____ vaccine was used to p	BCG	Polio vacc	Leprosy v	Rabies vaccine		
The Lepra bacillus was first obs	Jenner	Behring	Pasteur	Hansen		
The diphtheria bacillus was first	Pasteur	Hansen	Klebs	Loeffler		
Typing in Pneumococcus may b	serum typi	Quellung	Bamboo-s	stickland		
Corynebacterium exists in a ___	Rod	Cocci	Varied	Spindle shaped		
On repeated subculture Pneumo	smooth-ro	rough-smo	smooth	rough		
is always a seconda	meningitis	hemorrhag	paralysis	Broncho-pneumonia		
The strain used to produce the d	Bacillus C	Park Willi	Park Willi	Bacillus Calmette 8 strain		
The diagnostically important co	Bile solub	serum solu	symptoms	pathogeicity		
The incubation period of Lepra	5 to 6	2 to 5	4 to 9	1 to 3		
The BCG vaccine used to preve	Fernandez	Pasteur	Jenner	Hansen		
Preventation of anthrax in anima	prophlaxi	treatment	active imn	antibiotics		
reaction is useful for the	M'Fadyear	Quellung	Nagler	immunization		
Staphylococci are lysed under th	Penicillin	streptomy	amoxycilli	dapsone		
is a typical of Staphycocca	pus	edema	focal supp	impetigo		
Streptococcal sore throat is com	sore throa	Strep thro	infection	disease		
named the strains Stap	Jenner	Behring	Rosenbach	Klebs		
Typical Staphylococci are seen	pus	blood	stool	urine		
is the selective medium use	PLET	blood agar	Mac-conk	PDA		
In cultures Bacillus were arrang	chain	Bamboo-s	cluster	thread-like		
The of Corynebacterium	exotoxin	antitoxin	size	shape		
type of complications a	fever	headache	paralytic	nausea		
Food poisoningby Clostridium p	fish	egg	soup	meat		
Pneumococci were first noticed	Nagler	Robert Ho	Pasteur an	Kleb and Loeffler		
The toxin produced by virulent	exotoxin	endotoxin	antitoxin	toxin		

The drug of choice for treating g	Penicillin	streptomy	metranida	Amoxycillin		
The irregularly stained boat or l	citron bod	pleomorpl	irregular b	boat bodies		
The arrangement of diphtheria b	V shaped	L shaped	Chinese	Bamboo- stick		
The causative agent of gas gang	streptococ	micrococc	Clostridiu	Corynebacterium		
Clostridium species can grow w	PLET	Robertson	PDA	Mac-conkey		
_____ dyes are used to stain Pn	Acidic dye	Basic dyes	Neutral dy	Aniline dyes		
The most important antigen of P	Type spec	Type spec	Type spec	Type specific antibody		
When sputum is not available	blood	serum	serum coa	egg		
is an antigeniccompone	lipopolysa	teichoic ac	lactate	iron		
Staphylococcus cause_____	Tumour	Boils	Lesion	Fever		
Staphylococcus were first obser	Vonreckli	Ogsten	Passet	Pasteur		
Staphylococcus citreus produce	Red	Blue	Brown	Yellow		
Non virulent Staphylococcus is	Water	Hospital	Skin	Hair		
Mannitol is fermented by _____	Virulant	Avirulant	Commens	Normal flora		
Oil paint appearance is seen in_	Nutrient b	Nutrient a	Blood aga	Blood broth		
In intoxication the disease is cau	Toxoid	Tetroid	Enzyme	Toxin		
Alpha haemolysis means _____	Complete	Irregular	Partial	Nolysis		
Beeta haemolysis means _____	Complete	Irregular	Partial	Nolysis		
Selective media for Staphyloco	Manittol s	Mac conk	Blood aga	LJ agar		
Streptococcus shows _____	Alpha	Beta	Gamma	delta		

