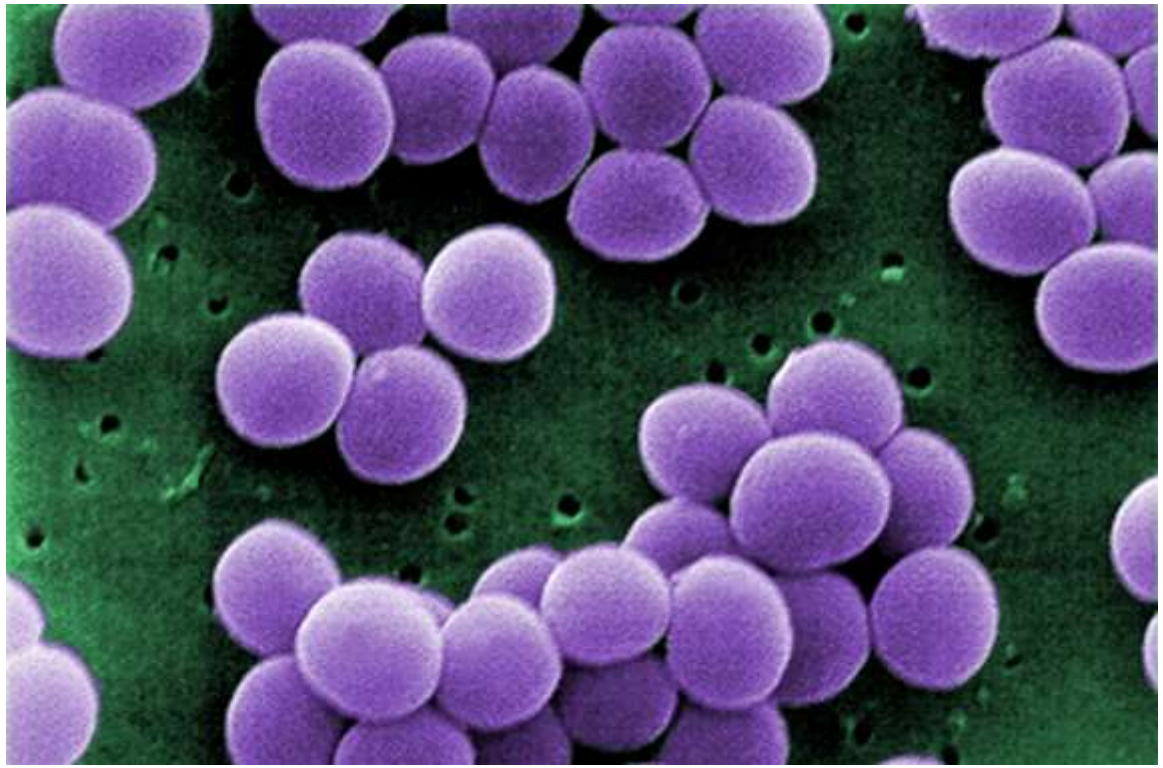
॥ स्वास्थ्य-सेवा-सुशिक्षणम् ॥
MIMER MEDICAL COLLEGE

Skin, soft tissue infection, Staphylococcus



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Learning objectives

At the end of the session, the students will be able to understand:

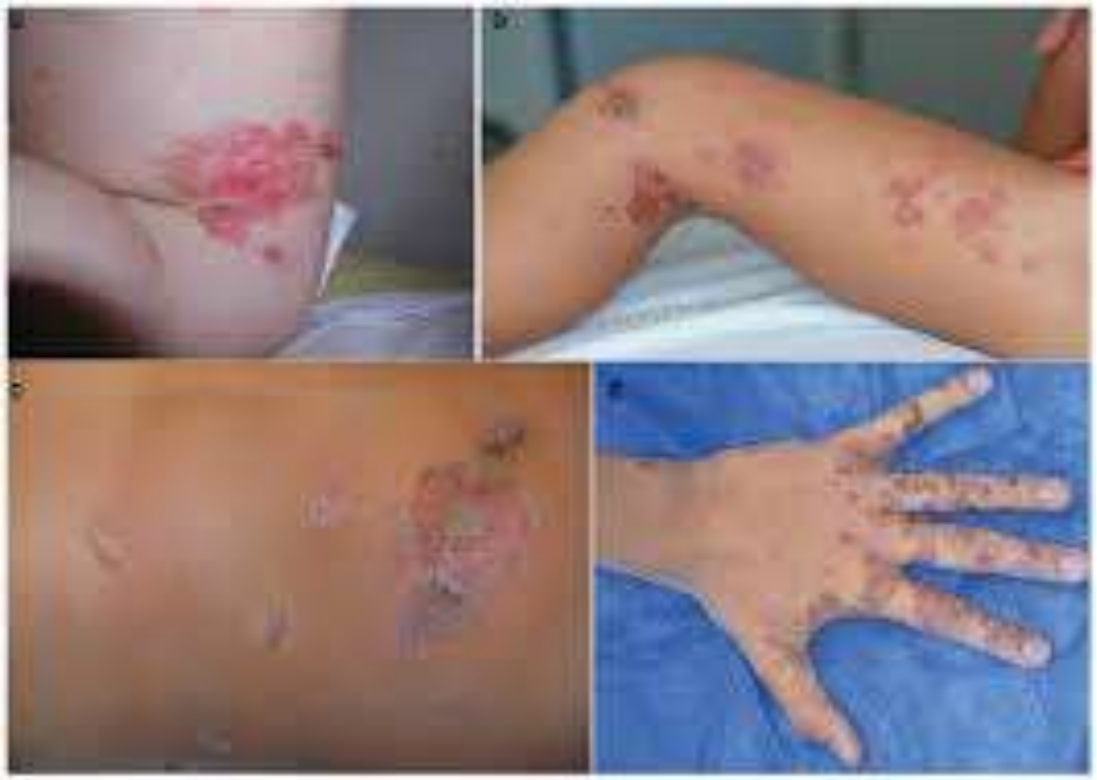
- Virulence factors, pathogenesis, clinical manifestations, lab diagnosis and treatment of *S.aureus* infections.
- Drug resistance in *S.aureus.*
- Coagulase negative staphylococcal infections

