COLLEGE NAME & CODE : Periyar Arts College, Cuddalore-01 & 105
COURSE NAME & CODE : III B.Sc., Microbiology & U26
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SUBJECT TITLE & CODE : MEDICAL BACTERIOLOGY & BMB52

UNIT-III

- Classification,
- morphology, cultural characteristics, pathogenicity, epidemiology, laboratory diagnosis, treatment, prevention and control of diseases caused by:
  - Staphylococci,
  - Streptococci,
  - Pneumococci,
  - Neisseriae(Gonococci & Meningococci),
  - Corynebacterium,
    - Mycobacterium,
    - Clostridium,
    - Bacillus,
    - Pseudomonas
    - Haemophilus.

STAPHYLOCOCCUS

- Staphylococci are Gram positive cocci that occur in grape-like structure.
- Cause localized suppurative lesions in human (penicillin resistance).
- Sir Alexander Ogston, a Scottish surgeon, he actually named it because of its role in abscesses and suppurative lesions.
- Strains from pyogenic lesions were found to produce golden yellow colonies.
- Strains from normal skin – white color colonies on solid media.
- Rosenbach named *Staph. aureus* & *Staph. albus*.
- The most important property of them is virulence & production of an enzyme called coagulase and to a lesser extent fermentation of mannitol.

Staphlylococcus aureus, Staphylococcus epidermidis and *Staphylococcus epidermidis*

Based on the above properties, *Staphylococcus* is classified into two groups.

i. Coagulase Positive - *Staphlylococcus aureus* & *Staphylococcus pyogenes*
(Coagulase test - Postive,
Ferment mannitol,
Pathogenic strain)

STAPHYLOCOCCUS AUREUS

MORPHOLOGY

- G +ve spherical cocci, approximately 1 µm in dm arranged characteristically in grape-like clusters.
- Cluster formation is due to cell division occurring in three planes, with daughter cells tending to remain in close proximity.
- They may also be found singly in pairs & in short chains of ¾ cells, especially when examined from liquid cultures.
- Never forms chain like structure.
- Non-motile, non-spore forming.
- Few strains possess microscopically visible capsules, particularly in young cultures.

CULTURAL CHARACTERISICS

- Aerobic and facultative anaerobes.
- Optimum temperature being 37 ˚C & pH 7.4 – 7.6 but readily grows on ordinary media within a temperature range of 10-42˚C.

- On Nutrient Agar (24 hr incubation), colonies are large (2-4 mm dm), circular, convex, slightly opaque & easily emulsifiable. Most of the strains produce 'golden yellow pigment'. The pigment is believed to be a lipoprotein allied to carotene.
- On Blood agar, most of the strains are hemolytic (β hemolytic), especially when incubated under CO_2.
- Uniform turbity in liquid media.
- Selective media: Mannitol Salt Agar (MSA).

BIOCHEMICAL REACTION

- ✓ They ferment a number of sugars producing acid but no gas.
- ✓ Reduce Nitrates to Nitrites, Liquify gelatin, Hydrolyse urea, Catalse - Positive,
- ✓ MR & VP – Positive, Indole – Negative

RESISTANCE

- They are most resistant, non-sporing bacteria.
- Isolated from pus after 2-3 months.
- They can withstand 60˚C for 30 minutes.
- Resist 1% phenol for 15 min.Mercury perchloride 1% solution kills them in 10 min.
- Resistant to lysozyme.
- Penicillin resistance - Produces β lactamase (Penicillinase) which inactivates penicillin by splitting the β-lactum ring.

PATHOGENICITY & VIRULENCE

The virulence factor include the following

A. <u>Cell Associated Polymers:</u>

Peptidoglycan
Teichoic acid
All these polymer makes the pathogen more efficient in causing the disease.

B. <u>Cell Surface Proteins:</u>

Protein-A – Chemotactic, antiphagocytic & anticomplementary effects.
Clumping factors: "Bound Coagulase" which is responsible for the 'slide' or 'coagulase test'.

C. <u>Extracellular Enzymes:</u>

Coagulase
Lipases
Hyaluronidase

D. <u>Toxins:</u>

Staph. aureus produce exotoxin which are as follows

Cytolytic toxins
Enterotoxins
TSST

Exfoliative toxins

PATHOGENESIS

They produce disease either by multiplying or by producing toxic enzymes. The most common bacterial infections of this are as follows.

a) Skin & Soft Tissues:

i. Folliculitis – inflammation of hair follicles
ii. Cellulitis - inflammation of soft & connective tissues.
iii. Furuncle & Carbuncle – Necrotising infection of skin & subcutaneous tissue composed of a cluster of furuncle with multiple draining sinuses.

b) Musculoskeletal:

i. Osteomyelitis – Bone inflammation
ii. Arthritis – Joint inflammation

c) Respiratory:

i. Tonsillitis
ii. Pharyngitis
iii. Sinusitis
iv. Otitis media

d) Endovascular:

i. Bacteremia
ii. Septicemia

e) CNS:

i. Abscess (A cavity formed by liquefaction necrosis within solid tissues)
ii. Meningitis

f) Urinary Infection:

i. Urinary isolates are to be considered significant even with low colony counts, as they may be related to bacteremia.

LABORATORY DIAGNOSIS

i. Specimen collection

The specimens to be collected depend on the type of lesion

e.g. Pus from suppurative lesions

Sputum from respiratory infections

ii. Culture

Plating the specimen for culturing the *Staphylococcus* sp. on blood agar and after overnight incubation, the organisms are cultured on selective media.

iii. Microscopy

Pure culture of the pathogen is grown in a broth medium.

iv. Biochemical tests

To confirm the Staphylococcus, coagulase test must be done.

