Review on the principle of General Veterinary Microbiology

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Summary: Microbiology is the study of microscopic size of bacteria, fungi and virus but not parasite. Bacteria generally can be gram positive or gram positive bacteria this done via the procedure gram stain laboratory technique. Bacteria are prokaryotes that are no true nuclear membrane. Gram positive bacteria retain blue color and gram negative bacteria retain pink or red color in laboratory measurement. Prokaryotic cell have circular DNA, one chromosome, reproduce by mitotic/binary fission, whereas eukaryotic cell have linear DNA, more than one chromosome.

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1. Introduction

Microbiology is the study of living organisms of microscopic size, which includes bacteria, fungi and the infectious agent at the borderline of life that are called viruses. It is concerned with their form, structure, reproduction, metabolism and classification. Why study microbiology? Because they are part of the human and animal environment and important to their health and activity. Subdivision of microbiology (Albano *et a*l., 2007).

Bacteriology, the study of bacteria, Mycology, the study of fungi and Virology, the study of virus

Bacteria; it is simple in structure and unicellular without,: nuclear membrane, mitochondria, golgi bodies, endoplasmic reticulum and Reproduce by asexual, binary fission and covered by cell wall (protection of internal structure, maintenance of shape). Bacteria have cell wall composed of muramic acid. Bacteria Prokaryotic cell but fungi have eukaryotic cell. Prokaryotic cell have circular DNA, one chromosome, reproduce by mitotic/binary fission, whereas eukaryotic cell have linear DNA, more than one chromosome. Most bacteria do have capsule used for protection and virulence of pathogenic bacteria and resistance to antibiotic. Have flagella (organ of locomotion), cell m/m (composed of phospholipids and small amount of protein) which have to control inflow and out flow of metabolites.

Gram negative cell wall composed of lipoprotein layer which connects outer m/m with peptidoglycans and phospholipid outer layer responsible for toxin portion and surface antigen. Gram positive cell wall have thick peptidoglycan layer includes teichoic and teichuronic acids responsible for surface antigen.

2. Gram staining procedures

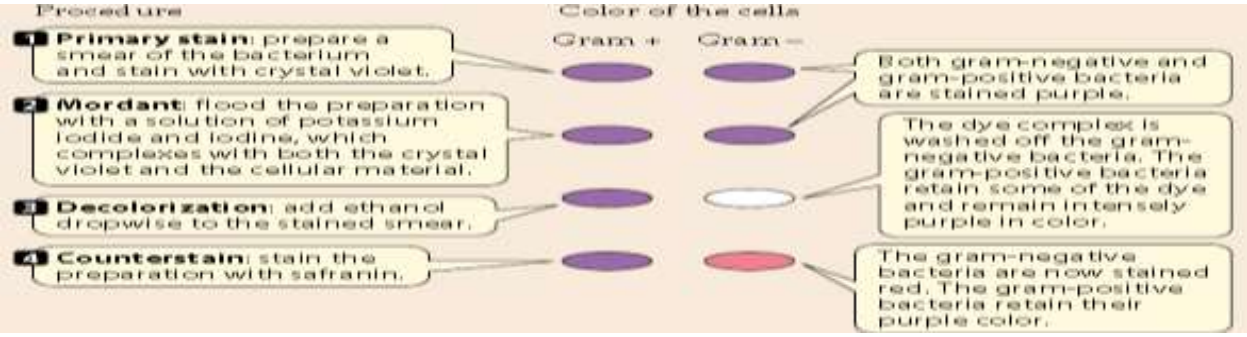


Figure 1: gram stain procedures to identify gram positive and gram negative bacteria.

Virus; smallest infectious particle consists of either DNA or RNA. Fungi are complex structure having/eukaryotic cells/Nucleus, Mitochondria, Golgi bodies and Endoplasmic reticulum.

