**STAPHYLOCOCCUS** and other related species  
*(Gram positive cocci, catalase positive)*


**General information about Gram positive cocci**
- Second only to the *Enterobacteriaceae* as the cause of human infection
- Clinically significant genera include *Staphylococcus*, *Streptococcus* and *Enterococcus*
- Can be recovered from almost any clinical specimen
- Organism is found on a variety of fomites and in dirt/dust on floors and walls
- Infection is most commonly spread by direct contact with an infected person or penetration of the skin or mucous membranes
  - Infection will elaborate an inflammatory response
- **Suppurative / Pyogenic / Purulent reaction**: inflammatory response to infections with GPC resulting in the accumulation of pus: a mixture of active or inactive neutrophils, other inflammatory cells, bacterial cells and extravascular fluid.
- Can produce pathogenic effects by producing toxins or enzymes.
  - Toxin: protein substance produced by some pathogenic bacteria that is highly toxic to other living organisms (a poison).

**General Information for Staphylococcus species**
- Normal flora of skin and mucous membranes of man and animals
- Most are facultative anaerobes (can use either aerobic respiration and/or fermentation depending on the availability of oxygen, does not solely depend on aerobic respiration for growth)
- Grow on any nutrient media that contains peptone
- Inhibited by media that contains crystal violet dye or very high conc. of bile salts
- Abundant growth at 18-24 hr.
- Non-motile, non-sporeforming

Common human pathogens:  
- *Staphylococcus aureus*  
- *Staphylococcus epidermidis*  
- *Staphylococcus saprophyticus*  
- *Staphylococcus lugdunensis*

**Identification of Staphylococcus and other related species (Genus Micrococcus and Genus Staphylococcus):**

- The role of colony morphology, microscopic and growth characteristics in the clinical laboratory (Chapter 8)
  - Colony morphology plays a significant role in presumptive identification of organisms
  - Trained microbiologist are able to differentiate between potential pathogens and non-pathogens in different body sites
    - Presumptive identification: cost effective, quality control
    - Correlation with original specimen gram stain

- Growth characteristics of *Staphylococcus* species:
  - Most will grow on primary isolation (routine) media:
    - Blood agar and Chocolate agar
  - No growth on MacConkey Agar
    - Often used as primary isolation media; selective for gram negative
  - These characteristics are useful during culture interpretation when determining what type of organism (gram positive vs. gram negative) has been isolated, therefore useful in deciding what biochemical test should be performed for identification.
General microscopic and biochemical characteristics of Genus Staphylococcus and Genus Micrococcus

- **Gram stain:** GPC in singles, pairs, tetrads and/or clusters
  - *Staphylococcus* is derived from Greek word *staphyle* meaning “grape-like clusters”
  - Gram stain provides clues that we have isolated a staph (tetrads, clusters)

- **Catalase test:**
  - The catalase test is used to detect the catalase enzyme which is able to breakdown hydrogen peroxide into oxygen and water, resulting in a rapid production of bubbles.
  - A colony is placed on a clean glass slide and a few drops of 3% hydrogen peroxide are placed directly on the colony. A positive result shows rapid production of bubbles.
  - Genus *Staphylococcus* and Genus *Micrococcus* are catalase positive
  - The catalase test differentiates *Staphylococcus* and *Micrococcus* from *Enterococcus* and *Streptococcus* species which are catalase negative.
  - **Note:** Red blood cells contain pseudocatalase. False positive catalase tests can occur when taking colonies from media containing blood.

Methods for Identification of *Staphylococcus* species:

- **Slide coagulase**
  - Detects clumping factor (formerly referred as cell-bound coagulase)
  - Clumping factor directly converts fibrinogen to fibrin causing agglutination
  - Heavy suspension of organism is made on glass slide and mixed with drop of plasma
  - Agglutination indicates a positive test
    - Indicates *Staphylococcus aureus*
    - Some species of Coagulase negative *staphylococcus* can be positive
  - Negative results should be confirmed (colony morphology, tube coagulase)

