General Information

- Second only to the Enterobacteriaceae as the cause of human infection
- Can be recovered from almost any clinical specimen
- Organism is found on a variety of fomites, and in dirt and 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 with contaminated objects
- Infection will elaborate an inflammatory response
  
  **Suppurative / Pyogenic / Purulent reaction:** inflammatory response to infections with GPC resulting in the accumulation of pus
  
  **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).

Family  
**Micrococcaceae**

**Identification:**
- **Gram morphology:** Gram-positive cocci
- **Catalase production:** Positive

**Growth characteristics:**
- Most will grow on primary isolation (culture set-up) media: Blood agar or Chocolate plates
- No growth will be seen on MacConkey Agar often used for primary isolation in urine, wounds, respiratory, stool, genital and blood cultures.
- 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.

Genus:  
**Staphylococcus**
**Micrococcus**
**Stomatococcus**
**Planococcus**
MICROCOCCUS SPECIES

General Information
- Normal flora on 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>
<tr>
<td>Furazolidone disk (100 ug/ml)</td>
<td>Resistant (&lt; 10mm)</td>
</tr>
<tr>
<td>Lysostaphin disk (200 ug/ml)</td>
<td>Resistant (&lt; 15mm)</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 SPECIES

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
- Colonies are opaque, smooth and circular with abundant growth at 18-24 hr.

Identification:
- Range in color from gray-white to white to cream to yellow
- Staphylococci are gram positive cocci, usually in clusters
- Non-motile and non-sporeforming
- Glucose fermenters
- Catalase positive
- Bacitracin resistant (<10 mm)
- Most are Furazolidone (>10 mm) or Lysostaphin (>15 mm) sensitive
- Modified oxidase negative
- Common pathogens: *Staphylococcus aureus*  
  *Staphylococcus epidermidis*  
  *Staphylococcus saprophyticus*
**Staphylococcus aureus**

**Identification:**

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>Staph. aureus</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colony morphology</strong></td>
<td>Opaque, smooth, raised, entire (smooth or regular border), white-golden (cream), most are beta hemolytic</td>
</tr>
<tr>
<td><strong>Gram morphology</strong></td>
<td>GPC in clusters, pairs, short chains or singly</td>
</tr>
<tr>
<td><strong>Catalase</strong></td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Glucose fermentation (OF)</strong></td>
<td>Fermenter</td>
</tr>
<tr>
<td><strong>Bacitracin susceptibility</strong></td>
<td>Resistant</td>
</tr>
<tr>
<td><strong>Coagulase (Free)</strong> *</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Clumping Factor (Bound Coagulase)</strong> *</td>
<td>Positive</td>
</tr>
<tr>
<td><strong>Manitol fermentation</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>DNase production</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>Novobiocin susceptibility</strong></td>
<td>Sensitive</td>
</tr>
<tr>
<td><strong>Salt tolerance</strong></td>
<td>Growth</td>
</tr>
</tbody>
</table>

*Tube Coagulase* = Detects both free (extracellular) and bound coagulase. Slide Coagulase = Detects bound coagulase “Clumping factor”. Clumping factor is bound to the bacterial cell wall and reacts directly with fibrinogen so that if precipitates on the staphylococcal cell causing the cells to clump. Latex agglutination = Detects both “Clumping factor” and Protein A that can also be produced by *S. aureus*. Protein A binds to the Fc portion of immunoglobulin to cause clumping of the Latex reagent.

**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

**Enzymes:**

- **Catalase:** inactivates toxic H$_2$O$_2$ and free radicals within phagocytic cell
- **Coagulase:** inhibits phagocytosis/bactericidal activity, activates fibrin clot formation
- **Staphylokinase** (fibrinolysin): breaks down fibrin clots allowing spread of infection to surrounding tissue (The possession of this enzyme can lead to false negative tube coagulase test due to lysis of the clot formation. This stresses the importance of reading tube coagulase at 4 hours)
- **Hyaluronidase:** hydrolyzes hyaluronic acid in connective tissue allowing spread of infection to surrounding tissue
- **DNase:** degrades DNA
- **Penicillinase (Beta-lactamase):** hydrolyzes the beta-lactam ring of penicillins/cephalosporins
  - Inducible: produced only in presence of beta-lactam antimicrobials
  - Constitutive: produced continually

**Exo-toxins**

- **Hemolysins:** lyse RBC’s and other cells
- **Leukocidin:** lyses WBC’s and macrophages
- **Exfoliatin:** cleaves a layer of the epidermis resulting in sloughing of the skin
- **Enterotoxin:** heat stable exotoxins (proteins A–E) that acts on the intestinal mucosa to cause vomiting and diarrhea. **TSST-1** (enterotoxin **F**) causes massive stimulation of immune system and is associated with Toxic Shock Syndrome
Clinical Significance

*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.

