Staphylococci

The most clinically important species of Staphylococci include *Staphylococcus aureus*, *S. epidermidis* and *S. saprophyticus*. They are Gram-positive cocci; usually arranged in clusters; non-motile; catalase positive; non-sporing; grow over a wide temperature range (10–42 ˚C), with an optimum of 37 ˚C; aerobic and facultatively anaerobic; grow on simple media.

Classification

1 Colonial morphology: ***S. aureus*** colonies are **grey** to **golden yellow**; ***S. epidermidis*** and ***S. saprophyticus*** colonies are white. Staphylococci may produce haemolysins, resulting in haemolysis on blood agar.

2 Coagulase test: ***S. aureus*** possesses the enzyme **coagulase**, which acts on plasma to form a clot. Other staphylococci (e.g. ***S. epidermidis*** and ***S. saprophyticus***) do not possess this enzyme and are often termed, collectively, 'coagulase-negative staphylococci' (CoNS). There are three methods to demonstrate the presence of coagulase:

(a) tube coagulase test: diluted plasma is mixed with a suspension of the bacteria; after incubation, clot formation indicates S. aureus

(b) slide coagulase test: a more rapid and simple methodin which a drop of plasma is added to a suspension of staphylococci on a glass slide; visible clumping indicates the presence of coagulase.

(c) latex agglutination test: cells are mixed with coated latex particles; visible agglutination provides simultaneous detection of staphylococci containing coagulase and/or protein A.

3 Deoxyribonuclease (DNAase) production: ***S. aureus*** possesses an enzyme, DNAase, which depolymerises and hydrolyses DNA; other staphylococci rarely possess this enzyme.

4 Protein A detection: ***S. aureus*** possesses a cell-wall antigen, protein A; antibodies to proteinA agglutinate ***S. aureus*** but not other staphylococci.

5 Novobiocin sensitivity: useful for differentiating between species of coagulase-negative staphylococci; ***S. saprophyticus*** is novobiocin resistant and ***S. epidermidis*** is sensitive.

Table 3.1 Main characteristics of staphylococci

Characteristic	*S. aureus*	*S. epidermidis*	*S. saprophyticus*
Coagulase	+	–	–
Deoxyribonuclease	+	–	–
Novobiocin	S	S	R
Colonial appearance	Golden-yellow	White	White
Body sites which may be colonised	Nose Mucosal surfaces Faeces Skin	Skin Mucosal surfaces	Periurethra Faeces
Common infections	Skin (boils, impetigo, furuncles, wound infections) Abscesses Osteomyelitis Septic arthritis Sepsis Infective endocarditis Prosthetic device-related infections	Prosthetic device-related infections e.g. artificial valves, heart, intravenous catheters, CSF shunts	Urinary tract infections in sexually active young women

+, present; –, absent; CSF, cerebrospinal fluid, S, sensitive R, resistant.

Morphology and identification

On microscopy, ***S. aureus*** is seen as typical Gram-positive cocci in 'grape-like' clusters. It is both coagulase and DNAase positive. Other biochemical tests can be performed for full identification.


S. aureus colonies on a blood agar plate

Pathogenicity

S. aureus causes disease because of its ability to adhere to cells, spread in tissues and form abscesses, produce extracellular enzymes and

exotoxins, combat host defences and resist treatment with many antibiotics.

Adhesins

S. aureus has a wide range of adhesins known as MSCRAMMs (microbial surface components recognizing adhesive matrix molecules), which mediate adherence to host cells.

Pathogenicity factors produced by *S. aureus*

Factor	Effect
MSCRAMMs	Mediate adherence to host cells
Protein A	Evade host defence/inhibits phacocytosis
Fibronectin-binding protein	Mediates binding to fibronectin
Fibrinogen-binding protein	Clumping factors
Capsule	Evade host defences
Coagulase	Generates protective fibrin layer around *S. aureus*
Staphylokinase	Fibrinolysis
Proteases	Degrade antibacterial proteins and matrix proteins
Lipases	Promote interstitial spreading of microorganism
Hyaluronidase	Degrades hyaluronic acid
α-Haemolysin	Lyses erythrocytes, damages platelets
β-Haemolysin	Degrades sphingomyelin/toxic for cells
Leukocidin/leucotoxin	Lyse white blood cells
Exotoxins, e.g. enterotoxins	Food poisoning with profuse vomiting
Superantigens, e.g. TSST, exfoliative toxin	Toxic shock syndrome, scalded skin syndrome

NB: Toxin production varies between strains of *S. aureus*.