A. Coagulase tests

They are of 2 types

a. Tube coagulase test

It is used to detect 'free coagulase'

b. Slide coagulase test

B. Phosphatase Test
C. Antibiotic sensitivity test
D. Bacteriophage Typing
E. Plasmid profile
F. DNA finger printing
G. Ribotyping
H. PCR based analysis for genetic pleomorphism.

TREATMENT

- Benzyl penicillin is the most effective antibiotic
- Cloxacillin is preferred
- For life-threatening conditions, Vancomycin is recommended.

STREPTOCOCCI

- Streptococci are G+ve cocci, occur in chains.
- Causes pyogenic lesions with the tendency to spread.
- Non-suppurative lesions, acute rheumatic fever & glomerulonephritis.

MORPHOLOGY

- 0.5-1.0 µm in dm
- Occur in chains / chains are longer in liquid medium than in solid.
- They are non-spore forming & non-motile.
- Some strains are Strep. pyogenes & some strains may possess capsules.
- Strains of Gp A & C – Hyaluronic acid
  Gp B & D – Polysacharide.

CULTURAL CHARACTERISTICS

- They are aerobes & facultative anaerobes.
- Opt temp for growth is 37°C.
- They grow only on media enriched with blood / serum.
- On Blood agar

  Small, circular, semi-transparent, low convex discs with an area of clear hemolysis around them.

BIOCHEMICAL CHARACTERS

- Produces acid but not gas / ferment several sugars.
- Catalase –ve.
- Not soluble in 10% bile.

RESISTANCE

- Destroyed at 54°C for 30 min.
- They are inactivated by antiseptics.
- Resistant to crystal violet.
- Sensitive to bacitracin.

ANTIGENIC STRUCTURE

1. **Capsule:**.
2. **Cell wall:**

a) Inner Layer:

- Made up of peptidoglycan.
- Responsible for cell wall rigidity.
- Exhibits pyrogenic & thrombolytic activity.

b) Middle Layer:

- Group specific carbohydrate. Serological grouping of Streptococci depends on C carbohydrate antigen.

c) Outer layer:

- Protein + Lipoteichoic acid.
- *Strept. pyogenes* can be classified based on the M, T, R proteins.

3. Pili / Fimbriae:

- Project through the capsule in Gp A Streptococci.
- Made up of M protein partly & covered with lipoteichoic acid.
- Helps in attachment to the epithelial cells.

TOXIN & OTHER VIRULENCE FACTORS

A. Hemolysin
B. Pyrogenic exotoxin
C. Streptokinase (Fibrinolysin)
D. Deoxyribonuclease (Streptodornase)
E. Nicotinamide adenine dinucleotidase (NADase)
F. Hyaluronidase
G. Serum opacity factor.

PATHOGENECITY

I. RESPIRATORY INFECTIONS

a) Streptococcal Sore Throat (SST)
b) Scarlet Fever
c) Otitis Media

II. SKIN & SOFT TISSUE INFECTIONS

a) Cellulitis

b) Impetigo (Streptococcal pyoderma)

c) Erysipelas

d) Toxic Shock like Syndrome

OTHER SUPPURATIVE INFECTION

- It causes abscess in internal organs such as brain, lungs, liver, kidney, etc.,
- **Septicemia**:

  Microorganisms. & its products in blood.

NON-SUPPURATIVE COMPLICATIONS

a) Acute Glomerulo Nephritis (AGN) (Bright's disease)

b) Acute Rheumatic Fever (ARF)

LABORAORY DIAGNOSIS

1. **Specimen**
   - Pus, CSF, Throat swab, Blood – for direct evidence
   - Serum for antibody demonstration
2. **Gram staining**
   - Smears prepared from pus often show Gram positive cocci. This may be single or in pairs rather than definite chains.
3. **Culture**
   - Throat swab is collected & is transported in Pike's medium (Blood agar containing 1 in 1000000 crystal violet & 1 in 16000 sodium azide) then they are plated on blood agar & incubated at 37°C anaerobically or under 5-10% CO_2, as hemolysis develops better under these conditions.
4. **Bacitracin Sensitivity**
   - *Strep. pyogenes* are highly sensitive to bacitracin.
5. **ASO Typing**
   - ASO test is used to diagnose Rheumatic fever & Glomerulonephritis.
   - ASO titer higher than 200 units are considered to significant.
   - In acute rheumatic fever (ARF), titres are high but in AGN titres are low.

PNEUMOCOCCI

Streptococcus pneumoniae

- Diplococci/ capsulated/ flame (Lancet) shaped.
- India-ink – capsule demonstration.
- Previously called as *Diplococcus pneumonia*e.
- Responsible for pneumonia.

MORPHOLOGY

- It is a G+ve cocci with one end broad or rounded & the other end pointed, presenting a flame shaped/ Lancet shaped appearance.
- It occurs in pairs (Diplococci) with the broad ends in opposition.
- They are capsulated enclosing each pair.
- Non-motile, non-spore forming.

CULTURAL CHARACTERISTICS

- They are aerobes & facultative anaerobes. Grows only in enriched media.
- Opt temp for growth is 37°C & optimum pH is 7.8
- On Blood agar

  Colonies are small, dome-shaped glistening with an area of greenish discoloration around them (β-hemolysis). Draughtsman or Carom coin appearance (Fig.)

BIOCHEMICAL REACTIONS

- Ferment several sugars forming acid only.
- They ferment inulin.
- Fermentation can be tested in Hiss's serum water or serum agar slopes.
- They are Catalase as well as Oxidase negative.

RESISTANCE

- Readily destroyed by heat (TDP 52 ºC for 15 min) and antibiotics.
- Sensitive to most antibiotics beta lactams being the drug of choice.

