STAPHYLOCOCCI

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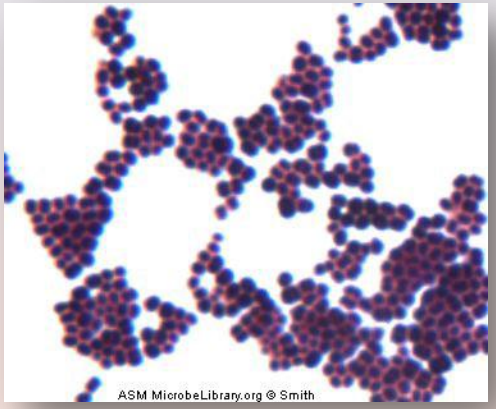
General characteristics

- Gram-positive spherical cells arranged in irregular clusters
  - *staphylé*: a bunch of grapes
- May also appear as single cells, pairs or short chains

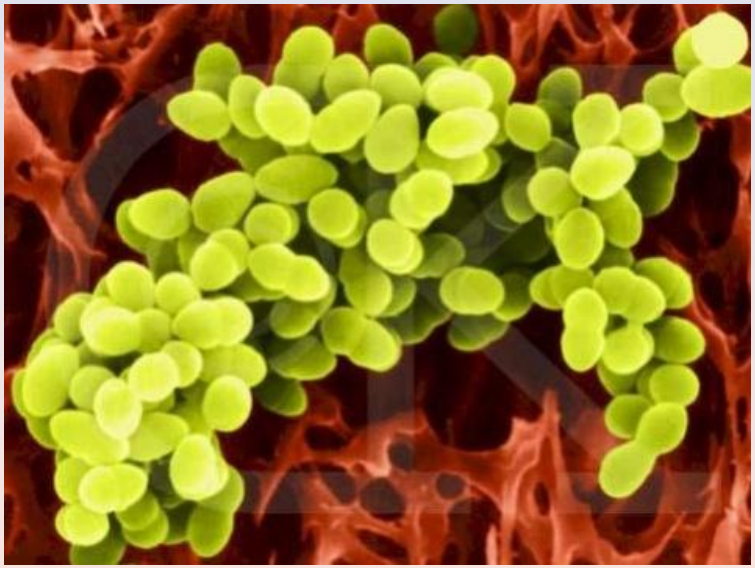


General characteristics

- Nonmotile
- Facultatively anaerobic
- Do not form spores
- Produce catalase
- Resistant to drying, heat and high concentration of salt
  - Can grow in media containing 10% of NaCl
  - Can grow at temperature of 18°C-40°C
- Produce pigments that vary from white to yellow
- Slowly ferment many carbohydrates; produce lactic acid
- Some are members of the normal flora

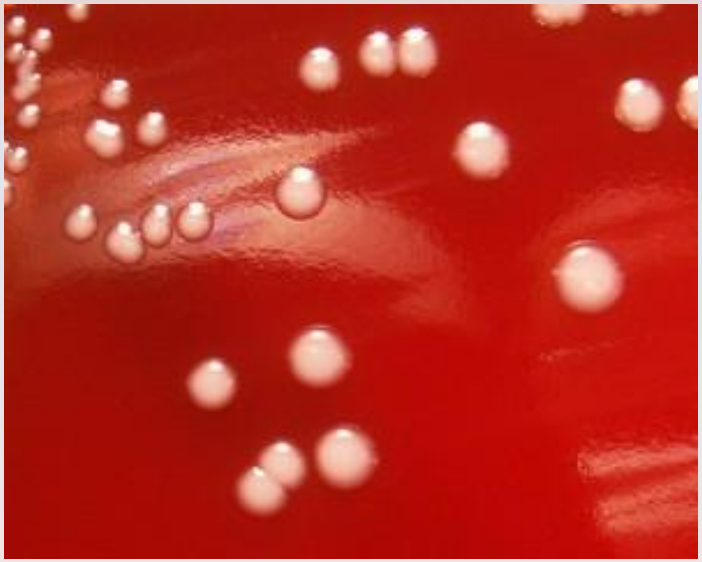
General characteristics

- The genus *Staphylococcus* has 45 species
- The species most commonly associated with human diseases:
  - S. *aureus*
  - S. *epidermidis*
  - S. *haemolyticus*
  - S. *lugdunensis*
  - S. *saprophyticus*



Morphological characteristics

- Grow readily on most bacteriologic media
- Grow most rapidly at 37°C but form pigment best at room temperature (20-25°C)
- Colonies are smooth, round, raised and glistening

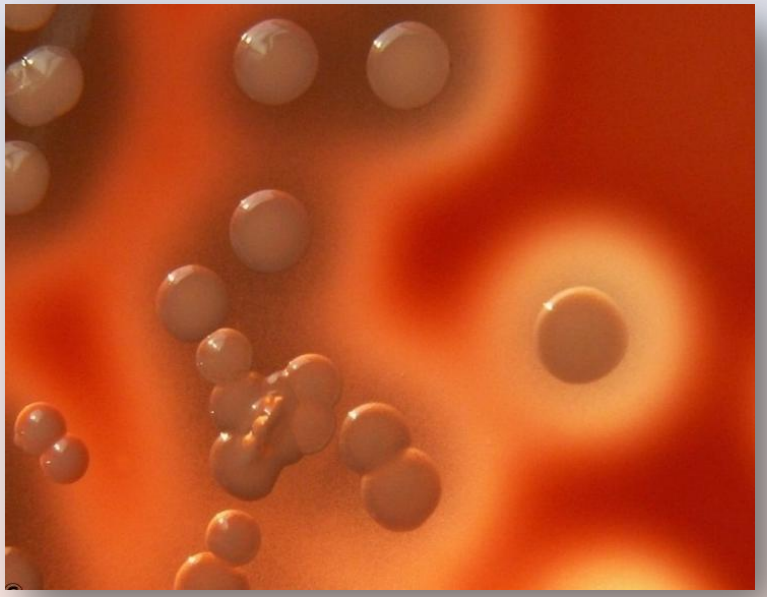


Morphological characteristics

- S. *aureus* usually form gray to deep golden yellow colonies
  - *aureus*: golden

Morphological characteristics

- S. *aureus* produces β-hemolysis when grown on 5% sheep blood agar



Staphylococcus aureus virulence factors

Structural components

- Capsule and slime layer
- Peptidoglycan
- Teichoic acids
- Protein A
- Clumping factor

