STAPHYLOCOCCUS

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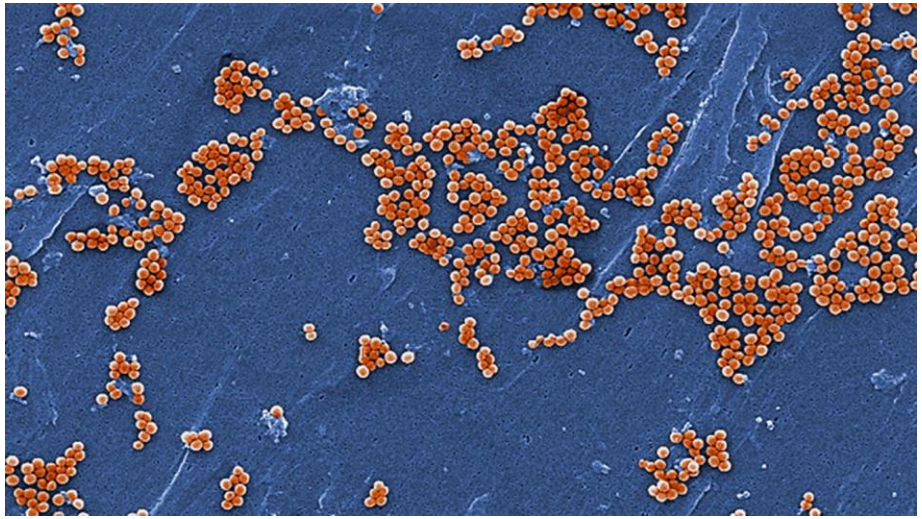
Staphylococcus

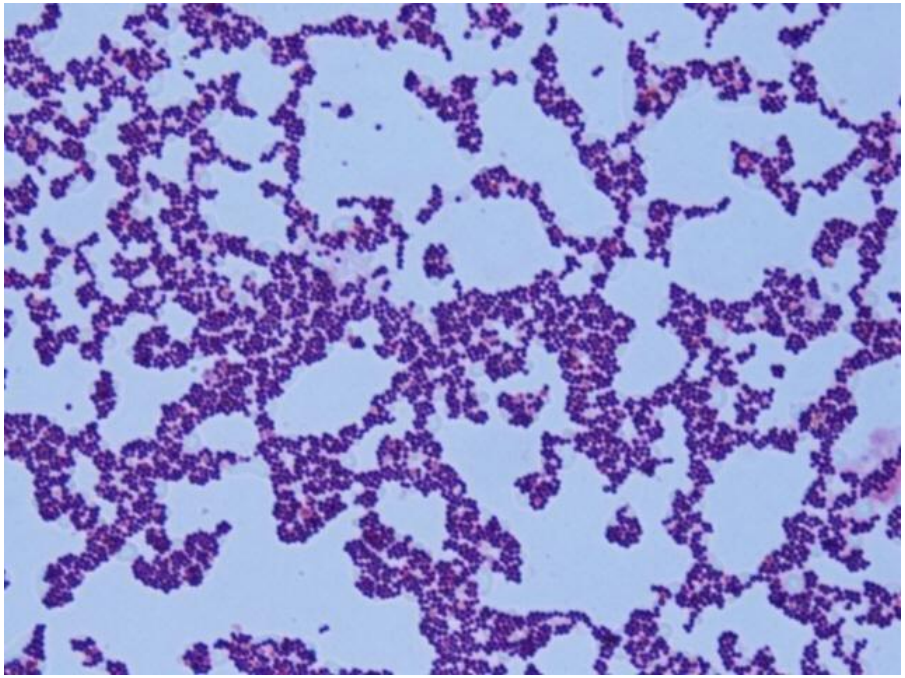


- Domain: Bacteria
- Kingdom: Bacteria
- Phylum: Firmicutes
- Class: Bacilli
- Order: Bacillales
- Family: Staphylococcaceae/ Micrococcaceae
- Genus: Staphylococcus
- Species: *Staphylococcus aureus*
- Binomial name: *Staphylococcus aureus*
  - Rosenbach 1884

History

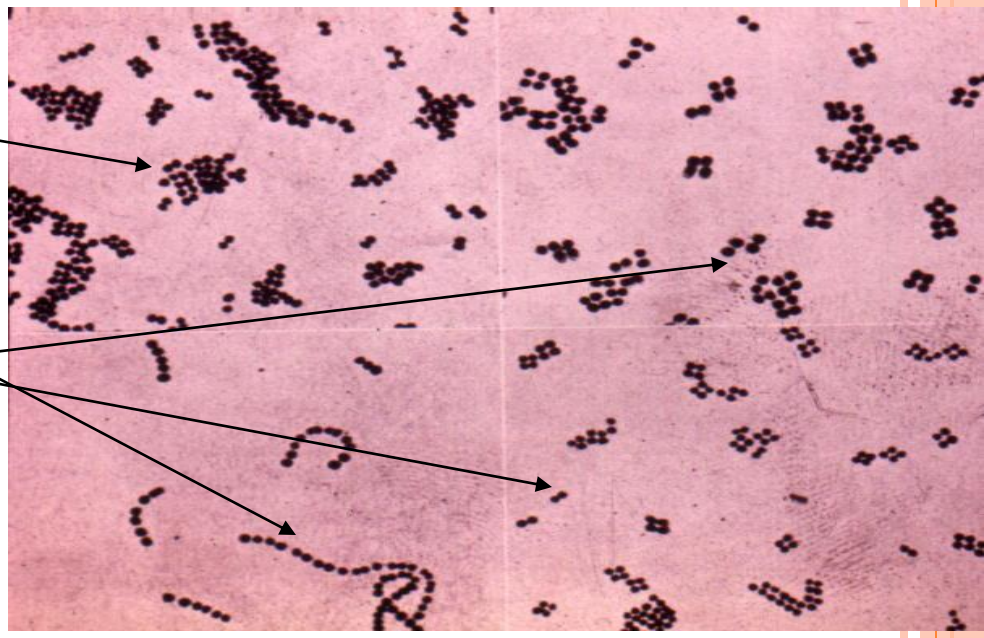
- Staphylococci were first observed in human pyogenic lesions by Von Recklinghausen in 1871.
- Pasteur in 1880 obtained liquid cultures of cocci from pus and produced abscesses by inoculating them into rabbits.
- Sir Alexander Ogston, a Scottish surgeon in 1880 who established conclusively the causative role of the coccus in abscesses and other suppurative lesions.
- He also gave the name Staphylococcus (*Staphyle, in Greek meaning 'bunch of grapes': Kokkos, meaning a berry*) due to the typical occurrence of the cocci in grape like clusters in pus and in cultures.

