

Musculoskeletal Module

Practical Microbiology Session

2025-2026

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Outlines

- Link common bacterial SSTI clinical conditions to their causative organisms
- Describe appropriate specimen collection methods for SSTIs
- Interpret Gram stain findings from SSTI specimens
- Identify culture characteristics and biochemical tests of
 - *Staphylococcus aureus*
 - *Streptococcus pyogenes*
 - *Pseudomonas aeruginosa*

Clinical-Lab Bridge - Common Pathogens in Bacterial SSTIs

Clinical Condition	Primary Organism(s)
Folliculitis	<i>S. aureus</i> / <i>Pseudomonas aeruginosa</i>
Furuncle / Carbuncle	<i>S. aureus</i>
Impetigo (non-bullous)	<i>S. aureus</i> , <i>S. pyogenes</i>
Impetigo (bullous)	<i>S. aureus</i> (exfoliative toxin)
Erysipelas	<i>S. pyogenes</i> (Group A Strep)
Cellulitis	<i>S. aureus</i> , <i>S. pyogenes</i>
Necrotizing Fasciitis Type I	Polymicrobial (anaerobes + aerobes)
Necrotizing Fasciitis Type II	<i>S. pyogenes</i>

Specimen Collection for SSTIs

- **Purulent infection:**
 - **Superficial infections** (Folliculitis, Impetigo):
 - Swab from the base of the lesion after removing crust/pus
 - **Deep/loculated infections** (Furuncle, Carbuncle, Abscess):
 - **Aspirate pus** using needle and syringe (preferred over swab)
 - Swab is acceptable only after incision and drainage
- **Non-Purulent infections** (Cellulitis, Erysipelas):
 - Swab is **often unhelpful** (low yield)
- **Necrotizing Fasciitis:**
 - Surgical tissue biopsy / deep tissue sample (gold standard)
 - Blood cultures (mandatory — high rate of bacteraemia)

