LECTURE NOTES

For Medical Laboratory Technology Students

Medical Bacteriology

Ethiopia Public Health
Training Initiative

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PREFACE

Text book on Medical Bacteriology for Medical Laboratory Technology students are not available as need, so this lecture note will alleviate the acute shortage of text books and reference materials on medical bacteriology.

Since it comprises most of the contents of course outline on medical bacteriology to nursing, pharmacy and environmental science students, it can be used as a main learning material to these category of students.

This lecture note gives emphasis on the knowledge and procedures of medical bacteriology to common pathogens in our country.

At last but not least, the quality of this lecture note is kept updated by continous comments made by users of this lecture note.

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ABBEREVIATIONS

. AIDS……. Acquired immunodeficiency syndrome
. AFB ……. Acid fast bacilli
. ATP ……. Adenosine triphosphate
. CO_2…… Carbon dioxide
. CSF…… Cerebrospinal fluid
. CNS …… Central nervous system
. DNA …… Deoxy ribonucleotide
. DNase …… Deoxy ribonucleotidase
. GIT …… Gastrointestinal tract
. HIV …… Human immunodeficiency virus
. HPF …… High power field
. IP …….. Incubation period
. LGV …… Lymphogranuloma venereum
. NADase …….. Nicotinamide adenine dinucleotidase
. NB …… Notta Bonne
. O_C ……. Degree of Celsius
. P^H ……. Hydrogen ion concentration
. RBC …… Red blood cell
. RNA …… Ribonucleotide
. RPR …….. Rapid plasma reagin
. SS agar …… Salmonella-Shigella agar
. STD …….. Sexually transmitted disease
. UTI …… Urinary tract infection
. VDRL …… Venereal disease research laboratory test
. WBC …… White blood cell

CHAPTER ONE

Learning Objective

- At the end of the lesson, the student should be able to:

1. Identify the structure of bacterial cell
2. Do simple and differential staining methods
3. Describe the essential nutrients required for bacterial growth
4. Describe the mechanisms of genetic variation in bacterial cell
5. Identify the chemical meanses of sterilization and disinfection, and their effect on bacterial cell
6. Do and interpret the result of anti-microbial sensitivity testing in vitro

1.1 INTRODUCTION TO MICROBIOLOGY

Microbiology is a subject which deals with living organisms that are individually too small to be seen with the naked eye.

It considers the microscopic forms of life and deals about their reproduction, physiology, and participation in the process of nature, helpful and harmful relationship with other living things, and significance in science and industry.

Subdivision of microbiology

Bacteriology deals about bacteria.

Mycology deals about fungi.

Virology deals about viruses.

History of Microbiology

Man kind has always been affected by diseases which were originally believed to be visitations by the gods and meant to punish evil doers.

Hippocratus, father of medicine, observed that ill health resulted due to changes in air, winds, water, climate, food, nature of soil and habits of people.

Varro (117-26 BC)said a theory that disease was caused by animated particles invisible to naked eye but which were carried in the air through the mouth and nose into the body.

Fracastorius (1500 G.C.) proposed that the agents of communicable disease were living germs, that could be transmitted by direct contact with humans and animals, and indirectly by objects ; but no proof because of lacking experimental evidence.

Antony Van Leeuwenhoek (1632-1723 G.C.), father of Microbiology, observed “animalcules” using simple microscope with one lens.

He was the first who properly described the different shapes of bacteria.

Although Leeuwenhoek was not concerned about the origin of micro-organism; many other scientists were searching for an explanation for spontaneous appearance of living things from decaying meat, stagnating ponds, fermenting grains and infected wounds.

On the bases of this observation, two major theories were formulated.

1. Theory of Abiogenesis
2. Theory of Biogenesis

Theory of Abiogenesis deals with the theory of spontaneous generation; stating that living things originated from non-living things.
Aristotle (384-322 BC): The founder of a theory spontaneous generation.
He observed spontaneous existence of fishes from dried ponds, when the pond was filled with rain.
Francesco Redi (1626-1697): He is the scientist who first tried to set an experiment to disprove spontaneous generation.

- He put the meat in a bottle and covered it with a gauze.
- He observed that the flies laid eggs from which the maggots developed.
- He said maggots did not developed from meat but from flies egg.

Theory of Biogenesis states that life comes from pre-existing life.
Louis Pasteur (1822-1895 GC) was the scientist who disproved the theory of abiogenesis.
He designed a large curved flask (Pasteur goose neck flask) and placed a sterile growth broth medium. Air freely moved through the tube; but dust particles were trapped in the curved portion of flask.
Microbial growth in the broth was not seen.
Therefore Pasteur proved that micro-organisms entered to substrates through the air and micro-organisms did not evolve spontaneously.

Major contribution of Louis Pasteur

1. Microbial theory of fermentation
2. Principles and practice of sterilization and pasteurization

3. Control of diseases of silk worms
4. Development of vaccines against anthrax and rabies.
5. Discovery of streptococci

The germ theory of disease

The complete establishment of the germ theory of disease depended on the work of a German scientist, Robert Koch (1843-1910).

Major achievements of Robert Koch

1. Discovery and use of solid medium in bacteriology
2. Discovery of causative agents of tuberculosis and cholera.
3. Koch's phenomenon
4. Koch's postulates

Koch's postulates: proof of germ theory of disease

A micro-organism can be accepted as a causative agent of an infectious disease only if the following conditions are satisfied.

1. The micro-organism should be found in every case of the disease and under conditions which explain the pathological changes and clinical features.
2. It should be possible to isolate the causative agent in pure culture from the lesion.
3. When such pure culture is inoculated into appropriate laboratory animal, the lesion of the disease should be reproduced.
4. It should be possible to reisolate the bacterium in pure culture from the lesion produced in the experimental animal.
5. Now a days additional postulate is mentioned i.e.

Specific antibody to the bacterium should be detectable in the serum during the course of the disease.

It has not been possible to fulfil every one of Koch's postulates, but by adhering to them as closely as possible, serious errors have been prevented.

Exceptions to Koch's postulates

1. Many healthy people carry pathogens but do not exhibit symptoms of the disease.
2. Some microbes are very difficult or impossible to grow in vitro(in the laboratory) in artificial media. Eg. Treponema pallidum
3. Many species are species specific. Eg. Brucella abortus cause abortion in animals but no report in humans.
4. Certain diseases develop only when an opportunistic pathogen invades immunocompromised host.

1.2. THE MICROBIAL WORLD

TAXONOMIC CLASSIFICATION OF ORGANISMS

TAXONOMY is the science of organisimal classification.

Classification is the assignment of organisms (species) into anorganised scheme of naming .idealy these schemes are based on evolutionary relationships (i.e the more similar the name, the closer the evolutionary relationships). Thus, classification is concerned with:-

1. The establishment of criteria for identifying organisms & assignment to groups (what belongs where)

2. The arrangement of organisms into groups of organism of organism (e.g. At what level of diversity should a single species be split in to two or more species?).
3. Consideration of how evolution resulted in the formation these groups.

TAXON:-

- A group or category of related organisms.

Two key characteristics of taxa are:

-Members of lower level taxa (e.g. Species) are more similar to each other than are members of higher level taxa (eg.Kingdom or domain).

-Member of specific taxa are more similar to each other than any are to members of different specific taxa found at the same hierarchical level (eg. Humans are more similar to apes, i.e., comparison between species, than either is similar to, for example, Escherichia coli). Thus once you know that two individuals are member of the same taxon, you can inter certain similarities between the two organisms.

NOTE that taxa are dynamic, changing as our knowledge of organism and evolutionary relationships change

BINOMIAL NOMENCLATURE

- Organisms are named using binomial nomenclature (viruses are exceptions)
- Binomial nomenclature employs the names of the two level taxa, genus and species, to name a specie. Binomial nomenclature includes:

i. Genus comes before species (e.g., *Escherichia coli*)
ii. Genus name is always capitalized (e.g*., Escherichia*)
iii. Species name is never capitalized (e.g., *coli)*
iv. Both names are always either italicized or underlined (e.g *Escherichia coli)*
v. The genus name may be used alone, but not the species name (i.e saying or writing "*Escherichia "* alone is legitimate while saying or writing " *coli"* is not)

Strain

a) A strain in some ways is equivalent to a breed or subspecies among plants or animal. Strain is the level below the species
b) Two members of the same strain are more similar to each other than either is to an individual that is a member of a different strain, even if all three organisms are members of the same species

Bacterial species

- A bacterial species is defined by the similarities found among its members. Properties such as biochemical reactions, chemical composition, cellular structures, genetic characteristics, and immunological features are used in defining a bacterial species. Identifying a species and determining its limits presents the most challenging aspects of biological classification for any type of organism.

- A formal means of distinguishing bacterial species is by employing a dichotomous key to guide the selection of test used to efficiently determine those bacterial properties most relevant to bacterial identification

The five kingdom system

The five kingdom system was first proposed in 1969 and is showing its age

The five kingdoms include:

i. Plantae (the plants)
ii. Fungi (the fungi)
iii. Animalia (the animals)
iv. Protista (the unicellular eukaryotes)
v. Monera (the prokaryotes)

Kingdom of Monera

Three categories:

- Eubacteria

Are our common, everyday bacteria, some of which are disease – causing; also the taxon from which mitochondria originated.

- Cyanobacteria

Are photosynthetic eubacteria, the taxon from which chloroplast originated

- Archaeobacteria

Are distinctive in their adaptation to extreme environments (e.g., very hot, salty, or acidic) though not all archaeobacteria live in extreme environments.

These distinctions are more phenotypic than they are evolutionary (i.e., a cyanobacteria is a eubacteria, and neither is an archaebacteria).

Kingdom Protista

Protista like Monera consist mostly of unicellular organisms. Distinctively, however, the members of Kingdom Protista are all eukaryotic while the mebers of kingdom Monera are all prokaryotic. Some members of protista are multicellular, however Kingdom protista represents a grab bag, essentially the place where the species are classified when they are not classified as either fungi, animals or plants.

Kingdom Fungi

Unlike pprotists, the eukaryotic fungi are typically non – aquatic species. They traditionally are nutrients absorbers plus have additional distinctive features. They do exist unicellular fungi, which we call yeast

DOMAIN

The domain is a taxanomic category that, depending on point of view, is either above the level of kingdom or supercedes the kingdom. The domain system contains three members

- Eukaryotes (domain Eukarya)
- Eubacteria (domain Bacteria)
- Archaebacteria (domain Archaea)

Viral classification

Classification of viruses is not nearly as well developed as the classification of cellular organisms. Today viruses tend to be classified by their chemical, morphological and physiological attributes (e.g. genome = DNA vs RNA, virion particle = enveloped vs non enveloped and myriad details of their intracellular infection cycles). Binomial nomenclature is not employed to name viruses; instead viruses are named by their common names (e.g., Human Immunodeficiency Virus a.k.a HIV)

Dichotomous key

A means of assigning an organism to a specific taxonomic category typically involves the use of specific criteria that may posed as questions (e.g. What does the organism look like etc.). Relevant criteria may be arranged as a dichotomous key. In a dichotomous key questions are arranges hierarchically with more general questions are asked first, with questions becoming more specific asked subsequently

EUKARYOTIC CELL

Eu- true

Karyote- nucleus

The eukaryotic cell has a true membrane bound nucleus, usually containing multiple chromosomes, a mitotic apparatus, a well defined endoplasmic reticulum and mitochondria.

PROKARYOTIC CELL

Pro- primitive

Karyote- nucleus

The prokaryotic cell possesses naked DNA with out associated basic proteins, divides amitotically by binary fission and bounded by a semi rigid cell wall.

Table 1.1. The distinguishing features between Eukaryotic cell and Prokaryotic cell

Features	**Prokaryotic cell**	**Eukaryotic cell**
.Size	1μm	10μm
. Nuclear membrane	Absent	Present
. Chromosome	Single	Multiple
. Nucleolus	Absent	Present
. Histones	Absent	Present
. Sexual reproduction	Absent	Present
. Cytoplasmic ribosomes	70s	80s
. Mitochondria	Absent	Present
. Endoplasmic reticulum	Absent	Present
. Lysosomes	Absent	Present.

. Micro filaments and tubules	Absent	Present
. Site of oxidativre phosphorylation	Cell membrane	Mitochondria
. Site of photosynthesis	Cell membrane	Chloroplast
. Peptidoglycan	Present	Absent
. Cell membrane composition	Phospholipids & Proteins	Sterols

Bacterial Cell

General property:

- Typical prokaryotic cell
- Contain both DNA and RNA
- Most grow in artificial media
- Replicate by binary fission
- Almost all cotain rigid cell wall
- Sensitive to antimicrobial agent

1.3. STRUCTURE OF BACTERIA

Bacterial structure is considered at three levels.

1. Cell envelope proper: Cell wall and cell membrane.
2. Cellular element enclosed with in the cell envelope: Mesosomes, ribosomes, nuclear apparatus, polyamies and cytoplasmic granules.
3. Cellular element external to the cell envelope: Flagellum, Pilus and Glycocalyx.

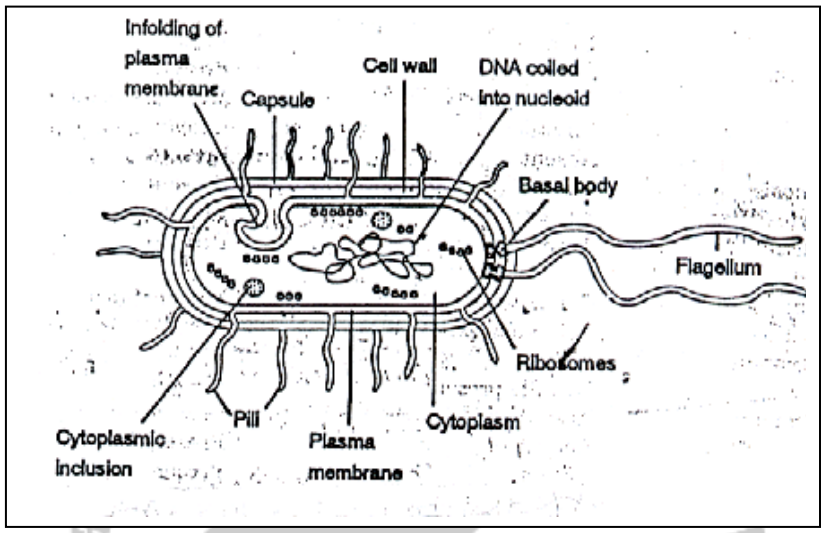


Fig. 1.1 Ultrastructure of Bacteria

1. Cell envelope proper

A. Cell wall

Multi layered structure and constitutes about 20% of the bacterial dry weight.

Average thickness is 0.15-0.5 μm.

Young and rapidly growing bacteria has thin cell wall but old and slowly dividing bacteria has thick cell wall.

It is composed of N-acetyl Muramic acid and N-acetyl Glucosamine back bones cross linked with peptide chain and pentaglycine bridge.

Components of cell wall of Gram negative bacteria

1. Peptidoglycan
2. Lipoprotein

3. Phospholipid
4. Lipopolysaccharide

Components of cell wall of Gram positive bacteria

1. Peptidoglycan
2. Teichoic acid

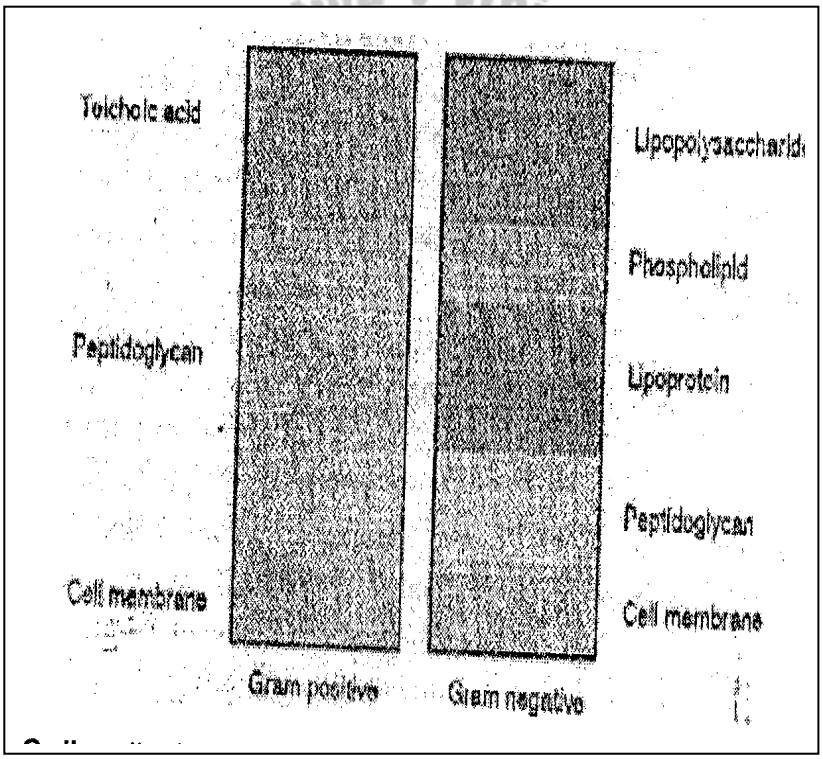


Fig. 1.2 Cell wall of Gram Positive & Gram Negative Bacteria

Functions of cell wall

1. Provides shape to the bacterium
2. Gives rigidity to the organism

3. Protects from environment
4. Provides staining characteristics to the bacterium
5. Contains receptor sites for phages/complements
6. Site of action of antibody and colicin
7. Contains toxic components to host

Bacteria with defective cell walls

Bacteria with out cell wall can be induced by growth in the presence of antibiotics and a hypertonic environment to prevent lysis.

They are of three types:

1. **Protoplasts**: Derived from Gram-positive bacteria and totally lacking cell walls; unstable and osmotically fragile; produced artificially by lysozyme and hypertonic medium: require hypertonic conditions for maintenance.

2. **Spheroplast**: Derived from Gram-negative bacteria; retain some residual but non-functional cellwall material; osmotically fragile;produced by growth with penicillin and must be maintained in hypertonic medium.

3. **L- forms**: Cell wall-deficient forms of bacteria usually produced in the laboratory but sometimes spontaneously formed in the body of patients treated with penicillin; more stable than protoplasts or spheroplasts , they can replicate in ordinary media.

B. Cell membrane

Also named as cell membrane or cytoplasmic membrane

It is a delicate trilaminar unit membrane .

It accounts for 30% of the dry weight of bacterial cell.

It is composed of 60% protein, 20-30% lipids and 10-20% carbohydrate.

Function of cell membrane

1. Regulates the transport of nutrients and waste products into and out of the cell.
2. Synthesis of cell wall components
3. Assists DNA replication
4. Secrets proteins
5. Carries on electron transport system
6. Captures energy in the form of ATP

2. Cellular element enclosed with in the cell envelope

A. Mesosomes

Convoluted invagination of cytoplasmic membrane often at sites of septum formation.

It is involved in DNA segregation during cell division and respiratory enzyme activity.

B. Ribosomes

Cytoplasmic particles which are the sites of protein synthesis.

It is composed of RNA(70%) and proteins(30%) and constitutes 90% of the RNA and 40% of the total protein.

The ribosome monomer is 70s with two subunits, 30s and 50s.

C. Polyamines

They are of three types

. Putrescin

. Spermidine

.Spermine

It is found in association with bacterial DNA, ribosomes and cell membrane.

Function of polyamines

1. Antimutagenic.
2. Prevent dissociation of 70s ribosome into subunits.
3. Increase resistance of protoplast lysis.

D. Cytoplasmic granules

. represent accumulated food reserves.

Nature of granules

. Glycogen

. Poly-beta hydroxy butyrate

. Babes-ernst (Volutin)

E. Nuclear apparatus

Well defined nucleus and nuclear membrane , discrete chromosome and mitotic apparatus are not present in bacteria ; so nuclear region of bacteria is named as nuclear body, nuclear apparatus and nucleoid.

Bacterial genome consists of single molecule of double stranded DNA arranged in a circular form.

Besides nuclear apparatus, bacteria may have extra chromosomal genetic material named as plasmids.

Plasmids do not play any role in the normal function of the bacterial cell but may confer certain additional properties(Eg. Virulence, drug resistance) which may facilitate survival and propagation of the micro- organism.

3. Cellular element external to the cell envelope

A. Glycocalyx (capsule and slime layer)

Capsule is gel firmly adherent to cell envelope.

Slime is gel easily washed off from cell envelope.

All bacteria have at least a thin slime layer.

Capsule is composed of polysaccharide and protein(D-Glutamate of *Bacillus anthracis)*

Features of capsule

1. Usually weakly antigenic.
2. Not necessary for viability.
3. Endows virulence.
4. Protects from phagocytosis.
5. Capsulated strains are invariably non-motile.
6. Visualized by negative staining and capsule staining.
7. Detected by quellung phenomenon.

B. Flagellum

It is the organ of locomotion in bacterial cell and consists of thee parts. These are .The filament

. The hook

. The basal body

The basal body and hook are embedded in the cell surface while the filament is free on the surface of bacterial cell.

Their presence in bacterial cell is detected by

- . Hanging drop preparation
- . Swarming phenomenon on surface of plate agar
- . Motility media
- . Special staining methods
  - . Silver impregnation methods
- . Dark –field microscopy
- . Electron microscopy

Size: 3-20μm in length and 0.01-0.013μm in diameter.

It is composed of protein named as flagellin.

The flagellar antigen in motile bacterium is named as H (Hauch) antigen.

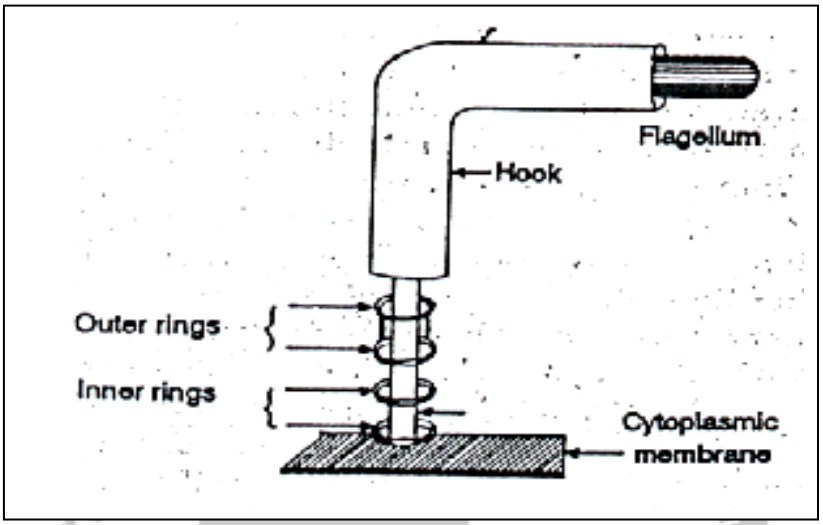


Fig. 1.3 Components of Bacterial Flagellum

Flagellar arrangements

1. Atrichous: Bacteria with no flagellum.
2. Monotrichous: Bacteria with single polar flagellum.
3. Lophotrichous: Bacteria with bunch of flagella at one pole.
4. Amphitrichous: Bacteria with flagella at both poles.
5. Peritrichous: Bacteria with flagella all over their surface.

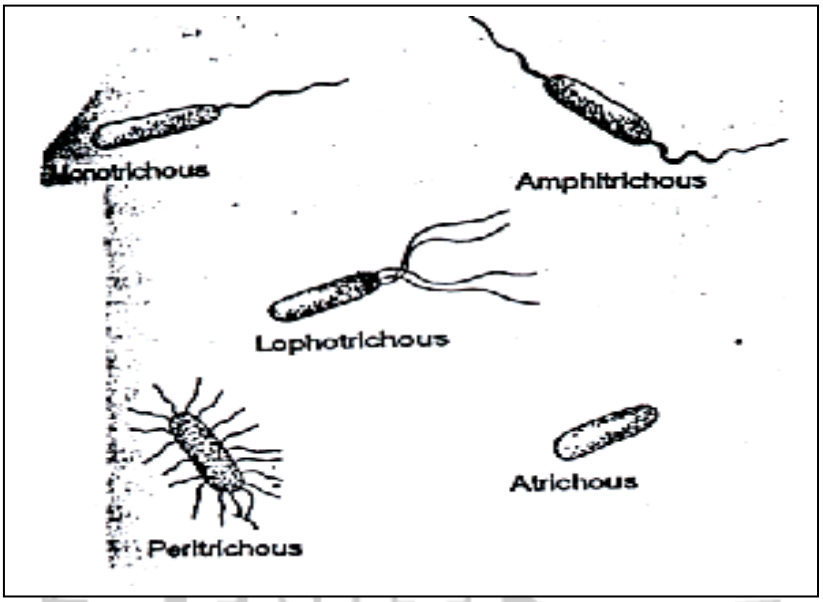


Fig. 1.4 Flagellar arrangements

Endoflagella (axial filament)

It is the organ of motility found in periplasmic space of spirochetes.

C. Pili (fimbriae)

It is hair like structure composed of protein (pilin)

Two types (Based on function)

. Common pili: The structure for adherence to cell surface.

. Sex pili: The structure for transfer of genetic material from the donor to the recipient during the process of conjugation.

Table 1.2. Comparison between flagella and pili

Character	Flagella	Pili
. Size	Large	small
. Thickness	+++	+
. Origin	Cell membrane	Cell wall
. Organ of locomotion	+	–
. Organ of adhesion	–	+
. Required for conjugation	–	+

D. Spores

Resting cells which are capable of surviving under adverse environmental conditions like heat, drying, freezing, action of toxic chemicals and radiation.

Bacterial spore is smooth walled and oval or spherical in shape.

It does not take up ordinary stains.

It looks like areas of high refractivity under light microscope.

It is significant in spread of disease and indicator of sterility of materials.

Spores are detected by

. Simple staining methods

. Special staining methods

Arrangements of spores

1. No bulging of cell wall
   . Oval central
   . Oval sub terminal

. Spherical central

2. Bulging of cell wall
   . Oval sub terminal
   . Oval terminal
   . Spherical terminal
   . Free spore

1.4. Classification of bacteria

Bacterial classification depends on the following characteristics.

1. Morphology and arrangement
2. Staining
3. Cultural characteristics
4. Biochemical reactions
5. Antigenic structure
6. Base composition of bacterial DNA

Morphology and staining of bacteria are the commonly used characteristics to classify bacteria.

1. Morphology of bacteria

When bacteria are visualized under light microscope, the following morphology are seen.

1. Cocci (singular coccus): Round or oval bacteria measuring about 0.5-1.0μmb in diameter.They are found insingle, pairs, chains or clusters.

2. Bacilli (singular bacillus): Stick-like bacteria with rounded, tepered, square or swollen ends; with a size measuring 1-10μm in length by 0.3-1.0μm in width.
3. Coccobacilli (singular coccobacillus): Short rods.
4. Spiral: Spiral shaped bacteria with regular or irregular distance between twisting.
   Eg. Spirilla and spirochaetes

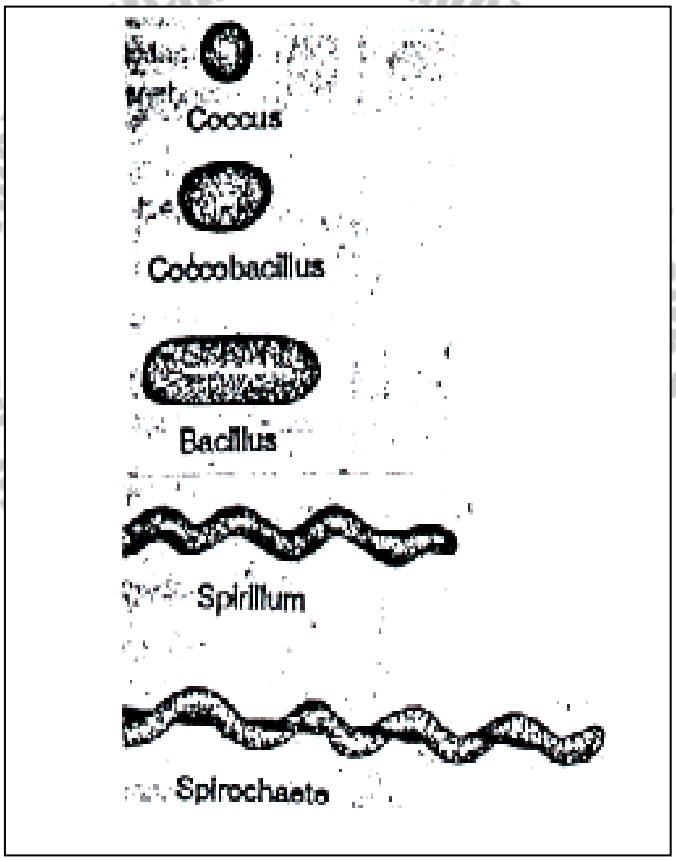


Fig. 1.5 Morphology of Bacteria

2. Staining of bacteria

Bacterial staining is the process of coloring of colorless bacterial structural components using stains (dyes).The principle of staining is to identify microorganisms selectively by using dyes, fluorescence and radioisotope emission.

Staining reactions are made possible because of the physical phenomena of capillary osmosis, solubility, adsorption, and absorption of stains or dyes by cells of microorganisms.

Individual variation in the cell wall constituents among different groups of bacteria will consequently produce variations in colors during microscopic examination.

Nucleus is acidic in character and hence, it has greater affinity for basic dyes. Whereas, cytoplasm is basic in character and has greater affinity for acidic dyes.

There are many types of affinity explaining this attraction force:

1. hydrophobic bonding
2. reagent-cell interaction
3. reagent-reagent interaction
4. ionic bonding
5. hydrogen bonding
6. covalent bonding

Why are stains not taken up by every microorganism?

Factors controlling selectivity of microbial cells are:

1. number and affinity of binding sites
2. rate of reagent uptake
3. rate of reaction
4. rate of reagent loss (differentiation or regressive staining)

Properties of dyes

Why dyes color microbial cells?

Because dyes absorb radiation energy in visible region of electromagnetic spectrum i.e., “light”(wave length 400-650). And absorption is anything outside this range it is colorless. E.g., acid fuschin absorbs blue green and transmit red.

General methods of staining

1. Direct staining

Is the process by which microorganisms are stained with simple dyes. E.g., methylene blue

2. Indirect staining – is the process which needs mordants.

A mordant is the substance which, when taken up by the microbial cells helps make dye in return, serving as a link or bridge to make the staining recline possible.

It combines with a dye to form a colored “lake”, which in turn combines with the microbial cell to form a “ cell-mordant-dye-complex”.

It is an integral part of the staining reaction itself, without which no staining could possibly occur. E.g., iodine.

A mordant may be applied before the stain or it may be included as part of the staining technique, or it may be added to the dye solution itself.

An accentuator, on the other hand is not essential to the chemical union of the microbial cells and the dye. It does not participate in the staining reaction, but merely accelerate or hasten the speed of the

staining reaction by increasing the staining power and selectivity of the dye.

Progressive staining

- is the process whereby microbial cells are stained in a definite sequence, in order that a satisfactory differential coloration of the cell may be achieved at the end of the correct time with the staining solution.

Regressive staining

- with this technique, the microbial cell is first over stained to obliteratethe cellulare desires, and the excess stain is removed or decolorized from unwanted part.

Differentiation (decolorization)

- is the selective removal of excess stain from the tissue from microbial cells during regressive staining in order that a specific substance may be stained differentiallyh from the surrounding cell.

Differentiation is usually controlled visually by examination under the microscope

Uses

1. To observe the morphology, size, and arrangement of bacteria.
2. To differentiate one group of bacteria from the other group.

Biological stains are dyes used to stain micro-organisms.

Types of microbiological stains

. Basic stains

. Acidic stains

. Neutral stains

NB: This classification is not based on P^H of stains.

Basic stains are stains in which the coloring substance is contained in the base part of the stain. The acidic part is colorless. Eg.

Acidic stains are stains in which the coloring substance is contained in the acidic part of the stain. The base part is colorless. It is not commonly used in microbiology laboratory.

Eg. Eosin stain

Neutral stains are stains in which the acidic and basic components of stain are colored.

Neutral dyes stain both nucleic acid and cytoplasm. Eg. Giemsa stain

Types of staining methods

1. Simple staining method
2. Differential staining method
3. Special staining method

1. Simple staining method

It is type of staining method in which only a single dye is used.

Usually used to demonstrate bacterial morphology and arrengement

Two kinds of simple stains

1. Positive staining: The bacteria or its parts are stained by the dye.

Eg. Carbol fuchsin stain
Methylene blue stain
Crystal violet stain

Procedure:

. Make a smear and label it.

. Allow the smear to dry in air.

. Fix the smear over a flame.

.Apply a few drops of positive simple stain like 1% methylene blue, 1% carbolfuchsin or

1% gentian violet for 1 minute.

. Wash off the stain with water.

. Air-dry and examine under the oil immersion objective.

2. Negative staining: The dye stains the background and the bacteria remain unstained. Eg. Indian ink stain Negrosin stain

2. Differential staining method

Multiple stains are used in differential staining method to distinguish different cell structures and/or cell types. Eg. Gram stain and Ziehl-Neelson stain

A. Gram staining method

Developed by Christian Gram.

Most bacteria are differentiated by their gram reaction due to differences in their cell wall structure.

Gram-positive bacteria are bacteria that stain purple with crystal violet after decolorizing with acetone-alcohol.

Gram-negative bacteria are bacteria that stain pink with the counter stain (safranin) after losing the primary stain (crystal violet) when treated with acetone-alcohol.

Required reagents:

. Gram's Iodine

. Acetone-Alcohol

. Safranin

Procedure:

1. Prepare the smear from the culture or from the specimen.
2. Allow the smear to air-dry completely.
3. Rapidly pass the slide (smear upper most) three times through the flame.
4. Cover the fixed smear with crystal violet for 1 minute and wash with distilled water.
5. Tip off the water and cover the smear with gram's iodine for 1 minute.
6. Wash off the iodine with clean water.
7. Decolorize rapidly with acetone-alcohol for 30 seconds.
8. Wash off the acetone-alcohol with clean water.
9. Cover the smear with safranin for 1 minute.
10. Wash off the stain wipe the back of the slide. Let the smear to air-dry.
11. Examine the smear with oil immersion objective to look for bacteria.

Interpretation:

. Gram-positive bacterium ……Purple

. Gram-negative bacterium ……..Pink

B. Ziehl-Neelson staining method

Developed by Paul Ehrlichin1882, and modified by Ziehl and Neelson

Ziehl-Neelson stain (Acid-fast stain) is used for staining Mycobacteria which are hardly stained by gram staining method.

Once the Mycobacteria is stained with primary stain it can not be decolorized with acid, so named as acid-fast bacteria.

Reagents required:

. Carbol-fuchsin

. Acid-Alcohol

. Methylene blue/Malachite green

Procedure for Ziehl-Neelson staining method

1. Prepare the smear from the primary specimen and fix it by passing through the flame and label clearly
2. Place fixed slide on a staining rack and cover each slide with concentrated carbol fuchsin solution.
3. Heat the slide from underneath with sprit lamp until vapor rises (do not boil it) and wait for 3-5 minutes.
4. Wash off the stain with clean water.
5. Cover the smear with 3% acid-alcohol solution until all color is removed (two minutes).
6. Wash off the stain and cover the slide with 1% methylene blue.for one minute.
7. Wash off the stain with clean water and let it air-dry.

8. Examine the smear under the oil immersion objective to look for acid fast bailli.

Interpretation:

Acid fast bacilli……..Red

Back ground……Blue

Reporting system

0 AFB/100 field ……No AFB seen

1-2 AFB/ 300 field…….. Scanty

1-10 AFB/100 field……1+

11-100AFB/100 field……2+

1-10 AFB/field…… ……3+

>10 AFB/field…… ….4+

NB: AFB means number of acid fast bacilli seen.

3. Special stains

a. Spore staining method
b. Capsule staining method

a. Spore staining method

Procedure:

1. Prepare smear of the spore-forming bacteria and fix in flame.
2. Cover the smear with 5% malachite green solution and heat over steaming water bath for 2-3 minutes.
3. Wash with clean water.
4. Apply 1% safranin for 30 seconds.

5. Wash with clean water.
6. Dry and examine under the oil immersion objective.

b. Capsule staining method: Welch method

Procedure:

1. Prepare smear of capsulated bacteria.
2. Allow smear to air-dry; do not fix the smear.
3. Cover the smear with 1% aqueous crystal violet for 1 minute over steaming water bath.
4. Wash with 20% copper sulfate solution. Do not use water.
5. Dry and examine under the oil immersion objective.

1.5. CULTIVATION OF BACTERIA IN CULTURE MEDIA

Culture media

It is the media containing the required nutrients for bacterial growth.

Uses: . Isolation and identification of micro-organisms
. Performing anti-microbial sensitivity tests

Common ingredients of culture media

. Peptone
. Meat extract
. Yeast extract
. Mineral salts
. Carbohydrates
. Agar

. Water

Peptone: Hydrolyzed product of animal and plant proteins: Free amino acids, peptides and proteoses(large sized peptides).

It provides nitrogen; as well carbohydrates, nucleic acid fractions, minerals and vitamins.

Meat extract: supply amino acids, vitamins and mineral salts.

Yeast extract: It is bacterial growth stimulants.

Mineral salts: these are: Sulfates as a source of sulfur.

. Phosphates as a source of phosphorus.

. Sodium chloride

. Other elements

Carbohydrates: Simple and complex sugars are a source of carbon and energy.

.Assist in the differentiation of bacteria.

Eg. Sucrose in TCBS agar differentiates vibro species.

Lactose in MacConkey agar differentiates enterobacteria.

Agar: It is an inert polysaccharide of seaweed.

It is not metabolized by micro-organism.

Property

. It has . high gelling strength

. high melting temperature(90-95 ^{o}c)

. low gelling temperature

. It forms firm gel at 1.5% W/V concentration.

. It forms semisolid gel at 0.4-0.5% W/V concentration.

Uses:

. Solidify culture media

. May provide calcium and organic ions to inoculated bacteria.

Water

Deionized or distilled water must be used in the preparation of culture media.

Types of culture media

1. Basic /Simple / All purpose media

It is a media that supports the growth of micro-organisms that do not require special nutrients.

Uses :

. To prepare enriched media

. To maintain stock cultures of control bacterial strains

. To subcuture pathogenic bacteria from selective/differential medium prior to performing biochemical or serological tests.

Eg. Nutrient Broth

Nutrient Agar

2. Enriched media

Media that are enriched with whole blood, lyzed blood, serum, special extracts or vitamins to support the growth of pathogenic bacteria.

Eg. Blood Agar

Chocolate Agar

3. Enrichment media

Fluid media that increases the numbers of a pathogen by containing enrichments and/or substances that discourage the multiplication of unwanted bacteria.

Eg. Selenite F broth media

Alkaline peptone water

4. Selective media

Media which contain substances (Eg. Antibiotics) that prevent or slow down the growth of bacteria other than pathogens for which the media are intended.

Eg. Modified Thayer –Martin Agar

Salmonella-Shigella(SS) agar

1. Differential media

Media to which indicator substances are added to differentiate bacteria.

Eg. TCBS Agar differentiates sucrose fermenting yellow colonies of Vibrio cholerae to non-sucrose fermenting blue colonies other Vibrio species.

NB: Most differential media distinguish between bacteria by an indicator which changes color when acid is produced following carbohydrate fermentation.

2. Transport media

Media containing ingredients to prevent the overgrowth of commensals and ensure the survival of pathogenic bacteria when specimens can not be cultured soon after collection.

EG. Amies transport media

Stuart media

Kelly-Blair media

Choice of culture media

The selection culture media will depend on:

1. The major pathogens to be isolated, their growth requirements and the features by which they are recognized.
2. Whether the specimens being cultured are from sterile sites or from sites having normal microbial flora.
3. The cost, availability and stability of media.
4. The training and experience of laboratory staff in preparing, using and controlling culture media.

Forms of culture media

1. solid culture media
2. semisolid culture media
3. Fluid culture media

1. solid culture media

. Plate cultures in petri dishes

. stab/slope cultures in tubes and bottles

Uses: Description of bacterial colonies

- size : diameter in mm
- Out line : circular, entire, wavy, indented

- Elevation: flat, raised, low convex and dome shaped.
- Transparency: transparent, opaque, and translucent.
- Surface: smooth (mucoid) and shiny, rough and dull.
- Color: colorless, white, pink, and pigmented
- changes in medium

Eg. Hemolysis in Blood Agar

Blackening of medium due to hydrogen sulfide production.

2. Semisolid culture media

Uses:

. as an enrichment media

. as motility media

3. Fluid culture media

Bacterial growth in fluid media is shown by a turbidity in the medium.

Uses :

. as an enrichment media

. as biochemical testing media

. as blood culture media

Preparation of culture media

Culture media contains essential ingredients for microbial growth requirements.

For successful isolation of pathogens, culture media must be prepared carefully.

Most culture media are available commercially in ready –made dehydrated form.

The major processes during preparation of culture media

- Weighing and dissolving of culture media ingredients
- Sterilization and sterility testing
- Addition of heat-sensitive ingredients
- Dispensing of culture media
- pH testing of culture media
- Quality assurance of culture media
- Storage of culture media

1. Weighing and dissolving of culture media ingredients

Apply the following while weighing and dissolving of culture media ingredients

- Use ingredients suitable for microbiological use.
- Use clean glass ware, plastic or stainless steel equipment.
- Use distilled water from a glass still.
- Do not open new containers of media before finishing previous ones.
- Weigh in a cool, clean, dry and draught-free atmosphere.
- Weigh accurately using a balance.
- Wear a facemask and glove while weighing and dissolving toxic chemicals.
- Do not delay in making up the medium after weighing.

- Add powdered ingredients to distilled water and mix by rotating or stirring the flask.
- Stir while heating if heating is required to dissolve the medium.
- Autoclave the medium when the ingredients are dissolved.

2. Sterilization and sterility testing

Always sterilize a medium at the correct temperature and for the correct length of time as instructed in the method of preparation.

Methods used to sterilize culture media

A) . Autoclaving

B) . Steaming to 100 ^{O}C

C) . Filtration

A) Autoclaving

Autoclaving is used to sterilize most agar and fluid culture media.

B) Steaming at 100 ^{O}C

It is used to sterilize media containing ingredients that would be inactivated at temperature over 100 ^{O}C and re-melt previously bottled sterile agar media.

C) Filtration

It is used to sterilize additives that are heat-sensitive and can not be autoclaved.

Sterility testing

The simplest way to test for contamination is to incubate the prepared sample media

At 35-37 ^{O}C for 24 hours. Turbidity in fluid media and microbial growth in solid media confirm contamination.

3. Addition of heat-sensitive ingredients

Refrigerated-heat sensitive ingredients should be warmed at room temperature before added to a molten agar medium.

Using an aseptic technique, the ingredients should be added when the medium has cooled to 50 ^{O}C, and should be distributed immediately unless further heating is required.

4. pH testing

The pH of most culture media is near neutral, and can be tested using pH papers or pH meter.

5. Dispensing of culture media

Media should be dispensed in a clean draught-free room using aseptic technique and sterile container.

Dispensing agar media in petridish

- Lay out the sterile petridishes on a level surface.
- Mix the medium gently by rotating the flask or bottle.
- Flame sterilize the neck of flask or bottle.
- Pour 15 ml of medium in each petridish.
- Stack the plates after the medium has gelled or cooled.

- Store the plates in a refrigerator.

NB: Agar plates should be of an even depth and of a firm gel.

The surface of the medium should be smooth and free from bubbles.

6. Quality control

- Inoculate quarter plates of the medium with a five hours broth culture for each control organism.
- Use a straight wire to inoculate and wire loop to spread the inoculum.
- Depending on the species, incubate aerobically, CO_2-enriched atmosphere and anaerobically at 35-37 ^{O}C for 24 hours.
- Examine for the degree of growth, morphology and other characteristics of microbial colonies.
- Record the result of each control species and compare to your standard reading.

Storage of culture media

- Dehydrated culture media and dry ingredients should be stored at an even temperature in a cool dry place away from direct light.
- Plates of culture media, and additives like serum, blood and antimicrobials in solid form require storage at 2-8 ^{O}C.
- Antimicrobials in solution form should be stored at –20 ^{O}C.
- All culture media and additives should be labeled with the name and date of preparation.

Inoculation of culture media

When inoculating culture media, an aseptic technique must be used to prevent contamination of specimens and culture media, and laboratory worker and the environment.

Aseptic technique during inoculation of culture media

- Decontaminate the workbench before and after the work of the day.
- Use facemask and gloves during handling highly infectious specimens.
- Flame sterilize wire loops, straight wires, and metal forceps before and after use.
- Flame the neck of specimen and culture bottles, and tubes after removing and before replacing caps and plugs.

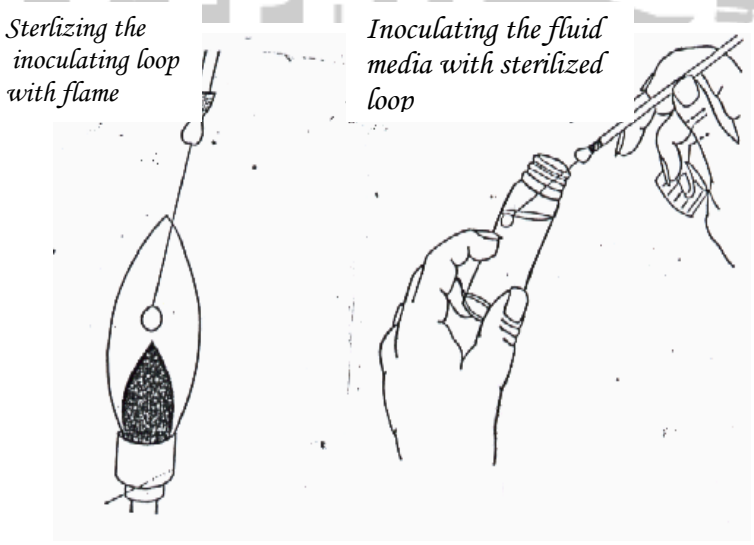


Fig. 1.6 Aseptic inoculation technique

Inoculation of media in petridishes

The inoculation of media in petridishes is named as 'plating out' or 'looping out'.

Before inoculating a plate of culture media, dry the surface of the media by incubating at 37 ^{O}C for 30 minutes.

To inoculate a plate, apply the inoculum to a small area of the plate ('the well') using sterile wire loop and then spread and thin out the inoculum to ensure single colony growth.

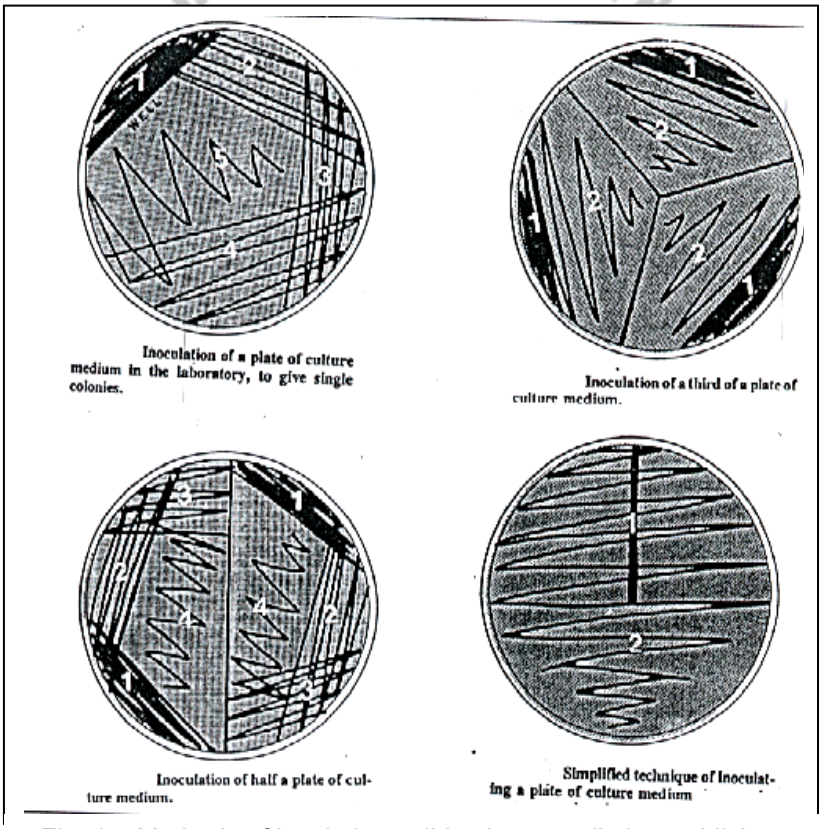


Fig. 1.7 Methods of ioculating solid culture media in petridishes

Inoculation of butt and slant media

To inoculate butt and slant media, use a sterile straight wire to stab into the butt and then streak the slant in a zigzag pattern.

Inoculation of slant media

To inoculate slant media, use a straight wire to streak the inoculum down the center of the slant and then spread the inoculum in a zigzag pattern.

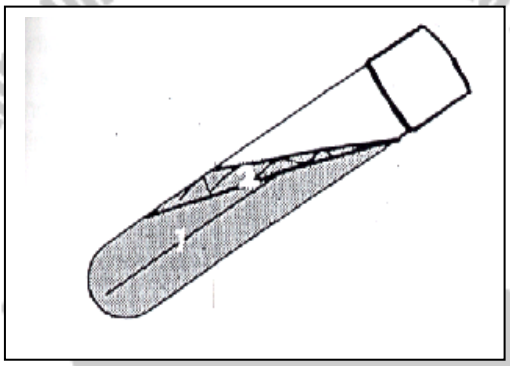


Fig. 1.8 Inoculation of slant and bath media

Inoculation of stab media

To inoculate stab media, use a straight wire to stab through the center of the medium and withdraw the wire along the line of inoculum.

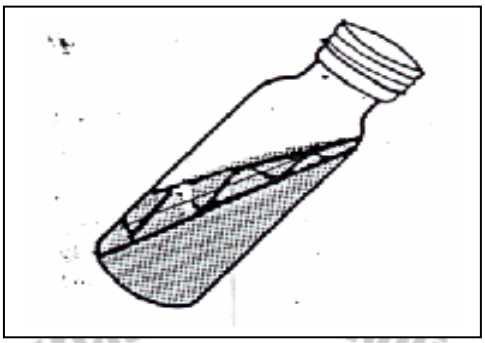


Fig. 1.9 Inoculation of slant media

Inoculation of fluid media

To inoculate fluid media, use straight wire or wire loops.

Incubation of cultures

Inoculated media should be incubated as soon as possible.

Optimal temperature, humidity and gaseous atmosphere should be provided for microorganisms to grow best.

The temperature selected for routine culturing is 35-37 ^{O}C.

Some pathogens require CO_2-enriched atmosphere to grow in culture media, and the simplest way to provide CO_2-enriched atmosphere is to enclose a lighted candle in an airtight jar which provides 3-5% CO_2 by the time the candle is extinguished.

Anaerobic atmosphere is essential for the growth of strict anaerobes, and the techniques for obtaining anaerobic conditions are the following:

. Anaerobic jar with a gas generating kit.

. Reducing agents in culture media.

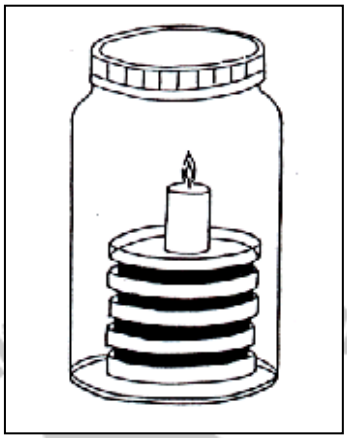


Fig. 1.10 Co2-enriched atmosphere

1.6. BACTERIAL NUTRITION

Bacteria, like all cells, require nutrients for the maintenance of their metabolism and for cell division.