- Ogston had noticed that non-virulent staphylococci were also present on skin surfaces.
- Most staphylococcal strains from pyogenic lesions were found to produce golden yellow colonies, and the strains from normal skin, white colonies on solid media.
- In 1884, Rosenbach named them *Staphylococcus aureus* and *Staphylococcus albus* respectively. Later *S. albus* was renamed as *Staphylococcus epidermidis*

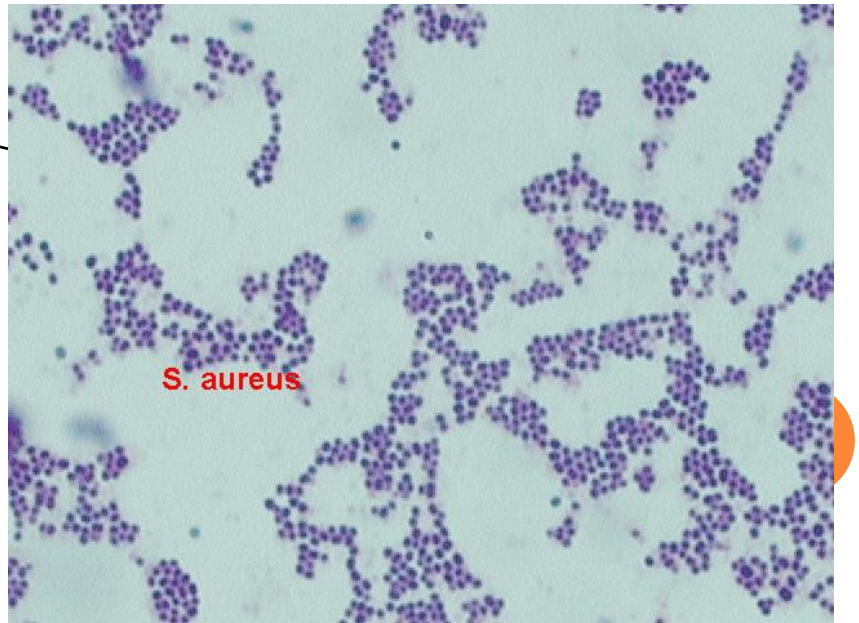


MORPHOLOGY

- *Staphlococcus* sp
- *Streptococcus* sp
- *Diplococcus* sp
- *Micrococcus sp*

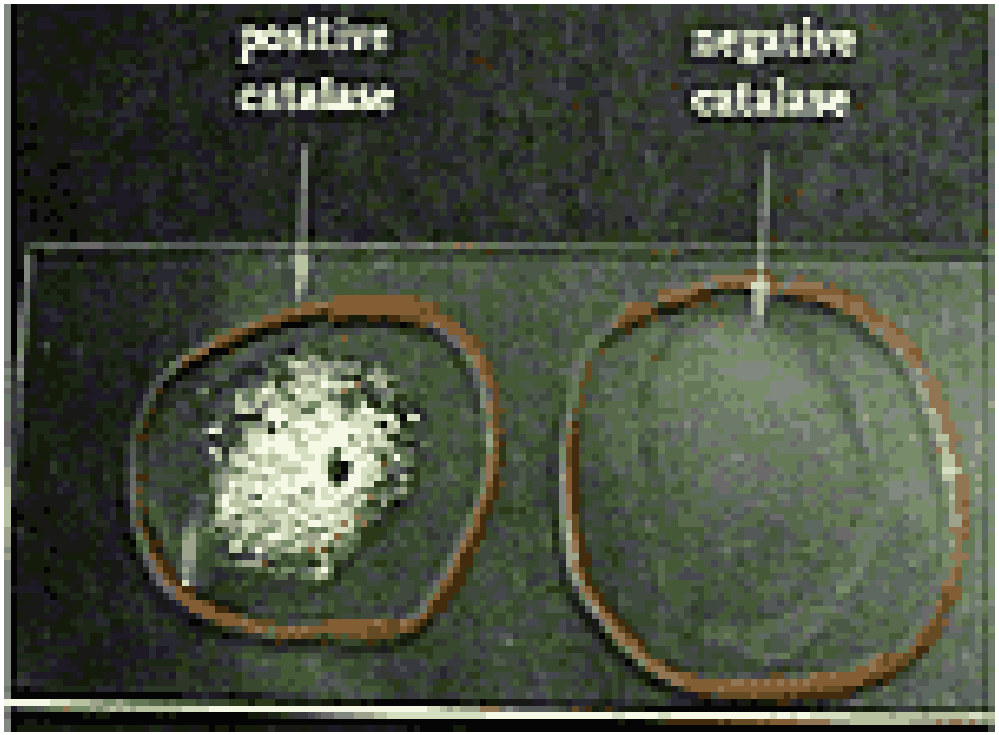


- *Staphylococcus* colony gram stained



General Properties

- Gram positive, 1µm size, catalase positive (*Streptococcus* catalase negative), oxidase negative, aerobic/facultative aerobic, non-motile, non-spore forming.
- *S. saccharolyticus* and *S. aureus* subsp. *anaerobious* are anerobic and catalase negative
- They are capable to divide in any plane so arranged as bunch of grapes
- Generation time is 20 minutes
- Nitrofurantoin susceptibility test (Susceptible: *Staphylococcus*/ Resistant: *Micrococcus*)