Classifications of SKIN AND SOFT TISSUE INFECTIONS (SSTIs)

- SSTIs can arise from invasion of organisms through skin due to breach in the anatomical barrier or from the hematogenous route, secondary to any systemic infection.

- Infection of dermis and epidermis
- Infection of skin appendages such as hair follicle, sebaceous gland, sweat gland and nail
- Infection of fascia (e.g. necrotizing fasciitis)
- Wound infections - surgical site infection, bite wound infection, burn-wound infection and infections of sinuses and fistula
- SSTI due to vascular injury and neuropathy: Diabetic foot ulcer and decubitus ulcer
- Cutaneous manifestations of systemic diseases
- Lymphadenitis and lymphangitis.

STAPHYLOCOCCAL INFECTIONS



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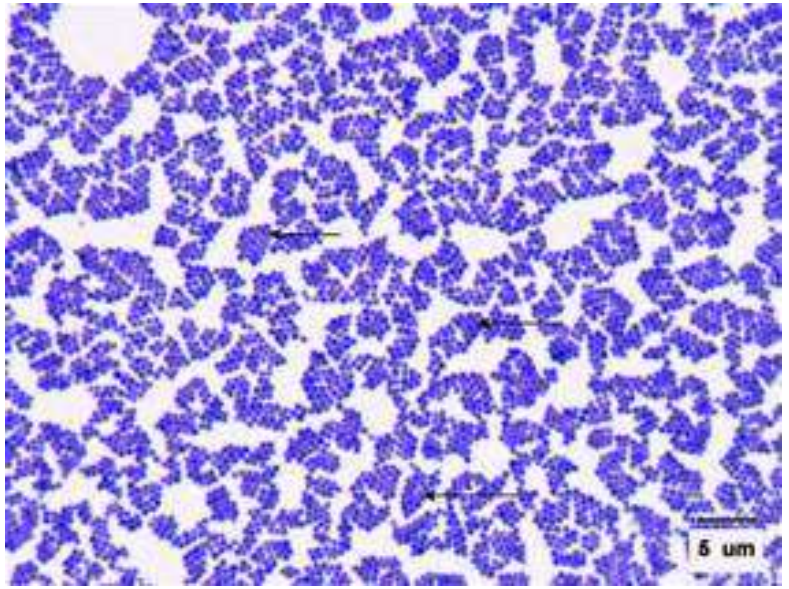
INTRODUCTION

- Gram-positive cocci - classified into two families—
- **Micrococcaceae :**
  - ✓**catalase positive**, gram-positive cocci arranged in tetrads or clusters
  - ✓*Micrococcus* and *Staphylococcus*.
- **Streptococcaceae:**
  - ✓catalase negative,
  - ✓gram-positive cocci, arranged in pairs or chains.

- Family Micrococcaceae comprises of genera— *Micrococcus* and *Staphylococcus*.
- *Micrococcus* species - skin commensals - not associated with human infections. They are 1–1.8 µm size, arranged in tetrads

