Staphylococcaceae Micrococcaceae

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In this lecture, you are expected to:

- Describe *Micrococcaceae & Streptococcus* in terms of their general properties and pathogenesis
- Discuss the identification characteristics of *Micrococaceae* in terms of morphology, culture media, colony characteristics, biochemical test, and serological test
- Explain the principles/concepts of different identification techniques used

In this lecture, you are expected to:

Enumerate the appropriate specimen submitted in the laboratory for isolation

- Classify culture media according to: indications for use, contents, purpose, etc.
- Prepare and perform quality control of culture media before use.
- Identify *Staphylococcus*, *Micrococcus* and *Streptococcus* from specimens
- Analyze case/s related to topic

Family Micrococcaceae

- Includes 4 genera:
  - *Planococcus* – free living saprophyte
  - *Micrococcus* – free living saprophyte
  - *Stomatococcus* – normal flora on surface of primates and other mammals
  - *Staphylococcus* – normal flora on surface of primates and other mammals
  - All, except *Planococcus* have been isolated from clinically significant sources

- Propensity to cause disease:
  - *Stomatococci* are part of the normal oral flora and are now an emerging pathogen in immuno-compromised patients.
  - *Micrococci* become pathogens when they are accidentally introduced into a susceptible host.
  - *Staphylococci* have long been recognized as important human pathogens. The most commonly isolated pathogenic species in order of pathogenicity are *S. aureus, S. epidermidis, and S. saprophyticus.*

- Morphology and General Characteristics
  - Gram positive cocci which may lose the ability to retain their Gram positive staining characteristics with age.
  - May occur singly, in pairs, tetrads (common for *Micrococci*), or in clusters (*Staphylococci* – staphyle means bunch of grapes and this arrangement is due to the tendency of the organism to divide in different planes)

Micrococcus vs Staphylococcus

	Micrococcus	Staphylococcus
General Characteristics		
Oxygen Requirements		
Aerobic Growth		
Anaerobic Growth		
Carbohydrate Utilization (OF Medium)		
Open Tube		
Closed Tube		

Micrococcus vs Staphylococcus

	Micrococcus	Staphylococcus
Antibiotic Susceptibility		
Bacitracin		
Furazolidone		
Lysostaphin		
Modified Oxidase Test (Microdase Test)		
Growth on Furoxone- Tween 80- oil red O agar		

Division pattern determines arrangement

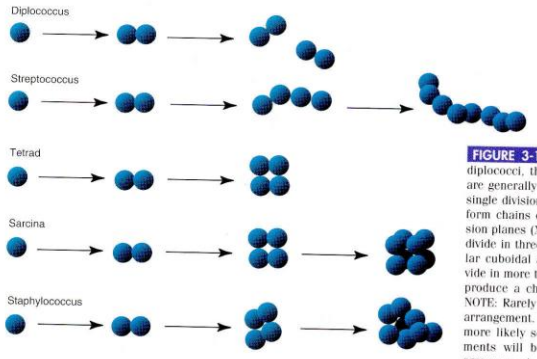


FIGURE 3-12 Division patterns among cocci. In diplococci, there is a single division plane and cells are generally found in pairs. Streptococci also have a single division plane, but the cells remain attached to form chains of variable length. If there are two division planes (X and Y), the cells form tetrads. Sarcinae divide in three planes (X, Y and Z) to produce a regular cuboidal arrangement of cells. Staphylococci divide in more than three planes (X, Y, Z and oblique) to produce a characteristic grapelike cluster of cells. NOTE: Rarely will a sample be composed of just one arrangement. The more complex the arrangement, the more likely scattered examples of simpler arrangements will be found. Look for the most common arrangement.

Staph sp. arrangement

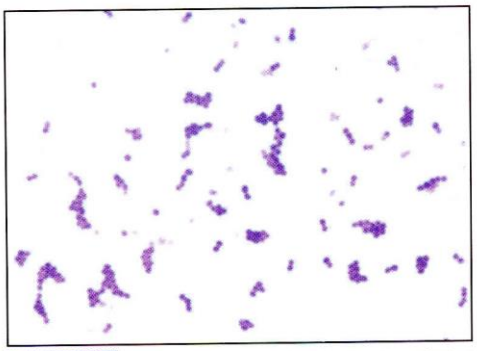


FIGURE 3-18 *Staphylococcus aureus* from broth culture showing the staphylococcus arrangement of cells. *S. aureus* is a common opportunistic pathogen of humans. The stain was crystal violet (X1000).