- **Tube coagulase**
  - (Detects staphylocoagulase which reacts with coagulase-reacting factor (CRF)
  - CRF is a thrombin-like molecule
  - Staphylocoagulase and CRF combine to indirectly convert fibrinogen to fibrin
  - A suspension of organism is suspended and incubated with plasma at 37° C
  - Clot formation within 4 hours indicates a positive test
    - **Positive test indicates Staphylococcus aureus**
    - Some species of Coagulase negative *staphylococcus* can be positive
  - Negative tubes should be held overnight at room temp.
    - **Note:** Some species possess enzyme (Fibrinolysin) that can cause dissolution of clot after prolonged incubation

- **Latex agglutination**
  - Various rapid kits available that utilize plasma-coated carrier particles, usually latex
  - Plasma detects clumping factor by reacting directly with fibrinogen
  - IgG detects Protein A
    - Protein A binds with Fc portion of IgG
  - High specificity and sensitivity than traditional slide test
  - Commonly used in clinical laboratories
  - Most often used to screen catalase + colonies that morphologically resemble *Staph. aureus*
  - Should be performed from non-selective media such as sheep blood agar; media with high salt content can have false positive results
  - Organism is emulsified in drop of reagent and rotated on slide
  - Agglutination within 20 secs indicates positive test
    - **Indicates Staphylococcus aureus**
    - Some species of Coagulase negative *staphylococcus/Micrococcus* can be positive
• **Novobiocin susceptibility**
  o Usually performed on urine isolates that are coagulase negative
  o Determines resistance to Novobiocin
  o Presumptive identification of *S. saprophyticus*
  o Uses 5-ug Novobiocin disk
  o Lawn of growth is prepared using 0.5 McFarland of the organism
  o Disk placed on the inoculum and incubated overnight
  o Zone sizes measured
    ▪ *Staphylococcus* saprophyticus – resistant (< 16 mm)
    ▪ Other Coagulase negative *staphylococcus* species – sensitive (>15 mm)

• **PYR hydrolysis**
  o Tests for the ability to hydrolyze the substrate L-pyrrolidonyl-alpha-naphthylamide
    ▪ *Staphylococcus aureus* is PYR negative
    ▪ *Staphylococcus lugdunensis* is PYR positive
    ▪ Other species will vary in their reaction

• **Rapid Ornithine (decarboxylase activity)**
  o Used to identify *Staphylococcus lugdunensis*
  o Modified conventional decarboxylase medium detect decarboxylase activity in 2 to 4 hours
  o Development of a dark purple to violet color in the medium (darker than the uninoculated reference tube) is a positive reaction
    ▪ *Staphylococcus lugdunensis* is rapid ornithine positive
    ▪ Other Staphylococcus species are negative (except rare strains of *S. epidermidis*)

• **Polymyxin B resistance**
  o Used to identify *Staphylococcus lugdunensis*
  o Determines resistance to polymyxin B
  o Useful to identify clinically significant Staphylococcus species
  o Uses 300 unit Polymyxin B disk
  o Lawn of growth is prepared using 0.5 McFarland of the organism
  o Disk placed on the inoculum and incubated overnight
  o Zone sizes measured
    ▪ Resistant (< 10 mm)
    ▪ Sensitive (≥10 mm)
  o *Staph. lugdunensis* is resistant as well as a few other species.

• **Automated and rapid multitest systems**
  o Vary in accuracy for some species
  o Most systems identify *S. aureus*, *S. epidermidis* and *S. saprophyticus* accurately

**Confirmatory tests for identification of Staphylococcus aureus**
• Single most commonly used test in the identification of *Staph aureus* is the coagulase tests.
  o Weak or questionable results may need to be confirmed as the isolate may indeed be coagulase negative staphylococcus species
  o Colony morphology is very important tool in the identification of *Staphylococcus aureus*
  o Questionable results will need to be confirmed with further tests
  o Tests to confirm:
    ▪ DNase
      ▪ Used in conjunction with other tests
      ▪ *Staphylococcus aureus* is DNase positive
    ▪ PYR
      ▪ *Staphylococcus aureus* is PYR negative
    ▪ Automated and rapid multitest systems
      ▪ Biotypes determine the organisms identity
    ▪ Molecular methods
Identification methods used to differentiate the genus *Micrococcus* from the Coagulase negative staphylococcus (Genus *Staphylococcus*):

- **Modified oxidase test (Microdase) (page 645 Koneman)**
  - Filter paper disks impregnated with tetramethyl-p-phenylenediamine dihydrochloride in dimethyl sulfoxide (DMSO)
  - DMSO makes the cells permeable to the reagent
  - Colony of growth is rubbed on disk
  - Development of blue-purple color with 30 seconds is positive test