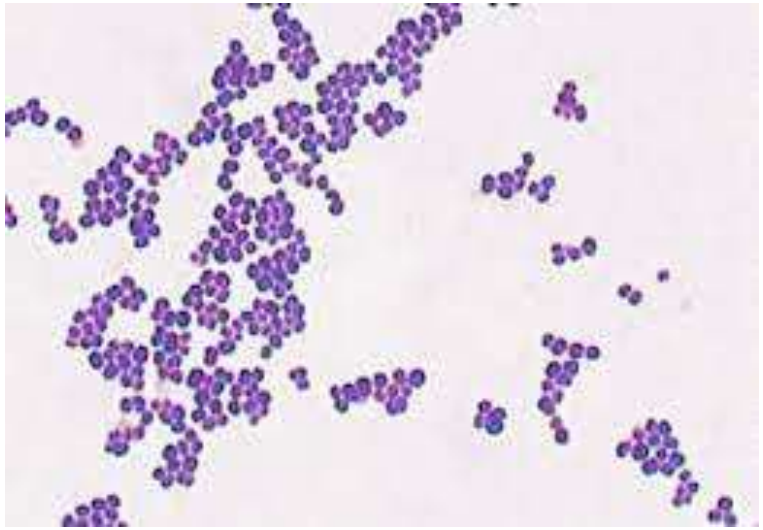
Staphylococcus

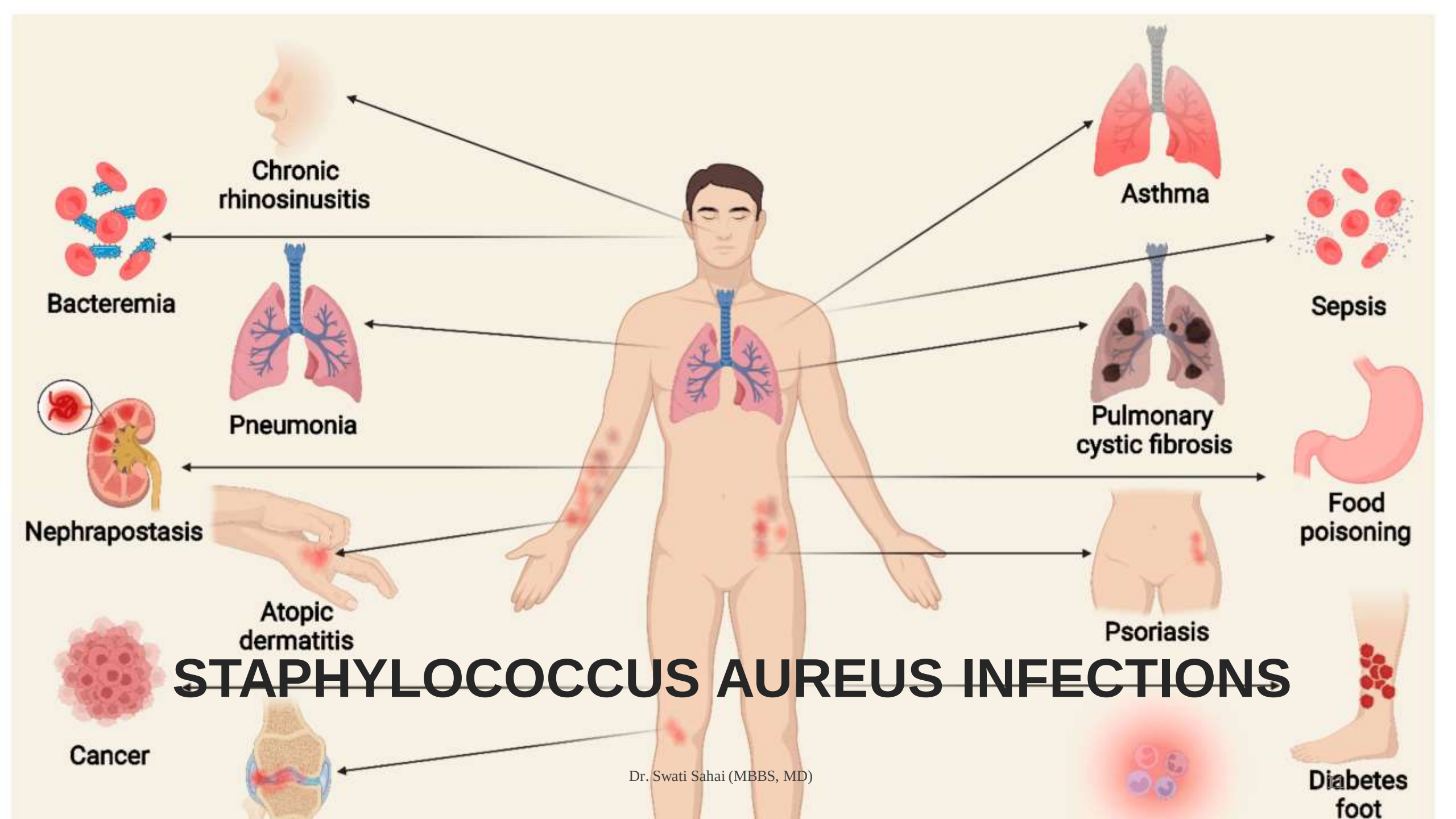
- *Staphylococcus* species - arranged in clusters
- *S. aureus* - **most pathogenic species** - produces an enzyme coagulase - forms the basis of coagulase test
- Other species do not produce coagulase: coagulase-negative staphylococci (CoNS) - rarely pathogenic to man



History

- *Staphylococcus* - named after the Greek word, *Staphyle* means ‘a bunch of grapes’ and *kokkos* means ‘berry shaped’; by Sir Alexander Ogston (1880).
- Rosenbach (1884) named the species *S. aureus*—based on the characteristic golden yellow pigmentation of its colonies.





STAPHYLOCOCCUS AUREUS INFECTIONS

STAPHYLOCOCCUS AUREUS INFECTIONS

- *Staphylococcus aureus* - catalase positive, coagulase positive, facultative anaerobe, non-motile, non-sporing and occasionally capsulated.
- Spherical cocci -1 µm in diameter.
- Arranged in grape-like clusters – due to cell-division in *S. aureus* - occurs in multiple planes with daughter cells remain attached together

- Produces golden yellow pigmentation on nutrient agar and β-hemolytic colonies on blood agar
- Most virulent species among staphylococci; produces infections- range from localized pyogenic infections to life-threatening systemic infections in man

Virulence Factors

Table 51.1: Virulence factors of *Staphylococcus aureus.*
Cell wall associated factors
Peptidoglycan Teichoic acid Cell surface adhesins, e.g. clumping factor Protein A
Toxins
Membrane active toxins • Hemolysins—α, β, γ, δ • Leukocidin (or panton valentine toxin)
Epidermolytic toxin (exfoliative toxin) Enterotoxins Toxic shock syndrome toxin
Extracellular enzymes
Coagulase Heat stable thermonuclease Deoxyribonuclease Staphylokinase (fibrinolysin) Others—hyaluronidase, lipase, and protease

CELL WALL ASSOCIATED FACTORS

- **Peptidoglycan:** Similar to other gram-positive bacteria, the peptidoglycan layer of *Staphylococcus* is thicker.
  - ✓ It confers rigidity to the cell wall and maintains the shape
  - ✓ It induces inflammatory response and also has endotoxin-like activity.
- **Teichoic acid:** It is made up of ribitol phosphate polymers, helps in adhesion of cocci to mucosal surfaces and inhibits opsonization.