Micrococcus sp. arrangement

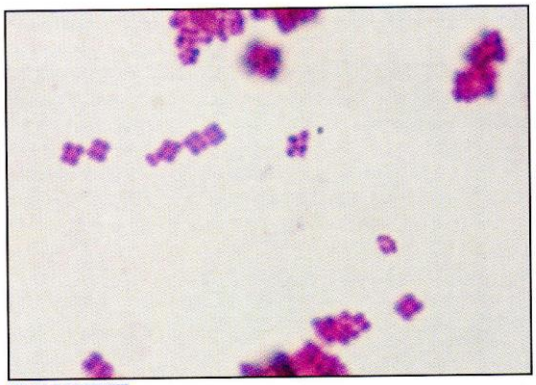
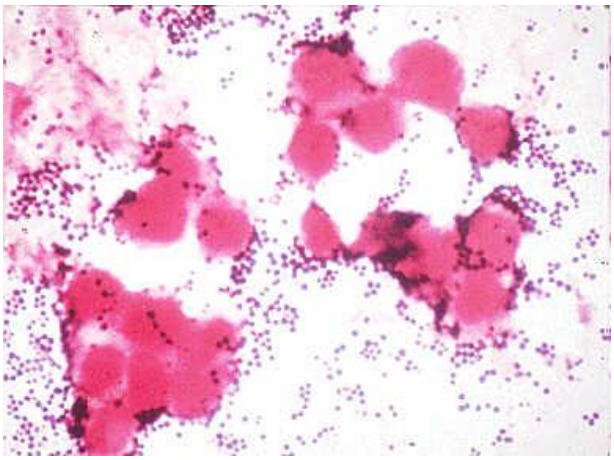
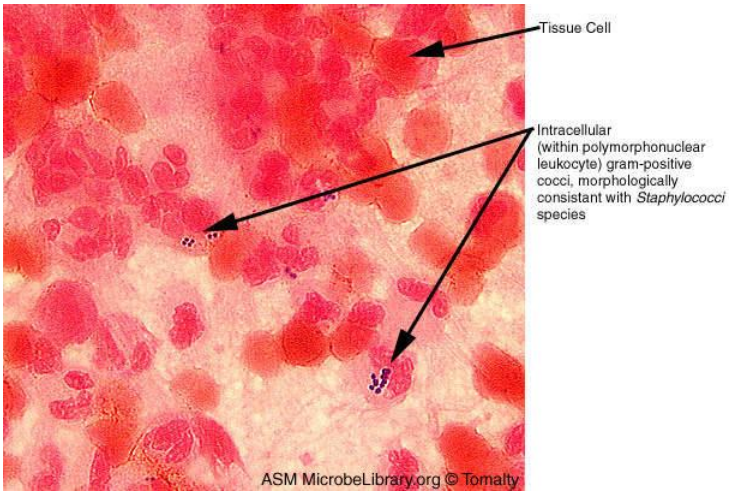


FIGURE 3-16 Tetrads of *Micrococcus roseus* stained with carbolfuchsin (X2640).

Staph in tissue

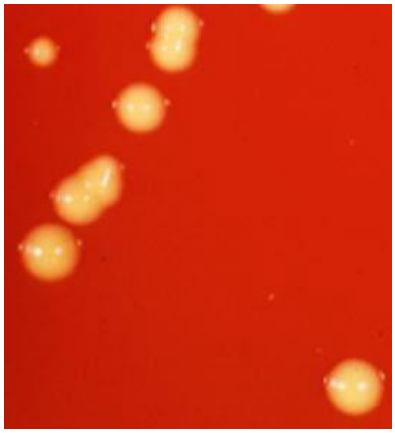


Staph in tissue



- Will grow on most lab media that will support the growth of Gram positive organisms. Within 24 hours smooth, circular colonies with a buttery consistency will grow.
  - *S. aureus* classically has a golden or yellow pigmentation, but many clinical isolates have a creamy or white pigmentation.
  - *S. epidermidis* produces white colonies
  - *M. luteus* produces colonies with bright yellow pigmentation

