MYCOBACTERIA

Learning objectives

To know in detail about,

- Classification of Mycobacteria
- Morphology, cultural and biochemical characteristics of Mycobacteria
- Cultivation methods of Mycobacteria
- Pathogenesis of tubercle formation
- Isolation and identification of Mycobacteria
- Explain the Acid fast staining and tuberculin tests

SYSTEMATICS

Domain	Bacteria
Phylum	*Actinobacteria*
Class	*Actinobacteria*
Subclass	*Actinobacteridae*
Order	*Actinomycetales*
Suborder	*Corynebacterineae*
Family	*Mycobacteriaceae*
Genus	*Mycobacterium*

- The genus includes animal and human pathogens as well as saprophytic members often referred to as atypical, anonymous, opportunistic, tuberculoid and MOTT (Mycobacteria other than typical tubercle) bacilli.

CLASSIFICATION

Classification of Mycobacteria (Tubercle Bacilli)

I. Slowly growing mycobacteria

- *Mycobacterium tuberculosis* causes human tuberculosis in human and dogs.
- *Mycobacterium bovis* causes bovine tuberculosis in many animal species and also cause tuberculosis in human
- *Mycobacterium africanum* causes human tuberculosis.
- The human type (*Mycobacterium tuberculosis*) is primarily a pathogen for man.
- But can cause disease in cattle, pigs, dogs, monkeys, parrots and other species.
- The bovine type (*Mycobacterium bovis*) is a common cause of disease in domestic animals particularly cattle, pigs, cat, dogs and horse.
- The avian type (*Mycobacterium avium*) is primarily a pathogen for birds. But can cause disease in cattle, sheep, goat and pigs.

II. Atypical mycobacteria

- Runyon (1959) grouped the atypical mycobacteria on the basis of pigmentation, colonial morphology and growth rate.
- The photochromogens will produce pigment only if exposed to light. The scotochromogens are those that produce yellowish orange pigments in the dark.
- The slow growing mycobacteria are those that require over 7 days incubation and rapid growers are those requiring less than 7 days.
    - Slowly growing photochromogens
        - *Mycobacterium kansasi, Mycobacterium marinum, Mycobacterium simiae*
    - Slowly growing scotochromogens
        - *Mycobacterium gordonae* (tap water scotochromogens)
    - Slowly growing non-chromogens
        - *Mycobacterium avium* (Avian tuberculosis)
        - *Mycobacterium intracellulare*
        - *Mycobacterium paratuberculosis* (Johne's disease – chronic hypertrophic enteritis in cattle)
        - *Mycobacterium lepraemurium* (Feline leprosy)
    - Rapid growing mycobacteria
        - *Mycobacterium phlei* (timothy grass bacillus)
        - *Mycobacterium smegmatis*

III. Non-cultivable mycobacteriae

- *Mycobacterium leprae*
    - *Addition to this the unspecified acid-fast bacilli such as Mycobacterium senegalense and Mycobacterium farcinogens were isolated from Bovine farcy.*

HISTORY

- The generic name *Mycobacterium* (fungus bacterium) was proposed by Lehmann and Neumann (1896).
- The first member of this genus to be identified was the lepra bacillus discovered by Hansen (1868) – Hansen bacillus.

- Koch (1882) isolated the mammalian tubercle bacillus and proved its causative role in tuberculosis by satisfying Koch‘s postulates.
- The acid-fast property of *Mycobacterium* was discovered by Ehrlich (1882).
- Johne (1895) described Johne‘s bacillus - *Mycobacterium paratuberculosis*.

HABITAT

- It has a worldwide distribution. The usual habitats of the great majority of the cultivable mycobacteria are water and watery habitats, marshes, wet soil, streams, lakes, rivers.
- The source of the pathogenic mycobacteria is usually infected animals.
- *Mycobacterium bovis* is excreted in respiratory discharges, faeces, milk, urine and semen.
- *Mycobacterium avium* and *Mycobacterium paratuberculosis* are shed in faeces and *Mycobacterium tuberculosis* mainly in respiratory discharges.
- The atypical mycobacteria are widespread in soil, pastures, grass and water.
- A few are commensals in animals and may infect them.

MORPHOLOGY

- Mycobacteria are slender rods of varying lengths that sometimes show branching filamentous form resembling ‗fungal mycelium‘.
- Hence, the name mycobacteria, meaning fungus like bacteria.
- Although cytochemically Gram positive, the Mycobacteria do not take up the dyes of the Gram stain because the cell walls are rich in lipids – Mycolic acid.
- Once a dye has been taken up by the cells they are not easily decolourised, even by acid-alcohol. Mycobacteria are therefore called as acid-fast bacilli.
- They are usually straight or slightly curved rod occurring singly, pairs or in small groups. The morphology varies from cells of species to species.
- *Mycobacterium tuberculosis* is often arranged in serpentine cords.
- *Mycobacterium kansasi* is distinct banded or beaded appearnce, while
- *Mycobacterium avium* is often almost coccoid.
- In clinical materials they may appear as bundle of faggots. They are non-motile, non-sporing and non-capsulated.