Gram Stain Interpretation

Gram-Positive Cocci in CLUSTERS → *Staphylococcus* species

- Looks like "grape-like" clusters

Gram-Positive Cocci in CHAINS → *Streptococcus* species

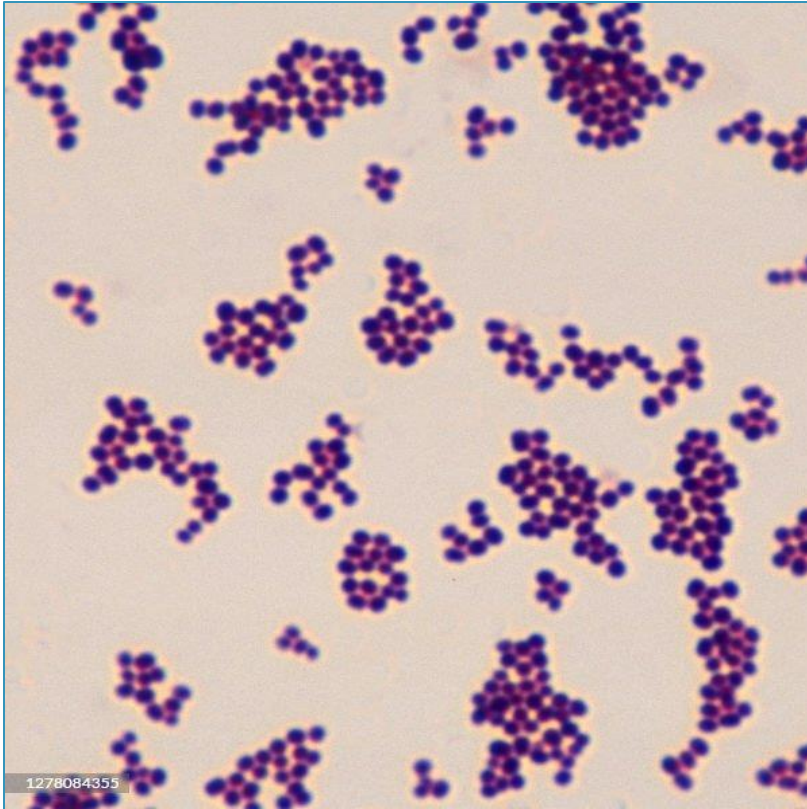
Mixed flora → Polymicrobial / NF type I

- May include anaerobes

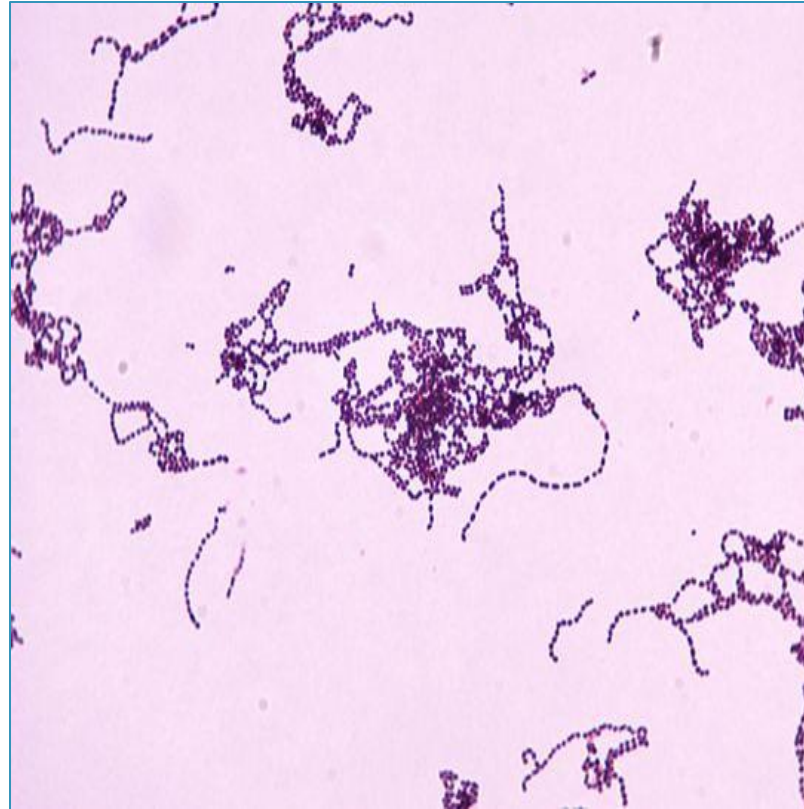
Gram-Negative Bacilli (rods) → *Pseudomonas aeruginosa* or Enterobacteriaceae