- **Glucose utilization**
  - Glucose/dextrose reagent with pH indicator
  - Determine if organism is oxidative, fermentative, or biochemically inert in its utilization of glucose
  - Oxidative or fermentative organisms produce acid byproducts
  - Prolonged incubation
  - **Two tube or one tube methods available**
    - One-tube modification method
      - One tube is stabbed with organism, not covered, and incubated
      - Color change on top of the tube only indicates oxidative use of glucose
      - Color change in the entire tube indicates fermentation
      - No color change or development of a blue color on the top of the tube indicates the organism is inert

- **Bacitracin susceptibility**
  - Lawn of growth is prepared
  - Disk placed on the inoculum and incubated overnight
  - Zone sizes measured
    - *Staphylococcus* species – resistant and growth up to the edge of the disk
    - *Micrococcus* species – sensitive, zones 10 mm or greater
Staphylococcus aureus

General information:
- Most clinically significant species of Staphylococci.
- Can be recovered from almost any clinical specimen
- Causes both mild and life-threatening disease
- Important cause of nosocomial infection
- Most commonly used test in the identification of Staphylococcus aureus is the coagulase test.
- Based on colony morphology, catalase and coagulase reaction the identification of Staphylococcus aureus can be made. Colony morphology plays an important role in the rapid identification of this species.

Identification:

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Staph. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>Opaque, smooth, raised, entire (smooth or regular border), white-golden (cream), most are beta hemolytic</td>
</tr>
<tr>
<td>Gram morphology</td>
<td>GPC in clusters, pairs, short chains or singly</td>
</tr>
<tr>
<td>Catalase</td>
<td>Positive</td>
</tr>
<tr>
<td>Glucose fermentation (OF)</td>
<td>Fermenter</td>
</tr>
<tr>
<td>Bacitracin susceptibility</td>
<td>Resistant</td>
</tr>
<tr>
<td>Modified Oxidase</td>
<td>Negative</td>
</tr>
<tr>
<td>Slide or Tube Coagulase</td>
<td>Positive</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>Positive</td>
</tr>
<tr>
<td>DNase production</td>
<td>+</td>
</tr>
<tr>
<td>Mannitol fermentation</td>
<td>Positive</td>
</tr>
<tr>
<td>Salt tolerance</td>
<td>Growth</td>
</tr>
</tbody>
</table>

Virulence factors of S. aureus:

Capsule:
- Possessed by some strains of Staphylococcus aureus thought to inhibit phagocytosis, may promote adherence to host cells and prosthetic devices

Clumping factor:
- Cell-bound material able to bind fibrinogen

Enzymes
- **Catalase (page 631, Koneman)**
  - Inactivates toxic H₂O₂ and free radicals within phagocytic cell
- **Coagulase (page 631, Koneman)**
  - May inhibits phagocytosis/bactericidal activity, activates fibrin clot formation
- **Fibrinolysin (page 631, Koneman)**
  - Breaks down fibrin clots allowing spread of infection to surrounding tissue
  - Enzyme responsible for dissolution of clot in the coagulase test during prolonged incubation at 35°C
- **Hyaluronidase**
  - Hydrolyzes hyaluronic acid in connective tissue allowing spread of infection of surrounding tissue
- **Penicillinase (Beta-lactamase)**
  - Hydrolyzes the beta-lactam ring of penicillins/cephalosporins
    - Inducible: produced only in presence of beta-lactam antimicrobials
    - Constitutive: produced continually
- **DNase**
  - Degrades DNA
Exotoxins produced by *Staphylococcus aureus*:

- **Hemolysins**
  - Lyses erythrocytes
  - Lethal effects on cells

- **Exfoliative toxin (or epidermolytic toxin)**
  - Cause the epidermal layer of skin to slough off
  - Responsible for staphylococcal scalded skin syndrome

- **Panton-Valentine Leukocidin**
  - Lethal to polymorphonuclear leukocytes
  - Contributes to spread of infection by suppressing phagocytosis
  - Causes severe cutaneous infections and necrotizing pneumonia

- **Enterotoxins**
  - Heat-stable exotoxin
    - Not destroyed by heating of food
  - Responsible for clinical feature of food poisoning
  - Cause diarrhea and vomiting in humans
  - Superantigens – activate strong over-reactive immune response