Staph aureus colonies



Staph epidermidis colonies

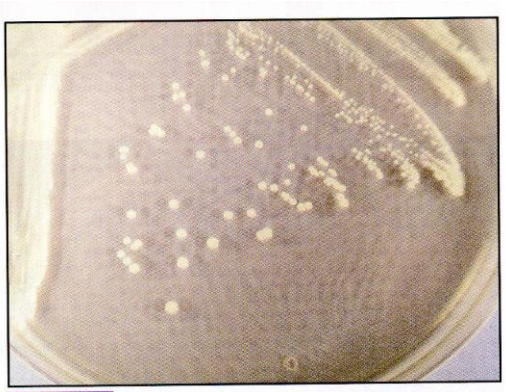
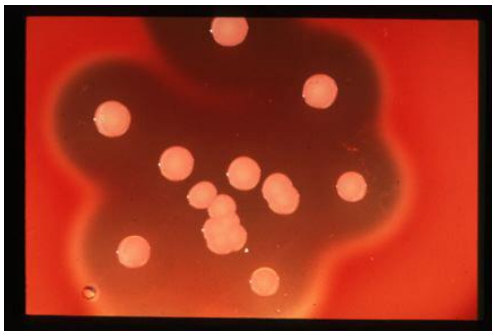


FIGURE 1-2 White, raised, circular and entire colonies of *Staphylococcus epidermidis* viewed from above with reflected light.

Micrococcus luteus colonies

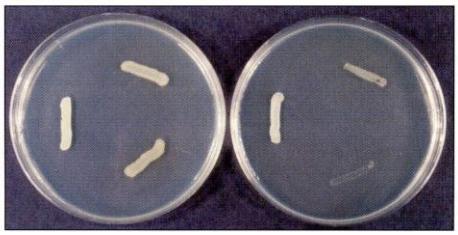
FIGURE 1-3
Convex yellow colonies of *Micrococcus luteus* as seen from above.

- On blood agar *S. aureus* produces _____. Other *Staph. species* produce alpha or gamma hemolysis.

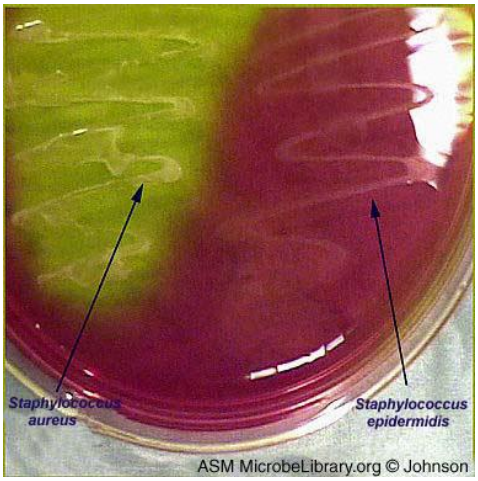


S. aureus

- Selective media may be used to isolate *Staph.* from specimens likely to be contaminated with other bacterial flora.
  - _____– inhibits Gram negative bacteria



- _____ - inhibits Gram negative bacteria
- _____ - high salt (7.5%) inhibits the growth of most other organisms, but *Staph.* are facultative halophiles and can grow in up to 10% salt.
  - also contains mannitol and the pH indicator _____. If an organism growing on MSA ferments mannitol, the acid produced turns the colonies yellow. *S. aureus* ferments mannitol and *S. epidermidis* does not.



- Biochemical identification
  - *Stomatococcus* versus all others in the family
    - *Stomatococci* are catalase
    - *Micrococci* and *Staphylococci* are catalase

Catalase test

$$2H_2O_2 \xrightarrow{\text{Catalase}} 2H_2O + O_2\uparrow$$

Hydrogen Peroxide

FIGURE 5-15 Catalase is an enzyme of aerobes and facultative anaerobes that converts hydrogen peroxide to water and oxygen gas.