Bacterial structural components and the macromolecules for the metabolism are synthesized from the elements. The four most important elements of bacteria are carbon, hydrogen, oxygen and nitrogen.

Carbon

Organisms require a source of carbon for the synthesis of numerous organic compounds that comprise protoplast.

Depending on their requirements, bacteria can be classified as

1. Autotrophs: Free-living, non-parasitic bacteria which use carbondioxide as carbon source.

The energy needed for their metabolism can be obtained from:

. Sun light-Photoautotrophs

. Inorganic compounds by oxidation-Chemoautotrophs

2. Heterotrophs: Parasitic bacteria require more complex organic compounds as their source of carbon and energy.

Human pathogenic bacteria are heterotrophs.

The principal source of carbon is carbohydrate which are degraded either by oxidation, in the presence of oxygen, or by fermentation, in the absence of oxygen, to provide energy in the form of ATP.

Hydrogen and oxygen

- Obtained from water.
- Essential for the growth and maintenance of cell.

Nitrogen

- Constitutes 10% of dry weight of bacterial cell.
- Obtained from organic molecules like proteins and inorganic molecules like ammonium salts and nitrates.

NB: Main source of nitrogen is ammonia, in the form of ammonium salt.

Growth factors

Growth factors are organic compounds that are required by micro-organisms in small amounts which the cell can not synthesize from other carbon source.

These are aminoacids, purines and pyrimidines, and vitamins.

Prototrophs: Wild-type bacteria with normal growth requirements.

Auxotrophs: Mutant bacteria, which require an additional growth factor not needed by the parental or wild type strain.

1.7. BACTERIAL GROWTH

It is an orderly increase in all the components of an organism.

It is an increment in biomass.

It is synchronous with bacterial cell reproduction.

Generation time

It is the time taken for the size of a bacterial population to double.

Bacteria grow by taking nutrients and incorporate them into cellular components; then bacteria divide into two equal daughter cells and double the number.

Bacterial growth phases

The pattern in cell numbers exhibited by bacterial population obtained after inoculation

Of a bacterium into a new culture medium.

The normal bacterial growth curve has four phases.

1. Lag phase

The period of adaptation with active macro molecular synthesis like DNA, RNA, various enzymes and other structural components.

It is the preparation time for reproduction; no increase in cell number.

2. Exponential(log) phase

The period of active multiplication of cells.

Cell division precedes at a logarithmic rate, and determined by the medium and condition of the culture.

3. Maximal stationary phase

The period when the bacteria have achieved their maximal cell density or yield.

There is no further increase in viable bacterial cell number.

The growth rate is exactly equal to the death rate.

A bacterial population may reach stationary growth when one of the following conditions occur:

1. The required nutrients are exhausted
2. Inhibitory end products are accumulated
3. Physical conditions do not permit a further increase in population size

4. Decline phase

The period at which the rate of death of bacterial cells exceeds the rate of new cell formation.

There is drastic decline in viable cells.

Few organisms may persist for so long time at this period at the expense of nutrients released from dying micro-organisms.

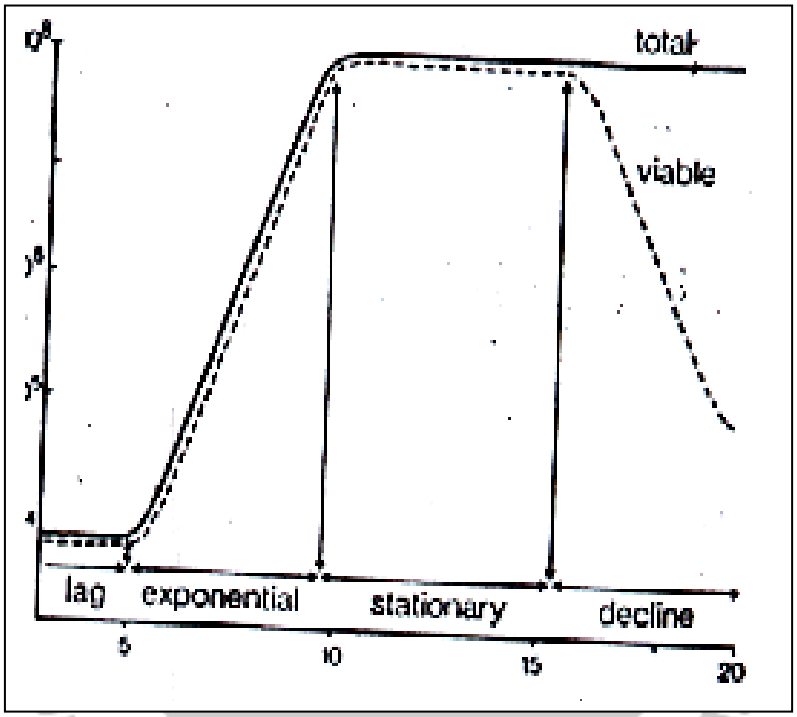


Fig. 1.11 Bacterial growth curve

Quantitative measurement of bacterial growth

Bacterial growth is measured by determining number of bacteria.

The common measuring methods are

1. Viable plate count
2. Direct count
3. Turbidimetric method

1. Viable plate count

The most common method of estimating bacterial growth which involves counting the number of bacterial colonies grown on solid media after incubation of the inoculated media for 18-24 hours.

Procedure

- The sample is serially diluted.
- The suspension is inoculated on solid media by surface spread technique i.e. the suspension is spread
- The plate is incubated for 18-24 hrs to allow the bacteria to grow and form colonies.
- The concentration of bacteria in the original sample can be determined by counting the visible colonies multiplied by the dilution factor.

$$\text{Number of colonies} = \frac{\text{Number of colonies} \times \text{dilution factor}}{\text{Volume of sample}}$$

NB: The statistically significant plate count is between 30 and 300 colonies.

Less than 30 colonies on a plate are not accepted for statistical reasons.

Greater than 300 colonies on a plate are too close to distinguish as an individual colony forming unit (too numerous to count).

Limitation of viable plate count: It selectively in favor of a certain group of bacterial population.

2. Direct count

It involves direct microscopic counting of bacteria in the sample using counting chamber.

It is relatively quick and does not need the sample to be incubated.

Procedure

. Serial dilution of the sample

. Fill known area and volume of the counting chamber with the sample

. Total number of bacteria in the sample per unit volume is equal to No. of bacteria in the sample X the No. of squares X dilution factor.

3. Turbidimetric method

It is the method of determination of bacterial growth in liquid media.

Bacterial growth increases the turbidity of liquid to absorb light.

The turbidity of the suspension is determined by spectrophotometer.

Factors influencing bacterial growth in vitro

Not all bacterial species grow under identical environmental conditions. Each bacterial species has a specific tolerance range for specific environmental parameters.

Out side the tolerance range environmental conditions for a bacteria to reproduce, it may survive in dormant state or may lose viability.

Rates of bacterial growth are greatly influenced by the following environmental parameters.

. Nutrition

. Temperature

. Oxygen

. P^H

. Salinity

. Pressure

. Light radiation

1. Nutrition

The following nutrients must be provided for optimal bacterial growth.

- Hydrogen donors and acceptors
- Carbon source
- Nitrogen source
- Minerals: sulfur and phosphorus, trace elements
- Growth factors: amino acids, purines, pyrimidines and vitamins.

2. Temperature

Temperature tolerance range: The minimum and maximum temperature at which a micro-organism can grow; which is different in different species of bacteria.

Optimal growth range of temperature: The temperature at which the maximum growth rate occurs; and results in the shortest generation time of bacteria.

Based on different optimal growth temperature requirement, bacteria are divided into:

Optimal growth temperature

. Psychrophilic bacteria .15-20 ^{o}c; grow best at low T^{0} range

. Mesophilic bacteria .30-37^{0}c; grow best at middle T^{0} range

. Thermophilic bacteria .50-60^{o}c; grow best at high T^{0} range

NB: Most human pathogens and many of the normal flora of human bodies have an optimal temperature of 37^{0}c; There fore they are mesophilic bacteria.

3. Oxygen

Base on oxygen requirements and tolerance, bacteria are divided classified as:

. Obligate aerobes

. Obligate anaerobes

. Facultative anaerobes

. Microaerophiles

- Obligate aerobic bacteria grow only when free oxygen is available to support their respiratory metabolism.

They obtain ATP by using oxygen as a final electron acceptor in respiration.

- Obligate anaerobic bacteria grow in the absence of oxygen; exposure to oxygen kills anaerobes.
- Facultative anaerobic bacteria grow in the presence or absence of oxygen.

They obtain ATP by fermentation or anaerobic respiration

- Microaerophilic bacteria grow best at reduced oxygen tension; high oxygen tension is toxic to them.

4. Hydrogen ion concentration

It is a measure of acidity and alkalinity.

P^H<7 is acidic

P^H =7 is neutral

P^H>7 ia alkaline

- Neutrophilic bacteria grow best at near neutral P^H value.
- Acidicophilic bacteria prefer to grow at low P^H value (acidic medium).
- Alkalinophilic bacteria prefer to grow at high P^H value (alkaline medium).
- Most pathogenic bacteria grow best at P^H of 6-8.

5. Salinity

Salt content of the medium affects bacterial growth.

Halophilic bacteria grow best at high salt concentration.

. Moderate halophiles require 3% salt concentration.

. Extreme halophiles require 15% salt concentration.

Most bacteia can not tolerate high salt concentration. High salt concentration disrupts membrane transport systems and denatures proteins of bacteria but halophiles have adaptive mechanisms to tolerate high salt concentration.

6. Pressure

Osmotic pressure: The pressure exerted on bacterial cell surface as a result of difference in solute concentration between the inside and out side of a cell.

Osmotolerant bacteria can grow in solutions with high solute concentration.

Osmophilic bacteria grow best at high hydrostatic pressure.

Hydrostatic pressure: The pressure exerted by the weight of a water column.

High hydrostatic pressures more than 200 atmosphere generally inactivates enzymes and disrupts membrane transport process.

Barotolerant bacteriacan grow at high hydrostatic pressure.

Barophilic bacteria grow best at high hydrostatic pressure.

7. Light radiation

Photosynthetic bacteria require light in the visible spectrum to carry out photosynthesis.

COMMON BIOCHEMICAL TESTS

Litmus milk reduction test:

Required: Litmus milk medium

Wire loop

Bunsen burner

Test bacteria

Method:

. Inoculate 0.5 ml of sterile litmus milk medium with the test bacteria.

. Incubate at 35-37 ^{O}c for up to 4 hrs.

. Observe for changes in color every 30 min.

Results:

. Change in color of medium from pink to white or pale is suggestive of enterococci

CAMP test (Christie, Atkins , Munich Paterson)

Principle: S.agalaciae produce protein named as camp factor, which interacts with staphylococci β-hemolysin on sheep red blood cell.

Method:

. Streak S.aureus isolate across sheep blood agar plate.

. Inoculate the test bacteria at right angle to staphylococci with out touching it.

. Incubate over night at 35-37 ^{O}c.

. Formation of an arrow-head shaped area of hemolysis indicates interaction of camp factor with staphylococci hemolysin.

Bacitracin test

Principle: Streptococcus pyogenes is sensitive to bacitracin but other kinds of streptocci are resistant to bacitracin.

Method:

. Streak a blood agar plate with the isolated organism.

. Place bacitracin disc in the streaked area.

. Incubate the plate for 24 hours at 37 0c.

. Examine the plate for a zone of no-growth around the disc.

No-growth around the disc..... S.pyogenes

Growth around the disc ..... Other streptococci

Optochin test

Principle: S. pneumoniae is sensitive to optochin disc unlike other alpha-hemolytic streptococci.

Method:

. Streak a blood agar plate with the isolated organism.

. Place optochin disc in the streaked area.

. Incubate the plate for 24 hours at 37 0c.

. Examine the plate for a zone of no-growth around the disc.

No-growth around the disc .....S.pneumoniae

Growth around the disc …otherAlpha-hemolytic streptococci

Carbohydrate utilization test

Method:

. Prepare saline suspension of test bacteria

. Add 0.1ml of bacterial suspension into each of four test tubes containing glucose, lactose, maltose and sucrose carbohydrate discs.

. Incubate in a water bath at 37^{O}c and examine at 30 min intervals for 5 hrs for change in color.

Result: Change in color from red to yellow-orange indicates carbohydrate utilization.

BILE SOLUBILITY TEST

This helps to differentate S.pneumoniae, which is soluble in bile and bile salts, from viridans streptococci which are insoluble.

Principle

A heavy inoculum of the test organism is emulsified in physiological saline to give a turbid suspension. The bile salt sodium deoxycholate is then added. The test can also be performed by adding the bile salt to a broth culture of the organism. The bile salt dissoles S.pneumoniae as shown by a clearing of the turbidity within 10-15 minutes. Viridans streptococci are not dissolved and therefore there is no clearing of the turbidity.

Required

Sodium deochollate100g/l

Physiological saline (sodium chloride, 8.5g/l)

Method

- Emulsify several colonies of the test organism in a tube containing 2ml of sterile physiological saline, to give a turbid suspension.
- Divide the organism suspension between two tubes.
- To one tube, add 2 drops of the sodium deoxycholate reagent and mix.

- To the other tube, add 2 drops of sterile distilled water and mix.
- Leave both tubes for 10-15 minutes.
- Look for a clearing of turbidity in the tube containing the sodium deoxycholate.

Results

Clearing of turbidity ---------- Probably
S.pneumoniae

No clearing of turbidity ----------organism is probably
Not S.pneumoniae

There should be no clearing of turbidity in the tube to which distilled water was added. If there is, repeat the test.

Note: Some strains of S.pneumoniae are not dissolved by bile salts, and very occasionally some strains of viridans streptococci give a positive tests.

Controls

Bile solubility positive control: Streptococcus pneumoniae.
Bile solubility negative control: Streptococcus faecalis.

CATALASE TEST

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

Principle

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water.

An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Care must be taken if testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the colony, a false positive reaction will occur. It is usually recommended, therefore, that catalase testing be performed from a blood free culture medium such as nutrient agar.

Required

a. Hydrogen peroxide, 3% H_2O_2

   Note: Shaking the reagent before use will help to expel any dissolved oxygen. False positive reactions may occur if the hydrogen peroxide contains dissolved oxygen.

Method

- Pour 2-3ml of the hydrogen peroxide solution into a test tube.

- Using a sterile wooden stick or a glass rod, remove a good growth of the test organism and immerse it in the hydrogen peroxide solution.

Note: A nichrome wire loop must not be used because this may give a false positive reaction.

- Look for immediate bubbling.

Results

Active bubbling ----------	Positive test Catalase produced
No release of bubbles ----------	Negative test No catalase produced

Note: if the organism has been cultured on an agar slope, pour about 1ml of the hydrogen peroxide solution over a good growth of the organism, and look for the release of bubbles.

Caution: performing the test on a slide is not recommended because of the risk of contamination from active bubbling.

If the rapid slide technique is used, the hydrogen peroxide solution should be added to the organism suspension after placing the slide in a petridish. The dish should then be covered immediately, and the preparation observed for bubbling through the lid.

Controls

Positive catalase control: Staphylococcus species.

Negative catalase control: Streptococcus species.

CITRATE UTILIZATION TES

This test is one of several techniques used to assist in the identification of enterobacteria. The test is based on the ability of an organism to use citrate as its only source of carbon and ammonia as its only source of nitrogen.

Principle

The test organism is cultured in a medium which contains sodium citrate, an ammonium salt, and the indicator bromo – thymol blue. Growth in the medium is shown by turbidity and a change in colour of the indicator from light green to blue, due to the alkaline reaction, following citrate utilization.

Required

Koser's citrate medium or Simmon's citrate agar.

Method

Using a sterile straight wire, inoculate 3-4ml of sterile Koser's citrate medium with a broth culture of the test organism.

Note: Care must be taken not to contaminate the medium with carbon particles, such as from a frequently flamed wire.

Incubate the inoculated broth at 35 – 37^{O}C for up to 4 days, checking daily for growth.

Results

Turbidity and blue colour ---------- Positive test
Citrate utilized

No growth ----------Negative test
Citrate not utilized

Controls

Positive citrate control: Klebslella pneumonlae

Negative citrate control: Escherichia coli.

COAGULASE TEST

This test is used to differentiate Staphylococcus aureus which produces the enzyme coagulase, from S.epidermidis and S.saprophyticus which do not produce coagulase.

Principle

Coagulase causes plasma to clot by converting fibrinogen to fibrin. Two types of coagulase are produced by most strains of S.aureus:

Free coagulase which converts fibrinogen to fibrin by activating a coagulase – reacting factor present in plasma. Free coagulase is detected by the appearance of a fibrin clot in the tube test.

Bound coagulase (clumping factor) which converts fibrinogen directlyto fibrin without requiring a coagulase – reacting factor. It can be detected by the clumping of bacterial cells in the rapid slide test. It is usually recommended that a tube test should be performed on all negative slide tests. A tube test must always be

performed if the result of the slide test is not clear, or when the slide test is negative and the Staphylococcus has been isolated from a serious infection.

Required

a. Undiluted human plasma (preferably pooled) or rabbit plasma. The plasma should be allowed to warm to room temperature before being used.

   Plasma from EDTA (ethylenediamine – tetra – acetic acid) or citrate anticoagulated blood is usually used.

   Note: Occasionally citrate-utilizing organisms such as Klebsilla can cause the clotting of citrated plasma in the tube test. This can be prevented by adding heparin to the citrated plasma. It is also possible for human plasma to contain inhibitory substances which can interfere with coagulase testing. Adequate controls must be included for both slide and tube tests.

Method for slide test (to detect bound coagulase)

Place a drop of physiological saline on each end of a slide, or on two separate slides.

Emulsiy a colony of the test organism in each of the drops to make two thick suspensions.

Note: Colonies from a mannitol salt agar culture are not suitable for coagulase testing. The organism must first be cultured on nutrient agar or blood agar.

Add a drop of plasma to one of the suspensions, and mix gently. Look for clumping of the organisms within 10 seconds.

No plasma is added to the second suspension. This is used to differentiate any granular appearance of the organism form true coagulase clumping.

Results

Clamping within 10 secs ---------- S.aureus

No clumping within 10 secs----------No bound coagulase produced.

Controls

Positive coagulase control: Staphylococcus aureus.

Negative coagulase control: Escherichia coli or

Staphylococcus epldermids

Method for tube test (detect free coagulase)

Dilute the plasma 1 in 10 in physiological saline (mix 0.2 ml of plasma with 1.8ml of saline).

Take three small test tubes and label:

T = Test organism (18-24h broth culture)

Pos = Positive control (18-24h staph. Aureus broth culture)

Neg = Negative control (sterile broth)

A suitable broth is brain heart infusion

Pipette 0.5ml of the diluted plasma into each tube.

Add 5 drops (about 0.1ml) of the test organism culture to the tube labeled 'T'.

Add 5 drops of the staph. Aureus culture to the tube labeled 'Pos'.

Add 5 drops of sterile broth to the tube labeled 'Neg'.

After mixing gently, incubate the three tubes at 35-37^{O}C. Examine for clotting after 1 hour. If no clotting has occurred, examine at 30minute intervals for up to 6 hours.

When looking for clotting, gently tilt each tube.

Most Staph, aureus strains produce a fibrin clot within 1 hour of incubation. There should be no fibrin clot in the negative control tube.

Results

Fibrin clot ---------- S. aureus

No fibrin clot ---------- No free coagulase produced

DEOXYRIBONUCLEASE (DNAse) TEST

This test is used to differentiate Staph. Aureus which produces the enzyme DNAse from other staphylococci which do not produce DNAse. It is particularly useful if plasma is not available to peform a coagulase test or when the results of a coagulase test are difficult to interpret.

Principle

Deoxyribonuclease hydrolyzes deoxyribonucleic acid (DNA).

The test organism is cultured on a medium which contains DNA. After overnight incubation, the colonies are tested for DNAse production by flooding the plate with a weak hydrochloric acid solution. The acid precipitates unhydrolyzed DNA. DNAse producing

colonies are, therefore surrounded by clear areas indicating DNA hydrolysis.

Required

a. DNAse agar plate
   Up to six organisms may be tested on the same plate.
b. Hydrochloric acid, 1 mol/l

Method

Divide a DNAse plate into the required number of strips by marking the underside of the plate.

Using a sterile loop or swab, spot – inoculate the test and control organisms. Make sure each test area is clearly labeled.

Incubate the plate at 36-37^{O}C overnight.

Cover the surface of the plate with 1mol/l hydrochloric acid solution. Tip off the excess acid.

58. Look for clearing around the colonies within 5minutes of adding the acid.

Results

Clearing around the colonies ----------DBAse positive strain.

No clearing around to colonies ----------DNAse negative strain.

Controls

Positive DNAse control: staphylococcus arureus .

Negative DNAse control: staphylococcus epidermidis.

HYDROGEN SULPHID (H_2S) PRODUCTION

The detection of hydrogen sulphide gas (H_2S) is used mainly to assist in the identification of enterobacteria and occasionally to differentiate other bacteria such as Bacteroides and Bruceila species. H_2S is produced when sulphur – containing amino acids are decomposed.

Use of Kligler iron agar (KIA) to detect H_2S

This medium is suitable for detecting H_2S production by enterobacteria. H_2S is detected by the ferric citrate contained in the medium.

Inoculate the test organism into KIA and incubate it at appropriate temperature over night.

Observe blacking of the medium

Lead acetate paper test to detect H_2S

When a sensitive technique for detecting H_2S production is required, the lead acetate paper test is recommended.

Inoculate a tube or bottle of sterile peptone water or nutrient broth with the test organism.

Insert a lead acetate paper strip in the neck of the bottle or tube above the medium, and stopper well.

Incubate the inoculated medium at 35-37^{O}C, and examine daily for a blackening of the lower part of the strip.

Results

Blackening ---------- Positive test H2S produced

No blackening ---------- Negative test No H2S produced.

Controls

Positive hydrogen sulphide control: proteus vulgaris

Negative hydrogen sulphide control: shigella species

INDOLE TEST

Testing for indole production is important in the identification of enterobacteria. Most sftrains of E.coli, P.vulgaris, P.rettgeri, M.morganll, and providencia species break down the amino acid tryptophan with the release of indole.

Principle

The test organism is cultured in a medium which contains tryptophan. Indole production is detected by Kovac's or Ehrlich's reagent which contains 4(P)-dimethylaminobenzaldehyde. This reacts with the indole to produce a red coloured compound. In the following method the use of the combined motility indole urea (MIU) medium is described. A Kovac's ragent paper strip is inserted in the neck of the tube, and indole production is indicated by a reddening of the strip. Indole is a volatile substance (easily vaporized). The tube must be well stoppered during incubation.

The indole test can also be performed by culturing the organism in tryptone water or peptone water containing tryptophan, and

detecting indole production by adding Kovac's or Ehrlich's reagent to an 18-24h culture.

Required

Motility indole urea (MIU) medium

MIU medium indicates whether an organism is motile or non-motile, indole positive or negative, and urease positive or negative.

Method

Using a sterile straight wire, inoculate 5ml of sterile MIU medium with a smooth colony of the test organism.

Place an indole paper strip in the neck of the MIU tube above the medium, and stopper the tube, incubate at 35-37^{O}C overnight.

Examine for idole production by looking for a reddening of the lower part.

Results

Reddening of strip ----------Positive test
Indoloe produced

Noered colour ----------Negative test
No Indoloe produced

Note: If the reaction is weak, confirm the result by adding 1ml of Kovac's regent to the culture. Examine for a red colouring of the surface layer within 10 minutes.

Controls

Positive indole control: Escherichia coli

Negative indoloe control: Enterobacter aerogenes.

Motility Test

This is shown by a spreading turbidity from the stab line or a turbidity throughout the medium (compare with an uninoculated tube).

Urease production

This is shown by a red-pink colour in the medium.

NITRATE REDUCTION TEST

This test is used to differentiate members of the Enterobacteriaceae that produce the enzyme nitrate reductase, from Gram negative bacteria that do not produce the enzyme.

The test is also helpful in differentiating Mycobacterium species as explained.

Principle

A heavy inoculum of the test organism is incubated in a broth containing nitrate. After 4 hours, the broth is tested fro the reduction of nitrate to nitrite by adding sulphanilic acid reagent. If nitrite is present, the acid reagent is diazotizex and forms a pink-red compound with alpha-naphthylamine. When nitrite is not detected it

is necessary to test whether the organism has reduced the nitrate beyond nitrite. This is done indirectly by checking whether the broth still contains nitrate. Zinc dust is added which will convert any nitrate to nitrate. If no nitrite is detected when the zinc dust is added, it can be assumed that all the nitrate has been reduced beyond nitrite to nitrogen gas or ammonia by a nitrate reducing organism.

Required

a. Nitrate broth
b. Sulphanilic acid reagent
c. Alphanaphthylamine reagent
d. Zinc dust

Method

Inoculate 0.6 ml of sterile nitrate broth with a heavy growth of the test organism.

Incubate at 35-37^{O}C for 4 hours.

Add 1 drop of sulphanilic acid reagent and 1 drop of alphanaphthylamine reagent.

Shake to mix and look for a red colour.

Results

Red colour ---------- Positive test
Nitrate reduced

If no red colour is produced, add a very small amount (knife point) of zink dust powder. Look again for a red colour and intrpret as follows:

Red colour ---------- Negative test
No reduction of nitrate
No red colour ---------- Positive test
Nitrate reduced

Controls

Positive nitrate reduction control: Escherichia coli. Negative nitrate reduction control: Pseudomonas aeruginosa.

OXIDASE TEST (Cytochrome Oxidase)

The oxidase test is used to assist in the identification of pseudomonas, Neisseria, Vibrio, and Pasteurella species, all of which produce oxidase enzymes.

Principle

A piece of filter paper is soaked with a few drops of oxidase reagent. A colony of the test organism is then smeared on the filter paper. If the organism is oxidase - producing, the phenylenediamine in the reagent will be oxidized to a deep purple colour. Occasionally the test is performed by flooding the culture plate with oxidase reagent but this technique is not recommended for routine use because the reagent rapidly kills bacteria. It can be useful, however, when attempting to isolate N.gonorrhoeae colonies from mixed cultures in the absence of a selective medium. The oxidase positive colonies must be removed and subcultured within 30 seconds of flooding the plate.

Important: Acidity inhibits oxidase enzyme activity. The oxidase test must not be performed, therefore, on colonies that produce fermentation on carbohydrate – containing media, such as sucrose fermenting V.cholerae colonies on TCBS medium, Subinoculation on nutrient agar is required before the oxidase test can be performed reliably. Non – fermenting colonies, however, can be tested. Colonies tested from a medium that contains nitrate may give unreliable oxidase test results.

Required

- **Oxidase reagent**

Freshly prepared

This is a 10g/l solution of tetramethyl –p-phenylenediamine dihydrochloride.

Note: Oxidase reagent is easily oxidized. When oxidized, it is blue in colour and must not be used.

Method

Place a piece of filter paper in a clean petri dish and add 2 or 3 drops of freshly prepared oxidase reagent.

Using a piece of stick or glass rod (not an oxidized wire loop), remove a colony of the test organism, and smear it on the filter paper.

Look for the development of a blue – purple colour within a few seconds.

Results

Blue-purple colour ---------- Positive test
(within 10 seconds) Oxidase produced

No blue – purple colour ---------- Negative test
(within 10 seconds) No oxidase produced

Note: ignore any blue – purple colour that develops after 10 seconds.

Controls

Positive oxidase control: Pseudomonas aeruginosa.
Negative oxidase control: Escherichia coli.

OXIDATION – FERMENTATION (O-F) TEST

This test is used to differentiate those organisms that oxidize. Carbohydrates (aerobic utilization) Such as Pseudomonas aeruginosa, from those organisms that ferment carbohydrates (anaerobic utilization) such as members of the Entero-bacteriaaceae.

Principle

The test organism is inoculated into two tubes of a tryptone or peptone agar medium containing glucose (or other carbohydrate) and the indicator bromothymol blue. The inoculated medium in one tube is sealed with alayer of liquid paraffin to exclude oxygen.

Fermentative organisms utilize the carbohydrate in both the open and sealed tubes and the colour of the medium changes from green to yellow. Oxidative organisms, however, are able to use the carbohydrate only in the open tube. There is no carbohydrate utilization in the sealed tube (medium remains green).

Although most genera of aerobic bacteria are either carbohydrate oxidizers or fermenters, the production of acid may be slow and therefore cultures are usually incubated for 7-14 days.

Required

a. Oxidation fermentation (O-F) medium
   Glucose, maltose, and sucrose O-F media are the most commonly used.
b. Sterile paraffin oil (liquid paraffin)

Method

Using a sterile straight wire, inoculate the test organism to the bottom of two bottles (or more if testing several carbohydrates) of sterile O-F medium. Use a heavy inoculum.

Cover the incculated medium in one of the tubes (or one from each carbohydrate pair) with a 10mm deep layer of sterile paraffin oil or molten wax.

Incubate the tubes at 35-37OC for up to 14 days. Examine daily for carbohydrate utilization as shown by acid production.

Results

Open tube	Sealed tube	Interpretation
Yellow	Green	Oxidative organism
Yellow	Yellow	Fermentative organism
Green or blue	Green	No utilization of carbohydrate

Controls

Oxidative control: Pseudomonas aeruginosa.

Fermentative control: Escherichia coli.

PHENYLALANINE DEAMINASE TEST

The test, which is also referred to as the Phenylpyruvic acid (PPA) test, is used mainly to assist in the identification of enterobacteria. It is based on the ability of bacteria such as Proteus specdies and some Providencia strains to break down phenylalanine (by oxidative deamination) with the production of phenylpyruvic acid. Y.enterocolitica (urease – producer is a phenylalanine negative.

Principle

The test organism is cultured on a slope of phenyiaianine medium. After overnight incubation, the deamination of phenylaianjine to phenyl – pyruvic acid is detected by adding iron III chloride (ferric chloride) which produces a green colour on the surface of the culture.

Required

a. Phanylalanine agar
b. Iron III chloride (ferric chloride),
   100g/l (10% w/v). The reagent must be freshly prepared.

Method

. inoculate a slope of phenylalanine agar with the test organism, and incubate at 35-37^{O}c overnight.

. Add 4 or 5 drops of the freshly prepared iron III chloride reagent to the culture, allowing the reagent to run down the slope.

. Look for a green colour on the slope.

Results

Green colour ----------Positive test
(Within 5 minutes) Phenylanine deaminated
No green colour ----------Negative e test
No deamination of phenylalanine

Controls

Positive PPA control: Proteus species
Negative PPA control: Escherichia coli.

TWEEN 80 HYDROLYSIS TEST

This test is used mainly to differentiate slow–growing Mycobacterium species as described in 44:1 species that hydrolize the detergent Tween 80 with the production of oleic acid are listed in Chart.

Principle

The test organism is incubated in a Tween 80 buffered substrate that contains the indicator neutral red. Tween hydrolysis is detected by a change in colour of the indicator from amber to pink – red due to the production of oleic acid.

Required

Tween 80 phosphate buffered substrate with neutral red.

*The substrate requires storage at 4^{O}c

Method

. Inoculate 4 ml of sterile Tween 80 phosphate buffered substrate with a loopful of growth of the test organism.

. Incubate at 35-37 ^{O}c for up to 18 days. Examine at 5,10, and 18 days for a change in colour of the substrate from amber to pink-red, as shown in colour.

Results

Pink-red substrate ---------- Positive test
Tween 80 hydrolyzed

No change in colour ---------- Negative test
No hydrolysis of Tween 80

Controls

Positive Tween hydrolysis control: Mycobacterium kansasii.

Negative Tween hydrolysis control: Use an unlnoculated tube of substrate.

UREASETEST

Testing for urease enzyme activity is important in differentiating entrobacteria. Proteus strains are strong urease producers. Y.enterocolitica also shows urease activity (Weakly a 35-37 ^{O}c) Salmonellae and shigellae do not produce urease.

Principle

The test organism is cultured in a medium which contains urea and the indicator phenol red. If the strain is urease-producing, the enzyme will beak down the urea (by hydrolysis) to give ammonia and carbon diaoxide. With the release of ammonia, the medium becomes alkaline as shown by a change iin colour of the indicator to red-pink.

The method described is that which uses the combined motility indole urea (MIU) medium, urease production can also be detected by culturing the organisms in Christensen's urea broth.

Required

a. Motility indole urea (MIU) medium.

Method

. Using a sterile straight wire, inoculate a tube of sterile MIU medium with a smooth colony of the test organism.

. Place an indole paper strip in the neck of the MIU tube above the medium. Stopper the tube and incubate at 35-37 ^{O}c overnight.

. Examine for urease production by looking for a red-pink colour in the medium as shown in colour.

Results

Red-pink medium---------- Positive test
Urease produced

No red-pink colour ---------- Negative test
No urease produced

Controls

Positive urease control: Proteus vulgaries.
Negative urease control: Eshcherlchle coli.

Note: After overnight incubation, y.enterocolitica gives a weak urease reaction and is non-motile at 35-37 ^{O}c. At room temperature (22-293^{O}c), the species is motile and shows a stronger urease reaction.

VOGES – PROSKAUER (V-P) TEST

This test is occationally used to assist in the differentiation of enterobacteria. K.pneumoniae, Vibrio cholerae blovar el tor, and some strains of Enterobacter, ferment glucose with the production of acetylmethylcarbinol (acetoin) which can be detected by an oxidation reaction.

Principle

The test organism is cultured in a glucose phosphate peptone water for 48 hours. Sodium hydroxide and a small amount of creatine are then added. Under alkaline conditions and exposure to the air, the acation produced from the fermentation of the glucose is oxidized to diacetyl which forms a pink compound with the creatine.

Required

a. Glucose phosphate peptone water.
b. Sodium hydroxide, 400g/l.
c. Creatine poweder.

Method

. Inoculate 2ml of sterile glucose phosphate peptone water with the test organism. Incubate at 35-37 ^{O}c for 48hours.

. Add a very small amount (knife point)of creatine and mix.

. Add about 3ml of the sodium hydroxide reagent and shake well,

Caution: The sodium hydroxide reagent is corrosive, therefore handle with care and do not mouth – pipette.

. Remove the bottle cap, and leave for 1 hour at room temperature. Look for the slow development of a pink – red.

Results

Pink – red colour ---------- Positive test
Acetoine produced

No pink – red colour ---------- Negative test
No acetoin produced

Controls

V-P Positive control: Enterobacter aerogenes or
Klebsiella pneumoniae

V-P Negative control: Escherichia coli.

1.8. BACTERIAL GENETICS

Genetics is the study of inheritance. Bacterial inherited characteristics are encoded in DNA.

Bacteria have two types of DNA that contain their genes. These are :

. Chromosome

. Extra chromosome: Plasmid

The bacterial chromosome is circular, double stranded DNA attached to bacterial cell membrane.

DNA replication in bacteria is semi-conservative i.e. each strand of DNA is conserved intact during replication and becomes one of the two strands of the new daughter molecules.

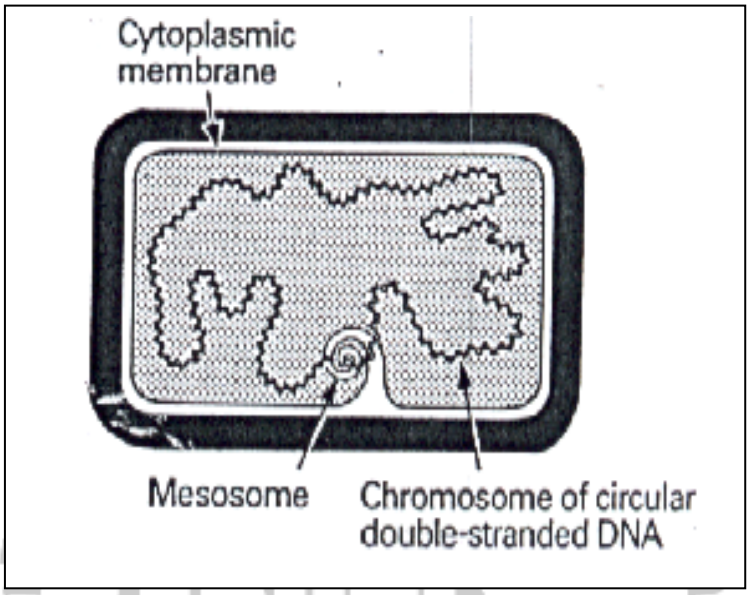


Fig. 1.12 Bacterial chromosome

Plasmids are self-replicating extra chromosomal DNA molecules.

It multiplies independent of the host cell.

Multiple copies of the same plasmid may be present in each bacterial cell.

Different plasmids are also often present in the same bacterial cell.

Plasmid types

There are many types of plasmid types. The following are examples.

a. R factors: Plasmids which contain genes that code for antibiotic resistance.

b. Col factors: Plasmids which contain genes that code for extracellular toxin (colicines) production that inhibit strains of the same and different species of bacteria.
c. F(fertility) factors: Plasmids that can recombine itself with the bacterial chromosome.

   It promotes transfer of the chromosome at a high frequency of recombination into the chromosome of a second (recipient) bacterial cell during mating.

Genetic variation in Bacteria

Mechanisms: Mutation and Gene transfer

1. Mutation: It is due to a chemical alteration in DNA.

It could be spontaneous or induced by chemical and physical meanses

Mutants are variants in which one or more bases in their DNA are altered; which are heritable and irreversible

Types of mutation

1. Substitution: Change of a single base.
2. Deletion: Los of a base.
3. Insertion: Addition of a base.

2. Gene transfer

There are three types of gene transfer that alter the DNA gene content of bacteria.

These are:

. Transformation

. Transduction

. Conjugation

1. **Transformation** occurs when fragments of exogenous bacterial DNA are taken up and absorbed into recipient bacterial cells.

Transformation of genes from one bacterium to another results in

. Change in pathogenicity of the bacterium.

. Change in antibiotic sensitivity pattern of bacterium.

Competence: The recipient bacterium must be competent to absorb the exogenous fragments of bacterial DNA.

Frequency: The frequency of transformation is low.

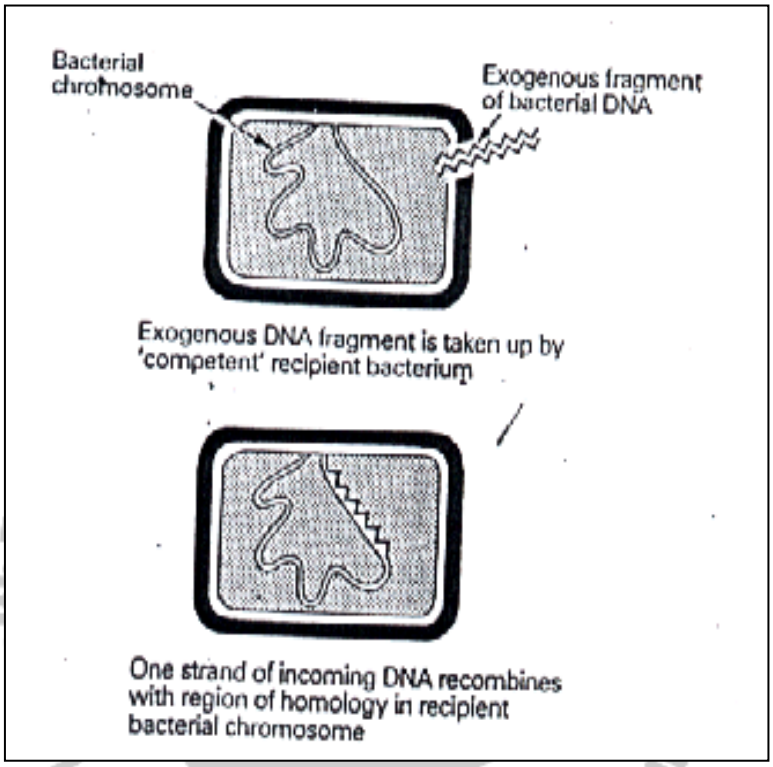


Fig. 1.13 Transformation; gene transfer by the uptake & subsequent recombination of a fragment of exogenous bacterial DNA

2. **Transduction** occurs when fragments of chromosomal DNA is transferred or transduced into a second bacterium by phage.

During phage replication, the bacterial DNA may be accidentally enclosed instead of the normal phage DNA, and when this particle which enclosed the bacterial DNA infects a second bacterial cell, the DNA from the first bacterium is released and incorporated into The chromosome of the second bacterium.

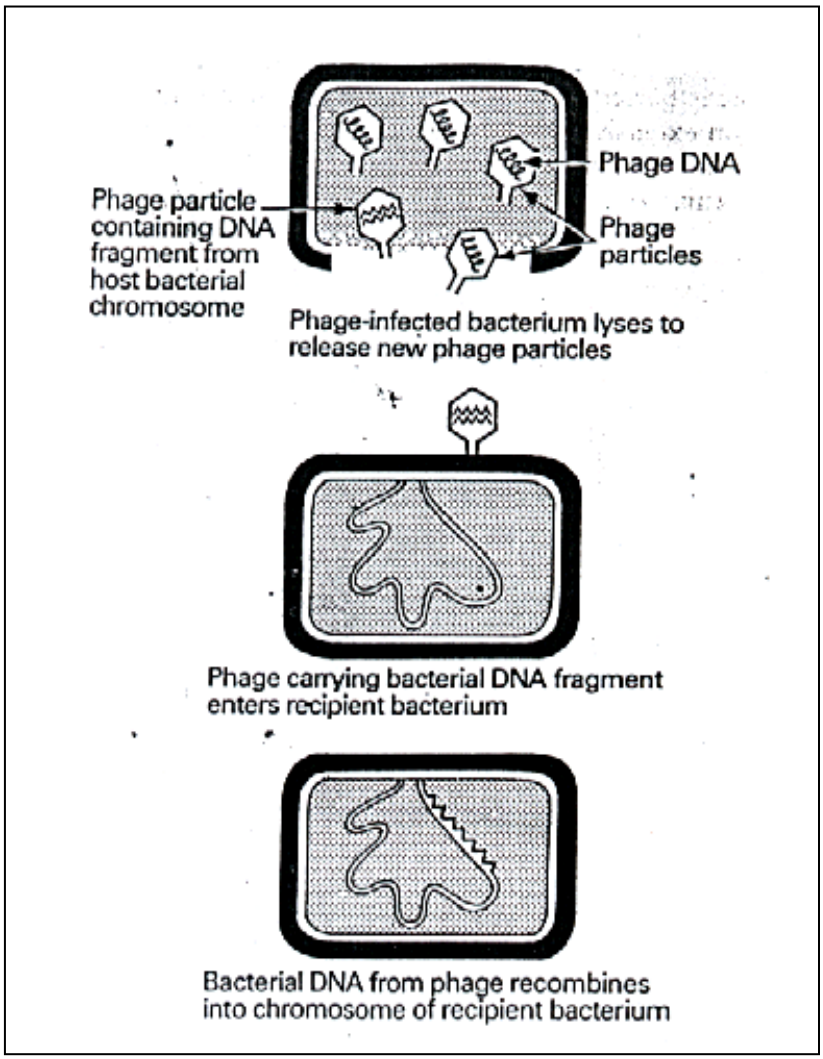


Fig. 1.14 Transduction; gene transfer from one bacterium to another via phage.

3. **Conjugation** occurs when plasmid DNA is transferred from donor to recipient bacterium by direct contact via a sex pilus.

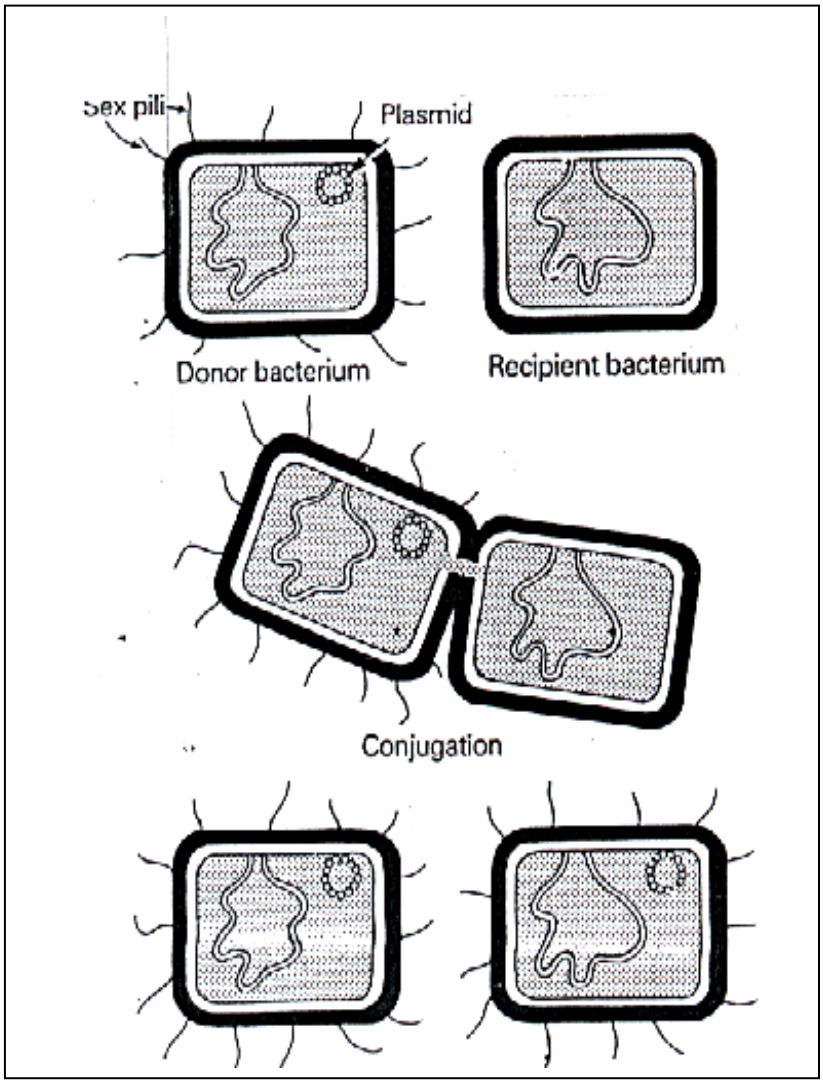


Fig. 1.15 Conjugation; plasmid gene transfer by conjugation

4. Transposition

Mechanism which enhances genetic flexibility among plasmids and bacterial chromosomes.

Transposons(Jumping genes) are segments of DNA that can transpose or move extremely readily, from plasmid to plasmid or from plasmid to chromosome(and viceversa).In this way, plasmid genes become part of the chromosomal component of genes.

When transposons transfer to a new site, it is usually a copy of the transposon that moves, the original transposon remaining in situ.

Transposons code for toxin production, resistance to antibiotics as wellas other fuctions.

1.9. STERILIZATION AND DISINFECTION

Sterilization: Destruction of all forms of microbial life including spores.

Disinfection: Destruction of microbes that cause disease; may not be effective in killing spores.

Antisepsis: destruction or inhibition of microorganisms in living tissue there by limiting or preventing the harmful effect of infection.

Sterilizing and disinfecting agents are divided into two groups.

These are:

1. Chemical methods of sterilization and disinfection
2. physical methods of sterilization and disinfection

1.9.1. Chemical methods of sterilization and disinfection

These chemical agents destroy any type of microbes with out showing any form of selectivity unlike antibiotics.

The efficacy of these agents depends on the following factors.

1. Concentration of the agent

   There is a relationship between the concentration of the agent and the time required to kill a given fraction of the microbial population.

2. Time of exposure

   Microbes are killed with a reasonable length of time with chemical agents.

3. p^H of the medium where action is to take place

   Hydrogen ion concentration determines degree of ionization of the chemical and bacterial surface charge.

   The non-ionized form passes through the bacterial cell membrane more readily than the ionized form.

4. Temperature

   Bactericidal potency of the chemical agent increases with an increase in temperature.

   An increase in 10^0c doubles the bacterial death rate.

5. Nature of the organism

   . Species of the bacteria

   . Growth phase of bacteria in culture

   . Presence of capsule, spore and other special structures

   . Number of bacteria in test system

6. Presence of extraneous materials

   Organic materials like serum, blood or pus makes chemicals inert that are highly active in their absence.

Classification of chemical methods of sterilization and disinfection

1. Chemical agents that damage the cell membrane
   . Surface active agents
   . Phenols
   . Organic solvents
2. Chemical agents that denature proteins
   . Acids and alkalies
3. Chemical agents that modify functional groups of proteins and nucleic acids
   . Heavy metals
   . Oxidizing agents
   . Dyes
   . Alkylating agents

1. Chemical agents that damage the cell membrane

Surface active agents

a. Cationic agents

   .Quaternary ammonium compounds (Quates)

   It causes loss of cell membrane semi permeability leading to loss of nutrients and essential metabolites. It as well denatures protein.

   - More active in Gram-positive bacteria than in Gram-negative bacteria.
   - More active at alkaline P^H

- Inactivated by organic materials.

b. Anionic agents

.Soaps and fatty acids

It causes gross disruption of cell membrane lipoprotein frame work.

. More active in Gram-positive bacteria than in Gram-negative bacteria.

. Active at acidic P^H

Phenolic compounds

Phenol is highly effective in Gram positive bacteria.

Clinically no more used because of its neurotoxic effect.

Currently used as a standard for measuring bactericidal potency of new chemicals i.e. phenol coefficient.

Phenol coefficient is the ratio of the concentration of the new chemical agent being tested to the concentration of the reference standard (phenol) required to kill in a specific time.

If phenol coefficient is less than one, the new chemical agent is less effective than phenol.

If phenol coefficient is equal to one, the new chemical agent is equal to phenol in efficacy.

If phenol coefficient is more than one, the new chemical agent is more effective than phenol.

Derivatives of phenol:

. Cresols e.g. Lysol, Creolin

. Halogenated diphenyl compounds eg. Hexachlorophene

It is more active on Gram-positive bacteria.

It is germicidal and anti-perspirant.

Organic solvents

Alcohol e.g. Ethyl alcohol, Isopropyl alcohol

. Disorganize cell membrane lipid structure.

. Denatures protein.

. Active against Gram-positive bacteria, Gram-negative bacteria and acid-fast bacilli.

Uses:

1. Potent skin disinfectants
2. Disinfects clinical thermometer

NB: Ethanol is potent at concentration of 70%.

Chemical agents that denature proteins

E.g. Acids and alkalies, Quates, Alcohol

. Causes conformational alteration of proteins (unfolding of polypeptide chain) resulting in irregular looping and coiling of polypeptide chain.

Acids like benzoic acid, citric acid and acetic acid are helpful as food preservatives: extending storage life of food products.

Chemical agents that modify functional groups of proteins and nucleic acids

Heavy metals

1. Mercurials : mercuric chloride – limited use because of toxicity.

   Organic mercurials – less toxic than inorganic mercuric salts.

   Used as antiseptics.

   E.g. Merthiolate

   Mercurochrome

2. Silver compounds

   E.g. Silver nitrate, Silver salfasalazine

Used as ophthalmic and wound (e.g. In burn patients) antiseptic.

Oxidizing agents

Converts functional –SH group into non-functional –S-S group.

1. Halogens e.g. Chlorine, Iodine

   a. Chlorine: inactivated by organic materials.

   Preparations and uses:

   - Chlorine: water disinfectant; the dosage is 0.5-1.0 PPM as a disinfectant.
   - Hypochlorite: sanitizing dairy and food processing industries, house holds and hospitals.
   - Organic or inorganic chloramine : effective water disinfectant acting by liberating chlorine.

   b. Iodine: effective skin disinfectant

   Preparations:

   . Aqueous Iodine

   . Iodine Tincture: 2% iodine and 70% ethanol.

   . Iodiphores (e.g. Betadine): Less toxic and less active than Aqueous iodine and iodine tincture.

2. Hydrogen peroxide (3%)

   Used for cleansing of wound, disinfecting medical-surgical devices and plastic contact lenses.

Dyes

E.g. Brilliant green

Malachite green

Crystal violet

. highly selective for Gram-positive bacteria.

