Gram positive Cocci

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Staphylococci and Streptococci constitute the main groups of medically important gram positive Cocci.

Staphylococcus :

Staphylococci are ubiquitous in nature , some species occurring as part of human flora like ***S. epidermidis*** .

Most are harmless and reside normally on the skin and mucous membranes of humans and animals.

The most important virulent species is *S. aureus* It is the most common cause of bacterial pyogenic infections and it is also important cause of food poisoning.

Classification

Classification of staphylococci depends on its ability to produce coagulase , an enzyme that causes plasma clotting.

***1-Staphylococcs aureus* is coagulase-positive pathogenic**

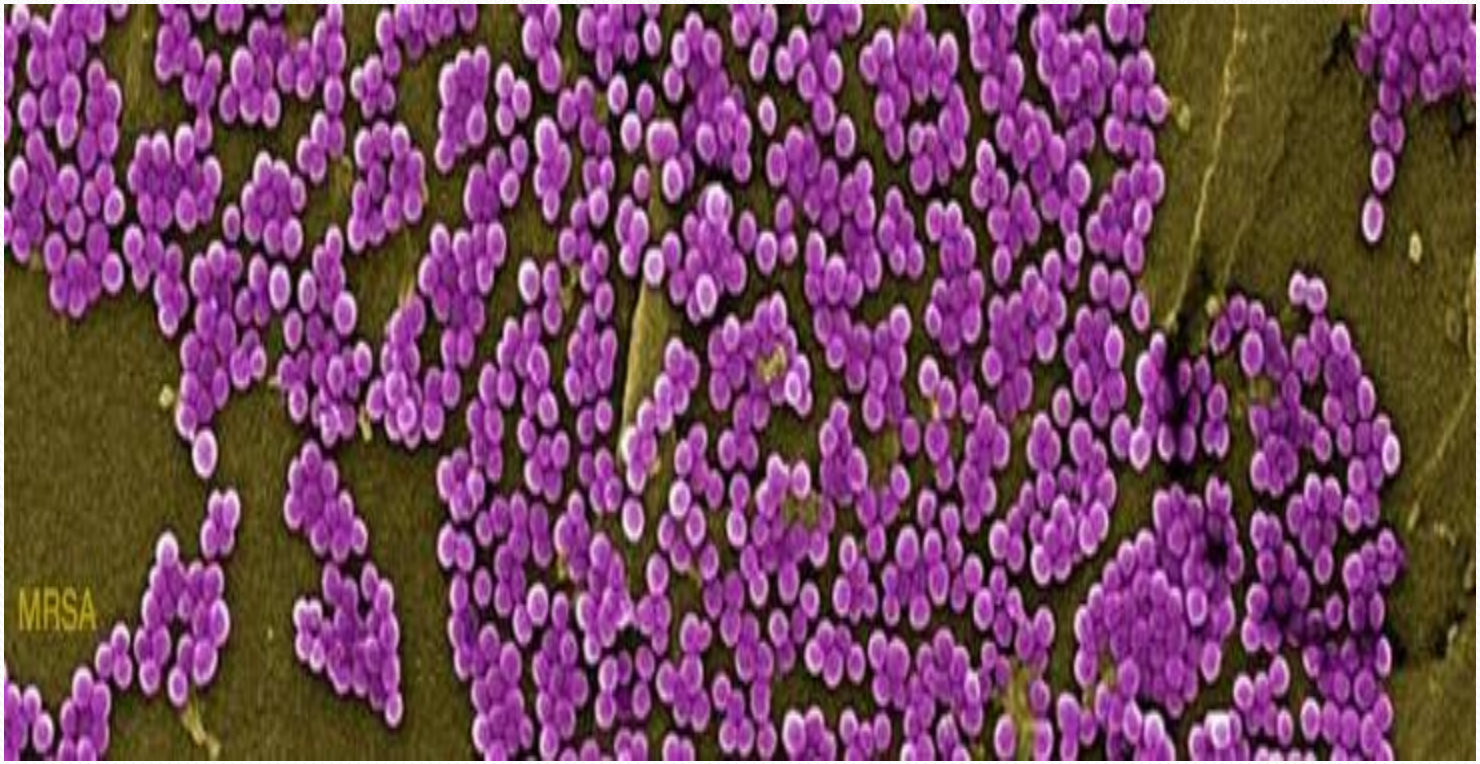
Species. It is the most common cause of bacterial pyogenic infections and it is also important cause of food poisoning and toxic shock syndrome.