- Consider in burn wounds, immunocompromised patients

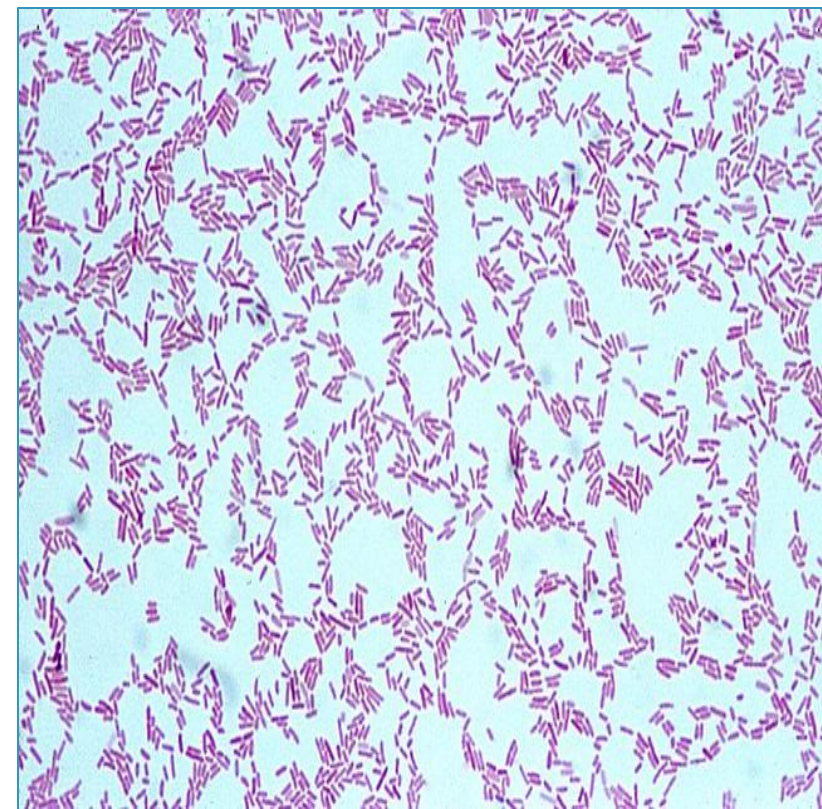
Gram Stain Interpretation



S. aureus

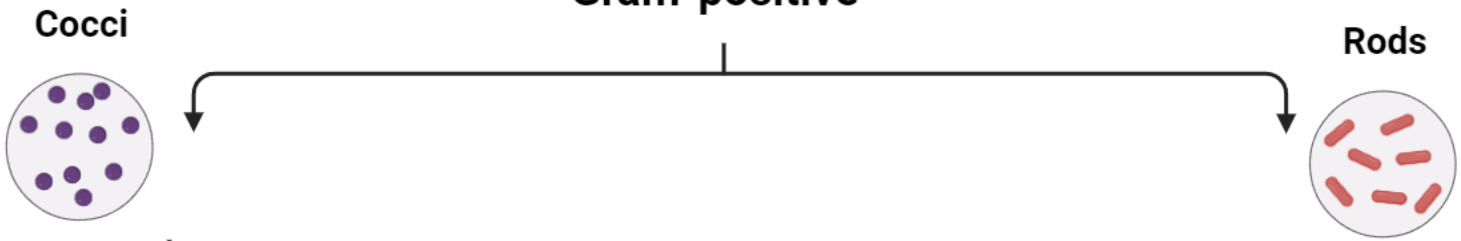


S. pyogenes

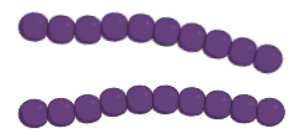


P. aeruginosa

Gram-positive



Catalase test



Streptococci



Staphylococci

Growth on sheep's blood agar



γ -hemolytic

Enterococcus



α -hemolytic

Capsule

S. pneumoniae

No Capsule

Viridans streptococci



β -hemolytic

Group A

S. pyogenes

Group B

S. agalactiae

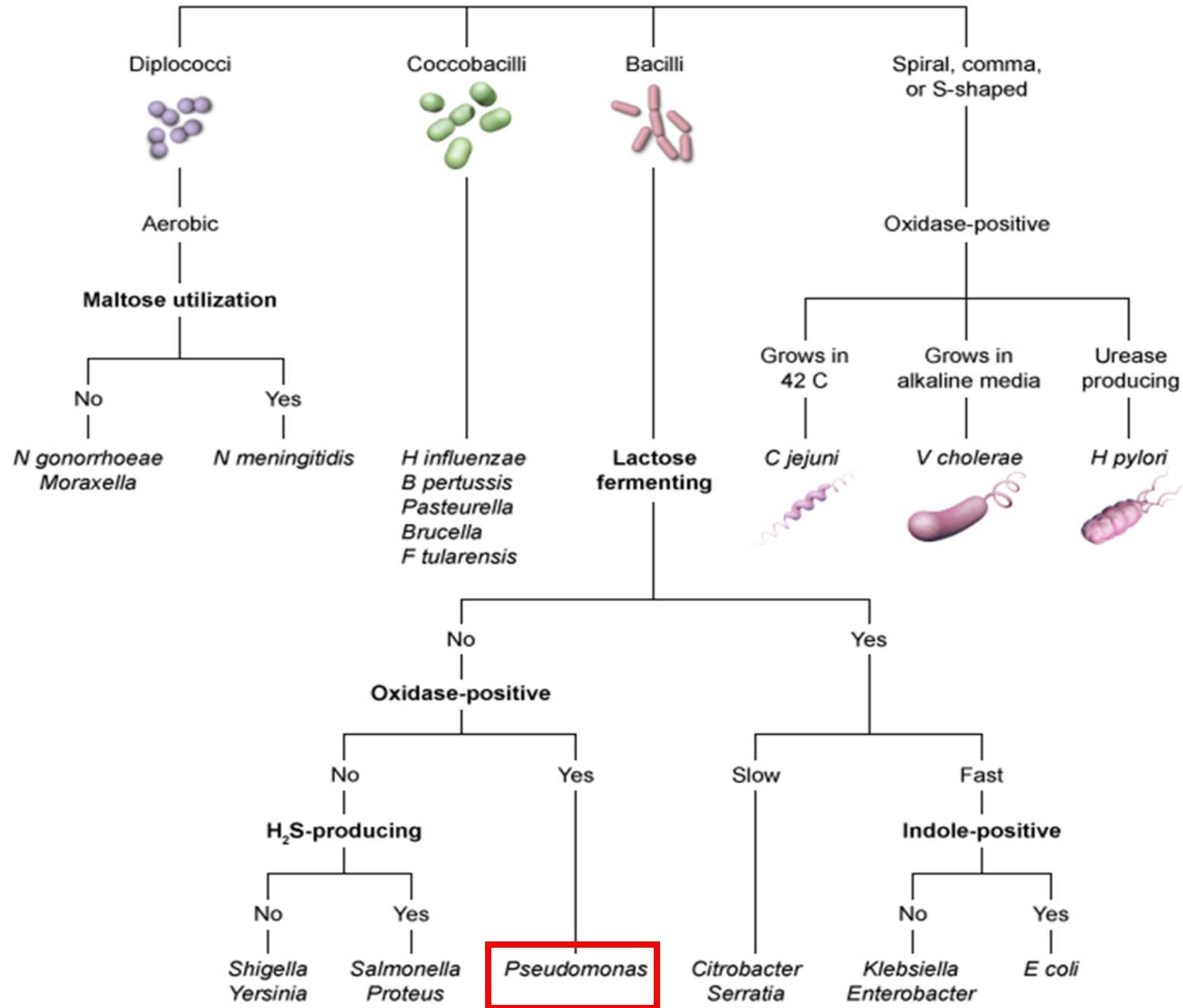
Coagulase test



S. saprophyticus
S. epidermidis

S. aureus

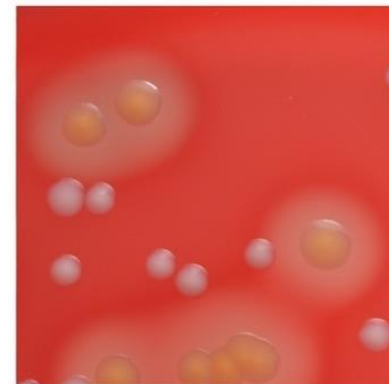
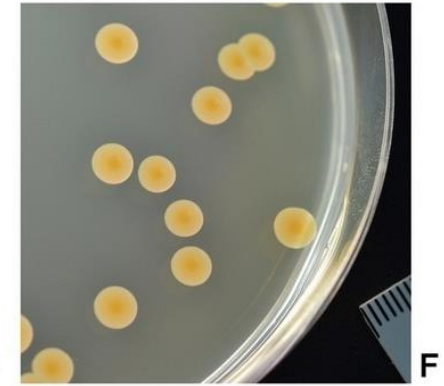
Gram-negative bacteria



S. aureus on Culture Media

1. Blood Agar (BA)

- **Golden-yellow** colonies
- Round, smooth, opaque, 2–3 mm after 24 hrs
- **Beta-haemolysis** (clear zone around colonies)



S. aureus on Culture Media

2. Mannitol Salt Agar (MSA)

- **Selective medium** - 7.5% NaCl inhibits most other bacteria
- **Differential medium**- Used to differentiate *S. aureus* from other Staphylococci
 - *S. aureus* **ferments** mannitol → yellow halo around colonies
 - Coagulase-negative Staph → pink/red colonies (**no colour change**)



S. aureus on Culture Media

3. DNase Agar

- **Principle:**

- DNase agar contains DNA.
- *S. aureus* produces DNase enzyme which breaks down the DNA.
- Adding HCl **precipitates intact DNA** (agar turns cloudy) → areas where DNA has been digested remain clear.
- Clear zone around colonies = DNA digested = DNase positive = *S. aureus*
- Opaque/cloudy up to the colony edge = DNA intact = DNase negative = not *S. aureus*
- **Confirmatory test**