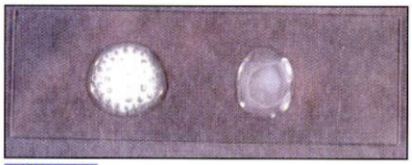


FIGURE 5-16 The catalase slide test in which visible bubble production indicates a positive result. *Staphylococcus aureus* (+) is on the left, *Enterococcus faecium* (−) is on the right.

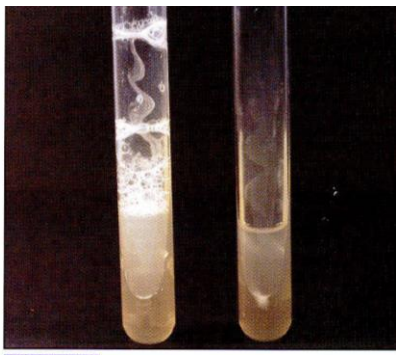


FIGURE 5-17 The catalase test may also be performed on a agar slant. *Staphylococcus aureus* (+) is on the left, *Enterococcu faecium* (−) is on the right.

- *Micrococci* versus *Staphylococci*
  - Oxidative/Fermentative (O/F) media
    - *Micrococci* are either oxidative or inert (asaccharolytic)
    - *Staphylococci* are fermentative

inert oxidative fermentative

- Modified oxidase test (contains DMSO that allows penetration of the thick G+ cell wall)
  - *Micrococci* are +
  - *Staphylococci* are –
- Lysostaphin - is a protease that breaks the glycine peptide linkages in the cell wall of *Staph. species*
  - *Micrococci* are resistant
  - *Staphylococci* are sensitive
- Bacitracin - .04 units
  - *Micrococci* are sensitive
  - *Staphylococci* are resistant

Bacitracin susceptibility

FIGURE 5-2 Bacitracin susceptibility on a sheep blood agar plate. *Staphylococcus aureus* (R) is on top and *Micrococcus luteus* (S) is below.

- Differentiation within the *Staph.* species
  - Coagulase test – in the presence of coagulase fibrinogen is converted to fibrin
    - *Staphylococcus aureus* is coagulase positive – there are other species of *Staph*. that are coagulase positive, but they are rare isolates from human infections (i. e., *S. intermedius* from canine bites). Therefore, a + coagulase test is usually sufficient for naming an isolate *S. aureus*.
    - All other *Staph species* are collectively called coagulase negative *Staph*. (CNS). These include *S. epidermidis* and *S. saprophyticus.*
    - There are two different coagulase tests
      - The slide test tests for bound coagulase
      - The tube test tests for free or extracellular coagulase.

Coagulase

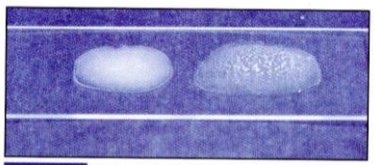


FIGURE 5-21 The coagulase slide test. Emulsions of *Staphylococcus aureus* (+) on the right and *S. epidermidis* (–) on the left were prepared in sterile saline. Agglutination of the coagulase plasma is indicative of a positive result.

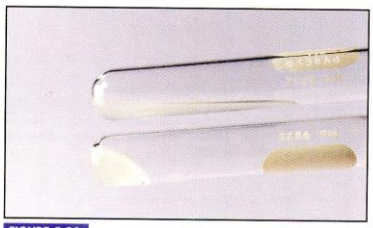


FIGURE 5-20 The coagulase tube test showing coagulase-negative *Staphylococcus epidermidis* above and the more pathogenic coagulase-positive *S. aureus* below. It is thought that coagulase increases virulence by surrounding infecting organisms with a clot which protects them from host defenses, such as phagocytosis and antibodies. This test was run for 24 hours.