2-Staphylococcs epidermidis is coagulase **negative staphylococcus species, it is a commensal of the skin but can cause severe infections in immune-compromised patients and those with central venous catheter.**

3-Staphylococcs saprophyticus is **another coagulase negative species that is part of the normal vaginal flora ,it is predominantly implicated in genitourinary tract infections in sexually-active young women.**

General characters:

Staphylococci generally stain dark gram positive cocci arranged in grapes or clusters. They are non spore forming and non motile organisms. They produce catalase enzyme which is one of the features that distinguish them from Streptococci.


MRSA

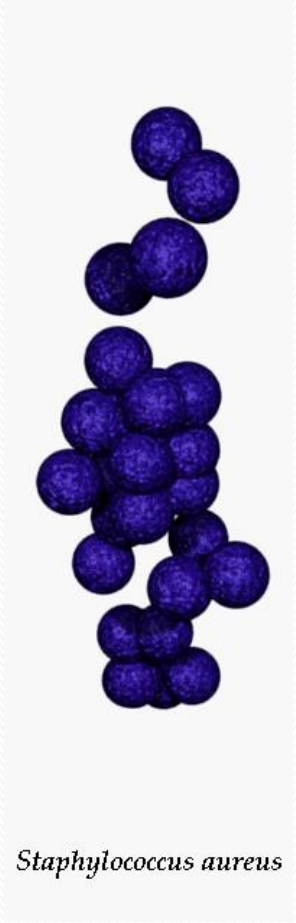
Growth characters

Staphylococci require various amino acids and growth factors , and routinely cultured on enriched media containing blood . It resist bile salts in intestine and media.

Regarding oxygen demand , Staphylococci are facultative anaerobic (They can grow under both aerobic and anaerobic conditions).

Staphylococcus aureus

It is the typical Staphylococcal pathogen , gram positive spherical in shape arranged in irregular clusters and some Cocci show pair , chain or individual arrangement under the microscope.



Staphylococcus aureus

Characters:

1-Nonmotile, non-spore forming facultative anaerobe
2-Fermentation of glucose produces mainly lactic acid

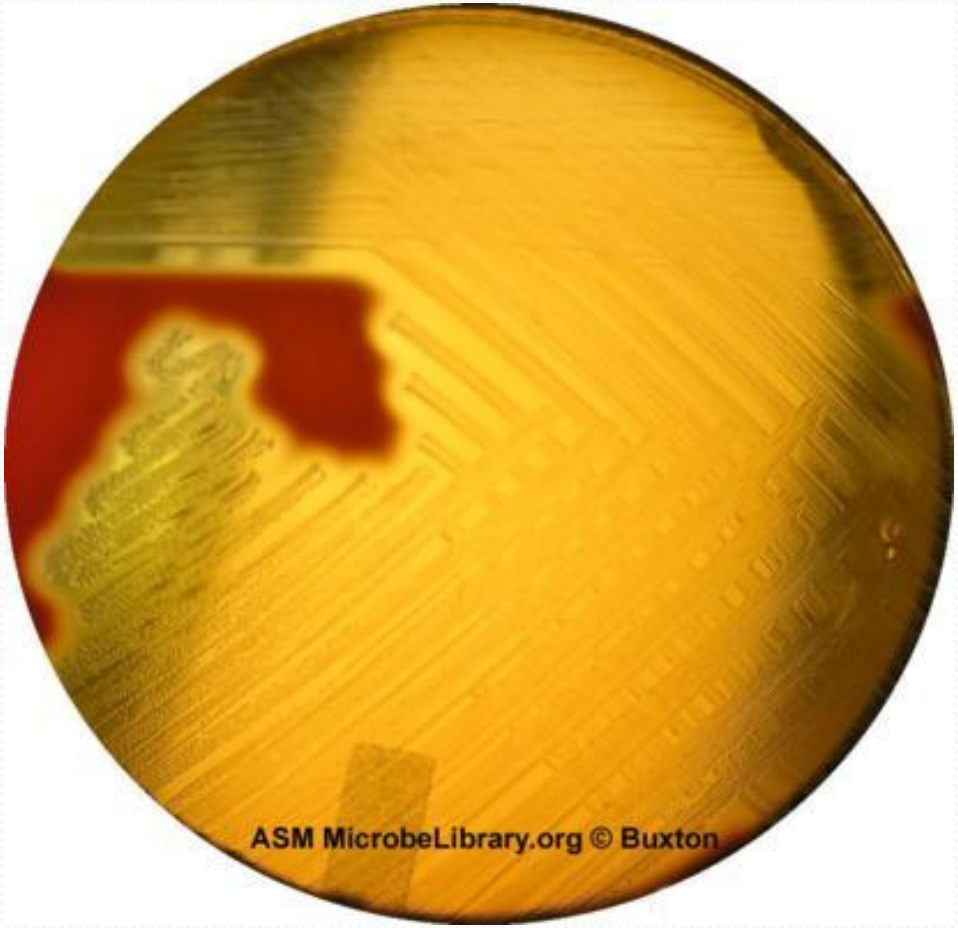
4-Ferments mannitol (distinguishes from *S. epidermidis*).