Catalase positive activity

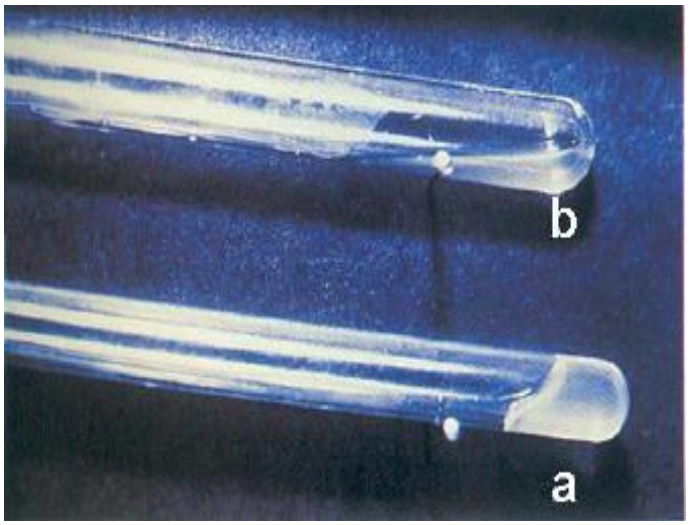
Name of Species

- **Coagulase Positive:**

Staphylococcus aureus, S. intermedicus, S. hyicus, S. schleiferi

- **Coagulase Negative:**

S. arlettae, S. capitis, S. caprae, S chromogenes, S. epidemidis, S. equorum, S. felis, S. gallinarum, S. haemolyticus, S. hominis, S. saprophyticus



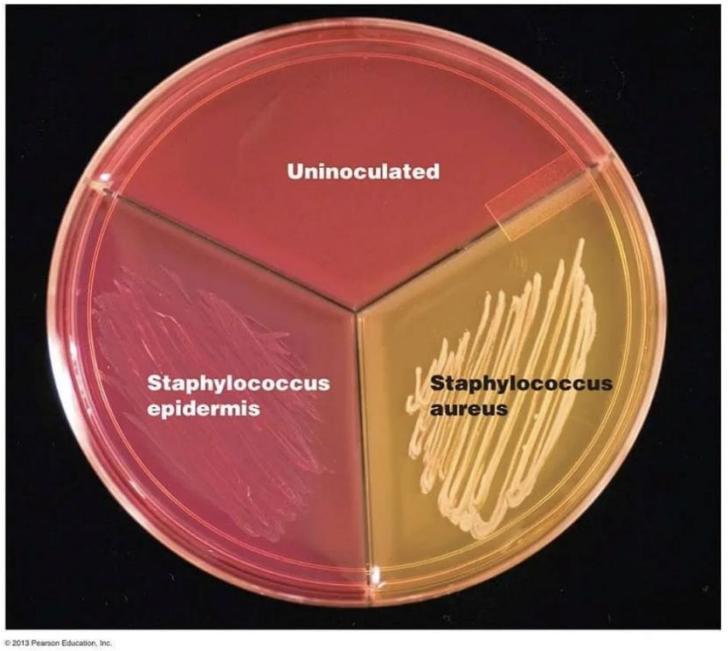
Usual Habitat

- Staphylococci are wide spread in nature although they are mainly found living on the skin, skin glands and mucous membrane of mammals and birds.
- They may be found in the mouth, blood, mammary glands, intestinal, genitourinary and upper respiratory tracts of these hosts.
- Skin surface
- Sebaceous glands, sweat glands
- Hair follicles
- Mucous membrane
- Nasal cavity
- 20–30% human population is carrier

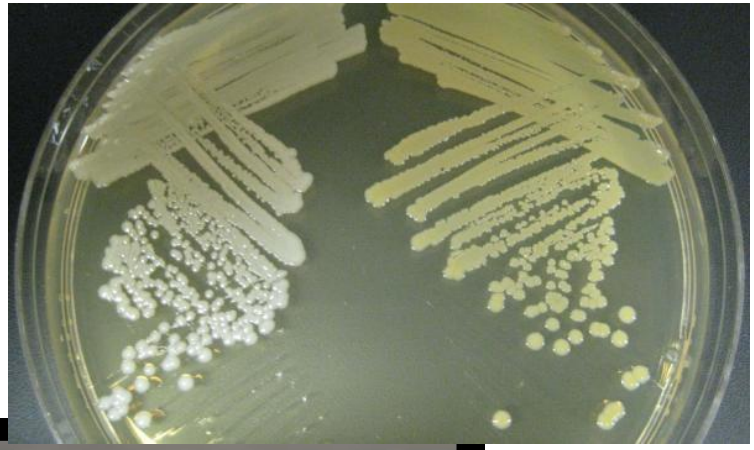
Cultural and Biochemical characteristics

- They grow readily on ordinary media within a temperature range of 10-42°C. Optimum temperature is 37°C and pH 7.4-7.6.
- On nutrient agar a typical 24hr *S. aureus* colonies are pigmented, smooth, entire, slightly raised, translucent and hemolytic on routine blood agar. Small colony variants (SCVs) of *S. aureus* produce colonies that are pinpoint in size, non-hemolytic and non-pigmented.
- In liquid medium, uniform turbidity is produced.
- Selective media used for isolating *S. aureus* contain 7.5-10% NaCl like salt-milk agar, ludlam's medium containing lithium chloride and tellurite.

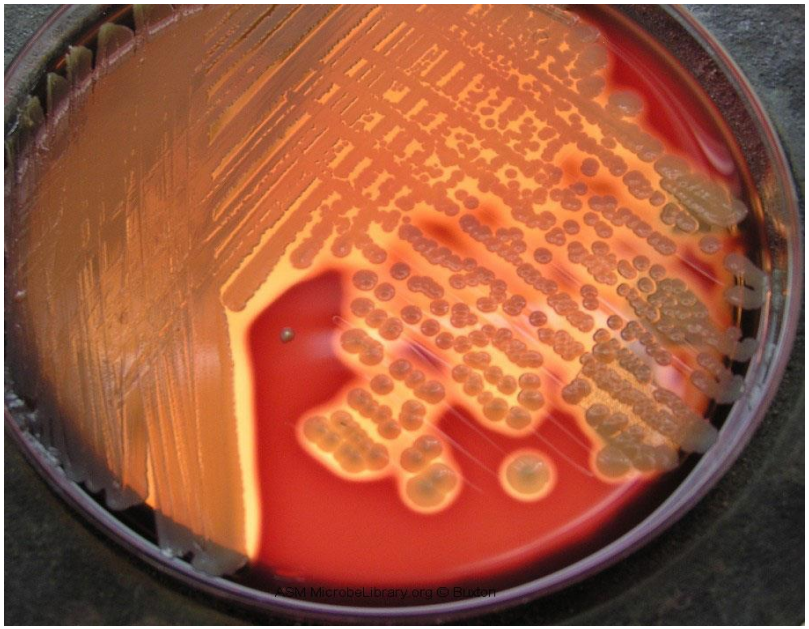
- **Mannitol salt agar**: prepared by 1% Mannitol sugar with high concentration salt (7.5% NaCl) and dye Phenol red. *Staphylococcus* spp. capable to ferment mannitol and produce yellow colonies
- **Maltose purple agar**: prepared 1% maltose with dye bromocresol purple. Use to differentiate maltose fermenting and non fermenting species.

