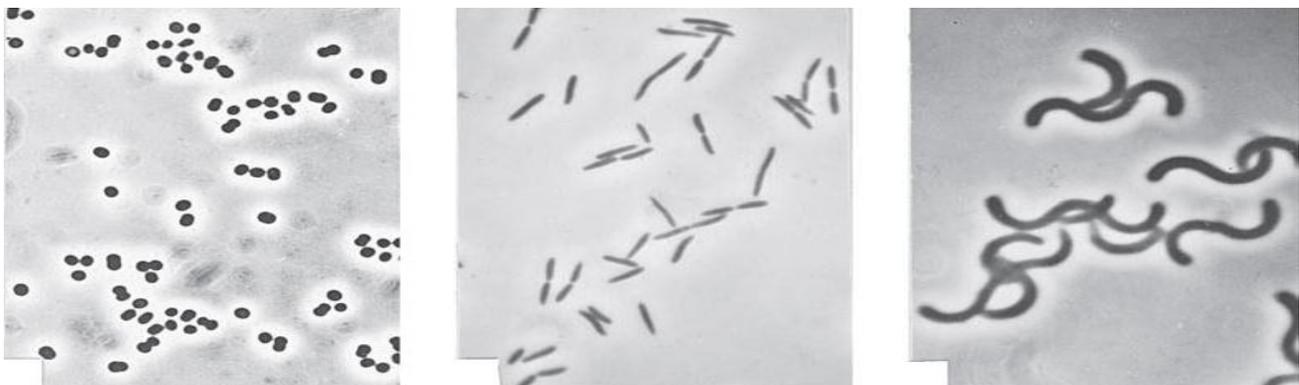


## Description of The Major Categories and Groups of Bacteria

### A. Gram-Negative Eubacteria

This is a heterogeneous group of bacteria. The **cell shape** may be **spherical, oval, straight or curved rods, helical, or filamentous**; some of these forms may be sheathed or encapsulated. Reproduction is by binary fission, but some groups reproduce by budding. Motility, if present, occurs by means of flagella or by gliding motility. Members of this category may be **phototrophic or nonphototrophic** bacteria and include **aerobic, anaerobic, facultatively anaerobic, and microaerophilic** species.



**Figure :** The cell shapes that occur among unicellular true bacteria.

A: Coccus. B: Rod. C: Spiral

### B. Gram-Positive Eubacteria

These bacteria have a cell wall profile of the gram-positive type; cells generally, but not always, stain gram positive. These cells may be encapsulated and can exhibit flagellar-mediated motility. Cells may be spherical, rods, or filaments; the rods and filaments may be non branching or may show true branching. Reproduction is generally by binary fission. Some bacteria in this category produce **spores** (eg, *Bacillus* and *Clostridium spp.*) as resting forms that are highly resistant to disinfection. The gram-positive eubacteria are generally **chemosynthetic heterotrophs**, and include aerobic, anaerobic, and facultatively anaerobic species.

### C. Eubacteria Lacking Cell Walls

These are microorganisms that lack cell walls (commonly called **mycoplasmas and making up the class Mollicutes**) and do not synthesize the precursors of peptidoglycan.

#### Gram positive cocci

#### ●●● The Staphylococci :

The staphylococci are gram-positive spherical cells, usually arranged in **grapelike** irregular clusters. Single cocci, pairs, tetrads, and chains are also seen in liquid cultures. They grow readily on many types of media and are active metabolically, fermenting carbohydrates and producing pigments that vary from white to deep yellow. Some are members of the normal microbiota of the skin and mucous membranes of humans; others cause suppuration, abscess formation, a variety of pyogenic infections, and even fatal septicemia. The pathogenic staphylococci often hemolyse blood, coagulate plasma, and produce a variety of extracellular enzymes and toxins.

Young cocci stain strongly gram positive; on aging, many cells become gram negative . Under the influence of drugs such as penicillin, staphylococci are lysed.

The most common type of **food poisoning** is caused by a heat stable staphylococcal enterotoxin. Staphylococci rapidly develop resistance to many antimicrobial agents, which consequently presents difficult therapeutic problems.

The genus *Staphylococcus* has at least 45 species. The four most frequently encountered species of clinical importance are:

- Staphylococcus aureus*,
- Staphylococcus epidermidis*,
- *Staphylococcus lugdunensis* , and
- *Staphylococcus saprophyticus*.

- ▶ *S. aureus* is **coagulase positive**, which differentiates it from the other species. *S. aureus* is a major pathogen for humans. Almost every person will have some type of *S. aureus* infection during a lifetime, ranging in severity from (food poisoning or minor skin infections to severe life-threatening infections.)
- ▶ **coagulase-negative** staphylococci are normal human microbiota and sometimes cause infection, often associated with implanted devices.