TOXIN & OTHER VIRULENCE FACTORS

a) The Capsular Polysaccharide
b) Pneumolysin
c) Autolysin

PATHOGENECITY

Pneumonia:

- *Step. pneumoniae* are one of the most common bacteria causing community acquired pneumonia (CAP) (both **Lobar pneumonia** & **Bronchopneumonia**).
- Also cause acute tracheobronchitis and empyema.
- Normal mucosal defense mechanism such as entrapment, expulsion and cough reflex, aided by the ciliary escalator effect, prevent the establishment of infection.
- But when normal defenses are compromised by viral infection, the bacteria penetrate the bronchial mucosa and spread through lung along peribronchial tissues and lymphatics.

Meningitis:

- Most serious of pneumococcal infections. It is usually secondary to infections like pneumonia, otitis media, etc.
- Pneumococcal meningitis is an infection of the covering of the brain and spinal cord.
- Symptoms include:

  Stiff neck, Fever, Headache

Suppurative Lesions:

- Empyema, pericarditis, otits media, sinusitis, conjunctivitis, suppurative arthritis and peritonitis.
- They are also responsible for Ocular infections like keratitis, dacrocystitis.

Bacteremia:

- Pneumococcal bacteria can invade the bloodstream, causing bacteremia, and the tissues and fluids surrounding the brain and spinal cord, causing meningitis which may be fatal.
- Symptoms include: Fever, chills, low alertness.

LABORAORY DIAGNOSIS

Specimen

- Sputum, Blood - for direct evidence, CSF & Urine – for antigen detection

Microscopy - Gram staining

- Smears prepared from pus, CSF, aspirated fluids often show Gram positive cocci.

Culture

- Sputum is inoculated on blood agar & incubated at 37º C under 5-10% CO_2. Blood culture in glucose broth.

Molecular methods

- PCR-based methods.

TREATMENT

- Antibiotic of choice is parenteral penicillin in severe cases and amoxicillin in milder ones.
- Many resistant strains originated which caused problems in treatment.
- Vancomycin – life threatening conditions.

GONOCOCCI

NEISSERIA GONORRHOEAE

MORPHOLOGY

- Capsulated Gram negative cocci (Diplococci)
- 0.5-1.0 µm in size, resembling the shape of coffee beans
- Non- spore forming, capable of moving using twitching motility
- Facultatively intracellular

CULTURAL CHARACTERISTICS

- *Neisseria* species are fastidious, require nutrient supplementation to grow in laboratory cultures.
- *N. gonorrhoeae* is usually isolated on Thayer-Martin agar (or VPN) agar with 3-7% CO_2.
- Thayer-Martin agar is a chocolate agar plate (heated blood agar) containing nutrients and antimicrobials (vancomycin, colistin, nystatin, and trimethoprim).

BIOCHEMICAL REACTIONS

- Oxidase : Positive
- Catalsae : Positive
- Nitrate : Negative
- DNase : Positive
- Ferment maltose with acid production

VIRULENCE FACTORS

- Pili: Piliated Gonococci – Virulent; Non-piliated Gonococci – Avirulent
- Endotoxin – Lipooligosaccharide (LOS) but weaker
- Outer membrane proteins (OMP)
- IgA protease

PATHOGENESIS

- Humans are the only natural hosts
- Acquire through sexual contact
- Adhesion on urethra or mucosal surface (pili)
- Cocci penetrate through intercellular spaces and reach the sub-epithelial connective tissue

- Incubation period = 2-8 days.

DISEASES

- *N. gonorrhoeae* causes following infections
    1. Gonorrhea
    2. Neonatal conjunctivitis (Ophthalmia neonatarum)
    3. Disseminated - Pelvic inflammatory disease (PID)

LABORATORY DIAGNOSIS

- It is frequently isolated from samples such as blood, urethral discharge in men, cervical discharge in females.

Gram staining

- The diagnosis is suggested by the finding of Gram negative bacteria bean shaped capsular diplococci.

Culture

- *N. gonorrhoeae* is cultured on Thayer-Martin agar incubated at 37 ºC in a 5% CO_2 atmosphere..
- *N. gonorrhoeae* grows rapidly producing small colonies, no hemolysis.

Oxidase test

- Violet-purple color indicates a positive result.

TREATMENT

- Penicillin
- Tetracycline (if beta-lactamase positive strain)
- Ceftriazone + Doxycyline (Erythromycin)
- Can use Cephalosporins and Fluoroquinolones

PREVENTION

- Transmission can be reduced by using latex barriers (e.g. condoms or dental dams) during sex and by limiting sexual partners.
- Condoms and dental dams should be used during oral and anal sex, as well.
- Spermicides, vaginal foams, and douches are not effective for prevention of transmission

MENINGOCOCCI
NEISSERIA MENINGITIDIS

MORPHOLOGY

- Capsulated Gram negative cocci (Diplococci)
- 0.5-1.0 µm in size
- Kidney shaped, flat sides adjacent
- Usually intracellular
- Non-motile, non-spore forming

CULTURAL CHARACTERISTICS

- Color : Bluish grey
- Shape : Round
- Size : About 1 mm
- Surface : Smooth
- Elevation : Convex
- Opacity : Translucent
- Consistency : Butyrous

BIOCHEMICAL REACTIONS

- Oxidase : Positive
- Catalsae : Positive
- Nitrate : Negative

ANTIGENIC PROPERTIES & VIRULENCE FACTORS

d) Capsular Polysaccharide

e) Outer membrane proteins

f) Pili

g) Toxin

h) Enzymes

TRANSMISSION

- Although dangerous disease, not easily spread.