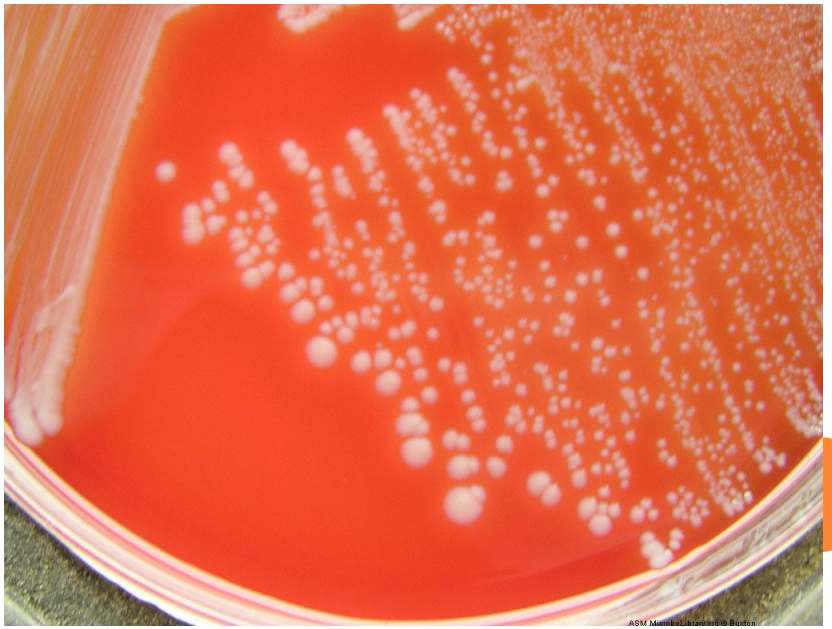


- Pigmentation: yellow/ golden yellow/ white
- *S. aureus* – yellowish or golden orange pigment.
- *S. albus* - white colonies.
- *S. citreus* - lemon yellow colour pigment

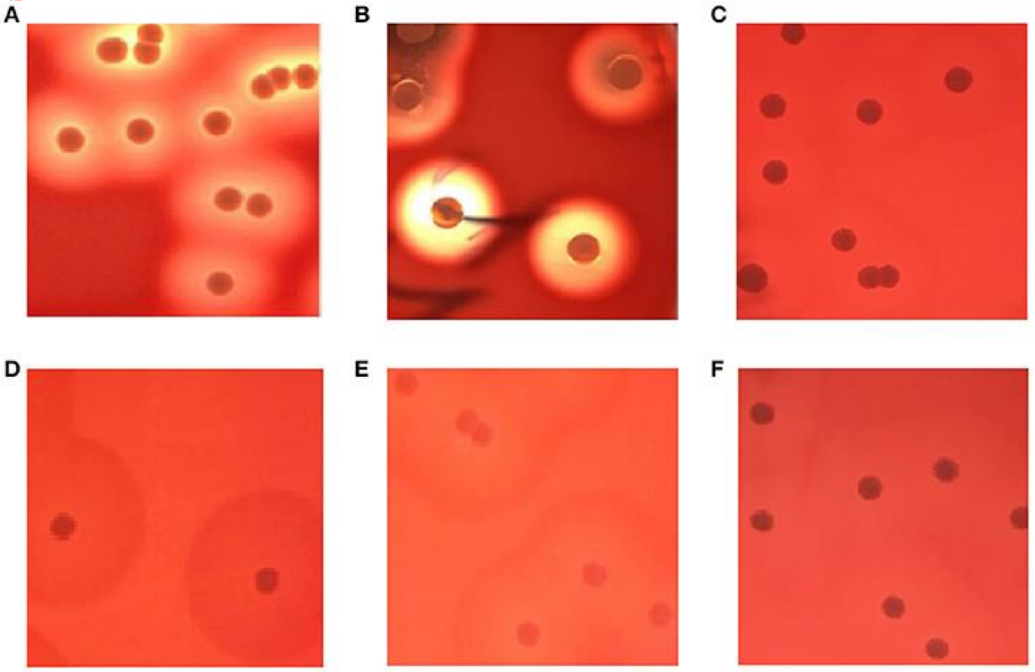


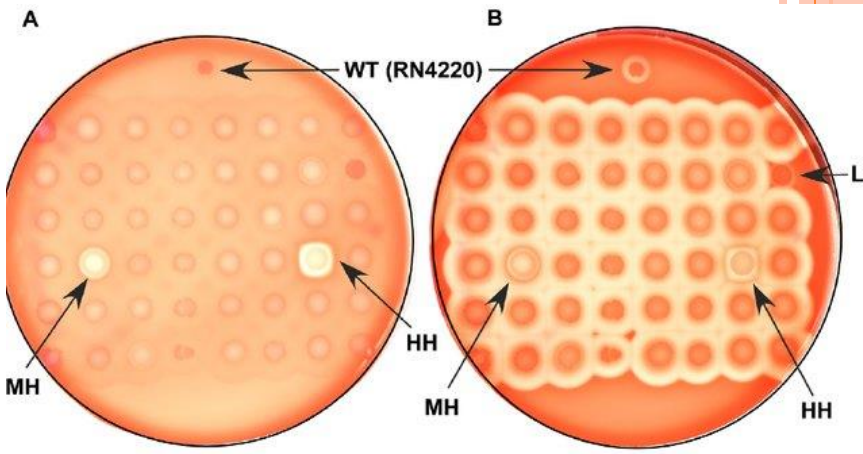
- **Haemolysin**: produce alpha, beta and gamma haemolysin (staphylococcus species showed **double heamolysis phenomena**)
- Alpha haemolysin: produced narrow zone of complete hemolysis
- Beta haemolysin: produced wider zone of partial or incomplete hemolysis
- Gama haemolysin: produced non hemolytic activity