- **Toxic Shock Syndrome Toxin 1 (TSST-1)**
  - Superantigen
  - Associated with toxic shock syndrome (both menstrual and non-menstrual cases)
  - Causes massive stimulation of immune system

**Protein A:**
- Binds Fc portion of immunoglobulin G (IgG)
- Blocks phagocytosis

Clinical Significance of *Staphylococcus aureus*:
*S. aureus* is normal flora (colonizers) of the various skin and mucosal surfaces. The invasive nature of the organism allows for infection to occur in various sites. Infections are usually suppurative (accumulation of pus). *S. aureus* is responsible for both mild and life-threatening infections.

Surface or Skin Infections:
- **Folliculitis**
  - Infection of hair follicle

- **Boils (furuncles)**
  - Usually an extension of folliculitis, infection involving surrounding skin and subcutaneous tissue resulting in large, raised, superficial abscesses, characterized by presence of pus

- **Carbuncles**
  - A mass of furuncles, may possess fever indicating systemic spread of infection

- **Impetigo**
  - Superficial skin infection seen primarily in children (differs from Impetigo caused by Streptococcus in that staphylococcal pustules are larger and are surrounded by a small zone of erythema)

Toxin Mediated Disease:
- **Scalded Skin Syndrome (SSS):**
  - Caused by exfoliatin/epidermolytic
    - Probably from a lesion distant from site of exfoliation
  - Usually seen in neonates and infants, and produces a burnlike effect on the skin

- **Toxic Shock Syndrome (TSS):**
  - Caused by TSST-1 toxin in both menstrual and non-menstrual cases
  - Characterized initially by high fever, rash and dehydration
  - Severe cases can develop hypotension and shock, cases can be fatal
  - Localized infection but toxin is systemic
  - Laboratory findings
    - Elevated leukocyte count with increased neutrophils
    - Culture of lesions may be positive, Blood cultures are usually negative
• **Food poisoning:**
  - Most commonly caused by *enterotoxin A and D*
  - **Heat stable toxins that are not destroyed by reheating foods**
    - Found in food that supports growth of *Staphylococcus* (potato salad, processed meats, custards, bakery goods)
    - Caused by ingestion of preformed enterotoxins
    - No detectable odor change or change in appearance
    - Cause vomiting and diarrhea 2-8 hrs. after ingestion
    - Lack fever and symptoms usually resolve within 24 to 48 hrs

**Other infections:**
- **Pneumonia**
  - Usually seen in the immunocompromised (elderly and young)
  - Predisposing factors usually present: viral infection, underlying disease, presence of foreign bodies, antibiotic therapy

- **Pseudomembranous enterocolitis**
  - Also known as antibiotic-associated colitis and occurs when the normal flora of the large bowel is altered. A severe acute inflammation of the bowel mucosa, with the formation of pseudomembranous plaques resulting in a watery diarrhea, abdominal cramps and fever.

- **Wound infections**
  - Usually due to injury of normal skin (trauma, burns, incisions)

- **Endocarditis/myocarditis**
- **Bacteremia/Septicemia**
- **Osteomyelitis**
  - Usually results from the spread of the organism via the bloodstream
- **Septic arthritis**
- **Nosocomial infections**

**Antibiotic Therapy:**
- **Susceptibility testing is performed following CLSI guidelines**
- Resistance is a concern.
  - Since *S. aureus* can possesses penicillinases susceptibility testing must be performed.
  - Routinely agents resistant to the enzyme penicillinase (methicillin, oxacillin, nafcillin) are used for treatment. Drugs of choice.
    - *S. aureus* can alter its binding sites to develop resistance to the penicillinase resistant antibiotics resulting in a **Methicillin Resistant Staphylococcus aureus (MRSA)**. MRSA is a concern in Nosocomial infections (Hospital acquired infections).
    - meca gene responsible for altering penicillin binding proteins
    - Oxacillin is used for detection of methicillin resistance.
    - Cefoxitin can be used to detect MRSA isolates (better inducer of the meca gene)
  - **Vancomycin = drug of choice for MRSA**