Coagulase

Other Coagulase-Producing Staphylococci

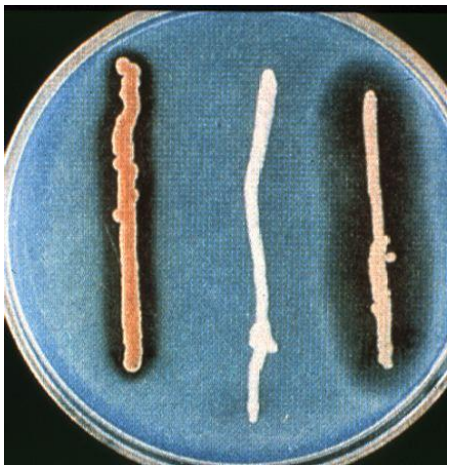
S. intermedius,
S. lutrae,
S. delphini,
Some strains of *S. hyicus*

Other Coagulase-Negative Staphylococci

S. warneri,
S. simulans,
S. schleiferi,
S. capitis,
S. hominis

- Mannitol fermentation
  - *S. aureus* and some *S. saprophyticus* are positive
  - *S. epidermidis* is negative
- DNAse
  - *S. aureus* is positive
  - *S. epidermidis* and *S. saprophyticus* are negative

DNAse test



Family Micrococcaceae

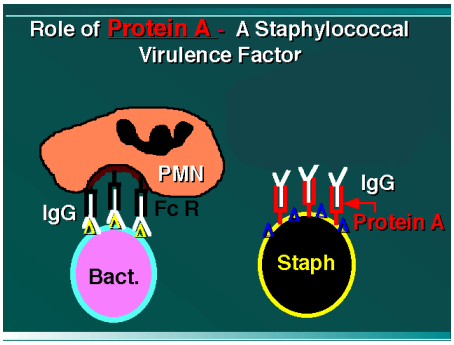
- Differentiation within the CNS
  - We usually only distinguish between the CNS on urinary tract isolates. For these isolates we want to distinguish between *S. epidermidis* and *S. saprophyticus.*
  - Novobiocin (5 ug disk)
    - *S. epidermidis* is sensitive, giving a zone of inhibition greater than or equal to 17 mm. in diameter
    - *S. saprophyticus* is resistant, giving a zone less than 17 mm. in diameter

Novobiocin susceptibility

FIGURE 5-62 Novobiocin disk test on a sheep blood agar plate. *Staphylococcus saprophyticus* (R) is on the left; *Staphylococcus epidermidis* (S) is on the right.

Mechanisms of pathogenicity

- _____ – binds to the Fc region of IgG and inhibits phagocytosis by preventing opsonization.



Mechanisms of pathogenicity

- Toxins
  - _____ – remember that *S. aureus* is beta hemolytic. It may produce 4 different hemolysins: α,β, γ, and δ. Except for β hemolysin, they all lyse leukocytes and other tissue cells as well as RBCs.
    - α toxin, in particular, may produce extensive tissue damage.
    - β hemolysin is known as hot-cold lysin because its hemolytic activity is enhanced when 37^0 C incubation is followed by 4^0 C incubation.

Mechanisms of pathogenicity

- _____ –acts exclusively on WBCs (PMNs and macrophages).
  - called the _____
  - lytic activity is due to an alteration of the activity of the Na+/ K+ pump
- Enterotoxins – *S. aureus* produces at least 6 distinct enterotoxins.
  - responsible for *Staph*. food poisoning.
  - heat stable and they act to stimulate neural receptors in the G.I. tract causing pain, vomiting, and diarrhea within 6 hours of ingestion.
  - symptoms are short lived.
  - Former enterotoxin type F (see below) is now known as _____

Mechanisms of pathogenicity

- _____ – it cleaves the upper layer of the epidermis, resulting in a condition called scalded skin syndrome.

Figure 26.2b Microbiology: An Evolving Science
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Mechanisms of pathogenicity

- _____
  - is pyrogenic (fever causing) due to IL-1 induction
  - causes erythroderma (red skin)
  - Causes enhanced susceptibility to endotoxin shock.
- Many of the effects of enterotoxins, exfoliative toxin and TSST-1 are due to their action as a superantigens.

Mechanisms of pathogenicity

- Enzymes
  - _____ – helps to wall the organism off from the host immune system. May also help by coating neutrophils with fibrin to protect the organism from phagocytosis. Many *Staph* infections are characterized by abscess formation.
    - Free or extracellular coagulase combines with a serum component to produce a thrombin-like activity to cleave fibrinogen to form a fibrin clot.
    - Bound coagulase binds to fibrinogen on cell surfaces converting it to fibrin, producing fibrin clots and causing agglutination of the bacterial cells (also called clumping factor)
    - High concentrations of coagulase can lead to intravascular coagulation, particularly in the lungs.