## Morphology and identification

### A. Typical Organisms

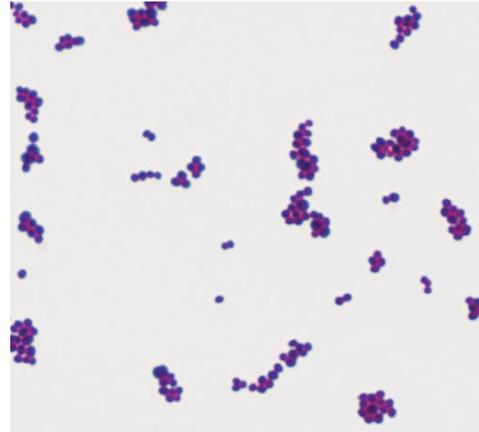
- Staphylococci are spherical cells about 1  $\mu\text{m}$  in diameter
- Arranged in irregular clusters .
- Single cocci, pairs, tetrads, and chains.
- Stain strongly gram positive.
- Are non-motile and do not form spores.

### B. Culture:

- Grow readily on most bacteriologic media under aerobic or micro aerophilic conditions.
- Colonies on solid media are round, smooth, raised, and glistening.
- *S. aureus* usually forms gray to deep golden yellow colonies.
- *S. epidermidis* colonies usually are gray to white on primary isolation.
- Many colonies develop pigment only upon prolonged incubation. No pigment is produced anaerobically or in broth.
- Hemolysis are produced by *S. aureus* and occasionally by other species. *Peptostreptococcus* and *Peptoniphilus* species, which are anaerobic cocci, often resemble staphylococci in morphology.



**FIGURE** Colonies of *Staphylococcus aureus* on a blood agar plate after 24 hours incubation. The yellow-gray colonies are 3 to 4 mm in diameter on the 10-cm plate. The colonies are surrounded by clear zones of hemolysis about 1 cm in diameter.



Gram stain of *Staphylococcus aureus* showing gram-positive cocci in pairs, tetrads, and clusters

### C. Growth Characteristics

- ▶ The staphylococci produce catalase, which differentiates them from the streptococci. Staphylococci slowly ferment many carbohydrates, producing lactic acid but not gas.
- ▶ Staphylococci are relatively resistant to drying, heat (they withstand 50°C for 30 minutes), and 9% sodium chloride but are readily inhibited by certain chemicals (eg, 3% hexachlorophene).
- ▶  $\beta$ -Lactamase production is common, is under plasmid control, and makes the organisms resistant to many penicillins
- ▶ Resistance to nafcillin, (and to methicillin and oxacillin) is independent of  $\beta$ -lactamase production.
- ▶ Plasmid-mediated resistance to tetracyclines, erythromycins, aminoglycosides, and other drugs is frequent in staphylococci.
- ▶ “Tolerance” implies that staphylococci are inhibited by a drug but not killed by it that is, there is great difference between minimal inhibitory and minimal lethal concentrations of an antimicrobial drug.

### D- Antigenic Structure

- ▶ Staphylococci contain antigenic polysaccharides and proteins as well as other substances important in cell wall structure.
- ▶ Peptidoglycan, a polysaccharide polymer containing linked subunits, provides the rigid exoskeleton of the cell wall.
- ▶ Teichoic acids, which are polymers of polyribitol–phosphate, are cross-linked to the peptidoglycan and can be antigenic.
- ▶ Protein A is a cell wall component of *S aureus strains* and is a bacterial surface protein that has been characterized among a group of adhesins called *microbial surface components recognizing adhesive matrix molecules* (MSCRAMMS).
- ▶ Most *S. aureus strains of clinical importance have* polysaccharide capsules, which inhibit phagocytosis by polymorphonuclear leukocytes.

### E- Enzymes and Toxins

Staphylococci can produce disease both through their ability to multiply and spread widely in tissues and through their production of many extracellular substances. Some of these substances are enzymes; others are considered to be toxins, although they may function as enzymes.

#### 1. Catalase

Staphylococci produce catalase, which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative.

#### 2. Coagulase and Clumping Factor

*S. aureus produces coagulase, an enzyme-like protein that* clots oxalated or citrated plasma. Coagulase binds to prothrombin; together they become enzymatically active and initiate fibrin polymerization.

**Clumping factor is another example of an MSCRAMM** that is responsible for adherence of the organisms to fibrinogen and fibrin. When mixed with plasma, *S aureus* forms clumps. Clumping factor is distinct from coagulase.

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### 3. Other Enzymes

Other enzymes produced by staphylococci include a hyaluronidase, or spreading factor; a staphylokinase resulting in fibrinolysis but acting much more slowly than streptokinase; proteinases; lipases; and  $\beta$ -lactamase.