**Detection of Methicillin (Oxacillin) Resistance Staphylococcus aureus**
- **Resistance is encoded by the meca gene – alters penicillin binding proteins**
- **Routine susceptibility testing is not adequate**
  - Heteroresistant strains that are both sensitive and resistant cells can coexist within a culture. Consequently, in oxacillin susceptibility tests some cells may appear susceptible and others resistant
  - Resistant strains grow more slowly
  - **Modification of in vitro susceptibility testing can be modified to enhance detection of MRSA.**
  - When performing **antimicrobial testing optimal detection of MRSA** is obtained by:
    - Preparing inoculum with direct method
    - Incubation at a cooler temperature (30-35° C)
    - Using media with a neutral pH (7.0 –7.4)
    - Final test reading after 24 hours of incubation
    - Supplementation of media with 2% NaCl
Other methods to detect MRSA based on detection of mecA gene and the new penicillin binding protein PBP2a
- Latex agglutination to detect PBP2a protein
  - Confirms MRSA
- Nucleic acid probes or PCR amplification for detection of mecA gene
  - Gold Standard for MRSA detection
- Cefoxitin Disk (30 ug)
  - Due to heterotypic resistance phenotype some MRSA may not be detected by MIC microdilution methods
  - A Cefoxitin disk has been proven to be a good inducer of the mecA gene which is responsible for methicillin resistance.

Differential and Screening media used for Staphylococcus aureus and/or MRSA
- Mannitol Salt agar
  - Selective and differential primary culture media
  - Used to recover and identify Staphylococci from mixed flora
  - High concentration of salts inhibits most gram-negative and gram-positive bacteria other than Staphylococcus species
  - Mannitol is the sole carbohydrate in medium
  - Organism ferments mannitol and changes pH indicator in the medium from phenol red to yellow
    - S. aureus typically will appear yellow, surrounded by a yellow zone
    - Subculture to a less selective media is preferred before identification
    - False positive latex results will occur if colonies are taking directly from the Mannitol Salt agar due to the high salt concentration in the medium
- CHROMagar or Spectra MRSA agar (Remel Instructions for Use)
  - Selective media that permits direct detection and identification of Methicillin Resistant Staphylococcus aureus (MRSA) through the incorporation of specific chromogenic substrates
  - Cefoxitin is incorporated in the CHROMagar to induce the mecA gene and allow for increased detection
  - CLSI recommends the use of cefoxitin when screening for MRSA as it good inducer of the mecA gene which is responsible for methicillin resistance.
- Mueller-Hinton agar with 4% NaCl and 6 ug Oxacillin (Oxacillin Screen agar)
  - Inoculated agar is incubated at 35°C for 24 hours and observed for growth
  - Used in the detection of Methicillin Resistant Staph. aureus

Coagulase Negative Staphylococci

Clinical Significance:
- Normal flora of the skin and mucous membranes.
- Coagulase Negative Staphylococci are increasingly associated with infection due to the widespread use of prosthetic devices, intravascular catheters, prolonged surgical procedures, and the presence of underlying disease and the incidence of immunocompromised hosts. Infections with this organisms are predominantly hospital acquired, associated with instrumentation procedures, immunosuppressive therapy and immunocompromised patients.
- Most frequently isolated Coagulase Negative Staphylococcus in clinical lab is Staph. epidermidis.
**Staphylococcus epidermidis**

**Preliminary Identification:**

<table>
<thead>
<tr>
<th></th>
<th><strong>Staphylococcus epidermidis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>Opaque, smooth, raised, entire (smooth or regular border), gray-white, non-hemolytic</td>
</tr>
<tr>
<td>Gram morphology</td>
<td>GPC in clusters, pairs, short chains or singly. Same as S. aureus (may be smaller in size)</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Glucose fermentation</td>
<td>Fermenter</td>
</tr>
<tr>
<td>Bacitracin susceptibility</td>
<td>Resistant</td>
</tr>
<tr>
<td>Slide or Tube Coagulase</td>
<td>Negative</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>Negative</td>
</tr>
<tr>
<td>Novobiocin susceptibility</td>
<td>Sensitive</td>
</tr>
</tbody>
</table>

For definitive identification of *S. epidermidis* additional biochemical testing would need to be performed. From the above reactions an identification of coagulase negative staphylococcus can be made.

