MYCOPLASMA

Learning objectives

To know in detail about,

- Mostimportant species of Mycoplasma and the mycoplasmal diseases of domesticanimals and poultry
- Morphology, cultural and biochemical characters of Mycoplasma
- Diene's staining method
- Difference betweeen L forms and Mycoplasma
- Diagnostic methods used to identify Mycoplasma

MORPHOLOGY

- Class: *Mollicutes* (Soft skin)

Domain	Bacteria
Phylum	*Firmicutes*
Class	Mollicutes
Order	Mycoplasmatales
Family	*Mycoplasmataeceae*
Genus	*Mycoplasma*
Order	*Acholeplasmatales*
Family	*Acholeplasmataeceae* (not required cholestrol)
Genus	*Acholeplasma*
Order	*Entomoplasmatales*
Family	*Spiroplasmataeceae*
Genus	*Spiroplasma*

- Family: *Mycoplasmataceae* -Sterol or Cholestrol requirement for their growth
- Genus

    - *Mycoplasma*
    - *Ureaplasma* (hydrolyses urea)
- Division: *Tenericutes*
    - The term mycoplasma that refers to the fungus-like forms of the branching filaments.
    - They are the smallest and simplest prokaryotic cells capable of self-replication.
    - The genome of mycoplasmas and ureaplasmas is the smallest for any self-replicating prokaryotic cells.
    - They are $5x10^8$ daltons compared to the average bacterial genome of 2.5 $x10^9$ daltons.
    - These organisms lack the genetic ability to form a cell wall and are enclosed in triple layerd plasma membrane composed of protein, glycoprotein, glycolipid and phospholipid.
    - As there is no rigid cell wall, the mollicutes are plastic, filterable andpleomorphic.
    - Mycoplasmas are parasitic and pathogenic for animals. More than 60 speciesof *Mycoplasma* are known to cause disease in variety of animals.

HISTROY AND HABITAT

History

- The first mycoplasmal species, the causative agent of bovine pleuropneumonia, wasfirst isolated by Nocard and Roux (1898).
- The species discovered later were called PPLO, because of their resemblance to theorganisms causing pleuropneumonia.

Habitat

- The Mycoplasmas occur worldwide as free-living saprophytes or as parasites of animals.
- Both pathogenic and non-pathogenic species are found as commensals on the mucous membrane of the URT, intestinal and genital tracts, on articular surfaces andin the bovine mammary gland.
- Outside the host, the pathogenic species can survive in microenvironments, protectedfrom sunlight, for several days.

MORPHOLOGY

- *Mycoplasmas* are the smallest and most pleomorphic

microorganism.

- They occur as cocci, spirals, flaments and rings. The characteristic feature is the complete absence of cell wall or cell wall precursors such as muramic acid and diaminopimelic acid.
- They stain poorly with Gram's stain (gives Gram –ve). Gives better resuts with Giemsa and other Romanowsky stain.
- The method of reproduction is not fully understood. But some divide by binary fission, or by budding.
- Some have a reproductive cycle by the development within the filaments of elementary bodies and their subsequent release by fragmentation and disintegrationof the filaments.
- The minimal reproductive unit is about 0.3 µm in d.m. but because the cells arepliable they are able to pass through a 0.22 µm membrane filter.
- Dark field and Phase contrast microscopy are recommended for studying themorphology of *Mycoplasma*.
- *M. mycoides* has a galactan capsule. They are generally non- motile and non-sporeforming.

CULTURAL, BIOCHEMICAL CHARACTERISTICS AND RESISTANCE

- Mycoplasmas are highly fastidious organisms and most require specific growth factors, an isotonic medium and the absence of inhibitory substances for growth.
- *Mycoplasma* and *Ureaplasma* require reduced sterol or cholestrol for their growth.
- Because the *Mycoplasma* are unable to synthesise purines and pyrimidines, they require complex media.
- The agar which contains bovine heart infusion, 20% horse serum, (Pooled serum from several animals), 10% yeast extract, 20ml of adenine dinucleotide, 50 units of penicillin and 0.25 mg of thallous acetate (inhibitory to Gram –ve and fungi) and theoptimal pH is 7.5.
- Most grow aerobically but some require N2 with 5% to 10% Co2 after 2 to 6 days ofaerobic incubation at 370C, colonies on solid media are 0.1 to 0.6 mm in d.m.
- under low power magnification, the colonies appear transparent, flat and often resemble a fried egg.
- Colonies grow into the medium and are difficult to remove from the agar surface.

- The colonies are best studied by stained with Diene's method
- A block of agar containing microcolonies is placed, colony side upwards, on a microscopic slide.
- A light film of Diene‘s stain (alcoholic solution of methylelne blue and azure) is placed on a coverslip and allowed to dry.

- This is then put, stain-slide downwards on the microcolonies on the agar block thenexamined under low power of microscope.
- The dense center of the microcolonies, which grow down into the agar, stain darkblue, the less dense peripheral zone, resembling surface growth, stains light blue.
- Most mycoplasmas are haemolytic in swine blood agar. Mycoplasmas may grow inchicken embryos (yolk sac route) and cell cultures.
- *Ureaplasma* produce tiny colonies (T-mycoplasma) than the conventional mycoplasma

Biochemical characters Mycoplasmas are chemoorganotrophs, the metabolism beingmainly fermentative.