**Surface or Skin infections:**
- **Folliculitis** = infection of hair follicle
- **Boils (furuncles)** = infection involving surrounding skin and subcutaneous tissue
  Characterized by presence of pus
- **Carbuncles** = a mass of furuncles
- **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 toxin*, usually seen in neonates and infants, and produces a burnlike effect on the skin
- **Toxic Shock Syndrome (TSS):** caused by *TSST-1 toxin*
  Initially characterized by fever, rash, and signs of dehydration. In extreme cases the disease is characterized by hypotension and shock, involvement of 3 or more organ systems, desquamation (shedding of the epidermis layer of the skin) of extremities within 2 wks of onset and negative results on blood, throat and CSF cultures.
- **Food poisoning:** most commonly caused by *enterotoxin A and B*
  - Found in food that supports growth of *Staphylococcus*
  - (potato salad, processed meats, custards, bakery goods)
  - Cause vomiting and diarrhea 2-8 hrs. after ingestion
  - Lack fever and symptoms resolve within 24 hrs.

**Other infections:**
- **Wound infections** = usually due to injury of normal skin (trauma, burns, incisions)
- **Pneumonia** = usually seen in the immunocompromised (elderly and young)
  Predisposing factors usually present: viral infection, underlying disease, presence of foreign bodies, antibiotic therapy
- **Endocarditis/myocarditis**
- **Bacteremia/Septicemia**
- **Osteomyelitis** = usually results from the spread of the organism via the bloodstream
- **Septic arthritis**
- **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.
- **Nosocomial infections**

**Antibiotic Therapy:**
- Since *S. aureus* can possesses penicillinase not all isolates can be treated by penicillin. *S. aureus* does not have a predictable pattern of sensitivity therefore susceptibility testing should be done.
- Routinely agents resistant to the enzyme penicillinase (methicillin, oxacillin, nafcillin) are used for treatment however *S. aureus* can also alter its binding sites to develop resistance to the penicillin resistant antibiotics resulting in a *Methicillin Resistant Staphylococcus aureus* (*MRSA*). *MRSA* is a concern in Nosocomial infections (Hospital acquired infections).
- **Vancomycin** = drug of choice for MRSA
Growth Characteristics of MRSA (page 81-82 Mahon)

- Sensitive and resistant strains can coexist within a culture
- Resistant strains may grow more slowly
- When performing **antimicrobial testing optimal detection of MRSA** is obtained by:
  - Using media with a neutral pH (7.0 – 7.4)
  - Incubation at a cooler temperature (30-35 C)
  - Adding 2-4% NaCl to the media
  - Incubating for a full 24 hrs.

**Coagulase Negative Staphylococci**

**Clinical Significance:** 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.

**Staphylococcus epidermidis**

**Identification:**

<table>
<thead>
<tr>
<th></th>
<th>Coagulase negative staph</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colony morphology</strong></td>
<td>Opaque, smooth, raised, entire (smooth or regular border), gray-white, non-hemolytic</td>
</tr>
<tr>
<td><strong>Gram morphology</strong></td>
<td>GPC in clusters, pairs, short chains or singly</td>
</tr>
<tr>
<td></td>
<td>Same as S. aureus (may be smaller in size)</td>
</tr>
<tr>
<td><strong>Catalase</strong></td>
<td>+</td>
</tr>
<tr>
<td>**Glucose fermentation (OF)</td>
<td>Fermenter</td>
</tr>
<tr>
<td><strong>Bacitracin susceptibility</strong></td>
<td>Resistant</td>
</tr>
<tr>
<td><strong>Coagulase production (Bound or Free)</strong></td>
<td>Negative</td>
</tr>
<tr>
<td><strong>Mannitol fermentation</strong></td>
<td>Negative</td>
</tr>
<tr>
<td><strong>DNase production</strong></td>
<td>Negative (w)</td>
</tr>
<tr>
<td><strong>Novobiocin susceptibility</strong></td>
<td>Sensitive</td>
</tr>
<tr>
<td><strong>Salt tolerance</strong></td>
<td>Variable</td>
</tr>
</tbody>
</table>

For identification of *S. epidermidis* additional biochemical testing would need to be performed. From these 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.