- Through droplets from mouth and nose.
- Transmission not necessarily gives rise to disease.
- 15% adults carry disease causing strains in nose and throat.

PATHOGENESIS

Bacteria reach the intracranial structures in one of the 3 ways

- Hematogenous spread
- Extension from the juxtacranial structures
- Iatrogenic sources

DISEASES caused

Meningitis, Meningococcemia, Pneumonia, Arthritis, Urethritis

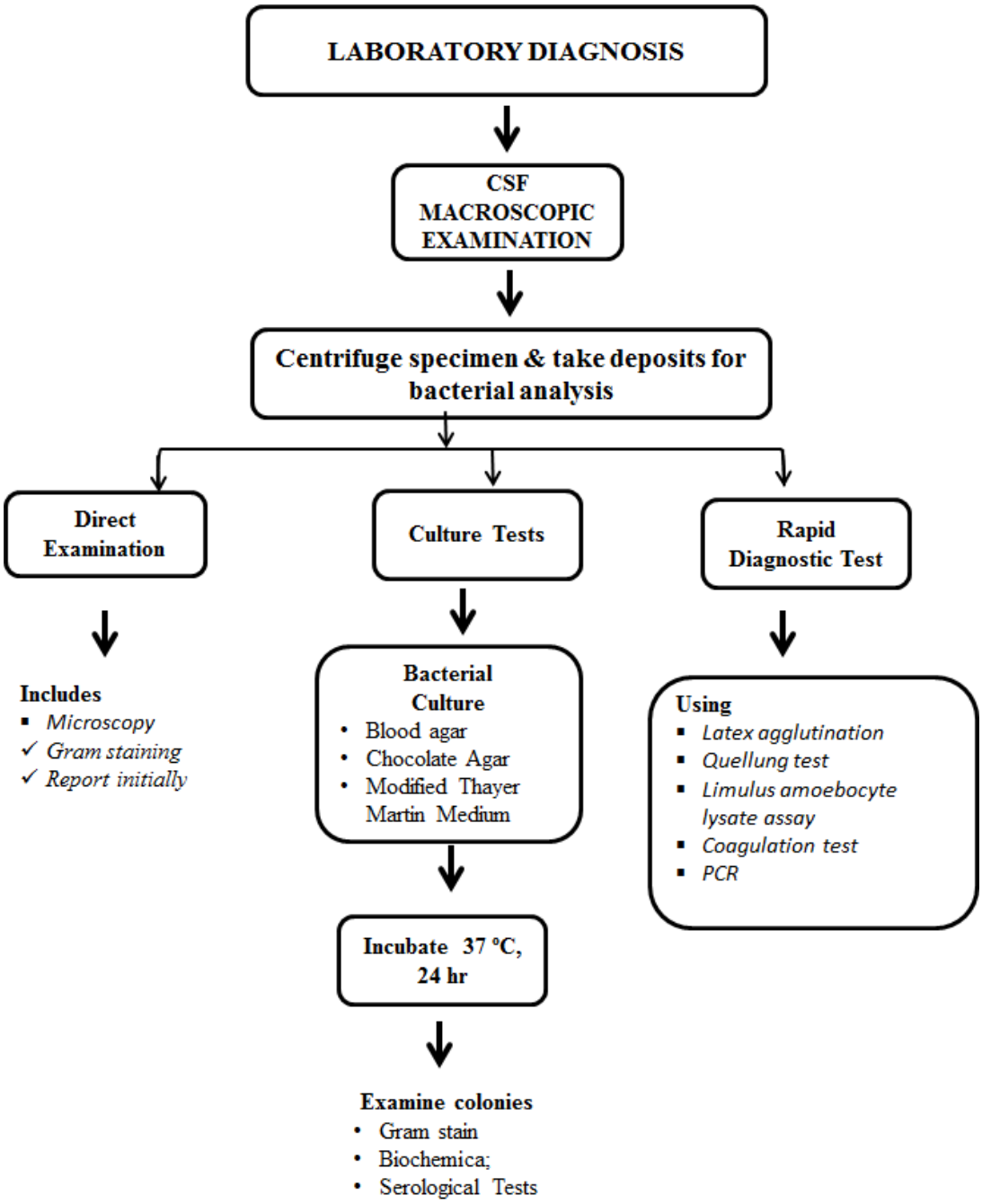
MENINGITIS

- Meningitis is an inflammation of leptomeninges within the subarachnoid space.
- **SIGNS & SYMPTOMS**

  Includes petechial rash (rapidly spreading),

  Photophobia (intolerance of bright light)

  Phonophobia (intolerance of noises)
- **Bacterial meningitis** can be classified as
  - Acute
  - Chronic
- Acute Meningitis: Onset of meningeal symptoms over the course of hours to days.
- Chronic Meningitis: Onset in weeks to months.
- Acute Pyogenic Meningitis: Infectious inflammatory infiltration of leptomeninges caused by bacteria.



TREATMENT

- Ceftriaxone / Cefotaxime
- Vancomycin
- Ampicillin

MYCOBACTERIUM TUBERCULOSIS

INTRODUCTION

Tuberculosis

- Tuberculosis is an Ancient disease
- Spinal tuberculosis in Egyptian Mummies
- History dates to 1550 -1080 BC
- Robert Koch – Discoverer of *Mycobacterium tuberculosis* (Koch's disease)
- It is a major human disease – affects healthy people
- Problems
    - association with AIDS
    - multiple-drug resistance
    - chronic disease
    - prolonged treatment

Classification of Mycobacteria

1. **Tubercle bacilli**
    a) Human – MTB
    b) Bovine – M. bovis
    c) Murine – M. microti
    d) Avian – M. avium
    e) Cold blooded – M. marinum
2. **Lepra bacilli**
    a) Human – M. leprae
    b) Rat – M. leprae murium
3. **Mycobacteria causing skin ulcers**
    a) M. ulcerans
    b) M. belnei
4. **Atypical Mycobacteria (Runyon Groups)**
    a) Photochromogens
    b) Scotochromogens
    c) Nonphotochromogens
    d) Rapid growers
5. **Johne's bacillus**
    M. paratuberculosis
6. **Saprophytic mycobacteria**
    a) M. butyricum
    b) M. phlei
    c) M. stercoralis
    d) M. smegmatis
    e) Others

Epidemiology

- One of the most serious infectious diseases in the developing countries
- *One third* of the world's population infected with *M. tuberculosis*
- *Thirty million* people have active disease
- *Nine million* new cases occur
- *Three million* people dies of the disease, each year.