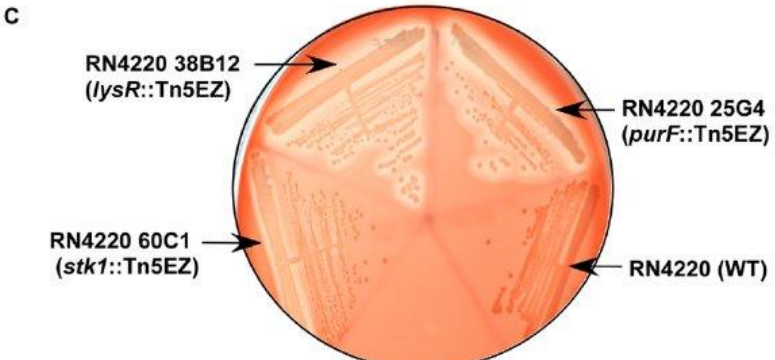




- **Hot- cold lysis phenomenon: In which** beta haemolysin producing strains start to produce alpha haemolysin during refrigeration at 4℃.
- ***S. hyicus* are non hemolytic**
- Capable of liberating **'V' factor** into the medium, which favours the growth of ***Haemophilus*** organism.

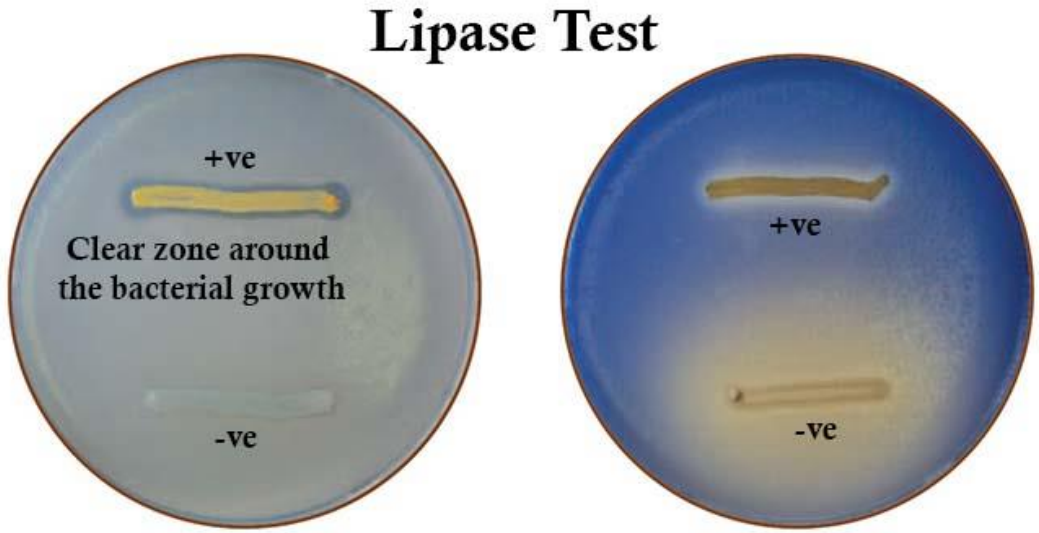




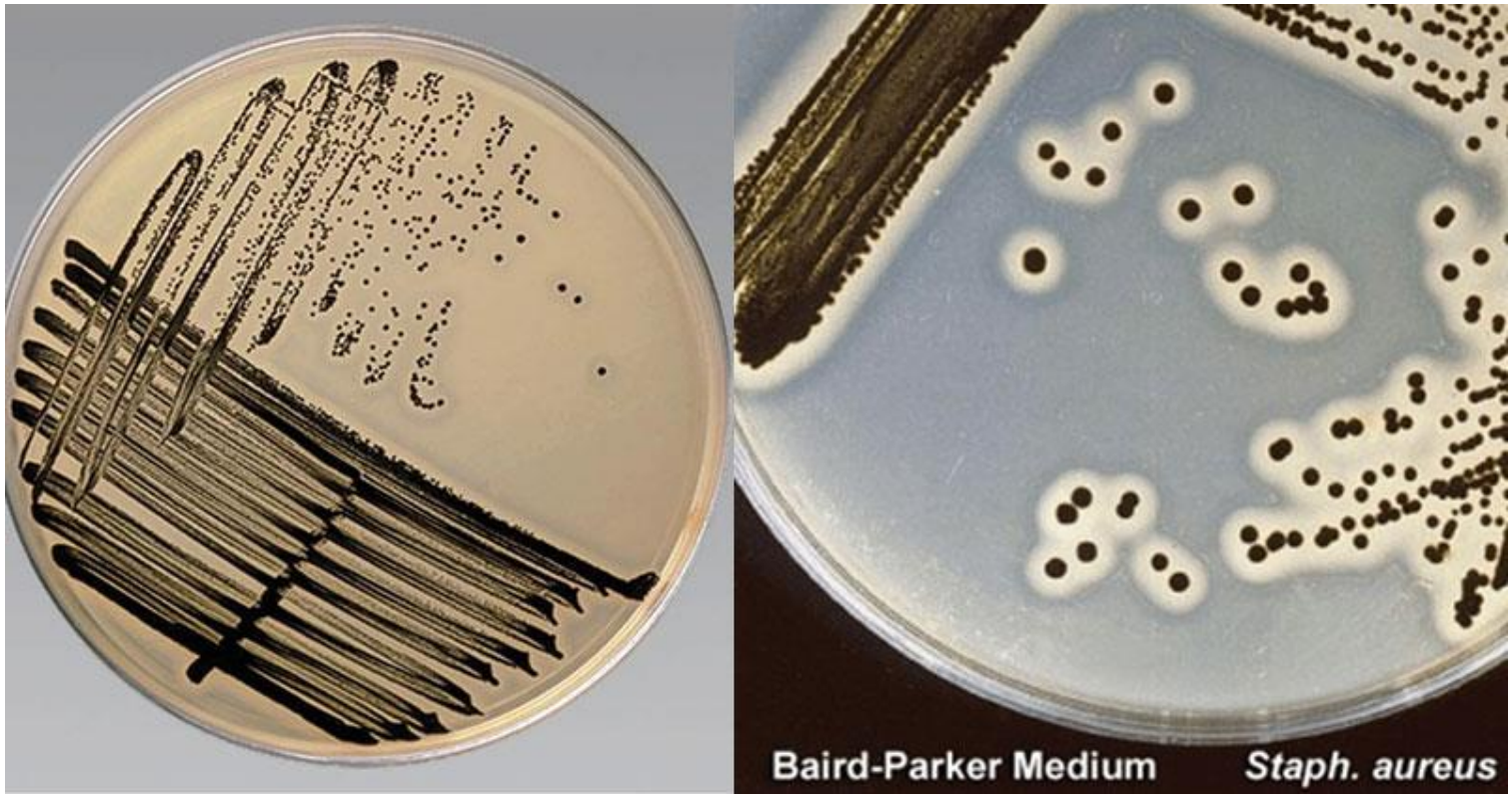


- **Coagulation phenomenon:** *Staphylococcus* spp. produce coagulase enzyme: it is found as two types free and bound coagulase (also known as clumping factor)
- Slide coagulase test can detect bound coagulase and tube coagulase test can detect free coagulase