Answer			
diplococcus			
Staphylococcus			
other staphylococci			
Staphylococcus aureus			
Staphylococcus aureus			
bacitracin			
hide porter's disease			
Staphylococcus aureus			
60C			
60			
46			
Multibaciliry disease			
dapsone			
BCG			
Hansen			
Klebs			
Quellung			
Varied			
smooth-rough			
Broncho-pneumonia			
Park Williams 8 Strain			
Bile solubility			
2 to 5			
Fernandez			
active immunization			
M'Fadyean's			
Penicillin			
focal suppuration			
sore throat			
Rosenbach			
pus			
PLET			
Bamboo-stick			
antitoxin			
paralytic			
meat			
Pasteur and Sternberg			
exotoxin			

metranidazole			
pleomorphs			
Chinese			
Clostridium perfringens			
Robertson's cooked meat			
Aniline dyes			
Type specific capsular polysaccharide			
serum coated laryngeal swabs			
teichoic acid			
Lesion			
Vonreckling hausen			
Yellow			
Skin			
Virulant			
Nutrient agar			
Toxin			
Partial			
Complete			
Manittol salt agar			
Beta			

2018

II B. Sc Microbiology –Medical Microbiology – 16MBU402
(2016-2019 Batch, IV semester)

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 fungal and 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 $\leq$ 2 hours.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

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 ampule and 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.

Decubitus ulcer – A swab is not the specimen of choice. Cleanse surface with sterile saline. Submit tissue or aspirate inflammatory material from the base of the ulcer in a sterile tube or anaerobic system. Transport time ≤2 hours.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

Ear

. 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. Obtain sample 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) by rolling 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.

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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 a sterile 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.

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2018

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 with remaining saline to clear secretions.

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

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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 laboratory immediately.

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 by PCR: For each 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 of collection.

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.

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2018

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 of three 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.

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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.

II BSc Microbiology (2016-2019 batch)					
MEDICAL MICROBIOLOGY - 16MBU402					
Unit IV Question	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**	
Which of the following is the be	E coli	Streptococ	Clostridiu	Salmonella	
_____is a common cause	Salmonell	Enterotoxi	Staphyloc	Klebsiella	
With reference to infections wit	Enteroagg	Enterohen	Enteroinva	Enterotoxigenic E coli is a c	
E coli produces _____	acid alone	acid and g	gas alone	No acid No gas	
Transmission of intestinal disea	airborne	blood cont	fecal-oral	animals	
E coli is an _____ in humans	Pathogen	predator	Parasite	commensal	
*E coli exhibits IMViC*_____	++--	--++	+-+-	++++	
The K antigen in E coli is comp	Protein	lipid		carbohydrate	
_____is known as travelers	EPEC	ETEC	EHEC	EAEC	
EHEC is also known as_____	EPEC	EAEC	VTEC	EXEC	
SIDS is seen in case of _____	*E coli*	*Klebsiella*	*Proteus*	*Pseudomonas*	
Klebsiella is_____	Non motil	motile and	Non motil	motile and non capsulated	
Klebsiella exhibits_____ c	Dry	mucoid	Pale	diffuse	
K pneumoniae is also known as	Jansen's b	Koch's ba	Friedlande	Escherich's bacilli	
Klebsiella exhibits IMViC____	++--	--++	+++	----	
The tribe proteae are_____	Fermentor	non ferm	Late ferme	early fermentors	
The proteae is classified into__	One	two	three	Four	
Proteus exhibits _____	Swarming	no	Fish in str	Darting	
The predominant aerobic bacter	Non spori	non acid f	viruses	Gram negative bacilli	
The clinical picture of dysentery	*Mycobacte*	*Pseudomo*	*Klebsiella*	*Shigella*	
Shigella is _____	Flagellate	sporing	capsulated	Non motile	
The selective medium used for S	Deoxycho	EMB	MSA	Martin Thayer	
The Shigella culture filtrates der	Hypersens	Lytic	Chemotox	Neurotoxicity	
The minimum infective dose for	10-50 baci	10-100 ba	100-1000	1-10 bacilli	
Bacillary dysentery has an incub	6 hours	1 day	1-7 days	more than 7 days	
The main features of bacillary d	Rice water	presence o	Abdomina	Loose scanty feces	
The infection with *Salmonella* i	Malaise	gastric ulc	Septicemi	Enteric fever	
*Salmonella is known as*_____	Eberth gaf	Shiga	Friedlande	Escherich	
Salmonella typhi is the causativ	Typhoid f	paratypho	Enteric fev	Malaise	
The incubation period of *Salmo*	6 hours	1 day	1-7 days	7-14 days	
The infective dose for *Salmonel*	1-10 bacil	100000 ba	10000000	10000 bacilli	
Vibrio is _____rods	Helical	Elongated	Twisted	Curved	
Vibrio cholerae was first isolate	Pasteur	Koch	Paccini	Boyd	
Vibrio is_____	Motile	Non motil	Slime	Capsulated	
Vibrio cholerae are_____ro	Gram posi	Non motil	Spore forr	curved, Cylindrical	
_____Is used as tran	Alcohol m	Acid	sea water	V R Medium	
Vibrio colonies may be easily ic	Biochemic	String test	Cultural cl	Coombs test	
Heiberg classified Vibrios into_	2	4	6	8	
The route of infection with Vibr	Oral	respiratory	Ingestion	inhalation	
*Pseudomonas is motile by*_____	polar	bipolar	peritrichou	Atrichous	
*Vibrio is motile by*_____	Atrichous	peritrichou	polar	Lopotrichous	
Glycocalyx is composed of ___	Protein	lipid	Polysacch	carbohydrate	
*Pseudomonas produces*_____	Ruby	lucosin	Pyocynani	Verdin	