Uses

. for treatment of dermatological lesions.

. for formulation of selective culture media.

Alkylating agents

E.g. Formaldehydde

Glutaraldehyde

Ethylene oxide

Formaldehyde

37% aqueous solution form is named as formalin.

Uses:

. Preservation of fresh tissues.

. Preparation of vaccines from bacterial surfaces, viruses and toxins.

. Bactericidal including spores.

Glutaraldehyde

. 10 times more effective than formaldehyde.

. cold sterling for medical-surgical instruments.

Ethylene oxide

. gaseous sterling chemical.

Use: sterilize medical-surgical devices that would be damaged by heat.

Antiseptic agents: Disinfectants that are applied on animate bodies.

Characteristics:

. Never be toxic to cells

. Never be corrosive

. Should never change nature of skin

Eg. Savlon

Alcohol(70%)

Iodine tincture

Iodophor

1.9.2. Physical methods of sterilization and disinfection

1. Heat: the most reliable and universally applicable method of sterilization.

Mechanism of action

. Dry heat – denatures protein.

. Moist heat – denatures and coagulates protein.

1.1. Dry heat : It is less efficient and requires high temperature and long period heating than moist heat.

Dry heat can be used by the following methods:

a. Incineration : It is an efficient method of sterilization and disposal of contaminated needles, syringes and cover slips at high temperature

b. Red heat : Inoculating wires, loops and points of forceps are sterilized by holding them in the flame of a Bunsen burner until they are red hot.

c. Flaming: Scalpels and neck of flasks, bottles and tubes are exposed for a few seconds, but it is of uncertain efficacy.

d. Hot Air Sterilizer (Oven): it is essential that hot air should circulate between the objects being sterilized and these must be loosely packed and adequate air space to ensure optimum heat transfer.

It is done by applying 160 ^{0}c for 1 hour.

Use: Sterilizes glassware, oils, greases, lubricants and powders.

1.2. Moist heat: It is preferred to dry heat due to more rapid killing.

Moist heat can be used by the following methods.

a. Boiling: It is not reliable method of sterilization. It is done by applying 100 ^{0}c for 30 minutes.

Used for sterilizing catheters, dressing and fabrics.

b. Tyndallization : Intermittent steaming (Fractional sterilization)

Steaming of the material is done at 100 ^{0}c for 30 minutes on three consecutive days.

The principle is that spores which survived the heating process would germinate before the next thermal exposure and then would be killed.

It is used for sterilizing heat sensitive culture media containing materials such as carbohydrates, egg or serum.

c. Pasteurization: It is the process of application of heat at temperature of

62 ^{0}c for 30 minutes(Holder method) or 72 ^{0}c for 15 seconds (Flash method) followed by rapid cooling to discourage bacterial growth.

Uses:

. Pasteurization of milk

. Preparation of bacterial vaccines.

d. Autoclaving : Steam under pressure

It is based on the principle that when water is boiled at increased pressure, hot saturated steam will be formed which penetrates and gives up its latent heat when it condenses on cooler objects.

Hot saturated steam in autoclaving acts as an excellent agent for sterilization because of:

1. high temperature
2. High latent heat
3. ability to form water of condensation
4. contraction in volume that occurs during condensation

Method of using an autoclave

- Add the correct volume of water to the autoclave.
- Place the bottles and tubes of culture media with caps loosened in the inner chamber of the autoclave.
- Secure the lid i.e. Open the air-outlet and close the draw-off knob.
- Adjust the safety valve to the required pressure and temperature.
- When the required pressure and temperature has been reached, begin timing.

NB: Most culture media are sterilized at a pressure of 15 lb/in^2, at a temperature of 121 ^{O}C for 15 minutes.

- At the end of sterilizing time, turn off the heat and allow the autoclave to cool naturally.

It destroys bacterial endospores and vegetative cells.

Uses: Sterilize solid and fluid culture media, gowns, medical and surgical equipment.

Time –Temperature-Pressure level relationship in moist heat sterilization (autoclaving)

Temperature	Time	Pressure level
121 ^{0}c	15 minutes	15 lb/inch^2
126 ^{0}c	10 minutes	20 lb/inch^2
134 ^{0}c	3 minutes	30 lb/inch^2

Methods of controlling sterilization

1. Recording of temperature and time of each sterilizing cycle.
2. Heat-sensitive autoclave tape fixed to the outside of each pack.

. Color change of autoclave tape from blue to brown-black indicates completesterilization.

3. Biological indicator : Use of paper strips impregnated with spores of Bacillus stereothermophilus

. Put the paper strip in the culture medium after autoclaving and observe for germinating bacteria to check for growth. In complete sterilization there should not be bacterial growth.

e. Freezing: Inactivation of living bacteria by cold.

It prevents active multiplication of bacteria by decreasing the metabolic activity of bacteria.

Lyophilization : Freeze-drying : Involves rapid freezing with subsequent drying.

Use:

. Preservation of microbial cultures.

. Preservation of vaccines.

f. Filtration : Mechanical sieving through membrane filters.

Uses:

. Sterilization of thermolabile parental and ophthalmic solutions, sera and plasma.

. Microbial evaluation of water purity.

. Viable counting procedures.

. Determination of viral particle size

g. Radiation : Ioning and ultra violet radiation

Ioning radiation includes χ ray, γ ray and β ray.

These induce break down of single stranded or sometimes double stranded DNA.

Ultra violet radiation has less quantum energy with low penetrating power than ionic radiation.

Spore forming bacteria are more resistant to ionic and ultra violet radiation than vegetative bacteria because of:

1. The spore coat confers protection.
2. DNA is in different state in spores.

Use: Sterilize surgical sutures, catheters, petridishes, culture media while dispensing and pharmaceutical products like hormones, enzymes and antibiotics.

It also sterilize biological safety cabinet (Laboratory rooms).

1.9.3. Anti-Microbial agents and Sensitivity Testing

Anti- Microbial drugs

Anti-microbial drugs include

. Antibiotics

. Chemical anti-microbials

Antibiotics:

Definition: Antimicrobial substances produced by living micro-organisms.

Chemical anti-microbials

Definition: synthetically produced anti-micorbial compounds.

Anti-microbial drugs show specific toxicity to microbial cells due to differences in cell envelope, protein and enzymes to host cells.

Mechanism of action of anti-microbial drugs

1. Those inhibiting cell wall synthesis, leading to cell lyses.
   - penicillin
   - cephalosporin
   - vancomycin

2. Those damaging cell membrane leading to loss of cell contents and then cell death.
   - polymyxin
   - Amphotericin B
3. Those inhibiting protein synthesis and then arresting bacterial growth
   - aminoglycosides
   - tetracycline
   - erythromycin
   - chloramphenicol
   - clindamycin
4. Those inhibiting nucleic acid synthesis
   4.1. Preventing DNA synthesis
   - Nalidixic acid
   - Quinolones
   4.2. Preventing RNA synthesis
   - rifampicin
5. Those inhibiting nucleotide synthesis
   - sulfonamide
   - trimethoprim

Resistance of bacteria to anti-microbial drugs

Production of enzymes that destroy or inactivate anti-microbials

Eg. B.lactamase destroying B-lactam ring of penicillin

1. Altering permeability of bacterial cell membrane

   Eg. resistance to polymixin and tetracycline

2. Developing an altered structural target for the drug

   Eg. resistance to aminoglycosides and erythromycin

3. Developing an altered metabolic pathway that bypasses the reaction inhibited by the drugs

   Eg. resistance to sulfonamides

4. Developing an altered enzyme that can still perform its metabolic function but is much less affected by the drug

   Eg. resistance to trimethoprim

Dangers of indiscriminate use of antimicorbial drugs

1. Wide spread sensitization resulting in hypersensitivity and anaphylactic reaction, and drug rashes.
2. Changing normal microbial flora leading to “super infection” due to over growth of drug-resistant micro-organism.
3. Direct drug toxicity

   Eg. renal and auditory nerve damage due to aminoglylosides toxicity.

4. Masking serious infection with out eradicating it.
5. Development of drug resistance by micro-organisms.

Anti-microbial sensitivity testing

Anti-microbine activity is measured in vitro in order to determine:

- the potency of an anti-microbial agent
- concentration of anti-microbial agent in body tissues or fluids
- the sensitivity of a given micro-organism to known concentrations of the drug

Measurement of anti-microbial activity

Techniques

- a diffusion technique
- a dilution technique

Diffusion Sensitivity Tests

It is the routinely used sensitivity test by most microbiology laboratories.

A filter paper disk containing measured quantities of drug is placed on a solid medium that has been seeded with the test organisms. The drug diffuses from the disk into the medium.

Following over right incubation, the diameter of the clear zone of inhibition surrounding the deposit of drug is taken as a measure of the inhibitory power of the drug against the particular test organism.

Bacterial strains sensitive to the drug are inhibited at a distance from the disk whereas resistant strains grow up to the edge of the disk.

In the kirby-Bauer technique, the zone of inhibition is measured and compared to a prepared scale, which correlates the zone of inhibition size with the minimum inhibition concentration (MIC).

NB: Minimal inhibitory concentration (MIC) is the lowest concentration of antimicrobial agent that is required to inhibit in vitro bacterial multiplication under specified conditions.
Minimal bactericidal concentration (MBC) is the least concentration of the anti-microbial required producing a sterile culture.

Dilution Sensitivity Tests

- Agar dilution tests
- Broth dilution tests

Graded amounts of antimcorbial agents are incorporated into liquid or solid bacteriology media. The media are subsequently inoculated with test bacteria and incubated.
The end point is taken as that amount of antimicobial agent required to inhibit the growth of the test bacteria (MIC) or to kill the test bacteria (MBC).
Nowadays the above tests are either time consuming or cumbersome, so the advent of microdilution both solution tests has simplified the method and permit a quantitative result to be reported, indicating the amount of a given drug necessary to inhibit or kill the test micro-organism.

Factors affecting anti-microbial activity in vitro

1. P^H of the environment
   - some drugs more active at acidic or alkaline P^H
2. Components of medium
   - media composition components enhance or inhibit bacterial growth.
3. Stability of drug
4. Size of inoculum: the larger the bacterial inoculum, the lower the apparent sensitivity of the organisms.
5. Length of incubation: short exposure of moisture to the drug inhibits their growth but does not kill them; longer exposure of moisture to a drug gives a chance for resistant motants to emerge.
6. Metabolic activity of moistures
   - actively and rapidly growing micro-organisms are more susceptible to drug action than those in the resting phase.

Techniques of routinely used antimicrobial sensitivity testing (disc diffusion tests)

Required:

- sensitivity testing media
- Anti-microbial discs
- Control strains
- Turbidity standard

Sensitivity testing media: The commonly used media is Mueller-Hinton agar.

For pathogens requiring enriched media like Neisseria gonorrhea, Heamophilus influenzae and Streptococcus pneumoniae, it is necessary to add blood to (heat it if needed) sensitivity testing agar.

Turbidity (Opacity) standard: This is a barium chloride standard against which the turbidity of the test inocula can be compared. The turbidity of the standard is equivalent to the turbidity of subcultured broth test micro-organism.

Method

- Emulsify several colonies of similar appearance of the test organism in a small volume of sterile nutrient both.
- Match the turbidity of the subculture against the turbidity standard.
- Apply a loopful of the test organism subculture to the sensitivity testing plate using a sterile loop.
- Spread the inoculum evenly across the plate using a sterile dry cotton wool swab.
- Allow the inocula to dry for a few minutes with the petridish lid in place.
- Place the anti microbial discs into the test organism in petridish using a sterile forceps or dispenser.

  NB: each disc should be pressed down on the medium and should not be moved once in place.
- Incubate the plate aerobically at 35-37 ^{o}c over night after 30 minutes of applying the discs.

- Read the tests and interpret as ‘sensitive (S)’, “ resistant (R) ”or “ intermediate (I)” comparing the chart of the sensitivity test**.**

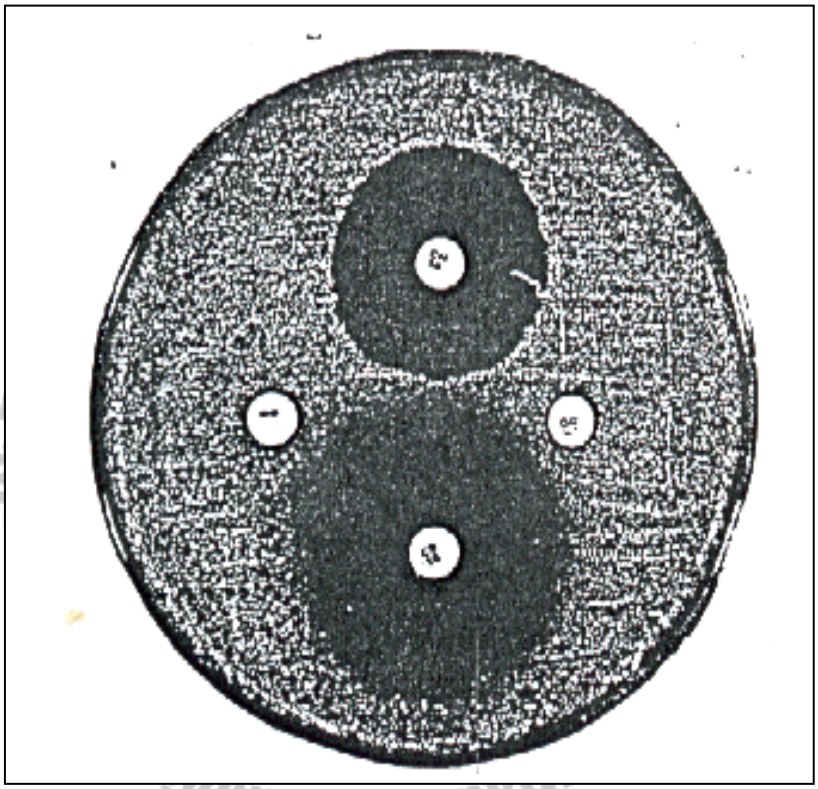


Fig. 1.16 Antimicrobial sensitivity test Media

Review Questions

1. Mention the function of bacterial cell envelope
2. Mention the procedure of gram's and Ziehl-Neelson's staining method
3. Discuss the different types of culture media
4. Label and describe each bacterial growth phase
5. Describe the types of gene transfer that alter the bacterial genome
6. List and describe factors that influence antimicrobial sensitivity testing in vitro

CHAPTER TWO

COLLECTION, TRANSPORT AND EXAMINATION OF SPECIMEN

- If pathogens are to be isolated successfully, the type of specimen, its collection,time and method of its dispatch to the laboratorty must be correct.
- Adequate information about the patient's condition and antimicrobial treatment must also be sent in the Specimen.

Type of specimen

The correct type of specimen to be collected will depend on the pathogens to be isolated.

For example**:** a cervical not a vagina**l** swab is required for the most successful isolation of *N.gonorrhoeae* from a woman.

:sputum not a saliva is essential for the isolation of respiratory pathogens.

Time of collection

- Specimens such as urine and sputum are best collected soon after a patient wakes when organisms have had the opportunity to multiply over several hours.
- Blood for culture is usually best collected when a patient's temperature begins to rise.

- ❖ The time of collection for most other speciemens will depend on the condition of the patient, and the time agreed between medical, nursing and laboratory personnel for the delivery of the specimen to the laboratory.
- ❖ Every effort must be made to collect specimens for microbiological investigation before antimicrobial treatment is started.

Collection techniques

- ♦ The laboratory should issue written instruction to all those responsible for collecting specimens including staff of wards, out patient clinics and health centres.
  - ♦ Precaution apply to the collection of most microbiological specimens:
    1) Use a collection technicnque that will ensure a specimencontains only those organisms from the site where it was collected.
       - If contaminating organisms are introduced in to a specimen during its collection or subsequent handling, this may lead to difficulties in the interpretation of cultures and delays in the issuing reports.
       - A strictly sterile (aseptic) procedure is essential when collecting from site that are normally sterile.

Example: collection of blood, CSF or effusions

This teachings is necessary not only to prevent contamination of the specimen but also protect the patient.

2) Avoid contaminating discharges or ulcer material with the skin commensals. The swabs used to collect the specimens must be sterile and the absorbent cotton wool from which the swabs are made must be free from antibacterial substances.

3) Collect specimens in sterile, leak- proof, dry containers, free from all traces of disinfectant.

Container must be clean but need not be sterile for the collection of feaces and sputum.

4) Those responsible for collecting specimens should report any abnormal features, such as coldiness in a specimen which should appear clear, abnormal coloration or presence of pus, blood, mucus or parasites.

Labelling of specimens and sending of a request form

Each specimen must be accompanied by a request form which gives:

- The patient's name, age (whether an infant, child or adult**),** number, and ward or health center**.**
- Type of specimen and the date and time of it collection.
- Investigation required
- Clinical note giving details of the patient's illness, suspected disease, and any antimicrobial treatment that may have been started at home or in the hospital.

Specimen containing dangerous pathogen

- Those delivering, receiving and examining specimens must be informed if a specimen is likely to contain highly infection organisms. Such specimen should be labelled **HIGH RISK**, and whenever possible carry a warning symbols such as red dot, star or triangle
- Specimen which should be marked as **HIGH RISK** include:
  - Sputum likely to contain *M. tuberculosis*
  - Faecal specimen that may contain *V. cholerae* or *S.typhi*
  - Fluid from ulcers postules that may contain *anthrax bacilli* or treponemes
  - Specimens from patient with suspected HIV infection, hepatitis, viral haemorrhagicfever, or plague.

Preservatives and transport media for microbiological specimen

- In general, specimens for microbiological investigations should be delivered to the laboratory as soon as possible.
  This will help to ensure that pathogens are living when they reach the labratory.
- When a delay in delivery is unavoidable,
  For example, when transporting a specimen from health center to a hospital laboratory, a suitable chemical preservative or transport culture medium must be used.

- ❖ This will help to prevent organisms from dying due to enzyme action, change of pH, or lack of essential nutrients.

  Example. Amies transport medium widely used and effective in ensuring the survival of pathogens like the more delicate organisms such as *Neisseria gonorrhoeae*.

Cary - Blair medium in used as transport medium for faeces that may contain *Salmonella, shigella, campylobacter or vibro species.*

Example of Preservatives include boric acid added to urine and cetylpyridinium- chloride sodium chloride (CPC-Nacl) added to sputum for the isolation of M.*tuberculosis*.

Transport of microbiological specimens collected in a hospital

- Specimen should reach to the laboratory as soon as possible or a suitable preservative or transport medium must be used.
- Refrigeration at 4-10 ^{0}c can help to preserve cells and reduce the multiplication of commensals in unpreserved specimens.
- However, specimens for isolation of *Haemophilus*, S. *pneumoniae* or *Neisseria species*, must never be refrigerated because cold kills these pathogens.

- ❖ If specimen are to be mailed, the regulations regarding the sending of “pathological specimens” through the post should be obtained from the post office and followed exactly. When dispatching microbilogical speciemens:

1) Keep a register of all specimens dispatched.
   Record Name
   Number
   Ward or health centre of the patient.
   Type of specimen
   Investigation required
   Date of dispatch
   Method of sending the specimen (eg. Mailing, hand –delivery etc.)
2) Ensure that the specimen container is free from cracks, and the cap is leak proof. Seal round the container cap with adhesive tape to prevent loosening and leakage during transit.
3) If the container is glass tube or bottle, use sufficient packaging material to prefect a specimen.

If the specimen is fluid, use sufficient absorbent materiel to absorb it.

4) Mark all specimen that may contain highly infectious organism **"HIGH RISK"**

5) Dispatch slides in a plastic slide continer or use slide carrying box.
6) Label speciments dispetched by mail, "FRAGILE WITH CARE- PATHOLOGICAL SPECIMGN".

Collection, Transport and examination of sputum

Possible pathogens

Gram positive	**GRAM NEGATIVE**
Streptococcus pnemoniae	*Haemophilus influenzae*
Staphylococcus aureus	*Klebsiella pneumoniae*
Streptococcus pyogenes	*Pseudomonas aerugnosa*
	Proteus species
	Yersina pestis

Sputum commensals

- Sputum as it is being collected passes through the pharynx and the mouth. Therefore, it becomes contaminated in the small number of commensal organisms from the upper respiratory tract and mouth

Gram positive	**Gram negative**
Staphyloccus aureus	*Neisseria*
Staphyloccus epidermidis	*Branhamella catarrhac's*
Streptococcus Viridans	*Haemophilus influenzae*
Streptococcus pnemoniae	*Fusobacteria*
Enterocci	*Coliforms*
Diphtheroids	
Yeast-lke fungi	

In a hospital with a microbiology Laboratory.

1. Give the patient a clean (need not be sterile), dry, wide-necked, leak- proof container and request him or her to cough deeply to produce a sputum specimen

   Note: The specimen must be sputum, not saliva.

Sputum is best collected in the morning, soon after the patent wakes and before any mouth-wash is used.

If the patient is young child and it is not possible to obtain sputum, gastric washings can be used for the isolation of M.*tuberculosis* but not for other respiratory

2. Label the container and fill the request form.
3. If pneumonia or bronchopneumonia is suspected, deliver the sputum to the laboratory with as little delay as possible.
   - Because organisms such as S.*pneumoniae* and H. *influenza* require culturing as soon as possible.
   - If the specimen is for the isolation of M.tuberculosis, ensure it is delivered to the laboratory within 2 hours or kept at 4 ^{O}c until delivery is possible.
   - If pneumonia plague is suspected: Deliver the sputum to the laboratory as soon as possible. Make sure that the specimen is marked **HIGH RISK**.

For dispatching to a microbiology laboratory

Collect the sputum in a container as usual.

Depending on whether the sputum is for the isolation of *pneumonia* and bronchopneumonia or M.*tuberculosis*, proceed as follows:

Pneumonia and Bronchopneumonia pathogen

- ❖ Collect a purulent part of the sputum on a cotton wool swab, and insert in a container of Amies transport medium. Label the container using a lead pencil
- ❖ Amies will help the pathogens to survive and from being overgrown by fast-multiplying commensas.
- ❖ Make a smear on slides for gram staining and fix using heat or alcohol and send the swab and the request form to reach to the laboratory.

M.*tuberculosis*

- Make a smear of the sputum on slides for Ziel-Neelsen staining, from the most pumlent materials.
- Fix with alcohol.

If pneumonia plague is suspected:

- Send the swab of the sputum in cary-Blair transport medium to reach the microbiology laboratory.

  Caution: Y. pestis is highly infectious organism.

Laboratory examination of sputum

1. Describe the appearance of the specimens

- Purulent: Green- looking with pus and mucus.
- Mucopurulent: Green- looking with pus and Mucus
- Mucoid: Mostly mucous
- Mucosalivary: Mucus with a small amount of saliva
- Bloody: should be reported.

2. Examine the specimen microscopically.

Gram smear

Look for pus cells and bacteria

- Gram positiveve diplococci that could be *S. pneumoniae*
- Gram positive cocci that could be *S. aureus*
- Gram negative rods that could be *H. influenzae*
- Gram negative capsulated rods that could be K. *pneumoniae*.

Additional investigation

Look for:

- **saline preparation**, if paragonimiasis is suspected
- **Eosin paration**, if asthma or other allergic condition is suspected.
- **Potassium hydroxide (KoH) preparation**, if fungal infection is suspected (yeast cells, Nocardia species, actinomycetes).

- **Giemsa smear**, if histoplasmosis or pneumonic plague is suspected.

3) Culture the specimen

To obtain as pure a culture as possible of a respiratory pathogen it is necessary to reduce the number of commensals inoculated.

Ways of reducig commensal numbers include washing the sputum free from saliva or liquefying and diluting it.

Blood agar and chocolate agar

- Wash a purulent part of the sputum in about sml of sterile physiological saline.
- Inoculate the washed sputum on plates of blood agar and chocolate (heated blood) agar.
- Add an optochin disc to the chocolate agar plate. This will help to identity *S. pneumonia*.
- Incubate the blood agar plate aerobically and the chocolate agar in a co_2 enriched atmosphere at 35-37^{0}c for upto 48 hours, examining for growth atfter ovemight incubation.

Additional:

Lowenstein Jensen medium, if pulmonary tubercles is suspected.

- About 20 minuets before culturing, decontaminate the specimen by mixing equal volumes of sputum and

sodium hydroxide (NaoH) 40 g/l or (4% W/v) solution. Shake at intervals to homogenize the sputum.

- Using a sterile pasteur pepitte, inoculate 200 Nl (0.2ml) of the well-mixed homogenized sputum on a slope of acid Lowenstein Jensen medium. Allow the specimen to run down the slope.
- Incubate at 35^0c –37^0c in a rack placed at an agle of about 45^0c to ensure that the specimen is incontact with the full length of the slops.
- After one week, place the slope in an upright position and continue to incubate, examining twice a week for groth.

4. Examine and report the culture

Examine the blood agar & Chocolate agar culture for:

S. pneumonia

S. aureus

H. influezae

K. pneumoniae

Pse. Aeruginosa

Examin Lowenstein Jensen e culture for:

M.. *TUBERCULOSIS*

Collection, transport and examination of mouth and throat specimens

Possible pathogens

Gram positive	**Gram negative**
Streptococcus pyogenes	*Vincent's* organisms
Other *beta-haemolytic streptococci*	
Corynebacterium diphtheriae	

Virus: Respiratory viruses, enteroviruses and herpes simplex virus type-I

Fungi: candida albicans.

- Pathogens in the upper respiratory tract such as *Bordetella, pertussis*, *streptoccus pneumoniae,* and *Neisseria meningitidis*, are usually more successfully isolated from pernasal and nasopharyngeal specimens.

Throat and moth commensals

Gram positive	**Gram negative**
Viridans streptococci	*Branhamella catarrhalis*
Non-*haemolytic streptococci*	*Neisseria Pharyngitidis*
Streptococcus pneumonia	*Fusobacteria*
Staphylococcus epidermidis	*Coliform*
Micrococci	
Lactobacilil	

Collection and Transport of Throat and Mouth swabs

- Whenever possible it should be collected by a medical officer or experienced nurse.

In a hospital with a microbiology laboratory

1. In a good light and using the handle of a spoon to depress the tongue, examine the inside of the month.

Look for inflammation, and the presence of any membrane, exudate or pus.

- With diphtheria – a greyish- yellow membrane (later becoming grayish Green-black and smelly) can often seen over the soft palate and backwards on to the phargedl wall.
- With streptococcal sore throat, the tonsil are inflammed and often coverd in a yellow spots.
- With infectious mononucleosis, the tonsils may be covered with a white exudate
- With *C. albicans* infection, patches of white exudate may be seen attached in places to the mucosal membrane of the month.

2. Swab the affected area using a sterile cotton. Taking care not to contaminate the swab with saliva, return it to its sterile container.
    - Before swabbing the patient must not treated with antiboitics or antiseptic month-washesr (gargles) for 8 hours.

- For children, swabbing may causes obstruction child's airway instead blood for culture should be collected.

3. Within 2 hours of collection, deliver the swab with a request form to the laboratory

Dispatching to the microbiology laboratory

1. Using a sterile swab or silica gel collect a specimen from the infected area.
2. Taking care not to contaminate the swab, return it to its tube.
   Seal with adhesive tape and label using a lead pencil.
3. Send the swab with its request form to reach the microbiology laboratory within three days.

Laboratory Examination of throat and month swabs

1. <u>culture the specimen</u>

Blood agar

- Inoculate the swab on a plate of blood agar.
- If the swab is received in silica gel (eg. from health centre), moisten it first with sterile nutrient broth and then inoculate the plate.
- Add a bacitracin disc. This will help in the identification of *S. pyogenen.*
- Incubate the plate preferably anaerobically or in a co_2 enriched atmosphere overnight at 35-37^{0}c

- Beta-haemolytic streptococci produce larger Zones of haemolysis when incubated anaerobically.
- A minority of Group A streptococcus strains will only grow if incubated anaerobically.

Additional

Modified Tinsdale medium and tellurite blood agar if diphtheria is suspected.

- If in an area where diphtheria occurs or the disease is suspected, inoculate the swab on modified Tinsdale medium and tellurite blood agar.
- Incubate the plates aerobically at 35-37^{0}c for up to 48 hours, examing for growth after overnight incubation.

Sabouraud agar if thrush is suspected

- Inoculate the swab on sabourad agar
- Incubate at 35-37^{0}c for up to 48hours checking for growth after overnight incubation

2. Examine the specimen microscopically

Gram smear

Examine the smear for pus cells and Vincent's organisms:

- Vincent's organism are seen as Gram negative spirochaetes (B. vincenti)

And fusiform rods, (L. buccalis).

- If thrush is suspected, look for Gram positivee yeast-like cells.
- If diphtheria is suspected, look for Gram positive pleomorphic rods
- Commensal diphtheroids, however, are strongly Gram positive and Unlike

C.diphtheriae, they show little variation in size or shape.

Additional

Albert stained smear

Examine the smear for bacteria that that could be C. diphtheriae:

- Most strains of C. diphtheriae contain dark-staining volutin gtanules.
- The pleomorphic rods tend to join together at angles giving the appearance of Chinese letters.

3. Examine and report the cultures.

Blood agarculture

- look for beta-haemolytic colonies that could be group Astreptococcu(S. pyogenes)

or a beta-haemolytic streptococcus belonging to another lancefield group such as group C or G.

- Most group A strains show sensitivity to bacitacin.

Reporting of the throat swab cultures:

- If a B-haemolytic streptococcus sensitive to bacitracin is isolated, report the culture as “S. pyogenes presumptive group A isolated, Lancefield group to be confirmed.
- If a B-haemolytic streptococcus that is not sensitive to bacitracin is isolated (confirm that the colonies are streptococci), report the culture as ”Beta-haemolytic streptococcus isolated, lancfield group to follow”.

Additional:

Modified Tinsdale medium (MTM) and tellurite blood agar (TBA) Cultures.

- On MTM, c. diphtheriae produces grey-black raised colonies surrounded by a dark brown area.
  -If there is no growth, reincubate to the plate for further 24 hours.
- Examine the TBA plate for grey or grey-black colonies measuring 0.5-2 mm in diameter.

Sabouraud agar culture ***Look for candid albicans***

Collection transport and examination of Nasopharyngeal aspirates and Nasal swabs

Nasopharyngeal Aspirates and perinasal swabs

Possible pethogens

Grampositive	**Gram negative**
Streptococcus pneumonia	*Haemophylus influenzae*
Corynebacterium diphtheriae	*Neisseria meningitidis*
	(carriers)
	Bordetella pertussis
	Bordetella parapertussis
	Klebsiella species

Also M. leprae

Viruses: Respiratory viruses and entrovruses.

Anterior Nasal Swabs

Possible pathogens

- Most anterior nasal swabs are examined to detect carriers of pathogens

Gram positive	**Gram negative**
S. aureus	N. meningitidis
S.pyogenes	H. influenzae (mostly non capsulate)

Nasopharyngeal Aspirates, perinasal and Anterior Nasal swabs Commensals

Gram positive	**Gram negative**
S. viridans	*Neisseria species*
S. pneumoniae	*Haemophilus species*
Entrococci	
Staphylococcus species	
Micrococci	

Collection and Transport of upper Respiratory Tract specimens

Nasopharyngeal aspirates: Gently pass a sterile catheter through one nostril as far as the nosopharynx.

: Attach a sterile syringe to the catheter, and aspirate a specimen of mucopus.

: Dispense the specimen into a small sterile container.

: Label and deliver with a completed request form to the laboratory as soon as possible.

Pernasal swabs for the culture of B. pertussis:

If whooping cough is suspected and the nasal passages are clear, collect a penasal swab as follows:

1. Using a sterile cotton or alginate wool swab attached to an easily bent pieces of wire, gently pass the swab along the floor of one nostril directing the swabdown wards and backward as far as the Nasopharynx.
2. Taking care not to contaminate the swab, replace it in its sterile container.
3. Label, and deliver immediately to the laboratory with a request form.

Note:- B. *pertusis* does not survive well on a swab

-It must be cultured as soon as possible.

-If plating cannot be performed at the bedside, the swab should be placed in special transport medium

-If it is not possible to collect a per nasal swab, a less satisfactory way of isolating B. *pertussi*s is to hold the plate of culture medium in front of the child's month during a coughing attack.

Anterior nasal swabs to detect carriers:

1. Using a steile cotton wool swab moistened with sterile peptone water, gently swab the inside surface of the nose.
2. Taking care not to contaminate the swab, replace it in its sterile container.

3. Label, and within 2 hours deliver the swab with a request form to the laboratory.

Laboratory examination of upper respiratory tract specimen

1. culture the specimen

Blood agar and chocolate agar

- To detect H*. influenzae,* N. *meningitidis*, and S*. aureus* carrier:
  - Inoculate the swab on chocolate (heated blood) agar.
  - Incubate the plate in carbondioxide enriched atmosphere at at 35^0 $^-37^0$c for up to 48 hours., examinig for growth after overnight incubation.
- To detect S. pyogenes and S. aureus carriers:
  - Inoculate the swab on blood agar.
  - Incubate the plate preferably anaerobically at 35^0 $^-37^0$c overnight (if for the isolations of S. aureus only, incubate aerobically).

Additional

Charcoal Cephalexin blood agar if whooping cough is suspected

- Inoculate the swab over the entire surface of a plate of charcoal cephalexin blood agar (CCBA).

- Incubate the plate aerobically in a moist atmosphere (in a plastic bag or polythene container with a wet piece of cotton wool) at 35-37^{0}c for up to 6 days,
- Examining for growth after about 48 hours incubation.

Culture of swab received in bordetella transport medium:

- Inoculate the swab on a plate of CCBA
- Return the swab in its container and incubated it at 35-37^{0}c
- Examine the plate for growth after 48hoursrs incubation.

❖ If no growth is seen, inoculate a second plats of CCBA with the incubated swab.

- Reincubate the first plats for a further four nights.
- Examining for growth every 24 hours.
- The second plate for up to 6 days.

2. Examine and report the cultures

Blood agar and chocolates agar cultures(routine)

Look for coloniess that could be

H. influenzae

Neissena mengitdis

S. aureus

S. pyogenes (Group A)

Additional

Charcoal ceplea lecin blood agar (CCBA) culture

The examination of a CCBA culture for *berdetela* species and the identification of *B. pertussis* and *B. parapertussis*.

Collection, Transport and examination of Ear Discharges

Possible pathogens

Gram positive	**Gram negative**
S. aureus	*P. aeruginossa*
S.pyogenes	*H. influenzae*
Other beta-haemolytic streptococci	*Klebsiella specia*
S. preumoniae	*proteus species*
	E.coli and other coliforms
	Bacteriodes species

Fungi: Aspergillus species especially A. niger, candida species, and occasionally various species of dermatophyte or phycomycete.

A fungal infection of the ear is called otomycosis

- External Ear infection are more commonly caused by:
  - *S. aureus*
  - *S. pyogenes*
  - *P. aeruginos.*

Middle ear infection or otitis media are commonlycaused by :

s. pneumoniae

s. pyogenes

and other B- *haemolytic streptococci*

H.*influenzae*(esp. in children)

coliforms

Klebsiella spenil

S. aureus

P. aeruginosa

- Chronic ear infection are often caused by Bacteroides species or fungi

Commensals

The middle ear is normally sterile. The following organisms may be found as commensals in the external ear:

Gram positive	**Gram negative**
Viridans streptococci	*Escherichia coli and*
other coliforms S.epidermidis	
Bacillus species	
Corynebacterium species	

Collection and Transport of Ear Discharges

1. Collect a specimen of the discharge on a sterile cotton.
2. Place it in container of Amies transport medium, breaking off the swab stick to allow the bottle top to be replaced tightly.
3. Make a smear of the discharge on a slide (for Gram staining).
4. Label the specimens and send them with its request form to the laboratory Within 6 hours.

Laboratory examination of Ear Discharges

1. culture the specimen

Blood agar and Macconkey agar

Inoculate the specimen on blood agar and macconkeg agar
Incubate both plates aerobically at 35-37^{0}c overnight.

Additional: Chocolate agar if the patient is a child: Inoculate the specimen on chocolate (heated blood) agar for the isolation of H. influenza.

Incubate the plate in a carbon dioxide enriched atmosphere at 35-37^{0}c for up to 48 hours, examining for growth after overnight incubation.

Blood agar (Kanamycin) for anaerobic incubation if the infection is chronic

Inoculate the specimen on blood agar, preferably that which contains Kanamycin to inhibit the growth of commensals.

Incubate the plate anerobically for up to 48hours, checking for growth after overnight incubation.

Sabouraud agar if a fungal infection is suspected

Inoculate the specimen on sabouraud agar, and incubate at room tempreture for up to 6 days.

2. Examine the specimen Microscopically

Gram smear

- Make an evenly spread of the specimen on a shide.
- Allow the smear to air-dry in a safe place.
- Fix and stain by Gram technique.
- Look for pus cells and bacteria

  - Gram positive cocci that could be S.aureus
  - Gram positive streptococci or diplococi that could by streptococci pathogens

- Gram negative rods that could be H.influenzae, p. aeroginosa, klebsiella specis, proteus species, E.coli or other s.
- Gram positive yeast cells that could be candida species.
- Small numbers of Gram positive cocci, streptococci, rods and also Gram negative rods may be seen in smears of ear discharges because these organisms form part of the normal microbial flora of the external ear.

Additional:

Potassium hydroxide preparation if a fungal infection is suspected

- Mix a small amount of the specimen with a drop of potassium hydroxide, 200g/l (20%W/v) on a slide, and cover with a coverglass.
- After 10 minutes, or when the preparation has cleared sufficiently, examine microscopically using 10x or 40x objective.

Look for:

- Brnaching septate hyphae with small round spores, that could be Aspergillns speies
- Pseudohyphae with yeast cells, that could be candida specis (Gram positive)

- Branching septate hyphae, that could be a species of der matophyte
- Branching aseptate hypae, that could be a species of phycomycete.

3. Examine and Report the culture

Blood agarand MacConkey **Examine chocolate agar**

S. aureus *H. influenza*

B-haemolytic streptococcus

E.Coli

Profeus spese

P. aeruginosa

S. pneumonia

examine sabouroud agar for:

examine the anaerobic blood agar mete for: fungi elements

Bacteriode species

Collection, transport and examination of eye specimens

Possible pathogens

Gram positive	**Gram negative**
Staphylococcus aureus	*Nesseria gonorrhoeae*
Streptococcus pneumonae	*Haemophilus influenzae*
Streptococcus agalactiae (Group – B)	*Haemophilus aegyptius*
Streptococcus Pyogenes (Group – A)	*Pseudomonas aeroginosa*
Other B- hemolytic streptococci	*Moraxella lacunata*
	Erterobacteria

Chlamydiae*: C trachomatis*

Viruses: Adenoviruse, herpesviruses and enteroviruses

Note:

1. Inflammation of the the delicate membrane lining the eyelid and covering the eyeball conjunctiva is called conjunctivitis.Bacterial conjunctivitis is mainly caused *by S. aureus, H. influenzae, H.aegyptius, S. Pneumoniae* and occasionally by *Moraxella lacunata*.
2. Eye infection of new born infants can be caused by:
    - *N. gonorrhoeae*, transmitted as the infant passes down the birth canal. It causes a severe purulent conjunctivitis that can lead to blindness if not treated. Infection usualy develops within 72hours of birth.

- *Streptococcus Group B (S.agalactiae*) and other *B-hemolytic streptococci* that can be transmitted during birth.
- *C. trachomatis*, that is transmitted during birth and causes conjuctivitis 5-12 days after birth.
- *S. aureus* that is acquired after birth (commonly referred to as "sticky eyes")

3. *C. trachomatis* serotype A, B and C cause endemic trachoma, a major cause of blindness especially in children and also in adults in rural areas of developing countries.
4. *P. aerogunisa* eye infections are frequently caused by the use of contaminated eye drops.
5. Herpes simplex virus can cause severe inflammation of the cornea (Keratitis)

Commensals

- That may be found in the eye discharges:

Gram positive	**Gram negative**
Viridans streptococci	*Non-pathogenic neisseriae*
Staphylococci	*Moraxella speires*

Collection and transport of eye specimen

- Eye specimen should be collected by medical officer or experienced nurses. Conjunctival scrapings to detect *C. trachomatis* must be collected by medical person.

- Specimen from the eye must be cultured as soon as possible after collection because the natural secretions of the eye contain antibacterial enzymes.

Procedure

1. Using a dry sterile cotton wool swab, collect a specimen of discharge (if an inflant, swab the lower conjunctival surface).
2. Inoculate the discharge on the following culture media:
    - Blood agar
    - Chocolate (heated blood) agar
    - MNYC *N. gonorrheae* selective medium, if the patient is a newborn infant.
3. Make a smear of the discharge on slide (frosted-ended) for staining by the Gram technique. If *C. trachomatis* infection of the newborn is suspected, make a second smear for staining by thr Giemsia technique and label the plate and slide.
4. As soon as possible, deliver the inoculated plates and smear(s) with request form to the laboratory.

Laboratory examination of the eye specimen

1. Culture the specimen

Routine:

Blood agar and chocolate agar

- Inoculate the eye discharge on blood agar and chocolate (heated blood) agar.

- Incubate the blood agar aerobically at 35-37^{0}C overnight.
- Incubate the chocolate agar plate (CAP) in a CO_2 enriched atmosphere for 48hours, checking for growth after overnight incubation.

Additional

MNYC selective medium if gonococcal conjunctivtis is suspected (infant less than 3 weeks old).

- Inoculate the discharge on the plate
- Incubate at 35-37^{0}C in a CO_2 enriched atmosphere overnight.

Loeffler serum slope if Moraxella infection is suspected:

- Inoculate the eye discharge on a loeffler serum slope.
- Incubate at 35-37^{0}C overnight.

2. Microscopically examination

Routine:

Gram smear

Look for:-

- Gram negative intracellular diplococci that could be *N. Gonorrhoeae*. If found, a presumptive diagnosis of gonococcal conjunctioitis can be made A cervical swab from the mother should also be cultured for the isolation of *N.gonorrhoae*.

- Gram positive streptococci or diplococci that could be streptococci pathogens.
- G ram positive cocci that could be *S.aureus*
- Gram negative rods that could be Haemophilus species.

Additional

Giemsa smear if C. trachomatis infection is suspected:

1) Fix the air-dried smear by covering it with methanol for 3 minutes.
2) Dilute the Giemsa stain in the buffered water
   C. trachomatis, dilute the stain 1 in 40:
   - Fill asmall cylinder to the 19.5 ml (mark with buffered water)
   - Add 0.5ml Giemsa stain to 20ml mark.
   Eg. 0.5ml + 19.5ml
   For Other organisms, 1ml + 19ml
3) Stain the smear with diluted Giemsa in dish
   C. trachomatis, stain 1 ½ - 2 hours
   For other organisms, stain 25-30 minutes
4) Wash the slide from the dish and rise the smear with buffered water. And let the smear to air-dry and examine with oil immersion objective.

Result

- ✓ trachomatis inclusion bodies ---------- Blue-mauve to dark purple. Depending on the stage of development; If the inclusion body is more mature, it will contain ---- red-mauve stiaing elementary particles.

- ✓ Chlamydia bodies must be differentiated from bacteria that may infect the eye such as neisseriae, Hemophilus species, staphylococci, streptococci, and moraxella species.
- ✓ All these bacteria, with the exception of Hemophilus species, stain dark blue in Giemsa stained smear, but Haemophilus rods stain pale blue.
- ✓ Report the smear as "chlamydial inclusion bodies present" or "No chlamydial inclusion bodies seen".

Examine and Report the cultures

Blood agar and chocolate agar

Look for colonies that could be:

Haemophylus Influenzae

Staphylococcus aureus

Beta- helmolytic streptococci

Streptococcus Pneumoniae

Pseudomonas aeruginosa

Loeffler serum slope culture

Look for Moraxella lacunata

Additional

MNYC Medium culture

- Look for N. gonorrheae
  - ✓ . oxidase positive.

- ✓ . CHO utilization test can also confirm the organism
- ✓ . The organism should also be tested for B-lactamase production.

	Glu	lact	mal	suc
N.gonorrhoeae	A	-	-	-
N.meningtidis	A	-	A	-

Glu =glucose, Lac= lactose, mal=maltose, suc,= sucrose, A= acid produced

Acid produced (yellow or orange yellow)

Collection, transport and examination of skin specimen

Possible pathogens

Gram positive	**Gram negative**
S. aureus	*Escherichia coli*
S. pyogenes	*Proteus*
Enterococci	*Pseudomonas aeruginosa*
Anaerobic streptococci	*yersinia pestis*
Bacillus anthracis	*Vincent's organisms*
Corynebacterium ulcerans	

Also *M. leprae*
M. ulcerans
T. Pertenue
T. Carateum

Virus: pox viruses and herpesviviruses
Fungi: Ringworm
parasite: Leishmania spps
: onchocerca volvulus
:D. medinensis

Commensales

Gram positive	**Gram negative**
Staphylococci	*Escherichia Coli and* other coliforms
Micrococci	
Anaerobic cocci	
Viridans streptococci	
Enterococci	
Dephtheoids	

Collection of skin specimens and ulcer materials

1. Using a sterile dry cotton wool swab, collect a sample of discharge from the infected tissue.

   If there is no discharge, use swabmoistened with sterile physiological saline to collect a specimen.

   Insert the swab in a sterile tube.

   - If the tissue is deeply ulcerated and necrotic (full of dead cells); Aspirate a sample of infected material from the side wall of the ulcer using a sterile needle and syringe.
   - Fluid from pustules and blisters: Aspirates a specimen using a sterile needle and syringe.
   - Serous fluid from skin ulcers, papillomas or papules, that may contain treponemes:
     - Collect a drop of the exudates directly on a clean cover glass and invert on a clean slide.

- Delivery immediately the speciemen to the laboratory for examination by dark-field microscopy.

2. If the specimen has been aspirated, transport the needle and syring in a sealed water proof container immediately to the laboratory.

Laboratory examination of skin specimens

1) Culture the specimen

Blood agar and MacConkey

- Inoculate the specimen
- Incubate both plate aerobically at 35-37^{0}C overnight.

Additional:

Sabourand agar if a fungal infection is suspected

- Inoculate to agar plate
- Send to a Mycology Reference laboratory.

Modified Tinsdale Medium (MTM), if cutaneous diphitheria is suspected:

- Inoculate the sample for isolation of C. Ulcerans
- Incubate aerobically at 35-37^{0}C for up to 48hours, examining the growth after overnight incubation.

Blood agar and MacConkey agar at room temperature, if bubonic plague is suspected:

- Inoculate the specimen
- Incubate both pletes aerobically at room temperature far up to 48hours.

- Examination for growth after overnight incubation.
  - ✓ Y. pestis is a highly infectious organism.

  Maximum care should be taken

Lownstein Jensen (LJ) Meduim if Buruli ulcer is suspected:

- Decontaminate the swab by immersing it in sodium hydroxide 40g/l (4% W/v) solution for 10 minutes.
- Inoculate the decontaminated specimen on two slopes of acid LJ medium.
- Incubate one slope at 35-37^{0}C as described for culture of M. tuberculosis and incubate the other slope at 32^{0}C for up to 8 weeks.

2.) Microscopical examination

Routine:

Gram Smear:

Look for:

- Gram positive cocci that could be *S. aureus*
- Gram positive streptococci that could be *S. pyogenes* or other streptococci.
- Gram negative rods that could be *p. aeroginosa*, *proteus speices, E.coli* or other coliforms.
  - ✓ **If tropical ulcer is suspected**, look for vincent's organisms
  - ✓ **If cutaneus anthrax is suspected**, look for large Gram variable rods lying in chains that could be *B. anthracis*.

- Examine also a polychrome methylene blue stained smear.

✓ **If bubonic plague is suspected**, look for Gram negative coccobacilli that could be Y. pestis.

Additional:

Potassium hydroxide preparation, if ringworm or other superficial fungi infection is suspected.

For detection of ringworm:

Giemsa techniques or wayson`s techniques,if bubonic plague is suspected.

Polychrome Loefler methylene blue smear if cutaneous anthrax is suspected.

- Fix with potassium permanganates 40g/l solutions for 10 minutes.
- Look for chain of large blue-stained capsules characteristic of *B. anthracis*.

Ziel-Neelsenstained smear if buruli ulcer is suspected

examine for acid fast bacilli.

Dark-field microscope to detect treponemes

- look for motile treponeme if yaws or pinta is suspected

Examine and report the culture

Blood agar and MacConkey agar cultures

Look for: *S. aureus* Aditional investigations

S. pyogenes **sabouraud agar**

P. aeniginosa -if fungal infection is suspected

Enterococci	**Lowenstein Jensen**
Proteus species	**-**if Buruli ulcer is suspected
Escherichia coli	**MTM**
	- if cutaneous diphtheria is suspected

Collection, Transport and Examination of urogenital specimens possible pathogens

Urethral swabs

- *N. gonorrhoeae*
- *S. Pyogenes*
- *Ureaplasma urealyticum*
- *Chlamydia trachomatis* and
- Occassionally *Trichomonas vaginalis*

Cervical swabs from non-puerperal women:

- *N. gonorrhoeae*
- *S. pyogenes*
- *Other B.hemolytic streptococci*
- *Chalmydia trachomatis and*
- *herpes simplex virus*

Cervical swabs from women with puerperal sepsis or septic abortion:

- *S. pyogenes*
- *other B – haemolytic*
- *streptococci*

- *anaerobic streptococci*
- *enterococci*
- *S. aureus*
- *clostridium pertfringes*
- *Listenia monocytogenes*
- *Bacterioles species*
- *protens species*
- *E. Coli & other coliform*

Vaginal Swabs:

T. vaginalis

candida species

Gardnerella vaginalis (Haemophilus vaginalis)

Neisseria gonorrhoeae.

Fluid and pus from genital ulcers

T. pallidium

C. trachomatis

Calymmatobacterium granulomatis (Donovania granulomatis)

H. ducreyi.

Collection and transport of urogenital specimen

- Amies medium is the most efficient medium for transporting urethral, cervical and vaginal swabs.

Specimen required for diagnosis of gonorrhoea

Male patients:

. Smears of urethral discharge

. Rectal swab from homosexual patient

Female patients

. Smears of mucopus from the cervix and urethra

Note: N. gonorrhoeae infects the mucaus membranes of the cervix, not the vagina.

The pathogen is, therefore, more likely to be isolated from a cervical swab than from a vaginal swab.

Collection of Urethral specimens

1. cleans the urethral opening using a swab moistened with sterile physiological saline.
2. Gently massage the urethra from above downwards, and collect a sample of pus on a sterile cotton wool swab.
    - The patient should not have passed urine preferably for 2hours before the specimen is collected.
3. Make a smear of the discharge on a slide for staining by the Gram technique and label the specimen.

Collection of Cervical specimen

1. Moisten a vaginal speciemen with sterile warm water and insert into the vagina.
2. Cleause the ceroix using a swab moistened with sterile physiological sahrie.
3. Pass a sterile cotton wool swab into the endocervical canal and gently rotate the swab to obtain a specimen.

- Amies transport
- Make a smear of the cervical mucopus for Gram stainingl and lable the specimen

Collection of vaginal specimen

- Using sterile swab, collect a sample of vaginal discharge.
  - Amies
  - Smear and label the sample

Laboratory examination of urogenital specimen

Routine culture

- **MNYC medium**

Incubate in moist CO_2 atmosphere

⇒*Neisseria gonorroeae*

Microscopy

⇒Gram smear:

Look for pus cells and bacteria

Suspected gonorrhoeae:

Look for intracellular gram negative diplococci

vaginitis:

Look for yeast cells, and epithelialcells in the gram variable coccobacili

Suspected **puerperal sepsis or septic abortion**

Look for gram positive rods, streptococci, cocci and gram negative rods.

