

# Mycoplasma to pass through bacterial membrane filters

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MYCOPLASMA INTRODUCTION? Mycoplasma coming under the class Mollicutes. There are 9 genera in the class Mollicutes. Thus class Mollicutes have 3 families are Mycoplasmataceae, Acholeplasmataceae, Anaeroplasmataceae .

From that 9 genera , 5 are veterinary importance (Mycoplasma, Ureaplasma, Acholeplasma, Anaerplasma, Asteroplasma) . There are 100 spp in the Mycoplasma genus . First Mycoplasma identified in 1890 was Mycoplasma mycoides subsp mycoides . Similar types of Mycoplasmas were subsequently identified called as Pleuro Pneumonia Like Organisms (PPLO) ? Generally Mycoplasma are Prokaryotes, have capable of replication.

Pleomorphic organisms which will appear as spherical , filaments . They do not have cell wall so they cannot synthesize peptidoglycan. However they have 3 layered flexible outer membrane which will cause the flexibility property of that organism .

Flexibility : Allows to pass through bacterial membrane filters (0.22 to 0.45 $\mu$ )

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Sensitive to heat , desiccation, detergents but they resistant to

penicillin HABITAT: -Found on mucosal surfaces of conjunctiva , nasal cavity, oro-pharynx , intestinal, genital tract . these are extracellular organisms.

Generally host specific in nature PATHOGENESIS: -Parasitic mycoplasmas tend to adhere firmly to host' s mucous membrane (adhesin) .

There they produce haemolysins, proteases, nucleases, other lethal factors that leads to death of cells. Some mycoplasmal organisms have

predilection site in mesenchymal cells -joints, serous cavities.

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Respiratory tract and lungs – frequent site of the pathogenic organisms. It destroys the cilia of respiratory tract thereby causes 2° bacterial invasion.

Latency can occur in that microbial pathogenicity. Stress, intercurrent infection & age predisposes the disease. Infections may be chronic or low grade and they are exogenous or endogenous.

**LABORATORY DIAGNOSIS:** -  
Specimens: Samples are fragile in nature, it should be kept at refrigerated condition and delivered to a laboratory within 24 – 48 hours of collection. Samples: Mucosal scrapings, tracheal exudates, aspirates, pneumonic tissue from the edge of lesion cavity or joint fluids, mastitis milk isolation: -  
Culture media: Mycoplasma are fastidious organisms, facultative anaerobes, 5-10% CO<sub>2</sub>. It requires enriched media for growth. Basic medium is a good quality beef infusion with supplements pH of the medium – 7.

2 to 7. 8. Commercially available agar or broth (supplemented with horse serum 20% and yeast extract with amino acid).

Penicillin – inhibition of gram +ve. Thallous acetate – inhibition of gram -ve, fungi. Specimens should be inoculated into 2 broths and onto (2 agar plates 1 for mycoplasma, 1 for urea plasm). Fluid material (fetal fluids, exudates) – directly inoculated into broth and agar medium. Some specimens (semen, joint fluids, tissues) contain inhibitors of mycoplasmas. Both undiluted specimen & ten fold dilutions in mycoplasmal broth should be cultured.

**IDENTIFICATION:** -  
Differentiation from bacterial L forms: Bacteria temporarily failed to form cell walls (L forms) can produce microcolonies similar to the mycoplasmas. Staining microcolonies with Diene's stain – aids in

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differentiation between L and mycoplasmal colonies . Mycoplasmal colonies retain stain Lform decolorise within 15 mins COLONIAL MORPHOLOGY: - Microscopically: Fried egg colonies? Diene ' s stain - recognizes microcolonies ? Inoculated agar plates placed in a humid atm. at 37°C? umbonate micro colonies when illuminated obliquely? Microcolonies - fried egg appearance IDENTIFICATION OF THE GENUS: -Sensitivity to digitonin ???? Mycoplasma and urea plasma are sensitive to digitonin? Done by digitonin disc applied on the agar media ? Positive - Zone of inhibition should be 5 mm or more IDENTIFICATION OF SPECIES: -Fluorescent antibody staining: To identify M. dispar and Urea plasm- bronchial epithelium of calves FA (direct and indirect) for staining mycoplasmal colonies : q For recognizing mixed cultures q commonly used in avian mycoplasmas? Enzyme linked immunoperoxidase: Porcine bronchial epithelium -M. hyopneumoniae ? AGID- Using known antisera to detect mycoplasmal ag ? ELISA - ag identification with known antisera ? Species specific DNA probes are available? Biochemical tests ? glucose fermentation , arginine hydrolysis , phosphatase activity SEROLOGICAL TEST Antibiotic susceptibility: Although it develops resistant to antimicrobial drugs? So ABST not usually performed? Tylosin, tetracyclin, tiamulin, fluroquinolones used for treatment ? Specific pathogen free (SPF ) programmes ? Established for poultry and pig herds? 2 phases in these programmes ???? Detection of infections and culling or isolation of affected animals? Followed by serological monitoring of the flocks to demonstrate continued freedom from infection CBPP? CHARACTERISTIC STANCE - Head, neck, extended and elbow abducted Postmortem

lesions ???? Lungs - marbled appearance? Grey , red consolidated lobules alternate irregularly with pink emphysematous lobules? Chronic : fibrinous encapsulation of necrotic foci(viable mycoplasmas)? Break down of capsules is major factor in the persistence and spread of CBPP? Joints - fibrin in synovial space & articular cartilage erosion CCPP: -? Highly contagious? incubation period : 6-10 days ? Transmission ???? Direct contact? Carrier animal may exist? PM lesions ???? Granular lung appearance Fibrinous pneumonia CRD: -? Highly versatile and successful pathogen? Once infected, it remains for life? Transmission ???? Vertical transmission? Economically significant disease ? PM lesions: ? sinusitis, conjunctivitis, tracheitis with excessive mucous , air sacculitis , pneumonia , synovitis, osteomyelitis