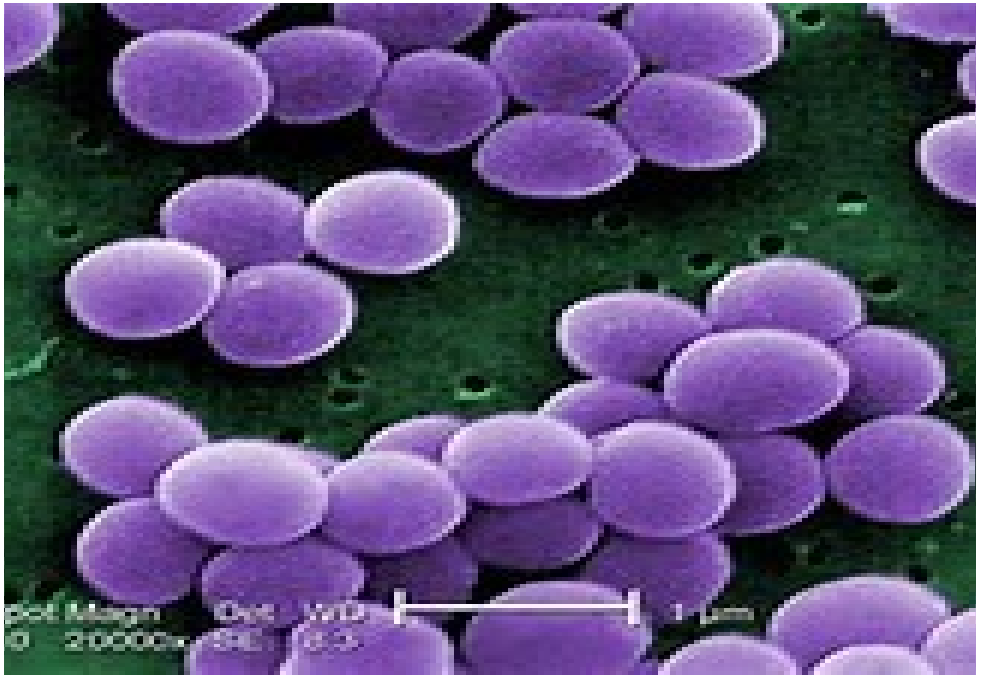
Gram Positive Cocci



Staphylococci

- Staphylococci are members of the Staphylococcaceae family
- **Gram positive cocci-**---Occurs singly, in pairs, and/or clusters--"staphle" means "bunches of grapes" (Greek)
- **Catalase positive, non-motile, non-spore forming**
- **Aerobic or facultative anaerobic**—except for *S. saccharolyticus* which is an obligate anaerobe.
- Usually, **colonizers of skin and mucosal surfaces--** infection occurs when this normal flora takes over and gets into the body through a cut or trauma

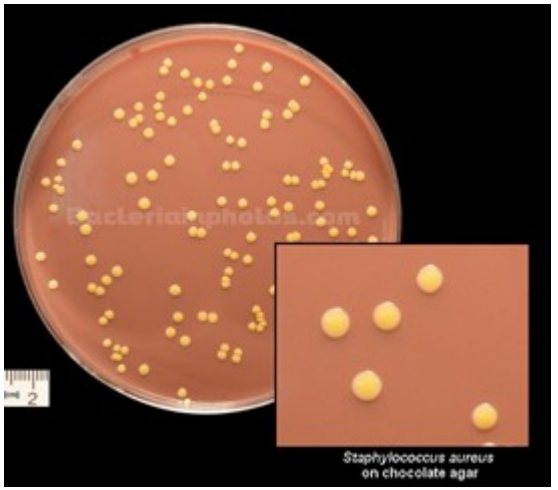
Staphylococcus growth on Media Agar

- Media:
  - Grows on sheep blood agar (SBA) and chocolate agar (Choc)
  - Selective media: mannitol salt agar (MSA), Columbia colistin-nalidixic acid agar (CNA), phenylethyl alcohol agar (PEA), and CHROMagar Staph aureus.
  - After 18-24 hours, colonies are medium size and appear cream colored, white, or light gold, and “buttery-looking”
  - Some species are **beta hemolytic**

Coagulase Positive Staph

Staphylococcus aureus

- Causes a variety of infections from cutaneous infections to life threatening systemic illnesses + nosocomial infections
  - Primary reservoir is the nares (20-40% of humans)
  - *S. aureus* is regarded as an opportunistic pathogen
  - S. aureus produces 2 forms of coagulase: bound and free
- After 18-24 hours incubation on SBA, *S. aureus* can produce β-hemolytic creamy buttery looking colonies. Can even produce a yellow pigment on Choc agar.