MORPHOLOGY

- SHAPE – long, slender, straight or slightly curved rod
- SIZE - Thin straight rods 0.4 - 3 µ
- Non-spore forming, Non-motile, non-capsulated, rod shaped
- Very slow growing

CULTURAL CHARACTERISTICS

- It includes non-selective & selective media. There are three general formulation that can be used for both, they are:
    - Semisynthetic agar media
    - Inspissated egg media
    - Broth media
- *M. tuberculosis* has unusually waxy walls, slow growers and among the most recalcitrant bacteria to treatment
- **Slow-growing**: generation time of 12 -18 hours (20-30 minutes for *Escherichia coli*)
- Colonies appear after 2 weeks or at 6-8 weeks
- **Hydrophobic** with a high lipid content in the cell wall
- **Ziehl-Neelsen Technique** of staining is employed for identification

RESISTANCE

- Not specifically resistant to heat: destroyed by 60 ºC in 20 min.
- In sputum: can survive 20-30 hr
- Relatively resistant to disinfectants, survives exposure to
    - 5% Phenol
    - 15% Sulphuric acid
    - 3% Nitric acid
    - 5% Oxalic acid
    - 4% NaOH

BIOCHEMICAL REACTIONS

- **Niacin Test** - Human MTB produces niacin when grown in egg medium
- **Aryl Sulphatase Test -** Enzyme Aryl sulphatase formed by only atypical mycobacteria
- **Neutral red Test** – Virulent strains of tubercle bacilli bind neutral red in alkaline solution while avirulent strains can not
- **Catalase peroxidase Test** – Most atypical mycobacteria are strongly catalase positive while MTB is weakly positive. MTB is strongly peroxidase positive while atypical mycobacteria are negative.
- **Nitrate reduction Test** – Positive in MTB and negative in *M. bovis*.

PATHOGENESIS

TUBERCULOSIS

- Tuberculosis (TB) is a contagious disease, like the common cold
- Only people who are sick with TB in their lungs are infectious

Mode of Transmission:

- Spreads through the air
- Cough, sneeze, talk or spit propels TB germs (bacilli) into the air
- ~ >500 droplets (Droplet Nuclei) are produced per cough
- The average TB patient generates 75,000 droplets per day before therapy

Mechanisms & Mode of Infection:

- inhaling a small number of bacilli (droplet infection – Pulmonary tuberculosis – *M. tuberculosis*)
- ingestion of milk from infected cattle (Intestinal tuberculosis – *M.bovis*)
- Contamination of abrasion – laboratory workers – skin infection
- They multiply in the alveolar macrophages

Basis of Tubercle formation (GRANULOMA):

- Tubercle is an avascular granuloma containing central zone of giant cells with or without caseation and peripheral zone of lymphocytes and fibroblasts
- Lesions may be exudative or productive
    a. Pulmonary tuberculosis
    b. Extra-Pulmonary tuberculosis

a. <u>Pulmonary tuberculosis</u>

- Infects lungs
- Distributed within macrophages
- Facultative intracellular pathogen
    - Inhibits phagosome-lysosome fusion
    - Resists lysosomal enzymes
- Others MTB factors
    - Mycobactin – siderophore
    - Cord factor – damages mitochondria

b. <u>Extra Pulmonary tuberculosis</u>

Bacteria on circulation leads to bacteremia leads to involvement of

• Gut	• Genito Urinary system	• Meningitis,
• Skin	• Bone marrow	• Gastro intestinal system
• Lymphnodes	• Spinal infection	• Arthritis

LABORAORY DIAGNOSIS

- Acid fast bacteria demonstration in sputum smear
- Culture on LJ media
- Biochemical identification
- Antibiotic sensitivity test
- Skin testing
    - Delayed hypersensitivity
    - Tuberculin
    - Protein Purified Derivative
- X-ray
- PCR

TUBERCULIN TEST (Mantoux Test)

- **Delayed hypersensitivity skin test** to assay
    - Cell mediated immunity (**CMI**) to tubercle bacillus
- **Material**: Purified protein derivative (PPD)
- **Dose**: 0.1 ml of PPD
- **Route of Administration**: Intradermal
- **Positive test**: Induration equal or greater than 10 mm

Develop 48-72 hr after injection

- **Negative test**: Induration less than 10 mm

  Develop 48-72 hr after injection
- An individual who has had a primary infection with tubercle bacilli develops induration, edema, erythema in 24-48 hours, and with very intense reactions, even central necrosis.

Interpretation of Tuberculin Test

- A **positive tuberculin test** indicates that an individual has been infected in the past
- In an individual who has not had contact with mycobacteria, no reaction
- It does not suggest that active disease or immunity to disease is present
- Tuberculin-positive persons are at risk of developing disease from **reactivation of the primary infection**, whereas **tuberculin-negative persons** who have never been infected are not subject to that risk, though they may become infected from an external source

TREATMENT

- Anti-tuberculous drugs
    - INAH (Isoniazid iso-nicotinyl hydrazide)
    - Rifampicin
    - Ethambutol
    - Pyrazinamide
- Multi-drug resistant tuberculosis
    - MDR TB is a global threat.