**Clinical Significance:**

- **Subacute bacterial endocarditis (SBE)** = usually associated to prosthetic heart valve

**Meningitis**

- Associated with prosthetic devices, shunts and catheters
due to the organisms capsule and slime substance

- **Bacteremia/septicemia**
- **Wound infections** Associated with immunocompromised patients (malignancies, burn, transplant, nosocomial)

- **Urinary tract infections**
- **Post-surgical infections** = acquired nosocomially from personnel or contaminated surgical devices

*S. epidermidis* poses a problem when interpreting positive blood cultures. The organism can be normal skin flora, if proper collection technique is not performed the blood cultures can be contaminated. Correlation with the number of blood cultures drawn, and infections in other sites can help in interpretation of culture results. If a blood culture is positive and catheter tip culture grow *S. epidermidis* as well then mostly like the organism is a pathogen rather than a drawing contaminant. This is also true of other organisms that can be interpreted as normal skin contaminants.

**Antibiotic Therapy**
- Generally more resistant than *Staphylococcus aureus*
- 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**

**Identification:**

<table>
<thead>
<tr>
<th></th>
<th><em>Staph. saprophyticus</em></th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Colony morphology</strong></td>
<td>Opaque, smooth, raised, entire, butyrous, glossy white-yellow, non-hemolytic</td>
</tr>
<tr>
<td><strong>Gram morphology</strong></td>
<td>GPC in clusters, pairs, short chains or singly</td>
</tr>
<tr>
<td><strong>Catalase</strong></td>
<td>+</td>
</tr>
<tr>
<td><strong>Glucose fermentation (OF)</strong></td>
<td>Fermenter</td>
</tr>
<tr>
<td><strong>Bacitracin susceptibility</strong></td>
<td>Resistant</td>
</tr>
<tr>
<td><strong>Coagulase production (Bound or Free)</strong></td>
<td>Neg</td>
</tr>
<tr>
<td><strong>Mannitol fermentation</strong></td>
<td>Variable</td>
</tr>
<tr>
<td><strong>Dnase production</strong></td>
<td>Neg</td>
</tr>
<tr>
<td><strong>Novobiocin susceptibility</strong></td>
<td>Resistant (&lt;16mm)</td>
</tr>
<tr>
<td><strong>Salt tolerance</strong></td>
<td>Growth</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.

**Clinical Significance**
- Urinary tract infections = 2nd to *Escherichia coli* as the cause of cystitis in young women.

**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)
**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. lugdunensis* 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
- 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 mucilanginosus**

**General Information**

- Normal oral flora, therefore commonly seen on respiratory cultures.
- 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
## Summary - Micrococcaceae

<table>
<thead>
<tr>
<th></th>
<th>Staph. aureus</th>
<th>Staph. epidermidis</th>
<th>Staph. saprophyticus</th>
<th>Micrococcus</th>
</tr>
</thead>
<tbody>
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<td>Colony morphology</td>
<td>Opaque, smooth, raised, entire, white-golden (cream), most are beta hemolytic</td>
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<td>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>Neg</td>
<td>Neg</td>
<td>Neg</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 (w)</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>Salt tolerance</td>
<td>Growth</td>
<td>Variable</td>
<td>Growth</td>
<td>N/A</td>
</tr>
</tbody>
</table>

For identification of *S. epidermidis* additional biochemical testing would need to be performed. From these reactions an identification of coagulase negative staphylococcus, not *S. saprophyticus* can be made.

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.
Summary - Micrococcaceae

Gram positive cocci

+ Catalase -

Micrococcaceae

<table>
<thead>
<tr>
<th>Modified oxidase</th>
</tr>
</thead>
<tbody>
<tr>
<td>Bacitracin</td>
</tr>
<tr>
<td>OF Glucose</td>
</tr>
</tbody>
</table>

Positive
Sensitive Oxidizer

Negative
Resistant Fermenter

Micrococcus

+ Coagulase - Latex agglutination

Staph. aureus

R Novobiocin S

1. Urine Cultures: *Staph. saprophyticus*
2. Other cultures: Coag neg Staph.

Coag neg Staph.