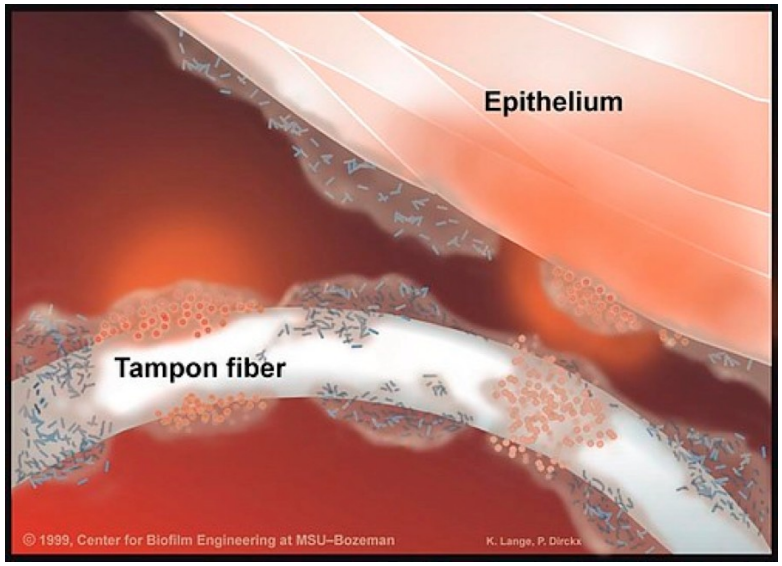
Virulence Factors:

Enterotoxins—food poisoning

- Heat-stable (stable at 100°C for 30 min) exotoxins that cause a variety of symptoms including diarrhea and vomiting.
- Enterotoxins A, B, and D most commonly cause food poisoning
- Enterotoxins B and C, sometimes G and I are associated with Toxic Shock Syndrome (TSS)

Virulence Factors: Toxic Shock Syndrome Toxin-1 (TSST-1)

- **Toxic Shock Syndrome Toxin-1 (TSST-1)**
- Causes nearly all cases of menstruating associated TSS
- Associated with approximately 50% of the non-menstruating associated TSS
- Previously called enterotoxin F
- Associated with tampon use: TSST-1 is absorbed through the vaginal mucosa, which leads to the systemic effect.

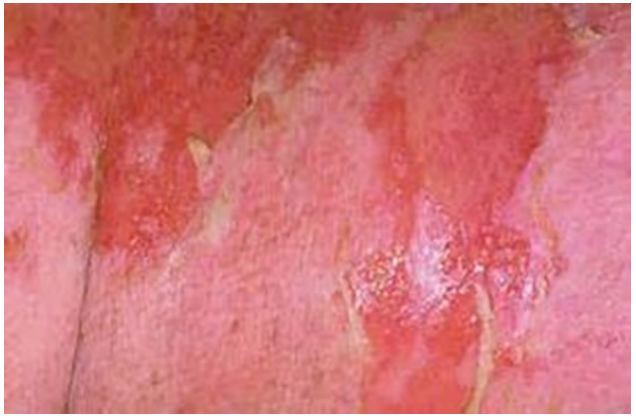


Virulence Factors: Cytolytic toxins

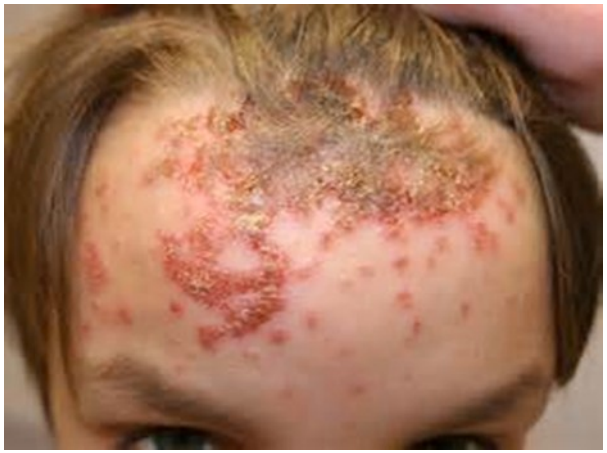
- Hemolysins and leukocidins are extracellular proteins cause hemolysis in RBCs and WBCs:
- **α-Hemolysin** lyses **erythrocytes**, damages **macrophages** and platelets, and causes severe tissue damage
- **β-Hemolysin** (aka sphingomyelinase C and "hot-cold lysin")
  - acts on sphingomyelin in the plasma membrane of erythrocytes.
  - "Hot-cold" feature is enhanced hemolytic activity on incubation at 37°C and 4°C.
- **γ-Hemolysin** is found only in association of Panton-Valentine leukocidin (PVL)
  - PVL is an exotoxin **lethal to WBCs** and **suppresses phagocytosis**
  - associated with Community acquired staph infections.
- **δ-Hemolysin** is found in both S. aureus and some CNS. Considered less toxic then α-Hemolysin and β-Hemolysin