Suspected **chanchroid**

Look for Gram negative coccobacilli showing bipolar staining

Additional culture

Blood agar (aerobic and anaerobic), macCokey agar,and cooked meat medium, if puerperal sepsis or septic abortion is suspected

Sabourand medium, if vaginal candidiasis is suspected and yeast cell not detected microscopically

Serum culture, if chancroid is suspected

⇒*H. ducreyi*

Microscopy

Sahlie preparation, if trichomoniasis is suspected.

Gemsa stained smear: If donovanosis is suspected

Dark field preparation, if syphilis is suspected.

Colleciton, transport and examination of cerebrospinal fluid

Possible pathogens

Gram positive

S. pneumonia

S. aureus

S. agalactiae (Group B)

Listeria monocytogenes

Bacillus anthracis

Gram negative

Neisseria meningitides

H.influenzae type b

Escherichia coli

Psendomonas aeraginosa

Proteus species

Salmonella species

⇒Also M. tuberculosis

Viruses:

Enteroviruses, especially echoviruses and coxsackieviruses. Rarely polioviruses may also be isolated from CSf.

Fungi: *Cryptococcus neoformans*

Parasites: Trypanosoma species

Naegleria fowleri

Acanthamoeba species and rarely the larvae of Angiostrongylus cantonensis and Dirofilaira immitis

Note:

1. Inflammation of the meninges (membranes that cover the brain and spinal cord) is called meningitis.

   Pathogens reach the meninges in the blood stream or occasionally by spreading from nearby sites such as the middle ear or nasal sinuses.

Meningitis is described as:

- Pyogenic (purulent), when the C.S.f contains mainly poly morphonuclear neutrophils (pus cell), as in acute meningitis caused by *N. meningitides, H. influenzae and S. pneumonae.*

- Pus cells are also found in the C.S.f in acute amoebic meningoencephalitis.
- Lymphocytic, when the C.S.f contains mainly lymphocytes, as in meningitis caused by viruses, *M. tuberculosis and C. neoformans.*
- Lymphocytes are also found in the C.S.f in trypanosomlasis meningoencephalitis and neurosyphilis.
- Eosinophilic, when the CSf contain mainly eosinophilus. This rare form of meningitis is caused by helminthes larvae such as Angiostrongylus cantonensis and Dirofilaria imitis
- Meningitis of the newborn (neonatal meningitis) is caused mainly by E.*coli, S. agalactiae (Group B), S. avreus, L. Monocytogenes.*
- Haemophilus meningitis occurs mainly in infants and young children.

Commensals

No normal microbial flora

Collection of Csf

- It should be collected by medical officer in aspectic procedure
- The fluid is usually collected from the arachnoid space. A sterile wide-bore needle is inserted between the 4^{th} and 5^{th} lumbar vertebrate and

C.s.f. is allowed to drip into a dry sterile container.

- A delay in examining C.S.f reduces the chances of isolating a pathogen.
- It will also lead to a falsely low glucose value due to glycolysis. If typanosomes are present, they will not be found because they are rapidly lyzed once the C.S.f has been withdrawn.

1) Take two sterile, dry, screw-cap containers and label one No 1 (first sample collected, to be used for culture), and the other No 2 (second sample collected, to be used for other investigations).
2) Collect about 1ml of C.S.f in container No 1 and about 2-3ml in container No2
3) Deliver immediately the samples with a request form to the laboratory.

Note: It is necessary to culture the C.S.f in the health center because pathogens such as *N.meningitidis, H. influenzae and S. pneumoniae*, are unlikely to survive transport to the bacteriology laboratory.

- If unable to perform a cell count and estimate the protein and glucose, transfer C.S.f sample No 2 to a screw-cap bottle containing sodium fluoride exalate and mix

This will preserve the cells and prevent the breakdown of glucose. Protein can also be estimated from a floride oxalated specimen.

Laboratory examination of C.S.f

- It should be performed without delay and should be reported micro-organism especially Gram smear. The fluid should be handled with special care because it is collected by lumbar puncture and only a small amount can be withdrawn.

Two samples of C.S.f

- It is usual to use sample No 1 for the culture, and sample No 2 for the cell caunt, microscopy and biochemistry.

This is because sample No 1 may contain blood (due to a traumatic lumbar puncture) which will affect the accuracy of the cell count and biochemical estimations.

I. Describe the appearance of the specimens

Report:

- Whether the C.S.f is clear, slightly cloudy, cloudy or definitely purulent (looking like pus).
- If the C.S.f is purulent or markedly cloudy, make immediately a smear for gram staining, and report it as soon as possible.
- Whether it contain blood and if so whether sample No 2 contains as much blood as sample no 1.

If blood is present in the C.S.f due to a traumatic lumbar puncture, sample No 1 will usually contain more blood than sample No 2.

If the blood is due to haemorrahage in the CNS, the two samples will probably appear equally blood-stained.

Following a subarachnoid haemorrhoge the fluid may appear xanthochromic, ie. Yellow-red (after centrifuting)

The fluid may also appear xanthromic if the patient is jaundiced or when there is spinal constriction.

- **Whether it contains clots**
  - Clotted C.S.f indicate an increase in fibrinogen.
  - It occurs when there is spinal construction
  - Clots can also be found in C.S.f. from patients with pyogenic meningitis (sterile beads to break the clot)
  - In tuberculosis meningitis, if the C.S.f is allowed to stand for several hours; skin (spider web clot) may form on the surface of the fluid.

This should be transferred to a slide, pressed out, alcohol-fixed, and stained by the Ziel-Neelsen method l.

2. **Perform a cell count (1:2) value $5x10^6$cell/l (sample No 2)**
3. **Test the specimen biochemically**
   - Glucose estimation ½ - 2/3 of that found in blood, i.e 2.5 – 4.0 mmol/l (45-72mg%)
   - Low Glucos →pyogenic meningitidis
   -Normal →viral miningitis.

-High glucose → hyperglycemia

- Total protein estimation and globulin test

Value: 0.15 – 0.40g/l (15-40mg%)

- A positive pandy's test for increase in total C.S.f protein in all form of meningitis.
- When the total protein exceeds 2.8/l (200mg%), the fibrinogen level in usually increased sufficiently to cause the C.S.f to clot. (sever pyogenic meningitis)

4. Culture the specimen (sample No 1)

It is necessary, if the fluid contains cells and, or, the protein concentration is abnormal.

Note: C.S.f should be cultured as soon as possible after collection.

If a delay is unavoidable, the fluid should be kept at 35-37^{0}C (never refrigerated).

If the C.S.f appears only slightly cloudy, centrifuge it in a sterile tube for 15-20minute and use the sediment for inoculating the plates.

Routine

Chocolate (heated blood) agar

Inoculate *-N. meningitides*

-S. Preumonia

-H. influential

Incubate in a CO_2 enriched atmosphere at 35-37^{0}C for 48hours, cheeking for growth after overnight incubation.

Additional

MacConkey and blood agar if the patiente is a newborn infant incubate both plate at 35-37^{0}C overnight

- *E.coli or other coliform*
- *S. agalacteae (Group B)*
- *Lesteriae monocytognes*
- *S. aureus*

Lowenstein Jensen medium if tuberculous meningitis is suspected

Sabourand agar if cryptococcal meningitis is suspected.

If capsulated yeast cells are seen in the microscopial preparations, inoculate a plate of sabouraud agar. Incubate at 35-37^{0}C for up to 72hours, cheeking for growth after overnight incubation.

5. Microscopy

The microscopical examination of C.S.f is required if the specimen appears abnormal, contains cells and, or, the total protein is raised with a positive pandy's test.

Routine:

Gram smear

- Gram negative intracellular diplococci that could be N. meningitidis.
- Gram positive diplococci or short streptococci that could be S. pneumoniae. It is often possible to see the capsules as unstained are as around the bacteria.
- Garm negative rods, possibly H. influezae
- Gram negative rods, could also be E.coli or other coliforms, especially if the C.S.f is from a newborn infant.
- Gram positive cocci in groups and singly, possible S.aureus.
- Gram positive streptococci, possibly S. agalacteae (G -.B)
- Gram positive yeast cells, C. neoformans.

Additional

Ziel-Neelsen – smear

M. tuberculosis

Indian ink preparation if *cryptococcal meningitis* is suspected:

Wet preparation to detect amoebae or Trypanosome

Giemsa stain to detect morulla cells or Burkett's lymphoma cells

These can be found when trypanosomes have invaded the CNS.

- They are larger than most lymphocytes, stain dark red mauve and contain vacuoles.
- Morula cells contain Igm and are throught to be degenerate plasma cells.

Collection, transport and Examination of Blood AND Bone marrow

Possible pathogens

Gram positive	**Gram negative**
S. aureus	*Salmonella typhi*
Viridans streptococci	*Other salmonella*
S. Pneumoniae	*Brucella species*
S. pyogenes	*H. influenzae*
Enterococci	*P. aeruginosa*
Anaerobic streptococci	*Klebsiella strains*
Clostridium prefringes	*E. coli*
	Proteus species
	Bacteriode species
	Neisseria meningitidis
	Yersinia pestis

Fungi: Candida albicans and other yeast Cells and other systemic mycosis

Parasites: Plasmodium species (malaria parasites)

Trypanosoma species

Wuchererila bancrofti

Brugia species

Loa loa

Leishmania donovani

Note:

1. The presence of bacteria in the blood is called bacteraemia. The term septicaemia refers to a severe and often fatal infection of the blood in which bacteria multiply and release toxins in to the blood stream.
2. Bacteria that are associated with neonatal septicaemia include E.coli and other coliforms, spaphylococci and B.haemolytic Group B streptococci.
3. In typhoid, salmonella typhi can be detected in the blood of 75-90% of patients during the first 10 days of infection and in about 30% of patients during the third week.
    - In chronic salmonellosis, bacteria are often more rapidly and successfully isolated from bone marrow, especially once antimicrobial treatment has been started.
4. Y. pestis can be isolated from the blood in septicaemia plague. The organism in highly infectious.

Commensal

Neither blood nor bone has a normal microbial flora.

Collection and culture of Blood and Borne marrow

- ✓ Blood and bone marrow require culturing immediately after collection, before clotting occurs.

Choice of culture media

- ✓ Because septicaemia is such a serious condition, it is essential to use media that will provide the fastest growth and isolation of as wide a range of pathogens as possible.
- ✓ The following media are suitable for routine culture of blood and bone marrow:
  - Tryptone soya (tryptic soy) diphasic medium
  - Thioglycollate broth medium

Tryptone soya (tryptic soy) diphasic medium

- ➢ A diphasic (two phase) medium is one that combines an agar slope with a broth medium.

Because the bacteria can be seen growing on the slope, the need to subculture on a solid medium every few days is avoided, thus reducing the risk of contamination.

- ➢ Tryptone soya diphasic medium consists of a tryptone soya agar slope and a tryptone soya broth to which is added Liquoid and P-aminobenzoic acid.

Liquoid: It is the commercial name for sodium polyanethol sulphonate. It prevents clotting of the blood and neutralize the natural bactericidal substances in fresh blood.

P. Aminobenzoic acid: This neutralizes the action of sulphonamides should these be present in the blood.

- ➢ Tryptone soya diphasic medium is suitable for the growth of a wide range of pathogens.

Incubation in CO_2 is required for the culture of brucella species. Strict anaerobes will not grow in this medium.

Thioglycollate broth

- This consists of a nutrient broth to which is added thiogiycollate to provide the condition necessary for the growth of anaerobes. Most aerobic bacteria will also grow in thioglycollates broth.
- Because liqoid is not added to this medium, a sufficient volume of broth must be used to prevent the blood from clotting and to dilute out the blood's natural bactericidal substances.

  The blood should be diluted at least 1 in 10 in the broth.

Examination of Blood and Bone marrow

1. Collect and culture the specimen

Blood

- It should be collected before antimicrobial treatement has been started and at the time the patient's temperature is beginning to rise.
- To increase the chance of isolating a pathogen, it is usually recommended that at least two specimens (collected at different times) should be cultured.
- Blood for culture must be collected as aseptically as possible.

2. Insert the needle through the rubber line of the bottle cap and dispense 5ml of blood into each culture bottle.

3. Gently mix the blood with the bruch.
    - The blood must not be allowed to clot in the culture media because any bacteria will become trapped in the Culture medium.

Incubate the inoculated media:

Thioglycollate broth

At 35-37^{0}C for up to 2 weeks, examining and sub-culturing

- Look for visible signs of bacterial growth such as turbidity above the red cell layer, colonies growing on top of the red cells ("cotton balls"), haemolysis, gas bubbles and clots.
- A sterile culture usually remains clear
- If there are signs of bacterial growth, subculture the broth and examine a gram stained smear for bacteria

Tryptone soya diphasic medium

At 35-37^{0}C for up to 4 weeks.

- Look for colonies on the agar slope (preferably using a hand lense), and signs of bacterial growth in the broth.
- Colonies of staphycococ, s .typhi, brucellosis and most coliforms can usually be seen easily. Where as colonies of pneumococci, S. pyogens, and Y.pestis are not easily seen.

- If growth is present subculture to blood agar plate, Chocolate agar plate, MacConkey

Collection, transport and examination of effusions (synovial, pleural, pericardial, ascitie and hydroceles fluids)

- An effusion is fluid which collects in a body cavities

Fluid which collects due to an inflammatory process is referred to as an **exudates** and that which forms due to a non-inflammatory condition is referred to as a **transudates**.

- If the effusion is all exudates, it is important to investigate whether the inflammatory process is all infective one.
- Effusions sent to the laboratory for investigation include:

Fluid	**Origin**
Synovial	From joint
Pleural	From the pleural cavity (space between the lungs and the inner chest wall)
Pericardial	From the pericardial sac (membranous sac surrounding the health)
Ascitic (peritoneae)	From the peritoneal (abdominal) cavity
Hydrocele	Usually from the sacs surrounding the tests.

1. **Synovitis** means inflammation of the synovial membrane (living of a joint capsule). It can be caused by bacteria,rheumatic disorder or injury.
2. Inflammation of a joint is called **arthritis.**
   The term polyarthritis is used when many joints are affected. Arthritis may be caused by bacteria (infective arthritis), rheumatoid arthritis, gout and pseudogout, osteoatrhtitus
3. The term pleural effusion is used to describe a non-purulent serous effusion which sometimes forms in pneumonia, tuberculosis, malignante disease etc
   Empyema is used to describe a purulent pleural effusion when pus is found in the pleural space.
4. Peritonitis means inflammation of the peritoneum, which is the serous membrane that lines the peritoneal cavity. Ascites refers to the accumulation of fluid in the pentional cary causing abdominal swelling.

Commensales

No microbial flora

Collection is carried out by a medical officer

- 2-3ml without anticogulent, to see whether clotting occurs.
- 9ml which contain 1ml sterile sodium citrate (3g/l (3% w/v) solution.
  - do the csf:
    - cell cout
    - protein estimate
    - microscopy
    - culture

CHAPTER THREE

Learning Objective

1. At the end of the lesson, the student shall be able to:
   - List the antigenic structure of bacteria
   - Apply the different chemical laboratory methods to identify the pathogenic bacteria
   - Develop the major classification scheme for gram-positive and gram-negative bacteria

GRAM POSITIVE COCCI

Genus Staphylococci
Genus Streptococci

2.1.1. GENUS: STAPHYLOCOCCI

Characteristics:

- Gram positive non spore-forming non-motile, spherical cells, usually arranged in grape-like clusters
- Single cocci , pairs, tetrads and chains are seen in liquid cultures
- Young cocci stain strongly gram-positive, on aging many cells become gram-negative
- The three main species of clinical importance
  . Staphylococcus aureus
  . Staphylococcus epidermidis

. Staphylococcus saprophyticus

Less common staphylococcal species

. Staphylococcus lugdenensis

. Staphylococcus hominis

. Staphylococcus warneri

- Can readily grow in ordinary media under aerobic and micro-aerophilic conditions
- grow most rapidly at 37 0c but form pigment best at room temperature of 20-25 oc
- Colonies in solid media are round, smooth, raised and glistening.
- Some of them are normal flora of the skin and mucus membrane of human, otrhhers cause suppuration abscess formation and fatal septicemia
- Produce catalase, which differentiate them from the streptococci.
- relatively resistant to drying , heat, and 9% Nacl, but readily inhibited by 3 % hexachlorophene

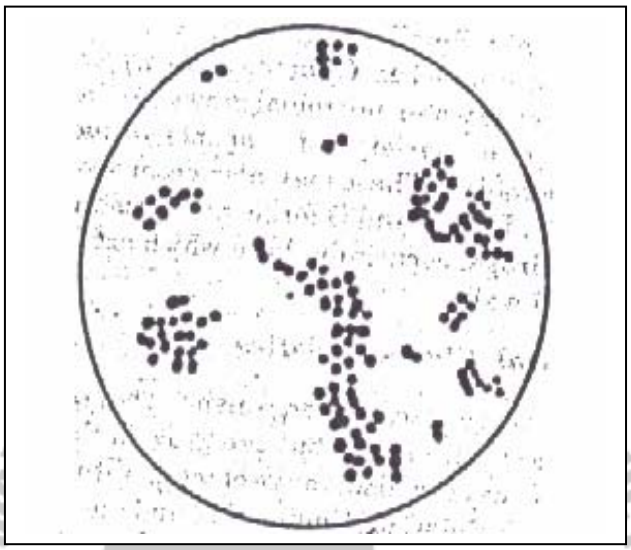


Fig 3.1 Staphylococci

Antigenic structure:

1. Peptidoglycan(Mucopeptide): Polysaccharide polymer which provide the rigid exoskeleton of the cell wall. It is important in the pathogenesis of infection like eliciting production of cytokines and opsonic antibodies; chemoattractant for polymorphs;and activate complement
2. Teichoic acid: Polymer of glycerol or ribitol phosphate
3. Protein A: Important in immunologic diagnostic test (coagglutination test).
4. Capsule: Anti-phagositic property
5. Enzymes

   . Catalase- Produced by staphylococci

   Converts H_20_2 into H_20 and 0_2

Catalase test differentiates staphylococci(catalase-positive) from streptococci(catalase-negative)

. Coagulase and clumping factor

. Coagulase clots oxidated or citrated plasma. Coagulase may deposit fibrin on the surface of organism and alter ingestion by phagocytic cells.

. Clumping factor: A surface compound that is responsible for adherence of the organism to fibrinogen and fibrin

Produced by Staphylococcus aureus

Determines Invasive potential of the organism.

Coagulase test differentiates S.aureus(coagulase-positive) from S.epidermidis (coagulase-negative)

. Hyaluronidase-Spreading factor

. Proteinases and lipases

. Staphylokinase- Fibrinolysin

. β-lactamase-Provides resistance of staphylococcus to β-lactam antibiotic like penicillin.

. Dnase: Deoxyribonucleotidase

. Nuclease

6. Toxins

. Exotoxins(α, β, γ, δ)

. Enterotoxin-Produced by S.aureus when grown in carbohydrate and protein foods.

Multiple (A-E, G-I, K-M) soluble heat-stable, gut enzyme resistant toxins which act on neural receptors

in the gut to stimulate vomiting center in the central nervous system. It is superantigen causing staphylococcal food poisoning

. Toxic shock syndrome toxin- Superantigen desquamative toxin Produced by S.aureus and Causes fever, shock, multiple-organ failure and skin rash.

. Exfoliative toxin-Epidermolytic superantigen produced by S.aureus and uses generalized desquamation of the skin (staphylococcal scalded skin syndrome).

Epidermolytic toxin A: Chromosomal gene product and heat stable

Epidermolytic toxin B: Plamid mediated and heat labile

. Leukocidin: S aureus toxin which kills WBCs by forming pores and incresing cation permeability

Clinical features:

. Folliculitis:Infection of one hair follicle.

. Curbuncle: Infection of multiple hair follicle and surrounding skin.

. Cellulitis: Infection of skin and subcutankeous tissue.

. Abscess formation: focal suppuration

. Mastitis: Infection of breast, especially in lactating mother

. Bulous impetigo: Crusted superficial skin lesion

. Pneumonia: Infection of lung parenchyma.

. Empyema: Accumulation of pus in pleural space

. Osteomyelitis: Infection of bone

.Endocarditis and meningitis: Infection of heart tissue and leptomeninges respectively.

. Food poisoning: Caused by enterotoxin produced by S.aureus

. Characterized by violent nausea, vomiting, and diarrhea

. Toxic shock syndrome: Caused by toxic shock syndrome toxin-1 produced by S.aureus

. Characterized by abrupt onset of high fever, vomiting, diarrhea, myalgia, scarlatiform rash,and hypotension with cardiac and renal failure in the most severe disease

. Occurs with in 5 days after the onset of menses in young women who use tampoons

. Staphylococcal scalded skin syndrome: Caused by exfoliative toxin produced by S.aureus.

S. saprophyticus: Relatively common cause of urinary tract infections in young women

S. epidermidis: occasional cause of infection often associated with implanted appliances and devices

Laboratory Diagnosis:

Specimen: Surface swabs, pus, blood, sputum, cerebrospinal fluid

Smear: Gram positive cocci in clusters, singly or in pairs.

Culture: Grow well aerobically and in a CO_2 enriched ordinary media at an optimal temperature of 35^0c-37^0c.

Colony appearance:

S.aureus: characteristically golden colonies.

frequently non-pigmented after over-night incubation.

hemolytic on blood agar plate.

7.5% Nacl containing media is used for mixed flora contaminated specimen

Mannnitol slt agar is used to screen for nasal carriers of S. aureus

S.epidermidis: white colonies, non-hemolytic

S.saprophyticus: may be white or yellow, non-hemolytic.

Biochemical reaction

1. Catalase test

Active bubbling……..Catalaseproducing Bacteria (Staphlococci)

No active bubbling……Non-catalase producing bacteria (streptococci)

2. Coagulase test

a. Slide test: To detect bound coagulase

Clumping with in 10 seconds…… S.aureus

No clumping with in 10 seconds……CONS(Coagulase negative staphylococci)

c. Tube test: To detect free coagulase

Fibrin clot…….S.aureus

No fibrin clot……CONS

Sensitivity testing:

Novobiocin sensitive……. S.aureus and S.epidermidis

Novobiocin resistant……...S.saprophyticus

Table 2.1 DIFFERENTIATION OF SPECIES

Organism	Colony appearance	Catalase Production	Coagulase Production	Novobiocin sensitivity	Hemolysison Blood agar
S. aureus	Golden	Positive	Positive	Sensitive	Positive
S. epidermidis	White-Gray	Positive	Negative	Sensitive	Negative
S. saprophyticus	White-Gray	Positive	Negative	Resistant	Negative

Treatment

Penicillin sensitive staphylococci……penicillin, ampicillin

Penicillin resistant staphylococci……cloxacillin, Nafcillin

Methicillin resistant staphylocicci…… Vancomycin

Prevention and control

Source of infection is shedding human lesions, the human respiratory tract and skin

Contact spread of infection occur in hospitals

Treatment of nasal carriers with topical antiseptics or rifampin and anti-staphylococcal drug

2.1.2. GENUS: STREPTOCOCCI

Characteristics:

- They are non-motile, non-sporulating, gram- positive facultative anaerobes
- Spherical or oval cells characteristically forming pairs or chains during growth

- Grow well on ordinary solid media enriched with blood, serum or glucose.
- Most streptococci grow in solid media as discoid colonies
- Capsular streptococcal strains give rise to mucoid colonies
- They are aerobic bacteria in which growth is enhanced with 10% carbondioxide.
- They are catalase-negative.
- They are widely distributed in nature and are found in upper respiratory tract, gastrointestinal tract and genitourinary tract as normal microbial flora.
- They are heterogeneous group of bacteria, and no one system suffices to classify them.
- The currently used classification is based on colony growth characteristics, pattern on blood agar, antigenic composition of group specific cell wall substance and biochemical reaction

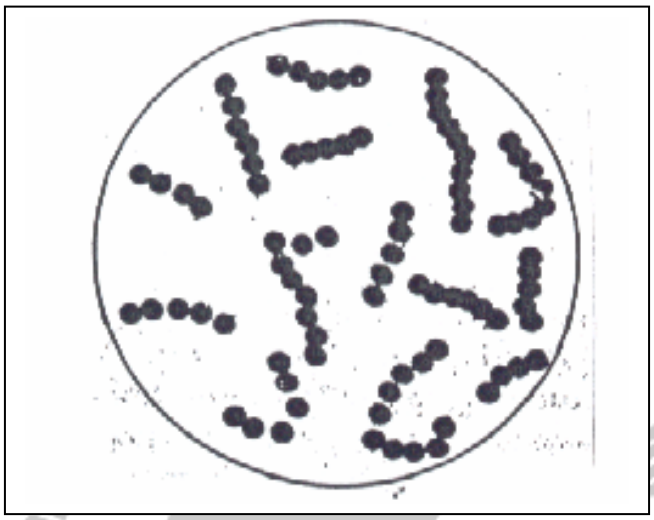


Fig. 3.2 Streptococci

Classification of streptococci: Based on

1. Colony morphology and hemolytic reaction on blood agar
2. Serologic specificity of the cell wall group specific substance and other cellwall capsular antigens
3. Biochemical reactions and resistnace to physical and chemical factors
4. Ecologic features

Table 2.2 Hemolytic reaction of streptococci:

Hemolysis	appearance	Designation	Example
.Complete	colorless, clear, Sharply defined zone	Beta(β)	S. pyogenes
.Partial streptococci	Greenish discoloration (reduced hemoglobin)	Alpha(α)	Viridans
.None	No change	Gamma(δ)	Enterococci

Lancefield grouping of streptococci:

Streptococci produce group specific carbohydrates(C carbohydrates) identified using group specific antiserum.

It is designated A-H and K-V.

The clinically important streptococci are grouped under A,B,C,D,F and G.

The main species and groups of medical importance

S. pyogenes…….. Lancefield group A

S. agalactiae…….. Lancefield group B

Enterococci……Lancefield group D

NB: Viridans streptococci and anaerobic streptococci are not grouped under Lancefield Classification.

Antigenic structure:

1. Group-specific cell wall antigen

Streptococcal cell wall obtained carbohydrate is the basis for serologic grouping of streptococci (Lancefield groups A-H, K-U)

2. M protein

They are found in hair-like projections of the streptococcal surfaceand determine virulence

Major virulent factor for group A streptococci.

There are two major structural classes of M protein(class I &class II) and

More than eighty serotypesof M protein of group A streptococci

The class I M protein may be a virulence determinant for rheumatic fever

Conserved antigenic domains on the class I M protein induce antibodies that cross react with cardiac muscle tissue

3. T substance: Acid and heat labile unlike M protein, and has no relation to virulence of streptococci.
4. R protein

Streptococcus pyogenes (Group A β-hemolytic streptococci)

The most pathogenic member of the genus

It is present as a commensal in the nasopharynx in a variable proportion of healthy individuals.

It produces different types of enzymes and exotoxins.

These are:

1. Streptokinase: Fibrinolysin.

   It is an active proteolytic enzyme which lyses fibrin by catalytic conversion of plasminogen to plasmin.

   Has been given intravenously for the the treatment of pulmonary edema and of arterial and venous thrombosis
2. Streptodornase: Streptococcal deoxyribonuclease

Mixtures of streptokinase and streptodornase are used in “enzymatic debridement”

3. NADase: Nicotinamide adenine dinucleotidase
4. Hyaluronidase: Spreading factor

   It degrades the ground substance of connective tissue (hyaluronic acid) and aids in spreading infectious micro-organoism
5. Hemolysins: Two types

   Streptolysin O and Streptolysin S

   Antistreptolysin O antibody titer > 1:200 todd: Supportive evidence for Acute reheumatic fever

2.3 Comparison of the streptolysins

Hemolysin	stability of oxygen	antigenic
Streptolysin O	No	Yes
Streptolysin S	Yes	No

6. Erythrogenic toxin: Pyrogenic exotoxins

It is responsible for the erythematous rash in scarlet fever.

Clinical features:

. Skin infection: Impetigo, cellulitis, erysipelas

. Scarlet fever

. Acute streptococccal sorethroat

. Ear infection: Acute otitis media and mastoiditis

.Puerperal fever: septicemia originating in the infected uterus

. Post-streptococcal diseases: Immunological diseases

1. Acute rheumatic fever

Immunological damage to the heart valves and muscle following

Streptococcal upper respiratory tract infection

It clinically presents with fever, malaise, migratory non-sppurative polyarthritis, carditis, erythema marginatum and subcutaneous nodules

2. Post streptococcal acute glomerulonephritis

Immunological damage to the kidney following infection of skin with streptococci

It clinically manifests with generalized body edema, elevated bloood pressure, protein and blood in the urine, bloood urea nitrogen retention and low complement level

. Necrotizing fascitis(Streptococcal gangrene): Extensive and rapidly spreading necrosis of skin and subcutaneous tissue

S. agalactiae

Clinical features

. Neonatal sepsis, pneumonia, and meningitis

. Septic abortion

. Puerperal sepsis

Enterococci

Clinical features

. Frequent cause of nosocomial infection

. Abdominal abscess

. Sub acute bacterial endocarditis

Viridans streptococci

Eg. Streptococcus mitis

Streptococcus mutans

Streptococcus salivarius

Streptococcus sanguis

Clinical features

. Sub acute bacterial endocarditis

Laboratory Diagnosis:

Specimen:

S. pyogenes- Throat swab, pus, blood

S. agalactiae- High vaginal swab of women; blood and cerebrospinal fluid of new born

Enterococci- Blood, pus

Viridans streptococci- Blood

Smear: Non-motile gram-positive cocci in chains

Culture, biochemical tests and sensitivity testing:

Grow in aerobic and anaerobic environment at a temperature of 35-37 ^{0}c.

Grow in ordinary media with shiny or dry colonies with grey-white or colorless appearance.

S. pyogenes- Shows clear zone of hemolysis in blood agar plate.

Does not grow in mac Conkey agar plate.

Bacitracin sensitive.

PYR-Positive

S. agalactiae- Shows clear zone of hemolysis in blood agar plate.

May show double zone of hemolysis when incubated anaerobically

Active in hippurate hydrolysis

CAMP test is used to identify the bacteria.

Viridans streptococci- Show greenish discoloration of blood agar plate.

Optochin resistant.

Not soluble in bile salts.

Do not ferment inulin.

Enterococci- Non-hemolytic or α hemolytic changes in blood agar plate.

Grow in the presence of 6.5% NaCl.

Grows in mac conkey agar

Identified by litmus milk reduction test.

PYR-Positive

Bile esculin-positive

Nutritionally Variant Streptococci (Nutritionally deficient streptococci)

Eg. Abiotrophia adjacens

Abiotrophia defectiva

. Require pyridoxal or cysteine for growth in blood agar

. Usually α hemolytic or non-hemolytic

. cause infective endocarditis

Treatment:

Streptococci other than enterococci …….. Penicillin/ Erythromycin

Enterococci ……. Penicillin + Gentamicin

Streptococcus pneumoniae

- Fastidious, lancet-shaped gram positive diplococci.
- Possess a capsule of polysaccharide that permits typing with specific antisera.
- Found as a normal flora in the upper respiratory tract.

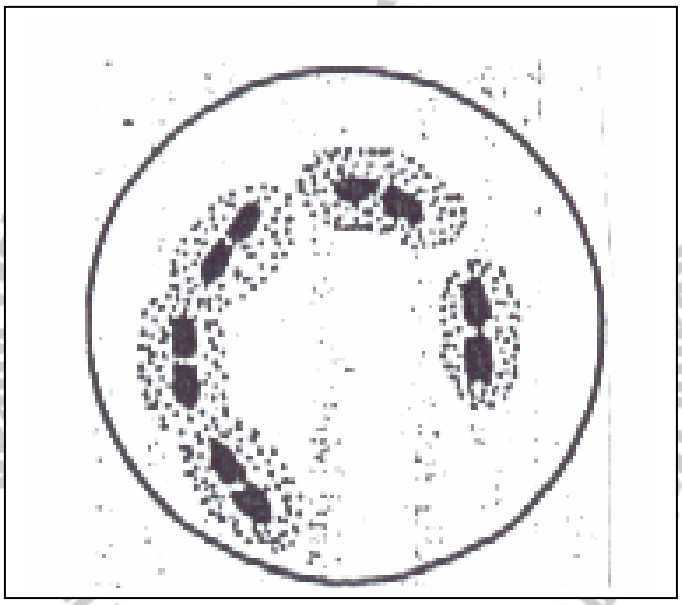


Fig. 3.3 Streptococcus pneumoniae

Antigenic structure:

. Capsular polysaccharide: Pathogenicity determinant with anti-phagocytic property. There are more than 80 serotypes of the bacteria based on capsular typing.

Identified by capsule swelling test (quellung reaction).

. C substance: Cell wall associated antigen

. Protein M antigen

. IgA_1 protease: Enzyme which cleaves IgA_1

Clinical features:

. Lobar Pneumonia

. Otitis media

. Sinusitis

. Bacteremia……..Meningitis

.Endocarditis

. Septic arthritis

Laboratory Diagnosis:

Specimen: Sputum, blood, cerebrospinal fluid, ear discharge and sinus drainage.

Smears: Lancet-shaped gram positive diplococci

Culture: Grow best in chocolate agar media in CO_2 enriched atmosphere.

- Shows α-hemolytic, draughts man colony appearance: Sunken centre colony due to spontaneous autolysis of older bacteria.
- Young colonies resemble dew-drops due to capsule.
- Bile soluble, ferment inulin.
- Optochin sensitive.

Serology: Quellung reaction

.Good for rapid identification of S.pneumoniae in fresh specimen.

Principle:

.Mix specific serotype of S.pneumoniae with specific anti-polysaccharide serum of the same serotype or with polyvalent anti-serum on a slide.

. Look for the appearance of capsule swelling under the 100X objective microscope

Treatment: Amoxicillin

Chloramphenicol

Thid generation Cephalosporins

Prevention and control:

Pneumococcal conjugate vaccine: Immunization of individuals with type specific polysaccharide vaccine

Biochemical reaction to diagnose streptococci

. Bile solubility test

. Litmus milk reduction test

. CAMP test

. Bacitracin test

.Optochin test

Table 2.4 Differentiation of streptococcus species

Species	Catalase test	Bacitracin test	.Optochin test Bile solubility test	Litmus milk reduction test	CAMP test
S. pyogenes	-ve	+ve	-ve	-ve	-ve
S. agalaciae	-ve	-ver	-ve	-ve	+ve
Enterococci	-ve	-ve	-ve	+ve	-ve
Viridans streptococci	-ve	-ve	-ve	-ve	-ve
.S.pneumoniae	-ve	-ve	+ve	-ve	-ve

GRAM POSITIVE SPORE FORMING RODS

Genus Bacillus

Genus Clostridium

2.2.1. GENUS: BACILLUS

Characteristics:

- Aerobic, non-motile,spore-forming, gram-positive chain forming rods.
- Bacillus species are ubiquitous saprophytes

  Important human pathogen

  B. anthracis

  B. cereus

Bacillus anthracis

- Major agent of bioterrorism and biological warfare
- Major pathogen of domesic herbivores that come in contact with humans

Antigenic structure:

- Capsule: Polypeptide of D-glutamic acid: Enables the organism to evade phagocytosis
- Polysaccharide somatic antigen
- Protein somatic antigen
- Anthrax toxin- Protein toxin: Complex of 3 protein factors

  Edema factor + Protective antigen = Edema toxin

  Lethal factor + Protective antigen = Lehtal toxin

Pathogenesis and Clinical feature:

Acquired by the entry of spores through injured skin incutaneous anthrax, or mucus membrane in intestinal anthrax, or inhalation of spores in the lung while handling skin and hides

There are four forms of anthrax.

1. Cutaneous anthrax (Malignant pustule): 95 % of anthrax presentation

   Characterized by a black necrotic lesion with a definite edematous margin onhands, arms, face or neck with regional lymphadenitis associated systemic symptoms.
2. Pulmonary anthrax (Wool sorter's disease):5% of anthrax presntation

Presents with substernal pain, cough with haemorrhagic mediastinitis and CXR-revealing mediastinal widening; and fatal if not treated early

3. ***Bacteremic anthrax: presents with clinical features of sepsis***
4. Intestinal anthrax: Presents with abdominal pain, vomiting, and bloody diarrhea

Bacteremic and intestinal anthrax are rare to occur

Laboratory diagnosis:

Specimen: Fluid or pus from skin lesion, Blood, sputum

Smear: Non-capsulated gram-positive rods with centrally located spores from culture

Large capsulated gram-positive rods with out spores from primary specimen.

Culture: Grows aerobically in ordinary media over wide range of temperature.

Non-hemolytic,large, dense, grey-white irregular colonies with colony margin of “Medussa Head” or “curled-hair lock” appearance due to composition of parallel chaining of cells.

Biochemical reaction:

Gelatin-stab culture: Gelatin liquefaction

Growth along the track of the wire with lateral spikes longest near the surface Providing “inverted fur tree” appearance.

Serology: ELISA has been developed to measure antibodies to edema toxin and lethal toxin

Positive result: 4-fold change or single titer > 1:32

Treatment:

Ciprofloxacin

Penicillin+ gentamicin or streptomycin

Prevention and control:

- Disposal of animal carcasses by deep burial or burning
- Decontamination (autoclaving) of animal product
- Protective clothing and gloves for handling potentially infected mateials
- Active immunization of domestic animals with live attenuated vaccine
- Immunize high occupation risk persons with anthrax toxoid

Bacillus cereus

General characteristics:

Exhibit motility by swarming in semisolid media

Produce β lactamase, so not sensitive to penicillin

Clinical features

1. Food poisoning

Pathogenicity determinant: Exotoxin

a.Emetic type food poisoning

IP is 1-5 hrs after ingestion of preformed toxin contaminating rice and pasta dishes

Characterized by nausea, vomiting, abdominal cramps, and self-limited with in 24 hrs

b. Diarrheal type food poisoning

IP is 1-24 hrs after ingestion of contaminated meat dishes with sporulating or preformed toxin

Characterized by profuse diarrheaand abdominal cramps. Fever and vomiting is uncommon

Lab. Diagnosis

>105 org/gm of food

Isolation of B.cereus in stool is not diagnostic since it is present in normal stool specimen

Treatment: Fluid replacement

Antibiotics not required

2. Ocular infection

Ocular disease following trauma from non-sugical penetrating objects

Manifests with keratitis, endophthalmitis, and panophthalmitis
Treatment: Clindamycin + Aminoglycosides

2.2.2. Genus: Clostridium

Characteristics:

- Clostridia are anaerobic, spore-forming motile, gram-positive rods.
- Most species are soil saprophytes but a few are pathogens to human.
- They inhabit human and animal intestine, soil, water, decaying animal and plant matter
- Spores of clostridia are wider than the diameter of organism and located centrally, subterminally and terminally
- Species of medical importance:
  - C. perfringens
  - C. tetani
  - C. botulinum
  - C.difficile

Clostridium perfringens

Characteristics:

- Capsulated, non-motile, short gram-positive rods in which spores are hardly seen.
- there are five toxigenic groups : A-E
- Human disease is caused by C. perfringens type A and C Pathogenicity determinant:

1. Enzymes: Digest collagen of subcutaneous tissue and muscle.
   . Collagenase
   . Proteinase
   . Hyaluronidase
   . Dnase
2. Toxins
   . PhospholipaseC (α toxin)
   It has lethal, necrotizing and hemolytic effect on tissue.
   It causes cell lysis due to lecithinase action on the lecithin which is found in mammalian cell membrane.
   . Theta toxin
   It has hemolytic and necrotic effect on tissue.
   . Enterotoxin

Clinical manifestation:

1. Clostridial myonecrosis: Gas gangrene

. IP(Incubation period) =1-3 days

Colonization of devitalized tramatized wound by C.perfringens spores, and organism germiation and release of toxins

Presentation: Muscle and subcutaneous tissue necrosiss and crepitation

Foul smelling wound discharge

Fever, toxaemia, hemolytic anemia, Shock

2. Clostridial food poisoning

It causes secretory diarrhea due to release of enterotoxin in the intestine

Self-limiting diarrhea similar to that produced by B. cereus

Laboratory diagnosis:

Specimen: Infected tissue, pus, vomitus, left over food, serum

Smear: Non-motile, capsulated, thick brick-shaped gram-positive rods in smears from tissue; spores are rarely seen.

Culture:

1. Blood agar medium

. β-hemolytic colonies are seen in blood agar in anaerobic atmosphere.

. Some strains produce double zone of hemolysis.

2. Cooked meat medium(Chopped meat-glucose medium)

Thioglycolate medium

. Saccharolytic property showing reddening of the meat with a rancid smell due to carbohydrate decomposition.

. Proteolytic property showing blackening of the meat with unpleasant smell due to protein decomposition.

. Formation of gas

Biochemical reaction:

. Nagler reaction: Lecithinase C activity- Opacity in the egg-yolk medium due to lecithin break down

Procedure:

1. Streak colonies of C. perfringens on egg-yolk agar.
2. Cover half of the medium with C. perfringens antitoxin.
3. Look for dense opacity production by the growth of C. perfringens; but no opacity on the area with antitoxin.

. Lactose fermentation: Reddening of the medium; red colonies when exposed to air.

. Litmus milk medium: “stormy- clot” formation due to acid and gas formation.

Identification of C.perfringens rests on colony form, hemolysis pattern, biochemical reaction, and toxin production and neutralization by specific antisera.

Treatment: Penicillin

Prompt and extensive wound debridement

Polyvalent antitoxin

Prevention and control

Early adequate contaminated wound cleansing and debridement

Closridium difficile

General characteristics:

. Not frequently found in the healthy adult, but is found often in the hospital environment

. Produce cytotoxins (A and B)

. Human feces are the expected source of the organism

Pathogenesis and clinical features:

Administration of antibiotics like ampicillin, clindamycin and cephalosporins results in killing of colonic normal flora and proliferation of drug resistant C.difficile and release of cytoxins

Clinically presents with pseudomembraneous colitis and manifests with fever, abdominal cramps, watery or bloody diarrhea leading to dehydration, septicemia and shock

Lab. Dignosis: Identification of toxin A and B in feces by latex agglutination test

Treatment:

Dicontinuation of offending drugs

Administration of metronidazole or vancomycin

Clostridium tetani

General characteristics:

- World wide in distribution in the soil and in animal feces
- Longer and thinner gram-positive rods with round terminal spores giving characteristic “drum-stick” appearance.
- There are ten antigenic types of c. tetani but all produce the same neurotoxin.
- The toxin has two components:
    1. Tetanospasmin: Neurotoxic property
    2. Tetanolysin: Hemolytic property

Pathogenesis and Clinical manifestation:

Infection of devitalized tissue (wound, burn, injury, umblical stamp, surgical suture) by spores of C.tetani → Germination of the spore and development of vegetative organism→ Neurotoxin release from vegetative cells → The toxin binds to receptors on the presynaptic membrane of motor neuron the retrograde axonal transport to the spinalcord and brain stem → Inhibition of inhibitory glycinergic and GABAergic secreting neurons → Spatic paralysis, muscle spasms and hyperreflexia

IP= 4-5 days to several wks

Tetanus classical presentation:

. Lock jaw or trismus

. Arched back or opistotonus

. Arm flexion and of leg extension

. Fever and sweating

. Muscle spasm and rigidity

Laboratory diagnosis: The bacteria can be cultured in a media with anaerobic atmosphere. Proof of isolation of C.tetani must rest on production of toxin and its neutralization by specific antitoxin

Diagnosis is exclusively by clinical picture and history of injury

Treatment:

Administration of penicillin

Proper wound debridement

Provision of tetanus antitoxin (TAT)

Prevention and control:

Avoid traditional application of mud or ash over the umbilical stump

Proper wound handling

Immunization with tetanus toxoid

NB: Since treatment of tetanus is not satisfactory, prevention is all important

Clostridium botulinum

General characteristics:

- Spores of C. botulinum are widely distributed in soil, they often contaminate vegetables, fruits and other materials.

- Produce a neurotoxin which is the most active known poison, and considered to be the major agent of bioterrorism and biologic warfare
- There are seven serotypes(A-G) of which A,B and E are the principal causes of human illness.

Pathogenesis and Clinical manifestation:

1. Food botulism

. IP = 18-24 hrs

. Route of entry is under cooked consumption of C. botulinum toxin contaminated spiced, smoked, vaccum-packed or canned food

.The toxin is absorbed from the gut and acts by blocking the release of acetylcholine at synapses and neuromuscular junction and manifests with flaccid paralysis and visual disturbance, inability to swallow, and speech difficulty

Death is secondary to respiratory failure or cardiac arrest

2. Infantile botulism

C.botulinum type A or B is usually implicated and affects infants when mixed feeding starts (after fourth month of life).

Ingestion and colonisation of the gut with C.botulinum, and production of toxin and adsorption of toxin leads to poor feeding, paralysis (floppy baby), and cranial nerve palsy.

Diagnosed by demonstration of the organism or toxin from the stool

3. Wound botulism

C.botulinum type A is usually implicated and caused by the production of toxin by C.botulinum in wounds.The symptoms are the same as those in food poisonig.

Laboratory diagnosis:

.Demonstration of toxin in patient's serum and left over food.

. Death of mice after intra-peritoneal injection of toxin.

Treatment:

Administration of intravenous trivalent antitoxin (A,B,E)

Mechanical ventilator for respiratory support

Prevention and control:

. Sufficient heating of cannaed foods before consumption

. Strict regularion of commercial canning

. Proper home canning methods

GRAM POSITIVE NON-SPORE FORMING RODS

GENUS CORYNEBACTERIA

Medical important species: Corynebacteria diphteriae

C. diphteriae

Characteristics:

Non-spore forming, non-capsulated, non-motile aerobe or facultative anaerobe gram-positive rods

Possess irregular swelling at one end that give them the "club-shaped" appearance

Possess granules (metachromatic granules) near the poles that give the rod a beaded appearance

It tends to lie in parallel (pallisades) or at acute angles to one another in stained smears, forming V,L , W shapes, so called Chinese-character arrangement

It has four four biotypes named as gravis, mitis, intermedius and balfanti based on growth characteristics and severity of disease produced

Pathogenesis and clinical features:

Found in nature in the respiratory tract, in wounds, or on the skin of infected person or normal carriers

Spread by droplets or by direct contact

The organism colonize the mucus membrane or skin abrasions and toxigenic C.diphteriae start producing exotoxin, possessing two components,fragment A and B. Fragment B transpots fragment A into the cell and fragment A inhibit polypeptide chain elongation by inactivating the elongation factor EF-2, required for translocation of polypeptidyl-tRNA from the acceptor to the donor site on the eukaryotic ribosome, leading to abrupt arrest of protein synthesis and result in cell necrosis and neurotoxic effect.

Diphteria toxin causes respiratory tract epithelial destruction tesulting in formation of necrotic epithelium with pseudomembrane formation over the tonsils, pharynx, and larynx. Distant toxic damage includes parenchymal degeneration and necrosis in heart muscle, liver, kidney, adrenal glands and peripheral and cranial nerves.

Wound/skin diphteria occurs chiefly in the tropics and forms membrane-covered wound that fails to heal. Systemic effect is negligible.

It clinically manifests with fever, sorethroat, suffocation (due to obstruction by upper respiratory tract pseudomembrane formation), arrhythemia, and difficulty of vision, swallowing and paralysis of upper and lower extremities.

NB: C.diphteriae var gravis tends to produce more severe disease than var mitis.

Laboratory diagnosis:

Specimen: Swabs from the nose, throat, or suspected lesion

Smears: Beaded rods in typical arrangement when stained with alkaline methylene blue or gram's stain

Culture: Small, granular,and gray, with irregular edges with small zone of hemolysis on blood agar

Selective media are necessary for isolation from cilincal specimens

Selective media

1. Loeffler's serum media: Grows rapidly with in 8 hrs. after inoculation and show typical appearance
2. Blood tellurite agar: Produce characteristic grey-black colonies due to their ability to reduce potassium tellurite to tellurium

Characteristics of C.diphteriae strains

C.diphteriae	Appearance in Loeffler's medium	Colonial type on Tellurite medium
Gravis strains	Club-shaped, few granules	Flat, drey with raised centre and irregular edge
Intermedius strains	Short irregularly staining rods	small, smooth colonies;

	with out granules but in Chinese character arrangement	grey-black with paler periphery
Mitis strains	Classic morphology with numerous granules and typical arrangement	Medium-sized,circular convex,glistening and black

Biochemical reaction: Acid production from a range of carbohydrate fermentation

Typing: Serotyping by agglutination tests, phage typing and bacteriocin typing have been used to subdivide strains of C.diphteriae foe epidemiologic studies

Toxin production: responsible for virulence; can be demonstrated by guinea pig inoculation or by gel precipitation test

1. Guinea pig inoculation: Inject suspension of the isolated strain of C.diphteriae into two guinea pig, one protected with diphteriaantitoxin.

Death in 2-3 days of the unprotected animal

2. Gel-precipitation (Elek) test: a filter paper strip previously immersed in diphteria antitoxin is incorporated into serum agar; the strain of C.diphteriae under investigation is then streaked onto the agar at right angles to the filter paper strip. Incubate at 37 ^{0}c for 1-2 days, and observe for lines of precipitation in the agar indicating toxin-antitoxin interaction.

Schick test: a skin test to demonstrate immunitydue to immunization or natural infection

Method: Intradermal injection of toxin into the anterior aspect of one forearm and heat-inactivated toxin into the other.

Objective: to detect susceptibility and hypersensitivity to diphteia toxin

Interpretation: Observe for erythema at the injection site at 36 hrs and 120 hrs. Reactions due to the toxin are slower and longer lasting than those resulting from hypersensitivity.

The schick test

Result	Test arm (toxin)		Control arm (Inactivated arm)		Interpretation	Immunization
	36hrs	120hrs	36hrs	120hrs		
-ve	-	-	-	-	Immune, not hypersensitive	Not required
+ve	+/-	+	-		Non-immune, Hypersensitive	Required
-ve/Pseudo	+	-	+	-	Immune, Hypersensitive	Not required
+ve/Pseudo	+	+	+	-	Non-immune, Hypersensitive	Single injection of low dose vaccine

Treatment: Penicillin/Erythromycin

Administration of diphteria antitoxin

Prevention and control:

Administration of Diphtheria toxoid

GENUS: LISTERIA

Most important species: Listeria monocytogenes

L. monocytogens

General characteristics:

. Widely present in plants, soil and surface water

. Zoonotic pathogen of domestic animals

. Non-sporulating, facultative anaerobe, intracellular. Gram positive rods

Antigenic structure:

. Listreriolysin(hemolysin)

Pathogenesis and clinical features:

Transmitted to humans through ingestion of poorly coooked meat and unpasteurized milk and milk products

1. Perinatal human listeriosis: Granulomatous infantisepticum
   . Early onset syndrome: Intrauterine sepsis
   . Late onset syndrome: Neonatal meningitis
2. Adult human listeriosis
   . Meningoencephalitis
   . Bacteremia

Lab. Diagnosis:

Specimen: Blood/ CSF

Culture: Grow in blood agar and demonstrate narrow zone of β-hemolysis

Produce “umbrella” growth pattern below the motility media surface at room $T^{0,}$ demonstrating motility at room temperature

Biochemical reaction: Catalase positive

Oxidase negative

Treatment:

. Ampicillin

. Erythromycin

. Cotrimaxazole

Prevention and control:

. Proper cooking of animal souce foods

. Pasteurization of milk and milk products

GENUS : ERYSIPELOTHRIX

Erysipelothrix rhusiopathiae

General characteristics:

. Slender, non-motile, Non-sporulating, gram-positive, facultative anaerobic rods

. Swine is major reservoir

Pathogenicity and clinical features:

Most human cases of disease are related to occupational exposure, i.e. direct inoculation from animals and animal products, like in fish handlers, fishers, butchers

1. Mild cutaneos form: Erysipeloid (Whale finger, seal finger)
2. Diffuse cutaneous form with systemic disease
3. Septic form: Bacteremia and endocarditis

Lab. Diagnosis:

Specimen: Blood

Culture: Shows α-hemolysis on Blood agar

Biochemical reaction:

. Catalase negative

. Produce acid from sugar fermentation

Treatment: Penicillin G

2.2. 2.3. GRAM NEGATIVE DIPLOCOCCI

2.3.1. GENUS: NEISSERIA

Characteristics:

. They are nopn-motile, gram-negative intacellular diplococci

. Rapidly killed by drying, sunlight, heat, and disinfectants

. Ferment carbohydrate produucing acid but not gas

. Each cocci is kidney-shaped with adjacent concave sides

. Grow best on complex media under aerobic conditions containing 5%co_2

. Oxidase positive.

