



بِسْمِ اللَّهِ الرَّحْمَنِ الرَّحِيمِ





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F. Micrococcacea

➤ The Gram-positive aerobic cocci include only one family; F. Micrococcaceae, and they conveniently divided into two groups based on catalase production:

1. Genera Micrococcus, Staphylococcus, and Rothia (Stomatococcus) are catalase-positive.
2. Genera Streptococcus, Enterococcus, Eremococcus, Gemella, Globicatella, Helococcus, and Vagococcus are catalase-negative.



G. Micrococcus



**G. Staphylo-
coccus**



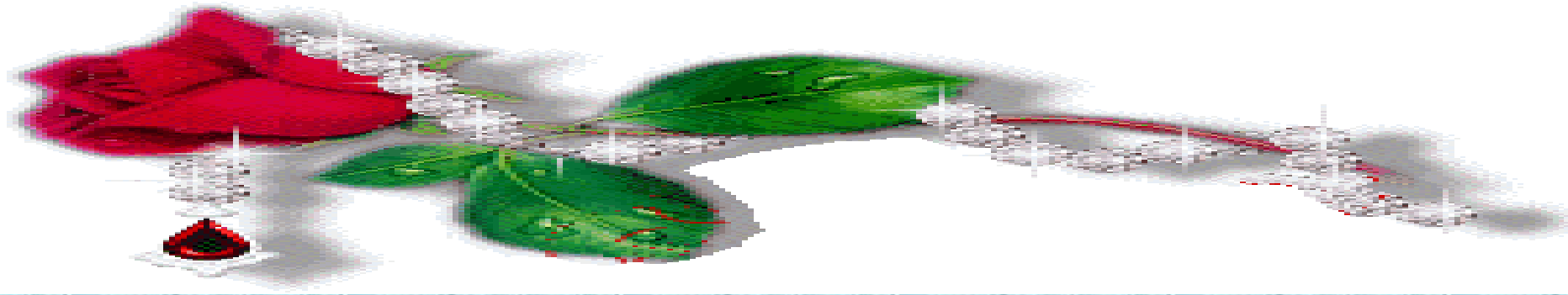
**G. Strepto-
coccus**



Others

G. Micrococcus

- It is non-pathogenic.??????
- Present in soil, air, dust, water and skin
- Have no uniform arrangement (irregular form).
- Larger in size (the largest cocci, 1.5- 2 μ in diameter).
- Not produce pigment on solid media.
- Catalase positive.
- Oxidase positive.
- Aerobic
- Oxidative (glucose $\xrightarrow{O_2}$ acid only)
- Non haemolytic on blood agar.



G. Staphylococcus

General Characteristics of *G. Staphylococcus*

- There are 40 recognized species of Staphylococci.
- They are Gram-positive cocci arranged mostly in grape-like clusters (bunches of grape).
- Most are harmless and reside normally on the skin and mm of humans and other organisms. Found worldwide, they are a small component of soil microbial flora.
- Produce endopigments (e.g. *S. aureus* → golden yellow
S. epidermidis → white)
- Aerobic or facultative anaerobic (ferment sugars).
- Catalase positive: Rapidly differentiates from *Streptococci*.
- They are halophilic bacteria (grow in 7.5% Na Cl)
- Some species grow in the presence of bile salts.

Species of Staphylococci of **importance** in veterinary medicine

- ❖ **S. aureus**: it is the most important pathogen.
 - Wound infections and abscess formation in man, animals and birds (sheep → lamb or tick pyaemia and Morel's disease)
 - Skin and mm infections in man and animals.
 - Acute mastitis.
 - Joint infections, especially in birds (Bumble foot)
 - Vaginal infections in dogs and horses, Scirrhus cord (Botryomycosis of the spermatic cord) in equines.
 - Food poisoning by enterotoxins in man and animals.
- ❖ **S. aureus subsp. Anaerobius**: isolated occasionally from ovine caseous lymphadenitis.
- ❖ **S. epidermidis**: Opportunistic pathogen; bovine mastitis, and skin abscesses in other animals.
- ❖ **S. intermedius**: Skin and ear infections in dogs, and occasional bovine mastitis.
- ❖ **S. hyicus**: Skin, milk of cattle, and avian arthritis.