Associated infections

. **Skin:** boils, impetigo, furuncles, wound infections, staphylococcal scalded skin syndrome;

. **Respiratory:** pneumonia, lung abscesses, exacerbations of chronic lung disease;

. **Skeletal:** most common cause of osteomyelitis and septic arthritis;

. **Invasive:** bloodstream infection, infective endocarditis, deep abscesses (brain, liver, spleen), toxic shock syndrome;

. **Gastrointestinal:** toxin-mediated food poisoning;

. **Device related:** indwelling catheters, prosthetic joints and heart valves.

Laboratory diagnosis

Laboratory diagnosis is by microscopic detection of the microorganism in clinical samples, direct isolation from the infected site or blood cultures, and detection of serum antibodies to staphylococcal haemolysin and DNAase. *S. aureus* can also be genotyped by molecular methods,including pulsed field gel electrophoresis (PFGE). Typing of *S. aureus* is useful in epidemiological studies.

Treatment and prevention

Antimicrobial agents, such as flucloxacillin, remain the first-line treatment for sensitive strains of ***S.aureus***.

S. epidermidis

. *S. epidermidis* is both coagulase and DNAase negative and is present in large numbers on the human skin and mucous membranes.

. *S. epidermidis* is a cause of bacterial endocarditis. It is also a major cause of infections of implanted devices such as cerebrospinal shunts.

. The microorganism colonises implanted devices by attaching firmly onto artificial surfaces. Some strains also produce a slime layer (glycocalyx), which appears to facilitate adhesion and protect the microorganism from antibiotics and host defenses. The increased use of implanted devices, particularly central venous catheters, has resulted in S. epidermidis becoming one of the most frequently isolated microorganisms from blood cultures. *S. epidermidis* occasionally causes urinary tract infections, particularly in catheterised patients. When isolated from hospitalized patients, *S. epidermidis* is often resistant to antibiotics such as flucloxacillin and erythromycin, necessitating the use of glycopeptide antibiotics (e.g. vancomycin).

S. saprophyticus

S. saprophyticus is both coagulase and DNAase negative and is frequently associated with urinary tract infections in sexually active young women, occasionally resulting in severe cystitis with hematuria.

Streptococci

Gram-positive cocci, Non-spore forming, non-motile, facultative anaerobes, characteristically form pairs or chains during growth.

Classification of Streptococci

The classification of streptococci into major categories has
been based on

(1) colony morphology and hemolytic reactions on blood agar.

(2) serologic specificity of the cell walls group-specific substance (Lancefield antigens) and other cell wall or capsular antigens.

(3) biochemical reactions and resistance to physical and chemical factors.

(4) ecologic features.

(5) molecular genetics have replaced phenotypic methods.

A. Hemolysis

1. Complete disruption of erythrocytes with clearing of the blood around the bacterial growth is called β-hemolysis (the human-pathogenic species *Streptococcus pyogenes*).

2. Incomplete lysis of erythrocytes with reduction of hemoglobin and the formation of green pigment is called α-hemolysis (*Streptococcus pneumoniae*).

3. Nonhemolytic- Streptococci (sometimes called γ- hemolysis).

Streptococci grown in blood culture showing Gram-positive cocci in chains.

B. Group-Specific Substance (Lancefield Classification)

This carbohydrate is contained in the cell wall of many streptococci and forms the basis of serologic grouping into Lancefield groups **A–H** and **K–U.**

C. Capsular Polysaccharides

The antigenic specificity of the **capsular polysaccharides** is used to classify some streptococci.

