MICROBIOLOGY I

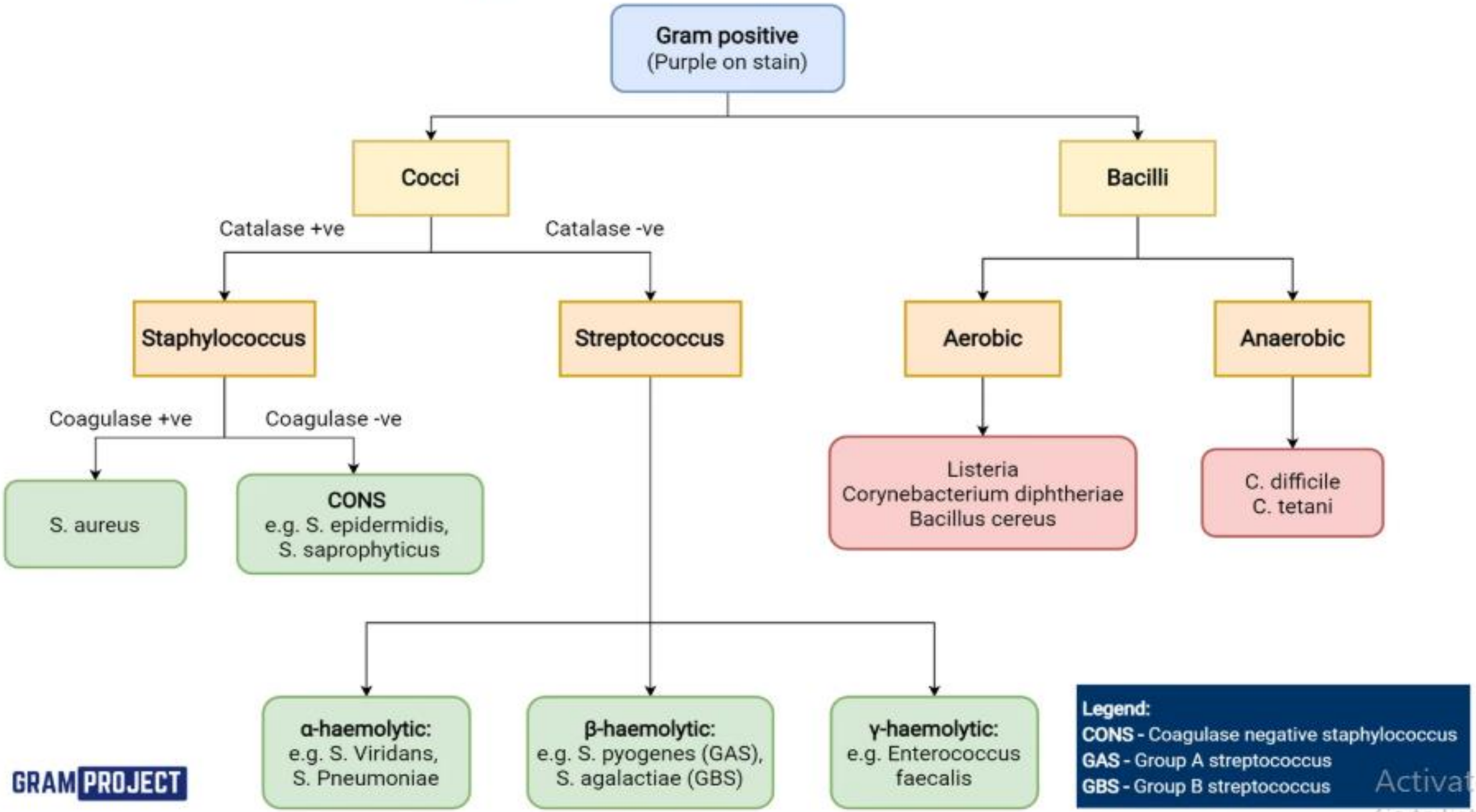
Pharmacy college

2nd stage

Lec 3

Gram Positive Cocci

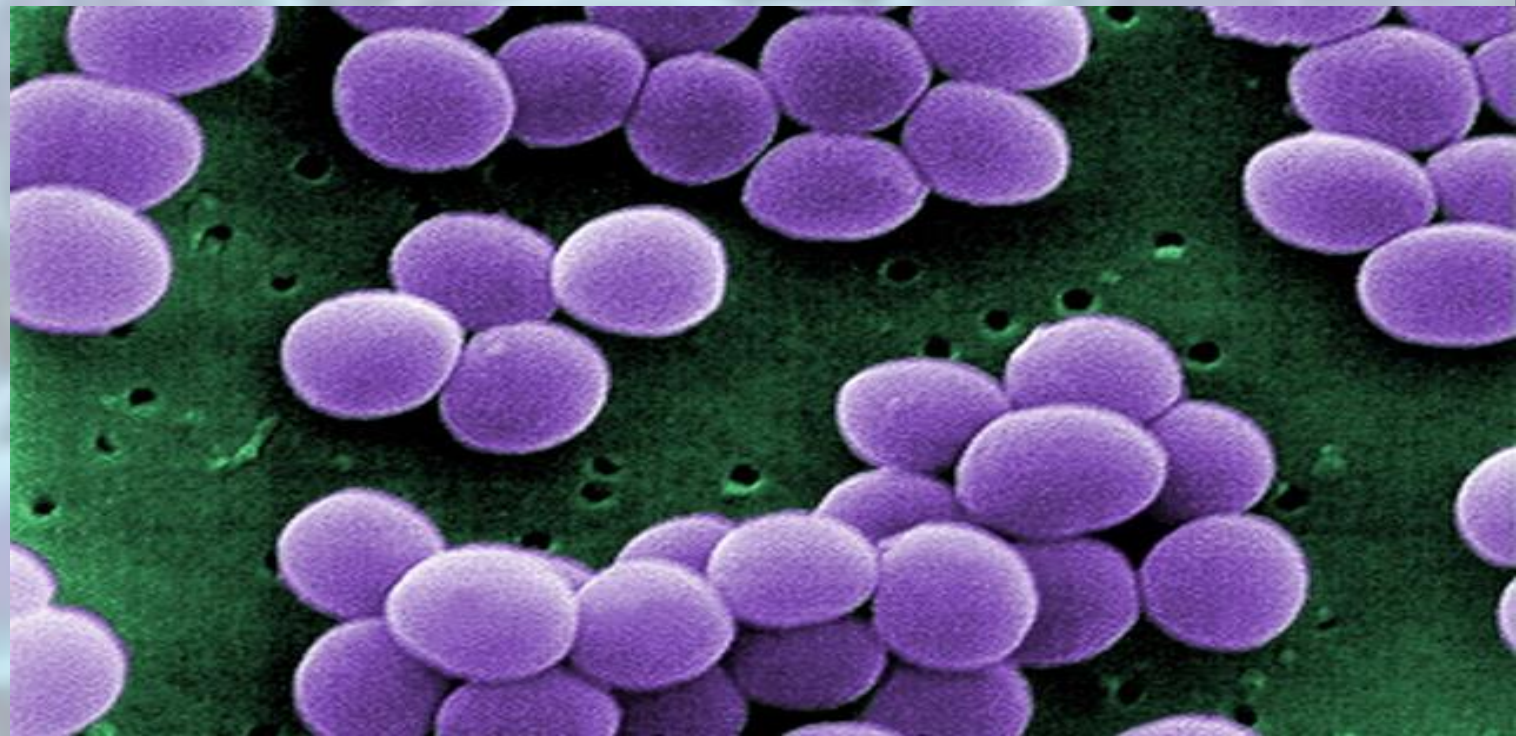
Dr. Noor Hassoon


Gram positive
(Purple on stain)
Cocci
Bacilli
Catalase +ve
Catalase -ve
Staphylococcus
Streptococcus
Aerobic
Anaerobic
Coagulase +ve
Coagulase -ve
S. aureus
CONS
e.g. S. epidermidis,
S. saprophyticus
Listeria
Corynebacterium diphtheriae
Bacillus cereus
C. difficile
C. tetani
α-haemolytic:
e.g. S. Viridans,
S. Pneumoniae
β-haemolytic:
e.g. S. pyogenes (GAS),
S. agalactiae (GBS)
γ-haemolytic:
e.g. Enterococcus
faecalis
Legend:
CONS - Coagulase negative staphylococcus
GAS - Group A streptococcus
GBS - Group B streptococcus
GRAM PROJECT

