Pathogenic Bacteria (practical)

Family: Micrococcaceae

Genus I: ***Staphylococcus***

S. aureus
S. epidermidis
S. saprophyticus
S. lugdunensis
S. warneri
S. hominis

Genus II: ***Micrococcus***

General Characteristics of Staphylococcus: The staphylococci are gram-positive cocci, spherical cells, usually arranged in grape-like irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. Staphylococci are non-motile and do not form spores. Oxidase negative and Catalase positive which differentiates them from the streptococci. Staphylococci are relatively resistant to drying, heat (they withstand 50 °C for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemicals, eg, 3% hexachlorophene. Some are members of the normal flora of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. Nasal carriage of *S aureus* occurs in 20–50% of humans.The pathogenic staphylococci often hemolyze blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins. Micrococcus species often resemble staphylococci. They are found free-living in the environment and form regular packets of four or eight cocci. Their colonies can be yellow, red, or orange.

Culture and Growth Characteristics: *Staphylococci* grow readily on many types of media under aerobic or microaerophilic conditions. They grow most rapidly at 37 °C but form pigment best at room temperature (20–25 °C). Colonies on solid media are round, smooth, raised, and glistening. And are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. *S. aureus* usually forms gray to deep golden yellow colonies. *S. epidermidis* colonies usually are gray to white on primary isolation. Various degrees of hemolysis are produced by *S. aureus* and occasionally by other species.

Pathogenesis: The three major species included. ***S. aureus, S. epidermidis and S. saprophyticus*** the last two species are a virulent however under special circumstances where a suitable portal of enter is provided, they become virulent.

S. epidermidis: may be etiological agent for skin lesion and endocarditis.
S. saprophyticus: has been implicated in some urinary tract infection (UTI).
S. aureus: are often cause:

1. Skin infection.
2. Abscess formation (pus- producing lesion).
3. Boils, acne and impetigo.
4. Infections of deeper tissue and organs include pneumonia, bacteremia, sepsis, endocarditis, meningitis osteomylitis and cystitis.
5. Enteritis due to enterotoxins contamination of food.
6. Toxic shock syndrome.

***Enzymes and Toxins*:**

1. **Catalase.**
2. **Coagulase and Clamping factor:** *S aureus* produces coagulase, an enzyme-like protein that clots oxalated or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization. Coagulase may deposit fibrin on the surface of staphylococci, perhaps altering their ingestion by phagocytic cells or their destruction within such cells.

   Coagulase + prothrombin

   Fibrinogen ⟶ Fibrin clot

   activation

   Clumping factor: is a surface *S aureus* compound that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *S aureus* forms clumps.

   Clumping factor

   Fibrinogen ⟶ Fibrin clot

3. **Nuclease (DNase):** extracellular enzyme which hydrolyse DNA or RNA to nucleotides which dissolve in acid. The enzyme act on phosphodiester bonds. DNase enzyme is medically important specially for ***S. epidermidis*** because it determines the pathogenicity of the strain. Normally ***S. epidermidis*** is non-pathogenic, it is normal floral of skin, and also can be isolated from upper respiratory tract, they are opportunistic and they can cause fatal infection if they reach the blood stream and colonized in heart causing endocarditis. Strain which produce DNase are pathogenic, non pathogenic strain do not produce DNase. All strain of ***S. aureus*** produce DNase**.**
4. **Hyaluronidase, or spreading factor.**
5. **Staphylokinase**
6. **Proteinases: (**gelatinase**)**
7. **Lipases.**
8. **β-lactamase:** which cause cleavage of β-lactam ring in the β-lactam antibiotics.
9. **Haemolysin:** lysis of RBCs.
10. **Lecocidin:** lysis of WBCs.
11. **Enterotoxin:** causes enteritis and food poisoning.
12. **Toxic Shock syndrome toxin and Exfoliative toxin**

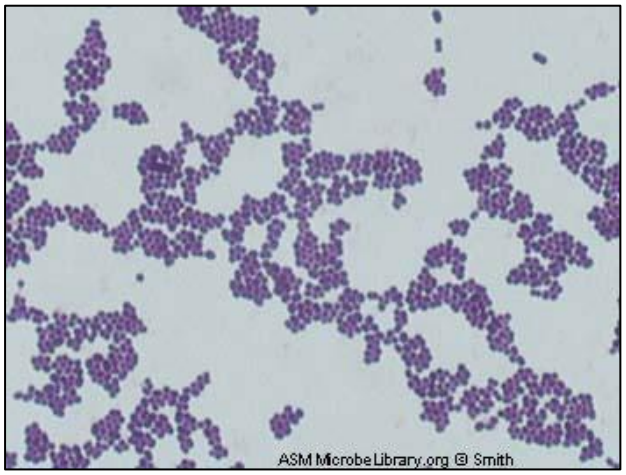
Specimens: Surface swab pus, blood, stool, sputum or tracheal aspirate, or spinal fluid for culture, depending upon the localization of the process.