- **Cell surface adhesins** are as follows:
  - ✓ Clumping factor/bound coagulase—it is a fibrinogen binding adhesin; responsible for slide coagulase reaction
  - ✓ Fibronectin binding adhesin
  - ✓ Collagen-binding adhesin.

- **Protein A (SpA):**
  - ✓has many **biological properties**, such as anticomplementary, chemotactic, mitogenic, inhibition of opsonization and induction of platelet damage
  - ✓**Mediates coagglutination reaction:** Protein A binds to Fc region of any IgG antibody, leaving Fab region free which binds to the corresponding antigen present in clinical samples. This test is obsolete now.
- **Microcapsule:**
  - ✓Some strains of *S. aureus* have polysaccharide microcapsule, which inhibits phagocytosis by neutrophils.
  - ✓The capsular polysaccharides are zwitterionic, i.e. they have both negative and positive charges, which is a feature that is critical for abscess formation

TOXINS

❖**<u>Membrane Active Toxins</u>**

- *Hemolysins*
- *Leukocidins/Panton Valentine Toxin*
- Epidermolytic/Exfoliative Toxin (ET)
- Enterotoxin: pre-formed toxin
- Toxic Shock Syndrome Toxin (TSST)

αhemolysin	Inactivated at 70°C reactivated at 100°C Lethal, leucocidal, dermonecrotic, cytotoxic and neurotoxic Lyses rabbit RBCs, but less active against sheep and human RBCs.
βhemolysin	Sphingomyelinase Lyses sheep RBC, but not human or rabbit RBC Exhibits hot-cold phenomenon
γhemolysin	Act together with leucocidin for hemolytic activity. Lyses rabbit, sheep and human RBCs
δhemolysin	Surfactant action Lyses rabbit, sheep, horse & human RBCs Lethal, leucocidal and dermonecrotic

Toxins	
Membrane active toxins • Hemolysins-α,β,γ,δ	
• Leucocidin/Panton valentine toxin (PVL)	F & S toxins + γ hemolysin →hemolytic and leucocidal (Synergohymenotropy) Expressed on MRSA
Epidermolytic toxin (exfoliative toxin)	Staphylococcal scalded-skin syndrome
Enterotoxins	Serotype A- MC, Superantigen Self limiting food poisoning IP<6 Hrs Preformed toxin →vagus stimulation
Toxic shock syndrome toxin	Super antigen Risk - abscesses, osteomyelitis, post-surgical wound infection

Toxic Shock Syndrome Toxin (TSST)

- **Risk factors:**
  - ✓reported from women using highly absorbent vaginal tampons.
  - ✓As a complictaion of staphylococcal abscess, osteomyelitis, and post-surgical, traumatic or burn wound infections

Pathogenesis

TSST-1 gets absorbed into circulation

↓

Being a superantigen, it stimulates the T-cells non-specifically

↓

Excessive production of cytokines

↓

Fatal multisystem disease

Clinical features

- Fever
- Hypotension
- Mucosal hyperemia
- Vomiting
- Diarhhoea
- Confusion
- Myalgia
- Abdominal pain
- Erythematous rashes
- Rapid involvement: liver, kidney, lungs, GIT, CNS

Case definition

- **Clinical criteria:**
  - ✓Fever
  - ✓Rash
  - ✓Desquamation
  - ✓Hypotension
  - ✓Multiorgan involvement (3 or more)
- **Laboratory criteria:**
  - ✓Negative blood culture for other pathogens
  - ✓Lack of evidence of other causes of infections

Treatment

- As toxin causes capillary leak; aggressive parenteral fluid replacement and vasopressor to reverse hypotension - initiated at the earliest
- Examine for and remove any colonized foreign body, e.g. vaginal tampon
- **Clindamycin** - drug for TSS (as it reduces the toxin synthesis).
- It is given along with anti-staphylococcal penicillin (e.g. cloxacillin) for MSSA or vancomycin for MRSA
- For clindamycin resistant cases: Linezolid - used for toxin supression.