Staphylococci

Introduction and General Characteristics

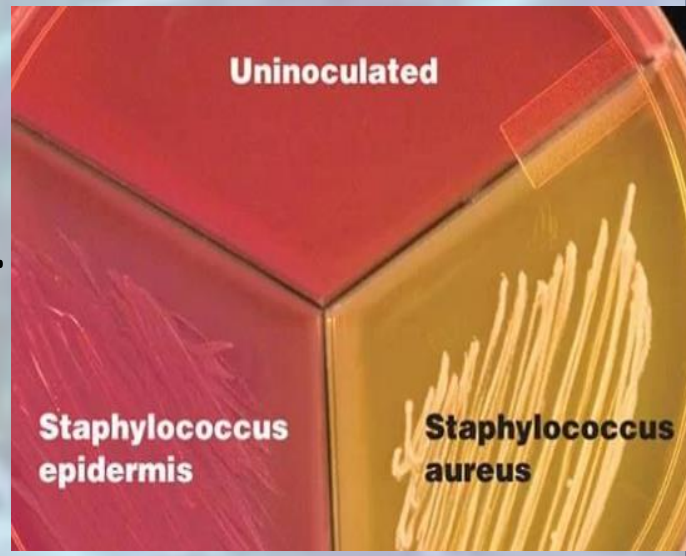
- The genus Staphylococcus comprises gram-positive cocci, stained dark violet .
- In clinical pharmacy and medical microbiology, differentiating these organisms rapidly is essential for establishing early targeted antimicrobial therapy.



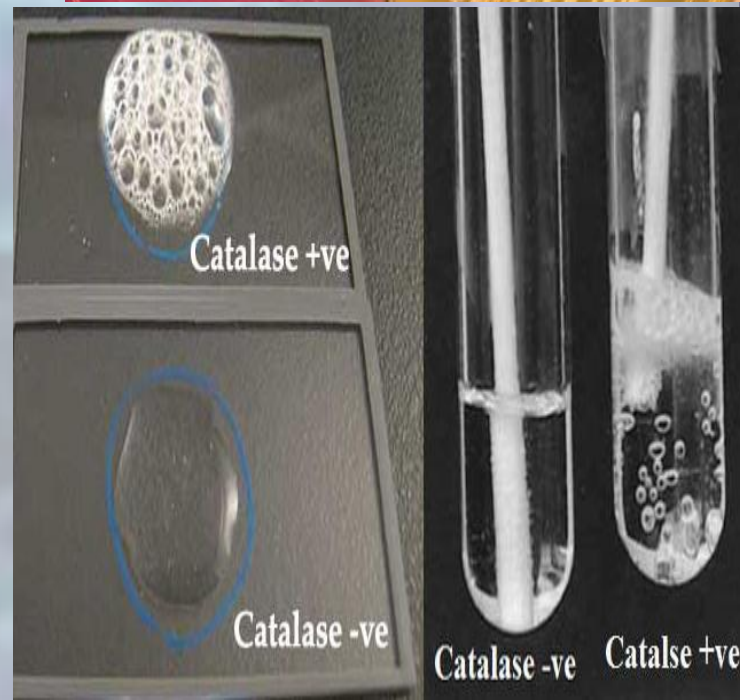
- **Cellular Morphology:**

Spherical cells, they characteristically divide in multiple perpendicular planes, forming irregular, grape-like clusters.

- **Metabolic Profile:**
- Facultative anaerobes, non-motile, and non-spore-forming.
- They are highly halotolerant, capable of growing in media containing up to 10% Sodium Chloride (NaCl), cultured in selective media like Mannitol Salt Agar (MSA).