CULTURAL AND BIOCHEMICAL CHARACTERISTICS

Cultural characteristics

- A comparatively slow growth rate is characteristic of the mycobacteria, with generation time ranging from 14-20 hours.
- Colonies appear only in about two weeks and sometimes may be delayed upto 6-8 weeks. Optimum temperature is 37°C and pH is 6.4 –7.0.
- *Mycobacterium tuberculosis* is an obligate aerobe while *Mycobacterium bovis* is microaerophilic. Growth is stimulated by 5-10% CO2.
- Tubercle bacilli do not have exact growth requirements. But they are highly susceptible to even traces of toxic substances like fatty acids in culture media.
- The toxicity is neutralized by addition of serum, albumin or charcoal.
- Several media, both solid and liquid, areavailable The egg based Lowenstein Jensen medium and Stone Brink's medium are most commonly used.
- Malachite green dye (0.025 g/100ml) is commonly used as the selective agent.

- *Mycobacterium tuberculosis*, *Mycobacterium avium* and many of the atypical mycobacteria require glycerol for growth. However glycerol is inhibitory to *Mycobacterium bovis,* while sodium pyruvate enhances its growth.
- On Lowenstein Jensen medium (i.e. glycerol containing media), *Mycobacterium tuberculosis* giving the characteristic rough, tough and buff colonies – is known as eugonic.
- The growth of *Mycobacterium avium* in this medium is also described as eugonic. *Mycobacterium bovis* has sparse, thin growth on glycerol containing media that is called dysgonic.
- *Mycobacterium bovis* however grows well on pyruvate containing media without glycerol (i.e. Stone brink's medium).
- Pigment formation is tested with young, well-developed colonies on Lowenstein Jensen medium.
- The cultures are exposed to a 100-watt, clear electric bulb, at a distance of 50 cm, for atleast an hour and then incubated again in darkness for a further 1-3 days.
- After this treatment the photochromogens will develop pigement.
- Many of the mycobacteria produce yellow/orange pigments while *Mycobacterium tuberculosis*, *Mycobacterium bovis* and *Mycobacterium avium* are non-chromogenic.
- In liquid media, the growth begins at the bottom, creeps up the sides and forms a prominent surface pellicle (mould like pellicle) that may extend along the sides above the medium.
- Virulent stains tend to form long serpentine cords on liquid media, while avirulent strains grow in a more dispersed fashion.
- Supplementation of media with mycobactin (extracted from non-mycobactin dependant isolates of *M.avium* subsp. *paratuberculosis*) is required for *M.avium* subsp. *paratuberculosis*.

Biochemical Properties

- They are oxidative. Atypical mycobacteria are catalse positive, while tubercle bacilli are peroxidase positive.
- Niacin production and nitrate reduction is only by *Mycobacterium tuberculosis*.
- Urease is reduced by *Mycobacterium tuberculosis* and *Mycobacterium bovis,* but not by avian strain.

RESISTANCE

- The Mycobacteria are resistance to physical influences and will retain their viability in soil and particles of dried faeces for many months.
- They are not specifically heat resistant; being killed at 60°C in 15-20 mts .
- Cultures may be killed by exposure to direct sunlight for two hours.
- Bacilli in sputum may remain alive for 20-30 hrs and in droplet nuclei for 8-10 days .
- They are relatively resistant to disinfectants i.e. exposure to 5% phenol, 15% H_2SO_4, 3% nitric acid, 5% oxalic acid and 4% NaOH.
- It is destroyed by tincture of iodine in 5mts and by 80% ethanol in 2-10 mts.

ANTIGENS AND TOXINS

- Many antigens have been identified in mycobacteria. Group specificity is due to polysaccharide and type specificity is due to protein antigen.
- They do not produce any exotoxins. The cell wall of the mycobacterium is composed of peptidoglycan, arabinogalactan and mycolic acid.

- In addition to this it contains wide range of lipids. The outer layer of the cell wall is composed of mycosides. (Peptidoglycolipids or Phenolic glycolipids).
- Mycosides are responsible for the control of cellular permeability, resistance to action of water-soluble enzymes, antibiotics and disinfectants.
- Cord factor (Trehalose – 6,6‘ dimycolate) and Wax D - inhibits chemotaxis, leukotaxis, responsible for delayed hypersensitivity
- Sulfatides- sulfur containing glycolipids –promote the survival of virulent tubercle bacilli within macrophages by inhibiting phagolysosome formation and avoiding exposure to hydrolytic enzymes present in the lysosomes.
- Virulence appears to reside in the lipids of the cell wall. Mycosides, phospholipids and sulpholipids are protecting the tubercle bacilli against phagocytosis.

