Pyogenic cocci staphylococci

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Pyogenic cocci

These organisms cause inflammatory process in human being, with the formation of pus for this reason they are known as pyogenic cocci.

The Important Pyogenic Cocci are

- Staphylococcus
- Streptococcus
- Pneumococcus

→ Gram positive

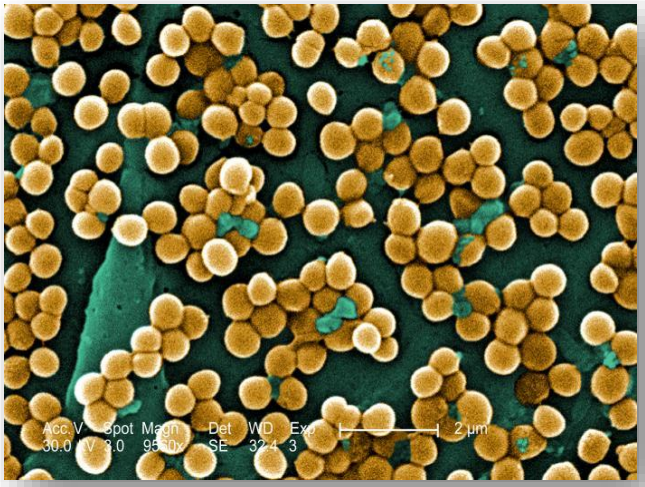
- Gonococcus
- Meningococcus

→ Gram negative

Staphylococcus

General characteristics

- Gram positive
- Spheres or ovoid bacteria
- 0.8-1 um in diameter
- Non motile
- Non sporulating

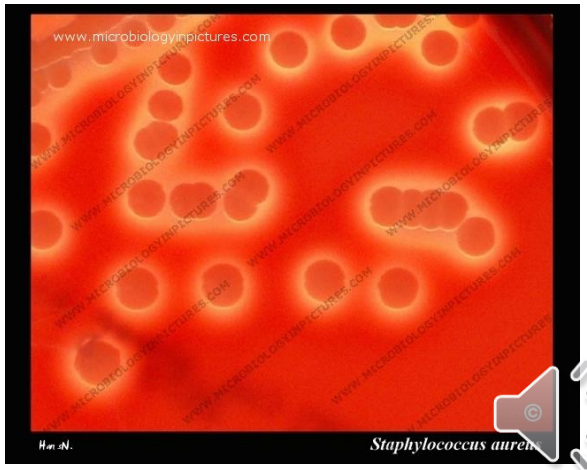


- Arranged in grape like clusters , in pairs or small groups.
- Grow on ordinary culture media and may produce pigment
- On blood agar the colony are small (1-2)mm

Smooth, Round, β- haemolytic

- Catalase positive

but some are negative.



Habitat

It is facultative anaerobes can live in difficult environments can survive

- **High salt media 10% Nacl**
- **Dryness environments**
- **Varity range of temperature (10-40)**

Habitat

- The staphylococci are seen normally on skin, in the nose, the intestinal contents, and in the saliva.
- Besides their presence in the suppuration, the organisms are present in air, milk and water.
- about 30-60% of healthy persons are nasal carriers of pathogenic staphylococci, and this percentage is more in hospital staff.

Scientific Classification

According to Rosenbach 1884

Kingdom: Bacteria

Phylum: Firmicutes

Class: Bacilli

Order: Bacillales

Family: Staphylococcaceae

Genus: Staphylococcus

Classification on the activities of bacteria

- Aerobic acid production
- Coagulase activity
- Haemolysin type
- Nitrate reuction
- Molecular method like ribotyping and

DNA hybridization

Coagulase activity

Staphylococcus divided into

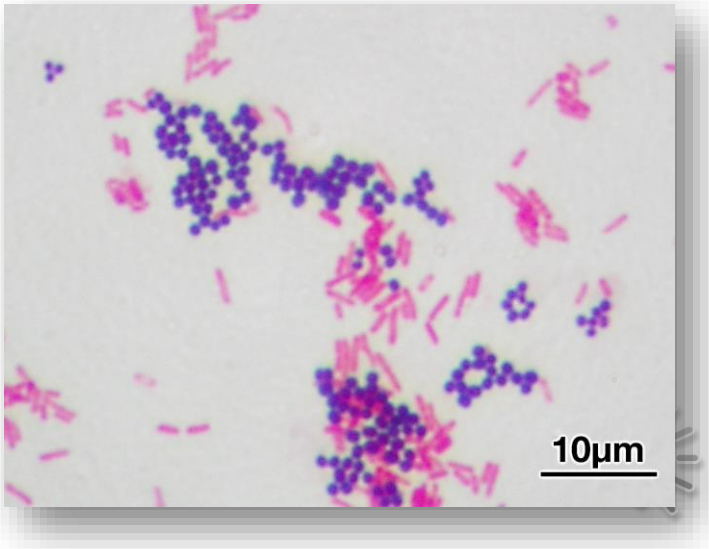
1. **Coagulases positive**
   Staph. aureus