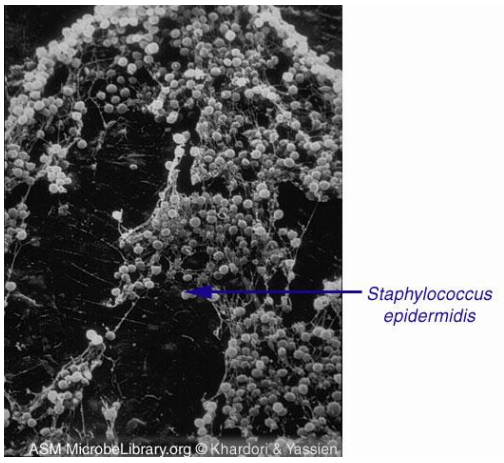
Mechanisms of pathogenicity

- Factors that help in dissemination
  - _____ – dissolves fibrin clots that the host may lay down during an inflammatory reaction to try to wall off the infection.
  - _____ – depolymerizes hyaluronic acid, the ground substance of tissues.
  - _____ – hydrolyzes lipids.
- _____ – breaks the beta lactam ring to inactivate penicillin.

Mechanisms of pathogenicity

- DNAse – degrades accumulated inflammatory exudate DNA from leukocyte disintegration helping the organism to spread (DNA is very viscous making dissemination more difficult)
- Slime production – this is an extracellular glycoconjugate that helps the organism to adhere to smooth surfaces and is produced by CNS as well as *S. aureus*.
  - This is important in allowing colonization of indwelling catheters, a major problem in hospitalized patients.

Slime production leading to colonization



- Clinical significance – *Staph* are ubiquitous and found as normal flora (NF) of man and other animals.
- CNS strains, usually *S. epidermidis*, are part of the NF of the skin
- *S. aureus* is part of the NF of the nasopharynx in 10-40% of the population. The percentage is higher in hospitalized patients.
- They are opportunistic (*S. epidermidis*) or facultative (*S. aureus*) pathogens.

Clinical significance

- Invasive infections
  - S. aureus can cause localized infections in nearly any area of the body. Local skin infections are the most common type of infection. Suppuration (pus production) is a hallmark of these infections.
    - Folliculitis is an infection of a hair follicle. If the hair follicle is an eyelash, the infection is commonly called a sty.

Folliculitis

Invasive infections

- Furuncle or boil – when folliculitis spreads to involve subcutaneous tissue.
- Carbuncle – is a series of interconnected furuncles
- Dissemination – when the organism spreads throughout the body to cause:
  - Bacteremia
  - Septicemia with lymphangitis
  - Osteomyelitis
  - Pneumonia
  - Meningitis
  - Endocarditis – acute or subacute (this can occur following a simple tooth extraction)

Septicemia with lymphangitis

FIGURE 23.3 Lymphangitis, one sign of septicemia. As tl

Bacterial endocarditis

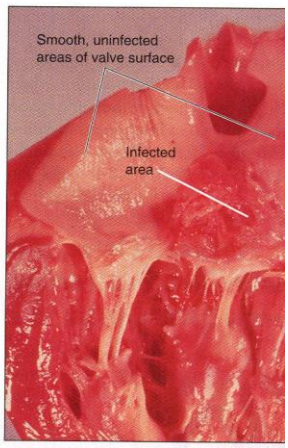
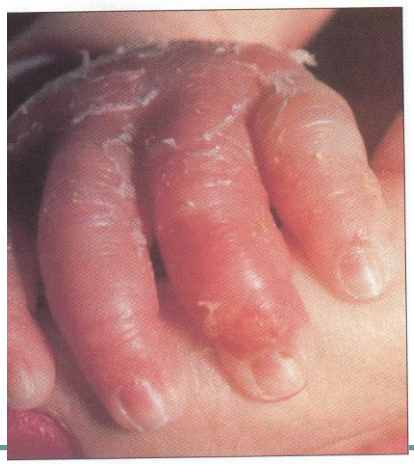