Staphylococcus aureus

General Characteristics of *S. aureus*

- It is a part of human and animals flora (skin and mm especially of the upper respiratory & digestive tracts).
- They are **Gram-positive cocci**, non-motile, non-sporulating arranged mostly in **grape-like clusters** (bunches of grape).
 - Medium sized (**0.8-1.2 μm in diameter**).
 - Some strains have polysaccharide **microcapsule**.
- The cell wall contain species specific capsular polysaccharide (the main component is **ribitol**) dividing *S. aureus* into 11 serotypes (types **5 & 8** are the most common clinical isolates).
- Produce carotenoid pigment (orange or golden yellow).
- Aerobic or facultative anaerobic. ➤ Ferment mannitol.
- Catalase positive. ➤ Coagulase positive.
- They are haemolytic on blood agar.
- Grow in 7.5% Na Cl (halophilic)
- May grow on MacConkey media. (????)

Pathogenicity: virulence factors

- Virulence of *S. aureus* is almost multifactorial. They include enzymes and toxins:
 1. Coagulase: conversion of fibrinogen to fibrin which may shield (coating) Staphylococci from phagocytic cells.
 - ✓ It clots plasma of rabbit or human because they contain **coagulase reacting factor (CRF)** but not fowl or mice plasma which devoid of CRF.
 - ✓ 2 forms of coagulase are formed:
 - Free coagulase: release in the medium, detected by tube test.
 - Bound coagulase (clumping factor): cell associated, detected by slide test.
 - ✓ More strains are +ve to tube test than to slide test.
 - ✓ *S. aureus*, *S. intermedius* and *S. hyicus* produce coagulase.

2. Haemolysins (cytolysins or hemotoxins):

- ✓ Four types are produced which are antigenically distinct:
- Alpha (α) haemolysin: potent haemolysin, it is related to *S. aureus* virulence (the more α haemolysin production the more virulent strain)
- Dissolve rabbit RBCs . - Heat labile; destroyed at 37°C.
- It causes spasms of smooth muscle and is dermonecrotizing and potentially lethal. It is the major toxin in gangrenous mastitis.
- Beta (β) haemolysin: potent haemolysin, it is mostly found in strains of animal origin.
- Dissolve sheep but not rabbit RBCs.
- It is a sphingomyelinase which damages cell membrane.
- Gamma (γ) haemolysin: has narrow haemolytic spectrum.
- Heat stable, inhibited by agar and cholesterol.
- Delta (δ) haemolysin: has broad haemolytic spectrum.
- Inhibited by phospholipid.

3. Leukocidin:

- ✓ Kills WBCs (granulocytes & macrophages) resulting in pus formation (pyogenic effect of *S. aureus*)
- ✓ It is composed of 2 heat-labile proteins.

4. Fibrinolysin (Staphylokinase):

- ✓ Degrades fibrin clots of human, rabbit and dog plasma (by converting plasminogen into plasmin).
- ✓ Helping the M.O to invade the tissue (spreading factor).

5. Exfoliative toxins (Exfoliatin A & B): (specific for epidermis)

- ✓ Responsible for desquamation in staphylococcal scalded skin syndrome in neonates and children (**SSSS**).

6. Enterotoxins (A, B, C₁, C₂, D, E and F): (Food poisoning)

- ✓ Group of powerful toxins responsible for food poisoning in man & animals (**especially A & B**).
- ✓ Heat stable; resist boiling for 30min, stomach HCl, pepsin & Trypsin.
- ✓ Associated with vomiting & diarrhea within 1-6hr (gastroenteritis).

6. Toxic shock syndrome toxins (TSST):

- ✓ It is identical to enterotoxin C & F.
- ✓ Induce excessive lymphokine production resulting in tissue damage.

7. Staphylococcal Protein A (SPA):

- ✓ It is surface component on most virulent strains of *S. aureus*.
- ✓ It binds FC portion of IgG resulting in coating of M.O by antibody and inhibits opsonization (**antiphagocytic**).
- ✓ Used in diagnostic laboratory Test (agglutination test).

8. Enzymes contribute to virulence:

- ✓ **Lipase**: degrades protective fatty acids on skin. Causing abscess in skin and s/c tissue.
- ✓ **Nuclease(DNase)**: degrades both DNA and RNA.
 - It is detected by streaking *S. aureus* in DNA agar media and incubate at 37C for 24h. Cover the plate with 1% toluidine blue.
 - In positive case, pink zones are shown around colonies.