- Most species utilize glucose or arginine as the major source of energy.
- Urea is not hydrolysed except by ureaplasmas.
- They are generally not proteolytic.

Resistance

- Mycoplasmas are more fragile because of the absence of cell wall.
- Drying, sunlight and the usual disinfectants readily kill them.
- They are resistant to penicillin and sulfonamides.

PATHOGENESIS

- Transmissions are usually veneral, vertical or by aerosols and manyimportant avianmycoplasmas are egg transmitted.
- Various stress factors predispose the mycoplasmal infection. They parasitic mycoplasma tends to adhere firmly to the host‘s mucous membranes with specific attachment structures.
  The organisms are extra cellular and produce haemolysins, proteases, nucleases andother toxic factors like capsular carbohydrates, ammonia that can lead to death of host cells or

to a chronic infection.

- Some pathogenic species have a predilection for mesenchymal cells lining joints andserous cavities.
- The respiratory tract and lungs are most frequent sites of infection.
- Mycoplasmas are capable of destroying the cilia of cells in the respiratory tract, thus predisposing secondary bacterial invasion.
- The fibrinous exudates frequently present in infections protects them from antibodyand antimicrobial drugs.

Host	Species	Disease
Poultry	• *M.gallisepticum*	• Chronic Respiratory Disease (CRD) and air sac disease in chicken • Infectious sinusitis in chicken
Turkeys	• *M.synoviae* • *M.meleagridis*	• Infectious synovitis • Airsacculitis and bursitis
Cattle	• *M.mycoides subsp mycoides* (Smallcolony type) • *M.bovis* • *M.bovigenitalium*	• Contagious Bovine Pleuro Pneumonia (CBPP) • Mastitis, arthritis, pneumonia, abortion and genital infection • Vaginitis, arthritis, seminal vesiculitis and mastitis
Goats	• *M.mycoides* subsp *Capri* • *M.mycoides subsp mycoides* (Large colony type)	• Contagious Caprine Pleuro Pneumonia (CCPP) • Pneumonia, septicaemia, polyarthritis and mastits
Sheep	• *M.agalactiae*	• Contagious agalactia
Pigs	• *M.hyorhinis* • *M.hyosynoviae* • *M.hyopneumoniae*	• Polyserositis and chronic arthritis in young pigs • Polyarthritis in adult • Enzootic pneumoniae of pigs

DIAGNOSIS

Specimens

- Mycoplasmas are fragile and specimens must be kept refrigerated anddelivered to laboratory within 24-48 hrs of collection.
- The samples may include mucousal scrapings, tracheal exudates, aspirates, and pneumonic tissue from the edge of the lesion, cavity or joint fluids and mastitic milk.
- Swabs should be submitted in transport medium.

Diagnosis

- Based on direct microscopy
  - FAT is most reliable than simple staining.
- By isolation and identification
  - The inoculation technique will vary according to the nature of the specimen.
    - Fluid materials such as foetal fluids and exudates can be inoculateddirectly into broth and spread over the surface of the agar medium.
    - Specimens such as semen, joint fluids and tissues may containinhibitors for mycoplasmas.
    - Make ten fold dilutions in mycoplasma broth and then culture.
    - Tissues, such as pieces of lung can be moved across the surface of anagar plate for inoculation.
    - Alternatively the tissue can be homogenized in broth, made ten folddilutions and inoculated.
  - Differentiation from bacterial L forms
    - Bacteria that have temporarily failed to form cell walls (L-form) can produce microcolonies similar to those of the mycoplasmas (the failure to form cell wall is often due to the bacteria being exposed to penicillin or other antimicrobial agents that affect cell wall formation).
    - The L-forms may also produce fried-egg colonies like mycoplasmas,but they differ in a number of respects.
    - L-forms resemble the parent bacteria biochemically and antigenically,they are not

filterable, the minimum reproductive unit being about 600nm, sterols are not required for their growth, they are non pathogenic and they show nucleic acid homology with parent bacteria.

- Differentiation between these two can be carried out by
  - Subculturing the suspected bacterial L-form on media without antibacterial substances should cause reversion of the L-forms with the formation of normal sized bacterial colonies.
  - Staining microcolonies with Diene's stain. Mycoplasmal colonies retain the stain indefinitely,whereas bacterial L-form microcoloniestend to decolourise in about 15 mts.

- Growth inhibition test
  - This test is based on the ability of antisera to specifically inhibit the growth ofhomologus species on solid media.
- Sensitivity to digitonin
  - This test reflects the requirement of cholestrol for growth.
  - *Mycoplasma* and *Ureaplasma* species are sensitive to digitonin whereas *Acholeplasma* are not.
  - Digitonin filter paper discs are commonly used.
- Serological test
  - Rapid plate agglutination test, HI test and AGID: for screening of avianmycoplasmas.

- CFT for CCPP and enzootic pneumonia. FAT, ELISA, LAT, species specific DNA probes are highly useful in the identification of mycoplasmas.

TREATMENT AND CONTROL

- Tetracycline, gentamycin, kanamycin, tylosin, erythromysin and quinolones are effective in some infections.
- Dipping hatching eggs in an antibiotic solution has been effective in producing chicksfree of *M.gallisepticum*.
- Cattle are vaccinated with live attenuated

M.mycoides subsp *mycoides* strain areuseful to prevent CBPP.

Live, attenuated and inactivated vaccines give partial protection against losses in eggproduction and infections due to *M.gallisepticum*.