EXTRACELLULAR ENZYMES

- Coagulase
- Other Enzymes
  - ✓Heat stable thermonucleases and DNase (deoxyribonuclease) : specific to *S. aureus*; not produced by any other staphylococcal species
  - ✓Staphylokinase (fibrinolysin) breaks down fibrin clots and may facilitate the spread of infection
  - ✓Hyaluronidase breaks down the connective tissue network
  - ✓Lipases and phospholipases breakdown the lipids

Pathogenesis

- **Colonization-**anterior nares, axilla and perineal skin
- **Introduction into the tissue -** minor abrasions or instrumentation→ adhere to tissue
- **Invasion**- with help of enzymes

- **Evasion of host defence mechanisms**
  - ➢Anti-phagocytic -microcapsule and Protein A
  - ➢Inhibition of leukocyte migration
  - ➢Intracellular survival -formation of small colony variants
- **Metastatic spread** - *S. aureus* spreads to various distant sites by hematogenous spread

Clinical Manifestations

Skin and soft tissue infections

S. aureus is one of the most common cause of various skin and soft tissue infections such as:

- **Folliculitis:** Infection of the hair follicle, characterized by a pus point surrounded by induration and erythema (Fig. 51.1A)
- **Furuncle (boil):** Painful pustular lesion in hairy, moist regions due to infection of the hair follicle that extends to become true abscess
- **Carbuncle:** Severe, painful lesion in the lower neck region, extending to the deeper subcutaneous tissue
- **Abscess:** Collection of pus, appears painful and swollen; surrounded by erythema (Fig. 51.1B)
- Mastitis and breast abscess (in nursing mothers)
- **Impetigo:** It mainly occurs in children, usually appears as red sores on the face, that bursts and develops into **honey-colored crusts**
- Surgical site wound infections (most common cause)
- Cellulitis (inflammation of skin and subcutaneous tissue)–usually develops around a wound or an ulcer
- **Hidradenitis suppurativa:** A recurrent follicular infection in areas rich in apocrine glands, such as the axilla
- **Botryomycosis:** It is mycetoma-like condition, characterized by subcutaneous swelling, sinuses, and discharge containing granules (Chapter 58)

Note:

- The pustular lesions of *S. aureus* infection usually heals quickly when the pus is drained. The fibrinous wall around the abscess tend to prevent spread of the organisms and therefore, care should be taken not to break the fibrinous wall by manipulation or trauma
- Predisposing factors to *S. aureus* cutaneous infections are—chronic skin conditions (e.g. eczema), skin damage (trauma, injections) or poor personal hygiene

Musculoskeletal infections

S. aureus is the most common cause of various conditions such as:

- Septic arthritis (most commonly involved joints are knees, shoulders, hips, and phalanges)
- Osteomyelitis (most commonly affected site in children is long bones and in adults is vertebrae)
- **Pyomyositis** (skeletal muscle infection): Mainly seen in tropical climates, but also occurs in HIV-infected patients
- **Abscess:** Psoas abscess and epidural abscess

Respiratory tract infections

- Ventilator-associated pneumonia in adults
- Septic pulmonary emboli
- Postviral pneumonia (e.g. influenza)
- Empyema and pneumothorax
- Pneumatocele (shaggy, thin-walled cavities in lungs) in neonates: *S. aureus* is the most common cause

Bacteremia and its complications

- Sepsis, septic shock
- Central line associated blood stream infection (CLABSI): *S. aureus* and CoNS are the leading causative agents
- Metastatic foci of infection involving kidney, joints, bone and lung
- Infective endocarditis:
  - Native-valve endocarditis (left-sided)—*S. aureus* is the most common cause
  - Prosthetic-valve endocarditis
  - Intravenous drug use associated endocarditis (right sided)—*S. aureus* is the most common cause
  - Nosocomial endocarditis—associated with increased use of central line

UTI (Urinary tract infection)

- Staphylococcal UTI and pyelonephritis usually occur secondary to bacteremia
- Rarely UTI is seen following instrumentation and insertion of catheter or implants

Toxin-mediated illnesses

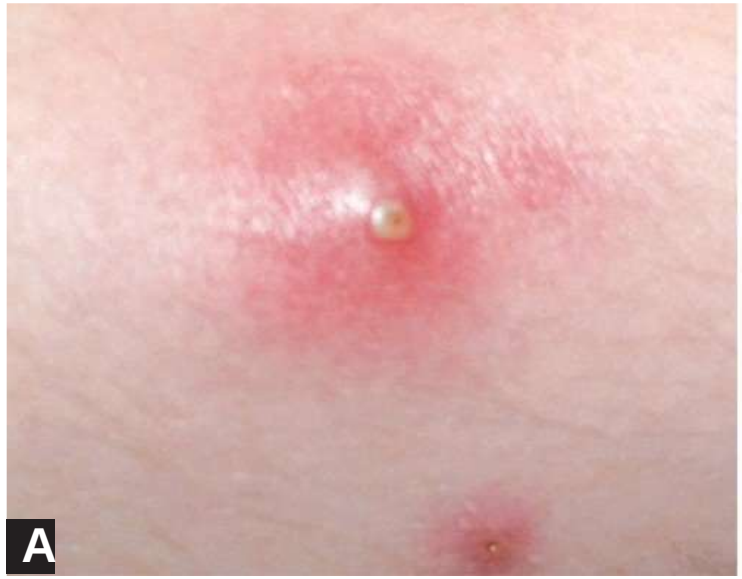
S. aureus causes the following toxin mediated diseases (as described earlier):