Laboratory diagnostic tests:

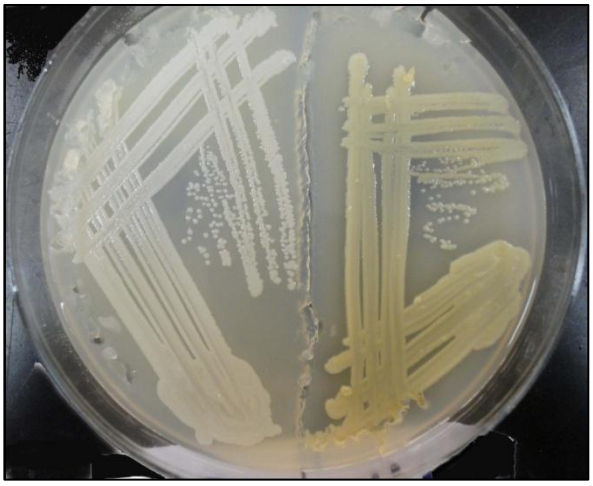
1. **Gram stain (**gram-positive cocci grape-like irregular clusters)

2. **Blood agar** (for haemolysis)
3. **Milk agar** (for pigments production)
4. **Staph 110** (selective media because it contains 7.5% NaCl).
5. **Mannitol salt agar (selective and differential media)** ***selective*** because it contains **7.5% NaCl.** ***Differential*** because it contains **mannitol sugar and phenol red as pH indicator** which differentiated between mannitol fermented Staphylococci e.g. *S. aureus* and mannitol non-fermented e.g. *S. epidermidis.*
6. **Gelatin liquefaction test**
7. **Catalase & Oxidase test**
8. **Coagulase test:** Citrated rabbit (or human) plasma diluted 1:5 is mixed with an equal volume of broth culture or growth from colonies on agar and incubated at 37 °C. A tube of plasma mixed with sterile broth is included as a control. If clots form in 1–4 hours, the test is positive.
9. **DNase test:** Heavily spot-inoculate DNase agar plate with Staphylococci bacteria over a 0.5-cm area, Incubate for 18 to 24 hours at 35°C. Then flood the DNase test agar plate with 1 N HCl. A zone of clearing around the colony indicates a positive DNase test. This clearing occurs because the large DNA molecule has been degraded the enzyme, and the end products dissolve in the added acid.

Test		*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Pigment		**Yellow, Golden**	**White**	**White, Light yellow**
Mannitol salt agar	**Growth**	+	+	+
	Fermentation	+	-	+/-
Staph 110		+	+	+
Coagulase		+	-	-
DNase		+	-/weak	-
Haemolysis		**β**	-/weak	-
Catalase		+	+	+
Gelatin liquefaction		+	-	-



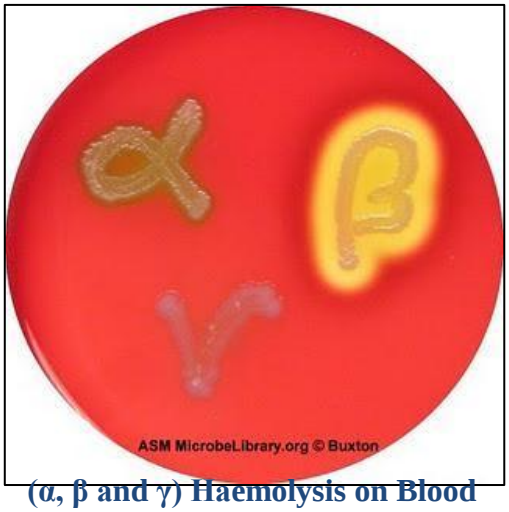
***Staphylococcus* Gr+ve cocci grape-like irregular clusters**