D. Biochemical Reactions

Biochemical tests include sugar fermentation reactions, tests for the presence of enzymes, and tests for susceptibility or resistance to certain chemical agents.

STREPTOCOCCUS PYOGENES

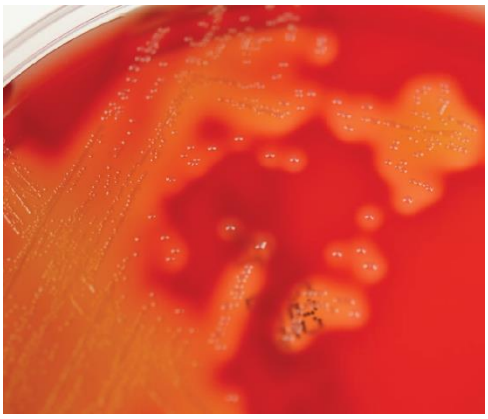
- important human pathogen.

- typically produces large (1 cm in diameter) zones of β-hemolysis around colonies greater than 0.5 mm in diameter.
- hydrolysis of L-pyrrolidonyl-β-naphthylamide.
- susceptible to bacitracin
- Streptococci are Gram-positive; however, as a culture ages and the bacteria die, they lose their Gram positivity and can appear to be Gram-negative; for some streptococci, this can occur after overnight incubation.
- Most group A strains produce capsules composed of hyaluronic acid.
- The hyaluronic acid capsule likely plays a greater role in virulence.
-*S. pyogenes* cell wall contains proteins (**M**, **T**, **R** antigens), carbohydrates (group specific), and peptidoglycans.
- Hairlike pili pili consist partly of **M** protein and are covered with **lipoteichoic acid**.

Culture

Growth of streptococci tends to be poor on solid media or in broth **unless** enriched with blood or tissue fluids. Nutritive requirements vary widely among different species.

Growth and hemolysis are aided by incubation in 10% CO2. Most pathogenic hemolytic streptococci grow best at 37°C. Most streptococci are facultative anaerobes and grow under aerobic and anaerobic conditions.



Group A β-hemolytic streptococci (*S. pyogenes*) after growth overnight on a 10-cm plate with 5% sheep blood agar. The small (0.5–1 mm diameter) white colonies are surrounded by diffuse zones of β-hemolysis 7–10 mm in diameter. (Courtesy of H Reyes.

Antigenic structure (M Protein)

This substance is a major virulence factor of *S. pyogenes*. **M** protein is a filamentous structure anchored to the cell membrane that penetrates and projects from the streptococcal cell wall.

Enzymes and Toxins

A. Streptokinase (Fibrinolysin)

Streptokinase is produced by many strains of group A β-hemolytic streptococci. It transforms the plasminogen of human plasma into plasmin, an active proteolytic enzyme that digests fibrin and other proteins, allowing the bacteria to escape from blood clots.

B. Deoxyribonucleases

Streptococcal deoxyribonucleases A, B, C, and D degrade DNA (DNases) and similar to streptokinase facilitate the spread of streptococci in tissue by liquefying pus.

C. Hyaluronidase

Hyaluronidase splits hyaluronic acid, an important component of the ground substance of connective tissue. Thus, hyaluronidase aids in spreading infecting microorganisms (spreading factor).

D. Pyrogenic Exotoxins (Erythrogenic Toxin)

Pyrogenic exotoxins are elaborated by *S. pyogenes*. There are three antigenically distinct streptococcal pyrogenic exotoxins (Spe): A, B, and C.

The streptococcal pyrogenic exotoxins have been associated with **streptococcal toxic shock syndrome** and **scarlet fever**.

E. Hemolysins

The β-hemolytic group A *S. pyogenes* elaborates two hemolysins (streptolysins) that not only lyse the membranes of erythrocytes but also damage a variety of other cell types.

Streptolysin O

-protein.

- hemolytically active in the reduced state (available– SH groups) but rapidly inactivated in the presence of oxygen.
- responsible for some of the hemolysis seen when growth occurs in cuts made deep into the medium in blood agar plates.
- combines quantitatively with antistreptolysin O (ASO), an antibody that appears in humans after infection with any streptococci that produce streptolysin O.