### 4. Hemolysins

1-  $\alpha$ -Hemolysin is a heterogeneous protein that acts on a broad spectrum of eukaryotic cell membranes.

2- The  $\beta$ -toxin degrades sphingomyelin and therefore is toxic for many kinds of cells, including human red blood cells.

3- The  $\delta$ -toxin is heterogeneous and dissociates into subunits in nonionic detergents. It disrupts biologic membranes and may have a role in *S aureus* diarrheal diseases.

4- The  $\gamma$  hemolysin is a leukocidin that lyses white blood cells and is composed of two proteins designated S and F.

### 5. Panton-Valentine Leukocidin

This toxin of *S aureus* has two components, It can kill white blood cells of humans and rabbits. The two components designated as S and F act synergistically on the white blood cell membrane as described for  $\gamma$  toxin.

### 6. Exfoliative Toxins

These epidermolytic toxins of *S aureus* are two distinct proteins of the same molecular weight. Exfoliative toxin A and Exfoliative toxin B is plasmid mediated and heat labile. These epidermolytic toxins yield the generalized desquamation of the staphylococcal **scalded skin syndrome** by dissolving the mucopolysaccharide matrix of the epidermis.

### 7. Toxic Shock Syndrome Toxin

Most *S aureus* strains isolated from patients with toxic shock syndrome produce a toxin called **toxic shock syndrome toxin-1 (TSST-1)**, which is the same as **enterotoxin** .

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## 8. Enterotoxins

There are multiple (A–E, G–J, K–R and U, V) enterotoxins that, similar to TSST-1, Approximately 50% of *S aureus* strains can produce one or more of them. The enterotoxins are heat stable and resistant to the action of gut enzymes. Important causes of food poisoning, enterotoxins are produced when *S aureus* grows in carbohydrate and protein foods. Ingestion of 25 µg of enterotoxin B results in vomiting and diarrhea. The emetic effect of enterotoxin is probably the result of central nervous system stimulation (vomiting center) after the toxin acts on neural receptors in the gut.

### Pathogenesis

Staphylococci, particularly *S epidermidis*, are members of the normal microbiota of the human skin and respiratory and gastrointestinal tracts. Nasal carriage of *S aureus* occurs in 20–50% of humans. Staphylococci are also found regularly on clothing, bed linens, and other fomites in human environments. The pathogenic capacity of a given strain of *S aureus* is the combined effect of extracellular factors and toxins together with the invasive properties of the strain.

Pathogenic, invasive *S aureus* produces coagulase and tends to produce a yellow pigment and to be hemolytic. Nonpathogenic, noninvasive staphylococci such as *S epidermidis* are coagulase negative and tend to be nonhemolytic. Such organisms rarely produce suppuration but may infect orthopedic or cardiovascular prostheses or cause disease in immunosuppressed persons.

### ► Diagnostic Laboratory Tests

#### A. Specimens:

Surface swab pus or aspirate from an abscess, blood, endonasotracheal aspirate, expectorated sputum, or spinal fluid for culture, depending on the localization of the process,

#### B. Smears:

Typical staphylococci appear as gram-positive cocci in clusters in Gram-stained smears of pus or sputum. It is not possible to distinguish saprophytic (*S epidermidis*) from pathogenic (*S aureus*) organisms on smears.

### C. Culture:

Specimens planted on blood agar plates give rise to typical colonies in 18 hours at 37°C, but hemolysis and pigment production may not occur until several days later and are optimal at room temperature. *S aureus* but not other staphylococci ferment mannitol. Specimens contaminated with a mixed flora can be cultured on media containing 7.5% NaCl; the salt inhibits most other normal microbiota but not *S aureus* so the Mannitol salt agar used as selective media for *S aureus* .

### D. Catalase Test:

This test is used to detect the presence of cytochrome oxidase enzymes. A drop of 3% hydrogen peroxide solution is placed on a slide, and a small amount of the bacterial growth is placed in the solution. The formation of bubbles (the release of oxygen) indicates a positive test result.

### E. Coagulase Test:

Coagulase-positive staphylococci are considered pathogenic for humans.

### F. Susceptibility Testing:

Resistance to **penicillin G** can be predicted by a positive test result for  $\beta$ -lactamase; approximately 90% of *S aureus* produce  $\beta$ -lactamase. Resistance to nafcillin (and oxacillin and methicillin) occurs in about 65% of *S aureus* and approximately 75% of *S epidermidis* isolates.

**Nafcillin** resistance correlates with the presence of *mecA*, the gene that codes for a penicillin-binding protein (PBP 2a) not affected by these drugs.