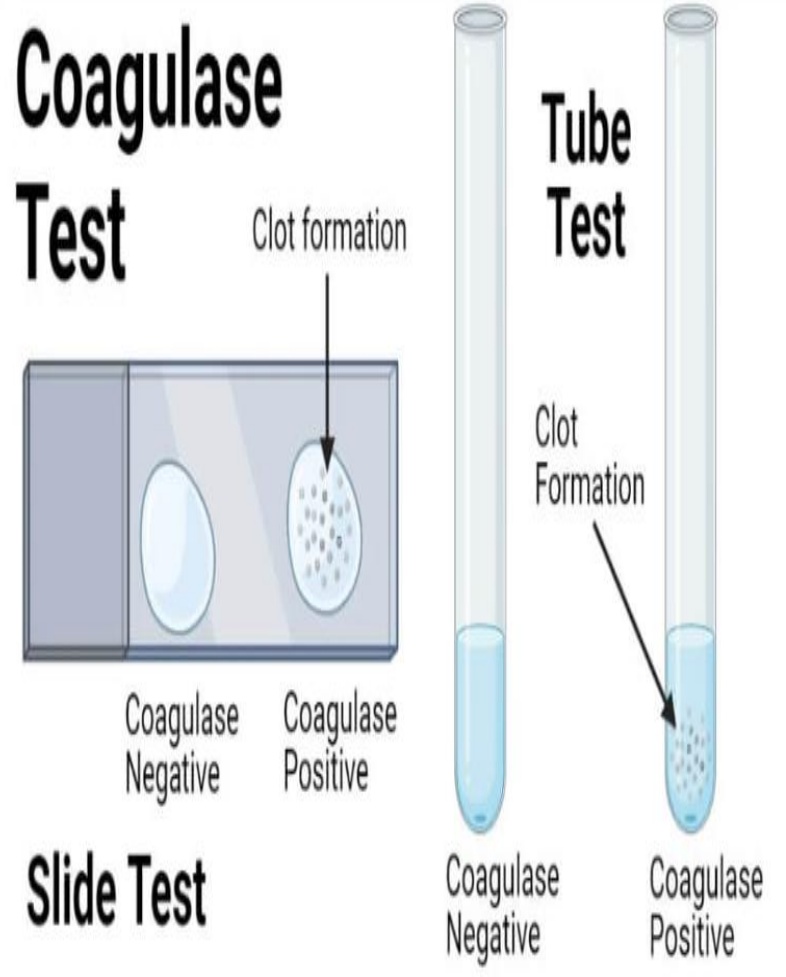
- **The Catalase Test:**
- **All** members of the genus produce the enzyme catalase, which catalyzes the breakdown of Hydrogen Peroxide into water and oxygen.
- This differentiates them from the catalase-negative Streptococcus genus.



Phenotypic Differentiation: CoPS vs. CoNS

From a clinical and diagnostic standpoint, the genus is divided by the presence or absence of the extracellular enzyme coagulase, which activates prothrombin to form fibrin clots.

Property / Species	Staphylococcus aureus (CoPS)	Staphylococcus epidermidis (CoNS)	Staphylococcus saprophyticus (CoNS)
Coagulase Production	Positive	Negative	Negative
Mannitol Fermentation	Positive (Turns MSA Yellow)	Negative (Stays Pink)	Variable (Usually Negative)
Novobiocin Susceptibility	Susceptible	Susceptible	Resistant
Primary Clinical Niche	Anterior nares, skin, pathological abscesses	Normal skin flora, medical devices, prosthetic hardware	Female periurethral tract, urinary tract flora



Staphylococcus aureus (Pyogenes)

- The most virulent species of staphylococcus is *S. aureus*, almost all isolates of which secrete coagulase.
- **Virulence Factors**

A. Cell-Wall Associated Structural Components

- Protein A: This prevents opsonization and subsequent phagocytosis by neutrophils.
- Fibronectin-Binding Proteins (FnBPA/B): Mediate bacterial adherence to host extracellular matrix components, facilitating tissue colonization.