S. aureus on Culture Media

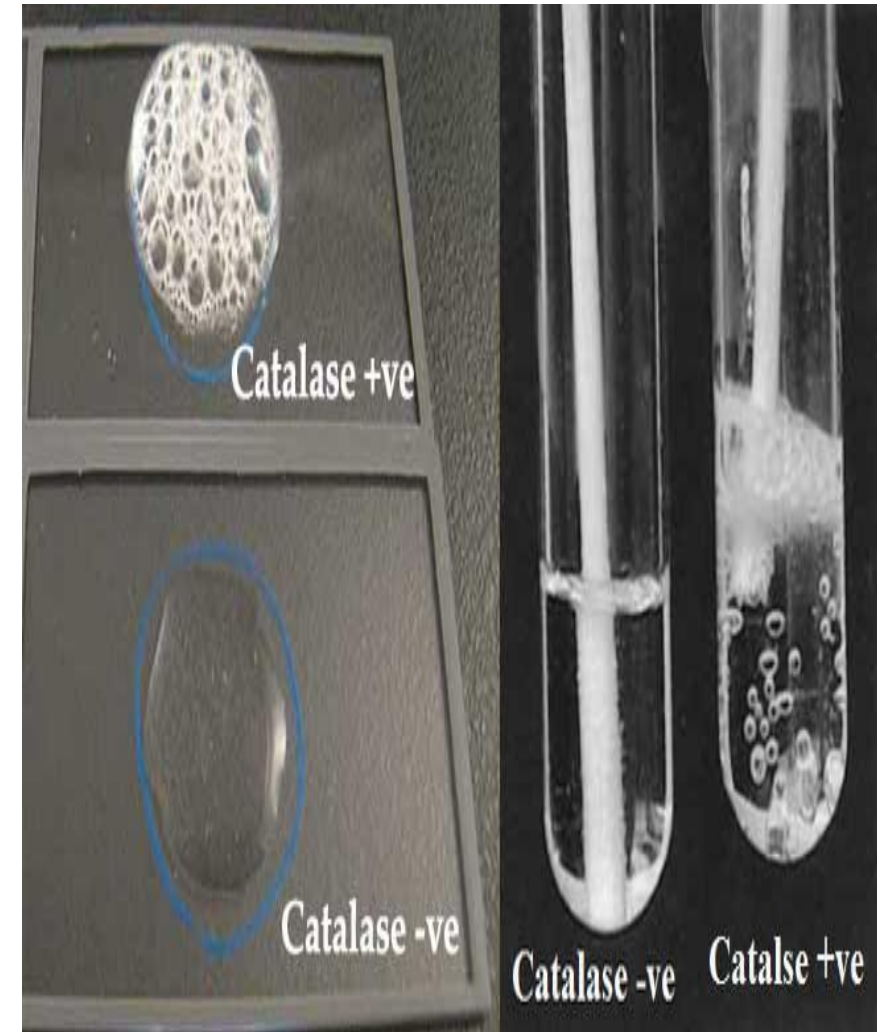
3. DNase Agar



S. aureus - Biochemical Identification

Catalase Test

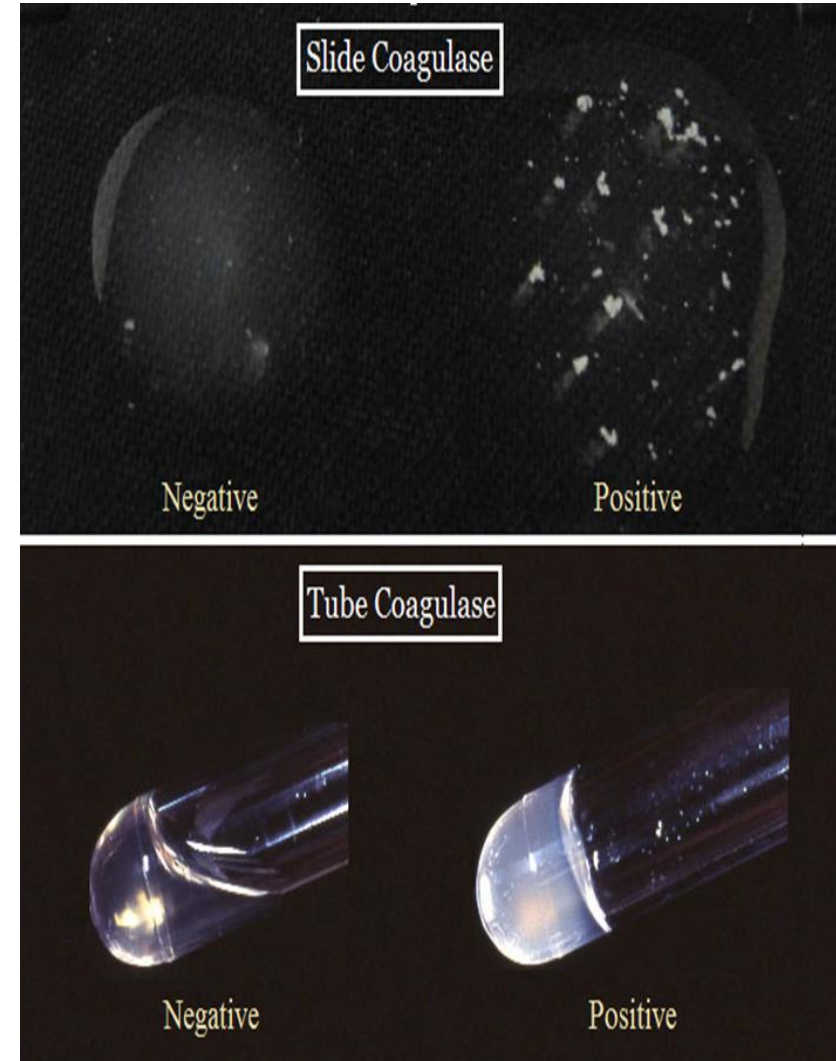
- **Principle:** Detects enzyme catalase that breaks down $\text{H}_2\text{O}_2 \rightarrow \text{H}_2\text{O} + \text{O}_2$
- **Method:** Add hydrogen peroxide to bacterial colony on slide and observe immediately
- **Result:**
 - A positive result is indicated by the rapid formation of bubbles
 - Separates **Staphylococcus** (Catalase +) from **Streptococcus** (Catalase -)