-This antibody blocks hemolysis by streptolysin O.

Streptolysin S

- agent responsible for the hemolytic zones around streptococcal colonies growing on the surface of blood agar plates.
- elaborated in the presence of serum—hence the name streptolysin S.

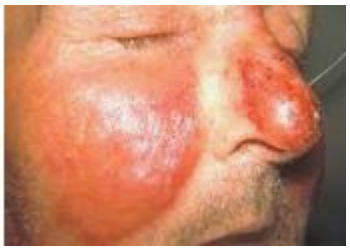
-not antigenic.

Most isolates of *S. pyogenes*produce both of these hemolysins. Up to 10% produce only one.

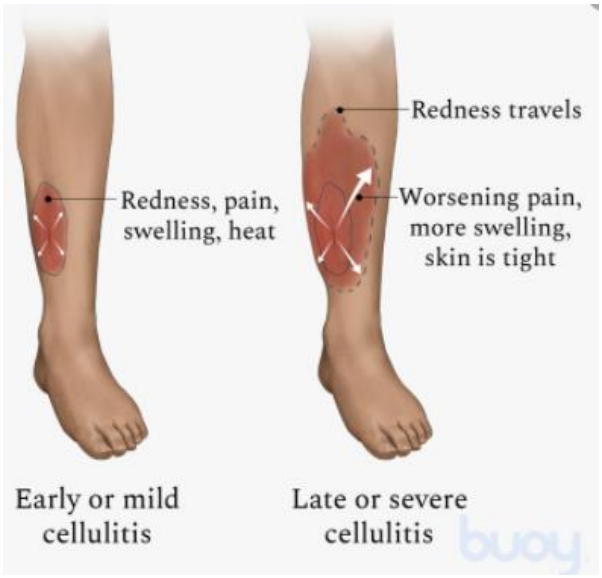
Pathogenesis and Clinical Findings

From the lymphatics, the infection can extend to the bloodstream.

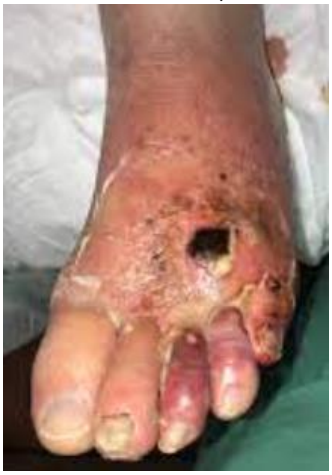
1. Erysipelas—If the portal of entry is the **skin**, erysipelas results. Lesions are raised and characteristically red.



2. Cellulitis—Streptococcal cellulitis is an acute, rapidly spreading infection of the skin and **subcutaneous tissues**.



3. Necrotizing fasciitis (streptococcal gangrene)—There is extensive and very rapidly spreading necrosis of the skin, tissues, and fascia.

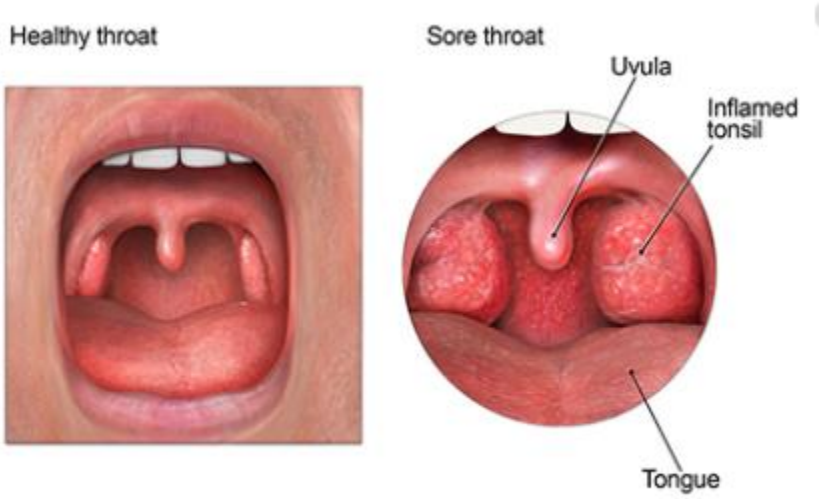


4. Puerperal fever—If the streptococci enter the uterus after delivery, puerperal fever develops, which is essentially a septicemia originating in the infected wound (endometritis).