CLOSTRIDIUM sp.,

Clostridium tetani

Clostridium botulinum

Clostridium perfringens

Clostridium difficile

CLOSTRIDIUM BOTULINUM

Categorization

Cell Wall: Gram Positive Shape: Spore-forming Rod

Metabolism: Obligate Anaerobe

Transmission

The causes of botulism tend to be slightly different in adults and infants.

Adult Botulism

C. botulinum spores may contaminate meats and vegetables that undergo packaging in cans. Within the anaeorbic environment of the can the spores can germinate, grow, and elaborate the Botulinum Exotoxin. Thus disease in adults is caused by ingestion of the pre-formed botulinum exotoxin while the actual bacteria may be dead.

Infant Boutlism

Food contaminated with C. botulinum spores, especially honey products, is ingested by infants. The spores germinate inside the infant's GI system, grow, and elaborate botulinum exotoxin. Thus disease in infants is caused by growing bacteria within the GI system which are actively elaborating exotoxin.

Virulence Factors

Botulinum Exotoxin:

Botulinum exotoxin is a neurotoxin which irreversibly blocks release of acetylcholine (Ach) from peripheral nerves at the synapse. The ingested toxin is absorbed and enters the blood which delivers it to peripheral nerve synapses. The gene for the botulinum exotoxin is carried on a lysogenic phage and is thus transmitted by transduction.

Clinical Consequences

Adult Botulism

Characterized by a descending paralysis which begins with diplopia and dysphagia. Paralysis continues with general muscle weakness ultimately resulting in respiratory depression and death. Importantly, this paralysis is characterized by flaccidity, rather than the rigor associated with *C. tetani*.

Infant Botulism

Infants first develop constipation followed by dysphagia and a generalized muscle weakness.

Treatment

Adult botulism: Antitoxin, an antibody which binds the botulinum toxin

Infant botulism: Supportive therapy as infants usually recover spontaneously

CLOSTRIDIUM PERFRINGENS

TRANSMISSION

Tissue Infection

C. perfringens spores are commonly found in the soil and can infect wounded tissue. The anaerobic, necrotic environment of wounded tissue allows spores to germinate and elaborate powerful exotoxins which promote further tissue necrosis. *C. perfringens* is no longer terribly common but was especially worrisome for soldiers in battlefields.

Gastrointestinal Infection

C. perfringens spores may exist in contaminated meat products and germinate in relatively anaeorobic environments of the GI system following ingestion.

VIRULENCE FACTORS

C. perfringens possesses nearly twenty different exotoxins and enterotoxins. Exotoxins include a variety which lead to tissue destruction and promote rapid necrosis.

CLINICAL CONSEQUENCES

C. perfringens can cause a variety of pathologies with a range of clinical seriousness. Tissue infections may begin as fairly localized infections but can also manifest as rapidly spreading necrosis. Gastrointestinal infection usually manifests as a self-limited bout of food poisoning.

Cellulitis

These are relatively well-localized infections of the skin's dermis. Fermentation of extracellular sugars by *C. perfringens* characteristically produces a large amount of gas which becomes trapped under overlying skin, giving it a crackly consistency described as "crepitation".

Necrotizing Fasciitis

C. perfringens can spread along fascial planes, leaving a wake of tissue necrosis. Spread can be rapid or slow and is often associated with overlying crepitation.

Clostridial Myonecrosis (Gas Gangrene)

Gas gangrene involves spread of *C. perfringens* into adjacent skeletal muscle and is usually the result of deep wound infections often associated with battlefield injuries. Infection of muscle is rapidly necrotic and produces large amounts of gas which is observed as extensive crepitation. Systemic failure of multiple organs is often observed in this setting.

Food Poisoning

Remarkably, despite the above, GI system infection of *C. perfringens* due to ingestion of spore-contaminated meats usually manifests as a bout of self-limited watery infectious diarrhea due to elaboration of enterotoxins with an incubation time of greater than 8h.

TREATMENT

Tissue Infections

Surgical debridement should be performed immediately. Hyperbaric oxygen is of dubious value but is occasionally performed with the notion that *C. perfringens* is an obligate anaerobe. When antibiotics are administered, natural penicillin can be used.

BACILLUS

Members of the genus Bacillus are ubiquitous, present in soil, dust, air and water and are frequently isolated as contaminants in bacteriological culture media. *B. anthracis*, the causative agent of anthrax, is the most important pathogen of the group. *B. cereus* can cause food poisoning. They are generally motile with peritrichous flagella except the anthrax bacillus which is non-motile.

BACILLUS ANTHRACIS

It is the causative agent of anthrax, a disease primarily of animals, and man gets infected secondarily.

MORPHOLOGY

Bacillus anthracis is a Gram positive, none acid-fast, non-motile, large (3-10 µm x 1-1.6 µm), rectangular, spore forming bacillus. The spores are refractile, oval and central in position and are of the same width as the bacillary body so that they do not cause bulging of vegetative cell.

In infected tissues, the bacilli are found singly, in pairs or in short chains, the entire chain being surrounded by capsule. The capsule is polypeptide in nature. In cultures, the bacilli are arranged end-to-end in long chains. The ends of the bacilli are truncated or often concave and somewhat swollen so that a chain of bacilli presents a bamboo-stick appearance.

CULTURE

Bacillus anthracis is an aerobe and facultative anaerobe, with a temperature range for growth being 12-45 ºC (optimum 35-37 ºC.