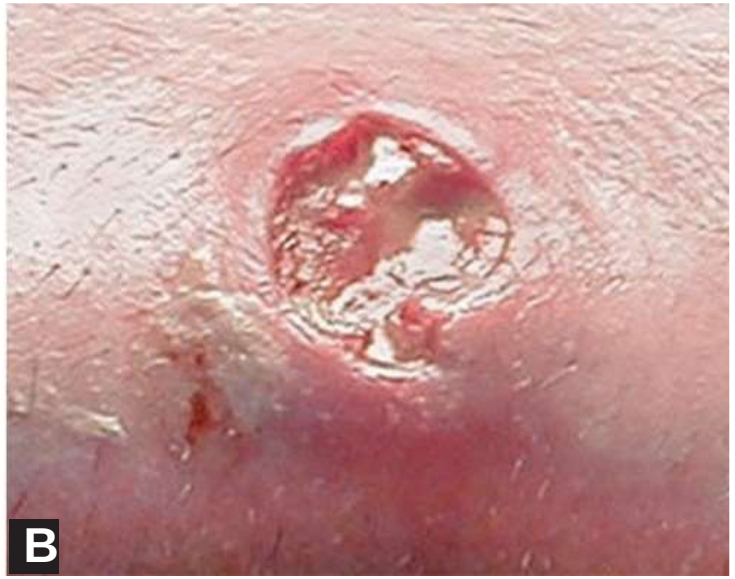
- Toxic shock syndrome
- Food poisoning
- Staphylococcal scalded-skin syndrome

Infections associated with CA-MRSA (Community associated methicillin-resistant *Staphylococcus aureus*)

Skin and soft tissues are the most common sites for colonization of CA-MRSA strains; about 5–10% of strains are invasive and can be life-threatening. Examples include:

- Necrotizing pneumonia
- Sepsis with Waterhouse-Friderichsen syndrome or purpura fulminans (*S. aureus* is rare cause; most commonly caused by meningococcus).
- Necrotizing fasciitis (*S. aureus* and *Streptococcus pyogenes* are the common causative agents)





A. Staphylococcal folliculitis; **B.** Staphylococcal abscess (ruptured).

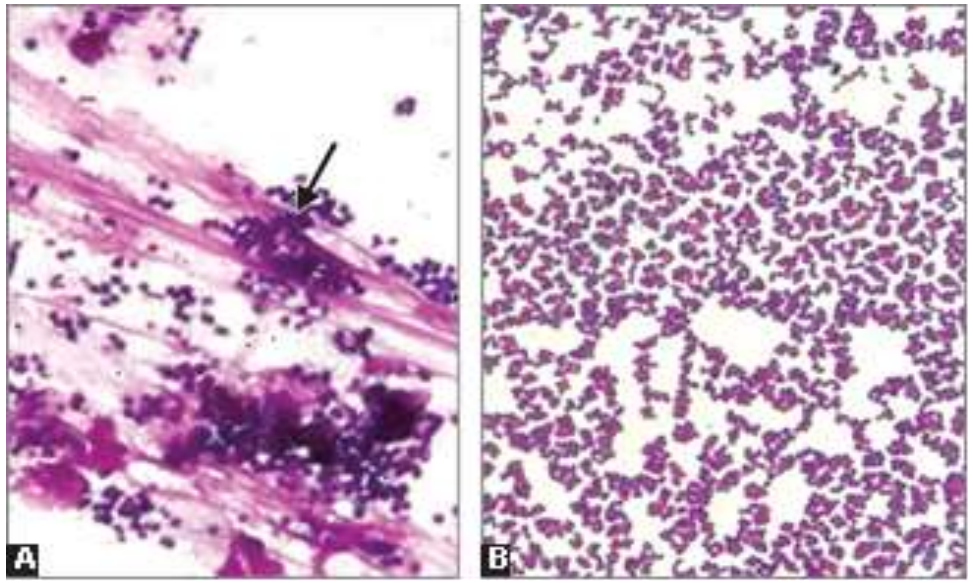
Epidemiology

- *Staphylococcus aureus* - part of normal human flora - 20–40% of healthy population are carriers of *S. aureus,* colonizing the organism persistently or transiently.
- **Most common site(s)** - anterior nares and oropharynx; followed by skin (abraded), vagina, axilla, and perineum.
- These colonization sites - reservoir for future infections

- Rate of colonization - higher among insulin dependent diabetics, HIV-infected patients, patients undergoing hemodialysis, injection drug users, and individuals with skin damage
- Overall, *S. aureus* - leading cause of healthcare associated infections.

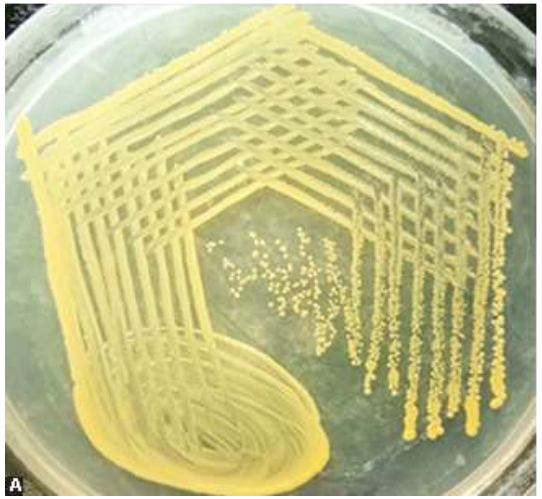
Laboratory diagnosis of S. aureus infections