✓ Esterase, Elastase, hyaluronidase, phospholipase: These enzymes also contribute to virulence.

9. Penicillinase (β lactamase) enzyme :

✓ Most strains of *S. aureus* are penicillin & cephalosporin resistant due to this enzyme.

• Methicillin-resistant *S aureus* (MRSA)

❖ Methicillin resistance is indicative of multiple resistance especially Methicillin and other β -lactams.

❖ Multiple antibiotic resistance is increasingly common in *S. aureus* and *S epidermidis*.

❖ Occurs due to mis use of antibiotics (lower doses, shorter course of treatment and/or use of antibiotic without sensitivity test).

❖ Methicillin-resistant *S aureus* (MRSA) causes outbreaks in hospitals (Nosocomial infections) and can be epidemic.

❖ Acquisition of *mecA* gene is responsible for mechanism of resistance to methicillin and other β -lactams antibiotics. This resistance is mediated by *mecA*-encoded alternative penicillin binding protein (PBP2a) which shows a reduced binding to β -lactams antibiotics. So, *mecA* is named an important sign of methicillin resistance.

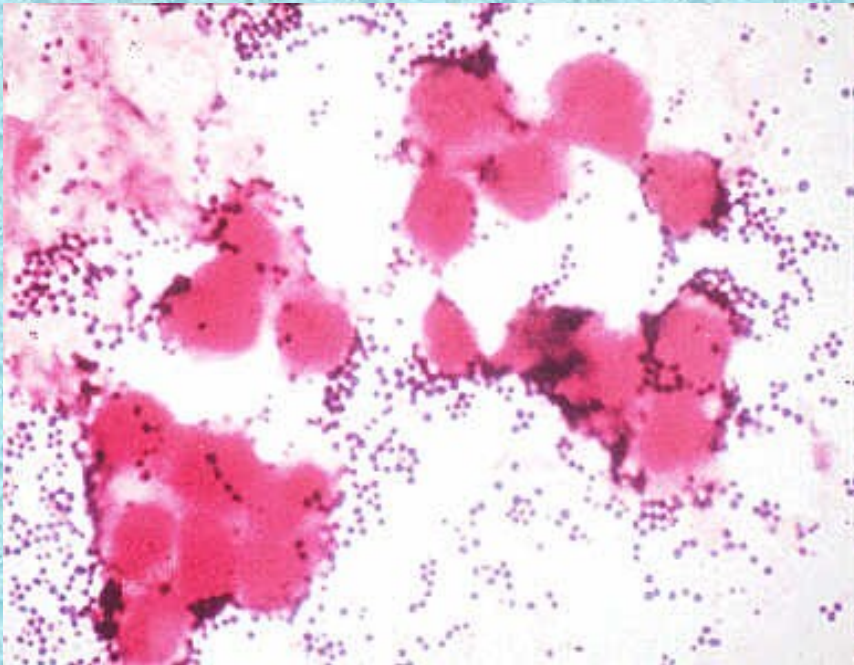
□ NB VRSA means vancomycin resistant *S aureus*