2. **Negative coagulates**
   Staph. epidermidis*, *staph. saprophyticus

Staphylococcus aureus

- **Staphylococcus are strongly gram positive, but in old cultures, individual cells may lose the a ability to retain the gram stain and thus appear gram negative.**

- **Some strains of *Staphylococcus aureus* are encapsulated during the early phase of their growth, but most show no capsule.**



Drug resistance

- Production of β-lactamase (95)%.
- Methicillin Resistant S. aureus (MRSA) is resistant to all β -lactams.
- Vancomycin resistant S. aureus (VRSA) to the glycopeptide antibiotic.
- Plasmid mediated resistance (gene tet, aminoglycoside, erythromycin)

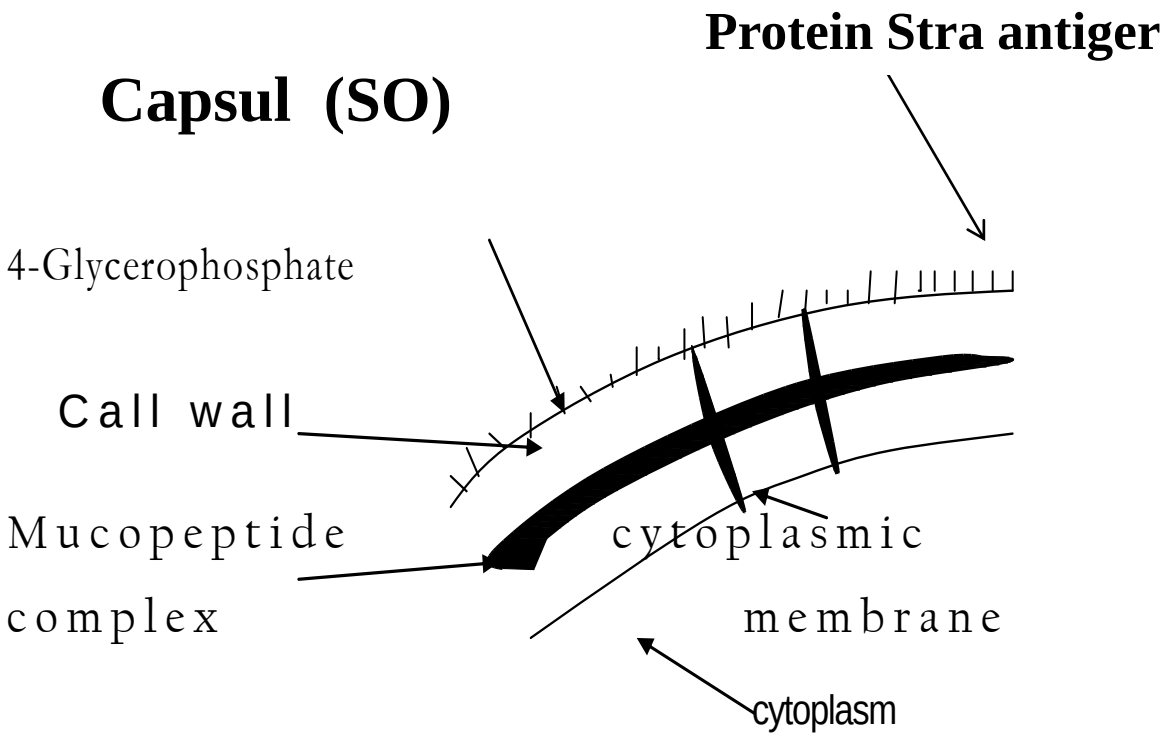
Biochemical reactions

- Staphylococcus ferment sugars and produce acid, with no gas. It ferments glucose, lactose, sucrose and mannitol .
- Liquefy gelatin.
- Catalase positive.