- **Direct smear microscopy:** Gram-positive cocci in clusters and pus cells

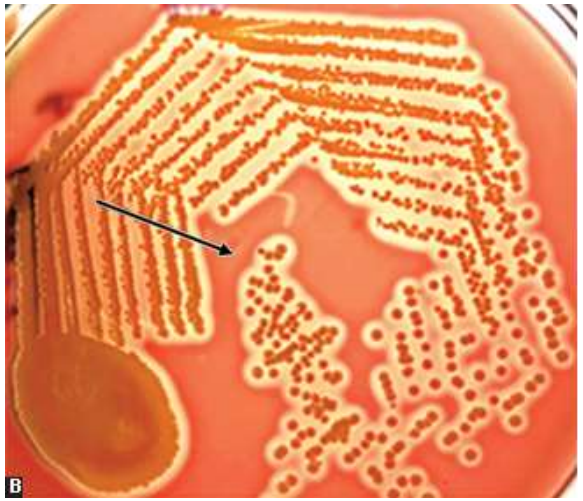


A. Direct smear: arrow showing gram-positive cocci in clusters with pus cells; **B.** Culture smear showing gram-positive cocci in clusters.

- **Culture**
  - ➢ Nutrient agar—golden yellow pigmented colonies
  - ➢ Blood agar—colonies with narrow zone of β-hemolysis
  - ➢ Selective media—such as mannitol salt agar, salt milk agar and Ludlam's medium
- **Culture smear microscopy:** Gram-positive cocci in clusters

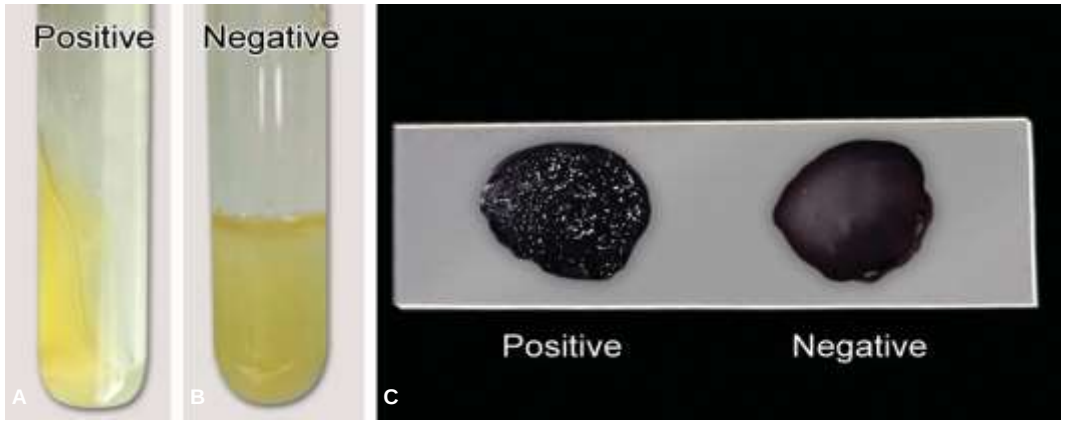


A. Nutrient agar–shows golden-yellow pigmented colonies;



B. Blood agar–arrow shows narrow zone of beta-hemolysis surrounding the colonies

- **Biochemical identification:**
  - ➢Catalase test-positive
  - ➢Coagulase test (slide and tube)—positive
  - ➢Protein A detection
- **Identification by automated systems** such as VITEK and MALDI-TOF
- **Antimicrobial susceptibility testing**



Coagulase test: **A.** Tube coagulase test (positive);
B. Tube coagulase test (negative); **C.** Slide showing coagulase test.

Treatment of *S. aureus* infections

- **Parenteral therapy for serious infections:**

Sensitive to pencillin	**DOC:** Penicillin G
Methicillin sensitive *S. aureus* (MSSA)	**DOC:** Anti-staphylococcal penicillins - nafcillin and cloxacillin *Note:* Vancomycin is inferior in terms of efficacy against MSSA, when compared to anti-staphylococcal penicillins; hence should not be prescribe for MSSA
Methicillin resistant *S. aureus* (MRSA)	**DOC:** Vancomycin (15-20 mg/kg bd) **Alternative drugs:** Discussed under MRSA treatment box

Empirical therapy (MRSA status not yet known):

- Vancomycin with/without an aminoglycoside (vancomycin is indicated only if MRSA risk is high or condition is serious, e.g. cardiac implant).

Oral therapy for skin and soft tissue infections:

Sensitive to methicillin	➢ Dicloxacillin ➢ Cephalexin/cefazolin
Resistant to methicillin (MRSA)	Clindamycin **Alternative drugs:** Cotrimoxazole, doxycycline, Linezolid

Drug Resistance in S. aureus

Production of β-lactamase Enzyme:

- β-lactamase or penicillinase enzymes cleave the β-lactam rings - organisms producing these enzymes develop resistance to β-lactam antibiotics.
- Resistance - **plasmid coded** – transferred between *S. aureus* strains by **transduction -** Produced by >90% of strains of *S. aureus*
- Resistance can be overcome by addition of β-lactamase inhibitors - clavulanic acid or sulbactam.

By Alteration of Penicillin-binding Protein (PBP):

- It is shown by MRSA strains of *S. aureus*.