Laboratory Diagnosis

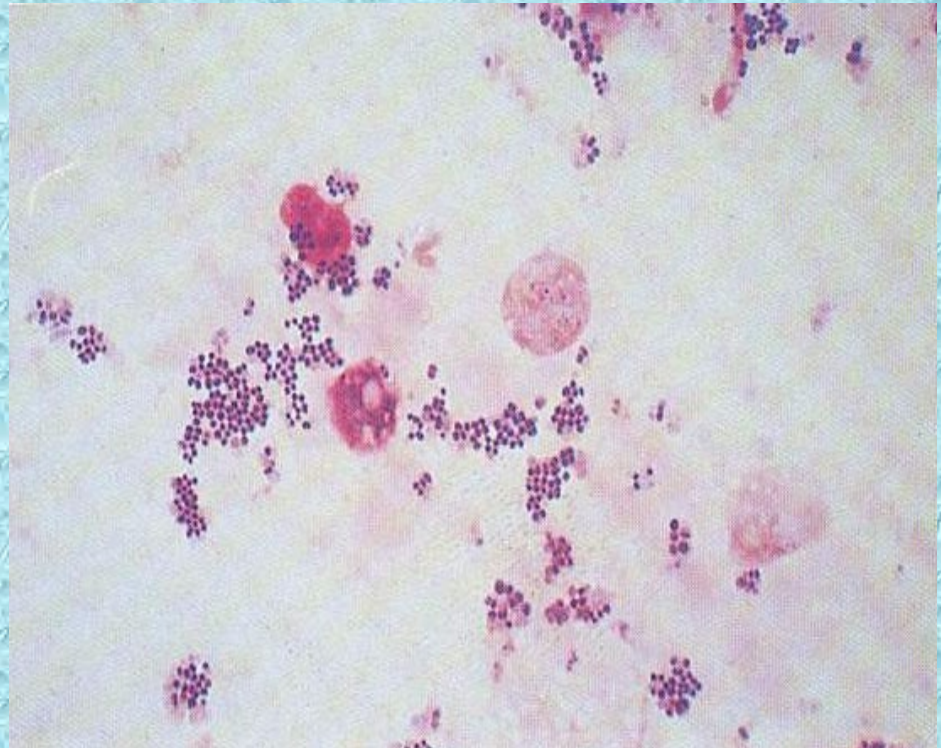
- **Specimen:** The type of specimen required for the diagnosis of *S. aureus* depends on the site of the infection. Samples may include abscess, mastitic milk, septic wound,...etc

Morphology

It is detected by examination of direct film from samples or culture.



Gram stain of *S. aureus* in pustular exudate





Culture characters




- ❖ Grow on ordinary media and may grow on MacConkey media.
- ❖ Aerobic and facultative anaerobic
- ❑ **Nutrient agar:** Colonies are round, smooth, glistening, up to 4mm in diameter ranged in colour from white to deep yellow.
- ❑ **Broth cultures:** it forms turbidity
- ❑ **Blood agar:** bovine RBCs are the best showing clear zone of β haemolysis. **Selective blood agar** containing **nalidixic acid** and **colistin**, is used to inhibit *Proteus* species and other Gram-negative contaminants.
- ❑ **Mannitol salt agar:** selective for Staphylococci contain 7% NaCl and mannitol. *S. aureus* can grow giving yellow colonies (mannitol fermenters) while other staphylococci give red colour (non-mannitol fermenters)
- ❑ **Baired Parker:** *S. aureus* colonies are black due to the reduction of tellurite. Halo-formation appears around the colonies due to proteolysis and lipolysis.



***S. aureus* on Baired Parker and MSA**

biochemical reaction

- It ferment mannitol  acid no gas (differentiate from other Staphylococci)
- Catalase +ve (differentiate from Streptococci)
- Gelatin liquefaction +ve
- DNase +ve
- Novobiocin sensitive
- **On Purple agar:** media containing bromocresol purple as a pH indicator and 1% maltose is used. *S. aureus* utilizes maltose and the acid produce changes in the medium and colonies from purple to yellow.

biochemical reaction

Slide and tube coagulase tests:

- In these tests, a suspension of Staphylococci is mixed with rabbit plasma either on a slide or in a small tube. The fibrinogen in rabbit plasma is converted to fibrin by coagulase
- The slide test detects the presence of a bound coagulase or clumping factor on the bacterial surface. A positive reaction is indicated by clumping of bacteria within 1 to 2 min.
- The tube test detects free coagulase or staphylocoagulase which is secreted by the bacteria into the plasma and a positive reaction is indicated by clot formation in the tube following incubation at 37C for 24 hrs.

Prepare diluted citrated rabbit plasma in sterile saline 1:10.

- Put 0.5 ml of the diluted plasma in 2 small test tubes.

- In one tube, add 5 drops of overnight broth culture of the tested bacteria and in the other tube, add 5 drops of saline (control negative).

- Incubate at 37C and examine after 1h and at intervals to 24hrs.

Toxins

haemolysins

leukocidin

Experimental infection (animal pathogenicity)

- inoculation of *S. aureus* in mice leads to death within 24-48hrs (**lethal toxin**).
- I/D injection in rabbit cause necrosis (**dermonecrotxin**).
- S/C or I/M injection in rabbit causing abscessation (**pyogenic**).

Agglutination tests

- This is important for detection A to D of enterotoxins and TSST.
- The tests are determined by clumping of the latex particles by the toxins present in the samples.
- Latex agglutination test is available commercially for rapid identification of *S. aureus*.