Pyocyanin is a _____________	Blue	green	Red	yellow		
Pyocynanin is soluble in______	Acid	Base	chloroforn	ether		
Fluorescin is_________ colored	Greenish y	Green	red	blue		
Pyorubin is__________colored	yellow	Green	red	blue		
Pyomelanin is________ colored	brown	red	Green	yellow		
Pseudomonas aeruginosa produ	Pyocyanin	melanin	rubin	verdin		
The term 'blue pus' is associate	*Proteus*	*Pseudomo*	*E coli*	*Vibrio*		
_________is used as selective r	Citrimide	EMB	DCA	MSA		
enteric fever is caused by _____	Salmonell	S paratyp	S enterica	Proteus		
EHEC strains are able to secrete	verotoxin	exotoxin	endotoxin	exfoliate toxin		
_________isolated influenza ba	Andrews a	Koch	Boyd	Pasteur		
The accessory factors required b	X and Y	X and V	X and Z	Y and Z		
_________ enzyme aids the inv	pectinase	elastase	protease	amylase		
__________is the drug of choic	Chloramip	Bacitracin	ceftazidim	Streptomycin		
selective media for Pseudomona	Mac conke	blood agar	PLET	Dettol agar		
*T pallidum causes*__________	Syphilis	typhoid	Tuberculo	pertusis		
T pallidum are highly sensitive	Acid	Antiseptic	drying	antibiotic		
Pseudomonas mainly causes__	Primary in	secondary	re- infectio	nosocomial infection		

Answer							
E coli							
Enterotoxigenic E coli							
Enteroinvasive E coli produces a disease similar to salmonellosis							
acid and gas							
fecal-oral route							
Parasite							
++--							
Polysaccharide							
ETEC							
VTEC							
E coli							
Non motile and capsulated							
mucoid							
Friedlander’s bacilli							
--++							
non fermentors							
three							
Swarming							
Gram negative bacilli							
Shigella							
Non motile							
Deoxycholate citrate agar							
Neurotoxicity							
10-100 bacilli							
1-7 days							
Loose scanty feces							
Enteric fever							
Eberth gaffky							
Typhoid fever							
7-14 days							
100000000 bacilli							
Curved							
Koch							
Motile							
curved, Cylindrical							
V R Medium							
String test							
6							
Oral							
polar							
polar							
Polysaccharide							
Pyocynanin							

Blue							
chloroform , Water							
Greenish yellow							
red							
brown							
Pyocyanin							
Pseudomonas							
Citrimide agar							
Salmonella typhii							
verotoxin							
Andrews and Laidlaw							
X and V							
elastase							
Chloramiphenicol							
Dettol agar							
Syphilis							
drying							
nosocomial infection							

2018

II B. Sc Microbiology –Medical Microbiology – 16MBU402
(2016-2019 Batch, IV semester)

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

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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.