5-Catalase positive

6-Coagulase positive

Cultural Characters:

The organism grow readily on most of lab media aerobically and facultative anaerobic. On blood agar it shows a zone of beta hemolysis due to its complete hemolytic activity on RBCs of blood agar .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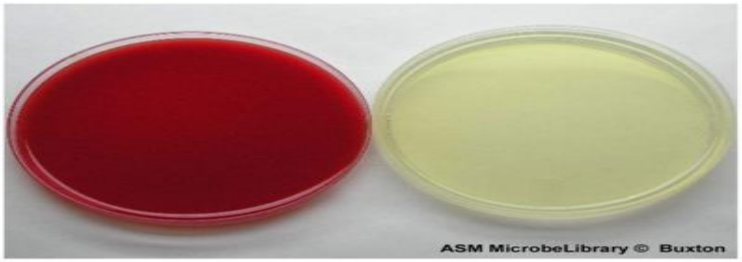
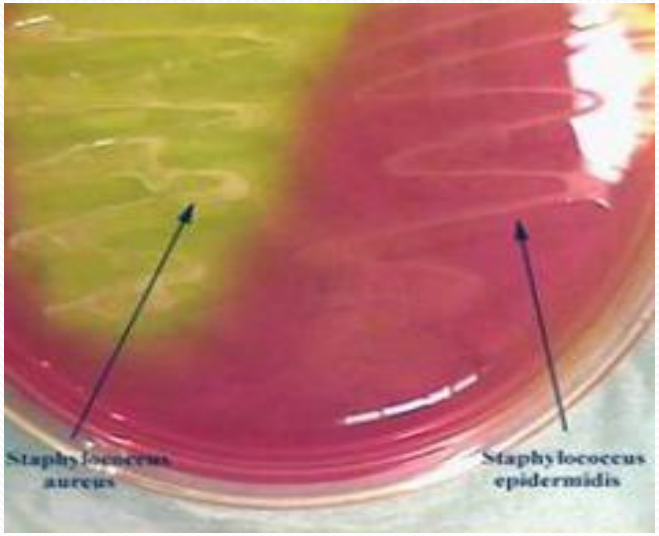
On nutrient agar and milk agar it produce golden colored colonies. It grow well on medium containing 5% NaCl like(Staph 110 medium , Mannitol Salt Agar) .



The optimal growth temperature is 37C and the most character is carbohydrate fermentation with acid production.



Biochemical Reactions:

1- It is Catalase positive bacterium.

Catalase enzyme is an enzyme found in most living cells that catalyzes the decomposition of hydrogen peroxide to water and oxygen and is found in almost all cells except certain anaerobic bacteria and Streptococci.

Coagulase enzyme

Coagulase: it is an enzyme which clots citrated or oxalated human or rabbit plasma.

Resistance:

- **Staphylococci are hardly being resistant to heat and drying , and thus can persist for long periods on fomites(inanimate objects), which can serve as sources of infection .**

- **It is sensitive for chemical disinfectants and antiseptics like phenol and tincture iodine .**

Pathogenic virulence factors

- Pathogenic virulence factors are the genetic, biochemical or structural factors that enable an organism to produce disease.
- The clinical outcome of an infection depends on the virulence of the pathogen and the opposing defense mechanism of the host

Antigenic and virulence factors:

S . aureus express many virulence factors like:

1-cell wall virulence factors :

a-Protein A it is a major component of the ***S. aureus*** cell wall . It acts strongly as antiphagocytic factor.

b- Peptidoglycan:

It is polysaccharide in nature , provides a rigid exoskeleton of cell wall . It plays a role in the pathogenicity of S. aureus through :

1-It stimulates pyrogenic factor, (interleukine -1).

