

MCB 301 – BACTERIOLOGY – LECTURE NOTE

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THE DIAGNOSIS OF INFECTIONS DISEASE

It is the physician's responsibility to suspect infectious disease in patients and to initiate studies to confirm or reject this suspicion. Patients with infectious disease may present with a variety of signs and symptoms, some overt and easy to recognize, others observe and possibly misleading.

The diffuse redness and swelling of the throat or tonsils, purulent discharges from wounds or mucous membranes, and the accumulation of pus in abscesses or body cavities, often resulting in pain, swelling, and increased heat to the area, are direct signs of infection calling for an immediate culture to establish the causative organism so that appropriate therapy may be started.

Cough, increased sputum production, burning on urination and dysentery are indirect signs that infection may involve deep organ systems. Fever, chills, flushing (i.e. vasodilation) and increase in pulse rate may be general.

Laboratory values suggesting the presence of infectious disease in patients with minimal or early symptoms include an elevation in the erythrocyte sedimentation rate, peripheral blood leucocytosis or monocytosis, and alterations such as elevations in gamma globulin or the presence of type specific antibodies.

Characteristics of Infectious Diseases

The infectious diseases have characteristic signs and symptoms signs are objective changes in the body, for example fever. On these basis of a disease can be recognized. Symptoms are the subjective changes for example pain, loss of appetite, etc. which are felt by the

patients. In a broad sense symptom is used for sign as well. In addition, a disease syndrome includes a set of signs and symptoms due to a particular disease; for example an AIDS patient experiences disease syndrome (*syndrome – a set of symptoms which occur together i.e. a symptom complex*).

Moreover, the characteristic symptoms of a disease develop during certain phases. The knowledge of the phases helps in the recognition of a disease. For example INCUBATION PERIOD which refers to time required after infection to the appearance of signs/symptoms. Incubation period varies from organism to organism. Second is the PRODRIMAL STAGE i.e. the period during which there is onset of signs and symptoms of a disease. Third, the PERIOD OF ILLNESS which is a phase during which the disease gets fully established and becomes most severe with characteristic signs and symptoms. The last characteristic phase is the period of decline when signs and symptoms disappear and the disease is recovered gradually. This stage is known as convalescence.

Transmission of Pathogens

For perpetration of disease and survival of the pathogen transmission from one host to the other occurs by any of the four main routes: air-borne, contact, vehicle and vector-borne.

1. **AIR BORNE TRANSMISSION:** The pathogens remain suspended in air and are transmitted through droplet which is small particles (1-4 m diameter) left from evaporation of large particles lies in the . The droplet mules remain in air for hours or days and carried to individuals because the pathogens cannot grow in air. Example of some air-borne diseases are chicken pox, flu, measles, mumps, viral pneumonia, diphtheria, pneumonia, tuberculosis, meningitis, etc.

2. **CONTACT TRANSMISSION:** Some of pathogens are spread when contact of the host is done with the reservoir of pathogen. In other words contact refers to person-to-person contact through touching, kissing or sexual contact. The diseases that spread through contact are humps and boils (through contact of oral secretions or body lesions), infection of staphylococcus (by nursing mothers), and AIDS and syphilis (through placenta, or blood to blood contact).
3. **VEHICLE TRANSMISSION:** Vehicle refers to inanimate materials such as utensils, towels, beddings, surgical materials, needles, food, water, etc. Bacterial spreading through food and causing food poisoning are *Staphylococcus aureus*, *Bacillus cereus*, *Escherichia coli*, *Vibrio cholera*, *Salmonella typhi*, *Clostridium difficile*, etc.
4. **VECTOR-BORNE TRANSMISSION:** A living organisms that transmit a pathogen is known as vector such as vertebrates (e.g. dogs, cat, bats, goat, sheep, etc.) or arthropods (e.g. flies, mites, insects, ticks, etc.). For example, flies carry flagella on their feet from faeces to food materials. Moreover, when pathogen does not undergo morphological and physiological changes within the vector is called harborage transmission e.g. the plague pathogen, *Yersinia pestis*. When the pathogen undergo morphological changes within the vector, it is called biological transmission e.g. *Plasmodium vivax*.

Control of Infectious Diseases

Since the infectious diseases are spread by several agents and cause epidemics, it can be controlled by one or several measures. This involves the breaking of the links of disease cycle, eliminating the reservoirs of the disease and making the individuals resistant (i.e. immunization).

1. **Breaking the links of disease cycle:** The pathogens survive on inanimate or animate for some time from where they are transmitted to suitable host. Therefore, if links between

two stages of disease cycle are broken further spread of the pathogen does not occur. This includes general sanitation methods.

- a) pasteurization of milk (b) destruction of vectors by spraying insecticides
 - c) chlorination of water supply (d) inspection of food and individuals handling it.
2. **Elimination of source of infections:** The source of infection can be eliminated by (a) adopting quarantine (legal prohibition of entry of goods, animals, etc. from one country to other or one state to other within a country) and isolating the carriers (b) destruction of animal reservoir (e.g. the cattle infected with foot and mouth disease virus are killed in other countries) (c) treatment of sewage (to check water-borne transmission of pathogens) and (d) use of chemicals by individuals to eliminate the pathogens.
3. **Immunization of Individuals:** For increasing the level of immunity (vaccination) mass immunization programmes are very good means of controlling infectious diseases such as polio, whooping cough, pertuency or tetanus (DPT), etc.