Physiology and growth of bacteria: Requires adequate nutrition, temperature, moisture, optimum pH and oxygen for multiplication. **Media for bacterial growth:** Fluid media; bacteria grow very well in 3-4 hrs, cannot study colonial morphology**,** Solid media used to study colonial morphology, for isolation of bacteria/24-48hrs/. Different types of media are: a) Basal media (nutrient agar), b) Enriched media (blood agar) and c) Differential media (macConkey agar)**.** Agar it is solidifying agent.

Factors influencing growth are: Temperature, optimum temperature for bacterial growth b/n 30 -37 degree centigrade, Based on temperature three groups of bacteria: a) **Psychrophilic** b/n 0-25 mostly water and soil bacteria, b) **Mesophlic** grow on b/n 20-40 °C most disease causing bacteria and c) **Thermophilic** grow b/n 50-60 °C. Moisture and light hydrogen ion concentration (Albano *et a*l., 2007).

3. Microscopy and micro organism

Materials are clearly seen by naked eyes if their diameter is greater than **100 micro** meters, so that microscope is necessary to see bacteria/cocci 1micro meter, baccili 2-10 micro meter. Viruses are more smaller and used **electron microscope** to see. Type of microscope: **Compound microscope**; have more than one lens. Total magnification power is calculated as multiplying magnification power of objective lens by magnification power of ocular lens. **Dark field microscopy**: The condenser used in an ordinary light microscope causes light to be concentrated and transmitted directly through the specimen. **Phase contrast microscope**; have special condenser and objective lenses. **Fluorescent microscope**; uses ultra violet light instead of white light. **Electron microscope**; uses beam of electron.

4. Classification of bacteria;

Classification of bacteria;based on shape: **Cocci**-spherical-streptococus, staphylococcus, **Bacilli** –rods-bacillus, clostridium, **Spiral** –spiral-spirochaetes and **Filamentous** –complex form-corenybacteria

Classification of bacteria; based on cell wall structure: Gram positive and Gram negative. Classification of bacteria; based on cellular respiration: a) **Aerobic**-requires oxygen-bacillus cereus, b) **An aerobic**-cannot tolerate oxygen – clostridium spp and c) **Facultative an aerobic**-grow in the absence or presence of oxygen staphylococci spp (Albano *et a*l., 2007).

5. Identification

In clinical microbiology it can identified at generic or spp level using two tests: primary identification test and secondary identification tests: primary identification test are: Cellular morphology(cocci, bacilli), Gram stain(-ve,+ve) and Acid fast stain, Catalase and oxidase tests, Motility test, Growth or absence of growth on macConkey agar, Oxidation fermentation test and KOH test.Secondary identification tests are: Once the bacterium has been identified at generic level by primary test, it can be also identified at spp level by; Coagulase test, CAMP test, Indole test, Methyl red test and Citrate test (Albano *et a*l., 2007).

6. *Staphylococcus species*:

Morphologically: Gram positive cocci, irregular clusters arrangement resembling bunches of grapes with their average diameter ≈ 1.0µm in diameter, Characteristics: Facultative anaerobes (fermentative), Catalase +ve, Oxidase-ve , and non-motile, The pathogenic Staphylococci : *S. aureus, S.*

intermedius and S. hyicus (most strains) are coagulase -positive. Coagulsae test correlates with pathogenecity due to surface proteins for colonization of host tissues, factors inhibiting phagocytosis (capsule, immunoglobulin binding protein A) and toxins that damage host tissues. The two commonly coagulase –ves isolates : *S. epidermidis and S. saprophyticus occurs commensals in the environment. They are considered as opportunistic infection causing agents in humans but non-pathogenic in animals.* Growth: on nutrient and blood agars, but not on MacConkey agar *(*Saha *et al*., 2006).

7. Staphylococci VS other Gram-positive cocci

Micrococci - non-pathogenic, Gram-positive cocci , Confused with coagulase-negative staphylococci Distinctive feature (oxidative in the O-F test, xidase-positive (variably) , differ from *staphylococci in susceptibility to bacitracin and furazolidone resistance and* Colonies: can be white, but often pigmented- from grayish-yellow through cream, to pink (Al-Harbi and Uddin, 2004).