PATHOGENESIS

Lesions

- Infection is usually by inhalation and ingestion. The mucociliaryclearance by mucus and epithelial cilia in the upper respiratory passages provides defense against infection.
- However, microorganisms on small particles (1-4 µm in size), such as, dust and water droplets reach alveolar spaces.
- In previously unexposed animals, local multiplication of the mycobacteria occurs and the resistance to phagocytic killing allows continued intra cellular and extra cellular replication.
- Infected host cells with mycobacteria can reach local lymphnodes and from there may pass to the thoracic duct with general dissemination.
- After 10-14 days, CMI responses develop and activated macrophages are able to kill some mycobacteria.
- The aggregation of macrophages contributes to the formation of a tubercle, and a fibrous layer may encompass the lesion.
- Caseous necrosis due to the cell death and tissue destruction occurs at the center of the lesion and this may proceed to calcification or liquefaction.
- Once CMI is established, the lymphatic spread is retarded but occurs via the erosion of bronchi or blood vessels to new area.
- Haematogenous spread may produce miliary tuberculosis (in deer). This involves multifocal tubercle formation in an organ.

PATHOGENECITY

Cattle

- Tuberculosis consists of a characteristic lesion – the tubercle. This is an avascular granuloma composed of a caseous necrosis in a central area encircled by a zone of epitheloid cells, and a peripheral zone of lymphocytes, granulocytes and fibroblasts.
- Calcification may be present in the necrotic centers.
- An outer boundary of fibrous tissue is usually present between the lesions and normal tissue.
- Tubercle lesions are more commonly present in the lymphnode, lungs and pleura.
- Military tuberculosis resembling millet seeds with similar lesions in various organs can be formed by the haematogenous route.

Sheep and Goats

- Similar lesions as that of cattle are seen.

Horses

- Common sites are liver, spleen and lungs. In pigs, the skeleton, especially vertebrate and long bones, are common sites.

Birds

- The grayish white granulomatous lesions are found in the liver and they are also present in the intestines, spleen and bone marrow.

DIAGNOSIS, CONTROL AND PREVENTION

Specimens to be collected

- Specimens from live animals include aspirates from cavities, lymph nodes, biopsies, tracheobronchial lavages and centrifuged deposit from about 50 ml of milk in the case of suspected tuberculous mastitis. With dead animals, collect fresh and fixed (10% formalin) samples of lesions.

Diagnosis

- Based on history, signs and post mortem lesions
- Direct microscopy
    - The Ziehl-Neelsen (acid-fast) stain is used to stain smears from lesions and other specimens.
    - Organisms are appearing as slender, often beaded, red staining rods against a blue background.
    - Smears stained by fluorescent dyes (auramine, acridine orange or fluorochrome) allow the mycobacteria to be seen more easily if relatively small numbers are present.
- Isolation
    - Several preliminary procedures are necessary in order to recover the comparatively slow growing mycobacteria
    - Selective decontamination to reduce significantly the number of fast growing contaminating bacteria.
    - Digestion or liquefaction of mucus is necessary. Mucin-trapped mycobacteria in specimens, such as broncho-tracheal exudates, may not be available for growth in cultures.
    - If mycobacteria are present, they must be concentrated by centrifugation.
    - For decontamination, the ground-up specimens must be treated with decontaminating agents, such as 5% oxalic acid, 4% sodium hydroxide followed by neutralization of the acid or alkali or 20% antiformin can be used and subsequently cultured in stone brink or Lowenstein Jensen media.
- By animal inoculation

Inoculated suspected material	***Mycobacterium tuberculosis***	***Mycobacterium bovis***	***Mycobacterium avium***
Rabbits (I/V)	+	++	++

Guinea pig (S/C)	++	++	-
Chicken (I/V)	-	-	++

- Bacteriophage typing: A, B, C, I
- Tuberculin test (Intra dermal, double intra dermal, ophthalmic tests in cattle andwattle test in case of poultry)
- Gamma interferon assay, Gas liquid chromatography, PCR, ELISA for detecting circulating antibodies, FAT , LTT assays can also be used in the diagnosis

Control and prevention

- Treatment and vaccination are inappropriate in control programmes for cattle.
- In many countries, tuberculin testing followed by isolation and slaughter of reactorshas been implemented as the basis of national eradication schemes.