Enzymes

- Catalase
- Coagulase
- Hyaluronidase
- Fibrinolysin
- Lipases
- Nuclease

Toxins

- Cytotoxins
- Exfoliative toxin
- Toxic shock syndrome toxin
- Enterotoxins

Structural components

- Capsule and slime layer
- Peptidoglycan
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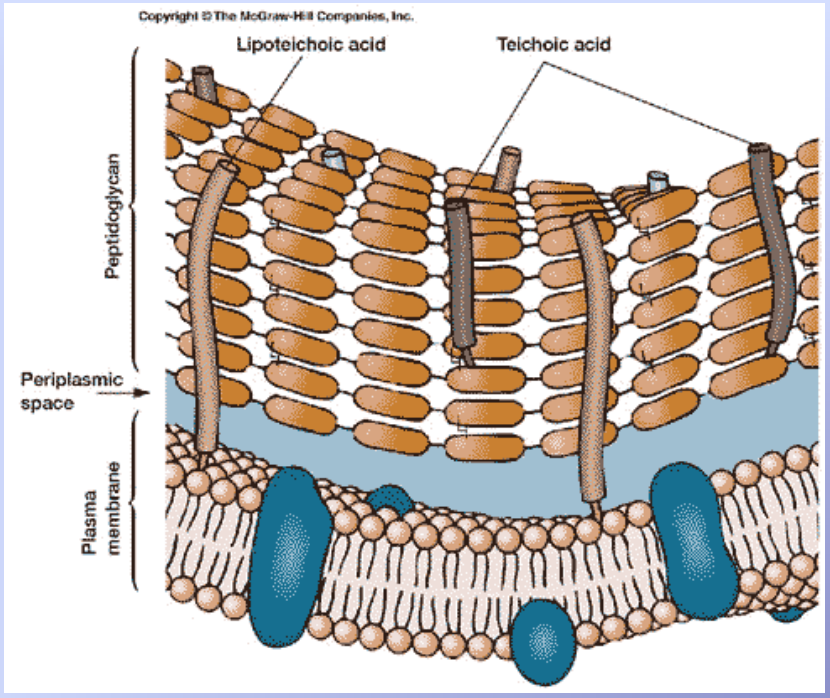
- Clumping factor

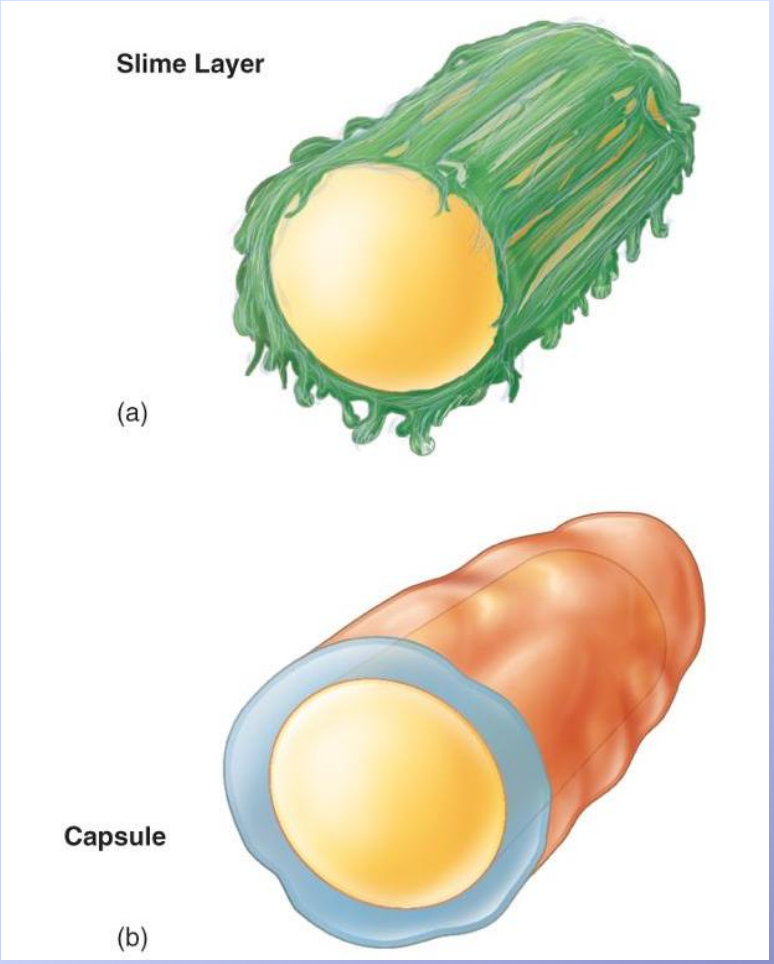


Structural components

Capsule and slime layer

Capsule:

- Polysaccharide capsule
- Inhibits phagocytosis by PMNs

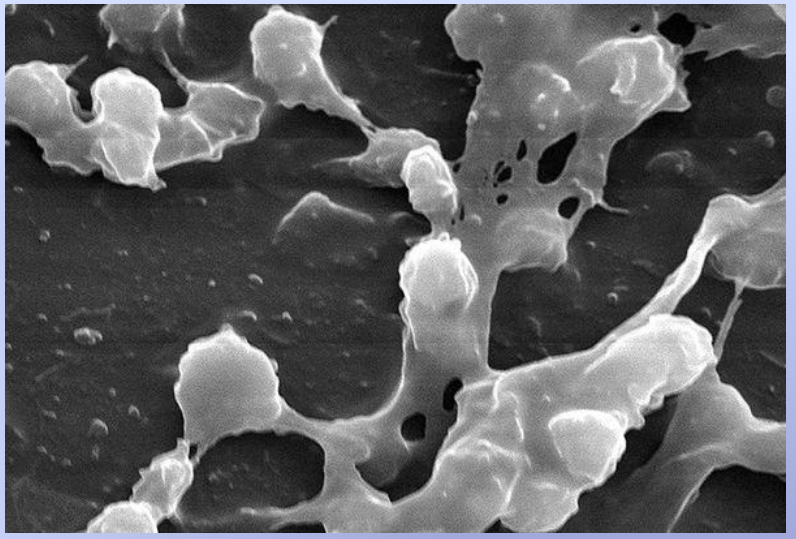


Structural components

Capsule and slime layer

Slime layer:

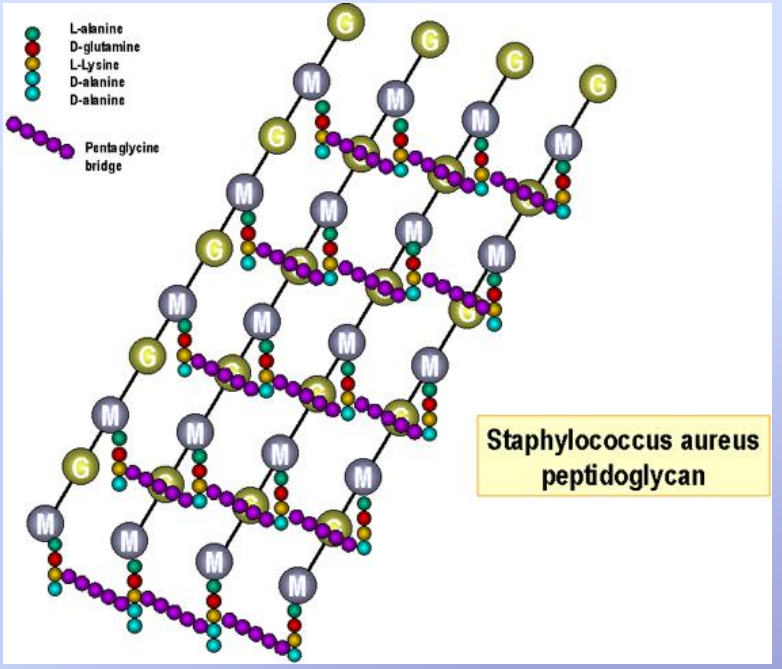
- Loose bound, water-soluble film
- Consists of monosaccharides, proteins and small peptides
- Binds bacteria to **tissues** and **foreign bodies** (catheters,and etc ...)