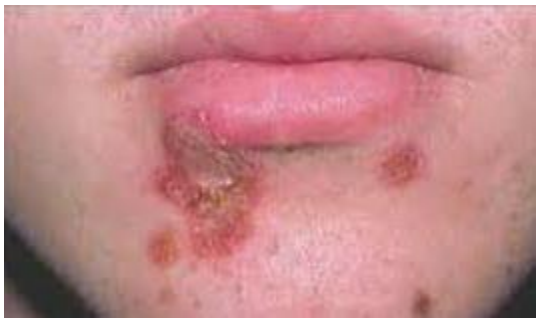
5. Bacteremia or sepsis—Infection of traumatic or surgical wounds with streptococci results in bacteremia, which can rapidly be fatal.

Local Infection

1. Streptococcal sore throat—The most common infection caused by β-hemolytic *S. pyogenes* is streptococcal sore throat or pharyngitis. *S. pyogenes* adheres to the pharyngeal epithelium by means of lipoteichoic acid-covered surface pili and by means of hyaluronic acid in encapsulated strains. *S. pyogenes* infection of the upper respiratory tract does not usually involve the lungs.



2. Streptococcal pyoderma—Local infection of superficial layers of skin, especially in children, is called impetigo. It consists of superficial vesicles that break down and eroded areas whose denuded surface is covered with pus and later is encrusted.



Streptococcal Toxic Shock Syndrome, and Scarlet Fever

Fulminant خاطف, invasive *S. pyogenes* infections with streptococcal toxic shock syndrome are characterized by shock, bacteremia, respiratory failure, and multiorgan failure. Death occurs in about 30% of patients.

Pyrogenic exotoxins A–C also cause **scarlet fever** in association with *S. pyogenes* pharyngitis or with skin or soft tissue infection.

Poststreptococcal Diseases (Rheumatic Fever, Glomerulonephritis)

1. Acute glomerulonephritis—This sometimes develops 1–5 weeks (mean 7days) after *S. pyogenes* skin infection (pyoderma, impetigo) or pharyngitis.

2. Rheumatic fever—This is the most serious sequela of *S. pyogenes* because it results in damage to heart muscle and valves. Certain strains of group A streptococci contain cell membrane antigens that cross-react with human heart tissue antigens.

Laboratory Diagnosis—Practical lecture.

Streptococcus pneumoniae

-is a member of the *S. mitis* group.

-Gram-positive diplococci, often lancet shaped or arranged in chains.

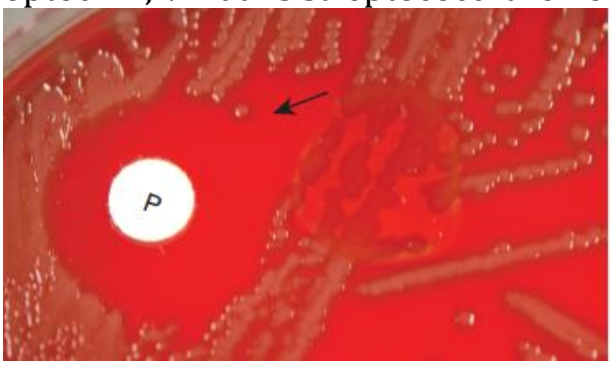
-possessing a capsule of polysaccharide that permits typing with specific antisera.