. The main species of medical importance are:

N. meningitidis

N.gonorrhoea.

N. gonorrhoea

Characteristics:

An obligate parasite of the human urogenital tract.

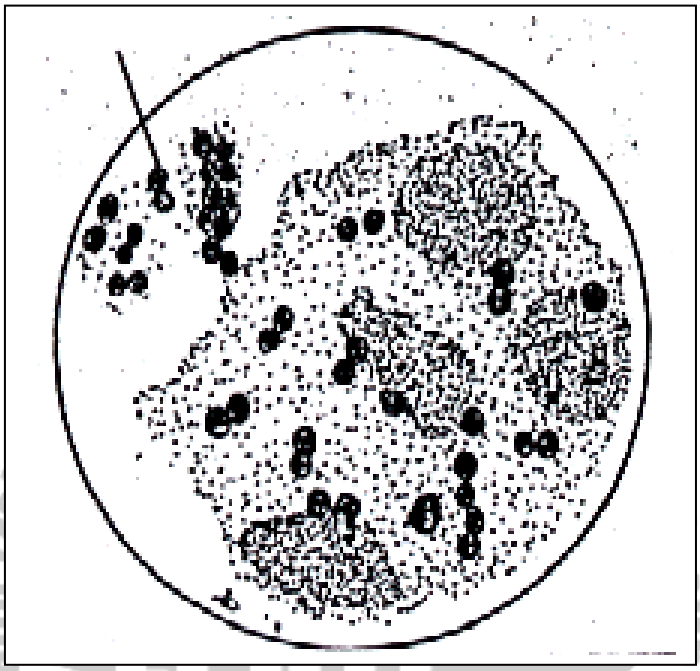


Fig. 3.4. Neisseria gonorrhoea

Antigenic structure: antigenically heterogeneous and capable of changing its surface structures.

1. Pili: Hair-like appendages extending from bacterial surface and enhance attachment to host cells and evade human defense.

   . The pilus of almost all strains of N. gonorrhoea are antigenically different, and a single strain can make many antigenically distinct forms of pilin.

2. Por (Protein I)

   . Pores on the surface of bacteria through which nutrients enter the cell.

3. Opa (Protein II)
   . Important for attachement of bacteria to host cells.
4. RMP (Protein III): Reduction-modifiable protein
   . It is associated with por in the formation of pores in the cell.
5. Lipooligosaccharide(LOS): Responsible to damage epithelial cells

Toxicity in gonococcal infection is largely gue to the endotoxic effects of LOS

6. LiP(H8): Gonococcal surface exposed Heat-Modifiable like Opa
7. Fbp(Iron binding protein):Expressed when there is limited available iron supply
8. IgA_1 protease:Splits and inactivates major mucosal IgA(IgA1)

Clinical manifestation:

Route of infection: Sexual contact

Male:

. Gonococcal urethritis

If complicated: Urethral stricture
Gonococcal epididymitis
Gonococcal epididymo-orchitis
Infertility

. Gonococcal suppurative arthritis

Female:

. Gonococcal cervicitis

. Gonococcal salpingitis

If compicated: Gonococcal tubo-ovarian abscess

Pelvic peritonitis

Infertility

Infant (When delivered through the infected birth canal)

. Gonococcal ophthalmia neonatorum

If untreated and complicated leads to blindness

Laboratory diagnosis:

Specimen: Urethral swab, cervical swab, eye swab

Smear: Gram-negative intracellular diplococci

More than five polymorphs per high power field.

Culture: Requires an enriched media like chocolate agar or thayer-martin agar.

. Grows best in CO_2 enriched aerobic atmosphere with optimal temperature of 35-37^{O}c.

.Fastidious- Dies with exposure to sunlight, room temperature and drying.

. Small glistening colonies.

.Culture of urethral exudate from men are not necessary when the gram stain is positive but culture should be done for women

Biochemical reaction**:** .Oxidase positive.

Ferment only glucose in carbohydrate utilization test

Serology: Antibodies to gonococcal pili and Omps can be Detected by immunoblotting, RIA or ELISA tests

Treatment: Gonorrhoea is difficult to treat because of resistance to lots of antibiotics, especially in developing countries.

Penicillinase-producing Neisseria gonorrhoea (PPNG) strains are resistant to penicillin.

Drug of choice: Ceftriaxone

Ciprofloxacin

Prevention and control

- Avoid multiple sexual partner
- Using mechanical protection methods (condom)
- Early diagnosis and prompt treatment of cases
- Contact tracing
- Screening of high risk population groups
- Ophthalmic ointment application of erythromycin or tetracycline to the conjunctiva of all new borns

Neisseria meningitidis

Characteristics:

- Gram-negative intra cellular diplococci.
- Present in the nasopharynx in 5-10% of healthy people.

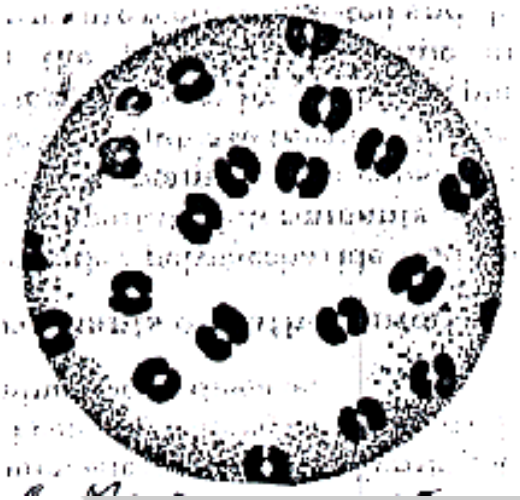


Fig. 3.5 *Neisseria meningitidis*

Antigenic structure:

. Capsular carbohydrate

It is important for serogrouping of meningococci and there are 13 serogroups. The most important serogroups associated with disease in humans are A, B, C, Y and W135.

. Outer membrane protein

Analogous to por protein of gonococci and responsible for the formation of por in the meningococcal cellwall

20 known serotypes

It is responsible for serotype specificity of meningococci.

. Lipopolysaccharide

Responsible for the toxic effects found in meningococcal disease

Clinical manifestation:

. Meningococcal meningitis

. Meningococcemia: Meningococcal septicemia

Table 2.5 Comparison features of meningococcal meningitis and meningococcemia

Features	**Meningococcal meningitis**	**Meningococcemia**
. Symptom/sign	Fever, headache, neck stiffness.	Fever, petechial rash, low blood pressure
. CSF appearance.	Cloudy.	Cloudy or clear
. Response to drugs.	Satisfactory.	Poor
. % during epidemics.	80-90% .	10-20%

Laboratory diagnosis:

Specimen: Cerebrospinal fluid, blood

Smear: Gram-negative intracellular diplococci

Culture: Transparent or grey, shiny, mucoid colonies in chocolate agar after incubation at 35-37^{O}c in a CO_2 enriched atmosphere.

Biochemical reaction: Oxidase positive.

Ferment glucose and maltose in carbohydrate utilization test.

Serology: Latex agglutination test/ Hemmagglutination test

Treatment: Penicillin

Penicillin-allergic patients are treated with third-generation cephalosporins or chloramphenicol

Prevention and control

. Chemoprophylaxis(Rifampin or minocycline) for house holds or close contacts

. Avoidance of over crowding

. Vaccination with polyvalent conjugate vaccine to high risk groups

NB: Meningococcal meningitis occurs in epidemics in Africa and named as Meningitis belt.

N. meningitidis serogroup A is the cause of African meningitis epidemic.

During epidemics, the carrier state rises from 5-10% to 70-80%.

Rifampicin is used as prophylactic drug to reduce the carrier state during epidemics and given to house hold and other close contacts.

2.6. Comparison features of N.gonorrhea and N.meningitidis

Features	**N.gonorrhea**	**N.meningitides**
. Site of infection	. Urethra/cervix	. Meninges
. Route of infection	. Sexual	. Inhalation
. Disease	Gonococcal urethritis /cervicitis	.Meningococcal meningitis /Meningococcemia
. Specimen of choice	.Urethral/Cervical swab	.Cerebrospinal fluid
. Biochemical reaction	.Oxidase positive . Ferment glucose only	.Oxidase positive . Ferment glucose and maltose

2.3.2. GRAM NEGATIVE COCCOBACILLI

GENUS: HAEMOPHILUS

Characteristics:

- This is a group of small gram-negative, non-spore forming, non-motile, pleomorphic bacteria that require enriched media for growth.
- Growth is enhanced in CO_2 enriched atmosphere.
- Present in upper respiratory tract as a normal microbial flora in healthy people.
- The group is fastidious requiring growth factors for isolation.

- The growth factors are X-factor(Hematin) and V-factor (Diphosphopyridine nucleotide).

. Requirement for growth factor helps for differentiation of species.

Growth factor required	Haemophilus species
X and V factor	H. influenzae, H. aegyptius, H. hemolyticus
X factor	H. ducreyii
V factor	H. parainfluenzae, H. parahemolyticus

. The main species of medical importance are:

H. influenzae
H. ducreyii
H. aegyptius

Haemophilus influenzae

Characteristics:

.Gram-negative cocobacilli.

. Fastidious bacteria requiring growth factors for isolation.

. Found in upper respiratory tract as normal flora in healthy people.

Antigenic structure

.Capsular polysaccharide

. There are six serotypes of H. influenzae, A-F.

. Capsular antigen type b is composed of polyribose ribitol phosphate.

. H. influenzae type b is the most common cause of disease in humans.

. It is the main virulence factor which provides anti-phagocytic property.

. Outer membrane protein

. Lipo-oligosaccharide

Clinical features: The bacteria causes disease most commonly in young children.

. Acute pyogenic meningitis
. Acute epiglottis
. Pneumonia
. Otitis media
. Siusitis
. Cellulitis
. Acute pyogenic arthritis

Laboratory diagnosis:

Specimen: Cerebrospinal fluid, sputum, blood, pus

Smear: Gram-negative short rods.

Culture: Chocolate agar contain both X and V factor; blood agar contain only X factor.

Satellitism test is used to identify H. influenzae in blood agar.

Methods:

. Mix a loopful of haemophilus growth in 2ml of sterile saline.

. Inoculate the bacteria suspension on a plate of blood agar using a sterile swab.

. Streak a pure culture of S. aureus across the inoculated plate which provides V-factor for H. influenzae.

. incubate the plate over night in a CO_2 -enriched environment at 35-37 ^{O}c.

. Look for growth and satellite colonies in next morning.

NB: Colonies are largest nearest to the S. aureus column of growth.

Serology: Quellung reaction (using specific antisera) Immunofluorescence stain

Treatment:

Ampicillin

Chloramphenicol

Cotrimoxazole

Third generation cephalosporins

H. ducreyii

- Slender, gram-negative, ovoid bacilli, slightly larger than H. influenzae.
- Bacteria enmass have configuration of 'shoals of fish".
- It causes chancroid (tender genital ulcer).
- Cultured in special enriched media (20-30% rabbit blood agar) with colonic morphology of small grey glistening colonies surrounded by zone of hemolysis.
- It is treated by erythromycin, cotrimoxazole and third generation cephalosporins.

H. aegyptius

It causes contagious conjunctivitis.

2.3.3. GENUS: BORDETELLA

Characteristics:

. Minute strictly aerobic non-motile gram-negative rods.

Bordetella species of medical importance:

B. pertussis

Antigenic structure:

. Pili: Adheres to ciliated epithelial cells of respiratory tract.

. Filamentous haemagglutinin: Adheres to ciliated respiratory tract.

. Pertussis toxin:

. Lumphocytosis promoting factor

. Histamine sensitizing factor

. Insulin secretion enhancing factor

. Adenylyl cyclase toxin

. Dermonecrotic toxin

. Hemolysin

. Tracheal cytotoxin: Inhibits DNA synthesis in ciliated respiratory epithelial cells.

. Lipipilysaccharide: Damages respiratory epithelial cells.

Clinical features:

Incubation period: 2 weeks

Route of transmission is respiratory from early cases and possibly carries.

It has three stages:

1. Catarrhal stage
2. Paroxysmal stage
3. Convalescence stage

During catarrhal stage, the patient is highlyinfectious but not very ill manifesting with mild coughing and sneezing.

During paroxysmal stage, the patient presents with explosive repetitive cough with characteristic ‘whoop’ upon inhalation leading to exhaustion, vomiting, cyanosis and convulsion.

During convalescence stage, the patient presents with prolonged cough.

Laboratory diagnosis:

Specimen: Saline nasal wash (Preferred specimen)

Nasopharyngeal swab or cough droplets on cough plate

Smear: Small, non-motile, capsulated, gram-negative cocobacilli singly or in pair, and may show bipolar staining.

Culture: Inoculate the primary specimen on Bordet-Gengue agar medium and incubate for 2-6 days at 37 ^{o}c in a moist aerobic atmosphere which produces small, raised, shiny, mucoid colonies.

Biochemical reaction:

. No growth on blood agar

. Oxidase positive

. Most of them are catalase positive

Serology: Direct fluorescent antibody test is most helpful, in identifying B. pertussis after culture on solid media.

Treatment: Erythromycin

Adminstration of erythromycin during the catarrrhal stage of disease promotes elimination of the organism and limits rate of transmission.

Treatment after the onset of paroxysmal stage does not alter the clinical course of the disease.

GENUS BRUCELLA

General characteristics:

- Gram-negative, non-motile, non-sporulating, zoonotic, obligate intracellular aerobic coccobacilli
- 3 major human pathogenic species

Species	Primary animal host
B.abortus	Cattle
B. melitensis	Goat / Sheep
B. suis	Swine
B.canis	Dogs

Antigenic structure:

. Lipopolysaccharide

. Superficial L antigen

Pathogenesis and clinical features:

Brucellosis is a zoonotic disease primarily affecting goat, sheep, cattle, buffalo,pigs and other animals, and transmitted to man by direct contact with infected tissue via skin and mucus membrane , and ingestion of infected milk and milk produts via intestinal tract

IP=1-6 wks

Brucellosis/ Undulant fever

2 stages of illness

1. Acute stage: Fever, malaise, sweating, hepatosplenomegally, lymphadenopathy

Associated with 80% spontaneous recovery

2. Chronic stage: Generally associated with hypersensitivity manifestations like fever, chest pain, and arthritis
   . High agglutinin titier

Complication: Brucella spondylitis(Vertebral brucellosis)

Lab. Diagnosis:

Specimen: Blood, Biopsy material (Bone marrow, Lymphnodes), serum Culture: Grow in blood agar, chocolate agar, or brucella agar incubated in 10% CO2 at 35-37 0C for 3 wks

Biochemical reaction:

- Non-hemolytic
- Catalase positive
- Oxidase positive
- Urease test positive
- Dye inhibition test positive

Serology: Agglutination test

- IgG agglutination titer >1:80 indicate active infection

Treatment:

- Doxycycline + rifampin
- Tetracycline + streptomycin

Prevention and control:

- Pasteurization of milk and milk products
- Reduction of occupational hazards
- Slaughter of all infected animals in dairy herds
- Vaccination of cattle with live attenuated strin of B. abortus

GENUS FRANCISELLA

Francisella tularensis

General characteristics:

Small, facutative intracellular, gram negative, non-motile pleomorphic rods

Antigenic structure:

Polysaccharide antigens

Protein antigens

2 major biogroups (Jellison type A and B)

Type A produce lethal disease in humans unlike type B

Pathogenesis and clinical features:

It is primarily a zoonotic disease and transmitted to human by biting arthropods, direct contact with infected animal tissue, inhalation of aerosols, or ingestion of contaminated food and water

Tularemia: 4 types

1. Ulceroglandular tularemia: Ulceration of arms and hands with lymphadenitis after tick bite or direct contact of broken skin with infected tissue or blood
3. Oculoglandular tularemia: Accidental contamination of the conjunctiva with infected droplets/aerosols
4. Pneumonic tularemia: Contracted through contaminated aerosols
5. Typhoidal tulsremia: Following ingestion of inadequately cooked food

Lab. Dignosis:

Specimen: Skin lesion, lymphnodes, sputum, conjunctival scrapings

Culture: grow in blood-cysteine-gextrose agar incubated at 37 0c under aerobic condition

Serology: Agglutination test

Single titer of ≥ 1:160 is highly suggestive of tularemia

Paired serum samples collected two weeks apart can show a rise in agglutination titer

Treatment:

Streptomycin or gentamicin

Tetracycline

Prevention and control:

Immunization of high risk persons (eg. Lab.personel handling the specimen) with live attenuated vaccine

GENUS PASTEURELLA

General characteristics:

- Gram-negative, non-motile, aerobic or facultative anaerobic coccobacilli with bipolar staining
- Grow in ordinary media at 37 0c
- Catalase positive
- Oxidase positive

Pasteurella multocida

- Occur in gasrointestinal and respiratory tract of many domestic and wild animals

- Most common organism in human wounds inflicted by bites from cats and dogs
- Treatment: Penicillin

  Tetracycline + flouroquinolones

2.4. GRAM NEGATIVE RODS

It comprises the following bacterial groups

1. **Oxidase negative**

. Enterobacteriaceae

a**. Lactose-fermenters**

. Escherichia spp.
. Klebsiella spp.
. Enterobacter spp.
. Citrobacter spp.

b**. Non-lactose fermenters**

. Salmonella spp.
. Shigella spp.
. Proteus spp.

2**. Oxidase Positive**

. Pseudomoas
. Vibrio
.Campylobacter
. Helicobacter

2.4.1. ENTEROBACTERIACEAE

Characteristics

Named, as well coliforms or enterobacilli:

. Found as normal flora in intestinal tract of humans and animals.

. Gram-negative, non-spore forming, aerobic and facultative-anaerobic bacteria.

. Most are motile.

. Grow over a wide range of temperature in ordinary media.

. All ferment glucose with acid production.

. Oxidase negative.

. Release endotoxin from their cell wall.

. Some release exotoxin.

Most of them have possessed three types of antigenes.

These are :

. H antigen-. Flagellar protein

. Found in the flagella.

. Possessed by motile enterobacteriaceae.

. Heat labile and sensitive to alcohol

May interfere with agglutination by O antisera

. K antigen- .Capsular polysaccharide or protein

. Surroundes the cell wall.

. Heat labile and may be associated with virulence

May interfere with agglutination by O antisera

. O antigen- .Outer membrane lipopolysaccharide.

. Found in the cell wall of enterobacteriaceae.

. Resistant to heat and alcohol, and usually detected by bacterialn agglutination

. Antibodies to O ags are usually IgM

GENUS:ESCHERICHIA

. Main species of medical importance is Escherichia coli.

Escherichia coli

Characteristics:

. Normal flora in human and animal gastrointestinal tract.

. Found in soil, water and vegetation.

. Most are motile; some are capsulated.

Clinical features:

. Urinary tract infection- cystitis, pyelonephritis

. Wound infection- appendicitis, peritonitis

. Neonatal septicemia and meningitis

. E.coli-associated diarrheal disease

1. Enteropathogenic E.coli(EPEC)

. causes outbreaks ofself-limiting infantile diarrhea

. they also cause severe diarrhea in adults

. antibiotic tretment shorten the duratin of illness and cure diarrhea

2. Enteroinvasive E.coli(EIEC)

. Non-motile, non-lactose fermenting E.coli invade the mucosa of the ileum and colon, and causes shigellosis-like dysentery in children in developing countries and travellers to these countries

3. Enterotoxigenic E.coli(ETEC)

. Colonization factor of the organism promote adherence to epithelial cells of small intestine followed by release of enterotoxin which causes toxin-mediated watery diarrhea in infants and young adults.

. It is an important cause of traveller's diarrhea

. Antibiotic prophylaxis can be effective but may increase drug resistance (Should not be uniformly recommended)

4. Entero haemorrhagic E.coli(EHEC)

   Cytotoxic verotoxin producing E.coli serotype O157:H7 causes haemorrhagic colitis (severe form of diarrhea), and hemolytic uremic syndrome characterized by acute renal failure, hemolytic anemia and low platelet count

5. Enteroaggressive E.coli(EAEC)

   .Adhere to human intestinal mucosal cells and produce ST-like toxin and hemolysin, and causes acute and chronic diarrhea in persons in developing countries

   . Produce food-borne illness in developed countries

Laboratory diagnosis:

Specimen: Urine, pus, blood, stool, body fluid

Smear: Gram-negative rods

Culture: Lactose-fermenting mucoid colonies on mac conkey agar and some strains are hemolytic on blood agar .

Biochemical reaction: Produce indole from tryptophan-containing peptone water.

Reduce nitrate to nitrite.

Serology: For serotyping (Epidemiologic information)

Treatment: Base on antibiotic sensitivity pattern

Genus: Klebsiella

Characteristics:

Non-motile, lactose-fermenting, capsulated, gram-negative rods.

Main species of medical importancce:

K. pneumoniae

K. rhinoscleromatis

K. ozenae

K.pneumoniae

It is found as a commensal in the intestinal tract, and also found in moist environment in hospitals.

It is an important nosocomial pathogen.

It causes:

. Pneumonia

. Urinary tract infection

. Septicaemia and meningitis (especially in neonates)

. Wound infection and peritonitis

K. rhinoscleromatis

It causes rhinoscleroma of nose and pharynx to extensive destruction of nasopharynx (hebra nose).

K.ozaenae

It causes ozena manifesting with foul smelling nasal discharge leading to chronic atrophic rhinitis.

Laboratory diagnosis of klebsiella species:

Specimen: Sputum, urine, pus, CSF, body fluid

Smear: Gram-negative rods

Culture: Large, mucoid, lactose-fermenting colonies on mac conkey agar, and shows stringy type growth when cultured in broth medium.

Serology: Capsular polysaccharide serotyping

More than 80 serotypes of K. pneumoniae recognized.

Treatment: Based on sensitivity testing

GENUS: ENTEROBACTER

It is gram-negative lactose fermenting motile rods, and found as a commensal in the intestinal tract of humans and animals and moist environments.

Medical important species is Enterobacter aerogens.

It produces mucoid colony resembling klebsiella on Mac Conkey agar.

Enterobacter aerogens is associated with urinary tract infection, wound infection and septicaemia in immunocompromised and chronically deblitated patients.

GENUS: CITROBACTER

It is gram-negative lactose fermenting motile rods, and opprtunistic pathogen.

Medical important species is Citrobacter freundii.

Citrobacter freundii is associated with urinary tract infection, wound infection and septicaemia in immunocompromised and chronically deblitated patients.

GENUS: SALMONELLA

Most isolates of salmonellae are motile

It grows readily on simple media

It never ferment sucrose or sucrose

Form acid +/- acid from glucose or mannose

Species of medical importance are:

- S. typhi
- S. paratyphi
- S. enteritidis

Clinical features:

1. Enteric fever

   It is caused by S.typhi and S.paratyphi, and transmitted by fecal-oral route via contaminated food and drinks

   Incubation period: 10-14 days

   Predisposing factors:

   .Reduced gastric acidity

   .Disrupted intestinal microbial flora

   .Compromised local intestinal immmunity

Both manifest with persistent fever, headache, malaise, chills, enlargement of liver and spleen, and skin rashes.

Paratyphoid fever is milder than typhoid fever

Complications:

- Intestinal perforation
- Lower gastrointestinal bleeding
- Dissenmination to different body organs including meninges and brain

Mortality rate

- Untreated cases: 10-15%
- Treated cases: < 1%

2. Bacteremia with focal lesions

Causative agent: S. choleraesuis

Manifests with blood stream invasion with focal lesions in lungs, bones and meninges

Intestinal manifestation are often absent

3. Gastroenteritis

It is caused by S. enteritidis

S. typhimurium

IP= 8-48 hrs

It manifests with initial watery diarrhea, and later bloody mucoid diarrhea associated with crampy abdominal pain and tenesmus.

Bacteremia is rare (2-3 % of cases)

It usually resolves in 2-3 days

Variables	Enteric fever	Septicemia	Enterocolitis
Ip	7-20 days	Variable	8-48 hrs
Onset	Insidious	Abrupt	Abrupt
Fever	Gradual rise	Rapid rise	usually low
Disease Duration	Several wks	Variable	2-5 days
GIT symptoms	Early Constipation /later diarrhea	Often none	Diarrhea at onset
Blood culture	+ve in 1st and 2nd wks	+ve during high fever	Negative
Stool culture	+ve in 2nd wk on	S/times +ve	+ve since onset

Laboratory diagnosis:

Specimen: 1. Blood, Bone marrow, stool, urine and serum for enteric fever.

. Blood – 80% positive in the first week.

. Stool- 70-80% positive in the second and third week.

. Urine- 20% positive in the third and fourth week.

. Serum for widal test- positive after the second week of illness.

2. Stool for gastroenteritis.

Gram reaction: Gram-negative rods

Culture: Bacteriologic methods for salmonella isolation

1. Differential medium

. For rapid isolation of lactose non-fermenters

Egs. EMB agar
Mac Conkey agar
Deoxycholate agar

2. Selective medium

. favor growth of salmonella and shigella over other enterobacteriaceae

Egs. SS agar
Hekton Enteric agar
XLD agar
Deoxycholate-Citrate agar

3. Enrichment cultures

. Inhibit replication of normal intestinsl flora and permit replication of salmonella

Egs. Selenite F broth
Tetrathionate broth

Non-lactose fermenting , H_2S producing colonies in
Mac conkey agar.

Biochemical reaction: Generally produce gas and acid from carbohydrate; except S.typhi which does not produce gas.

Serology: (wiedal test)

a. Tube dilution agglutination test

Used to determine antibody titers in patients with unknown illness

Method:

- Serial dilutions of unknown serum are tested against antigens from representative salmonella species.
- The highest diluted serum with positive result is taken as a tite

Interpretation of result

- High or rising titer to O antigen (≥ 1:160) suggests active infection.
- High or rising titer to H antigen (≥ 1:160) suggests past infection or immunization.
- High titer to the Vi antigen occurs in some cases

Causes of false positive Widal test

- Malria infection
- Other acute febrile illness
- Poor quality reagent

Causes of false negative widal test

- Spcimen collted after antibiotic adminastartion
- Specimen collted at early stage of diseases
- Technical errors

a. Slide agglutination test/ Kauffman-White system
   Used to identify unknown cultures with known sera

Required: Salmoella O and H polyvalent antiserum

Method:

. Mix known sera with unknown culture on a slide.

. Clumping occurs with in a few seconds in positive result

NB: Slide agglutination test is important for preliminary identification of culture

Treatment:

1. For cases
   Chloramphenicol
   Fluoroquinolones
   3rd generation cephalosporins
2. For carriers
   Ampicillin followed by cholecystectomy
   NB: salmonellae persist in gall bladder in chronic carriers

Prevention and control

- Sanity measures like hygenic food and drink handling, and avoid carriers from food handling until properly treated
- Provision of vaccine

Injectable acetone-killed S. typhi suspensions

Oral live, avirulent mutant strain of S. typhi in high endemic areas

GENUS: SHIGELLA

Species of medical importance are:

	Subgroups
S. dysenteriae	A
S. flexneri	B

S. boydii	C
S. sonnei	D

In developing countries, shigellosis (bacillary dysentery) is caused by S. flexneri and S. dysenteriae.

It is found in human intestinal tract as pathogen.

Pathogenesis and Clinical features:

Route of infection is fecal-oral route

Inoculum dose: 103 organisms

Pathogenicity determinant:

Toxins:

Endotoxin: irritate the bowel wall

Exotoxin: Enterotoxin and neurotoxin

S.dysenritiae type 1(shiga bacillus) produce heat labile exotoxin-mediated diarrhea

IP: 1-2 days

It causes shigellosis (bacillary dysentery) characterized by sudden onset of bloody mucoid diarrhea, abdominal cramp, tenesmus, fever, generalized muscle ache and weakness.

Complication: Dehydration

Electrolyte and acid-base disturbance

High prevalence: Poor sanitation

Poor personal hygiene

Polluted water supply

Young children are frequently affected.

Laboratory diagnosis:

Specimen: Stool,serum

Gram reaction: Gram-negative non-motile rods.

Culture: Non-lactose fermenting colonies on Mac conkey agar and SS agar.

Biochemical reaction: It produces acid but not gas from carbohydrate.

Serology: For serogrouping and serotyping.

It is not used to diagnose shigella infection

Treatment: Ciprofloxacin

Cotrimaxazole

Suppress acute clinical attacks of dysentry

Shorten the duration of symptoms

Prevention and cotrol:

Santirtary control of water, food and milk, sewage disposal and fly control

Antibiotic treatment of infected individuals

GENUS: PROTEUS

Proteus species are found in the intestinal tract of humans and animals, soil, sewage and water.

They are gram-negative, motile, non-capsulated , pleomorphic rods.

Species of medical importance:

P. mirabilis

P. vulgaris

Clinical features:

P. mirabilis

. Urinary tract infection

. Septicemia

. Abdominal and wound infection

. Secondary invader of ulcer, burn, pressure sores and chronic discharging ear.

P. vulgaris

. Important nosocomial pathogen.

.Isolated in wound infection and urinary tract infection.

Laboratory diagnosis:

Specimen: Urine, pus, blood, ear discharge

Smear: Gram-negative rods

Culture: Produce characteristic swarming growth over the surface of blood agar.

Ditching of culture media prevents spread of proteus species.

Non-lactose fermenting colonies in mac conkey agar.

Proteus species have a characteristic smell.

Biochemical reaction:

Proteus spp…….. Urease positive

P. vulgaris……... Indole positive

P. mirabilis…….. Indole negative

Serology: Cross react with Weil-fellix test

Treatment: Based on sensitivity testing.

GENUS YERSINIA

General characteristics:

- Animals are natural hosts of yersinia, and humans are accidental hosts of yersinia infection
- Short, pleomorphic microaerophilic or facultatively anaerobic gram negative rods exhibiting bipolar staining with special stains

Important human pathogens

Y. pestis

Y. pseudotuberculosis

Y. enterocolitica

Yersinia pestis

. Plague bacillus with gram negative, non-motile, facutatively anaerobe possessing bipolar granules

Antigenic structure:

LPS: Endotoxic effect

Envelope protein (Fraction I): Antiphagocytic property

V-W antigens: Plasmid gene-encoded virulence factor

Coagulase (produced at 28 ^{o}c; mice body T^{o})

Exotoxin (lethal for mice/unknown role in humans)

Bacteriocin (pesticin)

Pathogenesis and clinical features:

Rat flea (Xenopsylla cheopis) gets infected by biting an infected rodent → infected rat flea bites human (accidental host) → organism migrate to regional lymphnodes from the site of bite (bubonic plaque) and gets into the blood via lymphatics (septicemic plaque), or Primary pneumonic plaque results from inhalation of infective droplets, usually from an infected coughing person

IP=2-6 days

Human Plaque: 3 types

1. Bubonic plague: Fever, vomiting, painful lymphadenitis(buboes) in the groin or axillae
2. Pneumonic plague: Ip is 1-3 days
   Profuse mucoid or bloody expectoration with signs of pneumonia
3. Septicemic plague
   Fever, vomiting, diarrhea, hypotension, altered mentation, renal and heart failure, intra vascular coagulopathy

Lab. Diagnosis:

Specimen: Lymphnode aspirate, CSF, blood

Smears: Wright's stain, immunofluorescence stain, methylene blue stains, basic fuchsin stain

Wayson's stain to demonstrate bipolar granules

Cuture: Grow in blood agar or mac conkey agar

NB: All cultures are highly infectious and must be handled with extreme caution

Biochemical reaction:

Catalase positive

Oxidase negative

Serology: Fluorescent antibody technique using Y. pestis antisera

Prognosis: Mortaity rate is 50% (100% for pneumonic plaque)

Treatment:

Streptomycin

Tetracycline

Steptomycin + tetracycline or chloramphenicol

Prevention and control:

. Chemoprophylaxis for contacts of patients

. Formalin-killed vaccine for travellers to hyperendemic areas and high risk persons

Yersinia enterocolitica and Yersinia pseudotuberculosis

Non-lactose fermenting gram negative rods

Urease positive

Oxidase negative

Y. enterocolitica

. > 50 serotypes

. Y. enterocolitica Serotype 03, 08, and 09 cause human disease

. Human infection occurs by contaminated food and drinks from domestic animals or rodents

Y. pseudotuberculosis

. Six serotypes

. Y. pseudotuberculosis serotype 01 accounts for most human infection

. Human infection results from ingestion of food and drinks contaminated by animalfeces

Antigenic structure

. Inv (invasion) locus

. AIL (attachement invasion locus)

Pathogenesis and clinical feature:

Route of transmission: Contaminated food and drinks

Inoculum dose: 108-109 org

IP=5-10 days

Yersinosis: Enterocolitis

. Fever, abdominal pain, toxin and invasion-mediated diarrhea

. Usually self-limited disease

. Post-diarrheal diseases

. Arthritis

. skin rash/nodules

. Complication: Sepsis/ Meningitis

Lab. Diagnosis:

Specimen: Stool, blood, rectal swab

Culture: Grow in routine enteric media

Biochemical tests for species identification

Treatment:

Fluid replacement for enterocolitis (Antibiotics not required)

Cephalosporin (3^{rd} generation) + Aminoglycosides for sepsis/ meningitis

Prevention and control:

Conventional sanitary precautions

2.4.2. GENUS: PSEUDOMONAS

General characteristics:

. Gram-negative motile aerobic rods having very simple growth requirement.

. Can be found in water, soil, sewage, vegetation, human and animal intestine.

. Species of medical importance:

P. aeruginosa

P. pseudomallei

Pseudomonas aeruginosa

. Found in human and animal intestine, water, soil and moist environment
in hospitals.

. Primarily a nosocomial pathogen.

. Invasive and toxigenic, produces infections in patients with abnormal host defenses

Antigenic characteristic:

. Pili: Adhere to epithelial cells

. Exopolysaccharide: Anti-phagocytic property/ inhibit pulmonary clearance

. Lipopolysaccharide: Endotoxic effect

. Enzymes

.Elastases: Digests protein (elastin, collagen, IgG)

.Proteases

.Hemolysins

.Phospholipases C (heat labile): Degrade cytoplasmic membrane components

.

. Exotoxin A: Cytotoxic by blocking protein synthesis

Clinical features:

Pathogenic only when introduced into areas devoid of normal defenses eg. Breached mucus membrane or skin, use of IV line or urinary catheterization, neutropenia of any cause

. Urinary tract infection- chronic, complicated Urinary tract infection and associated with indwelling catheter.

. Wound infection of burn sites, pressure sores and ulcers.

.Septicaemia- "Ecthyma gangrenosum" skin lesion (haemorrhagic skin necrosis)

. Otitis externa- Malignant external ear infection in poorly treated diabetic patients.

. Pneumonia- Infection of the lung in patients with cystic fibrosis.

. Eye infection- Secondary to trauma or surgery.

Laboratory diagnosis:

Specimen: pus, urine, sputum, blood, eye swabs, surface swabs

Smear: Gram-negative rods

Culture:

.Obligate aerobe, grows readily on all routine media over wide range of temperature(5-42 ^{O}C).

. Bluish-green pigmented large colonies with characteristic "fruity" odor on culture media.

. Biochemical reaction:

. Oxidase positive

. Catalase positive

. Citrate positive

. Indole negative

. Produce acid from carbohydrate by oxidation, not by fermentation.

NB: identification of the bacteria is based on colony morphology, oxidase-positivity, characteristic pigment production and groth at 42 ^{O}c

Treatment: Ticarcillin or piperacillin and aminoglicosides
Aztreonam
Imipenem
Ceftazidime
Cefoperazone
Flouroquinolones

Prevention and control:

Special attention to sinks, water baths, showers and hot tubs Polyvalent vaccine tohigh risk groups.

2.4.3. GENUS: VIBRIOS

. Actively motile, gram-negative curved rods.
. Species of medical importance: Vibrio cholerae-01

Vibrio cholerae

Characteristics:

. Found in fresh water, shellfish and other sea food.
. Man is the major reservoir of V. cholerae-01, which causes epidemic cholera.
. Readily killed by heat and drying; dies in polluted water but may survive in clean stagnant water, esp. if alkaline, or sea water for 1-2 weeks.

Antigenic structure:

. O antigen

. Six major subgroups.

. All strains possess a distinctive O antigen and belong to subgroup I with subdivision into three serotypes; Ogawa, Inaba, Hikojima.

. Any serotype can be either Classical or ElTor biotype.

. ElTor biotype is more resistant to adverse conditions than Classical diotype of V. cholerae.

H antigen

. Little value in identification

Clinical features:

Route of infection is fecal-oral route.

After ingestion of the V.cholerae-01, the bacteria adheres to the intestinal wall with out invasion then produces an exotoxin causing excessive fluid secretion and diminished fluid absorption resulting in diarrhea (rice water stool) which is characterized by passage of voluminous watery diarrhea containing vibrios, epithelial cells and mucus; and result in severe dehydration.

Laboratory diagnosis:

Specimen: Stool flecks

Smear: Gram-negative motile curved rods

Motility of vibrios is best seen using dark-field microscopy.

Presumptive diagnosis: Inactivation of vibrios in a wet preparation after adding vibrio antiserum.

Culture:

1. TCBS (thiosulphate citrate bile salt sucrose agar) media Selectivemedia for primary isolation of V.cholerae.

. Observe for large yellow sucrose-fermenting colonies after 18-24 hrs of incubation.

2. Alkaline peptone water: Enrichment media for V.cholerae-01

   Growth on and just below the surface of peptone water with in 4-6 hours at room temperature as well as 37 ^{o}c.

Biochemical Reaction:

. Oxidase-positive.

. Ferment sucrose and maltose(acid; no gas).

. Do not ferment L-arabinose.

Treatment: Sensitive to tetracycline and chloramphenicol. Fluid and electrolyte replacement are the first line of management for cholera.

2.4.4. **GENUS: CAMPYLOBACTER**

Characteristics:

. Small, delicate, spirally curved gram-negative bacteria.

. Motile bacteria with single polar flagellum.

. Stricly microaerophilic bactria requiring 5-10% o_2 and 10% co_2 enriched environment.

. Oxidase and catalase positive.

Species of medical importance:

Campylobacter jejuni

Campylobacter coli

Campylobacter jejuni and Campylobacter coli

Characteristics:

- Gram-negative non-spore forming motile rods with comma, S or 'gull-wing' shapes.
- Requires selective media like skirrow's and Butzler's media for isolation of the bacteria from faecal specimen.

Antigenic structure:

. Lipopolysaccharide

. Cytopathic extracellular toxin

. Enterotoxin

Clinical features:

Inoculum dose: 10^4 organisms

Source of infection is contaminated food, drinks,and unpasteurized milk

The organism multiply in small intestine, invade the epithellium and produce inflammation

Campylobacter enteritis manifests with fever, headache, malaise, crampy abdominal pain and bloody mucoid diarrhea, and usually self-limited enteritis in a week period

Laboratory diagnosis:

Specimen: Stool

Microscopy: Typical 'gull-wing' shaped gram-negative rods.

Typical darting motility of the bacteria under dark field microscopy or phase contrast microscopy

Culture: Grow best at 420_c on selective media but can be cultured at 37 o_c.

Watery and spreading or round and convex colonies on solid media at low oxygen tension.

Biochemica;l reaction:

C jejuni …….. hydrolyzes hippurate.

C. coli …… does not hydrolyze hippurate.

Treatment: Erythromycin

Shorten the duration of fecal shedding of bacteria

Helicobacter pylori

General characteristics:

. Spiral-shaped gram negative, microaerophilic, motile rods with polar flagella

Antigenic structure:

Pili

Protease

Urease

Pathogenesis and clinical features:

Route of entry: Ingestion of contaminated food and drinks

Familial clustering of H. pylori infection occurs

. Type B chronic antral gastritis

. Peptic ulcer disease (gastric and duodenal ulcer)

. Gastric carcinoma

. Gastric lymphoma

Lab. Diadnosis:

Specimen: Gatric biopsy, serum

Smear: Giemsa or silver stain

Culture: Skirrow's media

Tanslucent colonies after 7 days of incubation

Biochemical reaction:

. Catalase positive

. Oxidase positive

. Urease positive

Serology:

. Detection of antibodies in the serum specific for H. pylori

. Detection of H. pylori antigen in stool specimen

Special tests:

. Urea breath test

Treatment:

Triple or quadruple therapy:

. Amoxicillin + clarithromycin/ metronidazole + Proton pump inhibitors (PPI (Omeprazole or lansoprazole))

or

.Metronidazole + Bismuth subsalicylate/ Bismuth subcitrate + Amoxicillin / Tetracycline + PPI

Prevention and control:

. Improving sanitary hygiene

GENUS: LEGIONELLA

L. pneumophila

General characteristics:

Fastidious, aerobic, gram negative intracellular rods

Ubiquitous in warm moist environment

Antigenic structure:

Complex surface antigens

>10 serogroups

L.peumophila serogroup 1 is the most common serogroup isolated in humans

Proteases

Phosphatases

Lipases

DNase

RNase

Major secretory protein (Metalloprotease): Possess cytotoxic and hemolytic property

Pathogenesis and clinical features:

Route of transmission: Inhalation of aerosols generated from contaminated cooling towers, heat exchange apparatus, shower water, tap water, and potable water following chlorination

1. Legionnaires disease: Pneumonic presentation with high fever, chills, dry cough, hypoxia, diarrhea, and altered mentation
2. Pontial fever: Fever, chills, malaise, headache, malaise, altered mentation

Laboratory diagnosis:

Specimen: Bronchial washing, Lung biopsy, Blood

Smears: DFA (direct flourescent antibody) staining

Silver staining

Cuture: Grow in BCYE (buffered charcoal-yeast extract) agar media

Biochemical tests:

Catalase positive

Oxidase positive

Hydrolyse hippurate

Serologic testing: Useful in the diagnosis of retrospective outbreaks of legionella infection

Treatment:

Erythromycin

Rifampin

Prevention and control

Hyperchlorination

Super heating of water

Infection caused by anaerobic bacteria

Anaerobic bacteria are found through out human body (Skin, mucous membrane, and gastrointestinal tract) as part of resident flora, and cause infection when contaminate normally sterile body sites

. Grow at low or negative oxidation-reduction potential

. Do not have cytochrome systems for oxygen metabolism

. Lack superoxide dismutase and catalase, and susceptible to the lethal effects of oxygen and oxygen radicals.

. Most anaerobic infections are caused by “moderately obligate anaerobes”, and polymicrobial in nature caused by combination of anaerobes, facultative anaerobes and aerobes.

Anaerobic bacteria found in human infections

1. Bacilli	Anatomic site
a. Gram negative	
Bacteroides fragilis group	colon
Prevotella melaninogenica	Mouth
Fusobacterium	Mouth/Colon
b. Gram positive	
Actinomyces	Mouth
Lactobacilli	Vagina
Propionibacterium	Skin
Clostridium	Colon
2. Cocci	
a. Gramnegative	
Veillonella	Mouth/Colon
b. Gram positive	
Peptostreptococci	Colon

clinical features:

Representative anaerobic infections	Commonly isolated anaerobic bacteria
Brain abscess	Peptostreptococci
Oropharyngeal infection	Actinomyces P. melaninogenica Fusobacterium
Pleuropulmonary infection	Peptostreptocci Fusobacterium P. melaninogenica B. fragilis
Intra-abdominal infection	
Liver abscess	Mixed anaerobes
Abdominal abscess	B. fragilis
Genital tract infection	
Pelic abscess	P. bivia P. disiens Peptostreptococci
Skin, soft tissue, and bone infection	Mixed anaerobic flora
Bacteremia	B. fragilis Clostridium

Peptostreptococci
Propionibacterium

Endocarditis B. fragilis

Diagnosis of anaerobic infection

1. Clinical
   . Foul smelling discharge due to short chain fatty acid products of anaerobic metabolism
   . Proximity to a mucosal surface
   . Gas in tissue due to production of co_2 and H_2
2. Lab. diagnosis
   . Grow most readily in complex media (Trypticase soy agar, Schaedler blood agar, Brucella agar, Brain-heart infusion agar) incubated at 35-37 ^{0}c in anaerobic atmosphere enriched with CO_2

Identificat ion of anaerobes is based on:

. Colony morphology
. Pigmentation
. Fluorescence
. Biochemical reaction
. Fatty acid production

Treatment:

Antimicrobials + Surgery
Clindamycin
Metronidazole
Cefotetan

Cefoxitin

Piperacillin

Penicillin

2.5. GENUS: MYCOBACTERIA

Characteristics:

- Non-spore forming, non-motile, aerobic, Acid-fast bacilli.
- Acid-fastness depends on the waxy envelope-mycolic acid of cell wall.
- More resistant to chemical agents than other bacteria.
- Once stained with primary stain, they resist decolorization by acid-alcohol.
- All bacteria are decolorized by acid-alcohol except Mycobacteria.

. Mycobacteria of medical importance:

M. tuberculosis

M. leprae

Mycobacterium tuberculosis

Characteristics:

. Strictly aerobic acid-fast bacilli.

. The main reservoir is an infected human.

Antigenic structure:

1. Lipids: Mycolic acid, waxes, phosphatides
   .responsible for acid-fastness, granuloma formation and caseation necrosis.
2. Proteins

. Elicits the tuberculin reaction and antibody production.

3. Polysaccharides

. Induce the immediate type of hypersensitivity.

Clinical manifestation:

Incubation period: 4-6 weeks.

Source of infection: Tuberculous patients

Route of infection: Respiratory- Inhalation of droplet nuclei

Ingestion of infected milk

Disease: Pulmonary and extrapulmonary tuberculosis

The disease generally manifests with low-grade persistent fever, night sweating, significant weight loss, fatigue and generalized weakness.

Laboratory diagnosis: Identification of M. tuberculosis

Specimen: Sputum; pleural, peritoneal and cerebrospinal fluid

Smear: Acid fast bacilli from primary specimen.

Cord forming acid-fast bacilli from culture.

Culture:

1. Semisynthetic agar media

. Middle brook 7H10 and 7H11

. Used for observing colony morphology, susceptibility testing, and as selective media

2. Inspissated egg media

. Lowenstein-Jensen medium

It is the ordinary selective media for tubercle bacilli

Raised, dry, cream colored colonies of tubercle bacilli after 3-6 wks of incubation

3. Broth media

Middle brook 7H9 and 7H12

. Support the proliferation of small inocula

Pigment production test:

. Leave the culture in the light for 2 hrs.

. Reincubate it at 35-37 ^{O}c over night.

. Reexamine the colonies for pigment production

. M. tuberculosis does not produce pigment in dark or light.

Incubation of a subculture at 25^{O}c

. M. tuberculosis will not grow at 25 ^{O}c.

Growth of the bacteria on Lowenstein-Jensen medium containing 4(P)- nitrobenzoic acid(PNB).

. No growth of M. tuberculosis on PNB media.

Biochemical reaction:

.Niacin test is Positive.

New techniques:

. Molecular probes (DNA probes)- It detects Mycobacterial RNA sequence.

. High-performance liquid chromatography

. Polymerase chain reaction

. Enzyme immunoassay

Treatment:

Anti-tuberculosis drugs

1. First-line drugs

. Isoniazid . Ethambutol

. Rifampin . Pyrazinamide

. Streptomycin

2. Second-line drugs
   . Kanamicin . Cycloserine
   . Capreomycin . Ofloxacin
   . Ethionamide . Para-aminosalicylic acid (PAS)

Preventionn and control:

. Prompt and effective treatment of patients with active tuberculosis and careful follow up of their contacts with tuberculin test and CXR

. Immunization with BCG (Bacillus-Calmette-Guerin) vaccine

. Pasteurization of milk and milk products

Mycobacterium leprae

Characteristics:

- Typical acid-fast bacilli, arranged in singly, parallel bundles or in globular masses.
- Not grown in non-living bacteriologic media.
- Characteristic lesions are grown in laboratory animals.

Eg. Foot pads of mice

Armadillos

Clinical features:

Incubation period is months to years.

Route of infection is through nasal mucus secretion.

Disease: Hansen's disease or leprosy.

The lesion involves the cooler parts of the body, Eg. Ear lobes.

Clinical triads: Anaesthetic skin patches

Peripheral neuritis

Presence of acid-fast bacilli from skin lesion

Two major types of leprosy

1. Lepromatous leprosy
2. Tuberculoid leprosy

Table 2.7. Comparison of the two types of leprosy

Characteristics	**Lepromatous leprosy**	**Tuberculoid leprosy**
1. Course	. Progressive	. Benign and non-progressive
2. Manifestation	. Nodular skin lesion	. Macular skin lesion
3. Involvement of nerve	. Slow and symmetrical	.Severe and asymmetrical
4. Cell mediated immunity	. Weak	. Strong
5. AFB from skin lesion	. Abundant	. Scanty
6. Lepromin skin test	. usually negative	. Usually positive

Laboratory diagnosis:

Specimen: Skin scrapings from the ear lobe.

Smear: Acid fast bacilli from the primary specimen.

Bacterial index (BI) indicates number of organisms present in a smear.

Number of M. leprae bacilli found in smears are related to type of leprosy and effect of drug therapy.

GRADING:

0…….No bacilli per field, count ≥100 fields

1+…….Average 1-10 bacilli per smear, count ≥100 fields

2+…….Average 1-10 bacilli per 10 fields, count ≥100 fields

3+…….Average 1-10 bacilli per 10 fields, count 25 fields

4+…….Average 10-100 bacilli per fields, count 25 fields

5+……. Average 100-1000 bacilli per fields, count10 fields

6+……. Average >1000 bacilli per fields, count10 fields

NB: Count BI for each smear and calculate the average to give an over all BI.

Morphologic index indicates percentage of viable bacteria in a smear.

It is used to judge the response of a patient to anti-leprosy drugs.

Viable bacilli stain clearly and evenly as solid red bacilli.

Non-viable bacilli stain poorly and unevenly as fragmented, beaded and granular red bacilli.

Methods of collecting skin smears and staining of M. leprae

Procedure:

. Explain to the patient the procedure you are going to do.

. Label the slide with the date, name and number.

. Fit the sterile scalpel blade in its scalpel holder.

. Cleanse the area from where the smear is to be taken using alcohol swab.

. When dry, hold fold of skin tightly between the thumb and forefinger until it becomes pale.

. Using the sterile blade, make a small cut through the skin surface, 5mm long and 2-3mm deep, where the bacteria is be found.

. Turn the scalpel blade until it is at a right angle to the cut.

. Make a small circular smear of the tissue juice

(Cover the cut with a small dressing)

. When the smears are dry, gently fix with heat.

. Cover the smear with the filtered carbol-fuchsin stain.

. Heat the stain until vapor just begins to rise; Don't over heat.

. Allow the smear heated stain to remain on the slide for 15 min.

. Wash off the stain with clean water.

. Decolorize the smear with 1% V/V acid-alcohol for 10 min.

. Wash off with clean water.

. Cover the smear with malachite green stain for 1-2 min.

. Wash off the stain with clean water.

. Wipe the back of the slide clean, and place in a draining rack for the smears to air-dry (protect from direct sun light).

. Examine the smear under microscope with 100X objective.

NB: The bacteria is not cultured in artificial media; but can be cultivated in vivo by inoculation into the foot pads of mice and Armadillos.

The animal develops slow-growing granulomas at the site of injection.