- Urease positive, they reduce nitrates to nitrites, liquefy gelatin and are MR, VP positive but indole negative.
- They are lipolytic when grown on medium containing egg yolk.
- They produce phosphatase which can be demonstrated by growing on nutrient agar containing phenolphthalein diphosphate.

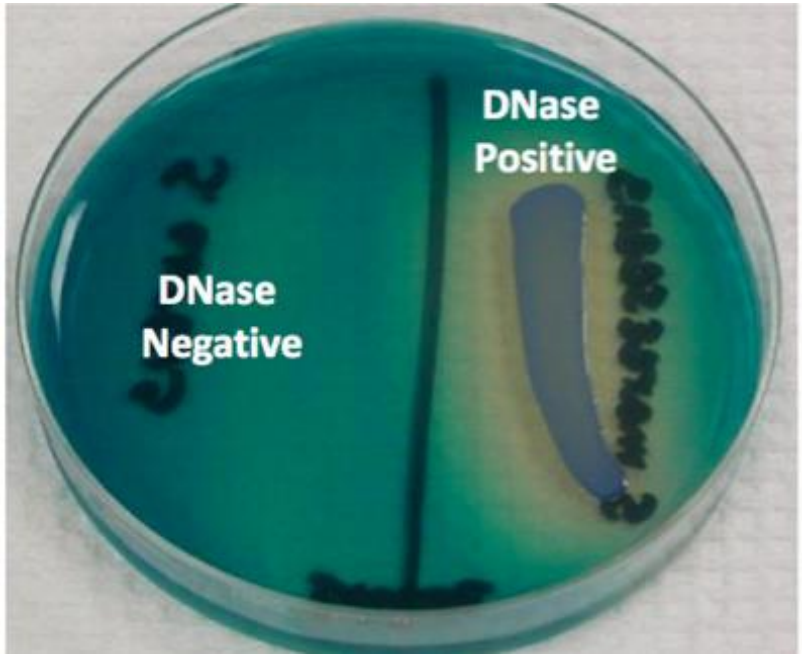


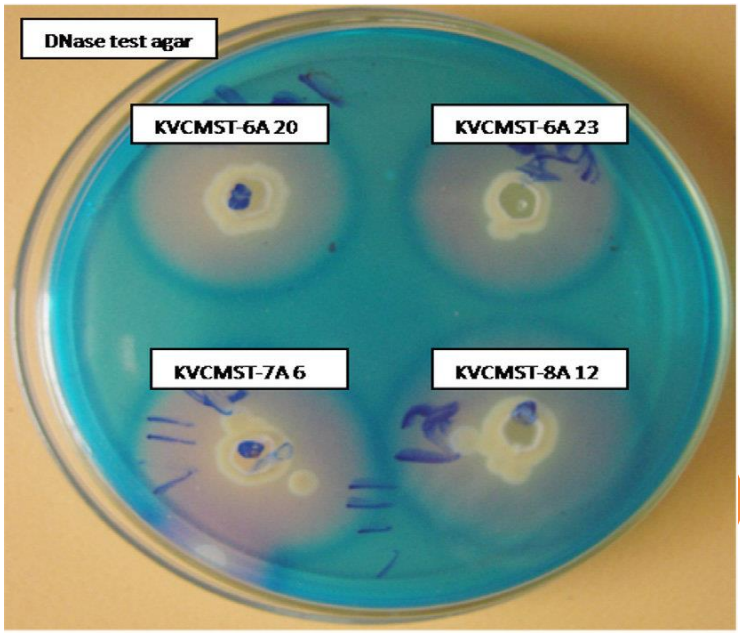
- In a medium containing potassium tellurite, tellurite is reduced and black colonies are produced.



