STAPHYLOCOCCI

Staphylococci are typical Gram-positive bacteria forming irregular clusters of cocci. Staphylococci are widespread in nature, although they are mainly found on the skin, skin glands and mucous membranes of mammals and birds, but can cause infection under certain circumstances.  

*S. aureus* is more pathogenic than the other common members of the genus, *S. epidermidis* and *S. saprophyticus*.  

*S. epidermidis* has been known to cause various hospital-acquired infections (such as prosthetic or indwelling devices), whereas *S. saprophyticus* is mainly associated with urinary tract infections in young females who are sexually active. Disease processes with *S. aureus* are numerous. The portal of entry is variable, since they gain access to the body via the skin, the respiratory tract or the genito-urinary tract.  

*Staphylococcus aureus* expresses many potential virulence factors:

1. **surface proteins** - promote colonization of host tissues
2. **leukocidin, kinases, hyaluronidase** - invasins that promote bacterial spread in tissues
3. **capsule, Protein A** - surface factors that inhibit phagocytic engulfment
4. **carotenoids, catalase** - enhance staphylococcal survival in phagocytes
5. **protein A, coagulase** - immunological disguises
6. **hemolysins, leukotoxin, leukocidin** - membrane-damaging toxins that lyse eucaryotic cell membranes
7. **TSST, ET** - exotoxins that damage host tissues or otherwise provoke symptoms of disease
8. inherent and acquired resistance to antimicrobial agents.

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**Fig. 1 Virulence determinants of Staphylococcus aureus.**

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1. S. - Staphylococcus
2. TSST - Toxic Shock Syndrome Toxin
3. ET - Exfoliatin Toxin
Staphylococci can cause many forms of infection:

1. *S. aureus* causes superficial skin lesions (boils) and localized abscesses in other sites.
2. *S. aureus* causes deep-seated infections, such as osteomyelitis and endocarditis and more serious skin infections (furunculosis).
3. *S. aureus* is a major cause of hospital acquired (nosocomial) infection of surgical wounds and, with *S. epidermidis*, causes infections associated with indwelling medical devices.
4. *S. aureus* causes food poisoning by releasing enterotoxins into food.
5. *S. aureus* causes toxic shock syndrome by release of superantigens into the blood stream.
7. Other species of staphylococci (*S. lugdunensis, S. haemolyticus, S. warneri, S. schleiferi, S. intermedius*) are infrequent pathogens.

![Fig. 2 Sites of infection and diseases caused by *Staphylococcus aureus*.](image)

Although strains of *Staphylococcus aureus* resistant to penicillin have caused infections for many years, isolates resistant to methicillin, oxacillin, and other β-lactams have become predominant—primarily in the last 20 years.

Diagnosis of staph infections begins with attempting to culture the bacteria from an infected site. Any area with pus, crusty drainage, or blisters should be cultured. Blood from patients with sepsis, toxic shock syndrome, or pneumonia should be cultured. Standard microbiological techniques include a positive coagulase test to identify staph. *S. aureus* lyases red blood cells in blood agar plates (hemolytic staphylococci) while *S. epidermidis* does not (nonhemolytic staphylococci). All staph should be further tested to see if the bacteria are...
resistant to the antibiotic methicillin (and other antibiotics) and thus determine if the organisms are 4MRSA.

**GRAM STAIN**

*Staphylococcus* is a genus of bacteria that is characterized by a round shape (coccus or spheroid shaped), Gram-positive (purple), and found as either single cells, in pairs, or more frequently, in clusters that resemble a bunch of grapes. The genus name *Staphylococcus* is derived from Greek terms (*staphyle* and *kokkos*) that mean "a bunch of grapes,"

![Fig. 3 S. aureus (left), S. epidermidis (right) - Gram stain.](image)

**STAPHYLOCOCCI - BLOOD AGAR CULTURE**

Blood agar is both differential and enriched medium. The blood that is incorporated into this medium is an enrichment ingredient for the cultivation of fastidious organisms. On blood agar, *S. aureus* usually displays a light to golden yellow pigment, whereas *S. epidermidis* has a white pigment and *S. saprophyticus* either a bright yellow or white pigment. However, pigmentation is not always a reliable characteristic. On blood agar, *S. aureus* is usually beta-hemolytic, *S. epidermidis* and *S. saprophyticus* are almost always nonhemolytic.

![Fig. 4 S. aureus (left) and S. epidermidis (right) - colonies on blood agar.](image)

4 MRSA – methicillin resistant *Staphylococcus aureus*
**S. aureus** - individual colonies on agar are round, convex, and 1-4 mm in diameter with a sharp border. On blood agar plates, colonies of *Staphylococcus aureus* are frequently surrounded by zones of clear beta-hemolysis. The golden appearance of colonies of some strains is the etymological root of the bacteria's name; aureus meaning "golden" in Latin. *Staphylococcus epidermidis* - showing γ-haemolytic, porcelin-white colonies as compared to *S. aureus* on the same medium. This clear distinction in colony color is not seen at all times.