Proof of the etiology of a Disease

The isolation of a particular organism from an infected person does not establish proof that it is the causative agent of the disease. It may exist in or near a lesion merely as normal flora or as a transient contaminant. In the early days of bacteriology some rules were laid down to establish whether or not an isolated organism was indeed the pathogen. These rules, called koch's postulate, can be summarized as follows:

1. The same organism must be found in all cases of a given disease.
2. The organism must be isolated and grown in pure culture from the infected person.
3. The organism from the pure culture must reproduce the disease when inoculated in to a susceptible animal.

4. The organism then must be isolated in pure culture from the experimentally infected animal.

Although these postulates are effective in determining the causative agents of most bacterial diseases, there are a few exceptions. For example, *Treponema pallidum* has been established as the causative agent of syphilis, but the organism has never been grown on artificial media. Similarly, *Mycobacterium leprae* has never been grown in the laboratory and, therefore, fits only the first postulate. Some vital diseases also fall into a category in which the last three postulates not always fulfilled.

How Pathogens Enter and Leave the Body

Each organism capable of producing disease has its own portal or portals of entry as well as a means of escaping from the host. The infectious agent in discharges from infected areas must be destroyed to prevent the transmission of the agent to a new host.

Microorganisms have been found to enter the body through the following areas:

1. Gastrointestinal tract via mouth. Examples include agent responsible for diseases such as typhoid fever, paratyphoid fever, dysentery, cholera, poliomyelitis, and infectious hepatitis, as well as food illnesses.
2. Respiratory tract via nose and mouth. This is the portal of entrance of all microbes causing respiratory diseases such as the common cold, measles, pneumonia and tuberculosis.
3. Skin and mucous membranes. Although the skin provides an effective protective barriers, minor breaks are undoubtedly always present that may allow the entrance of certain organisms. The staphylococcus that causes boil (furuncles) is one the more

frequent organisms entering in this way, however, the streptococci also may cause spreading skin infections.

4. Genitourinary system. The mucous membranes of the genital tract are the common site for invasion by venereal disease agents such as those causing syphilis and gonorrhoea. In addition, the urinary tract may be infected by microorganisms in the blood, or by their infection into the bladder during catheterization.
5. Blood. Those organisms that must be introduced directly into the blood in order to cause disease usually are transmitted from one individual to another by insect bites. The best known examples of diseases in this category are malaria and yellow fever – both transmitted by the mosquito. Other include the rickettsial diseases. Inadequate sterilization of needles and syringes can cause direct blood inoculation of hepatitis (particularly Hepatitis B) and Human immune deficiency virus (HIV).

The portals of exit for a disease agent are usually the same as their portal of entry. Thus, diseases of the respiratory tract are spread by way of secretion and excretions of the respiratory tract and mouth. In like manner, enteric infections (typhoid fever, polio myelitis, dysentery, etc.) leave the body by the intestinal tract and the spread through faecal contamination. Blood infections, which are spread by insects or contaminated needles and syringes, usually leave the individual in a similar manner, i.e. through direct contamination of a needle or syringe during the withdrawal of blood or the ingestion of the microorganism by a biting insect.

Staphylococci

The staphylococci are gram positive cocci. Staphylococci are isolated most frequently from skin, boils, abscesses, etc. In general, under the right conditions they can infect almost any tissue of the body. Some strains of *Staphylococcus aureus* produce a potent enterotoxin which can cause food poisoning in man.

The purp from boils, abscesses, or other infectious material is streaked over a plate of agar, preferably blood agar, because many of the staphylococci are hemolytic (produce hemolysins which lyse erythrocytes).

Microscopic examination of the isolated organism and a study of the pigmentation of the colony are usually sufficient for identification to species. The colonies of *Staphylococcus aureus* are golden in colour and 5100 medium, a selective medium for staphylococci, demonstrates this golden pigment very well. Colonies of *Staphylococcus epidermidis* are white. A final test used in the identification of staphylococci is the coagulase test which differentiates the highly pathogenic *Staphylococcus aureus* from the usually non-pathogenic *Staphylococcus epidermidis*. *Staphylococcus aureus* is coagulase positive, that is, will cause plasma to clot or coagulate. *Staphylococcus epidermidis* is coagulase negative.

The Streptococci

The streptococci are gram positive cocci which characteristically grow in chain formation and sometimes in pairs. They are non notice and non-spore forming. Many strains of beta-hemolytic streptococci produce capsules. The capsules are present only in the very young cultures. The streptococci in general are quite fastidious in their cultural requirements and need an enriched medium containing blood or serum.

From the standpoint of medical bacteriology streptococci are considered under the following headings:

1. Hemolytic streptococci (beta): Produce complete hemolysis or clear areas around the bacterial colony.

Group A – responsible for most of the streptococcal infections in man (*Streptococcus pyogenes*).