2-Chemotactic activity to polymorphs

2- extracellular toxins and enzymes :

A. Toxins:

1- Haemolysin : It is an exotoxin , its action usually directed to the red blood cell membrane leading to RBCs lyses . A clear zone of Beta hemolysis is seen around the Staphylococcal colony.

b- Leukocidin : This toxin kills polymorphs and macrophages.

c- Enterotoxins : they are imposed in food poisoning when ***Staphylococcus aureus*** contaminates food . They are of six types A,B,C,D,E & G , type A & B are most common . Enterotoxins are produced by 50% of all ***Staphylococcus aureus*** isolates .

d- **Toxic shock syndrome toxin : (TSS-1):** It is the cause of Shock syndrome . It is systemic infection with high fever , hypotension ,multi organ involvement and finally shock and death.

e- Exfoliative toxin (ET): It causes Scalded skin syndrome in newborns and children.

Enzymes :

1-Catalase enzyme:

It is an enzyme which cause hydrolysis of H_2O_2 into water and oxygen, this enzyme is very important for the differentiation between staphylococcus and Streptococcus

.

2-Coagulase: it is an enzyme which clot citrated or oxalated human or rabbit plasma.

Plasma ---Coagulase----------> clotting

Coagulase converts fibrinogen of plasma to fibrin , its catalyst is coagulase reacting factor CRF which is present in plasma . This is known as free coagulase.

Bound coagulase ,is attached to the bacterial cell , it is known clumping factor also.

It does not need CRF and fibrinogen for its action but it promotes bacterial cell clumping in the presence of plasma and it remains on the surface of the organism.

3-Lipase: It causes hydrolysis of lipoprotein in the blood and fat of sebaceous glands of the skin.

4-Hyalouronidase: It is known as spreading factor also, it also depolymerize intercellular hyaluronic acid .

5- B lactamase : I causes hydrolysis of beta lactam ring of penicillin molecule. It imposed in penicillin resistance.

6-Other enzymes like Phosphatase and gelatinase.

Pathogenesis:

pathogenesis depends on the combined actions of several virulence factors , so it is difficult to determine precisely the role of any given factor.

S. aureus **causes different infections in human , the typical staphylococcal lesion is known as furuncle or localized abscess.**

- **A common entry point into the body is a break in the skin , which may be minute needle stick or traumatic or a surgical wound.**
- **Another portal of entry is the respiratory tract. For example Staphylococcal pneumonia is frequent complication of influenza.**

Inflammatory steps:

The localized host response to Staphylococcal infection is inflammation . The following steps are usually involved in the formation of such infection.

1- A number of microorganisms enter the skin through hair follicle , they will grow and release toxins and enzymes . Released coagulase acts on plasma leading to clot formation around the lesion. Inflammatory cells attracted to the lesion surrounding it .

2-The center of the lesion undergo liquefaction , this lesion points to the surface to drain pus , hence the pyogenic infection . after pus drainage a granular tissue fills the cavity of the lesion .

From this site (site of local infection), a **serious consequences of Staphylococcal infection occur when bacteria invade blood stream resulting with Bacteremia or Septicemia .**

Septicemia: **It means the presence and persistence of pathogenic microorganisms and their toxins in the blood , it may be rapidly fatal.**

Bacteremia: It means the presence of viable bacteria circulating in the blood stream , it may result in seeding internal abscess , skin lesions or infections in the lung, kidney, heart skeletal muscles or meninges

Infections caused by *S. aureus* :

A. Local infections: The most common local infections are:

1- Impetigo: It is superficial localized skin infection common in children , highly infectious and show blister formation which will open and covered with pus.

2-Acne : Infection of the sebaceous glands of the skin particularly in adolescents . lipase enzyme plays a role in such infection.