Figure 2: Staphylococcus versus Micrococci

8. Natural habitat

Staphylococci occur Worldwide in mammals but strains distribution between species is limited. Majority of bacteria inhabiting the skin, skin glands and mucous membranes of humans, other mammals and birds. Coagulase +ve species - *S. aureus and S. intermedius.* Inhabit: distal nasal passages, external nares and skin especially near mucocutaneous borders such as the perineum, external genitalia and bovine udder; as transients in the GIT (Al-Harbi and Uddin, 2004).

9. Pathogenesis and pathogenicity

Staphylococci are pyogenic and are associated with abscess formation and suppuration. In chronic *Staphylococcal wound infections (*'botryomycosis') the lesion is granulomatous with pockets of pus throughout the tissue. The pathogenic Staphylococci produce a 'Battery' of toxins (21different toxins isolated) and enzymes but not fully understood: Enterotoxines(A-E): involved in human food poisoning. Exfoliatin produces Staphylococcal scalded skin syndrome (SSSS) in human infants and dogs.Human toxic shock syndrome (TSS) is caused by TSS toxine-1. Epidermolytic toxines =porcine exudative epidermitis and pyrogenic exotoxin may be produced.

Hemolytic toxins occur singly or in combination. Alpha toxin - *acts on membrane lipids, is hemolytic and mitogenic.* Beta toxin - *a phospholipase C prevalent in animal strains, produces broad zones of "**hot-cold lysis**" on sheep or cattle blood agar at 37°C, a partial hemolysis that occurs and goes to completion on further incubation at lower temperatures.* Delta toxin - *lyses cells of various species by a detergent like action.* Gamma toxin - *Little is known but has impact in the inflammatory process on corneal damage.*

Enzymes: Staphylococcal enzymes includes: Staphylokinase : is a plasminogen activator for plasmin fibrin dissolving activity, Coagulase - causes plasma coagulation in vitro and Hyaluronidase- fluid spreading factors- reduce hyaluronic acid viscosity *(*Saha *et al*., 2006).

10. Laboratory diagnosis

S**pecimens includes:** exudates, pus from abscesses, mastitic milk, skin scrapings, urine and affected tissue**s and Staining is** gram's staining directly from pus or exudates may show gram-positive cocci in the typical 'bunches of grapes' formation.

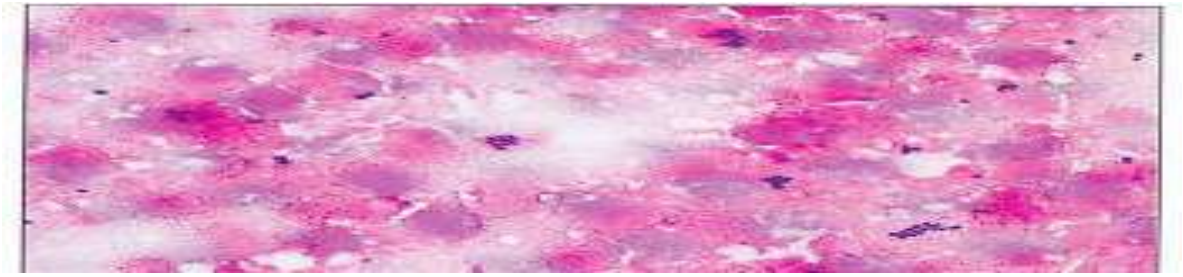


Figure 3: bunches of grapes' appearance of *Staphylococci.*

11. Identification

Colony characteristics: After 48 hrs of incubation, usually appear in 24 hrs, isolated colonies can reach 4mm in diameter. Colonies are often round, smooth, glistening and opaque. On blood agar - appear substantial and opaque when compared with Streptococci colonies: smaller, translucent, with B-hemolysis. **a) Pigmentation:** *S. aureus strains have a golden yellow pigment.* Colonies of *S. intermedius and S. hyicus are also non- pigmented (white).***b) Haemolysis:** haemolysins (alpha, beta, gamma and delta) can be produced singly, in combination or not at all. *S. aureus and S. intermedius are usually haemolytic and often produce both alpha-lysin and beta –lysin which exhibit double haemolysis.* I) β–lysin is clear and complete haemolysis immediately around the colony. II) α - lysin incomplete (partial) haemolysis. III) Gamma haemolysis is non-haemolytic (*S. hyicus) and* IV) Coagulase negative, Staphylococci haemolytic activity is commonly variable and very slows (Bairagi *et al*., 2002).