Group B, C, F, G, H, K and L – other – harmless inhabitants of mucous membranes or occasional incitants of human infection.

Group E, M and N – occur only in lower animals.

2. Viridans streptococci (alpha): Produces partial hemolysis or green areas around bacterial colony.

By biochemical characteristics they are divided into several species. The most common of which are *Streptococcus salivarius* and *Streptococcus mitis* both common throat organisms. Both can give rise to subacute bacterial endocarditis (SBE).

3. Non-hemolytic streptococci (gamma): No hemolysis whatsoever around bacterial colony.

4. Enterococci (Group D): The example of organism in this group is *Streptococcus faecalis*. They may be beta hemolytic, non-hemolytic or produce “greening” of blood agar. *Streptococcus faecalis* is identified by the presence of growth in media containing 0.5% sodium azide and 6.5% NaCl at 45°C. These conditions are lethal to other streptococcus species.

- Note:** (1) The typical beta hemolytic streptococcus colony is surrounded by a zone of clear hemolysis. Two types of beta hemolysis are released: (a) streptolysin O which is destroyed by atmospheric oxygen is therefore demonstrated only in deep colonies and (b) streptolysin S which is oxygen stable and is responsible for surface colony hemolysis.
- (2) Beta hemolysis alone does not mean that organism is group A streptococci from the other groups is their susceptibility to Bacitracin. A bacitracin disc is placed on a blood agar plate seeded with the unknown beta hemolytic streptococcus. Group A streptococci (*Streptococcus pyogenes*) are sensitive to bacitracin and a zone of growth inhibition will be produced around the disc. Non group A streptococci will fail to show growth inhibition around the bacitracin disc.

Diplococcus pneumonia

The pneumococci in their most typical form are encapsulated, gram positive diplococci. In pus, body fluid, sputums and body tissues they may be found in short chain and occasionally as individual cocci. Morphologically the pneumococci may be very hard if not impossible to distinguish from the streptococci.

On blood agar, the encapsulated organisms form round glistening unpigmented and mucoid colonies. The pneumococci colonies are surrounded by a zone of alpha hemolysis (green) and must be differentiated from alpha hemolytic streptococci. The various methods employed in the laboratory to differentiate *Diplococcus pneumonia* from alpha hemolytic streptococci are:

1. Optochin disc – contains ethylhydrocupreine hydrochloride, an antibacterial drug which inhibits the growth of pneumococci but not streptococci. This is the method most commonly used in diagnostic laboratories since it is quite simple and accurate.

2. Bile solubility – The pneumococci are bile soluble and when a suspension of the organisms is added to the bile salt, sodium deoxycholate, the organisms will lyse (turbidity will disappear). Streptococci are not bile soluble and will not lyse in the presence of sodium deoxycholate.
3. Mouse virulence – The encapsulated. Pneumococci are highly virulent for mice whereas alpha hemolytic streptococci are non virulent.
4. Insulin fermentation – pneumococci ferment the sugar insulin – most strains of streptococci do not.
5. Capsular swelling test – Quelling reaction: All virulent pneumococci have capsules and can be identified or differentiated on the basis of the reaction of their capsule with specific antibody. When the capsular antigen reacts with its specific antibody the capsule becomes readily visible under the microscope and appears to have enlarged or increased in size.

The Meisseria

They are gram negative diplococcic. The pathogenic species, *Meisseria gonorrhoeae* causes gonorrhoea, endocarditis, arthritis and conjunctitis in new born. Also *Meisseria meningitidis* cause cerebrospinal meningitis. Other species that are non pathogenic *Meisseria catarrhalis* and *Meisseria sicca*/

Messeiria organisms produce indophenols oxidase. This enzyme oxidizes dimethyl or tetra-methhyl p-pheonylenediamine hydrochloride to form a colored compound. When a drop of the reagent solution is placed on a colony of gonococcus, meinigococcus or some other Meisseria, that colony shows a color changes from pink to purple to blue black. *This test, therefore, can be used to detect oxidase positive colonies among the mixed growth.*

Confirmation is obtained by gram staining smear examination and biochemical tests.

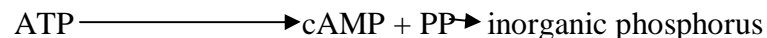
	Clue	Maltose	Lactose	Sucrose	Oxidase
<i>N. gonorrhoeae</i>	+	-	-	-	+
<i>N. meningitidis</i>	+	+	-	-	+

Also serologic test with known specific antigen could be used to confirm.

Escherichia coli

Although *Escherichia coli* is part of the normal flora of the intestinal tract, for years it was suspected of causing an occasional moderate to severe diarrhea in humans and animals. It is now established that various *E. coli* strains may cause diarrhea by either of two mechanisms: (1) by production of enterotoxins that indirectly cause fluid loss and (2) by the actual invasion of the epithelial lining of the intestinal wall, causing inflammation and fluid loss.