Antigenic structure

Fractional analysis of Staphylococcus reveals the following antigenic components

- **Teichoic acids and lipoteichoic acid**
- **Protein-A**
- **Capsular (microcapsule)**
- **Peptidoglycan: fever and inflammation**
- **Adhesins (binding and colonizing)**



Antigenic Structure of Staphylococcus

Toxins and enzymes:

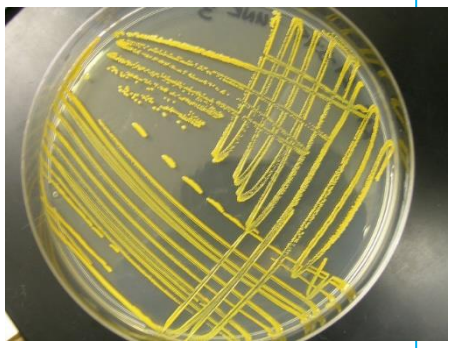
Cultural characters

- The *Staphylococcus aureus* grows simply on a variety of conventional nutrient media.
- Optimum growth temperature is 36 to 38C and with PH 4.8-9.4, best growth is at neutrality.
- They are aerobes and facultative anaerobes, common media used for the growth of these organisms are:

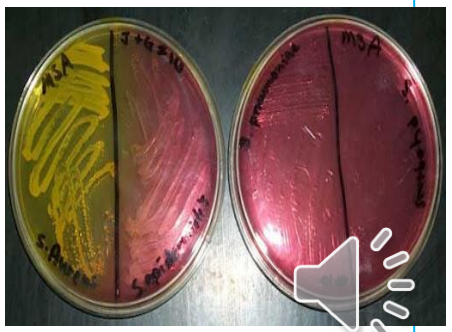
1-Nutrient broth

2-Nutrient agar

After 24 to 48 hours incubation at 37C, the colonies are 2-3 mm in diameter, round, convex, smooth, opaque, pigmented with a moist, glistening surface.



- **Golden-yellow in. Staphylococcus aureus**
- **White in Staphylococcus albus**
- **Lemon yellow in Staphylococcus citreus**



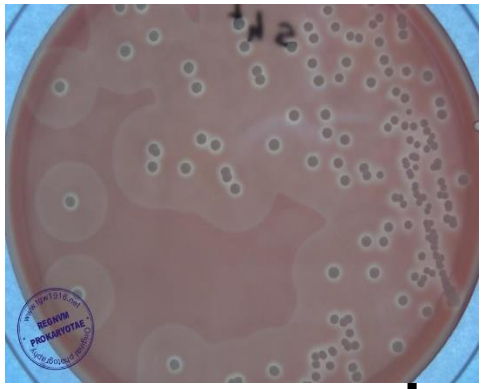
pigments are known as lipochromes, and do not dissolve in water, but are soluble, in ether, benzene, acetone and alcohol.

Note: no pigment is produced anaerobically or in a liquid media and it does not diffuse into the medium.

Note: The staphylococci grow in the presence of 10-15 % NaCl, in nutrient broth or nutrient agar acts as a selective medium and inhibit the growth of most gram negative bacilli.

3- Mlik agar

On this medium the pigment is more rapidly formed more intense and easily recognized, there is clear area around the colonies which indicates staphylococcal proteases.



4- Blood agar

Marked zone of Beta-haemolysis may appear around colonies on sheep or rabbit blood agar.

5- Egg yolk medium

The colonies may be surrounded by zones of opacity as a result of enzyme lipase.

6- Blood tellurite medium

The majority of coagulase positive staphylococcus are able to grow in the presence of tellurite.

Reduction of tellurite to tellurium occurs to give grayish black colonies.

Toxins and enzymes

The known metabolites of staphylococcus may be classified as:

A-Toxins

- α, β, γ, δ toxin (α, β, γ, δ haemolysins)
- Leucocidin
- Enterotoxin
- Toxic shock syndrome 1
- Exfoliative Toxins (ETs)

B-Non toxic metabolites

- Free coagulase
- Bound coagulase
- Staphylokinase
- Hyaluronidase
- Penicillinase
- Phosphatase
- Protease
- Lipase
- Gelatinase
- Catalase

Alpha(α)-hemolysin

it is haemolytic, leucocidal (kills macrophages and leucocytes), cytotoxic and dermoncrotic. The toxin is antigenic and toxoid is prepared by treating with formalin. This toxin lysis erythrocytes of rabbits, cows and goats but not of man.

Beta(β)-haemolysin

It is hot-cold haemolysin, producing slight lysis of erythrocytes at 37C, but extensive lysis at cold temperature. This toxin lysis Erythrocytes of sheep, ox and goats. Erythrocytes of rabbits and man are resistant. The toxin is antigenic.