Structural components

Peptidoglycan

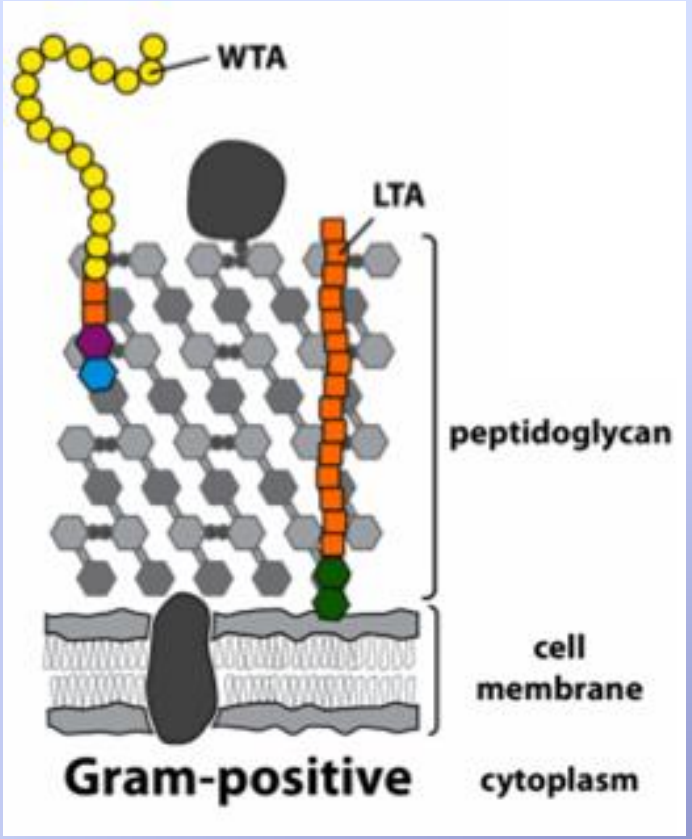
- Provides the rigid exoskeleton of the cell wall
- Stimulates;
  - Production of IL-1
  - Activation of complement
  - Aggregation of PMNs



Structural components

Teichoic acids

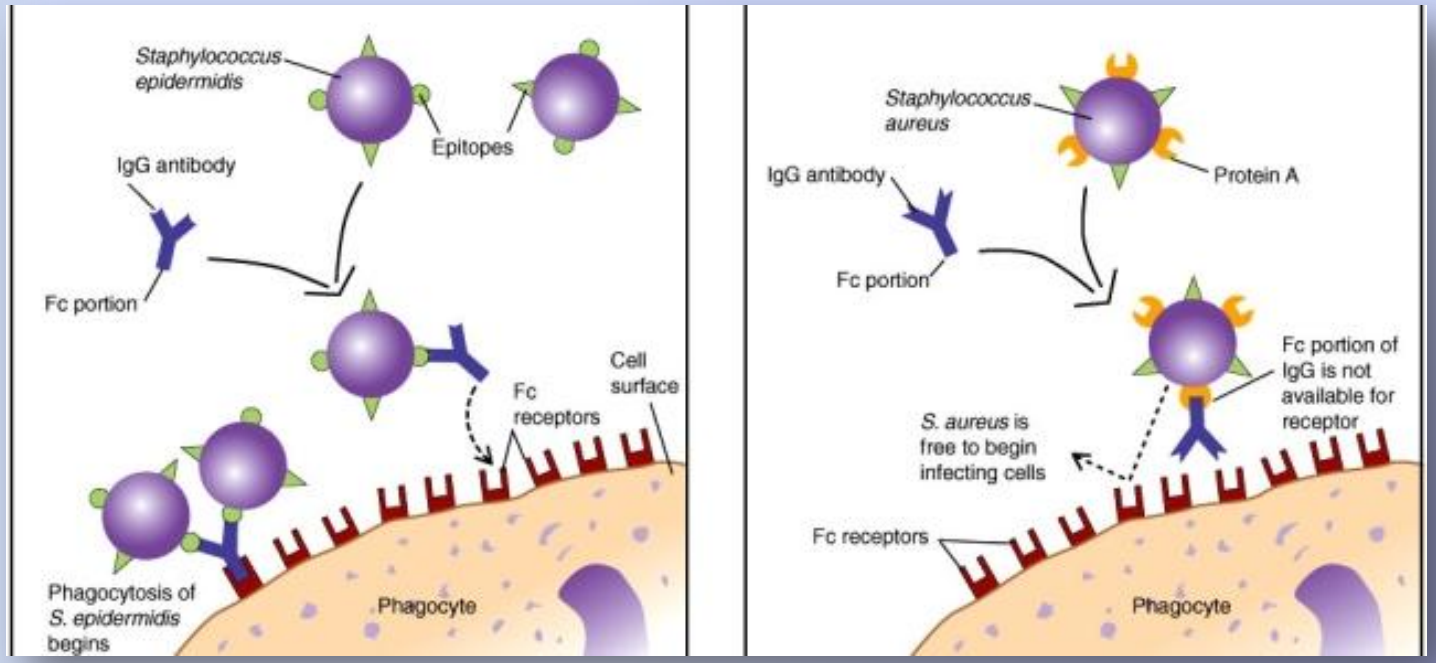
- Bound to peptidoglycan layer or cytoplasmic membrane (lipoteichoic acids)
- Species-specific
- Bind to fibronectin and mediate attachment to mucosal surfaces



Structural components

Protein A

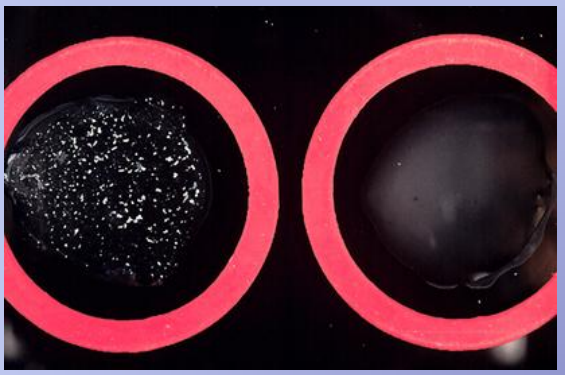
- Cell wall component of most *S. aureus* strains
- Binds to **Fc region** of IgG molecules
  - Prevents antibody-mediated immune clearance of the organism