Enterotoxin-producing *E. coli*, called enteropathogenic *E. coli*, produce one or both of two different toxins. One is a heat-stable toxin called ST and the other is a heat-labile toxin called LT. Both toxins cause diarrhea. LT, which is destroyed by heating at 65°C for 30 minutes, has been purified, and its mode of action is identical to that of cholera toxin. LT stimulates the activity of a membrane-bound adenylyl cyclase. This results in the conversion of ATP to cyclic AMP (cAMP) as shown below:



Extremely minute amounts of cAMP will induce the active excretion of Cl⁻ and inhibit the absorption of Na⁺, collating an electrolyte imbalance across the intestinal mucosa that causes the loss of copious quantities of fluid from the intestine.

Infection with pathogenic *E. coli* may cause a severe and sometimes fatal infection in newborns. The disease in adults, known by many names such as traveller's diarrhea or Montezumia's revenge may vary from a mild disease with several days of loose stools to a severe and fatal cholera like disease.

Invasion of the blood stream by *E. coli* may result in meningitis in the newborns, the debilitated patient, patients with leukemia or those receiving immunosuppressive drugs.

Klebsiella pneumonia

Set is also called "fried Lander's bacilli". Of interest is the frequency with which it predominates in fecal cultures following oral chemotherapy and participate in urinary tract infection. *Klebsiella pneumonia* contains a large capsule and the colonies on solid media are quite large and very mucoid due to the presence of the capsule.

Lactose – Negative Group of Enteric Bacilli

The lactose negative group is of major medical importance because it contains many human and animal pathogens as follows:

1. The proteus group – normal members of the intestinal tract which act as opportunists by producing urinary tract infection. Examples

Proteus mirabilis }
Proteus vulgaris } *swarmers*

Proteus morganii

2. *Pseudomonas aeruginosa* – Produces urinary tract and wound infections.

Produces two pigment

pyocyanin (blue pus) – soluble in chloroform

fluorescin (yellow) – Not soluble in chloroform but soluble in water

3. *Salmonella typhosa*. Typhoid fever which is an acute infection beginning in lymphoid tissue of the small intestine but usually developing as a generalized infection of the body can also cause perforation of the intestine if not treated.
4. Other salmonella species – Paratyphoid fever and gastroenteritis (salmonellosis). Sometimes it can cause septisemia.
5. Shigella species – contains species causing shigellosis (Bacillary dysentery). Note that *Entamoeba histolytica* which is intestinal parasite can cause dysentery referred to as amoebic dysentery.

They are classified as

group A – *Shigella dysenteriae*

group B – *Shigella flexneri*

group C – *Shigella boydii*

group D – *Shigella sonnei*

Brucellae

The members of the genus *Brucella* are the etiological agents of brucellosis or undulant fever which occurs principally in animals. Man is infected accidentally or indirectly through contact with infected animal or their excreta (slaughterhouse workers), the ingestion of unpasteurized milk and other dairy products made from raw milk.

Three species of *Brucella* are of medical importance. *Brucella abortus* causes abortion in cattle, *Brucella mellitensis* infects sheep, and *Brucella suis* infect swine. All three species can infect any of the above animals mentioned.

Pasteurella, Francisella, Yersinia

Four species in this group of microorganisms are important as etiological agents of infection in man

1. *Yersinia pestis*: the etiological agent of plague.
2. *Yersinia enterocolitica*: a causative agent – a severe enterocolitis infection with symptoms of dysentedy.
3. *Francisella tularensis*: The etiological agent of tularemia (Rabbit fever)
4. *Pasteurella multocida*: This infects man following cat or dog bites and scratches.

Haemophilus

This is also gram negative, nonspore forming rods. The genus Hemophilus contains known pathogens for man as well as nonpathogenic species that are part of the normal flora of the mucous membranes. The following is a list of the important pathogenic species of Hemophilus and disease they produce:

H. influenza – meningitis in children, pharyncytis

H. aegyptius – conjunctivitis (pink Dye)

H. ducreys venereal disease – chancroid

H. vaginalis – vaginitis, cystitis, cervicitis

H. parainfluenzae – Prare case of endocarditis

SATELLITISM: When *Hemophilus influenza* is cultured on blood agar in conjunction with *Staphylococcus aureus* as a “spot” inoculation on the surface of the plate, colonies of *H. influenza* will become distinctly larger is the area adjacent to the staphylococcus colony. This area of heavier growth is called the “satellite phenomenon”. The staphylococci elaborate the “v” factor which enhances the growth of *H. influenza*.

H. parainfluenzae can be easily differentiated from *H. influenza* by testing for satellitism on plane nutrient agar with a *Staphylococcus aureus*. *H. parainfluenzae* will give a “satellite phenomenon” on plain nutrient agar since it does not require X factor. *H. influenza* will not grow since it needs the X factor present in blood agar.

Note: (1) V factor is produced by staph aureus to show satellitism with all hemophilus

(2) X factor is present in blood

H. influenza – needs X factor to grow

H. parainfluenzae – does not need X factor to grow

Bordetella

This is also gram negative rod. The significant pathogenic species of Bordetella is *Bordetella pertussis*, the etiologic agent of whooping cough. *Bordelella parapetussis* produces a whooping cough like disease and *Bordelella bronchiseptica* is non pathogenic for man but is associated with causine distemper.