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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

4.1 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 susceptibility testing.
* It is low in sulphonamide, trimethoprim, and tetracycline inhibitors.
* It gives satisfactory growth of most nonfastidious pathogens.
* 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.

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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 0.005 ml of antibiotics to each disc.

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 cold disks.
* 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.

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* 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 bacterial inoculum.
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 described here.

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.

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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.
2. 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.

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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 zone diameter.
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.

4.2 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 -20^{o}C .Once taken out,they should not be refrozen or reused.

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

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

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 37^oC. Inoculate a tube containing 2ml broth with the organism and keep at $+4^oC$ 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.

Prepared by: Dr. S. Dinesh Kumar, Assistant Professor, Dept of Microbiology, KAHE

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 37^oC overnight. The tubes are also incubated overnight at 37^oC. 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 37^oC 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 or slow bactericidal activity.
- No growth- if the whole inoculum has been killed
- 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^{o}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 start the 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.

4.3 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 diffusion

Müeller -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

1. 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 discs on 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.
4. 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.

II BSc Microbiology (2016-2019 batch)					
MEDICAL MICROBIOLOGY - 16MBU402					
Unit V Question	**Opt 1**	**Opt 2**	**Opt 3**	**Opt 4**	
which of the following is non –	Entamoeb	Entamoeb	Balantidiu	Trichomonas vaginalis	
Amphotericin B is effective trea	Leishmani	Amoebias	Naegleria	Acanthamoeba infection	
Which of the following is not ar	Albendazo	Tetracycli	Trimethop	Pyrimethamine	
Which of the following is not a	Naegleria	Giardia	Leishmani	Dientamoeba	
Montenegro skin test used to be	Kala-azar	Hydatid di	Toxocaria	Cysticercosis	
Amoebiasis can mimic in clinica	Appendici	Schistoson	Toxocaria	Cysticercosis	
Entamoeba histolytica gains acc	Lymphatic	Portal syst	Direct inv	Through perineural space	
In case of hepatic amoebiasis ES	Normal	Almost alv	Below nor	Variable	
Meningoencephalitis due to Nae	Amphoter	Chloroqui	Metronida	Tetracycline	
Malabsorption seen in giardiasis	Mechanica	Poor enzy	Insolublisi	Direct invasion	
Which of the following use pseu	Ciliates	Amoebae	Flagellates	Microsporidia	
A host that harbours the larval o	Definite h	Intermedia	Reservoir	None of the abo	
Ribbon like helminth parasites a	Tapeworm	Flukes	Round wo	Amoeba	
The test for an accurate faecal e	Direct wet	Permanent	PCR	Serological test	
The best test to distinguish amo	Ultra soun	Good phys	CT scan	Serological evidence of E. histo	
There is 100 percent infection ir	100 or mo	1,000 or m	1,000 or m	100,000 or more	
A 'pear shaped' trophozoite is n	Entamoeb	Giardia lar	B.coli	Dientamoeba fragilis	
A 30-years –old male who is ana	Leishmani	Histoplasn	Toxoplasn	Toxocara canis	
Blood smears for suspected mal	Just after t	Before the	When the	At night	
After initiation of specific treatn	Within 6 h	Within 24	within a w	After one more febrile attack	
Which of the following is not a	Potassium	Iodine cry	Ethyl alco	Water	
All the following are advantages	Good over	Easy to pr	Long shel	Preserves trophozoites	
A series of 3 stool specimens fo	2 days	4 days	7days	10 days	
The aldehyde test is based on th	Altered W	Altered all	Decreased	. Antigen antibody complement	
"Eating at the same table" would	Parasitism	Symbiosis	Commena	Mutualism	
A chest X-ray in cases of pneur	Cavitation	Nodular d	Ground gl	Lobar infiltration	
Treatment of choice for vaginiti	Metronida	Thiabenda	Piperazine	Chloroquine	
The partner of a Patient who su	Only if sy	Only if ex	Always be	No need of treatment	
What percentage of males infect	Nil	5-10%	10-15%	50-60%	
Chagas' disease is usually treate	Nifurtimo	Niclosami	Spraziqua	Ivermectin	
Stains used for parasite observat	Gomori's	Iron hema	Both (a) &	Giemsa Stain	
Using blood films which of the	Trichomor	Entameob	Plasmodiu	Ascaris	
. …… Film allows	Thick	Thin	Hot	Cold	
…….. film is used to ide	Thick	Thin	Hot	Cold	
Cerebrospinal fluid is collected	Spinal	Bone mar	Liver	Spleen	
The adult worm of *Chlonorchis*	Intrahepat	Lung pare	Urinary bl	Lumen of Intestine	
The drug of choice for treating c	Pyrimetha	Metronida	Tetracycli	There is no effective treatment	
The drug of choice for balantidi	Metronida	Oxytetracy	Chloroqui	Albendazole	
Which of the following drug use	Invermect	Metronida	suramin	mefloquine	
Nucleus of Entamoeba coli has	Central ka	Peripheral	No karyos	Many karyosomes	
Parasites causing human infecti	Two	Three	Four	Five	
Protozoan parasites consists of .	Multiple	Single	Row	Double	
Protozoan parasites that infect h	Apicompl	Microspor	Kinetopla	Retortamonadida	