S. aureus - Biochemical Identification

Coagulase Test

- Principle: Coagulase is an enzyme that clots plasma (converts fibrinogen → fibrin)
- Differentiates:
 - *S. aureus* = coagulase POSITIVE
 - CoNS (*S. epidermidis*, *S. saprophyticus*) = coagulase NEGATIVE
- **Slide test (bound coagulase):** Clumping in 10 seconds
- **Tube test (free coagulase):** Clot formation in 4-24 hours (more reliable)



S. aureus - MRSA Detection

Why is MRSA important in SSTIs?

- MRSA is a leading cause of community-acquired SSTIs (CA-MRSA)
- Resistant almost to all beta-lactams (penicillins, cephalosporins, carbapenems)
- Carries the *mecA* gene → encodes altered PBP2a (low affinity for beta-lactams)

Missed MRSA = treatment failure

S. aureus - MRSA Detection

1. Cefoxitin Disk Diffusion (Recommended by CLSI)

- Cefoxitin is a better inducer of *mecA* than oxacillin
- Apply 30 μ g cefoxitin disk on Mueller-Hinton agar
- Incubate 35°C for 16–18 hrs
 - Zone of inhibition \leq 21 mm = **MRSA**
 - Zone of inhibition \geq 22 mm = **MSSA**
- **This is the standard phenotypic method used in most clinical labs**



S. aureus - MRSA Detection

2. Latex Agglutination for PBP2a

- Detects PBP2a protein directly from colony
- Rapid result (minutes)

3. Molecular Method (Gold Standard)

- PCR for *mecA* gene
- **Most definitive** but not available in all labs



MRSA

MSSA

S. pyogenes on Culture Media

1. Blood Agar (BA) — Primary Isolation Medium

- Small, translucent, pinpoint colonies (0.5–1 mm after 24 hrs)
- Surrounded by a wide, clear zone of **beta-haemolysis**

2. Does **NOT** grow on Mannitol Salt Agar

- GAS is inhibited by 7.5% NaCl
- This is a key differential point — if growth appears on MSA, **it is NOT *S. pyogenes***



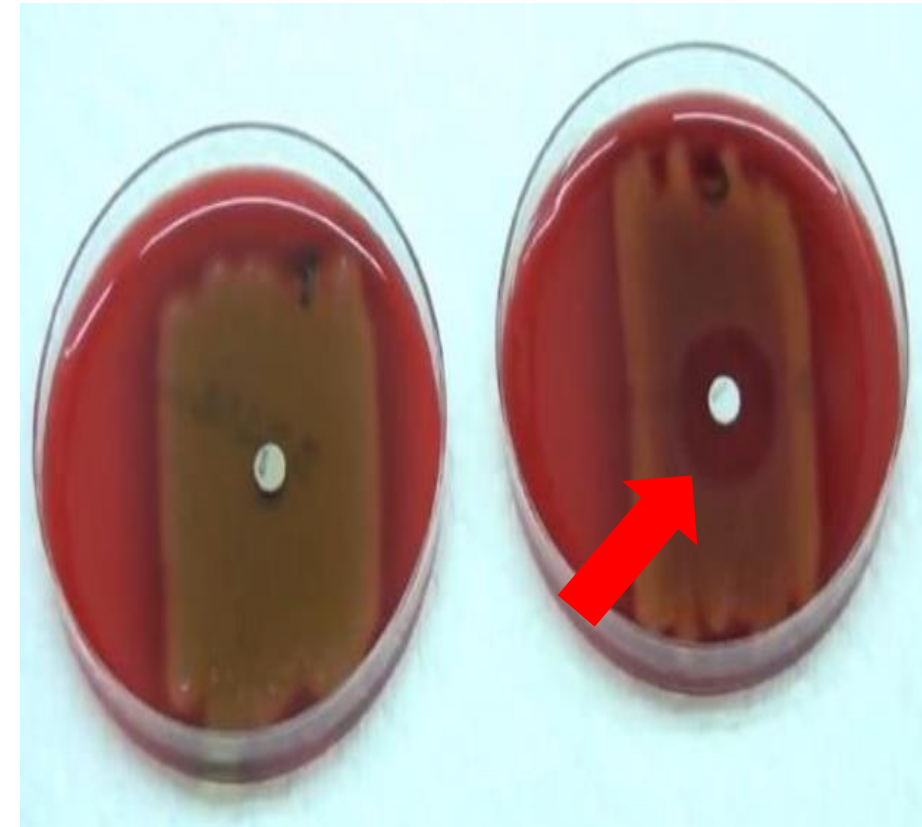
S. pyogenes — Biochemical Identification