C. Biochemical Reactions

Glucose, maltose and sucrose are fermented with production of acid only. Catalase is formed and nitrates are reduced to nitrites.

D. Pathogenesis

Anthrax is primarily a disease of animals like cattle and sheep, and less often of horses and swine, Infection occurs in susceptible animals by ingestion of the spores present in the soil. Direct spread of disease from animal to animal is rare. Infected animals discharge large number of bacilli from the mouth, nose and rectum. These bacilli sporulate in soil and remains as the source of infection. The disease is usually a septicaemic but may sometimes be localised.

Human Anthrax

- Humans are occasionally secondarily infected from diseased animals. There are three clinical type of disease based on route of infection
  cutaneous,
  pulmonary and
  intestinal
- All types lead to septicaemia and meningitis.
- Cutaneous anthrax follows entry of the spores through the abraded skin. The face, neck, hands and back are commonly affected sites.

E. Laboratory Diagnosis

1. Specimens

Swabs, fluid or pus from pustules; sputum and blood from pulmonary and septicaemic anthrax are generally collected. Laboratory personnel should take additional protective precautions against infection during handling the material.

2. Microscopy

Gram stained smear from the specimen shows often chain of large Gram positive bacilli. Capsule appears as a clear halo around the bacterium by India-ink staining.

3. Culture

Specimen is inoculated on nutrient agar medium and incubated at 37 ºC for overnight. Medusa head colonies appear on the medium. Smears made from these colonies show typical Gram positive spore bearing bacilli.

4. Animal Inoculation

White mouse or guinea pigs are injected with exudate or culture. Animal dies in 36-48 hours. Smears made heart blood and sputum show bacilli.

5. Serology (Ascoli's Thermoprecipitin Test)

The tissues are ground up in saline and boiled for 5 minutes and filtered. When this extract is layered over the anti-anthrax serum in a narrow tube, a ring of precipitate appears at the junction of two liquids within five minutes in a positive ease. It is mainly used for rapid diagnosis when the sample received is putrid and viable bacilli are unlikely to be found.

F. Treatment

Doxycilline and ciprofloxacin are used for treatment.

PSEUDOMONAS

PSEUDOMONAS AERUGINOSA

MORPHOLOGY

It is slender, Gram negative bacillus, 1.5-3 µm x 0.5µm, non-capsulated, non-sporing and is actively motile by a polar flagellum.

CULTURE

It is a strict aerobe and grows well on ordinary media like nutrient broth and nutrient agar, The optimum temperature for growth is 37 ºC, but growth occurs at a wide range of temperature 5 ºC to 42 ºC.

1. Nutrient Agar

Colonies are smooth, large, translucent, low convex, 2-4 mm in diameter. The organism produces a sweetish aromatic odour. There is greenish blue pigment which diffuses into the medium (Fig, 32. l).

2. Blood Agar

Colony characters are similar to those on nutrient agar. Many strains are haemolytic on blood agar.

PIGMENT PRODUCTION

Ps. aeruginosa produces a number of pigments which diffuse into surrounding medium. These pigments are:

1. Pyocyanin

2. Fluorescin (Pyoverdin)

3. Pyorubin

4. Pyomelanin

BIOCHEMICAL REACTIONS

Ps. aeruginosa derives energy from carbohydrates by oxidative breakdown rather than a fermentative metabolism. It utilises only glucose oxidatively with acid production. All strains of *Ps. aeruginosa* are oxidase positive and utilize citrate as the sole source of carbon. They are catalase positive and indole, MR, VP and H_2S tests negative. They reduce nitrates to nitrites and further to gaseous nitrogen. Important biochemical reactions are summarised below.

RESISTANCE

It is killed by heating at 55ºC for one hour. It is resistant to the chemical disinfeetants and can even grow in certain antiseptics like quaternary ammonium compounds, chloroxylenol and hexa-chlorophane. It is sensitive to 2% aqueous alkaline solution of glutaraldehyde and also to silver salts. Due to its sensitivity to silver salt, silver sulphonamide compounds have been applied as topical creams in burns.

PATHOGENESIS

It causes infections more common in patients with neutropenia, cystic fibrosis, burns and those on ventilators. It is the most important agent causing nosocomial infections. It is due to its resistance to common antibiotics and antiseptics that it establishes itself widely in hospitals. Equipment such as respirators and endoscopes, articles such as bed pans, and antiseptic or disinfectant solutions may be frequently contaminated. The other common infections caused by it are;

i. Urinary tract infections following catheterisation.
ii. Acute purulent meningitis following lumbar puncture,
iii. Post-tracheostomy pulmonary infection
iv. Septicaemia in patients who are debilitated due to malignancy or immune-suppressive therapy.
v. Wound and burn infections,
vi. Chronic otitis media and otitis externa
vii. Eye infections.
viii. Infantile diarrhoea.

LABORATORY DIAGNOSIS

1. Specimens

Pus, wound swab, urine, sputum, blood or CSF.

2. Culture

Specimens may be inoculated on nutrient agar, blood agar or MacConkey agar and incubated at 37 ºC for 18-24 hours.

3. Gram Staining and Motility

They are Gram negative bacilli and are actively motile.

4. Biochemical Reactions

The oxidase test is positive within 30 seconds. They are non-fermenter. They break down glucose oxidatively with acid production only,

5 Antibiotic Sensitivity Test

It is useful to select out proper antibiotic as multiple to antibiotics is quite common in *Ps. aeruginosa*.

TREATMENT

It is intrinsically resistant to most of the commonly used antimicrobial agents, Ciprofloxacin, piperacillin, ticarcillin, azlocillin, cefotaxime, ceftazidime, gentamicin and tobramycin are used in treatment of *Ps. aeruginosa* infections.