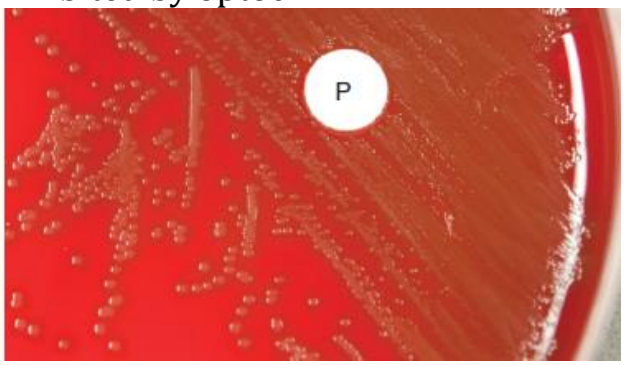
- In sputum or pus, single cocci or chains are also seen.

- With age, the organisms rapidly become Gram-negative and tend to lyse spontaneously.

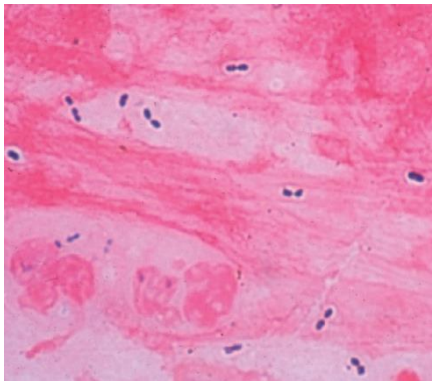
-Lysis of pneumococci occurs in a few minutes when ox bile (10%) or sodium deoxycholate (2%) is added to a broth culture or suspension of organisms at neutral pH. (Viridans streptococci do not lyse and are thus easily differentiated from pneumococci).

-On solid media, the growth of pneumococci is inhibited around a disk of optochin; viridans streptococci are not inhibited by optochin.





- capsule swelling test, or quellung reaction is very important in the diagnosis of this bacterium.



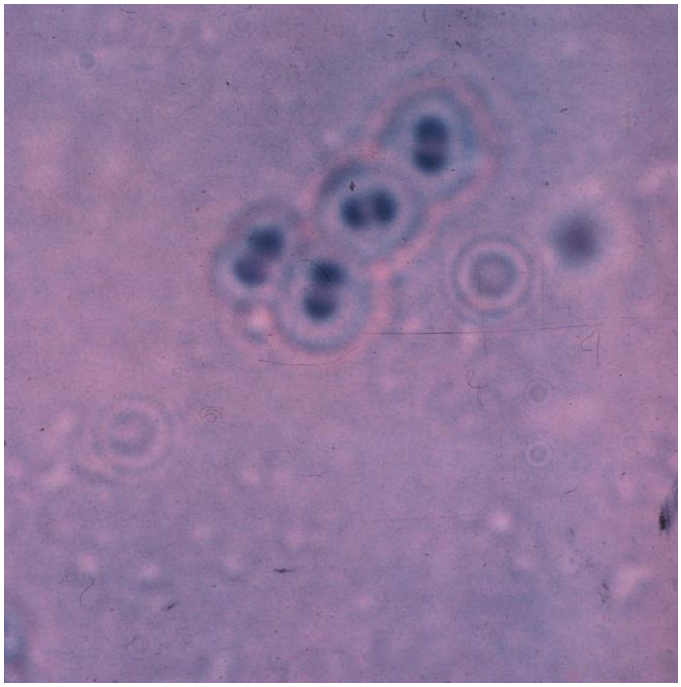
S. pneumoniae in sputum are seen as lancetshaped Gram-positive diplococci. Degenerating nuclei of polymorphonuclear cells are the large darker irregular red shapes (*arrow*). Mucus and amorphous debris are present in the background. Original magnification ×1000.

Culture

Pneumococci form small round colonies, at first domeshaped and later developing a central depression with an elevated rim. Other colonies may appear glistening because of capsular polysaccharide production. Pneumococci are α-hemolytic on blood agar. Growth is enhanced by 5–10% CO2.

Quellung Reaction

When pneumococci of a certain type are mixed with specific antipolysaccharide serum of the same type—or with polyvalent antiserum—on a microscope slide, the capsule swells markedly, and the organisms agglutinate by crosslinking of the antibodies.



Disease production

Pneumococci produce disease through their ability to multiply in the tissues. The virulence of the organism is a function of its capsule, which prevents or delays ingestion by phagocytes.