- **Catalase Test**

- Drop H₂O₂ on colony → no bubbles = **negative**
- *Streptococcus* = catalase **NEGATIVE**

- **Bacitracin Sensitivity**

- Place bacitracin disk on blood agar streaked with the isolate
- Any zone of inhibition = **sensitive** = **presumptive GAS**
- No zone = resistant = likely not GAS



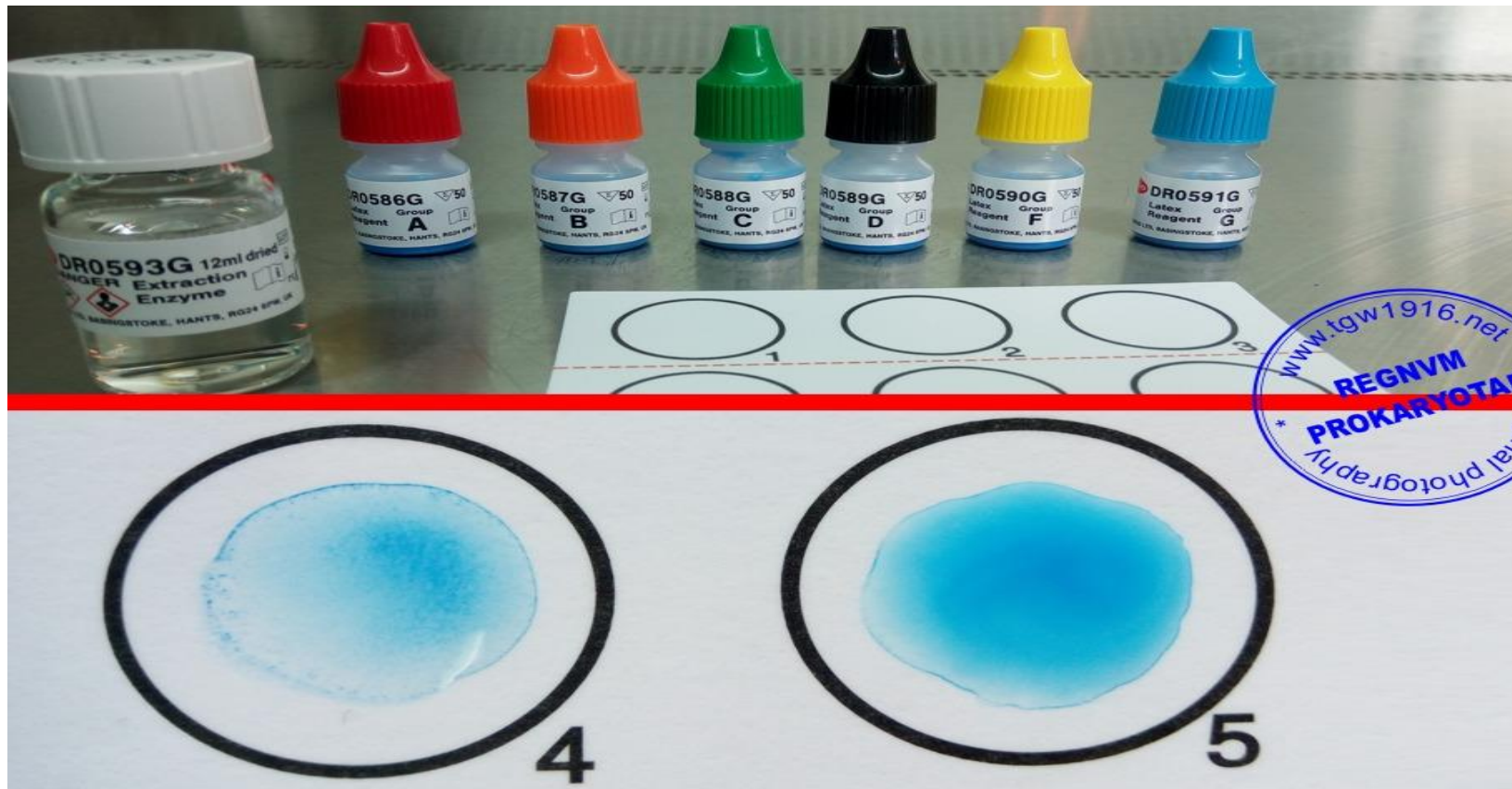
S. pyogenes — Biochemical Identification

Lancefield Grouping (Serological)

- Lancefield grouping is a serological method to classify catalase-negative, Gram-positive cocci (mainly Streptococcus) into groups A–U based on carbohydrate antigens in their cell walls.
- Latex agglutination using group-specific antisera
- *S. pyogenes* carries **Lancefield Group A** antigen (C carbohydrate of cell wall)
- Rapid result (minutes)

S. pyogenes — Biochemical Identification

Lancefield Grouping (Serological)



P. aeruginosa in SSTIs

When to think *Pseudomonas* in SSTIs?