B. Extracellular Enzymes (Tissue Degradation)

- Coagulase: Converts host fibrinogen to fibrin, coating the bacterial cell to evade immune detection.
- Hyaluronidase: Hydrolyzes hyaluronic acid in the host extracellular matrix, serving as a 'spreading factor' through tissues.
- Beta-Lactamase (Penicillinase): Secreted plasmid-encoded enzyme that hydrolyzes the beta-lactam ring of conventional penicillins, rendering them inactive (penicillins resistant).

C. Toxins and Super antigens

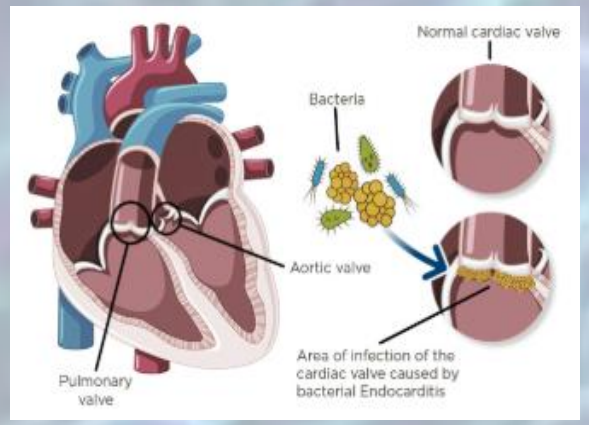
- Alpha-Hemolysin (alpha-toxin): Creates transmembrane pores in host cell membranes, causing osmotic lysis of erythrocytes and leukocytes.
- Toxic Shock Syndrome Toxin-1 (TSST-1): A potent superantigen that cross-links MHC Class II molecules on antigen-presenting cells directly to T-cell receptors without processing. This induces a massive, non-specific release of cytokines (Cytokine Storm), resulting in fever, hypotension, and multi-organ failure.
- Staphylococcal Enterotoxins (A-E): Heat-stable and acid-resistant toxins responsible for acute staphylococcal food poisoning via vagus nerve stimulation, its considered a superantigen also induces cytokine storm.

Clinical Infections:



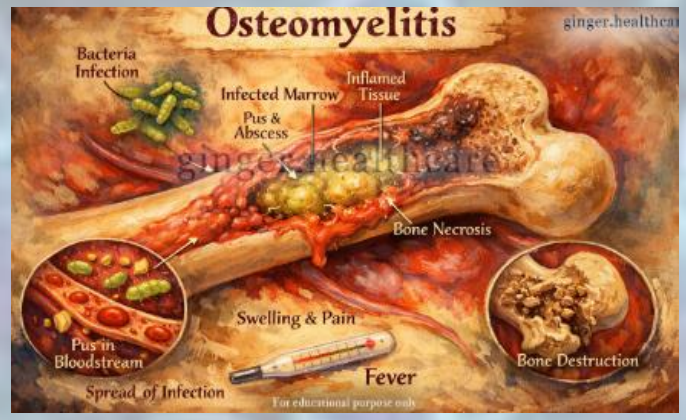
- Suppurative:

✓Deep skin abscesses (carbuncles).



✓Acute infective endocarditis (destroys healthy valves).

✓Osteomyelitis.





✓Necrotizing pneumonia.

- Toxin-Mediated:

✓Toxic Shock Syndrome (TSS).

✓Staphylococcal Scalded Skin Syndrome (SSSS).



✓Rapid food poisoning (2–6 hours).

Staphylococcus epidermidis (The Biofilm Specialist)

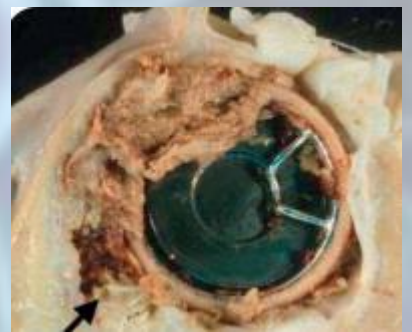
- **Key Virulence Factor:**
- Extracellular Biofilm Matrix:
- Facilitated by the ica operon.
- Anchors bacteria to synthetic plastics, creating a physical shield that blocks neutrophils and antibiotic penetration.