Treatment: Anti-leprosy drugs

. Dapsone

. Rifampicin

. Clofazimine

ACTINOMYCETES

. Most are soil saprophytes, but some are human pathogens responsible to cause actinomycosis nocardiosis and actinomycetoma

. Large group of gram positive bacilli with a tendency to form chains and filaments

. Related to mycobacteria and corynebacteria

. Endogenous members of the bacterial flora in the mouth and lower gastrointestinal tract

Actinomycosis

Chronic suppurative and granulomatous infection with interconnecting sinus tracts that contain sulfur granules

Etiology:

Actinomyces israeli

Actinomyces naeslundii

Characteristics:

. Gram positive, facultative anaerobe substrate filaments that grow in co_2 enriched condition

Pathogenesis and clinical features:

. Infection is initiated by trauma that introduces these endogenous bacteria into the mucosa

1. Cervico facial actinomycosis

Fluctuant mass with draining fistula in jaw area, and may extend to involve bone and lymphnodes in the head and neck

2. Thoracic actinomycosis

Resemle subacute pulmonary infection with extension to chest wall and ribs

3. Abdominal actinomycosis

May be secondary to ruptured appendix or ulcer with extensive involvement of abdominal organs

Lab. Diagnosis:

Specimen: Tissue, pus, sputum

Smear: Gram-positive filaments with lobulated sulfur granules

Culture: Thioglycolate broth or blood agar incubated anaerobically or co_2 enriched condition

Biochemical reacrion: Catalase positive/negative

Treatment: Penicillin

Clindamycin + Surgery

Erythromycin

Nocardiosis

Etiology: Nocardia asteroides complex

N. abscessus

N. farcinia

N. nova

Nocardia brasiensis

Nocardia otitidiscaviarum

Characteristics:

. Found world wide in soil and water, and opportunistic pathogen

. Aerobic gram positive, partially aci fast bacilli

Pathogenesis and cloinical features:

Route of transmission: Inhalation

Usual presentation is subacute or chronic pulmonary infection with dissemination to the brain and skin

Lab. diagnosis

Specimen: Sputum, pus, CSF, biopsy material

Smear: Gram positive, partially acid fast bacilli

Treatment: Cotrimoxazole + Surgery

Non-respoders to cotrimoxazole

Amikacin

Imipenem

Cefotaxime

Actinomycetoma

Slowly progressive, painless, destructive subcutaneous tissue infection

Etiology: Nocardia brasilensis

Streptomyces somaliensis

Actinomadura madurae

Treatment: Combination of streptomycin, cotrimoxazole and dapsone

6. SPIROCHETES

Characteristics:

. Long, slender, helically coiled, spiral or cork-screw-shaped gram-negative rods.

. Move by bending and rotating body movements.

. Spirochete consist of protoplasmic cylinder bounded by a cell wall and outer membrane. There is an axial filament or endoflagella between the cell wall and outer membrane.

Spirochetes of medical importance:

. Treponema

. Borellia

. Leptospira

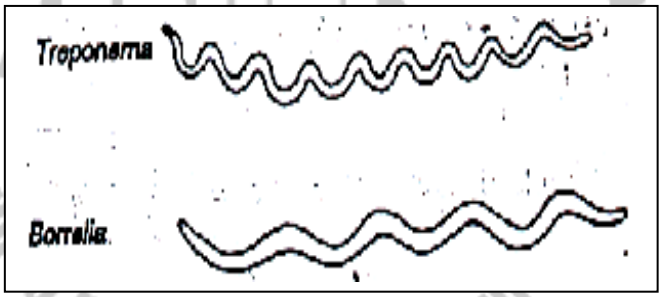


Fig. 3.6. Spirochetes

2.6.1. GENUS: TREPONEMA

Species of medical importance:

T. pallidum causes syphilis

T. pertenue causes yaws

T. carateum causes pinta

T. endemicus causes bejel

Treponema pallidum

Characteristics:

. Slender spiral, microaerophilic gram-negative rods.

. Not cultured in artificial media, in fertilized eggs and tissue culture, but the saprophytic Reiter strain grows in anaerobic culture

. Actively motile, rotating steadily around their endoflagella

. Remain viable in the blood or plasma store at 4 ^{0}c at least for 24 hrs (transmitted via blood transfusion)

Antigenic structure:

. Membrane proteins

. Outer sheath proteins

. Endoflagellar core proteins

. Cardiolipin

. Hyaluronidase

Pathogenesis and Clinical features:

Natural infection with T. pallidum is limited to the human host

Incubation period is 3-4 weeks.

Route of transmission is sexual contact.

A. Acquired syphilis

It has four stages.

1. Primary stage: Hard chancre: Clean-based, non-tender, indurated genital ulcer with inguinal lymphadenopathy.
1. Secondary stage: Manifests with generalized maculopapular rash condylomata lata and white patches

in the mouth.There may be syphilitic meningitis, nephritis, periostitis, hepatitis and retinitis.

Primary and secondary syphilis are rich in spirochete from the site of the lesion and patients are highly infectious.

2. Latent stage: Patients are symptom-free but relapse ca occur.

   Giagnosis is by serological test.

   Early latent stage: Relapse of symptoms and signs occur, and patients are infectious. It occurs with in 2 years of developing primary syphilis.

   Late latent stage: There is no relapse of symptoms and signs.

   Patients are not infectious. It occurs after 2 years of developing primary syphilis.

3. Tertiary stage: Manifesting with gumma(granulomatous lesion) in bone, skin and liver; meningovascular syphilis, syphilitic paresis, tabes dorsalis, syphilitic aortitis and aortic aneurysm.

Out come of acquired syphilis in untreated cases

1. One third of cases seems spontaneously cured during primary and secondary syphilis but no clear evidence
2. One third of cases become positive for serological tests of syphilis.

3. One third of cases develop tertiary syphilis.

B. Congenital syphilis

Route of transmission: Mother-to-child during gestation.

Out come: Abortion

Fetal death

Still birth

Early neonatal death

Organ damage: Congenital syphilis triad

. Interstitial keratitis

. Hutchison's teeth

. Deafness

Laboratory diagnosis:

Specimen: Tissue from skin lesion

1. Dark field microscopy

.Motile spirochetes in dark field illumination are observed.

2. Immunofluorescence stain

Procedure:

. Put tissue fluid on a glass slide.

. Fix and stain with fluorescein-labeled anti-treponeme serum.

. Observe fluorescent spirochetes in Immuno-fluorescence microscopy.

3. Serological tests for syphilis (STS)

Specimen: Serum

a. Non-treponemal antigen tests

Antigen- Cardiolipin from beef heart

1. Flocculation test –VDRL , RPR

. Positive after 2-3 wks of untreated syphilitic infection

. Positive result revert to negative with in 6-18 months of effective therapy of syphilitic infection

Principle: Antigen and antibody (Reagin) reaction results in clumping after aggitation

. It can give quantitative results, and valuable in establishing a diagnosis and in evaluating effect of treatment

2. Complement fixation test: Wasserman test; Kolmer test

Principle: Reagin-containing sera (mixture of IgM and IgA) fix complement in the presence of "cardiolipin-cholestrol-lecithin complex" antigen.

NB: Flocculation and Complement fixation tests are valuable in establishing diagnosis and in evaluation of chemotherapy effectiveness.

False-positive results in both tests can occur since the tests are non-specific.

b. Treponemal antibody tests

. Fluorescent treponemal antibody-absorption test (FTA-abs)

. Treponema pallidum- particle agglutination test (TP-PA)

. Treponema pallidum immmobilization test (TPI)

FTA-Abs test

Principle: The test employ indirect immunofluorescence, i.e combination of killed T.pallidum + patient's serum + Labelled antihuman gammaglobulin

. The first to bwcome positive in early syphilis, and remains posisive for several months after effective therapy

TP-PA test

Procedure:

. Sensitize T.pallidum antigens with gelatin particles

. Add diluted serum containing antibody to the sensitized gelatin particle in a microdilution tray

. Positive result when agglutination occurs

Treatment: Penicillin

Tetracycline

Erythromycin

Control measures: Treatment of cases and screen contacts

Practice safe sex with condoms

Health education

2.6.2. GENUS: BORELLIA

Borellia recurrentis: Causative agent of epidemic relapsing fever

Borellia duttoni: Causative agent of endemic relapsing fever

General characteristics:

. Highly flexible irregular spiral organism, and move by rottion and twisting

. Cultured in complex serum-rich artificial media and embryonated eggs.

. Famous in antigenic variation.

. Stain readily with bacterioigic dyes and blood stains

Pathogenesis and Clinical Features:

Disease	Reservoir	Vector
Epidemic RF	Human	Pediculus humanus (body louse)
Endemic RF	Rodents	Ornithodorus moubata (Ticks)

In epidemic RF, infection due to B.recurrentis occurs when abraded skin of the host is cantaminated with coelomic fluid of the lice which has been crushed on

In endemic RF, infection due to B. dutonni occurs by bite or by crushing the tick on the abraded skin, and occasionally by contact with the blood or tissue of infected rodents

Incubation period: 3-10 days

Clinical features of both types of relapsing fever is almost similar but epidemic relapsing fever is more severe and associated with high mortality rate,

Manifestation: Sudden onset of fever, headache, malaise for 3-5 days followed by an non-febrile period of one week. 3-5 relapses can occur with diminishing severity.

Conditions favoring disease transmission

. Overcrowding/ Poverty/ Famine/drought for epidemic RF

. High prevalence of tick in the locality for endemic RF

Laboratory diagnosis:

Specimen: Blood

Smear: Giemsa's stain / wright's stain

Seen as large, loosely coiled spirochetes

Culture: Cultured in serum-rich complex medium

Animal inoculation: Intraperitoneal inoculation rat with spirochete-containing blood, and examine the rat tail blood for spirochetes after 2-4 days

Treatment: Penicillin

Tetracycline

Control measures:

For epidemic RF

Delousing with insecticides

Improve personal and family hygiene

For endemic RF

Avoidance of exposure to ticks

GENUS LEPTOSPIRA

L. interrogans

More than 200 serovars

General characteristics:

. Tightly coiled, thin, flexible spiraled spirochetes forming one polared hooked ends

. Grow best in semisolid (Fletcher's or Stuart's) media under aerobic condition at 28-30 ^{O}c

. Can survive for weeks in alkaline PH water

. Fatty acid oxidation is major source of energy

Antigenic structure:

Lipopolysaccharide: Determine the specificity of human immune response to the organism and serologic classification of leptospirae

Pathogenesis and clinical features:

Essentially zoonotic infection and humans are accidental host

Source of infection is contaminated foood and water with leprospia spp.

IP=1-2 weeks

Leptospirosis is characterized by biphasic illness initially presenting with fever, prostration, jaundice,hemorrhage and nephritis followed by aesptic meningitis

Lab. Diagnosis

Specimen: Blood, CSF, urine, tissue, serum

Smears: Dark field examination

Fluorescein-conjugated antibodies staining

Immunohistochemical staining

Giemsa staining

Culture: Cultured in fletcher's semisolid media

Growth in the media is slow, requiring incubation for at least 8 wks

Animal inoculation: Intraperitoneal inoculation of young hamsters with spirochetal contaminated fresh plasma or urine

Demonstration of spirochetes after few days in peritoneal cavity

Serology: EIA/ Agglutination tets

High titers of aggglutinating antibodies after 5-8 wks of leptospiral infection

Treatment:

Doxycycline

Ampicillin/Amoxicillin

Prevention and control:

. Preventing exposure to potentially contaminated water

. Reducing contamination by rodent control

. Chemoprophylaxis: Using doxycycline during heavy exposure

2.7. GENUS: RICKETTSIAE

Characteristics:

.Obligate intracellular pleomorphic gram-negative coccobacilli occurring in single, pairs, short rods and filaments.

. Poorly stained in gram reaction.

. The organism stains red in macchiavello's stain.

. The organism stains blue in giemsa's stain.

. Grow in yolk sac of embryonated eggs, cell culture and laboratory animals.

. Destroyed by heat, drying and bactericidal chemicals.

Antigenic structure:

Group-specific antigens

Species-specific antigens

Clinical Features: Clinical illness is due to the invasion and multiplication of rickettsiae in the endothelial cells of small blood vessels. It manifests with fever, headache, malaise, skin rash and enlargement of liver and spleen.

The genus rickettsiae has three main groups based on their antigenic structure.

These are: Typhus group

Scrub typhus group

Spotted fever group

Table 2.8. Hosts and vectors of the medically important rickettsiae

Organism	Disease	Hosts	Vectors
1. Typhus group			
R. prowazeckii	Louse-borne typhus	Man	Body louse
R. typhi	Murine typhus	Rat	Rat flea
2. Scrub typhus			
R. tsutsugamushi	scrub typhus	Rodents	Mite
3. Spotted fever group			
R. conorii	African tick typhus	rodents, dogs	tick

R. rickettsi	Rocky mountain Spotted fever	Rodents, dogs	Tick
R. akari	Rickettsial pox	Mice	Mite

Species of medical importance in our country

R. prowazeckii

R. typhi

Rickettisia prowazeckii

. It causes epidemic or louse-borne typhus and the milder recrudescence form,

Brill-Zinser disease.

Clinical Features: It is transmitted by self-inoculation of the organism by scratching after bite by infected louse(Pediculous humanus corporis and pediculous humanus capitis). The illness manifests with sudden onset of fever, headache, malaise, prostration and skin rash.Epidemics of the disease are associated with overcrowding, cold weather, lack of washing facilities and fuel, famine and war.

Brill-Zinser disease (BZD) is recrudescence of infection in persons who have had classical typhus in the past.

Early IgG antibodies response rather than IgM antibodies and milder course of the disease is characteristic od BZD due to development of partial immunity

Rickettsia typhi

. It causes endemic or flea-borne typhus.

Clinical Features: It is transmitted to man when bitten by an infected rat-flea.

The disease is milder than louse-borne typhus and occurs in those individuals living or working in highly rat-infested area.

Laboratory diagnosis:

Specimen: Serum for serological tests

The serological tests to diagnose typhus are:

1. Indirect fluorescent antibody test
2. Complement fixation test
3. Weil –felix reaction: Some of the antigen of Proteus strain (OX-19, OX-2,OX-K) agglutinates withsera from patients with rickettsial diseases.

Treatment:

Tetracycline

Chloramphenicol

2.8. GENUS: MYCOPLASMA

Characteristics:

. Part of normal flora of human genital tract or oral cavity of healthy adults

. Formerly named as pleuropneumonia-like organism (PPLO).

. The smallest living micro-organism capable of free living in nature self-replicating on laboratory media

. Highly pleomorphic due to absence of rigid cell wall, instead bounded by a triple-layered “unit membrane”

. Completely resistant to penicillin and cephalosporin.

. Can reproduce in complex cell-free media.

. Have an affinity to mammalian cell membrane

. 14 species of mycoplama is identified in humansand classification of species is based on biochemicalo reaction and serological tests

Antigenic structure

Glycolipids (CF antigens)

Proteins (ELISA antigens)

Mycoplasma species of medical importance

Mycoplasma pneumoniae

Mycoplasma hominis

Ureaplasma urealyticum

Mycoplasma pneumoniae

Clinical features:

Route of transmission: Infected respiratory secretion

Infection is initiated after adherence of bacterial polar tip adhesin protein to respiratory epithelial cells

IP=1-3 wks

It is a major cause of pneumonia in young age groups (5-20yrs.)

Extra pulmonary manifestations:

Hemolytic anemia

Skin rashes/lesions

Meningoencephalitis

Myelitis

Neuritis

Myopericarditis

Arthritis

Laboratory diagnosis:

Specimen: Sputum

Culture: Cultured in semisolid media-enriched with yeast extract and serum, incubated aerobically for 7-12 days

Identification: Observe for “fried-egg” colonies embedded into the surface of the medium or inhibition of growth around discs impregnated with specific antisera.

Serology: Complement fixation test

Indirect Immunofluorescent test

Haemagglutination inhibition test

NB: Cold hemagglutinins titier ≥ 1:64 suggests M.pneumoniae infection

Treatment: Tetracycline

Erythromycin

NB: The above antibiotics produc eclinical improvement but do not eradicate the organism

Mycoplamas are resistant to penicillin, cephalosporins and vancomycin

Mycoplasma hominis and Ureaplasma urealyticum

. Found as a normal flora in the lower genital tract.

. Mycoplasma hominis causes genital infection and post-partum sepsis.

. Ureaplasma urealyticum causes non-gonococcal urethritis.

. Treatment is the same as M. pneumoniae.

2.9. GENUS: CHLAMYDIA

Characteristics:

. Obligate intracellular gram-negative bacteria.

. Reproduce by binary fission.

. Posses both DNA and RNA.

. Have cell wall and ribosomes.

. Sensitive to anti-microbial agents.

. Have enzyme systems and make their own proteins, lipids, nucleic acids and vitamins.

Three species of medical importance

C. tracomatis

C. pneumoniae

C. psittacii

NB: Chlamydia tachomatis is the main species of clinical importance in developing countries.

Developmental cycle of chlamydia

The infectious environmental stable particle, named as elementary body is ingested by a host cell. The elementary body is reorganized into reticulate body in the host cell which is specifically adapted for intracellular growth. The reticulate body grows and divides many times to form inclusions in the host cell cytoplasm.

With in 24-48 hours of developmental cycle, the reticulate bodies rearrange them selves into infective elementary bodies and released after host cell rupture.

Antigenic structure: Group-specific antigen

Species-specific antigen

Chlamydia trachomatis

. Stained with giamsa's and iodine stain.

. Appearance in giemsa's stain

Elementary body -------- Purple

Reticulate body---------- Blue

Host cell cytoplasm----- Blue

. Appearance in iodine stain

Brown inclusions in host cell cytoplasm because of glycogen matrix surrounding the particle.

. There are 15 serotypes of C. trachomatis.

C. trachomatis serotype A, B, C causes trachoma.

C. trachomatis serotype D-K causes genital infection.

C. trachomatis serotype L_1-L_3 causes lymphogranuloma venereum(LGV).

C. trachomatis serotype A, B, C causes trachoma.

Incubation period is 3-10 days

Route of transmission is through indirect contact like eye-to-eye by infected fingers or sharing towels.

It manifests as a chronic keratoconjunctivitis producing scarring and deformity of the eyelids, corneal vascularization and opacities which may lead to blindness.

It is associated with a low standard of living and poor personal hygiene.

Laboratory diagnosis:

Specimen: Conjunctival scraping from upper tarsal conjunctivae.

Smear: Giemsa's stain during early disease stage.

Culture: Mac coy cells or embryonated eggs

Serology: Immunofluorescent tests

Treatment:

Erythromycin

Tetracycline

Control measures:

. Improving hygienic standard.

. Treatment of cases with antibiotics.

. surgical correction of eyelid deformities.

C.trachomatis serotype D-K causes

1. Genital infection.

. Male ---------- non-gonococcal urethritis

Epididymitis

. Females------ Urethritis
Cervicitis
Pelvic inflamatory diseases

If complicated in females, it causes infertility and ectopic pregnancy.

2. Inclusion conjunctivitis resembling trachoma.

Transmission is by self-inoculation of the eye with infected genital secretion.

3. Neonatal inclusion conjunctivitis and neonatal pneumonia

Transmission is during passage through the infected birth canal.

Laboratorydiagnosis:

Specimen: Endocervical scraping

Culture: mac coy cells

Serology: Enzyme immunoassay for group-specific antigen.

Direct fluorescent antibody test for species- specific antigen.

C. trachomatis serotype L_1-L_3 causes lymphogranuloma venereum (LGV).

It is a sexually transmitted disease (STD) which is characterized by suppurative ingunal adenitis.

Complication: . . Elephantiasis of penis, scrotum or vulva due to lymphatic obstruction.

. Sinus formation at site of lesion.

Treatment

Tetracycline

Erythromycin

Chlamydia pneumoniae

Humans are the only known host

Produces sulfonamide-reisistant, round, dense, glycogen negative inclusions

Only one serovar has been demonstrated

Route of transmission: person –to-person transmission via Air borne

Clinical features:

Most infection are asymptomatic to mildly symptomatic

Symptomatic cases present with

- Chlamydial pneumonia
- Pharyngitis
- Sinusitis
- Otitis media

Laboratory dianosis:

Culture: Grows better in HL and Hep-2 cells incubated at 35-37 0c for 3 days

Intracellular inclusions are detected by fluoresccein staining with a genus and species specific antibodies or fluorescein conjugated C.pneumonia specific monoclonal antibodies

Serology: Micro immunofluorescence test

Most sensitive method for the diagnosis of C. pneumoniae infection

Single IgM titer of ≥ 1:16

Single IgG titer of ≥ 1:512

Four fold rise in either the IgM or IgG titers

Treatment:
- Tetracycline/doxycycline
- Macrolids: Erythromycin/Azithromycin
- Fluoroquinolones

Review Questions

- Describe laboratory methods of differentiation of staphylococcus species
- List the kinds of laboratory tests used to differentiate the different
- streptococcus species
- Mention the criteria used to identify N. gonorrrha and N.meningitidis
- Describe methods of collecting skin smears and staining of mycobacterium leprae
- Mention the diagnostic tests for syphilis at different stages of the disease

CHAPTER FOUR

Learning Objective

At the end of the session, the student should be able:

- To identify body tract defense mechanisms
- To identify the main pathogenic bacteria responsible to cause body tract

  Infection
- To list the specimen of choice in body tract infection
- To choose the appropriate culture media, biochemical and Serological tests to identify the pathogenic bacteria in body tract infection

4.1. HOST-PARASITE RELATIONSHIP

Definition: It is a beneficial or harmful relationship between the host and the parasite.

On the basis of their life habits, microorganism is classified as saprophytes or parasites.

Saprophytes : Mode of life of free-living organisms which obtain their nourishment from soil and water. Saprophytes in general do not require a living host.

Parasitism: An Adaptation to life on or in the bodies of higher organisms. The association may take one of the three forms:

1. Symbiosis: The ability to live in the tissues of the host with mutual benefit.
2. Commensalism: The ability to live on the external or internal surface of the body with out causing disease.

   "*Eating at the same table"*

3. Pathogenicity: The ability of an organism to cause disease.

The outcome of the host- parasite relationship depends on a balance between the virulence of the parasite and the resistance of the host.

Aggressive mechanisms of the parasite

1. Adherence factors

   . Pili: Hair-like appendages extending from the bacterial cell surface.

2. Invasiveness of micro-organism

   A high degree of bacterial invasiveness is usually associated with severe infection.

3. Bacterial toxins

   These are of two types.

   a. Exotoxins

   b. Endotoxins

Table 4.1 Characteristics of bacterial toxins

Character	**Exotoxin**	**Endotoxin**
. Composition	Protein	ipopolysaccharide
. Action	Specific	on-specific
. Antigenicity	Strong	Weak
. Effect of heat	Labile	Stable
. Produced by	Gm+ve&Gm-ve Bacteria	m-ve bacteria only.
. Converted to toxoid	Yes	No
. Mode of release from bacteria	Excreted by	released on bacterial death

	living cell	(Integral part of cell wall)

4. Enzymes

.Tissue degrading enzymes

. Collagenase: Degrade collagen, which is major protein of fibrous connective tissue.

. Hyaluronidase: (Early spreading factor) hydrolyzes hyaluronidic acid, which is the ground substance of connective tissue.

. Lecithinase: Splits lecithin of cell membrane into phosphorylcholine and glycerides

. Staphylokinase/Streptokinase (fibrinolysin)

5. Anti-phagocytic factors

. Protein A of Staphylococcus aureus

. M protein of Streptococcus pyogenes

6. Intracellular pathogenicity

E.g. Mycobacterium tuberculosis

Brucella spps.

Defense mechanisms of the host

1. Non-specific defense mechanisms

a. Skin

. Many layered impermeable barrier to invasion of the tissues by microorganisms from the environment.

.Infection is frequent when this barrier is breached.

E.g. Wounds, Burns

b. Mucus membrane: A single layer of epithelium and less protective than skin.

c. Lysozyme: An enzyme which lyses the mucopeptide (peptidoglycan) of the Gram-positive bacteria.

d. Normal flora: Prevents establishment of pathogenic bacteria.

Flushing of tears, urine and respiratory secretion

e. Tears: Keep the eye surface sterile due to lysozyme and flushing.

f. Respiratory secretion: Traps bacteria and constantly moves them upward propelled by cilia on the cells of the epithelium.

g. Urine: Voiding helps to flush out bacteria that have gained entry to the bladder.

h. p^H of body tract

Low p^H in stomach due to hydrochloric acid secretion kills ingested bacteria.

Low p^H in vagina due to lactic acid conferred by lactobacilli spp. prevents entry of pathogenic bacteria.

i. Phagocytosis: The process by which microorganisms are ingested and destrrroyed by phagocytic cells.

There are two types of phagocytic cells.

1. Neutrophil polymorphonuclear leukocytes (The polymorphs)
   . Produced and mature I bone marrow.
   . Short lived cells; circulate in the blood stream for six hours.
   . Act as an early defense against infection and are the “pus cells” seen in the exudate from acute infection.

. Perform only one phagocytic event.

2. Macrophages

. Produced in the bone marrow and found in blood stream as monocyte and in tissue as fixed macrophage.

. Long-lived cells

. Can perform many phagocytic events.

Major events in phagocytosis

1. Chemotaxis: Attraction of the phagocytic cell to the site of the organism.
2. Attachment: Adherence of the organism to the membrane of the phagocytic cell.
3. Ingestion of the micro-organism by pseudopods of the phagocytic cells.
4. Formation of phagosome and phagolysosome.
   Phagosome: The engulfed bacterium by a phagocyte.
   Phagolysosome: Fusion ofphagosome and lysozyme (bag of hydrolytic and proteolytic enzymes found in phagocytic cells).
5. Intracellular killing of microorganism.
6. Exocytosis (removal) of degraded and killed bacteria.

j. Complement system: Cascade of reactions mediated by complement components.

Complement components are a family of proteins present in serum.

Major functions of complement system

1. Liberation of complement fragments that attract phagocytic cells.
2. Promotes and enhances phagocytosis.
3. Induces inflammatory reaction

k. Others

1. Nutrition: Malnutrition predisposes to infection.
2. Age: The very old and the very young are particularly liable to infection.
3. Sex: May be attributes to hormonal influence.
4. Impairment of the host immune response
   . radiotherapy
   . Immunosuppressive drugs including steroids
   . Malignancy
   . HIV
5. Race
6. Climate
7. Occupation

2. Specific defense mechanisms

There are two main mechanisms by which the host mounts a specific immune response against bacterial infection. These are:

1. The humoral(antibody) response
2. The cell mediated response

The humoral response

Antibodies are proteins produced by B-lymphocytes in response to antigens (foreign substance which induces and binds with antibody).

Functions of antibodies

1. Neutralization of toxin
2. Promotion of phagocytosis
3. Bacterial Lysis

The cell mediated response

It is important in killing of intracellular pathogenic bacteria.

T-lymphocytes are population of lymphocytes conferring cell mediated immunity due to release of hormone-like mediators (lymphokines).

Functions of lymphokines

1. Inhibition of macrophage migration: Localizes macrophage to the site of infection.
2. Chemotactic attraction of lymphocytes, macrophages and polymorphs to the site of infection.
3. Mitogenic activity: Stimulation of unsensitized lymphocytes to divide.

4.2 Normal microbial flora

It denotes the population of micro-organisms that inhabit the skin and mucus membrane of healthy normal person.

There are two groups of normal flora. These are:

1. Resident normal flora
2. Transient normal flora

Resident normal floras are relatively fixed microorganisms regularly inhabiting the skin and mucus membrane of the normal host.

Transient normal floras are non-pathogenic or potentially pathogenic microorganisms that inhabit the skin and mucus membrane for a short period of time like hours, days and weeks.

Roles of (Resident) normal flora

1. Prevent colonization by pathogenic micro-organisms and possible disease through “bacterial interference”.
   a. Competition for nutrition with pathogenic bacteria
   b. Competition for binding sites with pathogenic bacteria.
   c. Mutual inhibition by their toxic metabolites.
   d. Mutual inhibition by bacteriocins.
2. Synthesis of vitamin K in the gastrointestinal tract
3. Aid in the absorption of nutrients in the small intestine.

NB: Normal flora can cause disease when the defense mechanisms of the body is breached or when the micro-organism is placed in the abnormal body site.

Normal flora of the skin

The skin is rich in resident bacterial flora, estimated at 10^4 microbes per square inch.

. Staphylococcus epidermidis

. Propionibacterium acne

. Peptostreptococci and peptococci

. Diphtheroids

. Alpha-hemolytic streptococci and non-hemolytic streptococci

Normal flora of the mouth and nasopharynx and upper respiratory tract

The upper respiratory tract is heavily colonized by normal flora but the lower respiratory tract is sterile.

Mouth

. Viridans streptococci
. Non-pathogenic Neisseria spp.
. Diphtheroids
.Anaerobes like Prevotella spp., Fusobacterium spp. and Capnophaga spp.
. Commensal Neisseria
. Spirochetes
. Actinimyces

Nasopharynx and upper respiratory tract

. Staphylococcus epidermidis
. Diphtheroids
. Alph-hemolytic streptococci
. Commensal Neisseria
. Pneumococci
. Haemophilus spp.

Normal flora of the gastrointestinal tract

The normal flora of the stomach, duodenum, jejunum and upper ileum is scanty but the large intestine is very heavily colonized with bacteria.

. Coliforms except salmonella spp., shigella spp., vibrio spp., yersinia spp. and campylobacter spp.

. Enterococci

. Anaerobes like bacteroides, bifidobacteria, anaerobic lactobacilli, clostridia and peptostreptococci

Feces contain enormous number of bacteria, which constitute upto one third of the fecal weight.

Normal flora of the genitourinary tract

For anatomical reasons the female genital tract is much more heavily colonized than that of the male.

a. Female
   . Vulva
      . Staphylococcus epidermidis
      . Diphtheroids
      . Coliforms
      . yeasts

   . Vagina
      . lactobacilli
      . Bacteroids
      . Diphrheroids
      . Group B beta-hemolytic streptococci
      . Mycoplasma spp.
      . Yeasts

b. Male and female distal urethra
      . Staphylococcus epidermidis
      . Diphtheroids

. Alpha-hemolytic and non-hemolytic streptococci

. Coliforms

Normal flora of the eye

. Diphtheroids (Corynebacterium xerosis)

. Staphylococcus epidermidis

. Commensal Neisseria

. Non-hemolytic streptococci

Normal flora of the external auditary meatus

It is an extension of skin normal flora and often profusely colonized.

. Staphylococcus epidermidis

. Diphtheroids

. Alpha-hemolytic and non-hemolyic streptococci

4.3. INFECTION OF SKIN AND WOUND

A. Infection of skin

Defense mechanisms of skin

. The layers of skin

. Enzymes in the skin

. Fatty acids in the skin

1. Superficial skin infection

a. Folliculitis

. Infection of one hair follicle by S. aureus

. Common in children.

b. Furuncle (Boil)

. Infection of many hair follicle by S.aureus

. It can be single or multiple.

. It may extend to cause cellulitis.

c. Curbuncle

. Infection of skin with hair follicle by S.aureus

. It may extend to cause cellulitis.

2. Deeper skin infection

a. Impetigo

. Vesicular skin lesion with honey-colored crusts which is caused by

S.aureus and S. pyogenes.

. It is common in young children and spread by contact.

b. Erysipelas

. Rapidly spreading cellulitis on the face and limbs.

. It is caused by S. pyogenes.

c. Cellulitis

. Acute inflammation of subcutaneous tissue.

. Furuncle and curbuncle may complicate to cause cellulitis.

. It is caused by S. aureus, S. pyogenes, Gram-negative rods, and anaerobes.

d. Skin ulcers

.A break in the continuity of skin is named as skin ulcer.

. It is caused by S. aureus, S.pyogenes, B.anthracis, M.ulcerans and C. diphtheriae.

B. Infection of wound

a. Soil contaminated wound
   . It occurs after car accident and war.
   . It is caused by gram-negative rods.
b. Gas gangrene
   . Extensive tissue destruction with necrosis of muscle, foul smelling discharge and gas under the skin.
   . It is mainly caused by C. perfringens.
c. Burns
   . Infection of burn is by P. aeruginosa, S. aureus, S. pyogenes and gram-negative rods.
d. Surgical wounds
   . Types
      . Clean wound
      . Clean contaminated wound
      . Contaminated wound
      . Dirty wound

Laboratory diagnosis:

Specimen: Swab from lesion, ulcer and discharge.

Smear: Gram staining from primary specimen or culture.

Culture: Blood agar medium and Mac Conkey agar medium

Biochemical and sensitivity testing for microbe identification.

Treatment: Based on sensitivity testing.

4.4. Infection of Respiratory Tract

Respiratory defense mechanisms

1. Mucociliary activity
2. Cough reflex
3. Secretory Ig A
4. Alveolar macrophages
5. Normal microbial flora

Infection of middle ear and sinuses

1. **Acute infection**
    a. acute otitis media
    b. acute sinusitis

Acute infections of middle ear and sinuses are often due to secondary bacterial invasion following a viral infection of respiratory tract.

a. Acute otitis media

Causative agent: H.influenzae
S.pneumoniae
M. catarrhalis

Source: Endogenous; normal flora of the oropharynx

Clinical features: fever, headache, earache, ear discharge red tympanic membrane, pus discharging ear

Lab. diagnosis:

Specimen: Ear discharge (pus)

Procedures: Gram staining, culture, biochemical testing, serological testing, sensitivity testing

Treatment: Amoxicillin/ampicillin
Co-trimoxazole

b**. Acute sinusitis**

Causative agent: H.influenzae
S.pneumoniae
S.pyogenes

Source: Endogenous: normal flora of the nasopharynx

Clinical features: Discomfort over the frontal or maxillary sinuses
Pain and tenderness of sinuses with purulent nasal discharge.

Lab. Diagnosis:

Specimen: Lavage/drainage of sinuses

Procedure: Gram staining, culture, biochemical testing, serological testing and sensitivity testing

Treatment: Amoxicillin/ampicillin
Co-trimoxazole

2. **Chronic infection**

a. Chronic suppurative otitis media

Long standing ear disease characterized by periods of exacerbation with profuse ear discharge and pain; and remission with relatively dry ear.

Risk factors: History of acute or chronic otitis media
Parental history of otitis media
Crowding

Causative agent: P. aeruginosa
S. epidermidis
Viridans streptococci

S. pneumoniae

Laboratory diagnosis:

Specimen: Swabs of pus from the ear

Procedure: Gram staining, culture, biochemical and serological test for microbe identification.

Treatment: Little role of oral antibiotic agents in the treatment of chronic suppurative otitis media.

Local and systemic antibiotics are given to treat the exacerbated symptoms.

Regular ear wicking is important for chronic suppurative otitis media.

b. Chronic sinusitis

Painful sinusesand head ache are prominent symptoms; often associated with mucoid or purulent nasal discharge and nasal obstruction.

Causal organisms are same as those implicated in acute sinusitis.

Laboratory diagnosis:

Specimen: Saline washings from the affected sinus

Procedure: Gram staining, culture, biochemical and serological test for microbe identification.

Treatment: Antibiotics often give disappointing results. If antibiotic is given, it should be guided by sensitivity pattern or “best-guess” basis.

Bronchitis

1. Acute bronchitis

It is an acute inflammation of the tracheobronchial tree generally self-limited and with eventual complete healing and return of function.

Etiology: Viruses: The commonest causative agents

Bacteria: M. pneumoniae

A. pneumoniae

B. pertussis

Predisposing factors:. Chronic bronchopulmonary diseases

. Environmental irritants like indoor air pollution and tobacco smoking

Clinical features: Symptoms of upper respiratory infection proceed acute infectious bronchitis.

Initially dry cough followed by productive cough with mucoid or mucopurulent expectoration, low grade fever and substernal chest pain. Frank purulent sputum suggests super imposed bacterial infection..

Laboratory diagnosis:

Specimen: Sputum

Procedure: Gram staining, culture, biochemical and serological test for microbe identification.

Treatment: Antibiotics are indicated when:

.There is concomitant chronic obstructive pulmonary diseases.

. Purulent sputum is present

. High grade fever persists and the patient is more than mildly ill.

Drug of choice: Tetracycline

Cotrimoxazole

2. Chronic bronchitis

It is defined as chronic productive cough for at least three months in each of two successive years.

Causative factors: Cigarette smoking

Air pollution

Exposure to noxious stimuli

Clinical features:

Chronic productive cough with mucoid expectoration, low grade fever, weakness, and occasional chest pain.

It is characterized by remission and exacerbation of symptoms; the commonly exacerbating condition is superimposed bacterial infection.

Bacteria that exacerbate chronic bronchitis are:

Streptococcus pneumoniae

Haempphilus influenzae

Mycoplasma pneumoniae

Branhamella catarrhalis

Laboratory diagnosis:

Specimen: Sputum

Procedure: Gram staining, culture, biochemical and serological test for microbe identification.

Treatment:

. To stop cigarette smoking

. Avoid exposure to noxious stimuli

. Treat the exacerbation with antibiotics like amoxicillin, cotrimoxazole.

Pneumonia

It is infection of the lung parenchyma.

Causative agents: S. pneumoniae
S. aureus
H. influenzae
M. pneumoniae
Viruses

Route of entry of microbes to the lung

. Aspiration of oral and gastric secretion

. Haematogenous spread from distant foci

.Direct inoculation and local spread from surrounding tissue

. Inhalation

NB: Aspiration is the major route of infection.

Clinical features: Sudden onset of fever, chills, sweating, and productive cough of purulent or blood streaking sputum and pleuritic chest pain.

Complications:

. Pleural effusion

. Lung abscess

. Septicemia

Laboratory diagnosis:

Specimen: Lower respiratory secretion

NB: Lower respiratory secretion is indicated by greater than 25 neutrophils and less than 10 squamous epithelial cells per high power field.

Procedure: Gram staining, culture, biochemical and serological test for microbe identification.

Treatment: Based on sensitivity pattern of offending micro-organism.

4.5. Infection of gastrointestinal tract

Host defense mechanisms to prevent GIT infection

- .Gastric acidity
- . Mucosal epithelium
- . Peristalsis
- . Normal flora
- . Luminal phagocytic cells
- . Copro antibodies

Microbial factors contributing to GIT infection

- . Invasiveness
- . Toxin production
- . Colonization factors

DIARRHEA

Definition: Diarrhea is a passage of three or more watery or loose stool in a day.

It is a common cause of death in under five children in developing countries.

Aetiology:

a. Microbial causative agents
   - . Viral
     - . Rota virus

. Norwalk virus

. Adeno virus

. HIV

. Bacteria

. Salmonella spp.

. Shigella spp.

. E.coli

. Campylobacter spp.

. Protozoa

. Giardia lamblia

. Entamoeba histolytica

. Cryptosporidium parvum

. Isospora belli

. Fungus

. Candida albicans

b. Non-microbial causative agents

. Congenital

. Congenital megacolon

. Enzyme deficiency

. Lactase deficiency

. Iatrogenic

. Antibiotic related enterocolitis

. Endocrine

. Diabetes mellitus, Hyperthyroidism

. Psychogenic

. Stress related gastroenteritis

Types of diarrhea

1. Based on duration of diarrhea
   . Acute diarrhea: Diarrhea occurring in less one week time.
   . Persistent diarrhea: Diarrhea occurring in two weeks time.
   . Chronic diarrhea: Diarrhea occurring in more than two weeks time.
2. Based on nature of diarrhea
   . Secretary diarrhea: Watery diarrhea
   . Invasive diarrhea: Bloody mucoid diarrhea
   .Osmotic diarrhea Eg. Non-absorbed purgative intake (Magnesium sulfate)
   . Motility diarrhea Eg. Hyperthyroidism

NB: Only secretary and invasive diarrhea are caused by microbes.

Examples of secretary diarrhea

1. V.cholerae and E.T.E.Coli cause diarrhea by production of an enterotoxin which activates the adenylcyclase of enterocytes to increase the cyclic AMP (CAMP) level, leading into increased secretion and decreased absorption of fluids and electrolytes in the intestinal wall.
2. Giardia lamblia causes diarrhea by physical coverage of the normal absorptive surface.
3. Viruses cause diarrhea by disrupting the normal absorptive surface.

Examples of invasive diarrhea

Shigella spp. Campylobacter spp.

Salmonella spp. E.hystolytica

E.I.E.coli

All of them cause extensive intestinal mucosal damage by invasion of the intestinal wall

Leading into bloody mucoid diarrhea.

Complication of diarrhea

1. Local
   . Intestinal wall perforation
   . Intestinal vessel erosion leading to bleeding
2. Systemic (Generalized)
   . Dehydration leading to hypovolemic shock
   . Septicaemia leading to septic shock

. Malnutrition due to loss of essential nutrients and electrolytes.

Laboratory diagnosis:

Specimen: Stool, rectal swab

Wet mount preparation: Pus cells, red blood cells, parasites and ova.

Culture: Mac Conkey agar, SS agar, TCBS agar, Campylo agar

Observe for colony appearance, pigmentation, hemolysis.

Biochemical reaction and serology: For species identification.

Treatment:

. Fluid and electrolyte replacement

. Provision of antibiotics

. Continue feeding

FOOD POISONING

It is an out break related to common meal.

Characteristics:

. Sharing same meal.

. Multiple cases

. Same clinical features

. Developing same clinical features more or less at the same time.

There are two types of food poisoning

1. Food intoxication: Illness is caused by ingestion of food with preformed toxin.
2. Food infection: Illness is caused by ingestion of food with micro-organism.

   Incubation period and severity of disease in food infection is determined by inoculum of micro-organism ingested.

Table 4.2. Examples of food intoxication

Organism	Incubation period	Clinical findings	Related food item
Bacillus cereus	1-6 hours	Vomiting, cramp	Rice, Pasta dishes
Staphylococcus aureus	2-4 hours	Vomiting	Meat, Salads
Clostridium botulinum	12-72 hours	Flaccid paralysis	Meat, Vegetables

Table 4.3. Examples of food infection

Organism	Incubation period	Clinical findings	Related food item
Bacillus cereus	6-24 hours	Watery diarrhea	Meat, Vegetables
Salmonella spp.	6-12 hours	Dysentry	Meat, Vegetables
Shigella spp.	12-48 hours	Dysentry	Variable

Laboratory diagnosis:

Specimen: Left over food, vomits, stool

Culture the specimen for microbial isolation.

Serological technique for toxin isolation.

Treatment: Depends on the causative agent.

4.6. INFECTION OF URINARY TRACT

Definition: The presence of significant numbers of micro-organisms any where in the urinary tract.

NB: Kidney and bladder are sterile at normal state.

Host defense mechanisms

. Micturition (Urine flow)

. Surface bladder mucosa

. Normal microbial flora

. Secretary IgA

. Menstrual flow only in females.

Aetiology:

The commonest causative agents of UTI are gram-negative rods.

These are: Escherichia coli
Pseudomonas aeruginosa
Klebsiella pneumoniae
Proteus spp.
Enterobacter aerogens

Other important causative agents: Enterococci
Staphylococcus saprophyticus

Routes of infection

1. Ascending route (passage of bacteria from urethra to bladder and kidney.)
2. Haematogenous route (source of infection is blood)

NB: Ascending route is the commonest route infection of the urinary tract.

Contributing factors for urinary tract infection

1. Age: Very young and very old individuals are more at risk for UTI.
2. sex: UTI is more common in females than males because females have short and wide urethra.

3. Instrumentation: Indwelling catheters and cystoscopic procedures
3. Neurogenic bladder dysfunction: Diabetes mellitus, Spinal injury
4. Obstruction: Congenital anomalies in youngs and prostatic adenoma, stricture and calculi in olds.
5. Underlying diseases: Diabetes mellitus, sickle cell disease
6. Vesico-ureteral reflex: Associated with recurrent acute pyelonephritis.

Clinical features:

1. Lower urinary tract infection: Infection of urethra and bladder which manifests with frequency of micturition, pain during micturition, blood-stained or cloudy urine, supra pubic tenderness.

Usually no fever.

2. Upper urinary tract infection: Infection of the kidney parenchyma and pylus which manifests with the lower UTI symptoms and signs, flank pain, fever and chills, nausea and vomiting, and flank tenderness.

Laboratory diagnosis

Specimen: Clean catched midstream urine

Catheterized urine

Suprapubic aspiration

Direct microscopic examination: WBCs, RBCs, Epithelial cells.

The presence of more than five WBCs and abundant epithelial cells per HPF supports infection of urinary tract.

Gram stain: The presence of one bacterium in Uncentrifuged gram stained urine confirms Urinary tract infection.

Culture: Blood agar medium, Mac Conkey agar medium

Interpretation of culture results

1. $\geq 10^5$cfu/ml of urine is significant to indicate UTI.
2. $<10^3$cfu/ml of urine indicates contamination of specimen.
3. 10^3-10^5cfu/ml of urine is uncertain.

   NB: 10^3-10^5cfu/ml of urine in symptomatic patient or suprapubic or catheterized specimen indicates UTI.

4.7. INFECTION OF GENITAL TRACT

Table 4.4. Genital tract infection manifests as either genital discharge or genital ulceration with or without inguinal lymphadenitis.

Causative agents	Diseases
1. Bacterial	
Neisseria gonorrhea	Gonorrhea
Chlamydia trachomatis	Urethritis, cervicitis, LGV
Ureaplasma urealyticum	Urethritis
Gardenella vaginalis	Vaginitis
Treponema pallidum	Syphilis
Haemophilus ducreyii	Chancroid
2. Viral	
Herpes simplex virus-2	Herpes genitalis
HIV	AIDS
Human papilloma virus	Genital warts, Cervical dysplasia
Molluscum contagiosum virus	GenitalMolluscum contagiosum
3. Protozoal	
Trichomonas vaginalis	Vaginitis, Urethritis
4. Fungal	
Candida albicans	Vulvovaginitis, Balanitis
5. Ectoparasites	
Phthirus pubis	Pubic hair louse infestation
Sarcoptes scabei	Scabies

Urethral and vaginal discharge

1. Urethritis

It manifests with urethral discharge, pain during urination and frequency of urination.

Types

a. Gonococcal urethritis

Causative agent: N. gonorrhea

Incubation period is 2-7 days.

It accounts for 1/3 of urethritis cases.

Clinical findings: Yellowish purulent discharge and dysuria.

b. Non-gonococcal urethritis

Causative agents: C. trachomatis ----50%

U. urealyticum ----30%

M. hominis

T. vaginalis

Incubation period is 2-3 weeks.

Clinical findings: White mucoid discharge

Laboratory diagnosis:

Specimen: Urethral discharge or swab (Before urination or antibiotics)

Wet mount: T. vaginalis

Gram stain: Gram-negative intracellular diplococci

Culture: Modified thayer-martin medium

Biochemical and serology: Species identification

2. Cervicitis / Vaginitis

It manifests with vaginal discharge.

Causative agents:

N. gonorrhea --- Mucopurulent vaginal discharge.

T. vaginalis---Profuse foaming purulent, sometimes greenish vaginal discharge.

Non-specific vaginitis --- Yellowish homogenous vaginal discharge.

It is caused by anaerobes and G. vaginalis

C. albicans --- whitish curd-like vaginal discharge with itching and erythema of vulva.

Laboratory diagnosis:

Specimen: Vaginal discharge

Wet mount: . Clue cells i.e., distorted vaginal epithelial cells coated heavily with gram-negative coccobacilli which are diagnostic of infection with G. vaginalis.

. Yeast cells or psedohyphae which indicates infection with C. albicans.

. Motile T. vaginalis

Gram stain, culture, biochemical and serology for species identification.

Treatment:

N. gonorrhea ----- Ceftriaxone, Ciprofloxacin

B. trachomatis, U. urelyticum and M. homonis ---- Tetracycline

T. vaginalis, G. vaginalis and anaerobes ---- Metronidazole

C. albicans ------ Nystatin, Myconazole, Clotrimazole

Table 4.5. Genital ulceration with or with out regional lymphadenopathy

Disease	Lesion	Inguinal lymphadenopathy
Syphilis	Non tender, indurated clean based genital ulcer	Non tender, non-suppurating rubbery bilateral lymphadenitis
Chancroid	Tender, non-indurated shallow ragged ulcer	Suppurative, tender ymphadenitis
LGV	Occasionally small non tender genital papules is seen	Painless suppurative lymphadenitis with multiple draining sinuses
Genital herpes	Tender, multiple grouped vesicular lesions coalesce to form an ulcer	Non-suppurative, tender, bilateral lymphadenitis

Laboratory diagnosis

Specimen: Scrapings from base of lesion

Serous exudate squeezed from lesion

Pus from lymph node

Direct microscopic examination: Motile spirochetes under dark-field microscopy.

Giemsa's stain: Intranuclear inclusions for genital herpes.

Gram stain: Short gram-negative rods for H. ducreyii.

Culture: Chocolate agar medium for H. ducreyii

Serology: VDRL, FTA-ABs test

Treatment:

Syphilis -------- Penicillin

Chancroid -------- Erythromycin, Cotrimoxazole

Lymphogranuloma venereum ----- Tetracycline

Genital herpes ------- acyclovir

4.8. INFECTION OF BLOOD

Bacteremia is defined as mere presence of bacteria in blood. It occurs in normal person after invasive medical procedures. The organism which causes bacteremia are less virulent and usually cleared from blood with in 3o min.

Complications of septicaemia

. Infective endocarditis

. Endotoxic shock

. Acute pyogenic meningitis

. Septic arthritis

Infective endocarditis

1. Acute endocarditis

. It occurs in normal and abnormal heart valves and tissues with fulminant course resulting in death in six weeks time if not treated.

Etiologic agent: S. aureus

S. pyogenes

S. pneumoniae

N. gonorrhea

2. Sub acute endocarditis

. It occurs in acquired or congenitally damaged heart valves with less fulminant course resulting in death after six weeks if not treated.

.Etiologic agent: Viridans streptococci

Enterococci

S.epidermidis

S.aureus

S.epidermidis

Laboratory diagnosis:

Specimen: Blood

. Amount needed is 2ml for a child and 10 ml for an adult to give 1:10 dilution of the specimen.

Culture: Blood culture

. Blood culture bottle should have 18 ml and 90 ml of broth for a child and an adult respectively.

NB: Numbers of culture required are 3-6 with in 24 hrs.

Time of incubation of blood culture is 7 days and subculture is done in first, third and seventh day of incubation.

If the appearance of blood culture is changed to cloudy, it indicates bacterial growth.

Interpretation of results

- Positive bacterial growth in three of blood culture broth ----- Definitive diagnosis
- Positive bacterial growth in two of blood culture broth----- Probable diagnosis
- Positive bacterial growth in one of blood culture broth----- Contamination

Common contaminants of blood culture

S. epidermidis

Corynebacterium spp.

Bacillus spp.

Treatment: Antibiotic should be started after collection of the specimen.

Medication should be given intravenously based on 'best guess basis'.

4.9. INFECTION OF CENTRAL NERVOUS SYSTEM

Defense mechanisms of CNS

- . Cranium (Bony covering of the brain)
- . Blood brain barrier

Route of infection

- . Haematogenous
- . Extension from middle ear, mastoids and sinuses
- . Congenital defects
  - . Meningomyelocele
  - . Spina bifida
- . Trauma
  - . Skull fracture
- . Intraneural pathway

Common infection of central nervous system

1. Meningitis

Definition: Meningitis is an inflammation of leptomeninges.

Types:

- . Bacterial (Pyogenic) Meningitis
- . Viral meningitis
- . Fungal meningitis

Bacterial meningitis

a. Acute form

Causative agents

. In new born --------- Escherichia coli

Streptococcus agalactiae

. In children ---------- Haemophilus influenzae

Streptococcus pneumoniae

Neisseria meningitidis

. In adults ------ Streptococcus pneumoniae

Neisseria meningitidis

Haemophilus influenzae

Clinical features:

. Sudden onset of head ache, fever, malaise, vomiting associated with neckand back stiffness, behavioral changes like irritability and drowsiness, convulsions and coma.

b. Chronic form

. Mycobacterium tuberculosis

. Partially treated acute pyogenic meningitis

It manifests with unexplained head ache of weeks to months duration associated with fever, weakness, neck and back stiffness, and behavioral changes.