A parasite that lines inside the b	Endoparas	Ectoparasi	Obligate p	Facultative parasit		
…………………. Technique is	Floatation	Sedimenta	Evaporatio	Concentration		
Which of the following causes a	Entamoeb	Entamoeb	Entamoeb	Naeglaria fowleri		
Which of the following causativ	Plasmodiu	Brugia ma	Wuchereri	Loa loa		
Ticks are the vectors in which o	Malaria	Babesiosis	Loiasis	Chagas' disease		
All the following parasites can b	Leishmani	Plasmodiu	Plasmodiu	Balantidium coli		
Which of the following requires	Taeniasoli	Diphyllob	Hymenole	Taenia saginata		
The invasion of tissue by parasit	IgM	Protein	Aminoaci	Peptidoglycan		
Carbolic acid is the name comm	Phenol	Methyl alc	Ethyl alco	Potassium dichromate		
Parasite F test is used for the dia	Filariasis	Falciparun	Amoebias	Cysticercosis		
Entero-test capsule is used for sa	Duodenal	CSF	Urogenita	Rectal smear		
Modified acid fast staining is us	Leishmani	Isospora b	Pneumoys	Entamoeba fragilis		
The lenth of adult worm of Dipl	2 metres	5 metres	10 metres	20 metres		
Chronic infecton with which of	Trypanosn	Trypanoso	Leishman	Leishmania donovani		
Adult worms of which of the fo	Loa loa	Dracuncul	Onchocer	Brugia malayi		
Amastigotes of leishmania are fo	Liver pare	In- vitro cu	Salivary g	Human mononuclear phagoecyt		
Cysts of all the following intesti	Entamoeb	Entamoeb	Iodamoeba	Entamoeba hartm		

Answer					
Entamoeba coli					
Amoebiasis					
Albendazole					
Naegleria					
Kala-azar					
Appendicitis					
Portal system					
Almost always elevated					
Amphotericin B					
Mechanical barrier to absorption					
Amoebae					
Intermediate host					
Tapeworms					
Permanent stained side					
Serological evidence of E. histolytica					
100 or more					
Giardia lamblia					
Leishmania donovani					
When the diagnosis is first suspected					
Within 24-48 hours					
Ethyl alcohal					
Preserves trophozoites					
10days					
Altered albumin: globulim ratio					
Commenalism					
Cavitation					
Metronidazole					
Always be treated					
10-15%					
Nifurtimox					
Both (a) & (b)					
Plasmodium					
Thick					
Thin					
Spinal					
Intrahepatic bile duct					
There is no effective treatment					
Oxytetracycline					
Invermectin					
Peripheral or eccentric karyosome					
Two					
Single					
Apicomplexa					

Endoparasite					
Sedimentation					
Naeglaria fowleri					
Loa loa					
Babesiosis					
Balantidium coli					
Diphyllobothrium latum					
IgM					
Phenol					
bFalciparum malaria					
Duodenal conte					
Pneumoystis carini					
10 metres					
Trypanosma cruzi					
Onchocerca volvulus					
Human mononuclear phagoecytes					
Iodamoeba butschlii					