Structural components

Clumping factor

- Outer surface of most strains of *S. aureus* contains clumping factor **(bound coagulase)**
- Fibrinogen ⟶ Fibrin
  - Staphylococci clump or aggregate
- Detection of this protein: Primary test for identifying *S. aureus*



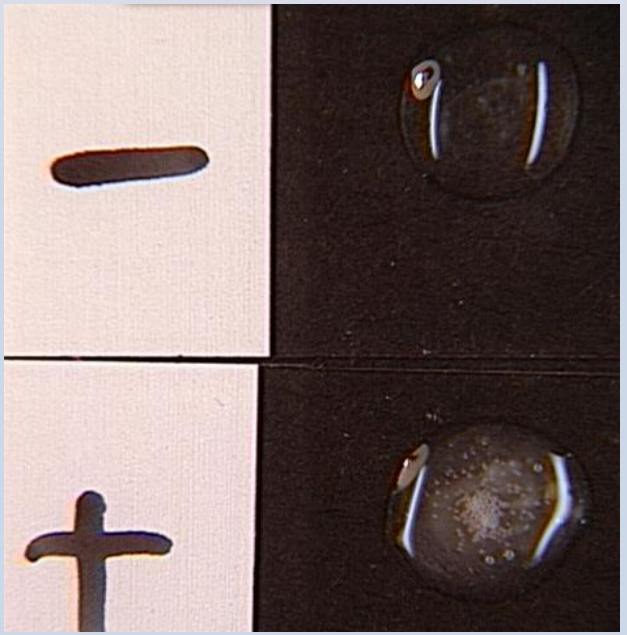
Slide coagulase test

Enzymes

- Catalase
- Coagulase
- Hyaluronidase
- Fibrinolysin (staphylokinase)
- Lipases
- Nuclease

Enzymes
Catalase

- Staphylococci produce catalase
  - Catalase converts hydrogen peroxide into water and oxygen
  - **Staphylococci: catalase (+)**
  - **Streptococci: catalase (-)**



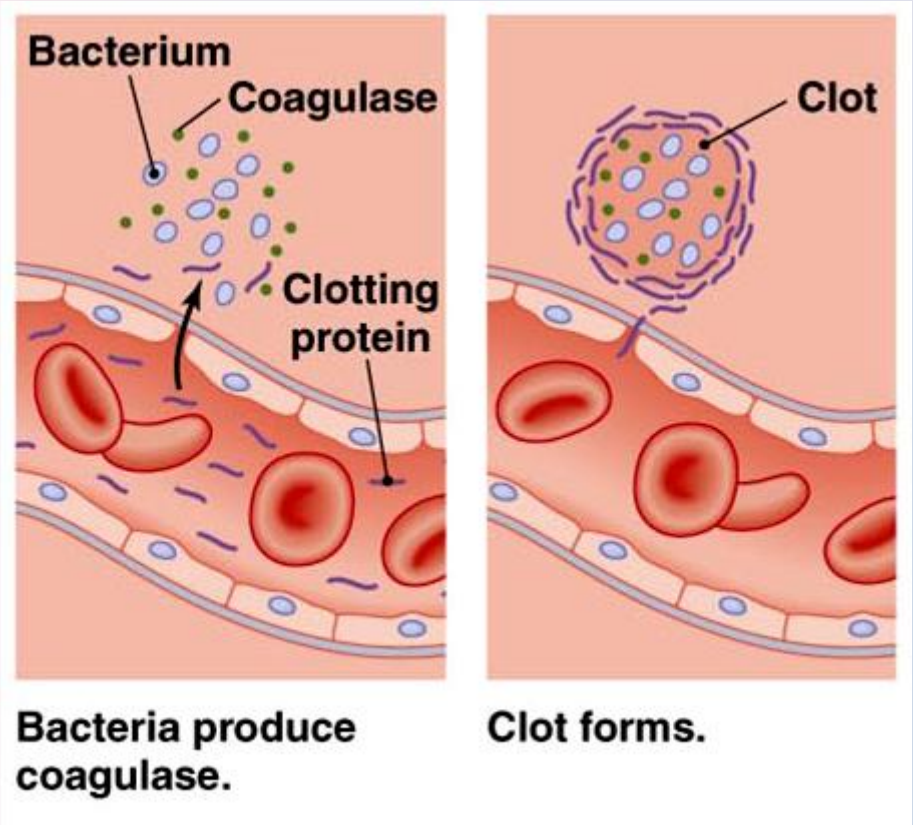
Catalase test

Enzymes
Coagulase

- *S. aureus*: Coagulase (+)
- Coagulase clots plasma
- Coagulase ⟶ deposit fibrin on the surface of staphylococci
  - Staphylococcal abscess ⟶ localizes the infection
  - Inhibits phagocytosis or destruction within phagocytic cells