3-Wound infection :

a-Surgical wound infection :

postoperative surgical wound infection may occur due to contamination with *S. aureus* from hospital atmosphere or from carriers like health staff workers . Hospital - acquired infection is known as nosocomial infection.

b-Traumatic wound infection:

Traumatic wounds of different types can be infected by different types of organisms like *S. aureus.*

4-Burn infections are also caused by Staphylococcus infections.

5-Boils or furuncles are subcutaneous abscesses often form around foreign bodies.

6- Deep localized infections: These may be metastatic from superficial infections or skin carriage, or may result from trauma ,like chronic or acute bone infections , joint infections , deep abdominal infection .

Systemic infections :

Pneumonia : *S. aureus* can cause severe necrotizing pneumonia.

Acute endocarditis : It is generally associated with intravenous drug abuse , caused by injection of contaminated preparations or by needles . Bacteria can be introduced into soft tissues and blood stream through injection without proper skin disinfection.

Diseases associated with toxin production:

- **Staphylococcal food poisoning : Staphylococcal gastroenteritis caused by ingestion of food contaminated with enterotoxin- producing *S. aureus* .**
- **Often contaminated food like bakeries , drinks and meat are source of food poisoning.**
- **symptoms such as nausea , vomiting and diarrhea are acute following short incubation period (less than six hours).**

- **Toxic shock syndrome** , it is associated with toxic shock syndrome toxin (TSST) secreted by some strains of ***S. aureus.***
- **Scalded skin syndrome** , it is associated with exfoliative toxin , this toxin causes destruction of certain skin cells which leads to peal off skin sheets.

Laboratory diagnosis :

Specimens: Specimens are different and depend on the site of infection, it might be :

wound swab , burn swab, pus from the lesion ,food or vomitus in food poisoning , sputum from pneumonic patient , urine from UTI , blood in case of septicemia. each specimen should be examined by:

Laboratory investigations:

1-Direct examination like grams stained smear .

2- Indirect examination through cultivation on suitable media like blood agar , mannitol salt agar and incubation of cultivated plates under 37c for 24 hrs. biochemical tests are supportive to reach final diagnosis of the organism like coagulase & catalase, tests.

3-Serotyping and phage typing , this test depends on the sensitivity of this organism to certain viruses known as bacteriophages. There are 22 phage types of *S. aureus*. Such typing is necessary for epidemiological studies and tracing sources of infection.

4-PCR test is rapid confirmative diagnostic test as well as it is applicable for the detection of plasmid and chromosomal antimicrobial resistance factors in causative bacterium .

Antimicrobial therapy:

Staphylococci are becoming increasingly resistant to many commonly used antibiotics including penicillins, macrolides such as erythromycin , tetracyclines and aminoglycosides.

Antibiotic of choice:

Methicillin (methicillin) and flucloxacillin are lactamase-resistant penicillins so are the antibiotics of choice in most staphylococcal skin infections. In spite of this affectivity ,there is now increasing methicillin resistance (MRSA).

Types of Antimicrobial Resistance

S. aureus **posses variety of antimicrobial resistance and susceptibility which falls into:**

1-plasmid mediated resistance to penicillins like penicillin G, Ampicillin , Ticarcillin due to beta lactamase production (penicillinase enzyme).

2-Methicillin , Naficilllin and oxacillin resistance is independent on beta lactamase secretion . Methicillin resistant *S. aureus* is known as MRSA while sensitive strain is known as MSSA. Mechanism for Naficillin resistance is based on lack of penicillin binding proteins in the organism.

3-Plasmid can carry also resistance for tetracycline , erythromycin and Aminoglycosides.

4-Tolerance indicates that *S. aureus* is not killed but inhibited by certain antibiotics when removed it will retain its activity.

Other types of Staphylococci :

Staphylococcus epidermidis **: It is one of skin bacterial normal flora, coagulase negative ,do not ferment mannitol and sensitive to novobiocin. It causes catheter associated infection particularly urinary tract infection (UTI).**

It may cause **endocarditis** after their introduction to the **blood stream** through contaminated syringe . these organisms may cause infection to the prosthetic device like artificial joint , prosthetic heart valve . This is due to production of extracellular slime material by this organism , **biofilm** formation acts as virulence factor.

***Staphylococcus saprophyticus* :**

It is frequent cause of cystitis in women, probably related to its occurrence as a part of normal vaginal flora. it can be differentiated from other coagulase negative species of Staphylococcus by its resistance to novobiocin