Bordetella pertussis require Bordet-Gengu (BG) wnebum for its isolation (special medium which contains penicillin to which *Bordetella pertussis* is resistant.

The Bacillus

The members of the genus Bacillus are widely distributed in nature and are common inhabitants of the soil. They are gram positive, spore-forming aerolysis organism.

The species representing the genus as a type is *Bacillus subtilis*, which ordinarily is nonpathogenic but may involve wounds producing low grade infections and may cause conjunctivitis.

The only member that is regarded as primary pathogen is *Bacillus anthrax*. Two forms of diseases may result in an infection with *Bacillus anthracis*.

- (a) Cutaneous anthrax also called “malignant pustule”.
- (b) Pulmonary anthrax also called “wool sorters disease”

CLOSTRIDIUM

The clostridia are gram positive rods. They are anaerobic.

Clostridial infections manifest themselves in two ways: (1) Toxemia due to the production of an exotoxin (2) Tissue necrosis due to the proliferation of proteolytic enzymes.

Clostridia that cause diseases of man are:

- a) *Clostridium tetani* – Tetanus
- b) *Clostridium perfringens* – Gas gangrene
- c) *Clostridium botulinum* – Botulism

Mycobacteria

Infection with mycobacteria usually occurs in the human, however the organisms can invade almost any tissue of the body. These organisms are gram positive, however the gram stain is never used in their identification. They are called “Acid Fast Bacilli” (AFB) because they resist decolorization with acid-alcohol after staining. The procedure most frequently used is the Ziehl-Neelsen (ZN) method which utilizes carbol fuchsin, acid alcohol and methylene blue or malachite green. With the exception of the genus *Nocardia*, only the mycobacteria are acid fast.

The most important pathogenic species for man are:

- (1) *Mycobacterium tuberculosis* – the etiological agent of tuberculosis
- (2) *Mycobacterium leprae* (Hansen’s bacilli) accepted as the etiological agent of leprosy.

Has not been cultured on laboratory media.

The Proteus Group

Members of the genus proteus are non lactose fermenters which are usually highly notice. Because of their extreme motility they tends to form a thin spreading film of growth over the entire surface of solid media making it highly difficult to isolate other bacteria that may be present. This swarming tendency can be prevented by cultivating the organism on a relatively dry surface of 5% agar medium or an ordinary 1-2% agar medium containing chloral hydrate (0.1%). Gram positive organism can be isolated from a swarming proteus by inoculating a blood agar plate containing sodium oxide (0.1%) which inhibit the growth of proteus organism but not gram positive organisms. Blood agar containing 0.25% pheny ethyl alcohol (PEA) can also be used for the same purpose. Charcoal agar will decrease swarming also.

Urease production is a distinguishing metabolic characteristic of proteus group and is very helpful in the identification of these organisms.

The species most commonly encountered in the clinical laboratory are *Proteus mirabilis*, and *Proteus vulgaris* which are spreaders or warmers and *Proteus morganii* and *Proteus rettgeri* which usually do not spread or swarm on solid media. These organisms may occasionally cause an acute enteritis particularly in children. They also cause urinary tract infections and may often become the dominant organism in mixed wound infections treated with antibiotics.

The antigenic structure of *Proteus vulgaris* is of particular medical interest because strains possessing certain O antigens (O x K, O x 2, o x 19) are agglutinated by the sera from patients with vickettsial diseases. This is known as the Weit-Felix Test.

SALMONELLA

Typhoid fever is an acute infection beginning in the lymphoid tissue of the small intestine but usually developing as a generalised infection of the body, particularly the lymphatic system. The symptoms of the disease are produced by endotoxins liberated by the disintegration of the bacilli.

The genus salmonella, of which there are more than 700 species or types, produce three distinct kind of infections: (a) Enteric or paratyphoid fever, a disease clinically indistinguishable from typhoid fever (b) A gastroenteritis commonly referred to as food poisoning and (c) septicemia.

Isolation and Identification of Salmonella

Isolation: Differential media such as Eosin methyl Blue (EMB) agar or MacConkey agar serve to distinguish colonies of lactose fermenting organisms (coliforms) from those negative for lactose. But EMB agar or MacConkey agar does not favour or selectively inhibit the growth of one enteric bacillus over another. And so, the isolation from fecal specimens of the occasional salmonella or shigella organism is rendered difficult by the overgrowth of the more numerous coliforms. This problem was solved by the introduction of selective media of lipids (SS (Salmonella-Shigella) agar is an example. The principal ingredient of SS agar are lactose bile salts, brilliant green dye and a pH indicator neutral red. It not only differentiates between lactose-fermenting and lactose-negative colonies, but it also inhibits most of the coliform organisms. The production of colourless colonies on EMB, MacConkey or SS agar is a property which the Salmonella share with several other genera. Therefore, identification of a salmonella organism requires the use of other methods such as biochemical and serologic tests.