Enzymes

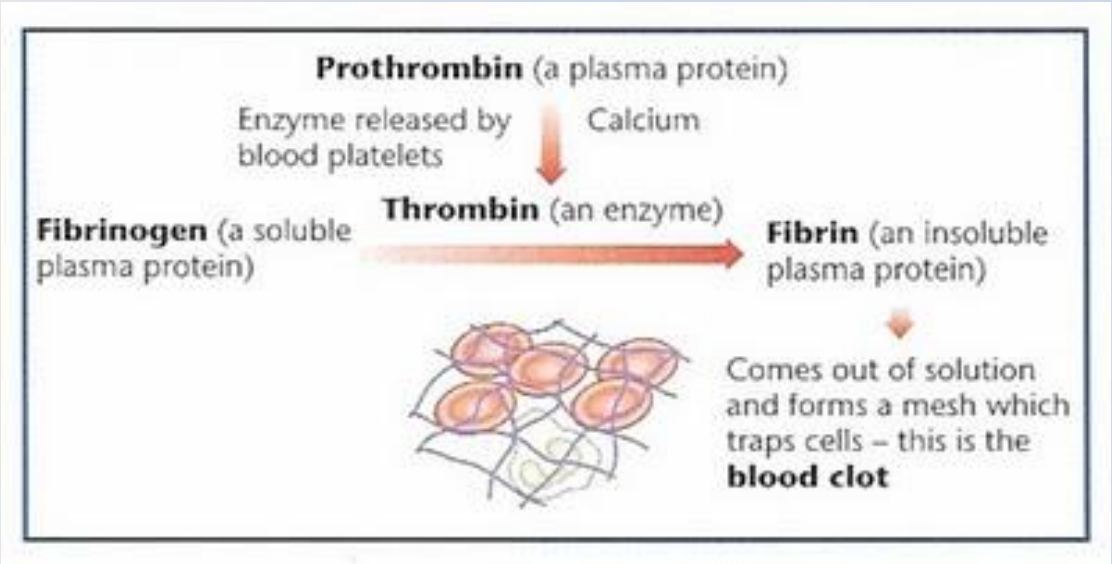
Coagulase



Tube coagulase test

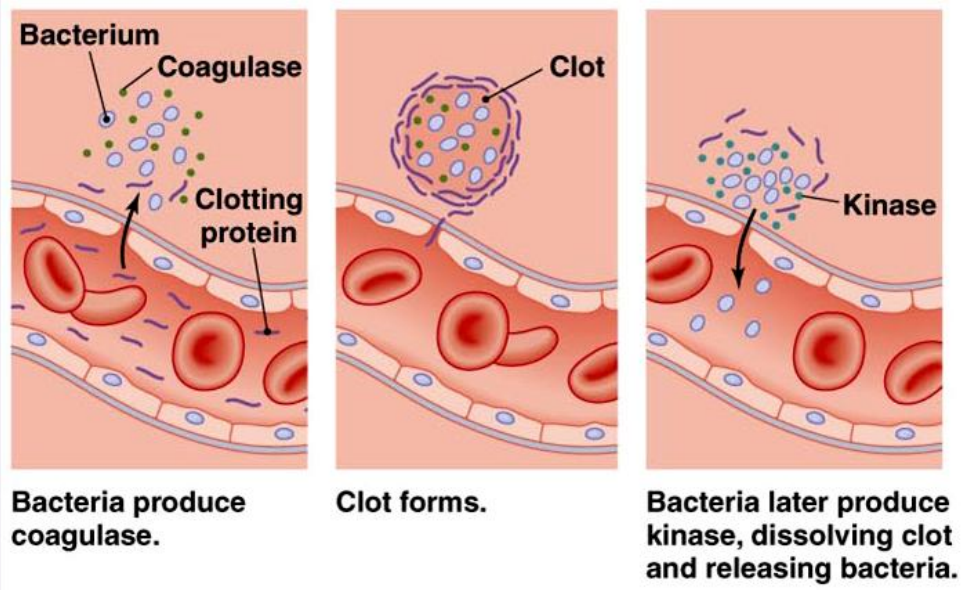
Enzymes
Coagulase

- Bound coagulase can directly convert fibrinogen to insoluble fibrin and cause clumping
- Extracellular coagulase first reacts with prothrombin, and conversion of fibrinogen to fibrin is catalysed



Enzymes
Spreading factors

- **Hyaluronidase:**
  - Hydrolizes hyaluronic acids (connective tissue)
- **Fibrinolysin (staphylokinase):**
  - Dissolves fibrin clots



Enzymes
Spreading factors

- **Lipases:**
  - Hydrolise lipids
  - Survival of staphylococci in the sebaceous areas of the body
- **Nuclease:**
  - Hydrolyze viscous DNA

Toxins

- Cytotoxins
  - Hemolysins
  - Panton-Valentine Leukocidine
- Exfoliative toxins
- Toxic shock syndrome toxin-1
- Enterotoxins

Toxins

Cytotoxins

- Hemolysins
  - Alpha (α)-toxin (α-hemolysin)
  - Beta (β)-toxin (sphingomyelinase C)
  - Delta (δ)-toxin
  - Gamma (γ)-toxin (γ-hemolysin)
- Panton-Valentine Leukocidin (PVL)

Toxins

Cytotoxins – Hemolysins

- Toxic to a variety of cells including leukocytes and erythrocytes
- **α-toxin:** important mediator of tissue damage in staphylococcal diseases
- The hemolysis on blood agar is particularly caused by **α-toxin**

Toxins

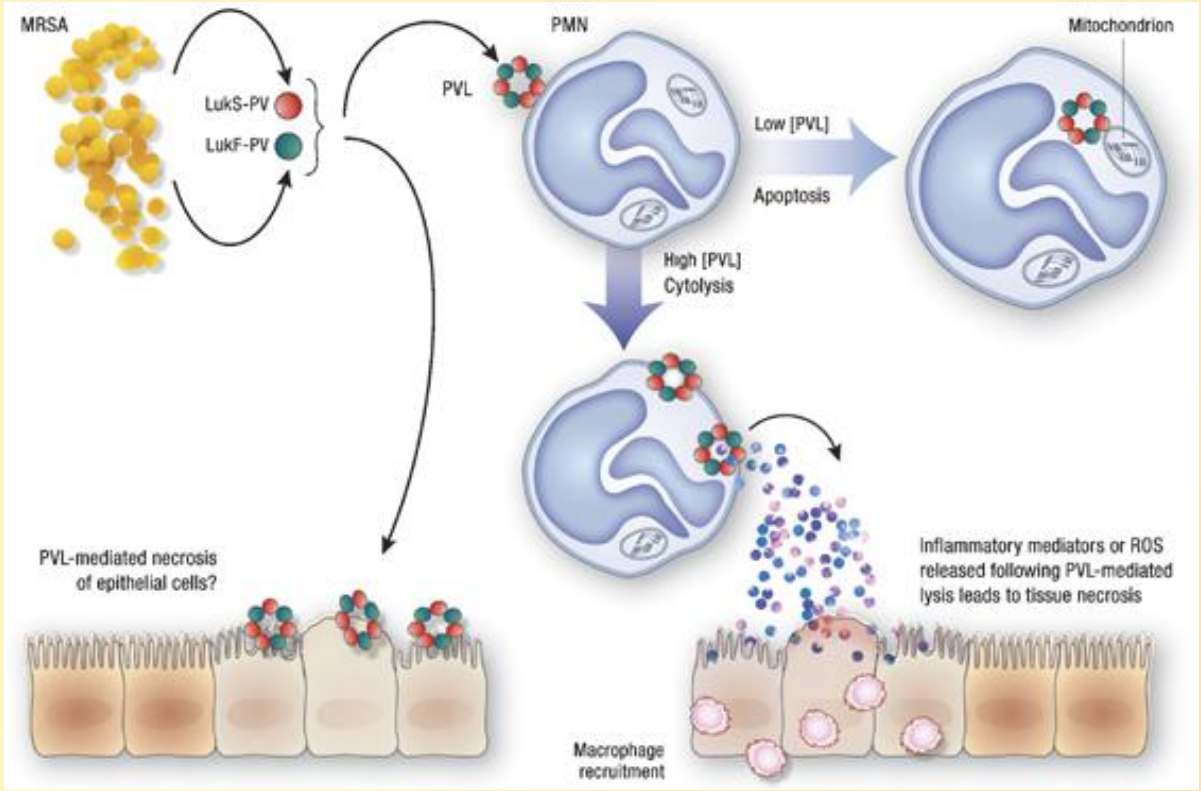
Cytotoxins – Panton-Valentine Leukocidin