### CATALASE TEST

Some bacteria contain flavoproteins that reduce oxygen (O₂), resulting in the production of hydrogen peroxide (H₂O₂) and, in some cases, an extremely toxic superoxide (O₂⁻). Accumulation of these substances will result in death of the organism as they are powerful oxidizing agents and destroy cellular constituents very rapidly unless they can be enzymatically degraded. These substances are produced when aerobes, facultative anaerobes, and microaerophiles use the aerobic respiratory pathway, in which oxygen is the final electron acceptor, during degradation of carbohydrates for energy production. A bacterium must be able to protect itself against such O₂ products or it will be killed. Many bacteria possess enzymes that afford protection against toxic O₂ products. Facultative anaerobes and obligate aerobes usually contain the enzymes superoxide dismutase, which has the ability to catalyze the destruction of superoxide, and either catalase or peroxidase, which catalyze the destruction of hydrogen peroxide as follows:

\[
\begin{align*}
\text{Superoxide dismutase} \\
2O_2^- + 2H^+ & \rightarrow O_2 + H_2O_2 \\
\text{Oxygen} & \quad \text{Hydrogen peroxide}
\end{align*}
\]

\[
\begin{align*}
\text{Catalase} \\
2H_2O_2 & \rightarrow 2H_2O + O_2 \\
\text{Peroxidase} & \quad \text{Water} \quad \text{Free Oxygen}
\end{align*}
\]

The inability of strict anaerobes to synthesize catalase, peroxidase, or superoxide dismutase may explain why oxygen is poisonous to these microorganisms. In the absence of these enzymes, the toxic concentration of H₂O₂ cannot be degraded when these organisms are cultivated in the presence of oxygen. Organisms capable of producing catalase rapidly degrade hydrogen peroxide which is a tetramer containing four polypeptide chains, which are usually 500 amino acids long. It also contains four porphyrin heme groups (i.e., iron...
groups) that will allow the enzyme to react with the hydrogen peroxide.
The enzyme catalase is present in most cytochrome-containing aerobic and facultative anaerobic bacteria. Catalase is the enzyme which has one of the highest turnover numbers compared to all other enzymes; one molecule of catalase has the ability to convert millions of molecules of hydrogen peroxide to water and oxygen in each second. Catalase production and activity can be detected either by adding the substrate H₂O₂ to an appropriately incubated (18- to 24-hour) tryptic soy agar slant culture or by slide test. Organisms which produce the enzyme break down the hydrogen peroxide, and the resulting O₂ production produces bubbles in the reagent drop, indicating a positive test. Organisms lacking the cytochrome system also lack the catalase enzyme and are unable to break down hydrogen peroxide, into O₂ and water and are catalase negative.
The catalase test is primarily used to distinguish among Gram-positive cocci. Members of the genus *Staphylococcus* are catalase-positive, and members of the genera *Streptococcus* and *Enterococcus* are catalase-negative.

**Procedure of catalase test (Slide Test)**

1. Transfer a small amount of bacterial colony to a surface of clean, dry glass slide using a loop or sterile wooden stick
2. Place a drop of 3% H₂O₂ on to the slide and mix.
3. A positive result is the rapid evolution of oxygen (within 5-10 sec.) as evidenced by bubbling (Fig. 5).
4. A negative result is no bubbles or only a few scattered bubbles.

![Catalase - slide test.](image)

**MANNITOL SALT AGAR CULTURE**

Mannitol salt agar (MSA) is both a selective and differential media used for the isolation of *Staphylococci* from mixed cultures.

**MSA components**

7.5% NaCl – selects for species of *Staphylococcus*. This concentration of salt is too high for most other bacteria to withstand and, therefore, inhibits their growth.

Mannitol – alcohol of the carbohydrate mannose. Mannitol fermentation produces acid end products which turn the medium yellow. Yellow indicates mannitol positive and no color change indicates mannitol negative.
**Phenol red pH indicator** – yellow in acid pH (the same indicator that is used in phenol red carbohydrate fermentation broths).

**Fig. 6 Mannitol Salt Agar.**

On MSA, only pathogenic *Staphylococcus aureus* produces small colonies surrounded by yellow zones. The reason for this color change is that *S. aureus* have the ability to ferment the mannitol, producing an acid, which changes the indicator color from red to yellow. The growth of other types of bacteria is usually inhibited. This growth differentiates *S. aureus* from *S. epidermidis*, which forms colonies with red zones.

**Expected Results**

1. On MSA, pathogenic *Staphylococcus aureus* ferments mannitol, thereby changing the colour of the medium from red to yellow.