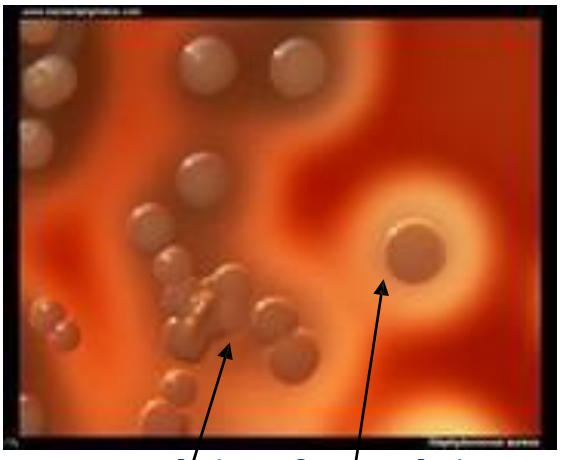
Pigments production on Milk agar

***S. epidermidis* & *S. aureus* on Mannitol salt agar**

***S. aureus* on Mannitol salt agar**

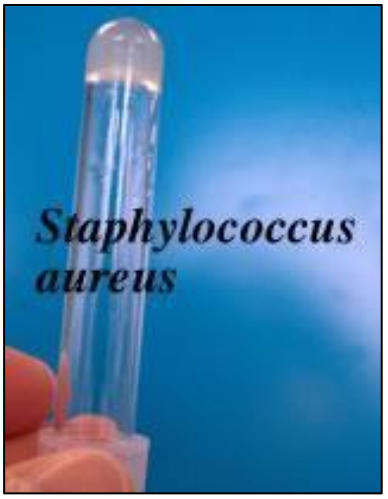


(α, β and γ) Haemolysis on Blood agar

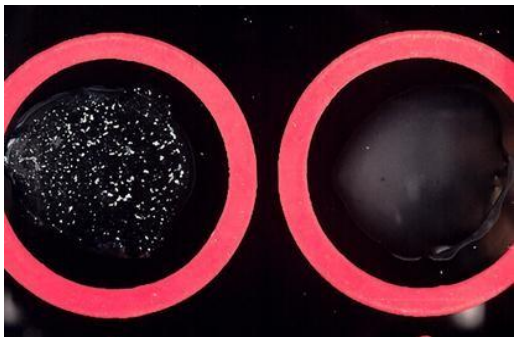


α- Haemolysis **β-Haemolysis**

Coagulase Test (tube test)



Positive Coagulase Test



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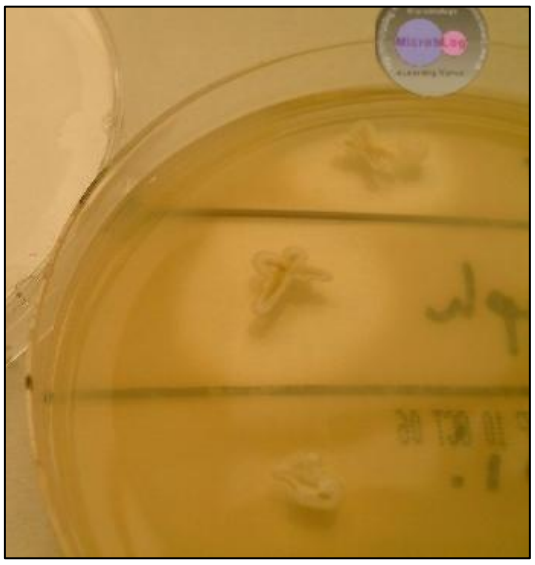
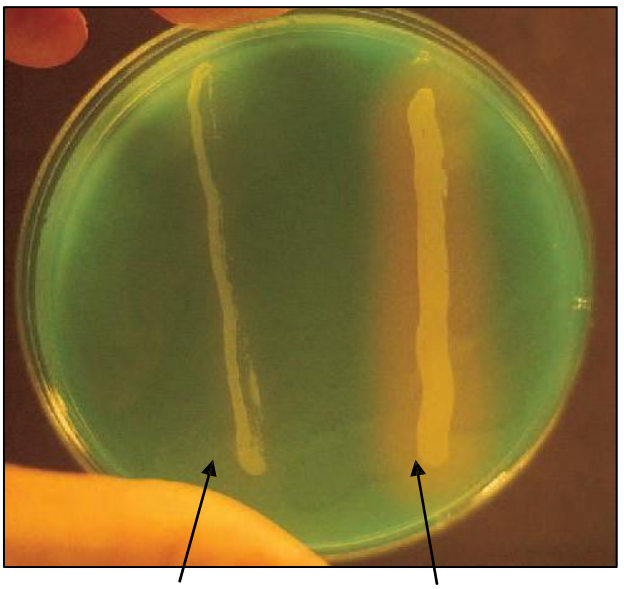
Clumping factor



DNase Test



S. epidermidis ***S. aureus***

DNase Tes