Delta(δ)-haemolysin

It is a protein and lysis erythrocytes of human and rabbits. The toxin is non antigenic.

Leucocidin

It is thermostable leucocytoxic substance and causes morphological changes in leucocytes of wide number of species.

Enterotoxin

This toxin is produced by few strains of Staphylococcus aureus enterotoxins are heat resistant and with stand boiling for 30min. Six distinct antigenic types of enterotoxins A,B,C,D,E,F have been recognized.

- Out of which A and B are the most important.
- Staphylococcal food poisoning results when food contaminated with entero-toxin producing. staphylococcus, having preformed toxin, is ingested.
- The common types of food usually responsible are cooked meat, fish, milk or milk products.
- Sufficient time should elapse between staphylococcal contamination of food and its consumption so as to produce enough enterotoxin.

The enterotoxin causes nausea, vomiting and diarrhea within 1.5 to 3 hours after ingestion of contaminated food.

Exfolative toxins (ETs)

This toxin is produced by some strains of Staphylococcus.

- It has special effect to produce exfoliation (Ritters syndrome), toxin epidermal necrolysis, impetigo and the clinical condition is called Staphylococcal scalded skin syndrome (SSSS) and is usually seen in young children below 4 years.

Free coagulase

- It is heat labile enzyme and also called extra-cellular coagulase
- Plasmas of many animal species are simply clotted by the free coagulase.
- The free coagulase does not act directly to convert fibrinogen to fibrin but rather interact with coagulase reacting factor in the plasma to produce complex called C.R.F coagulase.

C.R.F. + Coagulase → C.R.F. coagulase

This complex then replaces thrombin in the formation of fibrin.

C.R.F. coagulase + Fibrinogen → Fibrin (clot)

- **The plasma of guinea pig is deficient in coagulase reacting factor.**
- **Seven distinct antigenic varieties of coagulase have been described and the majority of human strains produce coagulase A.**
- **It is important that incubation time for free coauglase should not be prolonged for more than 2 hours, since on prolonged incubation some strains are capable to utilize citrate employed as anticoagulant and thus may result in spontaneous clotting of plasma and give false positive result.**
- **On continued incubation clot may be lysed by fibrinolysin produced by some strains .**

Bound coagulase

- When Staphylococcus aureus are mixed with undiluted plasma on a glass slide, thick clumps appear due to combination of fibrinogen in the plasma with a receptor present on the surface of the bacteria causing them to stick to gather.
- The factor causing this is the clumping factor or bound coagulase, which is attached to the surface of the organism and acts directly on the fibrinogen.
- It is protein and heat stable.
- All strains which produce bound coagulase also produce free coagulase but the reverse is not true.

- Only pathogenic staphylococcus produce coagulase, and coagulase production is test for the pathogenicity.
- Bound coagulase converts fibrinogen directly to fibrin without requiring an plasma factor.
- Coagulase promotes the virulence of the organisms by inhibiting phagocytosis, by laying down a fibrin barrier around the bacteria cell. Coagulase forms a barrier around the organisms and thus protects them from phagocytosis and bactericidal substance.
- It also helps in localizing the lesion.

Free coagulase	Bound coagulase
1- Secreted into the medium	1- Integral component of cell wall
2- Protein	2- Protein
3- Heat labile	3- Relatively heat stable
4- About 7 distinct antigenic varieties	4- Only one antigenic component
5- C.R.F. is essential	5- C.R.F. is not essential
6- Active against fibrinogen of wide range of animals	6- Active on fibrinogen of certain animal species

Staphylokinase (Müller's factor)

- It is also known as fibrinolysin and is formed by 70-90% of coagulase positive staphylococci.
- Staphylokinase acts on plasminogen and produces fibrinolysin.
- Clot dissolution by to the fibrinolytic enzyme.
- Staphylokinase is also responsible for the production of haemolytic area and proteolysis (clearing) which occur at a distance from colonies grown on blood agar.

Hyaluronidase

- It is also called spreading factor and produce by more than 90% of Staphylocceus aureus .
- It acts on hyaluronic acid (cementing substance) between connective tissue cells and dissociate them.

Penicillinase

- Some strains of staphylococcus are resistant to penicillin.
- Resistance is due to inactivation of penicillin by an enzyme.
- Penicillinase a (Beta-lactamase) that destroy the Beta-lactam ring of penicillin.