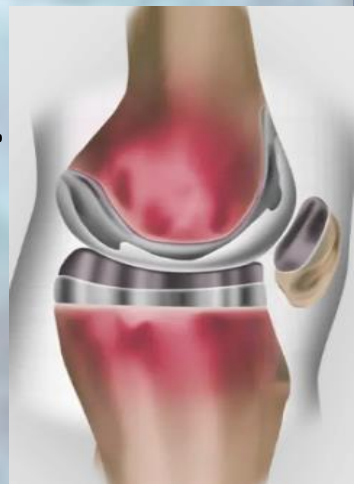
- **Clinical Infections:**
- Foreign-body infections:

✓Central venous catheter-related bloodstream infections (CRBSIs).

✓Prosthetic heart valve endocarditis.

✓Artificial joint/hardware infections.





Staphylococcus saprophyticus (The Uropathogen)

Key Virulence Factors:

- Surface Adhesions:

Proteins displaying specific binding affinity for human uroepithelial cells.

Clinical Infections:

✓Uncomplicated Urinary Tract Infections (UTIs): Known as **'honeymoon cystitis'**, the second most common cause of community-acquired UTIs in young, sexually active females.



Resistance Mechanisms

- Beta-Lactam Resistance (MRSA & MRSE) Mechanism:

Acquirement of the mecA gene, which encodes an altered Penicillin-Binding Protein known as PBP2a.

Impact: PBP2a has a profoundly low affinity for almost all standard β-lactams (penicillins, cephalosporins, carbapenems), rendering them ineffective.

- Glycopeptide Resistance (VISA & VRSA)

VISA: Driven by cell wall thickening, which traps vancomycin molecules before they reach the cytoplasmic membrane.

VRSA: Triggered by horizontal gene transfer of the vanA gene from Enterococci. This alters the terminal peptidoglycan cell wall target from D-Ala-D-Ala to D-Ala-D-Lac, preventing vancomycin binding.

Pharmacotherapy

1. MSSA (Methicillin-Susceptible): Anti-staphylococcal penicillins (Nafcillin, Oxacillin) or 1st-generation cephalosporins (Cefazolin).

2. MRSA / MRSE: Vancomycin , Linezolid , or Daptomycin.

3. *S. saprophyticus* (UTI): Nitrofurantoin, Trimethoprim-Sulfamethoxazole (TMP-SMX), or Fluoroquinolones.

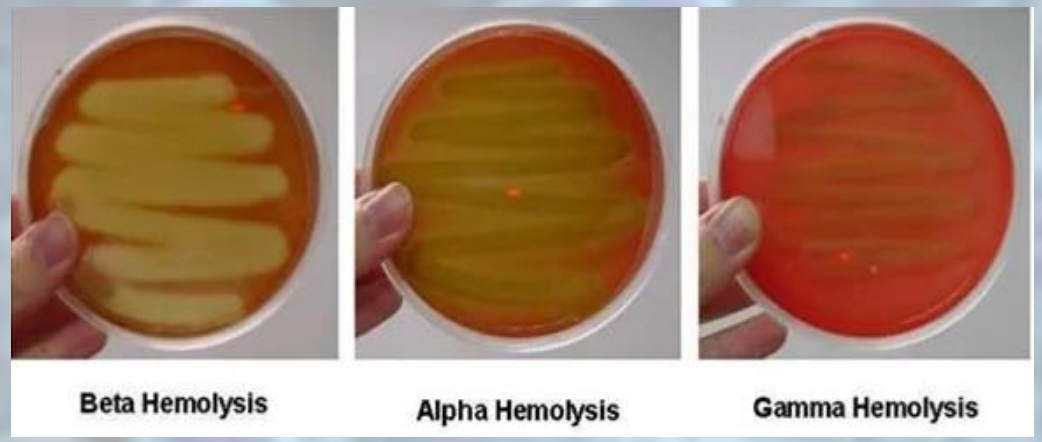
Streptococcus

- The genus Streptococcus comprises a diverse group of Gram-positive, catalase-negative cocci that characteristically arrange in pairs or chains.