Viral meningitis

The clinical features of viral meningitis are milder than bacterial meningitis.

Viruses causing viral meningitis are mumps virus, measles virus, coxsackie A and B virus, Entero viruses and echo virus.

Fungal meningitis

Eg. Cryptococcal meningitis

The clinical features of fungal meningitis is similar to that of chronic bacterial meningitis.

Complication of meningitis

. Cranial nerve damage
. Convulsions
. Brain abscess
. Obstructive hydrocephalus due to blockage of CSF drainage system.
. Subdural effusion of sterile or infected fluid.

Laboratory diagnosis:

Specimen: Cerebrospinal fluid by lumbar puncture

NB: Amount required for a child is 1-2 ml and for an adult is 2-4 ml.

The specimen should be processed with in one hour of collection.

Smears: Gram staining and Ziehl-Neelson staining of centrifuged specimen

Culture: Blood agar medium

Mac Conkey agar medium

Lowenstein-jensen medium

Sabouraud's agar medium

Biochemical, serology and sensitivity testing for species differentiation.

Normal CSF findings:

. Color ---------- Crystal clear, colorless

. Cells ---------0-5 per ml, all lymphocytes

. Microbiology---------- Sterile

. Protein---------- 15-40 mg per 100 ml.

. Glucose --------- 45-72 mg per 100 ml.

Table 4.6. Cerebrospinal fluid findings in meningitis

Meningitis type	Appearance	Cells per ml	Protein	Glucose
Acute pyogenic meningitis	Turbid	500-20,000 Predominantly Polymorphs	Markedly raised	Reduced or absent
Viral meningitis	Clear or Turbid	10-500 Predominantly Lymphocytes	Normalor slightly raised	Normal
Tuberculous Meningitis	Clear or Turbid	10-500 Predominantly Lymphocytes	Moderately raised	Usually reduced
Cryptococcal Meningitis	Clear or Turbid	10-500 Predominantly Lymphocytes	Raised	Reduced

Treatment: Antibiotic should be started based on 'best guess basis' after the CSF specimen was taken.

All medications for treating meningitis is given intravenously.

Encephalitis: It is an inflammation of brain substance.

It is commonly caused by viruses.

Brain abscess: It is an abscess with in the brain parenchyma.

It is a complication of meningitis, endocarditis and head injury.

4.10. INFECTION OF BONE AND JOINTS

The two main diseases are:

. Osteomyelitis: infection of bone.

. Septic arthritis: Infection of joints

Osteomyelitis

. Acute osteomyelitis

. Chronic osteomyelitis

Acute osteomyelitis

Infection of bone which occurs commonly under ten years old.

Distal femur, proximal tibia and proximal humerus are the classical sites.

Causative agents: S. aureus (80% of cases)

H. influenzae (especially in young children)

S. pneumoniae

S. pyogenes

Coliforms and Group B streptococci in new born.

Clinical features: Fever, bone pain, local tenderness and swelling, limitation of movement

Laboratory diagnosis:

Specimen: Blood culture, Pus from bone by needle aspiration

Gram reaction, culture, biochemical tests and serology for microbe identification.

Treatment: Antibiotics alone are usually effective if started early and continued for several weeks.

Surgery is needed if there is pus accumulation and bone destruction.

Chronic osteomyelitis

It manifests with bone pain, bone destruction with formation of sequestra and discharging sinuses.

The most common causal organism is S. aureus; others include M. tuberculosis,

S. typhi and Brucella species.

Laboratory diagnosis: Same as acute osteomyelitis

Treatment:

. Antibiotics for several weeks.

. Surgery is usually necessary for pus drainage and sequestra removal.

Septic arthritis

It is usually seen as a complication of septicemia or an extension of osteomyelitis.

Causative agents: S. aureus
S. pneumoniae
H. influenzae

Neisseria gonorrhea

Neisseria meningitidis

M. tuberculosis

Clinical features: The onset is sudden with fever, swelling and redness over the joint and severe pain which limits movement of the affected joint.

Laboratory diagnosis:

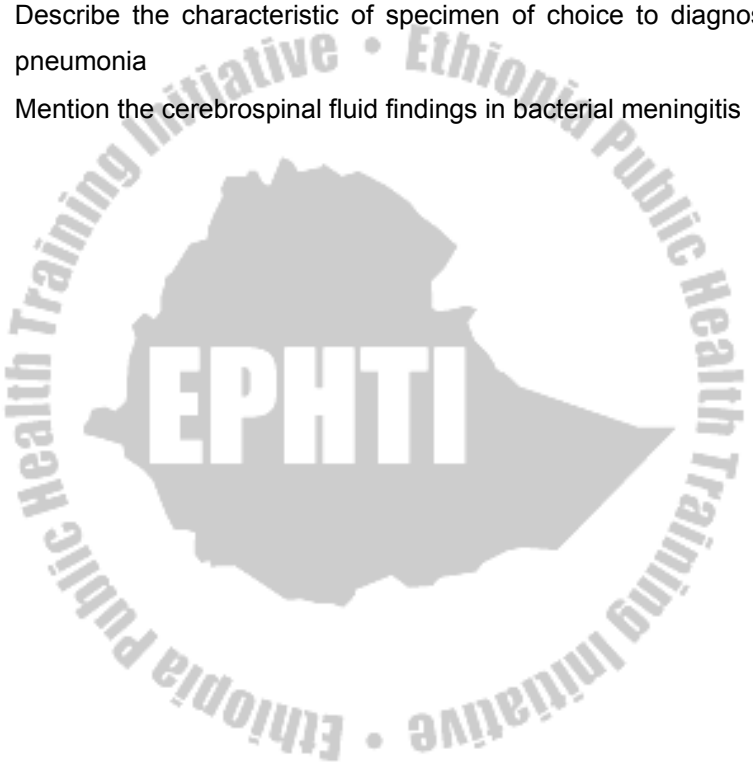
Specimen: Blood culture, joint aspirate

Gram reaction, culture, biochemical tests and serology for microbe identification.

Treatment: Antibiotic therapy based on “best-guess” basis, should be started as soon as diagnostic specimens have been taken.

Review Questions

- Discuss defense mechanisms of different body tracts
- Describe the interpretation of urine culture result
- Discuss the characteristics and types of food poisoning
- Describe the characteristic of specimen of choice to diagnose pneumonia
- Mention the cerebrospinal fluid findings in bacterial meningitis



CHAPTER FIVE

Learning objective:

At the end of the lesson, the student should be able to:

. Perform bacteriological analysis of water sample

Bacteriology of water

Good quality of water is odorless, colorless, tasteless and free from fecal pollution and harmful chemicals.

Human illness is caused by water supplies becoming contaminated from feces being passed or washed into rivers, streams, or being allowed to seep into wells.

Feces contain microorganisms like Escherichia coli, Streptococcus faecalis and Clostridium perfringenes, which contaminate safe water.

Determining whether a water supply is fecally polluted is to test for the presence of normal fecal organism.

Testing for normal fecal organisms as indicators of fecal pollution is a reliable way of determining whether water is bacteriologically safe to drink.

A single laboratory examination of any water does not justify the conclusion that the supply is safe for drinking so bacteriologic analysis of water should be performed at regular intervals.

Organisms used as indicators of fecal pollution are the coliform group particularly E. coli.

E. coli is a coliform organism, which is capable of fermenting lactose with the production of acid and gas at 37 ^{O}C and 44 ^{O}C in less than

48 hours, produces indole in tryptophan-containing peptone water and gives positive result in methyl red test.

When organisms of coliform group other than E.coli are found in a water sample,

Presence of S.faecalis confirms fecal pollution of water.

The presence of C. perfringenes in the absence of coliform group in water suggests fecal pollution of water at some remote date since cloostridial spores are capable of surviving in water for a long duration.

Collection of a water sample

Technique:

. Sample of water must be collected in sterile bottles.

. Hold the base of sterile bottle in one hand, remove the stopper and cover together with the other hand.

. Fill the bottle with sample water, replace the stopper and cover together.

Collecting a sample from tap:

. Remove any external fittings from the tap.

. Clean carefully the outside nozzle of the tap.

. Turn on the tap and allow the water to run for1 min.

. Sterilize the tap using the flame by igniting a piece of cotton wool soaked in alcohol holding with a pair of tongs.

. Allow the tap to cool by running the water for few seconds.

. Fill the sample bottle from a gentle flow of water, and replace the bottle cap.

Collecting a sample from an open well:

. Tie a sterile sample bottle on to a weighted length of rope; attach ½ Kg weighing stone as a weight below the bottle.

. Remove the cap from the bottle septically and lower the bottle into the well to a depth of one meter.

. Raise the bottle out of the well when no more bubbles raise to the surface.

. Replace the bottle cap and label the bottle.

Transport of water sample

Water sample should be placed in an insulated cold box immediately after collection, and should be processed with in six hours of collection.

Frequency of sampling

Population served	**Sampling interval**
< 20,000	Four weeks
20,000-50,000	Two weeks
50,000-100,000	Four days

Multiple tube technique for counting fecal coliforms

A 100 ml water sample is distributed (five 10 ml amounts and one 50 ml amount) in bottles of sterile selective culture broth containing lactose and an indicator.

After incubation, count the number of bottles in which lactose fermentation with acid and gas production has occurred.

Estimate the most probable number of coliforms in the 100 ml water by referring to probability tables.

Required:

. Bottles of sterile Mac Conkey broth (Purple)

. Water samples

	No. of bottles	ml of broth	strength of broth
Treated water sample	1	50	double
	5	10	double
Untreated water sample	1	50	double
	5	10	double
	5	5	single

NB: Double strength broth refers to broth made up using twice the normal amount of broth powder. Single strength broth contains the normal amount of broth powder.

Bottle broth contains an inverted durham tube for gas collection.

Method:

- Label the bottles.
- Mix thoroughly the sample of water by inverting the bottle several times.
- Remove the bottle cap and cover, flame the mouth of the bottle, and inoculate the bottles of sterile broth as follows:
- Add 50 ml of water to the bottle containing 50 ml of broth for treated and untreated water samples.
- Add 10 ml of water to each of five bottles containing 10 ml of broth for treated and untreated water samples.
- Add 1 ml of water into each of five bottles containing 5ml of broth in untreated water sample.

- Mix the contents of each bottle.
- Incubate the inoculated broth in a water bath at 44 Oc for 24 hours with the botles loosely capped.
- After incubation, examine and count each bottle which has produced acid and gas.
- Determine the most probable number (MPN) of fecal coliform bacteria in the 100 ml of treated and 105 ml of untreated water sample with referring to probablity tables.

NB: Acid production is shown by a change in color of the
Mac Conkey broth from purple to yellow and gas production by the collection of a bubble in the durham tube.

Interpretation of results

1. For treated water sample

. The E.coli count should never exceed 5 per 100ml.

E. coli count	**Comment**
0	Excellent
$\leq$ 5	Acceptable
> 5	Unacceptable

2. For untreated water sample

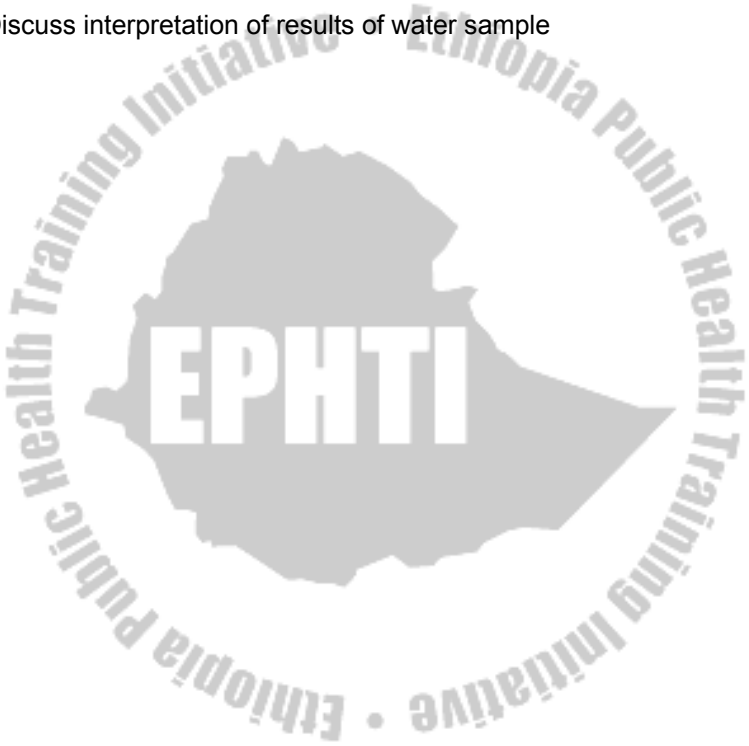
E. coli count	**Category**	**Comment**
0	A	Excellent
1-10	B	Acceptable

10-50	C	Unacceptable
>50	D	Grossly polluted

Review question

. Describe method of bacteriological analysis of water sample

. Discuss interpretation of results of water sample



CHAPTER SIX

Learning objective:

At the end of the lesson, the student should be able to:

- Know the simple, common and applicable methods
- Analysis difference food samples
- Know reasons for microbials food analysis

  →to meet certain set standards

  →to estimate the shelf – life of the product

  →to determine the quality of the food

  →to determine the safety of the food for public health

Food bacteriology

- Food are essential substance for life.
- They are also important vehicles for micro-organisms that cause food borne infections and intoxications.
- Food is essentially complex, and predicting whether, or how fast micro organisms will grow in any food is difficult.most food contains suffiecient nutrients to support microbial growth.
- To provide consumers with claimable qualities of food, these substance must be controlled, regulated and inspected from the microbiological view – point. Such activities are usually carried out by FAO, WHO, UNICEF, Federal state and other agencies.

- In Ethiopia the quality and safety of food have been controlled, regulated and inspected by the National Research Institute of Health and regional laboratories.

Sources of food contamination

Food may acquire their micro-organism from various sources and the following are the important sources.

1. Animals
   - Animals could be a source of contamination of food.
   - The surface of animals, the respiratory tract, the gastrointestinal tract, hides, Loofs and waste products of animals are important sources of contamination.
2. Plants
   - Food may get their microbial contamination from green plants.
   - The natural flora of growing plants includes pseudomonas, alcaligenes, bacissus, Micrococcus, coliform etc.
3. Sewage
   - Gastrointestinal pathogens, coliforms, Enterococci of untreated domestic sewage could be source of contamination of raw plant foods.
   - Sewage can also contaminate natural waters and contribute micro-organisms to shellfish, fish and other sea foods.

4. Soil
    - Soil is a very rich environment in microbes and is a major source of contamination of food.
    - Bacillus, clostridium, enterobacter, Escherichia, Micrococcus, Alkaligens, Flavobacterium, Pseudomonas, proteus, Aerobacter, molds and yeast are kinds of organisms that contaminate food from soil.
5. Air and water:- are also important source of food contamination.-

Factors influencing microbial activity in food

1. Nutrient found in foods
    - Organisms obtain their energy for carrying their metabolic activity mainly from the food.
2. Hydrogen ion concentration (pH)
    - The optimum pH for many microorganisms is near the neutral point of pH 7. However molds and yeasts as a rule are acid tolerant. This is one of the reasons why fungi are usually associated with acid foods especially fruits.
    - Many bacteria are not acid tolerant, accordingly, several acid like acetic, benzoic, propionic acids are used to preserve foods.

- It has been found that pH of 4.5 or below is lethal to salmonellae and staphylococci.

3. Oxidation reduction potential (O – R)
   - Organisms can be classified into aerobic and anaerobic based on their oxygen requirements. There fore, the reducing and oxidizing power of the food influences the type of organism that growth on it.
   - Foods with high oxidation potential favours the growth of aerobes and faculitative anaerobic organisms.
   - Foods with low oxidation potential favours the growth of anaerobic and faculitative anaerobic organism.
4. Growth inhibitors: - These are chemicals such as sodium chloride (NaCl), Nitrate, Nitrite, Sulphur dioxide and hypochlorites that are added to foods to the growth of certain organisms.
5. Temperature

It was found that E.coli, for example, requires the following periods for a cell to divide at different temperatures.

- 60minutes at 20^0C
- 40 minutes at 25^0C
- 29 minutes at 29^0C
- 17 Minutes at 37^0C
- 19 minutes at 40^0C
- 32 minutes at 45^0C
- No growth at 50^0C

It is clear that rate of growth is optimal at 37^0C, but decreases when the temperature is lowered below, or raised above 37^0C.

6. Water acitivity (a_W)
    - No microbial activity can occur unless water is available.
    - Water activity is the ratio between the vapour pressure of the food and that of pure water.
    - Pure water has a_W = 1.0 and a relative humidity of 100%. This means that a_W x 100 indicates the equilibrium relative humidity, which the particular food would produce if enclosed in a sealed container at a constant temperature.
    - Organisms have their own characterstic optimal a_W and range of a_W for their growth.

The lowest a_W values permitting growth of spoilage organisms are

- Normal bacteria	0.91	- xalophilic	0.77
- Normal yeast	0.88	- xalophilic fungi	0.65
-Normal moulds	0.80	- osmophilic yeasts	0.60

- As can be seen from the above values dry foods are most likely spoiled by moulds.
- Syrups and honey favours the growth of osmophilic yeasts.
- Moist neutral foods like milk and meats are spoiled by bacteria.

Microorganism of importance in food bacteriology

Micro-organisms of interest in food bacteriology are:

(i) Indicator organism(s):-

- An indicator organism or group of organisms is/are one whose numbers in a product reflects the success or failure of good manufacturing practices.
- Indicator organisms are bacterial groups (or species) whose presence in foods, above a certain numerical limits, is considered to indicate exposure of food to conditions that might introduce hazardous organisms and/or allow proliferation of pathogenic or toxinogenic species.
- They have value in assessing both the microbiological safety and quality of foods.
- Indicator organisms include coliforms and faecal streptococci.
- The best example of indicator organisms are the coliforms.
- Coliform group is defined to include all aerobic and faculitative anaerobic, gram negative, non-spor forming, rod shaped species which ferment lactose with the production of acid and gas within 48 hours at 35^0C
- Coliform are either part of the normal flora of intestinal tract of man and animals or found in environments such as soil and plants.
- Coliform that are commonly found in the intestinal tracts of man and animals (faeces) are called faecal

coliform and those coliforms that are normal inhabitants of soil and plants are called non faecal coliform.

- The recovery of coliform from food or water above a certain numerical limits implies that diseases causing organism may be present and the food is potentially dangerous for human consumption.

Differentiation of faecal from non faecal coliform:-

- In many laboratories differentiation of faecal coliforms from non faecal coliform is considered of limited value in determining the suitability of water or food for human consumption, as contamination with either type renders water or food potentially dangerous and unsafe from a sanitary stand point. However, differentiation may be advantageous under some conditions where the identity of specific members of the group present may indicate the source of pollution.
- Coliform such as a aerogenes and E. freundui only occessionally found in the intertinal contents of man and animans (6%). They are normally found in soil. Therefore, there presence in food or water is of doubtful sanitary significance.
- On the other hand E.coli, rarely found in the soil but constitute the predominant organisms in the intestinal contents of man and animals. Therefore, isolation E.coli in food may strongly suggest the

food has been polluted with feaces than isolation of A.aerogenes or E.freundii.

Methods used for differentiation of faecal from non faecal coliforms indole, methyl red, voges proskauer, citrate test (IMVic). IMvic test is one of the test used to differentiate faecal coliforms (E.coli) from non faecal coliforms (A.aerogenes and E.freundsi) as shown in the table below.

Organisms	**Indole**	**Methyl red**	**V – P**	**Sodium citrate**
E.coli type I	+	+	-	-
E.coli type II	-	+	-	-
E. freundii I	-	+	-	+
E. freundii II	+	+	-	+
A. aerogens I	-	-	+	+
A. aerogens II	+	-	+	+

Elevated temperature test

- In elevated temperature tests faecal coliforms are defined to be bacterial species that grow and ferment lactose with the production of acid and gas at temperatures higher than the normal (44-44.5^{0}C).
- E.coli type I and II grow and ferment lactose at elevated temperature while A. aeerogenes and

E. freundii fall to grow and ferment lactose at elevated temperature.

ii) **Index organisms**: is one whose presence implies the possible occurance of a similar but pathogenic organism.E. coliis used an index organism and its presence indicates possible presence of pathogenic enteriobacteriacea eg.salmonellae species.

iii) Food poisoning organisms

- those which cause the disease by infection
- those which produce toxin in food
    - ✓ Those which cause infection must grow in food in large numbers and cause infection when consumed together with food. The most most common microorganisms includes salmonella tyhimurrium, entropathogenicE.coli,Vibrio parahaemolyticus etc.
    - ✓ Those which cause intoxication must grow in food large numbers and produce enough toxin and when consumed together with food cause intoxication.The most common microorganism in this group are clostridium botulinium,staphylococcus and toxigenic fungi eg. Aspergillus flavus.

iv) Infectious microorganisms

Organisms whose presence in sall numbers in food and when consumed can cause infection. In this case the food acts as a vector but not necessarly as a growth medium.organisms in this group are, Vibrio choleraeO1, salmonella typhi, shigella sonnei,Bacillus anthracis, HepatitisB virus etc.

The spoilage micro-organisms

. The spoilage microorganisms include bacteria, yeasts and modlds that cause undesirable changes of the appearance, odour, texture or taste of the food. They are commonly grouped according to their type of activity or according to theiri growth reguirements.

Psychrophilic microorganisms

. Are those organisms capable of growing relatively rapidly at commercial refrigeration temperatures with out reference to optimum temperature for growth.

. Species of Pseudomans, Achromobacter, flavobacterium and Alcahigenes are examples of Psychrophilic bacteria.

. Many psychrophilic bacteria when present in large numbers can cause a variety of off flavoirs as well as defects in foods

. The presence of large number of psychrophilic bacteria in refrigerated foods such as dairy products, meat, poultry and sea food may reflect growth of initial population during storage and /or massive contamination at some point prior to or during refrigerated storage.

Thermoduric microorganisms

. Thermoduric organisms are those organisms which will survive so significant measure of heat treatment

. The thermophilic organisms not only survive the heat treatment but also grow at the elevated temperature

. Thermoduric bacteria are important with regard to milk and milk products as they may survive pastourisation temperature

The genera Micrococcus, Streptococcus primary the entrococci, Lactobacillus, Bacillus and Clostridium are recognized as containing some species which will qualify as thermoduric.

The thermoduric count may be useful as a test of the care employed in utensil sanitation and as means of detecting sources of organisms responsible for high bacterial count in pasteurized.

Lipolytic Microorganisms

Are those organisms capable of hydrolytic and oxidative deterioration of fats, mostly cream, butter, marganine, etc

The genera Pseudomans, Achromobacter, and staphylococcus among other bacteria, Rhizopus, Geotrichum, Aspergillus and penicillium among the moulds and the yeast genera Candida, Rhodotorula, and Hansenula contain may lipolytic species.

Proteolytic microorganisms

Proteolytic microorganisms are those microorganisms capable of hydrolyzing proteins producing a variety of odour and flavour defects

Proteolytic species are common among the genera Bacillus, Clostridim , Pseudomoans , and proteus.

Acid proteolytic organisms are those organisms which carry out protein hydrolysis and acid fermentation as streptococcus faecalis var. liquefaciens

Halophilic microorganisms

Haliphilic microorganisms are those organisms which require certain minimal concentrations of salt (NACl) for growth.

Slight halophiles grow optimally in media containing 2.5%. Most of the slight halophilic bacteria originate from marine environments

Marine psychrophilic bacteria of the genera pseudomonas, Moraxella. Acinetobacter, and Flavobacterium contribute to the spoilage of marine fish and shelfish

Moderate halophiles grow optimally in media containing 5.20% salt and most of the moderately halophilic bacteria involved in the spoilage of salted foods are gram positive species of the Bacillaceae and Micrococcaceae

The extreme halophiles grow optimally in media containing 20-30% salt

The extreme hamophiles are principally species of the genera Halobacterium and Halococcus which produce bright red or pink pigments, grow very slowly.

They have been incriminated in spoilage of fish, and hides preserved in sea salts.

Halotolerant organisms

Are those organisms capable of growth in salt concentrations exceeding 5%.

Some halotolerant microorganisms are involved in food spoilage while some others such as staphylococcus aureus and cl.perfringens are pathogenic.

Osmophillic microorganisms

Are those organisms tha grow in concentrated food products

Osmophillic microorganisms most commonly encountered in food industry are yeasts

They can grow in highly concentrated sugar solutions

They are frequently the cause of honeny, chocolate, candy,jams etc.

Almost all of the the known osmophillic yeasts are species of saccharomyces species.

Pectinolytic microorganism

Are those microorganisms capable of degrading pectins foun in fruites and vegetables.the destruction of the pectin can cause subsequent loss of jelly power of fruites and softening of the stored fruits and vegetables.

The pectinolytic organisms includes species of Achrobacterium, Aeromonas ,Arthrobacter, Bacillus , Enterobacer etc.

It also includes many yeasts and moulds

Acid producing microorganisms

An important group of acid producing bacteria in the food industry is the lactic acid bacteria

This group is subdivided into the genera streptococcus, Leuconostoc, Pediococcus and Lactobacillus.

The homofermentative species produce lactic acid from the available sugar, while the hetrofermentative types produce inadition to lactic acid, mainly acic acid, ethanol and CO_2.

Many sporeforming species belonging to the genera Bacillus and Clostridium are also important acid producers

Some mould and yeasts produce citric acid, oxalic acid,etc.

Yeasts and moulds

Yeasts and moulds can be responsible for spoilage of many types of foods

They often manifests themselves in foods of low pH, low moisture, high salt or sugare content, etc.

They are resistant to heat freezing, antibiotics

Mesophillic spore forming aerobes

The mesophilic, aerobic spore forming bacteria are all strains Bacillus species that grow at 35^0c but not at 55^0c.

They cause spoilage in canned low acid (pH > 4.6) foods is usually of the sour type.

Inadequate heat processing is commonly responsible since spores of mesophillic bacteria are moderately resistant to moist heat.

Thermophillic anaerobes

These organisms are obligatory anaerobes and are strongly saccharolytic, producing and abundant gas from different sugars they non hydrogen sulphid producers

They are responsible spoilage of canned food products.

Microbiological Examination of Food

Sampling

It is important to not that samples of foods collected for microbiological analysis should reflect the microbiological condition at the time of collection. This implies that

- Sampling should be carried out aseptically
- Samples should be protected against extraneous contamination
- Moreover, samples must be held under conditions that permit neither die off nor multiplication of the original microflora present in the food.

Definintion of terms

A lot

- Is a quantity of food produced and handled under uniform condition. This means that food produced within a batch or in a continuous process a food produced within a limited period of time
- The number of field samples collected are usually five. But for the investigation of food for salmonella the number of field sample is ten

Filed sample:

- The amount of material actually used in the analysis of food for microorganisms. The sample unit is recommended to be 25 g for all types of food

Microbiological criteria

A microbiological criteria is a microbiological value (eg. Number of microorganism per g of food) or a range established by use of defined procedures and includes the following information.

- A statement of the microorganism of concern and/or the toxin
- The analytical methods of their detection and quantification
- A plan refining the number of field sample to be withdrawn and the size of the sample unit
- The microbiological limits considered appropriate to the food
- The proportion of the sample units that should conform to these limits.

MICROBIOLOGICAL EXAMINATION OF DIFFERENT FOOD

For example Egg and egg products '

Types of contaminating microorganisms

- The shell of eggs are either sterile or harbour very low numbers of microorganisms at the time of ovipositor.
- It gets its bacterial contamination after oviposition from nesting material, dirt, and faecal matter.
- The flora of the egg shell is dominated by Gram-positive cocci. Whereas the Gram-negative rods are present in low numbers
- They penetrate more easily through the egg shell membrane and multiply more readily than do the Gram-positive cocci.
- The number of bacteria in liquid egg product depend upon the bacteriological condition of shell eggs used for breaking, plant sanitation and conditions under which the liquid may be stored.
- The most common bacterial genera found in liquid eggs are members of pseudomonas, Alcaligenes, Proteus and

Escherichia. These organisms are the main cause of spoilage resulting in characteristic off odours and off colours.

- The major pathogen associated with eggs and egg products is Salmonella.

Microbiological examination

Eggs can be given as liquid egg frozen egg, dried egg

Methods of analysis

a) Enumeration of mesophilic aerobic bacteria
b) Enumeration of coliforms
c) Detection of salmonella

Sampling plan and microbiological limit

Mesophilic aerobic bacteria should not be recovered from any of the five sample units examined, when the test is carried out according to the method described, in a number exceeding 10^6 per g, nor in a number exceeding $5x10^4$ per g from three or more of the five sample units examined (n=5, c=2,m=$5x10^4$,M=10^6)

n = the number of sample units comprising the sample

m = the threshold value for the number of bacteria; the result is considered to be satisfactory if the number of bacteria in all sample units does not exceed this value

M=is the maximum value for the number of bacteria, the result is considered to be unsatisfactory if the number of bacteria in one or more sample units is equal to or greater than this value.

C=is the number of sample units where the bacterial count may be between m and M. the sample is considered to be acceptable if the

bacterial counts of the other sample unit are equal to or less than the value of m Coliform bacteria should not be recovered from any of five sample units examined, when the test is carried out according to the method described, in a number exceeding 10^3 per g, nor in a number exceeding 10 per g from three or more of the five sample units examined (n=5, c=2, m=10, M=10^3).

Salmonella organisms should not be recovered from any of ten sample units examined when the test is carried out according to the method described; (n=10, c=0 m M=0).

METHODS OF MICROBIOLOGICAL ANALYSIS OF FOOD AND WATER

1. ENUMERATION OF MESOPHILIC AEROBIC BACRERIA (Aerobic plate count)

Principle

- This method is based on the assumption that the microbial cells present in a sample mixed with an agar medium each form visible, separated colonies.
- This is obtained by mixing decimal dilutions of the food sample homogenatte or warer with the medium
- After incubation of the plates at 35 ^{O}C 72 hours the number of mesophilic aerobic bacteria per g of food sample is calculated from the number of colonies Obtained in selected petridishes at levels of dilutions giving a significant result.

However, it should be borne in mind that this method, as all other methods, has some limitations

- Microbial cells often occur as clumps, clusters, chains, or pairs in foods, and may not be well distributed irrespective of the mixing and dilution of the sample.
- Consequently, each colony that appears of the agar plate may arise from a single cell or from groups of cell and Moreover, some microorganisms may fail to grow and form visible colonies on the agar medium as a result of unfavourable conditions of temperature, oxygen or nutrition, or because the cells are weak.
- Hence the colony count may nor reflect the actual number of viable bacteria in the food.

Apparatus and Glassware

a) Petri dishes 90-100mm, glass or plastic
b) Pipettes 1,5 and 10ml, graduated (total-flow)
c) Water bath, $45 \pm 1^{0}C$
d) Colony counter
e) Incubator, $35 \pm 1^{o}_{C}$

Culture media and diluent

a) Buffered peptone water (BPW)
b) Plate count agar(PCA)

Procedure

Preparation of food homogenate

- Weigh 25 g of the mixed sample aseptically into a sterile blender jar or into a stomacher bag and add 225ml of BPW.

- Blend the food at a speed of 15000-20000rpm for not more that 2.5 min or mix in the stomacher for 20 sec.

Dilution

a) Mix the food homogenate by shaking and pipette 1.0ml into a tube containing 9ml of the of the BPW, mix with a fresh pipette, and
b) From the first dilution, transfer with the same pipette 1.0ml to 2nd dilution tube containing 9ml of the BPW, mix with a fresh pipette, and
c) Repeat using a 3rd, 4th tube or more until the required numbers of dilutions are made.
d) Shake all dilution carefully.

Pour plating

a) Pipette 1.0ml of the food homogenate and of each dilution of the homogenate into each of the appropriately marked duplicate dishes

b) Pour into each petri dish 15ml of the PCA (kept at 45 ±1^{0}c in water bath) within 15 minute of the time of original dilution.
c) Mix the sample dilution and agar medium thoroughly and uniformly; allow to solidify.

Incubation

Incubate the prepared dishes, inverted, at 35±1^{0}C for 72±3hours.

Counting the colonies

Following incubation, count all colonies on dishes containing 30 -300 colonies and record the results per dilution counted.

Calculation

a) When the dishes examined contain no colonies, the result is expressed as; less than 1x10^1 bacteria per g or ml.
b) When the dishes (dilution 1in 10) contain less than 30 colonies, the result is expressed as: less than 3X10^2 (30x10=3x10^2).
c) When the colonies are more than 30, count the colonies in both plates of a dilution and calculate the average, retaining only two significant digits and multiply by the inverse of the

corresponding dilution to obtain the number of bacteria per g or ml.

Example: dilution 1/100 dish 1: 175 colonies

Dish 2: 208 colonies

Calculation: 175+208=383/2=191→190=x100

Result: $1.9x10^4$ bacteria per g of food.

ENUMERATION OF COLIFORM BACTERIA

Standard multiple tube fermentation technique (determination of the most probable number, **MPN**)

Principle

The standard tests are

- Presumptive test
- Confirmed test and
- Completed test

Presumptive

- Graduated amount of food are transferred to series of fermentation tubes containing lactose broth or lauryl sulphate tryptose broth of proper strength. It is usual practice to inoculate to five fermentation tubes
- The tubes are incubated at 35 ± 0.5^0c and examined at the end of 24±2 hours. Tubes ssowed no gas productions at the end of 24±2 hours are reincubated and examined at the end of 48±3hrs.

- The formation of gas in any of the tubes within 48±3 hours, regardless of the amount, constitutes a positive presumptive test.
- The absence of gas formation within that period constitutes a negative test, and no further tests need be performed.

Confirmed test

- All fermentation tubes showing gas production in presumptive tests within 48 hours at 35^0C shall be utilized in the confirmed test
- Eosin methylene blue(E.M.B) agar, Endo agar or brilliant green lactose bile broth fermentation tubes may be used in the test
- A loop-full of culture from each positive fermentation tubes is streaked over the surface of E.M.B agar or Endo agar. Development of typical colonies (nucleated, with or without metallic sheen) or atypical colonies (opaque, nonucleated mucoid, pink) the confirmed test may be considered positive
- If no colonies develop with in the incubation period the confirmed test may considered negative.

Brilliant green lactose bile broth (BGLBB)

- A loop-full of culture from each positive presumptive tube is transferred to brilliant green lactose bile broth fermentation tubes and incubated at appropriate temperature and time
- Presence of gas in any amount in the inverted vial within the incubation period may be positive confirmed test

Completed test

- Transfer typical or atypical colonies from E.M.B or Endo agar to lactose fermentations tubes and nutrient agar slants and incubate at appropriate temperature for a period not to exceed 48hours
- If Brilliant green lactose bile broth is used in the confirmed test, an E.M.B or Endo agar plate is streaked from each fermentation tube showing gas and all plates should be incubated at appropriate temperature and period

The purpose of the completed test is to determine

- The colonies developing on E,M.B or Endo agar are again capable fermenting lactose with the formation of acid and gas .
- Organisms transferred to agar slants show the morphological and tinctrial picture of members are the coliform group
- The formation of gas in any amount in the fermentation tube and the demonstration gram negative, non spore forming rods on the agar slants shall constitute a positive completed for members of the coliform group
- The absence of or failure to show the presence of rods answering to the above description in gas-forming culture shall constitute a negative completed group.

Apparatus and Glassware

a) Test tubes (18mmx180mm)
b) Durham tubes (10mmx75mm)
c) Pipettes 1(total-flow)
d) Incubators, 35 ± 1^0C, 37 ± 1^0C
e) Water bath, 45.5 ± 0.05^0C

Culture media and reagent

a) Brilliant-Green lactose bile broth 2% (BGLB)
b) Buffered peptone water
c) Indole medium and reagent
d) Koser's citrate
e) Lauryl sulphate tryptose broth (LST)
f) Levine's eosin methylene blue agar(L-EMB)
g) Voges-proskauer (VP) medium

Procedure

Preparation of food homogenate

- Wigh 25 gm of the mixed sample aseptically into a sterile blender jar or into astomacher bag and add 225ml of buffered peptone water(BPW)
- Blend the food at a speed of 15000 – 20000 rpm for not more than 2.5 minute or mix the stomacher for 20 seconds

Dilution

a) Mix the food homogenate by shaking and pipette 1.0ml into a tube containing 9ml of the of the BPW, mix with a fresh pipette, and
b) From the first dilution, transfer with the same pipette 1.0ml to 2nd dilution tube containing 9ml of the BPW, mix with a fresh pipette, and
c) Repeat using a 3rd, 4th tube or more until the required numbers of dilutions are made.
d) Shake all dilution carefully.

Inoculation

a) Inoculate each of 3 tubes of LST broth (containing inverted Durham tubes) with 1.0ml of the food homogenate (1 in 10).
b) Carry out the same operation from the first (1 in 100) and the second (1 in 1000) dilution tubes, using a new sterile pipette for each dilution.

Incubation

Incubate the LST tubes at 37±1^{0}C for 24 and 48 hours

Reading of enrichment tubes (presumptive test)

Record tubes showing gas production after 24hours, and re-incubate negative tubes for further 24 hours, and then record tubes showing gas production.

Confirmed test for coliforms

a) Transfer a loopful from each gas-positive tube of LST to a separate tube of BGLB broth.
b) Incubate the BGLB tubes at 35± 10C for 48hours.
c) The formation of gas confirms the presence of coliform bacteria. Recored the number of positive

tubes that were confirmed positive for coliforms.

Calculation (MPN)

Note the MPN appropriate to the number of positive tubes from the following table for example:

3 in 1:10, 1 in 1:100 and 0 in 1:1000 the table shows that MPN=43 coliforms/g or ml

MPN index and 95% confidence limits when 3 tubes are used

Number of positive tubes			MPN per g or ml	95% confidence limits	
1:10	1:100	1:1000		Lower	Upper
0	0	0	<3		
0	0	1	3	<0.5	9
0	1	0	3	<0.5	13
1	0	0	4	<0.5	20
1	0	1	7	1	21
1	1	0	7	1	23
1	1	1	11	3	36
1	2	0	11	3	36
2	0	0	9	1	36
2	0	1	14	3	37
2	1	0	15	3	44
2	1	1	20	7	89
2	2	0	21	4	47
2	2	1	28	10	150
3	0	0	23	4	120

3	0	1	39	7	130
3	0	2	64	15	380
3	1	0	43	7	210
3	1	1	75	14	230
3	1	2	120	30	230
3	2	0	93	15	380
3	2	1	150	30	440
3	2	2	210	35	470
3	3	0	240	36	1300
3	3	1	460	71	2400
3	3	2	1100	150	4800
3	3	3	>2400		

Differentiation of non-faecal coliforms faecal coliforms

a) Elevated temperature test

- Simultaneously with the confirmatory procedure using brilliant green lactose broth, transfer should be made from all positive presumptive tubes to EC broth medium.
- The inoculated EC tubes are incubated at 45.5 ^{o}C for 24hours, and gas formation is recorded and the bacterial density is estimated from the tables of MPN.

b) Run IMViC Test

- Streak E.M.B agar or Endo agar from each positive tubes in a way to obtain discrete colonies and incubate for 18-24 hours at 35 ^{o}C.
- Perform indole, methyl red, v-p and citrate tests (IMViC).

Classification of Colifroms by IMViC test

Indole	MR	VP	Citrate	Type
+	+	-	-	Typical E.coli
-	+	-	-	Atypical E.coli
+	+	-	+	Typical intermediate
-	+	-	+	Atypical intermediate
-	-	+	+	Typical E.aerogenes
+	-	+	+	Atypical E.aerogenes

Procedure

Preparation of food homogenate

Prepare as described above

Dilution

Prepare as described above

Inoculation

Inoculate three 10 ml portions of 1:10 dilutions into 10 ml each of double strength GSTB, and then inoculate three 1ml portions of 1:10, 1:100, 1:1000 and 1:10000 dilutions into single strength GSTB.

Incubation

Incubate broth tubes overnight at 35^0C.

Confirmation

a) After incubation, streak a loopful of the culture from the three highest dilutions of GSTB showing growth onto TCBS agar plates.

b) Incubate the plates for 18 hours at 35^0C.

c) The colonies of V.parahaemolyticus on TCBS appear round, 2-3mm in diameter with green or blue centres. V.alginolyticus

colonies are larger and yellow. Coliforms, proteus and enterococci colonies are small and translucent.

Biochemical identification

a) TSI: Streak the slant and stab the butt and incubate overnight at 35^0C. V.parahaemolyticus produces alkaline slant and acid butt, no gas and no H_2S (typical shigella-like reaction).

b) Motility medium: inoculate 4 tubes by stabbing. Diffuse circular growth occurs after 24hours incubation at 35^0C.

c) Make a Gram stain from growth on TSA slant.

d) Halophilic nature: inoculate 4 tubes of STB containing 0, 6,8and 10% NaCI, incubate; V.parahaemolyticus will grow well in 6 and 8% NaCI but not in 0 and 10% concentrations.

e) MR-VP test

f) Indole test

g) Carbohydrate fermentation: Inoculate one tube each of glucose, lactose, sucrose, maltose, mannitol, etc. from TSA saint. After incubation check for acid production.

h) Glucose fermentation: stab 2 subes of HLGB medium, overlay one tube with

sterile paraffin oil and incubate for 2 day at 35 °c. yellow coloration of both tubes indicates fermentation, in the tube without oil only indicates oxidation. V.paraphaemolyticus is a glucose fermenter producing no gas.

i) Cytochrome oxidase test: Allow 2-3 drops of alphanaphthol solution to flow over a fresh slant of V.parahaemolyticus or over a colony on a plate, then follow this by an equal amount of phenylenediamine solution. The development of dark blue colour within 2 minute is positive

j) LDC

k) Growth at 42^0C: incubate an inoculated TSB at 42^0C in a water bath for 24 hours.

The characteristics features of V.parahaemolyticus are:

a) Gram-negative curved rods
b) Cytochrome oxidase positive
c) Glucose oxidation/fermaentation (O/F) positive, no gas
d) Colony on TCBS typical blue-green in colour
e) TSI, alkaline slant, acid butt, no gas, no H_2S
f) Positive growth at 42^0C

g) Positive growth in 8% but not in 10%NaCl
h) Positive LDC
i) Negative VP
j) Negative sucrose

Calculation

When the blue green colonies on TCBS are finally identified biochemicalcally as vibrio parahaemolyticus, refer to the original positive dilutions of GSTB and apply the 3 tube MPN table for final enumeration of the organism.

ENUMERATION OF BACILLUS CEREUS

Principle

This method is based on surface plating technique using a medium containing egg yolk on which the colonies of B. cereus are recognized by being surrounded by zones of turbidity.

Apparatus and Glassware

a. Petridishes
b. Pipettes, 1 ml
c. Incubators, 20^0C, 30^0C, 35^0C

Culture media and reagents

a. Buffered peptone water
b. KG agar
c. Nitrate broth
d. Nutrient agar
e. Nutrient gelatin
f. VP medium

g. Gram stains

Procedure

Preparation of food homogenate

Prepare as described as above

Dilution

Prepare as described above.

Inoculation

Pipette 0.25ml of homogenate and dilutions of the homogenate on the surface of previously dried KG agar plates and spread with a sterile bent glass rod.

Incubation

Incubate the plates at 30^0C for 20-24hours

Counting of the colonies (presumptive B. cereus)

Count the colonies surrounded by a halo of dense precipitate (lecithinase activity) and calculate the total number per gram of specimen by multiplying by 4 and by the dilution factor.

Confirmation

a) From typical colonies make smear and stain with Gram and examine microscopically.
b) At the same time transfer some of the typical colonies to nutrient agar slants, incubate at 30^0C for 24hours and from the growth inoculate the following:
    i. Gelatine tube: examine for liquefaction after 24 hours incubation
    ii. Nitrate broth tube: after 24 hours incubation at 35 ^{0}C test for the reduction nitrate to nitrite

iii. VP medium

c) Characteristics of B.cereus

Glatine liquefication----------	+
Nitrate reduction ----------	+
Egg yolk reaction ----------	+
VP – reaction ----------	–
Gram stain ----------	+

Calculation

When the zone- forming colonies are confirmed microscopically and biochemically, their count gives the confirmed B.cereus.

ANNEX

Preparation of media

Presently, a wide range of culture media are available commercially in the form of dehydrated media. These media are simply reconstituted by weighing the required quantities and by adding distilled water, as per the manufacturer's instructions.

The pH determination can be conveniently done with the use of Lovibond comparator with phenol red indicator disc.

- Take two clean test tubes and add 5 ml of the medium to each of the tubes. One serves as a blank while phenol red indicator is added to the other tube.
- Compare the colour of the medium with the phenol red indicator at the appropriate pH marking.
- Add N/10 NaOH or N/10 HCl, drop by drop till the colour of the medium matches the colour of the disc at the required pH reading.
- Calculate the volume of the NaOH or HCL of 1/10 strength for 5 ml of the medium to get the required pH.
- Based on the calculation, the volume of 1N NaOH or IN HCl required for the total volume of medium can be calculated and added.
- Check the pH of the medium once again before use.

The quantity of agar given in the formulae of media may have to be changed depending upon the quality of agar used. The concentration varies from batch to batch and should be such that will produce a sufficiently firm surface on solidification. This can be tested by streaking with inoculating wire.

In some laboratories media are prepared by individual measurement of ingredients and then mixing the same. Hence the method of preparation is given likewise:

Nutrient broth

Meat extract	10.0 gm
Peptone	10.0 gm
Sodium chloride	5.0 gm
Distilled water	1000 ml

Mix the ingredients and dissolve them by heating in a steamer. When cool, adjust the pH to 7.5-7.6.

Nutrient agar

To the ingredients as in nutrient broth, add 15 gm agar per litre. Dissolve the agar in nutrient broth and sterilize by autoclaving at 121°C for 15 minutes. Prepare plates and slopes as required.

Glucose broth

Nutrientbroth 900ml
Glucose (10% solution) 100 ml

- Dissolve 9 gm glucose in distilled water and sterilize by tyndallisation.
- Add l00 ml of the glucose solution to 900 ml of sterile nutrient broth.
- Dispense 60 ml each in 100 ml pre-sterilized culture bottles.
- Sterilize by open steaming at l00°C for one hour.

Blood agar

Nutrient agar 100 ml
Sheep blood (defibrinated) 10 ml

- Melt the sterile nutrient agar by steaming, cool to 45°C.
- Add required amount of sheep blood aseptically with constant shaking.
- Mix the blood with molten nutrient agar thoroughly but gently, avoiding froth formation.
- Immediately pour into petri dishes or test tubes and allow to set.

Chocolate agar

The ingredients are essentially the same as in blood agar.

- Melt the sterile nutrient agar by steaming and cool to about 75°C.
- Add blood to the molten nutrient agar and allow to remain at 75°C after gently mixing till it is chocolate brown in colour.
- Pour in petri dishes or test tubes for slopes as desired.

XLD agar

Xylose	3.5gm
1lysine	5.0gm
Lactose	7.5gm
Sucrose	7.5gm
Sodiumchloride	5.0gm
Yeastextract	3.0gm
Sodiumdesoxycholate	2.5gm
Sodiumthiosulphate	6.8gm
Ferricammoniumcitrate	0.8gm
Phenolred	0.08gm
Agaragar	5.0gm
Water	1000 ml

Weigh the ingredients into a flask and add distilled water. Mix the contents well and steam it for 15 minutes (do not autoclave). Cool to 56°C and pour in plates.

Buffered glycerol saline

Glycerol	300	ml
Sodiumchloride	4.2	gm
Disodiumhydrogenphosphate	10.0	gm
Na2HPO4Anhydrous	15.0	gm
Phenolredaqueoussolution0.02percent	15.0	ml

Water 700 ml

- Dissolve NaCl in water and add glycerol.
- Add disodium hydrogen phosphate to dissolve.
- Add phenol red and adjust pH to 8.4.
- Distribute 6 ml in universal containers (screw -capped bottles of 30 ml capacity). Autoclave at 115°C for
- 15 minutes.

Loeffler serum medium

Nutrientbroth	100ml
Serum(sheeporhorseorox)	300ml
Glucose	1.0 gm

- Dissolve glucose in nutrient broth and sterilize at 121°C for 15 minutes.
- Add serum aseptically.
- Mix thoroughly but gently, avoiding froth formation.
- Distribute in sterile test tubes or quarter ounce screw-cap bottles.

- Inspissate the medium in a slanting position in a water inspissator at 82°C for two hours.
- In the absence of an inspissator, the medium may be coagulated by standing over the top of a steam sterilizer for 6-7 minutes.

Blood tellurite agar

Agar base

Meatextract	5.0 gm
Peptone	10.0 gm
Sodium chloride	5.0 gm
Agar	25.0 gm
Water	1000 ml

Dissolve the ingredients and adjust the pH to 7.6. Distribute in 100 ml quantities in a bottle and autoclave at 121°C for 15 minutes.

Glycerolated blood tellurite mixture

Sterile defibrinated sheep blood 14 ml
Sterileglycerol 6 ml
Sterile potassium tellurite solution
(1% in water) 4 ml

- Sterilize the glycerol in hot air oven at 160°C for 60 minutes and the tellurite solution by autoclaving at

- 115°C for 20 minutes. Mix the ingredients in a sterile flask, incubate for 1-2 hrs. at 37°C, then refrigerate.
- Haemolysis is complete after 24 hrs. The mixture keeps well in a refrigerator. One per cent solution of
- good quality tellurite is sufficient but 2% of some batches may be required.

Preparation of complete medium

Glycerolated blood tellurite mixture 24 ml
Agar base 100 ml

Melt the agar, cool to 45°C, add blood and tellurite and pour in sterile petri dishes.

M1. A-1 Medium
Tryptone 20 g
Lactose 5 g
NaCl 5 g
*Triton X-100 (Rohm & Haas) 1 ml
Salicin 0.5 g
Distilled water 1 liter

Dissolve ingredients in 1 liter distilled water. Adjust pH to 6.9 ± 0.1. Dispense 10 ml portions of single strength broth into 18 x 150 mm tubes containing inverted fermentation vials. For double strength broth, use 22 x 175 mm tubes containing inverted fermentation vials. Medium may be cloudy before sterilization. Autoclave 10 min at

21°C. Store in dark up to 7 days. (Commercially available A-1 medium is unacceptable.)

M2. Acetate Agar

Sodium acetate 2 g
NaCl 5 g
Mg SO_4 (anhydrous) 0.2 g
Ammonium phosphate1 g
K_2HPO_4 1 g
Bromthymol blue 0.08 g
Agar 20 g
Distilled water 1 liter

Add all ingredients except Mg SO_4 to 1 liter distilled water. Heat to boiling with stirring. Add Mg SO_4 and adjust pH. Dispense 8 ml portions into 16 x 150 mm tubes. Autoclave 15 min at 121°C. Incline tubes to obtain 5 cm slant. Final pH, 6.7

M3. Acid Broth

Proteose peptone 5 g
Yeast extract 5 g
Dextrose 5 g
K_2HPO4 4 g
Distilled water l liter

Dissolve ingredients and dispense 12-15 ml portions into 20 x 150 mm tubes. Autoclave 15 min at 121°C. Final pH, 5.0.

M4. AE Sporulation Medium, Modified (for *C. perfringens*)

(AE base is available commercially)

Polypeptone 10.0 g

Yeast extract 10.0 g

Na_2HPO_4 4.36 g

KH_2PO_4 0.25 g

Ammonium acetate1.5 g

Mg $SO_4 \cdot 7H_2O$ 0.2 g

Distilled water 1 liter

Dissolve ingredients and adjust to pH 7.5 ± 0.1, using 2 M sodium carbonate. Dispense 15 ml into 20 x 150-mm screw-cap tubes and sterilize by autoclaving for 15 min at 121°C. After sterilization, add 0.6 ml of sterilized 10% raffinose and 0.2 ml each of filter-sterilized 0.66 M sodium carbonate and 0.32% cobalt chloride ($CoCl_2 \cdot 6H_2O$) dropwise to each tube. Check pH of one or two tubes; it should be 7.8 ± 0.1. Just before use, steam medium for 10 min; after cooling, add 0.2 ml of filter-sterilized 1.5% sodium ascorbate (prepared daily) to each tube.