Phosphatase

- There is a close relationship between phosphatase activity virulence and coagulase activity.
- This is useful screen procedure to differentiate Staphylococcus aureus from Staphylococcus epidermidis.

Proteases

These enzymes are perhaps responsible for the rapid necrosis of tissues including bones.

Lipase

- 99.5% of coagulase positive strains are lipolytic.
- It is antigenic, but not related to pathogenicity.

Catalase

All the strains of staphylococcus, pathogenic and non pathogenic produce catalase and this is an important test to differentiate these organisms from streptococci.

Gelatinase

This enzyme liquefy gelatin and transform it to liquid state.

Pathogenicity

- The Staphylococcus aureus is present in the anterior noses of nearly 50% of the human population, but the incidence of disease is comparatively low.
- Every tissue and every organ is susceptible to invasion by the staphylococcus and the resulting disease is characterized by inflammation, necrosis and abscess formation.
- Staphylococcus lesions are localized in contrast to the spreading nature of lesions in streptococcus.

Positive coagulase

Infection of skin (pyoderma)

The most frequent area of involvement is the skin

- **Minor cutaneous abscesses**

are termed pustules

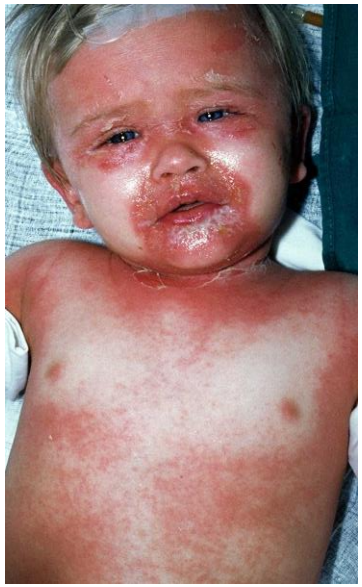
- **Larger ones furuncles (Boils) and carbuncle**

amore serious lesion which is usually occurs in the thick, collagenous tissue of the back of neck.

- **Intra epidermal lesions (Impetigo)**
- **More severe lesions (wounds Infection).**

Staphylococcus Scalded skin syndrome (SSSS)

Pemphigus neonatorum or Ritter's disease, is a dermatological condition caused by Staphylococcus aureus.



Gastrointestinal tract staphylococcus diseases

- **Pseudomembranous enterocolitis.**
- **Enteritis (oral therapy).**
- **Food poisoning, which may occur 1-6 hours after ingestion of infected food such as cooked meats, milk and milk products.**

Deeper infections

- Mastitis
- Osteomyelitis
- Endocarditis bronchopneumonia
- Neonatal meningitis

Toxic shock syndrome

➢ Epidemiological markers

- Biotyping
- Phage typing
- Antibiogram (antibiotic tests)
- Serotyping
- Molecular methods (PCR)

Sources of infection

- Patients
- Healthy carriers
- Animals

Prophylaxis

- No vaccine
- Many types of Antigenic structure
- Many toxins and route of actions

So to prevent infection just need cleaning

Coagulase Negative Staphylococci (CONS)

- **Infection of prosthetic devices**
- **Catheter (UTI)**
- **Bacteremia**

S. epidermidis

- **Staphylococcus epidermidis do not produce toxins and are usually harmless.**
- **Staphylococcus is a vary common cause of infection in the hospitals and infect new born babies, surgical patients and diabetic patients.**

Found on skin causes:

- Prosthetic infection
- Endocarditis
- Bacteremia
- UTI (hospitalized patients specially older people)
- Wound and burn infection
- Sensitive of novobiocin

S. Saprophyticus

- UTI in young women
- Resistance for novbiocin

Differential characters of staphylococcus

Characters	Staphylococcus aureus	Staph. Epidermidis
Colonial pigmantation	Yellow to golden	white
Coagulase	(+)	(-)
Mannitol fermentation	Acid	Negative
Haemolysin	Beta-haemolysis	Negative
lipase	positive	Negative
Deoxyribonucleasc	positive	Negative
Protein-A	present	Absent
Teichoic acids of the cell wall	Ribitol type	Glycerol type

Laboratory diagnosis

A - The material for diagnosis may depend on the type of lesion the common material referred to the laboratory may be:

swab, pus, urine, sputum, C.S.F, blood.

B - In food poisoning: food, vomit, faeces.

The morbid material is subjected to the following **routine for the isolation and identification of the organisms:**

- A) Smear
- B) Culture
- C) Mannitol fermentation
- D) Coagulase test
- E) Catalase test
- F) Phage typing

Thank you