- Leukotoxic but has **no hemolytic activity**
- Encoded on a mobile **phage**
- Majority of Community acquired – Methicillin resistant *Staphylococcus aureus* **(CA-MRSA)** strains: PVL (+)

Toxins

Cytotoxins – Panton-Valentine Leukocidin

- CA-MRSA is responsible for diseases including **necrotizing pneumonia**, severe sepsis and necrotizing faciitis
- PVL is related with **tissue necrosis**



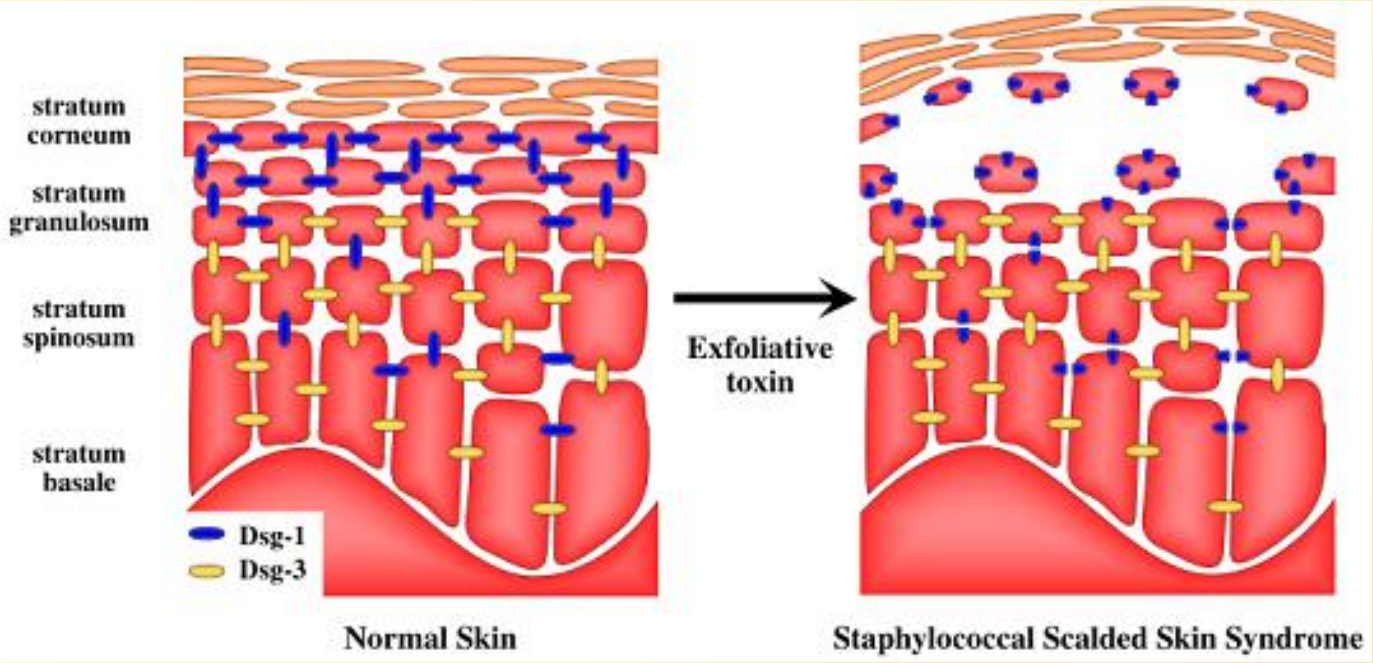
Toxins
Exfoliative toxins

- **Superantigens**
- Epidermolytic toxins
  - Epidermolytic toxin A (ETA): Phage associated; heat-stabile
  - Epidermolytic toxin B (ETB): Plasmid-mediated; heat-labile
- Lead to generalized desquamation of the **staphylococcal scalded skin syndrome (SSSS)**
  - Dissolve mucopolysaccharide matrix of epidermis

Toxins

Exfoliative toxins

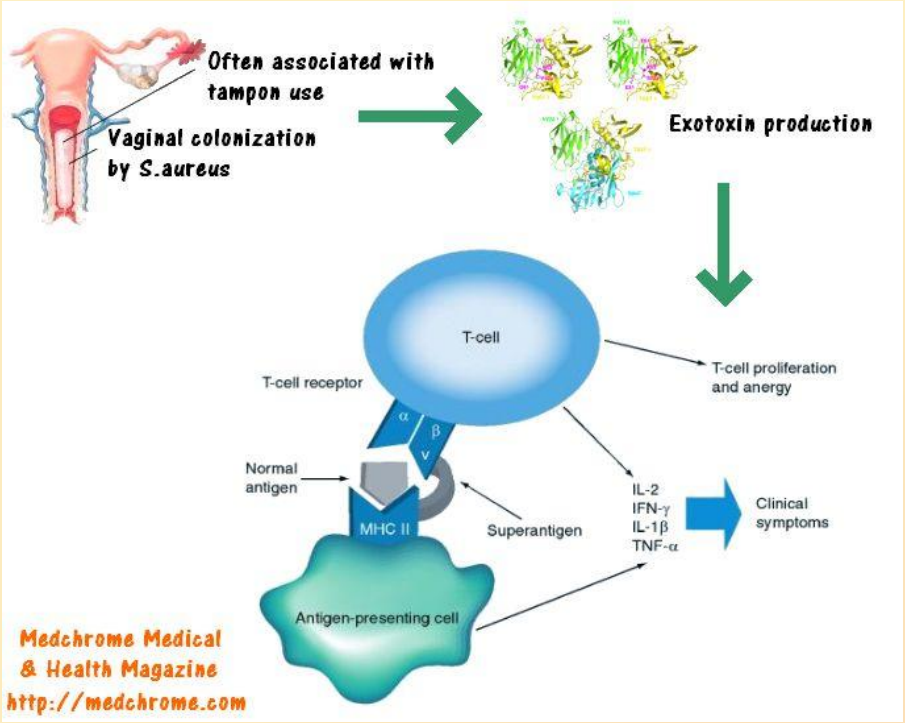
- Toxins lead to splitting of desmosomes in the stratum granulosum



Toxins

Toxic shock syndrome toxin-1 (TSST-1)

- **Superantigens**
- Heat- and proteolysis-resistant, chromosomally mediated
- Causes **toxic shock syndrome (TSS)**
  - Associated with fever, shock and multisystem involvement, including a desquamative skin rash



Toxins

Toxic shock syndrome toxin-1 (TSST-1)

- Toxic shock syndrome:
  - Menstruation-associated TSS – tampon use
  - Nonmenstruation-associated TSS – antibiotic treatment, hospital exposure
- For production of TSST-1
  - Low level of glucose
  - Temperature of 37-40°C
  - pH of 6.5-8
  - **Oxygen**
    - Tampon use
    - Proteolytic cleavage of menstrual blood (without tampon use)