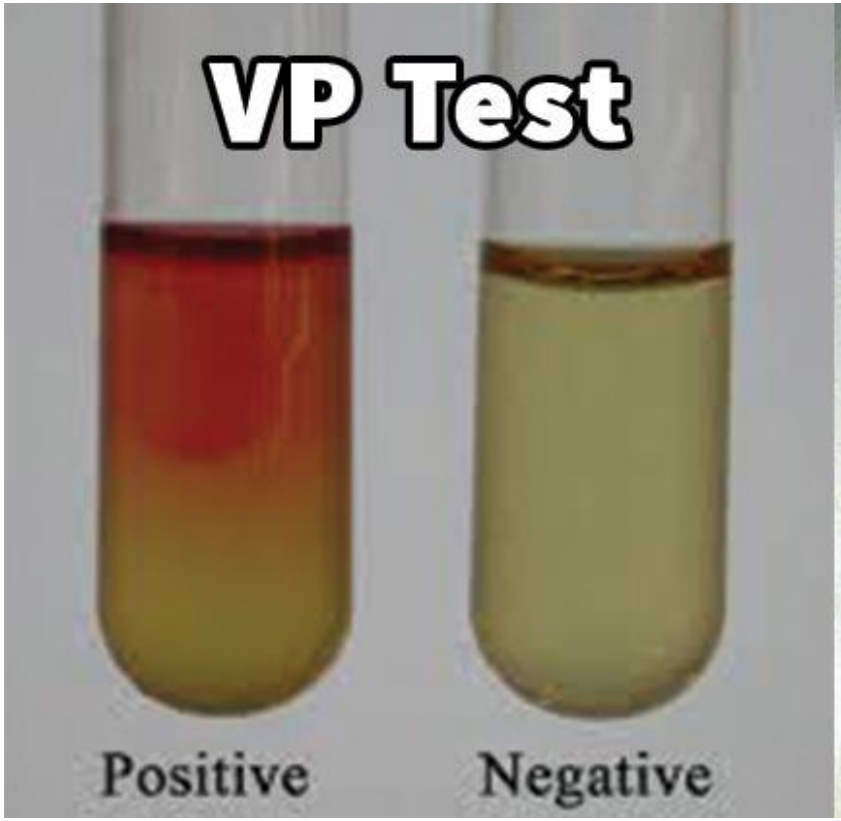
S. aureus Baird Parker Agar

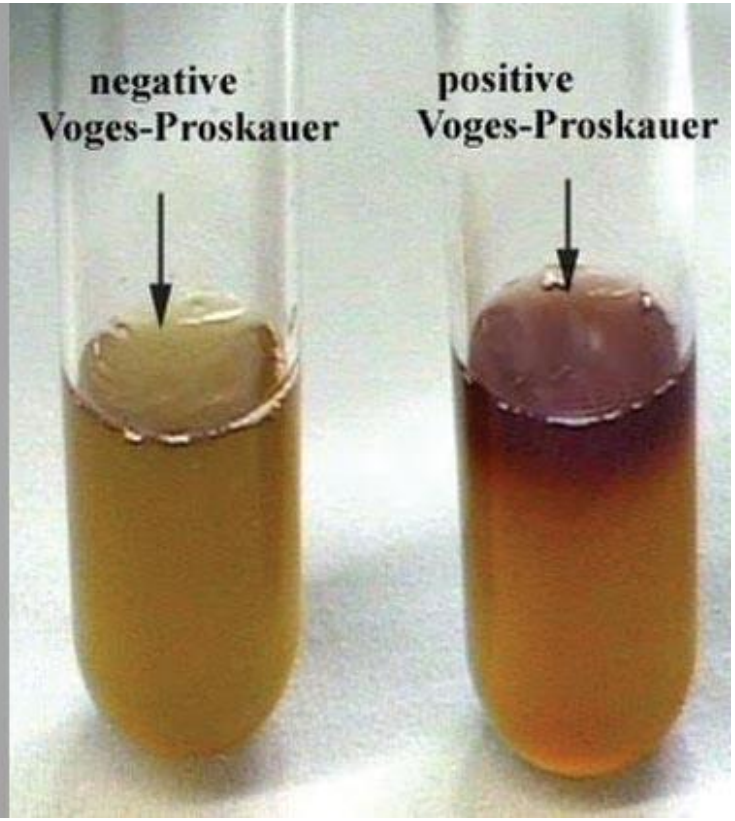
- **Heat Stable Nuclease**: A heat stable staphylococcal nuclease (thermonuclease (TNase)) that has endo and exonucleolytic properties and can cleave RNA or DNA is produced by most strains of *S. aureus*. TNase can be demonstrated by the ability of boiled cultures to degrade DNA in an agar diffusion test or detected by using metachromatic agar diffusion procedure and DNase toludene blue agar.





- **Acetoin Production (acetyl methyl carbinol):** In this biochemical reaction glucose converts in to pyruvic acid which can further be metabolized to produce acetoin (i.e., acetyl methyl carbinol or 3-hydroxybutanone) it can detect by conventional Voges-Proskauer test.





- **Antigens**

Carbohydrates

- The cell wall of *S. aureus* contains ribitol teichoic acid.

S. intermedius contains glycerol teichoic acid.

- Protein A - Present only in *S. aureus*.

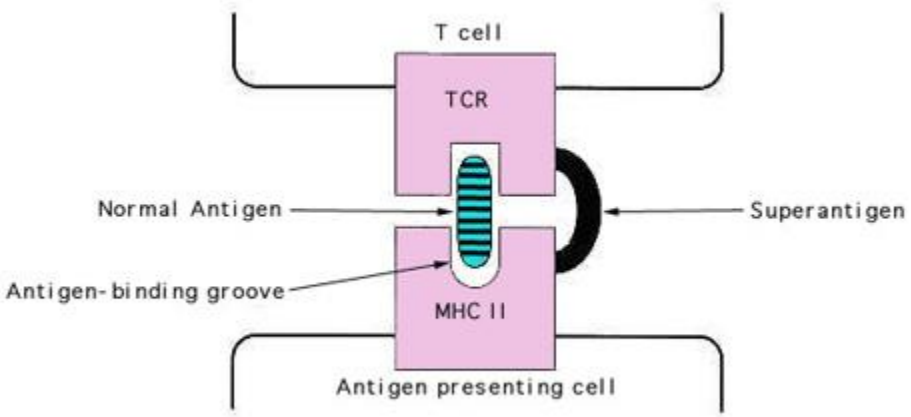
Micrococcaceae family differentiation

Organism	Appearance in stained smears	Coagulase production	Catalase production	Oxidase production	O-F test	Bacitracin disc (0.04 units)
Staphylococcus spp.	Irregular cluster	+/-	+	-	F	Resistant
Micrococcus spp.	Packets of four	-	+	+	O	Susceptible
Streptococcus and *Enterococcus* spp.	Chains	-	-	-	F	Resistant

Virulence Factors

1. Toxin/ Exotoxin: Toxins can be categorized in to 3 groups

A. Pyrogenic toxin super antigens: Super antigen activity that indudes toxic shock syndrome (TSS). This group includes TSST-1, which causes toxic shock syndrome and staphylococcal entero toxins which cause a form of food poisoning. They produce 6 serotypes of enterotoxins which cause diarrhea and vomiting.



B. Exfoliative toxins: are implicated in the disease staphylococcal scalded skin syndrome (SSSS)

C. Membrane damaging toxins: include ά toxin, ß toxin and ɣ toxin and the classical Panton-Valentine Leukodicin (PVL) factor.