**Fig. 7 Staphylococcus aureus  on Mannitol Salt Agar.**

2. *Staphylococcus epidermidis* grows on MSA, but does not ferment mannitol (media remains light pink in color, colonies are colorless).
COAGULASE TEST

Coagulases are enzymes that clot blood plasma by a mechanism that is similar to normal clotting. The coagulase test identifies whether an organism produces this exoenzyme. This enzyme clots the plasma component of blood. The only significant disease causing bacteria of humans that produce coagulase enzyme are *Staphylococcus aureus*. Thus this enzyme is a good indicator of the pathogenic potential of *S. aureus*.

In human host, the action of coagulase enzyme produces clotting of the plasma by converting fibrinogen to fibrin in the immediate vicinity of the bacterium as a means of protection by itself. The fibrin meshwork that is formed by this conversion surrounds the bacterial cells or infected tissues, protecting the organism from non-specific host resistance mechanisms such as phagocytosis and the anti staphylococcal activity of normal serum. This enables the bacterium to persist in the presence of a host immune response, which can lead to the establishment of infection. Thus, coagulase is described as a virulence factor( disease-causing factor) of *Staphylococcus aureus*. Citrate and EDTA (Ethylenediaminetetraacetic acid) are usually added to act as anticoagulants and prevent false-positive results. Most strains of *S.aureus* produce one or two types of coagulase; free coagulase and bound coagulase. Bound coagulase is localized on the surface of the cell wall and reacts with α- and β-chains of the plasma fibrinogens to form a coagulate. Free coagulase is an enzyme that is secreted extracellularly and bound coagulase is a cell wall associated protein. Free coagulase can be detected in tube coagulase test and bound coagulase can be detected in slide coagulase test.

Slide coagulase test may be used to screen isolates of *S.aureus* and tube coagulase may be used for further confirmation. There are seven antigenic types of free coagulase, but only one antigenic type of bound coagulase exists. Free coagulase is always heat labile while bound coagulase is heat stable.

In the test, the sample is added to rabbit plasma and held at 37° C for a specified period of time. Clot formation occurs within 4 hours is interpreted as a positive result and indicative of
a virulent *Staphylococcus aureus* strain. The absence of coagulation after 24 hours of incubation is a negative result, indicative of an avirulent strain.

**Detection of bound coagulase - Slide Test**

This method measures bound coagulase. The bound coagulase is also known as clumping factor. It cross-links the α and β chain of fibrinogen in plasma to form fibrin clot that deposits on the cell wall. As a result, individual coccus stick to each other and clumping is observed.

1. Divide the slide into two sections with grease pencil. One should be labeled as „test” and the other as „control”.
2. Place a small drop of distilled water on each area.
3. Emulsify one or two colonies of Staphylococcus on blood agar plate on each drop to make a smooth suspension.
4. The test suspension is treated with a drop of citrated plasma and mixed well with a needle.
5. Do not put anything in the other drop that serves as control. The control suspension serves to rule out false positivity due to auto agglutination.
6. Clumping of cocci within 5-10 seconds is taken as positive (Fig. 9).

![](image)

**Fig. 9 Slide Coagulase Test.**

Some strains of *S.aureus* may not produce bound coagulase, and such strains must be identified by tube coagulase test

**Detection of free coagulase - Tube Coagulase Test**

Most strains of *S.aureus* produce one or two types of coagulase; free coagulase and bound coagulase. Free coagulase is an extracellular enzyme which reacts with prothrombin and its derivatives. This method helps to measure free coagulase. The free coagulase secreted by *S.aureus* reacts with coagulase reacting factor (CRF) in plasma to form a complex, which is thrombin. This converts fibrinogen to fibrin resulting in clotting of plasma (Fig. 10).

1. Three test tubes are taken and labeled “test”, “negative control” and “positive control”.
2. Each tube is filled with 1 ml of 1 in 10 diluted rabbit plasma.
3. To the tube labeled test, 0.2 ml of overnight broth culture of test bacteria is added.
4. To the tube labeled positive control, 0.2 ml of overnight broth culture of known *S. aureus* is added.
5. To the tube labeled negative control, 0.2ml of sterile broth is added.
6. All the tubes are incubated at 37°C.
7. Positive result is indicated by gelling of the plasma, which remains in place even after inverting the tube.
8. If the test remains negative until four hours at 37°C, the tube is kept at room temperature for overnight incubation.

Fig. 10 Tube Coagulase Test.

The coagulase test is used to distinguish between pathogenic and nonpathogenic members of the genus Staphylococcus. All pathogenic strains of *S. aureus* are coagulase positive whereas the nonpathogenic species (*S. epidermidis*) are coagulase negative. While slide coagulase test is useful in screening, tube coagulase test is useful in confirmation of coagulase test. Samples must be observed for clotting within 24 hours. This is because some strains that produce coagulase also produce an enzyme called fibrinolysin, which can dissolve the clot. Therefore, the absence of a clot after 24 hours is no guarantee that a clot never formed. The formation of a clot by 12 hours and the subsequent disappearance of the clot by 24 hours could produce a so-called false negative if the test were only observed at the 24-hour time.

Sources:

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