**Virulence factors:**
- **Capsule** = promotes adherence to host cells and plastics
- **Extracellular Slime substance** = referred to as an adherence factor, allows the organisms to adhere to and form colonies on the surface foreign bodies such as catheter tips and other prosthetic devices (Teflon and plastics). Slime producing strains are able to inhibit immune functions including the action of lymphocytes and neutrophils (opsonization and phagocytosis). The ESS produces a biofilm that contains several layers of organisms that serves to protect the organism from antimicrobials. This requires the removal of the foreign body in order to provide a cure.
- **Biofilms** (Chapter 31 of textbook)

**Infections associated with Staphylococcus epidermidis:**
- **Subacute bacterial endocarditis (SBE)**
  - Usually associated with prosthetic heart valve
- **Meningitis**
- **Bacteremia/septicemia**
  - Associated with prosthetic devices, shunts and catheters and immunosuppressed patients
- **Wound infections**
  - Associated with immunocompromised patients (malignancies, burn, transplant, nosocomial)
- **Urinary tract infections**
  - Most common cause of hospital acquired infections
- **Peritonitis (page 640, Koneman)**
  - Characterized by abdominal pain, nausea, vomiting, fever and cloudy effluent after dialysis
  - Associated with patient with continuous ambulatory peritoneal dialysis
- **Post-operative surgical infections**
  - Acquired nosocomially from health care workers or contaminated surgical devices

**Interpretation of culture results:**
- **Normal skin flora**
  - Common contaminant in cultures such as blood or wound cultures

**Antibiotic Therapy**
- Generally more resistant than *Staphylococcus aureus*
- Routine susceptibility testing is performed following CLSI guidelines
- Susceptibility testing is done if presumed to be the cause of infection because the organism does not have a predictable pattern of susceptibility.
- Drug of choice: Methicillin
  - Vancomycin for methicillin resistant strains
**Staphylococcus saprophyticus**

**Preliminary Identification:**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><strong>Staph. saprophyticus</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>Opaque, smooth, raised, entire, butyrous, glossy white-yellow, non-hemolytic</td>
</tr>
<tr>
<td>Gram morphology</td>
<td>GPC in clusters, pairs, short chains or singly</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Glucose fermentation (OF)</td>
<td>Fermenter</td>
</tr>
<tr>
<td>Bacitracin susceptibility</td>
<td>Resistant</td>
</tr>
<tr>
<td>Tube or slide Coagulase</td>
<td>Negative</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>Negative</td>
</tr>
<tr>
<td>Novobiocin susceptibility</td>
<td>Resistant (&lt;16mm)</td>
</tr>
</tbody>
</table>

A coagulate negative Staphylococcus that is resistant to Novobiocin is indicative of *S. saprophyticus* in urinary tract cultures and no further identification is usually necessary. In other culture sites further testing would need to be performed if identification is needed since other species of *Coagulate negative staphylococcus* can be novobiocin resistant.

**Clinical Significance**

- Urinary tract infections = 2nd to *Escherichia coli* as the cause of cystitis in young women.
- Adheres well to the epithelial cells of the urogenital tract

**Antibiotic Therapy**

- Susceptibility tests are not routinely done due to lack of correlation between *in vitro* results and *in vivo* response
- Organism responds well to antimicrobials commonly used to treat uncomplicated urinary tract infections (nitrofurantoin, trimethoprim/sulfa, fluoroquinolones)

**Staphylococcus lugdunensis**

**Identification:**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th><strong>Staph. lugdunensis</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>Usually opaque, very white, non-hemolytic, can be beta hemolytic but develops later</td>
</tr>
<tr>
<td>Gram morphology</td>
<td>GPC in clusters, pairs, short chains or singly</td>
</tr>
<tr>
<td>Catalase</td>
<td>+</td>
</tr>
<tr>
<td>Glucose fermentation (OF)</td>
<td>Fermenter</td>
</tr>
<tr>
<td>Bacitracin susceptibility</td>
<td>Resistant</td>
</tr>
<tr>
<td>Slide Coagulase</td>
<td>Positive but clumpy</td>
</tr>
<tr>
<td>Latex agglutination</td>
<td>Positive but clumpy</td>
</tr>
<tr>
<td>Tube Coagulase</td>
<td>Negative</td>
</tr>
<tr>
<td>PYR hydrolysis</td>
<td>Positive</td>
</tr>
<tr>
<td>Rapid ornithine</td>
<td>Positive</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>Resistant</td>
</tr>
</tbody>
</table>