Present in menstruation without tampon use

Toxins

Enterotoxins

- **Superantigens**
- Important cause of **food poisoning**
- Staphylococcal enterotoxins A to R
- Enterotoxin A: most commonly associated with food poisoning
- **Heat-stabile** and **resistant to hydrolysis** by gastric and jejunal enzymes

Toxins

Enterotoxins

- Produced when *S. aureus* grows in carbohydrate and protein foods
- The toxins cause nonspecific activation of T cells and cytokine release
  - Release of inflammatory mediators from mast cells
  - Increase in intestinal peristalsis and fluid loss
  - Nausea and vomiting

Staphylococcal diseases

Staphylococcus aureus

- **Toxin-mediated diseases**
  - Scalded skin syndrome
  - Food poisoning
  - Toxic shock syndrome
- **Suppurative infections**
  - Impetigo
  - Folliculitis
  - Furuncles or boils
  - Carbuncles
  - Bacteremia, meningitis and endocarditis
  - Pneumonia and empyema
  - Osteomyelitis
  - Septic arthritis

Staphylococcal diseases

S. aureus > Toxin-mediated diseases

- **Staphylococcal scalded skin syndrome (SSSS)**
  - Disseminated desquamation of epithelium in infants
  - Blisters with **no organisms or leukocytes**
  - Low mortality rate

Staphylococcal diseases

S. aureus > Toxin-mediated diseases

- **Bullous impetigo**
  - Localized form of SSSS
  - Localized blisters are **culture positive**

Staphylococcal diseases

S. aureus > Toxin-mediated diseases

- **Staphylococcal food poisoning**
  - **Intoxication** rather than infection
  - Consumption of food contaminated with heat-stable enterotoxin
  - The most commonly contaminated foods:
    - Processed meats, custard-filled pastries, salad, potato salad, sandwich, milk and diary products, ice cream, poultry and egg products

Staphylococcal diseases

S. aureus > Toxin-mediated diseases

- **Staphylococcal food poisoning**
  - Results from contamination of the food by a human carrier
    - Individuals with staphylococcal skin infection
    - Carriers with asymptomatic nasopharyngeal colonization
  - Organisms grow in the food and release the toxin
  - Incubation period: **4 hours**
  - Rapid onset of severe vomiting, diarrhea, abdominal cramping
  - **No fever**
  - Antibiotic therapy is **not indicated**
  - Resolution within 24 hours

Staphylococcal diseases

S. aureus > Toxin-mediated diseases

- **Staphylococcal enterocolitis**
  - Enterotoxin A + cytotoxin
  - Watery diarrhea, abdominal cramps, fever
  - Primarily in patients who have received broad-spectrum antibiotics

Staphylococcal diseases

S. aureus > Toxin-mediated diseases

- **Toxic shock syndrome**
  - Multisystem intoxication
  - Fever, hypotension, and diffuse macular erythematous rash
  - High mortality without promt antibiotic therapy and elimination of the focus of infection

Staphylococcal diseases

S. aureus > Suppurative infections

- **Impetigo**
  - Localized cutaneous infection characterized by pus-filled vesicle on an erythematous base
  - Occurs primarily on the face and limbs
  - Can also be caused by *Streptococcus pyogenes*

Staphylococcal diseases

S. aureus > Suppurative infections

Folliculitis

Carbuncles

Furuncles (boils)

Staphylococcal diseases

S. aureus > Suppurative infections

- **Bacteremia, meningitis and endocarditis**
  - >50% of bacteremia ⟶ Surgical procedure or intravascular catheter
- **Pneumonia and empyema**
  - Necrotizing pneumonia ⟶ Septic shock and high mortality; PVL is an important virulence factor

Staphylococcal diseases

S. aureus > Suppurative infections

- **Osteomyelitis**
  - Destruction of bones
  - Hematogenous dissemination to bone, or a secondary infection

Staphylococcal diseases

S. aureus > Suppurative infections

- **Septic arthritis**
  - Painful erythematous joint with collection of purulent material in the joint space

Staphylococcal diseases

Coagulase-negative staphylococci

- Coagulase-negative staphylococci:
  - *S. epidermidis*
  - *S. haemolyticus*
  - *S. lugdunensis*
  - *S. saprophyticus*
  - ...

Staphylococcal diseases
Coagulase-negative staphylococci

- **Endocarditis of artificial heart valves**
- **Wound infections** ⟶ traumatic or surgical wounds; foreign bodies
- **Urinary tract infections**
  - S. *saprophyticus* ⟶ young sexually active women
  - Other CoNS ⟶ patients with urinary catheters
- **Catheter and shunt infections**
- **Prosthetic device infections**

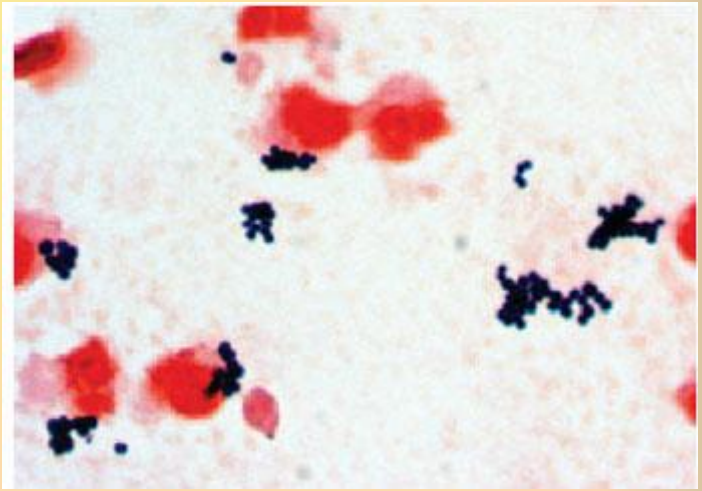
Laboratory diagnosis

- Microscopy
- Culture
- Catalase test
- Coagulase test
- Molecular tests

Laboratory diagnosis

Microscopy

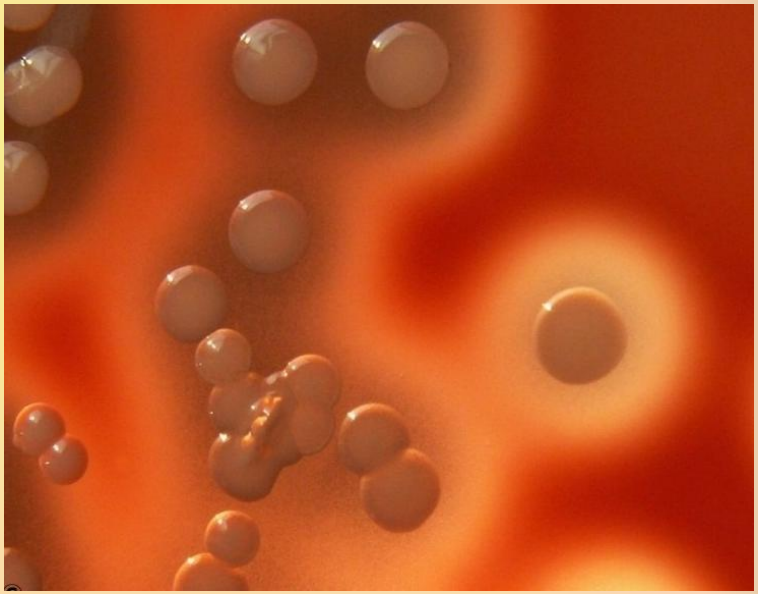
- Specimens
  - Surface swab pus, blood, tracheal aspirate, spinal fluid for culture
- Smears
  - Gram-positive cocci in clusters