Xylose-lysine deoxycholate agar (XLD) and Hektoen Enteric agar (HE) are two other selective media used for the isolation of salmonella and shigella from stool specimens. The percentage of isolations of salmonella and shigella obtained using either of these two media is much higher when compared with SS agar and for this reason XLD and HE agars have replaced SS agar in most well developed laboratories.

Identification: Certain of the biochemical tests are used as a screening method is that they serve to indicate the probable identify of an enteric bacillus. These are lactose fermentation, glucose fermentation, sucrose fermentation, production of hydrogen sulfide, breakdown of urea and citrate utilization. The first four characteristics are combined in one medium known as Triple Sugar Iron Agar (TSI). A gram negative bacillus that is negative for lactose, sucrose and urease but is positive for glucose and H₂S is a salmonella suspect. The final identification is established by serologic method in this instance by agglutination tests which is identification of an unknown organism of a salmonella by means of known antisera. As well a type of test also commonly used as the clinical laboratory in which the reagent are reserved is available. In this case, it is the agglutination of suspension (antigen) of known organism by an unknown (patient) serum demonstrates the presence of antibody specific for the antigen. Agglutination tests of this type constitute an important laboratory step in the diagnosis of salmonella infections (WIDAL TEST).

SHIGELLA

The genus shigella are the etiology agents of shigellosis or dysentery and in contrast to typhoid fever caused by *Salmonella typhi* or Salmonellosis caused by paratyphoid bacilli or other salmonella species which may cause generalized infection. Shigellosis is primarily an infection

involving the large intestine with the production of conditions that range from a mild diarrhea to a severe and toxic dysentery. To differentiate this disease from the dysentery caused by *Entamoeba histolytica*, shigella enteritis is known as bacillary dysentery or shigellosis and the other one is known as amoebic dysentery.

The shigella are morphologically indistinguishable from the salmonella. The growth requirements of the salmonella and shigella are so similar that isolation methods are of the same general nature. The dysentery bacilli however are more delicate. For example SS agar, which is somewhat valuable for the primary isolation of salmonella, is toxic for many shigella strains. Therefore, the less inhibitive EMB or XLD and HE agars are the media of choice for shigella.

A gram-negative bacillus originating from a colorless colony on EMB or SS agar is supported of being a shigella if it is H₂S-negative, urease-negative. Final identification of the shigella is carried out by specific agglutination test using “O” antisera. The shigella are classified as:

Group A (*Shigella dysenteriae*)

Group B (*Shigella flexneri*)

Group C (*Shigella boydii*)

Group D (*Shigella sonnei*)

MYCOBACTERIA

Infection with mycobacteria usually occurs in the lungs, however the organisms can invade almost any tissue of the body. These organisms are gram positive, however the gram stain is never used in their identification. They are called “Acid fast Bacilli (AFB) because they resist decolorization with acid-alcohol after staining. The procedure most frequently used is the Ziehl-Neelsen (ZN) method which utilizes carbol fuchsin, acid alcohol and the methylene blue or

malachite green. With the exception of the genus *Moriadia*, only the *Mycobacteria* are acid fast. The most important pathogenic species for man are:

1) *Mycobacterium tuberculosis* – the etiological agent of tuberculosis and (2) *Mycobacterium leprae* – the etiological agent of leprosy (Hansen bacilli) *M. leprae* has not been cultured on laboratory media.

Neither the tuberculin test nor any serologic test gives evidence of active disease due to tubercle bacilli. Only isolation of tubercle bacilli given such proof. Acid fast bacilli stain by Ziehl-Nelsen technic or fluorescence microscopic can give presumptive evidence of tuberculosis. Sputum is first treated with 2% NaOH are liquefied sputum is then neutralized and centrifuged. The sediment could be acid fast bacilli stained and also inoculated on Lo Westein-Jensen medium for isolation of *Mycobacterium tuberculosis* after incubation for 2-8 weeks.

The final diagnosis could be done by serologic test (complement-fixation) test, agglutination test with known antibody or fluorescent antibody technique.

The most widely use antituberculosis drugs at present are isoniazid (Isonicolusic and hydrazide, INH), ethambutol, rifampin and streptomycin.

VIBRIO

Vibrio cholera is a small, slightly curve gram-negative organism possessing a single polar flagellum. The organisms have many similarities to the members of the Enterobacteriaceae but can be readily differentiated by their positive oxidase reaction and their ability to grow at pH between 9.0 and 9.5. Growth is rapid on peptone agar, blood agar with pH near 9.0 or thioglycolate-citrate bile sucrose agar. A number of serologic types have been reported, based on antigenic differences in their O antigen. Of these, three strains have been given specific

names: Inaba Ogawa and Hikojima. Those strains which produce a soluble hemolysin have been designated as EI Tor strain of *Vibrio cholera*.

Cholera is spread as faecal-oral disease and is acquired by ingestion of faecally contaminated water and food. The organisms do not spread beyond the gastrointestinal tract, where they multiply to very high concentrations in the small and large intestine. Unlike the shigella, they do not penetrate the epithelial layer but remains tightly adhere to intestinal mucosa.