Reg. No. : ----------

[16MBU402]

KARPAGAM ACADEMY OF HIGHER EDUCATION

(Under Section 3 of UGC Act 1956)

COIMBATORE – 641 021

B.Sc. DEGREE EXAMINATION, JANUARY 201 8

DEPARTMENT OF MICROBIOLOGY

I INTERNAL TEST - FOURTH SEMESTER

MEDICAL MICROBIOLOGY

Time: 2 hours **Maximum: 50 marks**

Date / Session:

PART-A

Multiple Choice Questions No.1 to 20 **20 x 1 = 20 marks**

1_____ causes diarrhea, urinary tract infections, bacteremia and meningitis.

A. *Vibrio cholerae* B. *E.coli*

C. *Salmonella typhi* D. *Serratia*

2. Bacteria are sensitive to _____

A. Interleukins B. Interferons

C. Antibiotics D. Antitumours

3. _____media is used for cultivation of bacteria

A. Nutrient agar B. MacConkey agar

C. EMB agar D. MHA

4. The cocci which forms a bunch and irregular pattern are

A. *Staphylococci* B. diplococci

C. Tetracocci D. *Streptococci*

5. *Streptococcus pyogens* produces all of the following lesions, except

A. Impetigo B. Erysipeals

C. Boil D. Paronchia

6. Causative agent of Scarlet fever

A. *Staphylococcus aureus* B. *Streptococcus viridans*

C. *Streptococcus. pyogens* D. *Streptococcus mutans*

7. Rheumatic fever is most commonly caused by

a. *Streptococcus viridans* b. *Streptococcus pyogens*

c. *Staphylococcus aureus* d. *Klebsiella pneumoniae*

8. Penicillin is the drug of choice for

A. Scarlet fever B. Whooping cough

C. Brucellosis D. Cholera

9. Endotoxin produced by gram negative bacteria is present in

A. Peptidoglycan B. Lipopolysacharide

C. Teichoic acid D. Inner membrane

10. The bacterial culture prepared by pure culture method is

A. Inoculum B. Suspension

C. Dilution D. colonies

11. Isolation is

A. Purification of culture B. Introduction of inoculum

C. Separation of a single colony D. To grow microorganisms on surfaces

12. The condition required for autoclave

A. 121oC temp.and 15 lbs. pressure for 20 min.

B. 120oC temp.and 20 lbs. pressure for 30 min

C. 150oC temp.for 1 hr.

D. 130oC temp for 2 hr.

13. Lysozyme is effective against
    A. Gram negative bacteria B. Gram positive bacteria
    C. Protozoa D. Helminthes
14. Blood agar medium is
    A. Enrichment medium B. Enriched medium
    C. Selective medium D. Differential medium
15. Lyophilization means
    A. Sterilization B. Freeze-drying
    C. Burning to ashes D. Exposure to formation
16. Temperature used for hot air oven is
    A. 100oC for 1 hour B. 120oC for 1 hour
    C. 160oC for 1 hour D. 160oC for 1 hour
17. Agar is obtained form
    A. Brown algae B. Red algae
    C. Green algae D. Blue-green algae
18. A gram positive organism which produces swarming on culture medium is
    A. Salmonella B. Clostridium
    C. Staphylococci D. Proteus
19. Enhancement of virulence in bacteria is known as
    A. Pathogenicity B. Attenuation
    C. Exaltation D. Toxigenicity
20. Spores are killed by
    A. 70% alcohol B. Glutaraldehyde
    C. Autoclaving D. acid

Part B

Answer all the questions **3x2 = 6 marks**

21. Define infection and invasion
22. What is nosocomial infection?
23. How the diseases/ infections are spreaded?

Part C

Answer all the questions **3x8 = 24 marks**

24. A. Explain in short about normal microflora of human body
    Or
    B. Write in detail about processing of samples

25. A. Explain in short about host – pathogen interactions.
    Or
    B. Write in detail about the collection of clinical samples.

26. A. Give a detailed note on Streptococcal infections
    Or
    B. How the mycobacterium causes infection?

****