Laboratory diagnosis

Culture

- Staphylococci grow rapidly on nonselective media
- On 5% sheep blood agar *S. aureus* generates β-hemolysis **(α-toxin)**

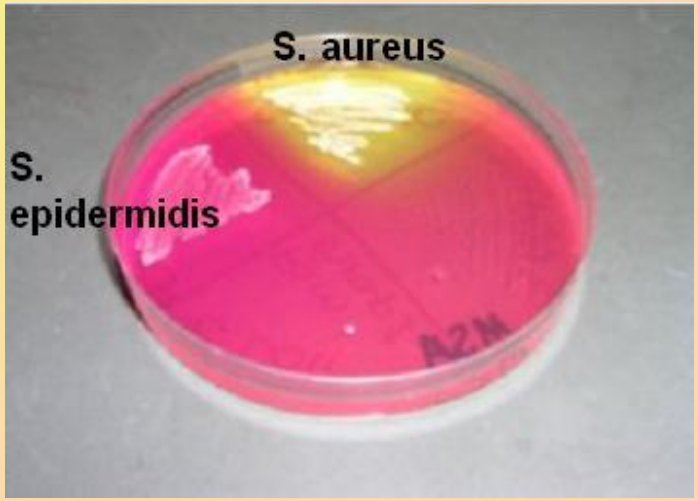


Laboratory diagnosis

Culture

- Mannitol-salt agar
  - S. *aureus* ferments mannitol ⟶ yellow colonies
  - NaCl inhibits most other organisms but not S. *aureus*

Mannitol-salt agar



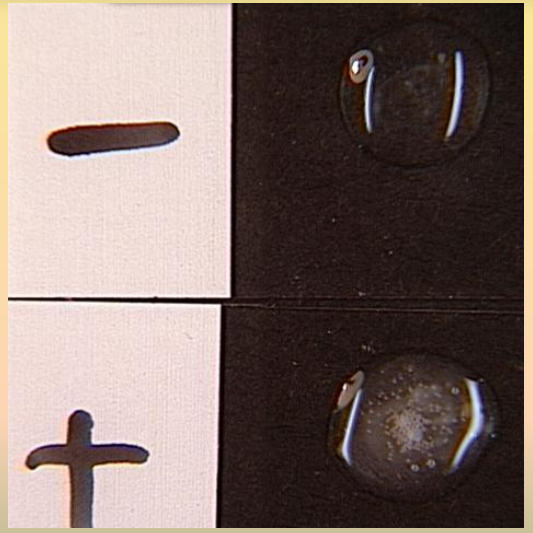
Yellow-colonies of S. *aureus* on mannitol salt agar

Laboratory diagnosis

Catalase test

- Differentiates **staphylococci** from **streptococci**
- Bacterial colony is added onto 3% H_2O_2 solution

$$2H_2O_2 \xrightarrow{\text{catalase}} 2H_2O + O_2$$



Laboratory diagnosis

Coagulase test

- **Tube coagulase test**
  - Detects **extracellular coagluase** enzyme
  - Bacterial colonies are added into 0.5 ml plasma
  - Incubation at 37°C, for 4 hours
  - Coagulation: Positive result

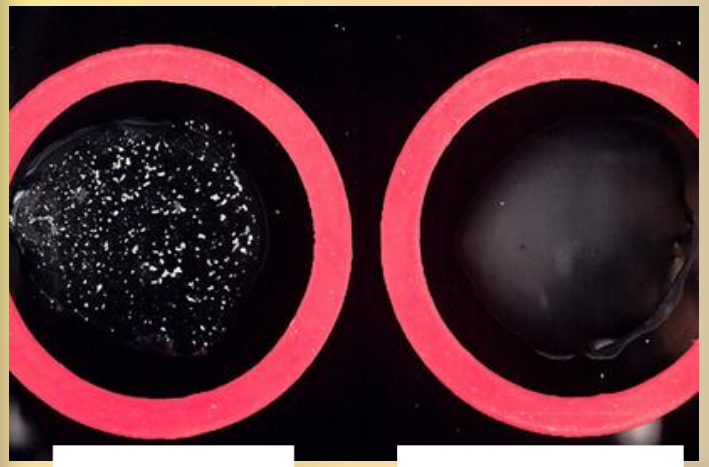
Negative result

Positive result (coagulation)

Laboratory diagnosis

Coagulase test

- **Slide coagluase test**
  - Detects the **clumping factor**
  - Bacterial colony is suspended in distilled water on a slide
  - 1-2 drops of plasma are dropped onto the suspension
  - The slide is rotated gently
  - Coagulation: Positive result



Positive result

Negative result

Treatment, Prevention and Control

- **Methicillin-resistant *Staphylococcus aureus* (MRSA)**
  - *mec A* gene ⟶ PBP ⟶ PBP 2A/ PBP 2' ⟶ Low affinity to β-lactam antibiotics ⟶ Peptidoglycan synthesis is not inhibited
  - Resistant to β-lactam antibiotics (methicillin, oxacillin, penicillin, and amoxicillin)
  - Also resistant to lincosamides, macrolides, aminoglycosides, etc…
  - Isolates should be tested for antimicrobial susceptibility

Treatment, Prevention and Control

- **Methicillin-resistant *Staphylococcus aureus* (MRSA)**
  - Glycopeptides (vancomycin and teicoplanin): current mainstay of therapy for MRSA infections
    - Vancomycin Intermediate *Staphylococcus aureus* (VISA)
    - Vancomycin Resistant *Staphylococcus aureus* (VRSA)
  - Quinupristin/dalfopristin, linezolid, tigecycline, daptomycin
    - Only approved for certain indication

Treatment, Prevention and Control

- **Nasal carriage**
  - The most common source of S. *aureus*
  - ~20% persistent carriers, ~30% intermittent carriers, ~50% non-carriers
  - Prophylaxis consisting of vancomycin and rifampin
  - Survey of high risk patients for anterior nares colonization
  - S. *aureus* can be transferred from nose to the other sites of body via hands
  - **Wearing gloves** and **washing hands** before and after patient contact

Treatment, Prevention and Control