(Ref: Textbook of MICROBIOLOGY (for Dental Student) – C.P.Baveja, Arya Publications, 2014)

HAEMOPHILUS

The genus Haemophilus contains non-motile, non-sporing, Gram negative bacalli and require one or both of two accessory growth factors (X and V) present in blood.

HAEMOPHILUS INFLUENZAE

MORPHOLOGY

It is a small (1.5µm x 0.3µm), Gram negative, non-motile bacillus showing considerable pleomorphism. It is non-spormg and non.acid-fast.

CULTURE

H influenzae has fastidious growth requirements. It grows better in aerobic than in anaerobic conditions, It requires enriched media such as blood agar or chocolate agar because the accessory growth factors known as X and V present in blood are essential for growth. The optimum temperature for growth is 35—37 ºC some strains require 5-10% It cannot grow on nutrient agar which lacks the accessory growth factors.

1. X Factor

2. V Factor

Satellitism

Although blood agar contains X and V factors, colonies of *H. influenzae* are small due to non.availability of V factor. After inoculating suspected Hvinfluenzae on a blood agar plate, *Staph. auerus* is streaked across the same blood agar plate and incubated at 3TC for 18-24 hours, The colonies of *H. influenzae* will be large and well developed alongside the streak of staphylococci while those further away from staphylococcal streak are smaller. This phenomenon is called satellitism and demonstrates that V factor is available in high concentration near the staphylococcal growth and only in smaller quantities away from it

BIOCHEMICAL REACTIONS

H. influenzae is catalase and oxidase positive, ferments glucose and galactose, reduces nitrate to nitrite. It does not ferment sucrose, lactose and mannitol.

ANTIGENIC STRUCTURE

Isolates occur in capsulated and non capsulated forms. Three major surface antigens are present-capsular polysaccharide, outer membrane protein (OMP) and lipooligosaceharide (LOS).

The capsulated strains produce a capsule which is polysaccharide in nature. On the basis of capsular material, Pitman divided *H. influenzae* into 6 serotypes—a, b, e, d, e, and f. Capsular serotype b strains are associated with most invasive infections. Non.capsulated strains cannot be typed and are called 'non-typable strains'.

VARIATION

Colonies of *H. influenzae* show a smooth to rough (S → R) variation accompanied by loss of capsule and virulence. Non-capsulated strains become capsulated by genetic transformation.

SENSITIVITY TO PHYSICAL AND CHEMICAL AGENTS

It is a delicate organism. It is readily killed by heat (55 ºC for 30 minutes), refrigeration (at 4 ºC), disinfectants and drying,

VIRULENCE FACTORS

1. Capsular polysaccharide:

It resists phagocytosis. Loss of capsule leads to loss of virulence.

2. Pili (Fimbriae): They help in attachment of organisms to epithelial cells.

3. Outer membrane proteins: They contribute in adhesion and invasion of host tissues.

4. IgA1 protease: *H. influenzae* produces a protease that specifically cleaves the heavy chain of IgA1.

PATHOGENESIS

It is obligate human parasite. The organism enters by respiratory route. The non-capsulated strains of *H. influenzae* are regular in the nasopharynx or oropharynx. Capsulated strains cause invasive infections, type b accounting for most cases. The following infections are caused by *H. influenzae*.

1. Meningitis

This is the most serious disease in children of 2 months to 2 years of age. The fatality rate is about in untreated patients. The bacilli reach the meninges from the nasopharynx, apparently through the blood

stream. Majority of the cases are due to type b strains,

2 Acute Epiglottitis

It is the second most common infection caused by *H. influenzae.* This is an acute inflammation of the epiglottis with obstructive laryngeal oedema, seen in children over two years old. This condition is always associated with bacteraemia and the organisms can be isolated from blood cultures.

3. Pneumonia

It typically occurs in infants.

4. Bronchitis

H. influenzae is an important pathogen in the acute exacerbations of chronic bronchitis.

5. Suppurative Lesions

Some of the suppurative lesions include septic arthritis, endocarditis, pericarditis and Otitis media.

LABORATORY DIAGNOSIS

1. Specimens

Depending upon the type of lesion. the following specimens may be collected:

Cerebrospinal fluid (CSF)

Blood

Throat swab

Sputum

Pus

Aspirates from joints, middle ears or sinuses etc.

2. Collection and Transport

Specimens should be collected in sterile containers and under all aseptic conditions. As *H. influenzae* is very sensitive to low temperature, therefore, clinical specimens should never be refrigerated. For optimal yield, specimens should be transported to laboratory without delay and inoculated on culture media immediately.

3. Direct Microscopy

(i) Gram staining

In meningitis, Gram stained smear of CSF shows pleomorphic Gram negative coccobacilli.

(ii) Immunofluorescence and Quellung reaction

These can be employed for direct demonstration of after mixing with specific type b antiserum.

(iii) Type b capsular antigen can also be detected in patient's serum, CSF, urine or pus

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4. Culture

(i) CSF culture

(ii) Blood culture

(iii) Sputum culture

5. Colony Morphology and Staining

After overnight incubation, small opaque colonies appear that show satellitism. A smear is made from colony and stained with Gram stain, it shows small Gram negative bacilli or coccobacilli,

TREATMENT

H. influenzae is susceptible to Sulphonamide, chloramphenicol, trimethoprim, ampicillin. tetracycline, ciprofloxacin, cefotaxime, ceftazidime. Cefotaxime or ceftazidime is the drug of choice for the treatment of *Haemophilus* meningitis.

PROPHYLAXIS

A purified type b capsular polysaccharide vaccine is used in children of 18-24 months. Vaccine is administered in two doses at an interval of months.

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