- Burn wound infections (most important association)
- Hot tub folliculitis
- Chronic wound infections (diabetic foot ulcers, pressure sores)
- **Green discoloration** of wound dressings or nail infections (green nail syndrome)

P. aeruginosa in SSTIs

Gram Stain Appearance

- Gram-negative bacilli (rods)
- Single or in pairs
- **If you see GNR from a burn wound or chronic ulcer → think Pseudomonas**



P. aeruginosa on Culture Media

1. Blood Agar

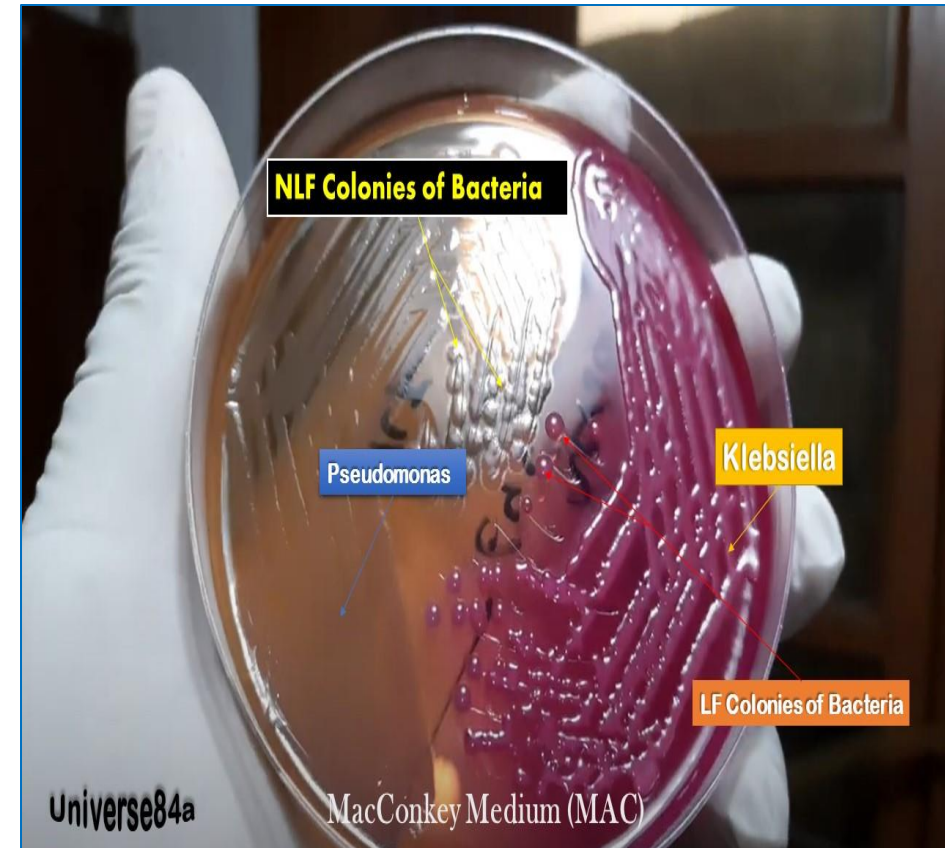
- Large, flat, spreading colonies with irregular edges
- Metallic sheen may be visible
- Beta-haemolysis
- **Distinctive grape-like or tortilla-like sweet odor**



P. aeruginosa on Culture Media

2. MacConkey Agar

- Contains:
 - Bile salts + Crystal violet dye → inhibit Gram-positive bacteria
 - Lactose + pH indicator
- Pale / colourless colonies = **non-lactose fermenter (NLF)**
- This helps distinguish from Enterobacteriaceae lactose fermenters (e.g., *E. coli* = pink)



P. aeruginosa on Culture Media

3. Nutrient Agar / Mueller-Hinton Agar

- **Pyocyanin** — blue-green pigment (diffuses into medium)
- **Pyoverdinin (fluorescein)** — yellow-green pigment (fluoresces under UV light)
- Pigment production is a hallmark — not all strains produce it, but when present it is almost diagnostic



Thank you