FIGURE 23.4 Acute bacterial endocardit

Clinical significance

- *Staph aureus* and *Staph saprophyticus* can cause urinary tract infections.
  - *Staph saprophyticus* is the second most common cause of urinary tract infections in sexually active young women.
- Toxigenic diseases
  - Food poisoning due to a heat stable enterotoxin. More common in foods with mayonnaise or custards.
  - Scalded skin syndrome – due to exfoliative toxin which initially causes a red rash followed by a peeling away of the skin in sheaths.
    - peeling usually occurs 2 times, but heals without scarring.
    - is more common in infants and young children.

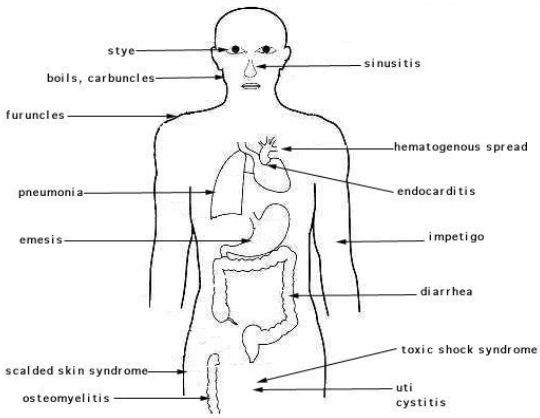
Scalded skin syndrome



Toxigenic diseases

- Toxic shock syndrome – the disease may occur in any individual, but most commonly starts as a vaginal infection in menstruating women using tampons.
  - is followed by a sudden onset of high fever, vomiting, diarrhea, red rash, and shock due to enterotoxin F (TSST-1).
  - This is followed 1-2 weeks later by desquamation, particularly of the palms of the hands and the soles of the feet.

Summary



- Antimicrobial susceptibility
  - 85% of all *S. aureus* now produce beta lactamase to inactivate penicillin.
    - Methicillin, a beta lactamase resistant penicillin may be used.
  - We now have methicillin resistant strains of *S. aureus* (MRSA).
    - Resistance is due to a change in the cell wall that leads to altered binding of antibiotics which include the cephalosporins, streptomycin, tetracycline, and sulfonamides as well as methicillin.
    - For individuals infected with these strains, vancomycin may be used.
      - _____ is given I.V. and requires hospitalization.

- Vancomycin resistant strains of *S. aureus* have now been reported!
- Other *Staph species* tend to be even more resistant to antibiotics than does *S. aureus*, so antimicrobial sensitivity testing is essential.

- For individuals with chronic infections of *S. aureus*, bacterial interference has been tried.
  - The individual is colonized with a *S. aureus* strain of low virulence with the idea that no superinfection will occur if the individual is already colonized.

Micrococcus sp

- GPC
- _____
- Nitrate _____
- Grows on 5% NaCl but not on 7.5% Nacl
- White or yellow pigment
- _____
- _____

Staphylococcus aureus

- GPC in _____
- Non-motile; Non-spore forming
- Grow on BAP as creamy, white, _____ colonies
- Golden yellow colonies in _____
- Can be isolated from cultures by use of medium with _____

Staphylococcus aureus

- Grows in MSA and ferments mannitol forming _____ colonies
- Facultative anaerobes
- Catalase _____
- Coagulase _____
- DNAse _____
- Phosphatase _____

Staphylococcus epidermidis

- _____ on blood agar plate
- Grows on MSA but does not ferment mannitol
- Catalase _____
- Coagulase _____
- DNAse _____
- Novobiocin _____

Staphylococcus epidermidis

- Clinical Significance
  - Part of the normal flora of the _____
  - Blood culture contaminant
  - Hospital acquired _____
  - Associated with

    _____

    _____

Staphylococcus saprophyticus

- _____ on blood agar plate
- Catalase _____
- Coagulase _____
- DNAse _____
- Novobiocin _____

Staphylococcus saprophyticus

- Clinical Significance
  - Part of the normal flora of the skin
  - Important cause of _____