Streptococcus have two overlapping **classification** systems:

1- Hemolytic Patterns on Blood Agar: Differentiates species via their capacity to lyse erythrocytes into:

- Alpha (partially hemolytic / green).
- Beta (completely hemolytic / clear zones).
- Gamma (non-hemolytic) phenotypes.



- **Lancefield Antigenic Grouping:** Subclassifies catalase-negative bacteria based on unique carbohydrate antigens embedded within their cell walls (Groups A through V).



Diagnostic and Clinical Comparison

Feature	*S. pyogenes* (GAS)	*S. agalactiae* (GBS)	*S. pneumoniae*	Viridans Group
Hemolysis	**Beta** (Clear)	**Beta** (Narrow clear)	**Alpha** (Green)	**Alpha / Gamma**
Lancefield / Key Marker	Group A **Bacitracin: Susceptible**	Group B **CAMP Test: Positive**	Non-groupable **Optochin: Susceptible**	Non-groupable **Optochin: Resistant**
Primary Niche	Skin, pharynx	Female genitourinary, GI tract	Nasopharynx	Oral cavity (dental plaque)
Key Virulence Factors	**M Protein**, Streptolysin O/S, Pyrogenic Exotoxins (SpeA/C)	Polysaccharide Capsule, Beta-hemolysin	**Polysaccharide Capsule**, Pneumolysin, IgA protease	Extracellular dextrans (biofilm synthesis)
Major Clinical Infections	Strep throat, Scarlet fever, Necrotizing fasciitis, Rheumatic fever	Neonatal sepsis, Neonatal meningitis, Endometritis	Community-Acquired Pneumonia, Otitis media, Meningitis	Dental caries, **Subacute Infective Endocarditis**
First-Line Formulary	**Penicillin V / G** or Amoxicillin	**Penicillin G** or Ampicillin (intrapartum)	Amoxicillin (high-dose); Ceftriaxone for systemic	Penicillin G ± Aminoglycoside

Pathogenicity Profiles

- ***Streptococcus pyogenes* (The Aggressive Pyogenic)**
- M Protein: The **primary virulence factor**. Extends from the cell wall to cleave C3b, preventing complement activation and phagocytic engulfment.
- Streptolysin O & S: Toxins that form structural pores in host cell membranes.
- Pyrogenic Exotoxins (SpeA, SpeC): Function as super-antigens to provoke massive, systemic cytokine release.
- **Clinical Diseases:** Suppurative infections include: Pharyngitis (Strep throat), Impetigo, Scarlet fever, and Necrotizing fasciitis.
- Post-streptococcal sequelae include: Acute Rheumatic Fever (ARF) and Acute Post-Streptococcal Glomerulonephritis (APSGN).

Streptococcus agalactiae (The Neonatal Threat)

Main virulence factor

- Capsular Shield: Utilizes a sialic-acid-rich polysaccharide capsule that suppresses host protective immunity.

Clinical Diseases:

- Asymptomatically colonizes 15-30% of healthy maternal urogenital tracts.
- Vertical transmission during delivery can lead to high-mortality (neonatal sepsis, pneumonia, and meningitis).

Streptococcus pneumoniae (The Encapsulated Diplococcus)

- **Virulence factors**

Polysaccharide Capsule: Highly antigenic and completely antiphagocytic. Loss of the capsule results in complete avirulence.

- Pneumolysin: An intracellular cytotoxin released upon bacterial autolysis that binds cholesterol, boring structural pores into ciliated respiratory epithelium.

Clinical Diseases:

The leading global cause of community-acquired pneumonia (CAP), acute bacterial meningitis, otitis media, and bacteremia.