Methicillin-resistant Staphylococcus aureus (MRSA)

- Methicillin resistance in *S. aureus* - mediated by a chromosomally coded gene called ***mecA gene***.
- Alters penicillin-binding protein (PBP) present on *S. aureus* cell membrane to PBP2a.
- PBP - essential protein needed for cell wall synthesis of bacteria.
- β-lactam drugs bind and inhibit this protein - inhibiting the cell wall synthesis

- The altered PBP2a of MRSA strains - less affinity for β-lactam antibiotics - MRSA strains are resistant to all β-lactam antibiotics
- **Risk factors:** poor hygienic conditions, close contact, contaminated material, and individuals with damaged skin
- **Types of MRSA:**
  - ✓Community acquired MRSA,
  - ✓Hospital Acquired MRSA

Types of MRSA	
Community-associated MRSA (CA-MRSA)	**Hospital-associated MRSA (HA-MRSA)**
These strains express *mecA* gene subtype IV, V, VI	These strains express *mecA* gene subtype I, II, III
They are usually more virulent and express several toxins such as Panton Valentine (PV) toxin	They are multidrug resistant (but their virulence is relatively low)
They cause invasive skin and soft tissue infections such as necrotizing fasciitis	They cause perioperative wound infections in hospitals and nosocomial outbreaks (hospital staff are the major carries)

Detection of MRSA:

- Antimicrobial susceptibility test - cefoxitin disk or oxacillin (MIC based method): These antibiotics - surrogate marker for the identification of MRSA
- PCR detecting *mecA* gene
- Latex agglutination test detecting PBP2a.

Treatment of MRSA infections

- Vancomycin - drug of choice for MRSA
- Alternative drugs include—teicoplanin, linezolid, daptomycin, telavancin and quinupristin/dalfopristin
  - ➢Linezolid is useful for skin and soft-tissue infection
  - ➢Daptomycin is indicated for endocarditis and complicated skin infections, not effective for pneumonia

- Even drugs - tetracycline, erythromycin or cotrimoxazole - also be effective in nonlife-threatening infections
- Antimicrobial susceptibility testing - before an alternative drug is used
- All β-lactam drugs should be avoided.
- 5th generation cephalosporins - ceftobiprole, ceftaroline and ceftolozane have shown some activity against MRSA.

Resistance to Vancomycin (VRSA and VISA)

- Erroneous and overuse of vancomycin - lead to the emergence of resistance to vancomycin.
- It may be of low grade resistance - VISA (vancomycin intermediate *S. aureus*) or high-grade resistance - VRSA (vancomycin-resistant *S. aureus*).

- The MIC (minimum inhibitory concentration) of vancomycin to VISA and VRSA isolates are 4–8 and >16 μg/mL respectively.
- The current guidelines recommend consideration of alternative drugs if vancomycin MIC is >1 μg/mL - treatment failure has been frequently observed beyond this MIC level.

- VISA - more frequently reported than VRSA
- **Mechanisms:** VRSA - mediated by *van A* gene , VISA - increase in cell wall thickness of *S. aureus*
- *van A* gene - acquired from a vancomycin-resistant strain of *Enterococcus faecalis* by horizontal conjugal transfer

- **Fitness cost:** Acquisition of a *van* gene - often associated with compensatory mutations in the genes responsible for survival - results in a reduced fitness of *S. aureus*.
- In contrast, ‘fitness cost phenomenon’ - not commonly observed in MRSA.
- **Treatment** of VRSA - based on antimicrobial susceptibility report.
- Linezolid, telavancin, daptomycin and quinupristin/dalfopristin - effective drugs.

Control Measures

- **Screening of MRSA carriers** among hospital staff - done when there is an outbreak. Mannitol oxacillin agar - preferred medium for this purpose.
- **Treatment of carriers** - topical 2% mupirocin (for nasal carriers) and chlorhexidine body bath (for skin carriers)

- **Stoppage of antibiotic misuse** in hospitals
- Ensure proper infection control measures **– hand hygiene** (most efficient way to prevent hospital spread), isolation of the patients and all other measures of contact precautions.

COAGULASE-NEGATIVE STAPHYLOCOCCAL INFECTIONS

- Staph.epidermidis
- Staph.saprophyticus
- Staph.lugdunensis
- **Staphylococcus schleiferi**

Staphylococcus epidermidis

- Most common CoNS (75–80%), isolated from clinical samples.
- It is present as normal flora on the skin, oropharynx and vagina.
- Its pathogenic role is greatly enhanced in presence of prosthetic-devices.

Pathogenesis: *S. epidermidis* involves a two-step process:

1. **Initial adhesion to the prosthetic device:** The surface adhesins of the organism bind to host serum or tissue constituents - fibrinogen or fibronectin, coated on the implanted prosthetic surfaces.

2. **Colonization:** *S. epidermidis* – produce extracellular polysaccharide material (glycocalyx or slime) - facilitates formation of a protective **biofilm** on the device surfaces

- **Manifestation:** *S. epidermidis* - most common cause of prosthetic-device related infections - endocarditis with insertion of valvular prosthesis and ventricular shunt infections.
- Also a common cause of stitch abscess
- Coagulase negative, but positive for phosphatase test.

Staphylococcus saprophyticus

- It causes urinary tract infection (UTI) in sexually active young women

Staphylococcus lugdunensis and Staphylococcus schleiferi

- Recently, these organisms have been associated with more serious infections - native-valve endocarditis and osteomyelitis.
- Their enhanced pathogenesis – due to expression of virulence factors -clumping factor (therefore, exhibit positive slide coagulase test) and lipase, which are usually absent in other CoNS.

THANK YOU