2. Iron uptake and Plasminogen activator: *Staphlokinase*

3. Immune evasion: chemotaxis inhibitory protein of Staphylococcus (chp), staphylococcal binder of immunoglobulin (sbi), staphylococcal complement inhibitor (scn), Protein A (spa)

4. Exoenzyme:

- Aureolysin (Protease; Zinc metalloproteinase)
- Hyaluronate lyase (Spreading factor: Degradation of hyaluronic acid, contribute to local dissolution of the extracellular matrix)
- Lipase & esterases (Degrade lipids)
- Staphopain (Cysteine protease)
- Staphylocoagulase
- V8 protease (Serine protease)
- Lysozyme (Hydrolyses the peptidoglycan in the cell wall of many bacteria)

5. Antiphagocytosis: Microcapsule Produced by over 90% of *S. aureus* strains. Two serotypes (5 and 8)

6. Adherence:

- Clumping factor: Mainly ClfA and ClfB, which bind to different sites in fibrinogen. ClfA binds to the γ-chain whereas ClfB binds to the α-chain
- Collagen binding protein
- Elastin binding protein
- Fibrinogen binding protein
- Fibronectin binding proteins
- Intercellular adhesion

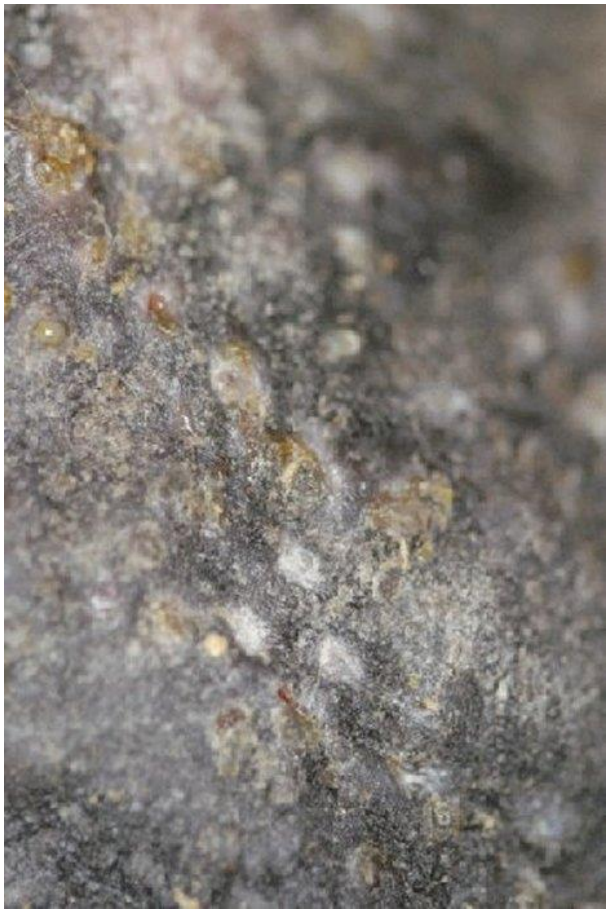
Diseases causes by *Staphylococcus* spp

Species	Hosts	Clinical conditions
Staphylococcus aureus	Cattle	Mastitis, udder impetigo
	Sheep	Mastitis Tick pyaemia (lambs) Benign folliculitis (lambs) Dermatitis
	Goats	Mastitis Dermatitis
	Pigs	Botryomycosis of mammary glands Impetigo on mammary glands
	Horses	Scirrhous cord (botryomycosis spermatic cord), mastitis
	Dogs, cats	Suppurative conditions similar to those caused by *S. pseudo-intermedius*
	Poultry	Arthritis and septicaemia in turkeys Bumble foot Omphalitis in chicks

Diseases causes by *Staphylococcus* spp

Species	Hosts	Clinical conditions
S. pseudintermedius	Dogs	Pyoderma, endometritis, cystitis, otitis externa, and other suppurative conditions
	Cats	Various pyogenic infections
S. hyicus	Pigs	Exudative epidermitis (greasy-pig disease) Arthritis
S. aureus subsp. anaerobius	Sheep	Lymphadenitis
S. schleiferi subsp. coagulans	Dogs	Otitis externa

- **Horse**: (**Botryomycosis**): Infrequent chronic granulomatous lesions involving the udder of the mare, cow and sow and the spermatic cord of horses.



- **Cattle**:

Mastitis: Staphylococcal bovine mastitis may be chronic, acute and peracute. Gangrenous mastitis due to a toxin is seen in postparturient cows.

- **Sheep**:

Tick pyemia in lambs occurs in 2-5 week old lambs, which is heavily infected with *Ixodes ricinus*.

Periorbital eczema is an infection due to abrasions, Staphylococcal dermatitis due to scratches from vegetation.

Poultry: (Bumble foot)

- A pyogranulomatous process of subcutaneous tissue of foot that can involve the joints.
- Staphylococcal arthritis and septicemia in turkeys, omphalitis – yolk sac infection, wing rot or gangrenous dermatitis infection in poultry.

Pig: (Greasy pig disease)

- Exudative epidermitis (greasy pig disease) is an acute generalized infection of suckling and weaned pigs caused by *S. hyicus*. This disease is characterized by excess sebaceous secretion, exfoliation and exudation.

- Dogs and Cats: Pyoderma is one of the most common skin diseases of dogs.
- In addition to this, Otittis externa and other suppurative conditions are caused by S. intermedius.
- Staphylococcal antigens produce intense inflammatory reaction and promote persistence of the bacteria.

Other Staphylococcal organisms

- *S. aureus sub sp.anaerobius* causes caseous lymphadenitis. They are anaerobic and catalase negative.
- *S. caprae* in goat's milk.
- *S. gallinarum* and *S. arlettae* - skin of chickens
- *S. lentus* in skin of sheep and goats.
- *S. equorum* in skin of horses
- *S. simulans* and *S. felis* - clinical specimens in cats
- *S. delphini* in skin of dolphins
- *S. aureus* in **Staphylococcal scalded skin syndrome (SSSS)** and Toxic shock syndrome (TSS) in humans.

Antimicrobial Resistance

- *Staphylococcus* spp has significant role in context of antibiotic resistance in the form of MRSA in (Methicillin resistant *Staphylococcus aureus*) / VRSA (Vancomycin resistant *Staphylococcus aureus*)

Diagnosis

Staphylococcus spp can diagnosed by various phenotypic and genotypic characteristics

- Primary and secondary biochemical test such as gram staining, catalase, oxidase, O/F, test
- Hemolysis and coagulase properties
- Colony characteristics and Growth on MSA and egg yolk Agar
- Absence of growth on Macconkey agar
- Phage typing

Further Readings

- Clinical Veterinary Microbiology 2nd Edition 2013 By Bryan Markey
- Veterinary Microbiology and Microbial Disease