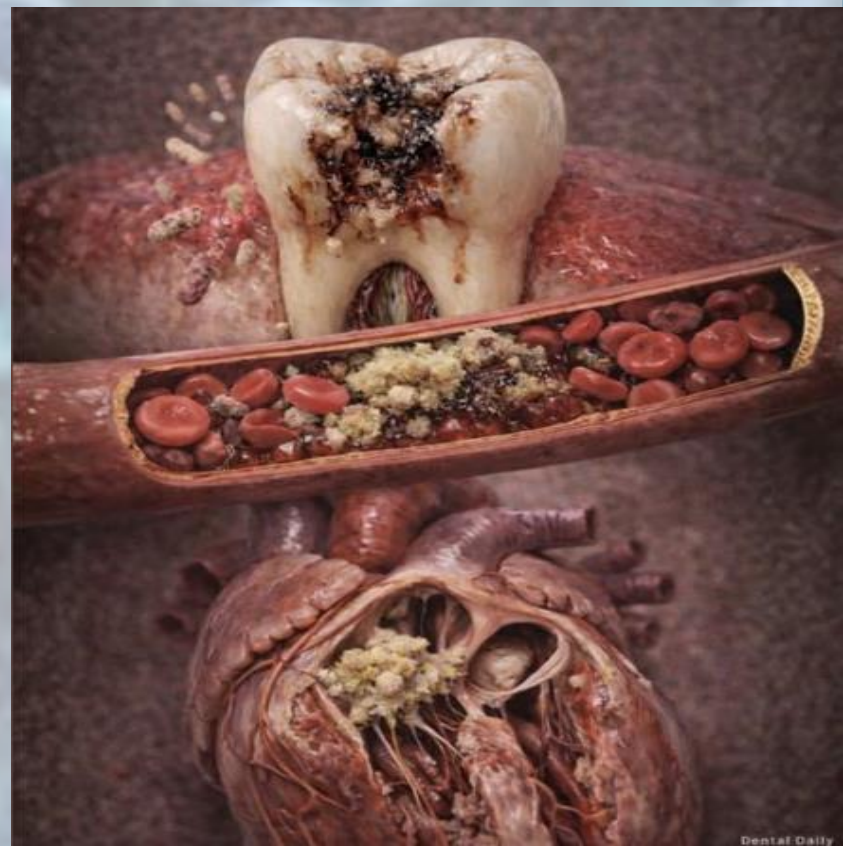
Viridans Group Streptococci (The Commensal Opportunist)

Main virulence factor

Dextran Production: Synthesizes extracellular polysaccharides (dextrans) from dietary sucrose, allowing firm adherence to dental enamel and damaged heart valves.

Clinical Diseases:

- Direct cause of dental caries (*S. mutans*).
- Following dental procedures, can enter the bloodstream and cause subacute infective endocarditis on pre-damaged or prosthetic valves.



Resistance Mechanisms & Pharmacotherapy

Unlike the staphylococci, the genus Streptococcus has remained remarkably unchanging in its therapeutic management, though critical pharmacodynamic nuances exist:

Beta-Lactam Status:

- GAS and GBS: Retain 100% universal susceptibility to Penicillin G and Amoxicillin. Penicillin remains the golden standard.
- S. pneumoniae: Displays (**altered target resistance**) via mutations in chromosomal genes encoding Penicillin-Binding Proteins (PBPs). This reduces binding affinity for beta-lactams but can often be overcome by rising doses (e.g., high-dose Amoxicillin or Ceftriaxone), unlike the complete failure seen in MRSA.

- **Macrolide Resistance:**
- For penicillin-allergic patients, macrolides (Azithromycin, Clarithromycin) are common alternatives. However, resistance is growing rapidly through two primary mechanisms:

1- Target Modification (MLS_B phenotype): Encoded by the erm gene, which methylates the 23S rRNA component of the 50S ribosomal subunit, completely blocking macrolide binding.

2- Efflux Pumps (M phenotype): Encoded by the mef gene, which actively pumps the drug out of the bacterial cytoplasm.


Thank
you!