**General Information**

- *S. lugdunensis* is PYR positive and *S. aureus* would be PYR negative
- *S. lugdunensis* is rapid ornithine positive (purple) other Coagulate negative staph are negative

**Clinical significance:**

- Endocarditis, septicemia, meningitis, skin and soft tissue infections, urinary tract infections, and septic shock
  - Particularly aggressive endocarditis, often requiring valve replacement
- Uses the same guidelines as *S. aureus* for interpretation of oxacillin susceptibility results
  - Important to identify to the species level to provide accurate reporting of antimicrobial susceptibilities when clinically significant
  - May possess mecA gene
General Information
- Normal flora of skin and mucous membranes
- Obligate aerobe, usually will not grow anaerobically
- Carotenoid pigments may give bright yellow or pink color to colony
- Non-motile and non-sporeforming.

Identification:

<table>
<thead>
<tr>
<th></th>
<th>Micrococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram morphology</td>
<td>Large GPC in pairs, tetrads, or masses</td>
</tr>
<tr>
<td>Colony morphology</td>
<td>Smooth, raised, opaque white, bright yellow, pink</td>
</tr>
<tr>
<td>Catalase reaction</td>
<td>+</td>
</tr>
<tr>
<td>Glucose fermentation (OF)</td>
<td>Oxidizer</td>
</tr>
<tr>
<td>Bacitracin disk (Taxo A = 0.04 U)</td>
<td>Sensitive (&gt;=10mm)</td>
</tr>
<tr>
<td>Modified oxidase</td>
<td>+</td>
</tr>
</tbody>
</table>

Clinical Significance
- Rarely produces disease
- May cause opportunistic infection in an immunocompromised host

Antibiotic therapy
- Standardized testing methods and therapeutic guidelines do not exist
- Appear to be susceptible to most beta-lactam antimicrobials

---

**Staphylococcus versus Micrococcus species**

<table>
<thead>
<tr>
<th></th>
<th>Staphylococcus species</th>
<th>Micrococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gram morphology</td>
<td>GPC in singles, pairs, and clusters</td>
<td>Large GPC in pairs, tetrads, or masses</td>
</tr>
<tr>
<td>Colony morphology</td>
<td>Opaque, smooth and circular, some are beta-hemolytic Range in color from gray-white to white to cream to yellow</td>
<td>Smooth, raised, opaque white, bright yellow, pink</td>
</tr>
<tr>
<td>Catalase reaction</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Glucose fermentation (OF)</td>
<td>Fermenter</td>
<td>Oxidizer</td>
</tr>
<tr>
<td>Bacitracin disk (Taxo A = 0.04 U)</td>
<td>Resistant (&lt;10 mm)</td>
<td>Sensitive (&gt;=10mm)</td>
</tr>
<tr>
<td>Modified oxidase</td>
<td>Negative</td>
<td>+</td>
</tr>
</tbody>
</table>
**The following is for information only and will not be tested at anytime in your theory exams. However it is useful information to have when you are in the clinical setting and evaluating cultures results.**

**Other Staphylococcus species**

- There are other species of *Staphylococcus* that may be clinically significant.
- Infections with *S. haemolyticus* and *S. lugdunesis* usually involve the implantation of medical devices or similar infections caused by *S. epidermidis*.
- They have gram morphologies similar to *Staphylococcus epidermidis*.
- Colony morphologies vary among the other species (white to grey-white, cream, opaque, smooth, raised, entire, non-hemolytic to beta-hemolytic (usually weak)).
- Various biochemical tests will differentiate the species
- Commercial systems have varying degrees of accuracy in identification
- Susceptibility patterns vary – may show multiple resistance patterns.
- Some animal isolates (*S. intermedius*, *S. hyicus*, and *S. delphini*) may be tube coagulase positive and should be considered in wounds involving animal bites.
- Coagulase negative species *S. lugdunensis* and *S. schleiferi* produce clumping factor and will be positive with the slide coagulase test or latex agglutination tests. However these species will be negative by tube coagulase test.

**NOTE:** Bacteriophage typing can be used as a means of further identification and classification of *Staphylococcus* species, especially *Staphylococcus aureus*. It is especially useful in epidemiological studies. It is performed in state and reference laboratories.