M5. Alkaline Peptone Agar

Peptone 10 g

NaCl 20 g

Agar 15 g

Distilled water 1 liter

Boil to dissolve ingredients. Adjust pH so that value after sterilization is 8.5 ± 0.2. Autoclave 15 min at 121°C. Solidify agar in tubes as slants.

M6. Alkaline Peptone Salt Broth (APS)

Peptone 10 g
NaCl 30 g
Distilled water 1 liter

Dissolve ingredients. Adjust pH so that value after sterilization is 8.5 ± 0.2. Dispense 10 ml into tubes. Autoclave 10 min at 121°C.

M7. Alkaline Peptone Water

Peptone 10 g
NaCl 10 g
Distilled water l liter

Adjust pH so that value after sterilization is 8.5 ± 0.2. Dispense into screw-cap tubes. Autoclave 10 min at 121°C.

M8. Anaerobe Agar

Base

Trypticase (tryptic) soy agar 40 g
Agar 5 g
Yeast extract 5 g
L-Cysteine (dissolved in 5 ml 1 N NaOH) 0.4 g
Distilled water l liter

Heat with agitation to dissolve agar. Adjust pH to 7.5 ± 0.2. Autoclave 15 min at 121°C. Cool to 50°C.

Hemin solution. Suspend 1 g hemin in 100 ml distilled water. Autoclave 15 min at 121°C. Refrigerate at 4°C.

Vitamin K_1 solution. Dissolve 1 g vitamin K_1 (Sigma Chemical Co., St. Louis, MO) in 100 ml 95% ethanol. Solution may require 2-3 days with intermittent shaking to dissolve. Refrigerate at 4°C.

Final medium. To 1 liter base add 0.5 ml hemin solution and 1 ml Vitamin K_1 solution. Mix and pour 20 ml portions into 15 x 100 mm petri dishes. Medium must be reduced before inoculation by 24 h anaerobic incubation in anaerobic glove box or GasPak jar.

M9. Anaerobic Egg Yolk Agar

Agar base

Yeast extract 5 g
Tryptone 5 g
Proteose peptone 20 g
NaCl 5 g
Agar 20 g
Distilled water 1 liter

Autoclave 15 min at 121°C. Adjust pH to 7.0 ± 0.2.

2 Fresh Eggs

Treatment of eggs. Wash 2 fresh eggs with stiff brush and drain. Soak eggs in 70% ethanol for 1 h. Crack eggs aseptically. Retain yolks. Drain contents of yolk sacs into sterile stoppered graduate and discard sacs. Add yolk to equal volume of sterile 0.85% saline. Invert graduate several times to mix. Egg yolk emulsion (50%) is available commercially.

Preparation of medium. To 1 liter melted medium (48-50°C) add 80 ml yolk-saline mixture (available from Difco as Bacto Egg Yolk Enrichment 50%), and mix. Pour plates immediately. After solidification dry 2-3 days at ambient temperature or at 35°C for 24 h. Check plates for contamination before use. After drying, plates may be stored for a short period in refrigerator.

M10. Antibiotic Medium No. 1 (Agar Medium A)

Gelatone or gelysate 6 g
Tryptone or trypticase 4 g
Yeast extract 3 g
Beef extract 1.5 g
Dextrose 1 g
Agar 15 g
Distilled water 1 liter

Autoclave 15 min at 121°C. Final pH, 6.5-6.6. Commercially available in dehydrated form as Difco Penassay Seed Agar or BBL Seed Agar.

M11. Antibiotic Medium No. 4 (Agar Medium B)

Tryptone or trypticase 6 g
Yeast extract 3 g
Beef extract 1.5 g
Dextrose 1 g
Agar 15 g
Distilled water 1 liter

Autoclave 15 min at 121°C. Final pH, 6.5-6.6. Commercially available in dehydrated form as Difco Yeast Beef Agar or BBL Yeast Beef Agar.

M12. Arginine-Glucose Slant (AGS)

Peptone 5 g
Yeast extract 3 g
Tryptone 10 g
NaCl 20 g
Glucose 1 g
L-Arginine (hydrochloride) 5 g
Ferric ammonium citrate 0.5 g
Sodium thiosulfate 0.3 g
Bromocresol purple 0.02 g
Agar 13.5 g
Distilled water 1 liter

Suspend ingredients in distilled water and boil to dissolve. Dispense into tubes (for 13 x 100 mm tubes use 5 ml). Autoclave 10-12 min at 121°C. After sterilization, solidify as slants. Final pH, 6.8-7.0.

M13. Bile Esculin Agar

Beef extract 3 g

Peptone 5 g

Esculin 1 g

Oxgall 40 g

Ferric citrate 0.5 g

Agar 15 g

Distilled water 1 liter

Heat with agitation to dissolve. Dispense into tubes, autoclave 15 min at 121°C, and slant until solidified. Final pH, 6.6 ± 0.2.

M13Bismuth Sulfite Agar (Wilson and Blair)

Polypeptone (or peptone)10 g

Beef extract 5 g

Dextrose 5 g

Na_2HPO_4 (anhydrous) 4 g

$FeSO_4$ (anhydrous) 0.3 g

Bismuth sulfite (indicator) 8 g

Brilliant green 0.025 g

Agar 20 g

Distilled water 1 liter

Mix thoroughly and heat with agitation. Boil about 1 min to obtain uniform suspension. (Precipitate will not dissolve.) Cool to 45-50°C. Suspend precipitate by gentle agitation, and pour 20 ml portions into sterile 15 x 100 mm petri dishes. Let plates dry about 2 h with lids partially removed; then close plates. Final pH, 7.7 ± 0.2 **DO NOT**

AUTOCLAVE. Prepare plates on day before streaking and store in dark. Selectivity decreases in 48 h.

M14. Blood Agar

Tryptone 15 g
Phytone or soytone 5 g
NaCl 5 g
Agar 15 g
Distilled water 1 liter

Heat with agitation to dissolve agar. Autoclave 15 min at 121°C. Cool to 50°C. Add 5 ml defibrinated sheep red blood cells to 100 ml melted agar. Mix and pour 20 ml portions into sterile 15 x 100 mm petri dishes. Final pH of base, 7.3 ± 0.2. Tryptic soy agar, tryptic soy agar blood base, or trypticase soy agar [soybean-casein digest agar (M152)] may be used as the basal medium. Commercially available sheep blood agar plates are satisfactory. For *Vibrio hollisae*, add NaCl to a final concentration of 1%.

M15. Blood Agar Base (Infusion Agar)

Heart muscle, infusion from 375 g
Thiotone 10 g
NaCl 5 g
Agar 15 g
Distilled water 1 liter

Heat gently to dissolve. Autoclave 20 min at 121°C. Final pH, 7.3 ± 0.2. Commercially available dehydrated heart infusion agars may be used.

M16. Blood Agar Base #2 (Difco)

Proteose peptone15 g
Liver digest 2.5 g
Yeast extract 5 g
NaCl 5 g
Agar 12 g
Distilled Water 1 liter

Autoclave at 121°C for 15 min. For blood agar, reduce water to 950 ml. Add 50 ml defibrinated (whole or lysed) horse blood and FBP (4 ml to agar + blood) after autoclaving and cooling to 48°C. Final pH, 7.4 ± 0.2.

M17. Brain Heart Infusion (BHI) Agar (0.7%)
(for staphylococcal enterotoxin)

Prepare a suitable quantity of brain heart infusion broth. Adjust pH to 5.3 with 1 N HCl. Add agar to give 0.7% concentration. Dissolve by minimal boiling. Dispense 25 ml portions into 25 x 200 mm test tubes. Autoclave 10 min at 121°C.

M18. Brain Heart Infusion (BHI) Broth and Agar

Formulations used by selected manufacturers are represented below; these media are normally available as pre-mixed dry powder.

Medium 1

Calf brain, infusion from 200 g

Beef heart, infusion from 250 g

Proteose peptone (Difco) or polypeptone (Bioquest) 10 g

NaCl 5 g

Na_2HPO_4 * 2.5 g

Dextrose 2.0 g

Distilled water 1 liter

Medium 2

Brain heart-infusion 6.0 g

Peptic digest of animal tissue 6.0 g

NaCl 5.0 g

Dextrose 3.0 g

Pancreatic digest of gelatin 14.5 g

Na_2HPO_4 *2.5 g

Distilled water 1 liter

Dissolve ingredients of Medium 1 in distilled water with gentle heat. Suspend ingredients of Medium 2 in distilled water and boil for 1 min to completely dissolve. For both Medium 1 and Medium 2, dispense broth into bottles or tubes for storage. Autoclave 15 min at 121°C. Final pH, 7.4 ± 0.2. Commercially available BHI is acceptable.

To prepare brain heart infusion *agar*, add 15 g agar to 1 liter BHI broth. Heat to dissolve agar before dispensing into bottles or flasks. Autoclave 15 min at 121°C.

For use with halophilic *Vibrio* spp., add NaCl to a final concentration of 2-3%.

M19. Brilliant Green Lactose Bile Broth

Peptone 10 g
Lactose 10 g
Oxgall 20 g
Brilliant green 0.0133 g
Distilled water 1 liter

Dissolve peptone and lactose in 500 ml distilled water. Add 20 g dehydrated oxgall dissolved in 200 ml distilled water. The pH of this solution should be 7.0-7.5. Mix and add water to make 975 ml. Adjust pH to 7.4. Add 13.3 ml 0.1% aqueous brilliant green in distilled water. Add distilled water to make 1 liter. Dispense into fermentation tubes, making certain that fluid level covers inverted vials. Autoclave 15 min at 121°C. Final pH, 7.2 ± 0.1.

M20. Bromcresol Purple Broth

Base

Peptone 10 g
Beef extract 3 g
NaCl 5 g
Bromcresol purple 0.04 g
Distilled water 1 liter

Dispense 2.5 ml portions of base solution into 13 x 100 mm test tubes containing inverted 6 x 50 mm fermentation tubes. Autoclave

10 min at 121°C. Final pH, 7.0 ± 0.2. Sterilize stock solutions of carbohydrates (50% w/v) separately by autoclaving or, preferably, by filtration (0.2 µm pore size). Add 0.278 ± 0.002 ml stock carbohydrate solution to 2.5 ml basal medium to give 5% w/v final carbohydrate concentration.

For use with halophilic *Vibrio* spp., add NaCl to a final concentration of 2-3%.

M21. Bromcresol Purple Dextrose Broth (BCP)

Dextrose 10 g
Beef extract 3 g
Peptone 5 g
Bromcresol purple (1.6% in ethanol) 2 ml
Distilled water 1 liter

Dissolve ingredients in distilled water. Dispense 12-15 ml into tubes. Autoclave 15 min at 121°C. Final pH, 7.0 ± 0.2..

M22. Egg Yolk Emulsion, 50%

Wash fresh eggs with a stiff brush and drain. Soak eggs 1 h in 70% ethanol. Drain ethanol. Crack eggs aseptically and discard whites. Remove egg yolks with sterile syringe or wide-mouth pipet. Place yolks in sterile container and mix aseptically with equal volume of sterile 0.85% saline. Store at 4°C until use. Egg yolk emulsion (50%) is available commercially.

M23. Heart Infusion (HI) Broth and Agar (HIA) (for *Vibrio*)

Beef heart, infusion from 500 g 1 liter

Tryptose 10 g
NaCl 5 g

Dissolve ingredients and dispense into tubes. For halophilic *Vibrio* spp. add an extra 15 g NaCl/liter. Autoclave 15 min at 121°C. Final pH, 7.4 ± 0.2. For heart infusion agar, add 15 g agar/L and boil to dissolve before dispensing and sterilizing.Commercially available from Difco.

M24. Kligler Iron Agar
Polypeptone peptone 20 g
Lactose 20 g
Dextrose 1 g
NaCl 5 g
Ferric ammonium citrate 0.5 g
Sodium thiosulfate 0.5 g
Agar 15 g
Phenol red 0.025 g
Distilled water 1 liter

Heat with agitation to dissolve. Dispense into 13 x 100 mm screw-cap tubes and autoclave 15 min at 121°C. Cool and slant to form deep butts. Final pH, 7.4 ± 0.2.Commercially available from Difco, BBL, and Oxoid.For use with halophilic *Vibrio* spp., add NaCl to a final concentration of 2-3%.
M25. Lysine Decarboxylase Broth (Falkow)
(for *Salmonella*)
Gelysate or peptone 5 g

Yeast extract 3 g
Glucose 1 g
L-Lysine 5 g
Bromcresol purple 0.02 g
Distilled water 1 liter

Heat until dissolved. Dispense 5 ml portions into 16 x 125 mm screw-cap tubes. Autoclave loosely capped tubes 15 min at 121°C. Screw the caps on tightly for storage and after inoculation. Final pH, 6.8 ± 0.2. For halophilic *Vibrio* spp., add NaCl to a final concentration of 2-3%.

M26. Lysine Decarboxylase (LDC) Medium
(for Gram-negative nonfermentative bacteria)
L-Lysine HCl 0.5 g
Dextrose 0.5 g
KH_2PO_4 0.5 g
Distilled water 100 ml

Dissolve ingredients. Adjust pH to 4.6 ± 0.2. Autoclave 15 min at 121°C. Aseptically dispense 1 ml portions to sterile 13 x 100 mm tubes.

M27. Lysine Iron Agar (Edwards and Fife)
Gelysate or peptone 5 g
Yeast extract 3 g
Glucose 1 g
L-Lysine hydrochloride 10 g

Ferric ammonium citrate 0.5 g
Sodium thiosulfate (anhydrous) 0.04 g
Bromcresol purple 0.02 g
Agar 15 g
Distilled water 1 liter

Heat to dissolve ingredients. Dispense 4 ml portions into 13 x 100 mm screw-cap tubes. Autoclave 12 min at 121°C. Let solidify in slanted position to form 4 cm butts and 2.5 cm slants. Final pH, 6.7 ± 0.2.

M28. MacConkey Agar
Proteose peptone or polypeptone 3 g
Peptone or gelysate17 g
Lactose 10 g
Bile salts No. 3 or bile salts mixture 1.5 g
NaCl 5 g
Neutral red 0.03 g
Crystal violet 0.001 g
Agar 13.5 g
Distilled water 1 liter

Suspend ingredients and heat with agitation to dissolve. Boil 1-2 min. Autoclave 15 min at 121°C, cool to 45-50°C, and pour 20 ml portions into sterile 15 x 100 mm petri dishes. Dry at room temperature with lids closed. DO NOT USE WET PLATES. Final pH, 7.1 ± 0.2.

M29. Motility-Indole-Ornithine (MIO) Medium

Yeast extract 3 g

Peptone 10 g

Tryptone 10 g

L-Ornithine HCl 5 g

Dextrose 1 g

Agar 2 g

Bromcresol purple 0.02 g

Distilled water 1 liter

Dispense 4 ml portions into 13 x 100 mm tubes. Autoclave 15 min at 121°C. Final pH, 6.5 ± 0.2.

M30. Motility Medium (for *B. cereus*)

Trypticase 10 g

Yeast extract 2.5 g

Dextrose 5 g

Na_2HPO_4 2.5 g

Agar 3 g

Distilled water 1 liter

Heat with agitation to dissolve agar. Dispense 100 ml portions to 170 ml bottles. Autoclave 15 min at 121°C. Final pH, 7.4 ± 0.2. Cool to 50°C. Aseptically dispense 2 ml portions to sterile 13 x 100 mm tubes. Store at room temperature 2 days before use.

M31. Motility-Nitrate Medium, Buffered (for *C. perfringens)*

Beef extract 3 g

Peptone (Difco) 5 g

KNO_3 1 g

Na_2HPO_3 2.5 g

Agar 3 g

Galactose 5 g

Glycerin (reagent grade) 5 ml

Distilled water 1 liter

Dissolve all ingredients except agar. Adjust pH to 7.3 ± 0.1. Add agar and heat to dissolve. Dispense 11 ml portions into 16 x 150 mm tubes. Autoclave 15 min at 121°C. If not used within 4 h, heat 10 min in boiling water or flowing steam. Chill in cold water.

M32. Motility Test Medium (Semisolid)

Beef extract 3 g

Peptone or gelysate10 g

NaCl 5 g

Agar 4 g

Distilled water 1 liter

Heat with agitation and boil 1-2 min to dissolve agar. Dispense 8 ml portions into 16 x 150 screw-cap tubes.Autoclave 15 min at 121°C. Final pH, 7.4 ± 0.2.

For *Salmonella*: Dispense 20 ml portions into 20 x 150 mm screw-cap tubes, replacing caps loosely. Autoclave 15min at 121°C. Cool

to 45°C after autoclaving. Tighten caps, and refrigerate at 5-8°C. To use, remelt in boiling water or flowing steam, and cool to 45°C. Aseptically dispense 20 ml portions into sterile 15 x 100 mm petri plates. Cover plates and let solidify. Use same day as prepared. Final pH, 7.4 ± 0.2.For use with halophilic *Vibrio* spp., add NaCl to a final concentration of 2-3%.

M33. Nitrate Broth
Beef extract 3 g
Peptone 5 g KNO_3 (nitrite-free) 1 g
Distilled water 1 liter

Dissolve ingredients. Dispense 5 ml portions into 16 x 125 mm tubes. Autoclave 15 min at 121°C. Final pH, 7.0 ± 0.2.

M34. Nitrate Broth, Enriched (CDC)
Infusion broth 25 g
KNO_3 (nitrite-free) 2 g
Distilled water 1 liter

Dispense 4 ml portions into 13 x 100 mm tubes with inverted Durham tubes. Autoclave 15 min at 121°C. Final pH, 7.3 ± 0.2.

M35. Oxidative-Fermentative (OF) Test Medium
Base
Peptone 2 g
NaCl 5 g
K_2HPO_4 0.3 g
Bromthymol blue 0.03 g

Agar 2.5 g
Distilled water 1 liter

Heat with agitation to dissolve agar. Dispense 3 ml portions into 13 x 100 mm tubes. Autoclave 15 min at 121°C.Cool to 50°C; pH, 7.1.

Carbohydrate stock solution. Dissolve 10 g carbohydrate in 90 ml distilled water. Sterilize by filtration through 0.22 µm membrane. Add 0.3 ml stock solution to 2.7 ml base in tube. Mix gently and cool at room temperature.Inoculate tubes in duplicate. Layer one tube with sterile mineral oil. Incubate 48 h at 35°C.For use with halophilic *Vibrio* spp., add NaCl to a final concentration of 2-3%.

M36. Selenite Cystine Broth
Medium 1 (modification of Leifson's formulation for selenite broth)
Tryptone or polypeptone 5 g
Lactose 4 g
Sodium acid selenite ($NaHSeO_3$) 4 g
Na_2HPO_4 10 g
L-Cystine 0.01 g
Distilled water 1 liter

Heat to boiling to dissolve. Dispense 10 ml portions into sterile 16 x 150 mm test tubes. Heat 10 min in flowing steam. DO NOT AUTOCLAVE. Final pH, 7.0 ± 0.2. The medium is not sterile. Use same day as prepared.

Medium 2 (North-Bartram modification)
Polypeptone 5 g

Lactose 4 g
Sodium acid selenite ($NaHSeO_3$) 4 g
Na_2HPO_4 5.5 g
KH_2PO_4 4.5 g
L-Cystine 0.01 g
Distilled water 1 liter

Heat with agitation to dissolve. Dispense 10 ml portions into sterile 16 x 150 mm test tubes. Heat 10 min in flowing steam. DO NOT AUTOCLAVE. Use same day as prepared.

M37. Sheep Blood Agar
Blood agar base (Oxoid No. 2) 95 ml
Sterile sheep blood, defibrinated 5 ml

Rehydrate and sterilize base as recommended by manufacturer. Agar and blood should both be at 45-46°C before blood is added and plates are poured. Commercial pre-poured sheep blood agar plates may be used.

M38. Shigella Broth
Base
Tryptone 20 g
K_2HPO_4 2 g
KH_2PO_4 2 g
NaCl 5 g
Glucose 1 g

Tween 80 1.5 ml
Distilled water 1 liter

Autoclave 15 min at 121°C. Final pH, 7.0 ± 0.2.

Novobiocin solution. Weigh 50 mg novobiocin into 1 liter distilled water. Sterilize by filtration through 0.45 µm membrane. Add 2.5 ml concentrate to 225 ml base.

M39. Simmons Citrate Agar
Sodium citrate*2 g
NaCl 5 g
K_2HPO_4 1 g
$NH_4H_2PO_4$ 1 g
$MgSO_4$ 0.2 g
Bromthymol blue 0.08 g
Agar 15 g
Distilled water 1 liter

Heat gently with occasional agitation. Boil 1-2 min until agar dissolves. Fill 13 x 100 or 16 x 150 mm screw-cap tubes 1/3 full. Autoclave 15 min at 121°C. Before medium solidifies, incline tubes to obtain 4-5 cm slants and 2-3 cm butts. Final pH, 6.8 ± 0.2.

M40. Sorbitol-MacConkey Agar
Peptone or gelysate 17.0 g
Protease peptone No. 3 or polypeptone 3.0 g
Sorbitol 10.0 g
Bile salts, purified 1.5 g

NaCl 5.0 g
Agar 13.5 g
Neutral red 0.03 g
Crystal violet 0.001 g
Distilled water 1 liter

Dissolve ingredients in distilled water by heating with stirring. Autoclave 15 min at 121°C. Final pH, 7.1 ± 0.2.

M41. Sporulation Broth (for C. perfringens)
Polypeptone 15 g
Yeast extract 3 g
Starch, soluble 3 g
$MgSO_4$ (anhydrous) 0.1 g
Sodium thioglycollate 1 g
Na_2HPO_4 11 g
Distilled water 1 liter

Adjust pH to 7.8 ± 0.1. Dispense 15 ml portions into 20 x 150 mm screw-cap tubes. Autoclave 15 min at 121°C.

M42. Thiosulfate-Citrate-Bile Salts-Sucrose (TCBS) Agar
Yeast extract 5 g
NaCl 10 g
Peptone 10 g
Ferric citrate 1 g
Sucrose 20 g
Bromthymol blue O.04 g

Sodium thiosulfate·5H_2O 10 g
Thymol blue 0.04 g
Sodium citrate·2H_2O 10 g
Agar 15 g
Sodium cholate 3 g
Distilled water 1 liter
Oxgall 5 g

Prepare in flask at least 3 times larger than required volume of medium. Add ingredients to warm distilled water and heat to dissolve. Bring just to boil, and immediately remove from heat. DO NOT AUTOCLAVE. Cool to 50°C and pour into petri dishes. Dry the plates overnight or at 37-45°C before use.

M42. Triple Sugar Iron Agar (TSI)

Medium 1	Medium 2
Polypeptone 20 g	Beef extract 3 g
NaCl 5 g	Yeast extract 3 g
Lactose 10 g	Peptone 15 g
Sucrose 10 g	Proteose peptone 5 g
Glucose 1 g	Glucose 1 g
$Fe(NH_4)_2(SO_4)_2$·6H_2O 0.2 g	Lactose 10 g
$Na_2S_2O_3$ 0.2 g	Sucrose 10 g
Phenol red 0.025 g	$FeSO_4$ 0.2 g
Agar 13 g	NaCl 5 g
Distilled water 1 liter	$Na_2S_2O_3$ 0.3 g

Phenol red 0.024 g

Agar 12 g

Distilled water 1 liter

These two media are interchangeable for general use.Suspend ingredients of Medium 1 in distilled water, mix thoroughly, and heat with occasional agitation. Boil about 1 min to dissolve ingredients. Fill 16 x 150 mm tubes 1/3 full and cap or plug to maintain aerobic conditions. Autoclave Medium 1 for 15 min at 118°C. Prepare Medium 2 in the same manner as Medium 1, except autoclave 15 min at 121°C. Before the media solidify, incline tubes to obtain 4-5 cm slant and 2-3 cm butt. Final pH, 7.3 ± 0.2 for Medium 1 and 7.4 ± 0.2 for Medium 2.

For use with halophilic *Vibrio* spp., add NaCl to a final concentration of 2-3%.

M150. Trypticase Novobiocin (TN) Broth
Trypticase soy broth 30 g
Bile salts No. 3 1.5 g
Dipotassium phosphate 1.5 g
Novobiocin 20 mg
Distilled water 1 liter

Dissolve all ingredients except novobiocin by heating and stirring; autoclave at 121°C for 15 min. Prepare stock solution of novobiocin by adding 20 mg monosodium novobiocin per ml of distilled water. Filter-sterilize. Make fresh stock each time of use, or store frozen at -

10°C in the dark (compound is light-sensitive) for not more than 1 month (half-life is several months at 4°C). Add 1 ml stock solution per liter of medium.

M43. Trypticase (Tryptic) Soy Agar
Trypticase peptone 15 g
Phytone peptone 5 g
NaCl 5 g
Agar 15 g
Distilled water 1 liter

Heat with agitation to dissolve agar. Boil 1 min. Dispense into suitable tubes or flasks. Autoclave 15 min at 121°C. Final pH, 7.3 ± 0.2. For use with halophilic *Vibrio*

M44. Tryptone (Tryptophane) Broth, 1%
Tryptone or trypticase 10 g
Distilled water 1 liter

Dissolve and dispense 5 ml portions into 16 x 125 or 16 x 150 mm test tubes. Autoclave 15 min at 121°C. Final pH, 6.9 ± 0.2.

M45. Tryptone Yeast Extract Agar
Tryptone 10 g
Yeast extract 1 g
*Carbohydrate 10 g
Bromcresol purple 0.04 g
Agar 2 g
Distilled water 1 liter

Dissolve agar with heat and gentle agitation. Adjust pH to 7.0 ± 0.2. Fill 16 x 125 mm tubes 2/3 full. Autoclave 20 min at 115°C. Before use, steam medium 10-15 min. Solidify by placing tubes in ice water.

*Glucose and mannitol are the carbohydrates used for identification of *S. aureus*.

M46. Tryptose Phosphate Broth (TPB)(for cell culture)
Tryptose 20 g
Dextrose 2 g
NaCl 5 g
Na_2HPO_4 2.5 g
Distilled water 1 liter

Sterilize by filtration through 0.20 µm membrane.

M47. Urea Broth
Urea 20 g
Yeast extract 0.1 g
Na_2HPO_4 9.5 g
K_2HPO_4 9.1 g
Phenol red 0.01 g
Distilled water 1 liter

Dissolve ingredients in distilled water. DO NOT HEAT. Sterilize by filtration through 0.45 µm membrane. Aseptically dispense 1.5-3.0 ml portions to 13 x 100 mm sterile test tubes. Final pH, 6.8 ± 0.2.

REAGENTS

R1. 4 M Ammonium Acetate
Ammonium acetate 308.4 g
Distilled water to make 1 liter

R2. 0.25 M Ammonium Acetate
Ammonium acetate 19.3 g
Distilled water to make 1 liter

R3. Basic Fuchsin Staining Solution

Dissolve 0.5 g basic fuchsin dye in 20 ml 95% ethanol. Dilute to 100 ml with distilled water. Filter if necessary with Whatman No. 31 filter paper to remove any undissolved dye. (TB Carbolfuchsin ZN staining solution, available from Difco Laboratories, is satisfactory.)

R4. 0.1 M Bicarbonate Buffer (pH 9.6)
Na_2CO_3 1.59 g
$NaHCO_3$ 2.93 g
Distilled water 1 liter

Store at room temperature for not more than 2 weeks.

R5. Bovine Serum Albumin (BSA) (1 mg/ml)
Nuclease-free bovine serum albumin 10 mg
Distilled water 10 ml

Place 0.5 ml portions into 1.5 ml plastic conical centrifuge tubes. Store frozen.

R6. 1% Bovine Serum Albumin in Cholera Toxin ELISA Buffer
Bovine serum albumin (BSA)1 g
ELISA buffer for (cholera toxin), pH 7.4 100 ml

Dissolve BSA in ELISA buffer. Aliquot and store at -20°C.

R7. 1% Bovine Serum Albumin in PBS
Bovine serum albumin (BSA)1 g
Phosphate-buffered saline, pH 7.4 100 ml

Dissolve BSA in PBS buffer. Aliquot and store at -20°C.

R8. Brilliant Green Dye Solution. 1%
Brilliant green dye 1 g
Distilled water (sterile) 10 ml

Dissolve 1 g dye in sterile water. Dilute to 100 ml. Before use, test all batches of dye for toxicity with known positive and negative test microorganisms.

R9. Bromcresol Purple Dye Solution. 0.2%
Bromcresol purple dye 0.2 g
Distilled water (sterile)100 ml

Dissolve 0.2 g dye in sterile water and dilute to 100 ml.

R10. Bromthymol Blue Indicator. 0.04%
Bromthymol blue 0.2 g
0.01 N NaOH 32 ml

Dissolve bromthymol blue in NaOH. Dilute to 500 ml with distilled water.

R11. Butterfield's Phosphate-Buffered Dilution Water
Stock solution
KH_2PO_4 34 g
Distilled water 500 ml

Adjust pH to 7.2 with 1 N NaOH. Bring volume to 1 liter with distilled water. Sterilize 15 min at 121°C. Store in refrigerator.

Dilution blanks
Take 1.25 ml of above stock solution and bring volume to 1 liter with distilled water. Dispense into bottles to 90 or 99 ± 1 ml. Sterilize 15 min at 121°C.

R12. Catalase Test
Pour 1 ml 3% hydrogen peroxide over growth on slant culture. Gas bubbles indicate positive test. Alternatively, emulsify colony in l drop 3% hydrogen peroxide on glass slide. Immediate bubbling is positive catalase test. If colony is taken from blood agar plate, any carry-over of red blood cells can give false-positive reaction.

Rl2a. Chlorine Solution, 200 ppm,
Containing Q.1% Sodium Dodecyl Sulfate

Commercial bleach (5.25% sodium hypochlorite) 8 ml
Distilled water containing 1 g sodium dodecyl sulfate 992 ml

Dissolve 1 g sodium dodecyl sulfate in 992 ml distilled water. Add 8 ml commercial bleach and mix well. Make immediately before use.

R13. 0.05 M Citric Acid (PH 4.0)
Citric acid (monohydrate)10.5 g
Double distilled water to make l liter

Dissolve citric acid in 900 ml distilled water. Adjust pH to 4.0 with 6 M NaOH and dilute to 1 liter. Store in refrigerator.

Rl4. Clark's Flagellar Stain
Solution A
Basic fuchsin, special 1.2 g
95% ethanol 100 ml

Mix and let stand overnight at room temperature.

Solution B
Tannic acid 3.0 g
NaCl 1.5 g
Distilled water 200 ml

Mix solutions A and B. Adjust pH to 5.0 with 1 N NaOH or 1 N HCl, if necessary. Refrigerate 2-3 days before use. Stain is stable l month at 4°C or may be stored frozen indefinitely (50 ml portions). To use, thaw stain, remix, and store at 4°C. Optimum staining time for each

batch varies 5-15 min. To determine staining time (after 2-3 days refrigeration at 4°C), stain a known flagellated organism on 3 or more cleaned slides for various times (e.g., 5, 10, 15 min). Mark best staining time on all containers.

IMPORTANT: Stain will not work unless slides are clean. To clean slides, soak them 4 days at room temperature in cleaning solution (either acid dichromate or 3% concentrated HCl in 95% ethanol). Rinse 10 times in fresh tap water and twice in distilled water. Air-dry at room temperature. Store in covered container.

Staining Procedure

To prepare suspension, pick small amount of growth from 18-24 h plate (equivalent to 1 mm colony). Do not pick up agar. Suspend gently in 3 ml distilled water. (Flagella can be knocked off.) Suspension should be faintly opalescent.

To prepare slide, pass cleaned slide through blue part of burner flame several times to remove residual dirt. Cool slide, flamed side up, on paper towel. Mark wax line across slide to give area 2.5 x 4.5 cm. Place large loopful of suspension in center of slide adjacent to wax line. Tilt slide, letting drop run down center of slide to end. If drop does not run evenly, slide is dirty. Discard it. Air-dry slide on level surface.

R15. Coating Solution for *V. vulnificus* EIA
Phosphate-buffered saline 100 ml
Triton X-100 (a polyoxyethylene ether)20 µl

Mix Triton X-100 with PBS, pH 7.4.

R16. Crystal Violet Stain (for Bacteria)

1. Crystal violet in dilute alcohol
Crystal violet (90% dye content) 2 g
Ethanol (95%) 20 ml
Distilled water 80 ml

2. Ammonium oxalate crystal violet.

Either solution is generally considered suitable as a simple stain to observe morphology.

R17. Disinfectants
(for preparation of canned foods for microbiological analysis)

1. Alcoholic solution of iodine
Potassium iodide 10 g
Iodine 10 g
Ethanol (70%) 500 ml

2. Sodium hypochlorite solution
*Sodium hypochlorite 5.0-5.25 g
Distilled water 100 ml

*Laundry bleach, which is 5.25% sodium hypochlorite (NaOCl), may be used.

R18. Dulbecco's Phosphate-Buffered Saline (DPBS)

NaCl 8.0 g

KCl 200 mg

Na_2HPO_4 1.15 g

KH_2PO_4 200 mg

$CaCl_2$ 100 mg

$MgCl_2 \cdot 6H_2O$ 100 mg

Distilled water 1 liter

Dissolve ingredients in water. Sterilize by filtration. Final pH, 7.2.

R19. 0.5 M EDTA

Na_2EDTA 186.12 g

Dissolve in 800-900 ml dH_2O . Adjust pH to 8.0 with 10 N NaOH.

Add distilled water to make 1 liter.

R20. EIA (*V. vulnificus*) Wash Solution

NaCl 87.65 g

Tween 205.0 ml

Dissolve ingredients in 10 liters of deionized water.

R21. Ethanol Solution, 70%

Ethanol, 95% 700 ml

Distilled wateradd to final volume of 950 m.

R22. Ferric Chloride. 10%

$FeCl_3$ 10 g

Distilled water 90 ml

R23. Formalinized Physiological Saline Solution

Formaldehyde solution (36-38%) 6 ml

NaCl 8.5 ml

Distilled water 1 liter

Dissolve 8.5 g NaCl in 1 liter distilled water. Autoclave 15 min at 121°C. Cool to room temperature. Add 6 ml formaldehyde solution. Do not autoclave after addition of formaldehyde.

R24. Gel Diffusion Agar. 1.2%

NaCl 8.5 g

Sodium barbital 8.0 g

Merthiolate (crystalline) 0.1 g

Noble special agar (Difco) 12.0 g

Distilled water 1 liter

Dissolve NaCl, sodium barbital, and merthiolate in 900 ml distilled water. Adjust pH to 7.4 with 1 N HCl and/or 1 N NaOH. Bring volume to l liter. Add Noble agar. Melt agar mixture in Arnold steamer. Filter in steamer, while hot, through 2 layers of analytical grade filter paper (e.g., No. 588, Schleicher and Schuell or equivalent). Dispense in small (15-25 ml) portions into 4 oz prescription bottles. Do not remelt more than twice.

R25. Gel-Phosphate Buffer

Gelatin 2 g

Na_2HPO_4 4 g

Distilled water 1 liter

Use gentle heat to dissolve ingredients. Sterilize 20 min at 121°C. Final pH, 6.2.

R26. Giemsa Stain

Giemsa powder 1 g
Glycerol 66 ml
Methanol (absolute) 66 ml
Distilled stain in glycerol by heating 1.5-2.0 h at 55-60°C. Add methanol. Store stain in tightly stoppered bottle at 22°C for at least 2 weeks. Dilute stock solution with distilled water (1+9) before use.

R27. Glycerin-Salt Solution (Buffered)

Glycerin (reagent grade) 100 ml
K_2HPO_4 (anhydrous) 12.4 g
KH_2PO_4 (anhydrous) 4 g
NaCl 4.2 g
Distilled water 900 ml

Distilled NaCl and bring volume to 900 ml with water. Add glycerin and phosphates. Adjust pH to 7.2. Autoclave 15 min at 121°C. For double strength (20%) glycerin solution, use 200 ml glycerin and 800 ml distilled water.

R28. Gram Stain

(commercial staining solutions are satisfactory)

Hucker's crystal violet

Solution A

Crystal violet (90% dye content) 2 g

Ethanol, 95% 20 ml

Solution B

Ammonium oxalate 0.8 g

Distilled water 80 ml

Mix solutions A and B. Store 24 h and filter through coarse filter paper.

Gram's iodine

Iodine 1 g

Potassium iodide (KI) 2 g

Distilled water 300 ml

Place KI in mortar, add iodine, and grind with pestle for 5-10 s. Add 1 ml water and grind; then add 5 ml of water and grind, then 10 ml and grind. Pour this solution into reagent bottle. Rinse mortar and pestle with amount of water needed to bring total volume to 300 ml.

Hucker's counterstain (stock solution)

Safranin O (certified) 2.5

Ethanol, 95% 100 ml

Working solution: Add 10 ml stock solution to 90 ml distilled water.

Staining Procedure

(Gram stain)Fix air-dried films of food sample in moderate heat. Stain films 1 min with crystal violet-ammonium oxalate solution. Wash briefly in tap water and drain. Apply Gram's iodine for 1 min. Wash in tap water and drain. Decolorize with 95% ethanol until blue color is no longer released (about 30 s). Alternatively, flood slides

with ethanol, pour off immediately, and reflood with ethanol for 10 s. Wash briefly with water, drain, and apply Hucker's counterstain (safranin solution) for 10-30 s. Wash briefly with water, drain, blot or air-dry, and examine.

R29. Endospore Stain (Schaeffer-Fulton)Solution A Malachite green10 g Distilled water 100 mlFilter to remove undissolved dye.**Solution B**Safranin O0.25 g Distilled water20 ml

R30. Hippurate Solution, 1%

Dissolve 0.1 g sodium hippurate in 10 ml distilled water. Filter-sterilize and store refrigerated or in 0.4 ml aliquots at -20°C. Commercial preparations are also available.

R31. Horseradish Peroxidase
(color development solution)

Solution A (horseradish)
HRP color development reagent 60 mg
Ice cold methanol 20 ml

Mix to dissolve. Protect from light and prepare fresh.

Solution B
Ice cold hydrogen peroxide, 30% 60 µl
Tris-buffered saline 100 ml

Prepare fresh before use. Mix ice cold solution A with room temperature solution B. Use immediately.

R32. 1 N Hydrochloric Acid

HCl (concentrated) 89 ml
Distilled water to make 1 liter

R33. Kovacs' Reagent

p-Dimethylaminobenzaldehyde 5 g
Amyl alcohol (normal only) 75 ml
HCl (concentrated) 25 ml

Dissolve *p*-dimethylaminobenzaldehyde in normal amyl alcohol. Slowly add HCl. Store at 4°C. To test for indole, add 0.2-0.3 ml reagent to 5 ml of 24 h bacteria culture in tryptone broth. Dark red color in surface layer is positive test for indole.

R34. 0.1 N Lithium Hydroxide

Lithium hydroxide (anhydrous) 2.395 g
Distilled water 1 liter

R35. Lugol's Iodine Solution

Potassium iodide (KI)10 g
Iodine 5 g
Distilled water 100 ml

Dissolve KI in about 20-30 ml of distilled water. Add iodine and heat gently with constant mixing until iodine is dissolved. Dilute to 100 ml

with distilled water. Store in amber glass-stoppered bottle in the dark.

R36. Methyl Red Indicator

Methyl red 0.10 g

Ethanol, 95% 300 ml

Distilled water to make 500 ml

Dissolve methyl red in 300 ml ethanol. Bring volume to 500 ml with distilled water.

R37. Methylene Blue Stain (Loeffler's)

Solution A

Methylene blue (90% dye content) 0.3 g

Ethanol (95%) 30 ml

Solution B

Diluted potassium hydroxide (0.01%) 100 ml

Mix solutions A and B.

R38. Nitrite Detection Reagents

A. Sulfanilic acid reagent

Sulfanilic acid 1 g

5 N acetic acid 125 ml

B. N-(l-naphthyl)ethylenediamine reagent

N-(l-naphthyl)ethylenediamine dihydrochloride 0.25 g

5 N acetic acid 200 ml

C. alpha-Naphthol reagent

alpha-Naphthol 1 g

5 N acetic acid 200 ml

To prepare 5 N acetic acid, add 28.75 ml glacial acetic acid to 71.25 ml distilled water. Store reagents in glass-stoppered brown bottles. To perform test, add 0.1-0.5 ml each of reagent A and either reagent B or reagent C (as specified in method) to culture grown in liquid or semisolid medium. Development of red-violet color with reagents A and B or orange color with reagents A and C indicates that nitrate has been reduced to nitrite. Since color produced with reagents A and B may fade or disappear within a few minutes, record reaction as soon as color appears. If no color develops, test for presence of nitrate by adding small amount of zinc dust. If color develops, nitrate has not been reduced.

Nitrate reduction test for enteropathogenic *E. coli*. To 3 ml of 18-24 h culture in indole-nitrite medium, add 2 drops each of reagents A and B.Red-violet color indicates that nitrate has been reduced to nitrite. Check negative tests by adding small amount of zinc dust; if red-violet color does not appear, nitrate has been reduced.

D. Alternative test reagents. 5-Amino-2-naphthylene sulfonic acid (Cleve's acid) and N,N-dimethyl-1-naphthylamine have been recommended as substitutes for preparation of reagent B. Absolute ethanol may be substituted for acetic acid in reagent C. However, comparative evaluations should be conducted before substitution of these alternative reagents.

R39. Oxidase Reagent

N,N,N',N'-Tetramethyl-p-phenylenediamine·2HCl 1 g
Distilled water 100 ml

This is the preferred reagent. Use freshly prepared. However, reagent can be used up to 7 days if stored in a dark glass bottle under refrigeration.Apply freshly prepared solution directly to young culture (24 h) on either agar plate or slant. Oxidase-positive colonies develop a pink color and progressively turn dark purple. If cultures are to be preserved, complete the transfer from plates to which reagent has been added within 3 min, since reagent is toxic to organisms.

R40. Peptone Diluent, 0.1%

Peptone 1 g
Distilled water 1 liter

Autoclave 15 min at 121°C. Final pH, 7.0 + 0.2.

R41. O.01 M Phosphate-Buffered Saline (pH 7.5)Stock solution (0.1 M)

Na_2HPO_4 (anhydrous) 12.0 g
$NaH_2PO_4 \cdot H_20$ 2.2 g
NaCl 85.0 g
Distilled water 1 liter

Dissolve ingredients in distilled water and bring volume to 1 liter. Dilute stock solution 1+9 in double distilled water. Mix well. Adjust pH to 7.5 with 0.1 N HCl or 0.1 N NaOH if necessary.

R42. 0.02 M Phosphate Saline Buffer (PH 7.3-7.4)

Prepare stock solutions of 0.2 M mono- and disodium phosphate in 8.5% salt solutions and dilute 1:10 for preparation of 0.02 M phosphate saline buffer.

Stock solution 1

Sodium phosphate dibasic anhydrous Na_2HPO_4 (anhydrous) (reagent grade) 28.4 g
NaCl (reagent grade) 85.0 g
Distilled water to make 1 liter

Stock solution 2

Sodium phosphate monobasic monohydrate NaH_2PO_4 H_2O (monohydrate) (reagent grade)4* 227.6 g
NaCl (reagent grade) 85.0 g
Distilled water to make 1 liter

To obtain 0.02 M phosphate-buffered saline (0.85%), make 1:10 dilutions of each stock solution. For example:

Stock solution 1 50 ml	Stock solution 2 10 ml
Distilled water 450 ml	Distilled water 90 ml
Approximate pH, 8.2	Approximate pH, 5.6

Using pH meter, titer diluted solution 1 to pH 7.3-7.4 by adding about 65 ml of diluted solution 2. Use resulting 0.02 M phosphate saline buffer solution in the lysostaphin susceptibility test on *S*. *aureus*.

NOTE: Do not titer 0.2 M phosphate buffer to pH 7.3-7.4 and then dilute to 0.02 M strength. This results in a drop in pH of approximately 0.25. Addition of 0.85% salt after pH adjustment also results in a drop of approximately 0.2.

R43. Physiological Saline Solution 0.85% (Sterile)

NaCl 8.5 g

Distilled water 1 liter

Dissolve 8.5 g NaCl in water. Autoclave 15 min at 121°C. Cool to room temperature.

R44 Potassium Hydroxide Solution. 40%

KOH 40 g

Distilled water to make 100 ml

R45. Saline Solution. 0.5% (Sterile)

NaCl 5 g

Distilled water 1 liter

Dissolve NaCl in water. Autoclave at 121°C for 15 min.

R46. Salts-Phosphate Buffered Saline Solution (Salts-PBS)

NaCl 121 g

KCl 15.5 g

$MgCl_2$ 12.7 g

$CaCl_2 \cdot 2H_2O$ 10.2 g

$NaH_2PO_4 \cdot H_20$ 2.0 g

$Na_2HPO_4 \cdot 7H_20$ 3.9 g
Distilled water 1 liter

Adjust pH to 7.4.

R47. Slide Preserving Solution

Prepare 1% acetic acid solution (10 ml glacial acetic acid, reagent grade + 990 ml distilled water). Add 1 ml glycerin to each 100 ml of solution.

R48. Sodium Bicarbonate Solution. 10%
Sodium bicarbonate 100 g
Distilled water to make 1 liter

Sterilize by filtration.

R49. 0.2 M Sodium Chloride Solution
NaCl 11.7 g
Distilled water to make 1 liter

Dispense in suitable containers. Autoclave 15 min at 121°C.

R50. 1 N Sodium Hydroxide Solution
NaOH 40 g
Distilled water to make 1 liter

Use for adjusting pH of culture media.

R51. 10 N Sodium Hydroxide Solution

NaOH 400 g

Distilled water to make 1 liter

R52. Standard Saline Citrate (SSC) Solution (20%)

NaCl (reagent grade) 175.3 g

Sodium citrate 88.2 g

Dissolve in 800 ml deionized water and adjust to pH 7 with 10 N NaOH. Bring volume to 1 liter.

6X SSC

NaCl (reagent grade) 52.6 g

Sodium citrate 26.5 g

Dissolve in 800 ml deionized water and adjust to pH 7 with 10 N NaOH. Bring volume to 1 liter.

3X SSC

6X SSC 500 ml

Deionized water 500 ml

2X SSC

6X SSC 333 ml

Deionized water 667 ml

R53. Tris-Buffered Saline (TBS) (PH 7.5)

Tris 2.42 g

NaCl 29.24 g
Double distilled water to make 1 liter

Dissolve ingredients. Adjust pH to 7.5 with HCl and bring volume to 1 liter.

R54.Tris-Buffered Saline (TBS), with Gelatin

1% solution
Gelatin 1 g
TBS, pH 7.5 100 ml

3% solution
Gelatin 3 g
TBS, pH 7.5 100 ml

Add gelatin to TBS at 40°C. Stir to dissolve. Cool to room temperature before use.

R55. Tris-Buffered Saline (TBS)-Tween
Tween 2050 F1TBS, pH 7.5 100 ml

Dissolve Tween 20 in TBS.

R56. Tris-Buffered Saline (TABS), 1% or 3% Gelatin, or Tween 20

Tris 2.42 g
NaCl 29.24 g
Distilled water 1 liter

Dissolve ingredients in distilled water by heating and stirring. Adjust pH to 7.5 with HC1. Autoclave 15 min at 121°C.

For 1% and 3% Gelatin-TABS, add 10 g and 30 g gelatin, respectively, to ingredients before autoclaving. Adjust final pH to 7.5 with HCl.

For Tween-TABS, add 0.5 ml Tween 20 to ingredient and adjust pH to 7.5 before autoclaving.

R57. Voges-Proskauer (VP) Test Reagents

Solution 1

alpha-Naphthol 5 g
Alcohol (absolute)100 ml

Solution 2

Potassium hydroxide 40 g
Distilled water to make 100 ml

Voges-Proskauer (VP) test. Transfer 1 ml of 48 h culture to test tube and add 0.6 ml solution 1 and 0.2 ml solution. Shake after adding each solution. To intensify and speed reaction, add a few creatine crystals to mixture. Let stand at room temperature. Read results 4 h after adding reagents. Development of eosin pink color is positive test.

GLOSSARY

Asexual reproduction: Reproduction in which sex cells are not involved; as by binary fission or budding.

Acute: Having rapid onset, severe symptoms and a short course.

Antigen: Foreign substance when gets into the body induces immune response.

Antibody: Endogenous glycoprotein, which reacts with antigen.

Blood Brain Barrier:

Chronic: Of long duration; denoting a disease with slow progression.

Convulsion: Paroxysms of involuntary muscular contraction and relaxation.

Congenital: Present at birth.

Dehydration: a condition resulting from loss of excessive body fluid.

Disease: Pathological condition of the body that presents with group of clinical symptoms and signs; and abnormal laboratory findings.

DNA: A nucleic acid consisting of deoxyribose, phosphoric acid and bases.

It is present in chromosomes of the nuclei of cells, is the chemical basisof heredity and the carrier of genetic information for living cells.

Endogenous: Produced or originating from with in a cell or organism.

Endoplasmic reticulum: Net work of membraneous tubules with in a cell and involved in transport of proteins synthesized on the ribosomes; and synthesis of lipids.

Electrolyte: An ionized salt in blood, tissue fluids and cells.

Fastidious: Requiring precise nutritional and environmental conditions for growth and survival.

Genome:

Hematogenous: Through the blood stream.

Histone: Positively charged protein that is part of chromatin in eukaryotic cells.

Hydrocephalus: Excessive fluid in the brain ventricles.

Iatrogenic: Any adverse mental or physical condition induced in a patient through the effects of treatment by a physician or surgeon.

Incubation Period: The time interval between exposure and development of disease.

Infertility: The inability or diminished ability to produce offspring.

Lysosome: Cell organelle that is part of the intracellular digestive system.

Microscopic: Can not be observed with naked eye.

Macroscopic: Can be observed with naked eye.

Microscope: Optical instrument that greatly magnifies minute objects.

Microorganism: Minute living body not seen with naked eye.

Mitochondria: Oval shaped cell organelles that contain the enzymes for aerobic stages of cell respiration and thus the site of ATP synthesis.

Microtubule/Microfilament: Tubular structures present in an eukaryotic cell and are important for maintaining rigidity; transporting substances in different directions with in a cell.

Nuclear membrane: A membrane enveloping nucleus of a living cell.

Nucleolus: Structure in the nucleus of a cell made of DNA, RNA, and protein.

It is the site of synthesis of ribosomal RNA(rRNA).

Purulent: Full of pus

Postulate: A supposition or view, usually self-evident that is assumed with out proof.

Pleural effusion: Fluid accumulated in pleural cavity.

Primary stain: The dye applied first in differential staining procedures.

Counter stain: The dye which stains the micro-organism or part of it after decolorization of the primary stain.

Mordant: It is a substance which facilitates the reaction of the primary stain with the material to be stained. It combines with the stain and then facilitate the reaction. Basic mordant reacts with acidic stain and acidic mordant react swith basic stain.

Decolorizer : It is a chemical added in differential staining procedure to selectively remove the stain from the materials that are not intended to be stained.

Pathogen : Organism that causes disease

Virulence : Degree of pathogenicity in causing disease which depends on

toxin production and invasiveness.

Invasiveness : The ability to penetrate in to the tissues, overcome the host defense, multiply and disseminate widely.

Toxicity: The capacity to damage the tissues.

Opportunistic : Normally harmless organism causing disease during lowered host resistance.

Infection: The result of breakdown in the host-parasite relationship and follows when the balance is tipped in favor of the parasite.

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