The foremost symptom of cholera is a severe diarrhea in which a patient may lose as much as 10 to 15 liters of liquid per day. The faces, containing mucus, epithelial cells and large number of vibrios, have been referred to as “rice water stools”. Death, which may occur in as many as 60% of untreated cases, results from severe dehydration and loss of electrolytes.

Diarrhea from *Vibrio cholera* is the result of the secretion of an enterotoxin, called cholera toxin, that stimulates the activity of the enzyme adenyl cyclase, which converts ATP to cyclic AMP (cAMP). This activity is identical to that described for one enterotoxin produced by the enteropathogenic *Escherichia coli*, however, the two toxins are not identical and antitoxins to one does not neutralize the other. The cAMP stimulates the septron of U⁻ and inhibits the absorption of Na⁺ resulting in a cupious fluid loss and an electrolyte imbalance.

The mortality of cholera can be reduced to less than 1% by the replacement of fluids and electrolytes. The abseroation that the inclusion of glucose in a salt solution permits oral replacement of electrolytes has made treatment of this disease (particularly in rural areas) much more effective.

The organisms can be viewed in stools, particularly with a dark-field microscopy. Fluorescently labeled antiserum can be used to confirm the identification of the observed organisms.

Control of cholera requires proper sewage disposal and adequate water sanitation. Immunization with killed *vibrio cholera* or formalin treated enterotoxin appears to give some protection and recovery from the disease imparts of an unknown degree or duration. Current research efforts are directed toward the preparation of a vaccine consisting of degraded enterotoxin which will stimulate IgA antibody production to the toxin.

HAEMOPHILUS

The Haemophilus is a Gram negative bacillus which require blood for growth as their genus name implies and blood agar is used routinely for their isolation in the laboratory. It has been known that in the blood, there are true factors, designated as the X and the V factors which are required for the growth of Haemophilus. These factors are hemin and nicotinamide adenine nucleotide (NAD). The hemin (X factor) is required for the organisms to synthesize their enzymes containing heme such as cytochromes and catalase. X factor or hemin, a heat stable substance present in erythrocytes is required by all haemophilus Sp. for growth. The thermolabile V factor or NAD is not required by all haemophilus Sp. For growth.

The genus haemophilus contains known pathogens for man as well as nonpathogenic species that are part of the normal flora of the mucous membranes. The following is a list of the important pathogenic species of Haemophilus and the disease they produce.

H. influenza serotype b: Serotypes other than b seldom cause disease. It cause meningitis in children, pharyngitis, tracheobronchitis.

H. aegyptius: conjunctivitis (pink eye)

H. chancroid: venereal disease – chancroid

H. vaginalis: vaginitis, cystitis, cervicitis

H. parainfluenzae: rare case of endocarditis – pneumonitis

Haemophilus influenza will grow on blood agar and chocolate agar. Growth is not luxuriant and particularly on blood agar, the fine delicate colonies may be overlooked.

SATELLITISM: When *H. influenza* is cultured on blood agar in conjunction with *Staphylococcus aureus* as a “spot” inoculation on the surface of the plate, colonies of *H. influenza* will become distinctly larger in the area adjacent to the *Staph.* Colony. This area of heavier growth is called the satellite phenomenon. The staphylococci elaborate the V factor which enhances the growth of *H. influenza*.

H. parainfluenzae can be easily differentiated from *H. influenza* by testing for satellitism on plain nutrient agar with a *Staphylococcus aureus*. *H. parainfluenzae* will give a satellite phenomenon on plain nutrient agar since it does not require X factor. *H. influenza* will not grow on plain nutrient sign since it needs the X factor present in blood agar.

Brucellae

The member of the genus *Brucella* are the etiological agents of brucellosis or undulant fever which occurs principally in animals. Man is infected accidentally or indirectly through contact with infected animal or their excreta (slaughter house workers), the ingestion of unpasteurized milk and other dairy products made from raw milk.

Three species of *Brucella* are of medical importance. *Brucella abortus* causes abortion in cattle, *Brucella mellitensis* infects sheep and *Brucella suis* infects swine. All three species can infect any of the above animals mentioned.

Bordetella pertussis

Bordetella pertussis is the causative agent of pertussis or whooping cough. This organism was first isolated and described by Bordet and Gengou and is sometimes called Bordet-Gengou bacillus. It is a small grown negative coccobacillus which is non motile and *Bordetella*

pertussis required Bordet-Gengou (BG) medium for its isolation (special medium which contains penicillin to which *Bordetella pertussis* is resistant).

The disease whooping cough is an acute infection of the respiratory tract involving both the trachea and the bronchi. It begins with a catarrhal stage characterized by sneezing and a mild but irritating cough. The paroxysms of coughing may be so severe that cyanosis, vomiting and convulsions follow and completely exhaust the patient. Endotoxins may be released from disintegrating organisms, causing local inflammation as well as fever. The organisms also excrete a hemagglutinin, and disrupted cells release a heat-labile toxin (called pertussin) which is responsible for some of the symptoms of whooping cough. The organisms do not invade the bloodstream but remain localized in the respiratory tract. The incubation period is usually seven to ten days.