**Stomatococcus (Rothia) mucilanginosus**

**General Information (page 661, Koneman)**

- Encapsulated GPC that is part of normal human respiratory flora
- Opportunistic pathogen in cases of endocarditis and septicemia in compromised patients and drug abusers
- Colony and gram morphology resembles *Staphylococcus*
- Shows strong adherence to the agar surface when you try to pick up the colony due to the presence of a capsule (colony will stand up like egg whites if teased with a stick)
- Catalase: variable (when positive, the reaction is weak)
- Doesn't grow on media with 5% NaCl
- Can be identified by commercial systems

Other sources:
3. CLSI M35-A2, Abbreviated Identification of Bacteria and Yeast; Approved Guidelines Second Edition
### Summary – Genus Micrococcus and Genus Staphylococcus

<table>
<thead>
<tr>
<th></th>
<th>Staph. aureus</th>
<th>Staph. lugdunesis</th>
<th>Staph. saprophyticus*</th>
<th>Micrococcus</th>
</tr>
</thead>
<tbody>
<tr>
<td>Colony morphology</td>
<td>Opaque, smooth, raised, entire, white-golden (cream), most are beta hemolytic</td>
<td>Usually opaque, very white, non-hemolytic, can be beta hemolytic but develops later</td>
<td>Opaque, smooth, raised, entire, butyrous, glossy white-yellow, non-hemolytic</td>
<td>Smooth, raised, opaque white, bright yellow, pink</td>
</tr>
<tr>
<td>Gram morphology</td>
<td>GPC in clusters, pairs, short chains or singly</td>
<td>Same as Staph. aureus</td>
<td>Same as Staph. aureus</td>
<td>Large GPC in pairs, tetrads</td>
</tr>
<tr>
<td>Catalase</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
<td>Pos</td>
</tr>
<tr>
<td>Glucose fermentation (OF)</td>
<td>Fermenter</td>
<td>Fermenter</td>
<td>Fermenter</td>
<td>Oxidizer</td>
</tr>
<tr>
<td>Modified oxidase</td>
<td>Neg</td>
<td>Neg</td>
<td>Neg</td>
<td>Pos</td>
</tr>
<tr>
<td>Bacitracin susceptibility (Taxo A 0.04U)</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Sensitive</td>
</tr>
<tr>
<td>Coagulase production (tube)</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>N/A</td>
</tr>
<tr>
<td>Clumping factor (slide or latex coagulase test)</td>
<td>Pos</td>
<td>Positive but clumpy</td>
<td>Neg</td>
<td>Neg (some strains can be positive with latex test)</td>
</tr>
<tr>
<td>Mannitol fermentation</td>
<td>Pos</td>
<td>Neg</td>
<td>Variable</td>
<td>N/A</td>
</tr>
<tr>
<td>Dnase production</td>
<td>Pos</td>
<td>Neg</td>
<td>Neg</td>
<td>N/A</td>
</tr>
<tr>
<td>Novobiocin susceptibility</td>
<td>Sensitive</td>
<td>Sensitive</td>
<td>Resistant (&lt;16mm)</td>
<td>N/A</td>
</tr>
<tr>
<td>Rapid Ornithine</td>
<td>Neg</td>
<td>Pos</td>
<td>Neg</td>
<td>N/A</td>
</tr>
<tr>
<td>Polymyxin B</td>
<td>Resistant</td>
<td>Resistant</td>
<td>Sensitive</td>
<td>N/A</td>
</tr>
</tbody>
</table>

*A coagulase negative Staphylococcus that is resistant to Novobiocin is indicative of *S. saprophyticus* in urinary tract cultures and no further identification is usually necessary. In other culture sites further testing would need to be performed if identification is needed since other species of *Coagulase negative staphylococcus* can be novobiocin resistant.
Identification Summary for Staphylococcal species
(Genus Staphylococcus and Genus Micrococcus)

Gram positive cocci

(+)

Catalase

(−)

Streptococcaceae and other related species

(+)

Coagulase

Staph. aureus

if colony morphology matches

(+)

Bacitracin or
Modified oxidase

or

Glucose utilization

Bacitracin Sensitive
Modified Oxidase Positive
Glucose Utilization Oxidizer

Micrococcus species

(Other)

Specimen Source

(Urine)

(+)

PYR

Coag neg Staph

(+)

Rapid ornithine

Staph lugu densis

(−)

Coag neg Staph

(−)

Novobiocin

Probable Staph. saprophyticus