12. The Streptococci and related cocci

The streptococci and enterococci are gram +ve cocci, forming pairs or chains of varying lengths. Size: about 1µm in diameter. Characteristics includes:Facultative anaerobes, Catalase and oxiadse-ve and Non-motile with exception of some strains. Streptococci are fastidious and require the addition of blood or serum to medium for growth. Enterococci tolerate the bile salts in macConkey agar and appear as small pin-point colonies on this medium. E.g E.faecalis.

13. Natural habitat

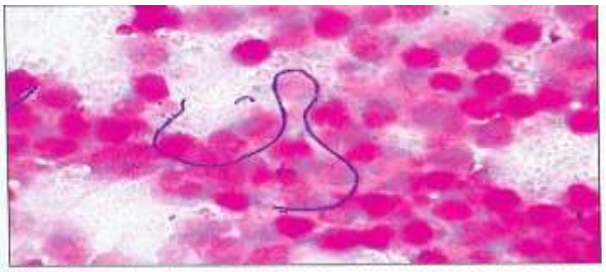
Streptococci are a worldwide in distribution.Most of veterinary interest streptococci are normal flora of mammalian mucous membranes (upper respiratory and lower urinogenital tracts.

14. Pathogenesis

Streptococci are also pyogenic bacteria associated with suppuration and abscess formation. Cellular products. Exotoxins of S. pyogenes include: hemolysins (streptolysins O and S), a phage-encoded pyrogenic toxin / erythrogenic toxin A /Pyrogenix toxin a responsible for the rash of scarlet fever of human. Enzymes include: hyaluronidase, DNAse, NAD+ glycohydrolase (NADase), protease, streptokinase (a fibrinolysin), The polysaccharide capsule of S. pyogenes, S. pneumonia and some strain of S.equi subsp.equi and S. agalactiae are anti-phagocytic. The cell wall M-protien of S. pyogenes and S.porcinus is also anti-phagocytic and in S.equi subsp.equi it may function as an adhesion (Bairagi *et al*., 2002).

15. Laboratory diagnosis

Specimens: exudates, pus, mastitic milk, skin scraping, CSF, urine and affected tissues. Direct microscopy: Smears from pus, exudates or centrifuged deposits of milk or urine be fixed and stained (gram's). *S. pneumoniae (the pneumonococcus) occurs as pairs of cocci.*



124 *S. equi* subsp.*equi* in a smear of pus from a case of strangles. The long chain of Gram-positive cocci is characteristic both of this bacterium and of the disease. (Gram stain, ×1000)

125 *S. pneumoniae* in a blood smear demonstrating pairs of cocci characteristic for this bacterium. (Leishman stain, ×1000)

Figure 4: bunches of grapes' appearance of *Streptococci.*

16. Isolation

The routine media includes sheep or blood agar. Mastitic milk samples can be involved on blood agar, Edwards medium and macConkey agar. 0.1 or 0.05 % **aesculin** to indicate aesculin hydrolysis- for group D: dark brown or black complexes with ferric citrate, allowing the test to be read. It is hydrolysis of esculin (a glucoside) into glucose and **esculetin**. Inoculated plates are incubated aerobically at 37oc for24-48 hrs (Bairagi *et al*., 2002).

17. Identification

Colonial morphology: Small colonies (about 1mm after 48 hrs incubation), Hemolysis on 5% sheep blood agar: alpha, beta, or gamma, in beta-haemolytic streptococci the colonies appear translucent and *S.equi subsp.equi and S. pneumoniae forms mucoid (*Saha *et al*., 2006).

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