Treatment is not entirely satisfactory, but several antibiotics are of value. Chloramphenicol, erythromycin or tetracycline may be used. Antibiotic therapy causes a reduction in the number of secondary infections, such as bronchitis and pneumonia, caused by other organisms.

The introduction of an effective vaccine has markedly reduced the incidence of whooping cough. Formerly almost every child had this disease during his early years of life. Today, cases are rare. The vaccine consists of killed encapsulated phase I organisms which are usually incorporated with diphtheria and tetanus toxoids. Because of the very high mortality of the disease in infants under one year of age, the vaccine should be administered by the second or third month of life.

NOTE: The encapsulated strain of *Bordetella pertussis* is of a single antigenic type and of maximum virulence. This strain has been designated as Phase I. After this strain is cultivated

in the laboratory on artificial media, the capsule is host as a result of mutation. These stepwise ranges have been designated as Phases II, III and IV; Phase IV is entirely avirulent.

BACTEROIDES

The genus *Bacteroides* includes a number of species but the one that is of clinical significance is *Bacteroides fragilis*. *Bacteroides* are gram-negative, nonsporulating, anaerobic bacilli, which constitute a major portion of the normal flora of the gastrointestinal tract and can be found on the mucus surfaces of the nose and throat. Occasionally they are isolated in blood culture or from the wounds and joint fluids. *Bacteroides* may be difficult to culture, requiring special media and prolonged incubation. Most strains grow well in fluid thioglycollate medium but may require much as 5 days to grow.

FUSOBACTERIUM

The fusiform bacilli are spindle shaped gram negative bacilli which may be curved or straight rods, usually with tapered ends. These organisms are fastidious in their growth requirements most species are strict anaerobes. They grow under the same general environmental conditions as the *Bacteroides* species and are found as normal flora in the mouth and intestinal tract.

The pathogenicity of the fusobacteria is somewhat limited and questionable although they have been found in association with necrotic and gangrenous conditions.

The one species *Fusobacterium fusiforme* is of interest to the medical laboratory because of its appearance in increased numbers in the fuso-spirochetal infection known as Vincent's angina/Trench mouth).

SPIROCHAETES

Spirochaetes differ from bacteria mainly in that they do not have a rigid cell wall. Most spirochaetes are so thin that they cannot be seen microscopically without staining.

The spirochaetes of medical importance are contained in the following three genera:

1. **Borrelia** – may vary in thickness and the coils are irregular and more open than the other forms. They display a “lashing” motility and live strictly parasitic existence.

2. **Treponema** – show more regularity in the coils and the spirals are more tightly wound. They display a more constant graceful motility than *Borrelia*. *Treponema* live a strictly parasitic existence.

3. **Leptospira** – the coils are uniform in size, very close together and hooked on one or both ends. Their motility is mainly rotary with some lashing and gliding movement taking place intermittently. They are found free-living in contaminated water.

Borrelia vincentii is the spirochaete partly responsible for fusso-spirochaetal infection i.e. Vincents angina-infection of the gums and pulmonary abscesses. Diagnosis is made by demonstrating the spinochaetes in blood films stained by Gemba's or Leishman's method, by examining fresh preparations by dark-background illumination (Darkfield exam) mice and rats and detecting the organisms in their blood after 48 hour.

Treponema pallidum is the etiological agent of syphilis. Their spirochete does not stain with Gemsa's or Leishman's stain. It is necessary therefore to utilize special staining techniques such as the silver impregnation method in which a precipitate is built up on the organisms which renders it visible. The organism is also visible under darkfield examination. Teponema for many of the diagnostic laboratory tests are grown in rabbit testicles.

Laboratory diagnosis of syphilis is based on serologic tests using lipid antigen. The particular antibody concerned, often referred to as "regain" can be measured by Wassermann reaction, which is a complement fixation reaction, or by one of several very similar precipitation (flocculation) reactions including the Kahn test and the UDRL (Venereal Disease Research Laboratory) test.

Leptospira icterohemorrhagiae and other species of *Leptospira* cause weils disease. Various sains of *Leptospira* may be cultured on flechers medium for primary isolation laboratory diagnosis is based on the serologic tests by agglutination method and Darkfield examination of blood and urine after 14-36 days of infection in which *Leptospira* are looked for.

Weil's disease classically takes the form of an acute illness with fever, conjunctivitis albuminuria, haemorrhages and jaundice. Some patients developed meningitis.

SPIRILLUM MINUS

Spirillum minus is a rigid cork screw like organism usually display two or three undulations and bipolar flagella. In the process of motion, which is darklike, it rotates around its long axis. It is gram negative, but Giemsa stain is preferred for smears.

The organism causes sodoku or rat-bite fever, which may follow the bite of a rat, mouse or other rodent. An ulcer forms at the site of the bite followed by generalized symptoms such as lymphadenitis, fever and rash.

Laboratory diagnosis is based on demonstration of the organism in the exudates of the primary and secondary skin lesion, in blood and in smear of regional lymph nodes are blood smears are stained with Giemsa stain.

The darkfield technique is excellent for the examination of wet preparations.