Virulence Factors: Exfoliative toxins

- Exfoliative toxins (aka epidermolytic toxin)
- Ritter disease (aka **Scalded Skin Syndrome**)-- illness characterized by red blistering skin that looks like a burn, scald, and/or slough off. Occurs in young children and newborns.
- **Bullous Impetigo-** is a cutaneous condition that characteristically occurs in newborn, and is caused by a bacterial infection, presenting with a large blister.



Ritter disease



Bullous Impetigo

Virulence Factors:

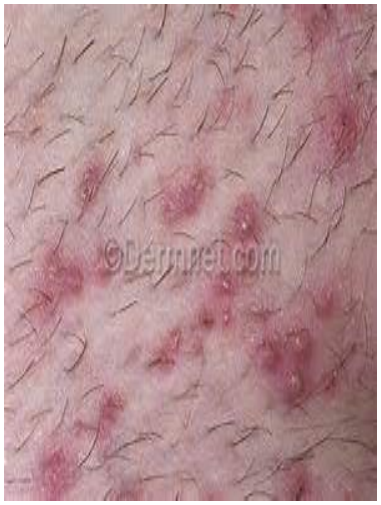
Protein A

- Found in the cell wall of S. aureus
- Has the ability to **bind the Fc receptor of IgG** to block phagocytosis

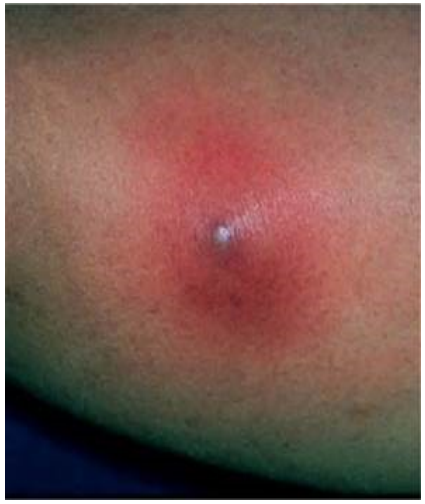
Enzymes

- **Coagulase-** helps prevent phagocytosis
- **Protease**-capable of destroying tissue.
- **Hyaluronidase**-enzyme hydrolyzes hyaluronic acid present in the intracellular ground substance that makes up connective tissue.
- **Lipase**-Acts on lipids present on the surface of the skin. Capable of destroying tissue.

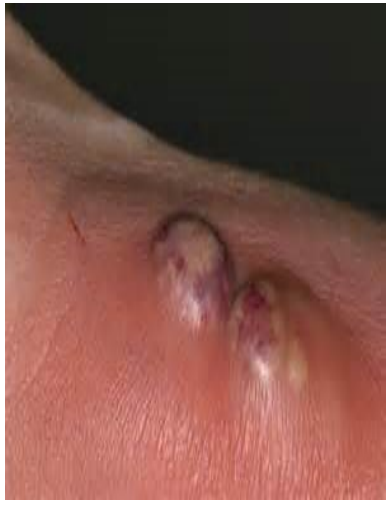
Infections caused by S. aureus: Skin and wounds Infections



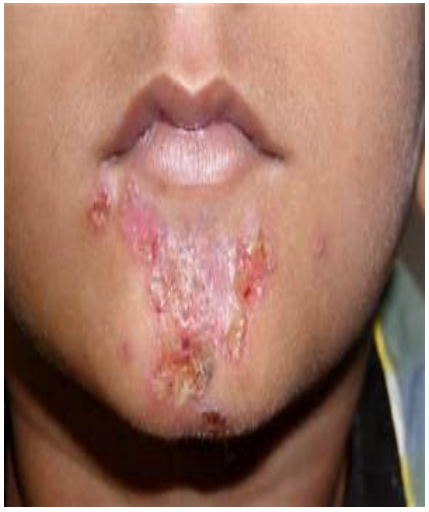
Folliculitis



Furuncles (boil)



Carbuncles (cluster of boils)

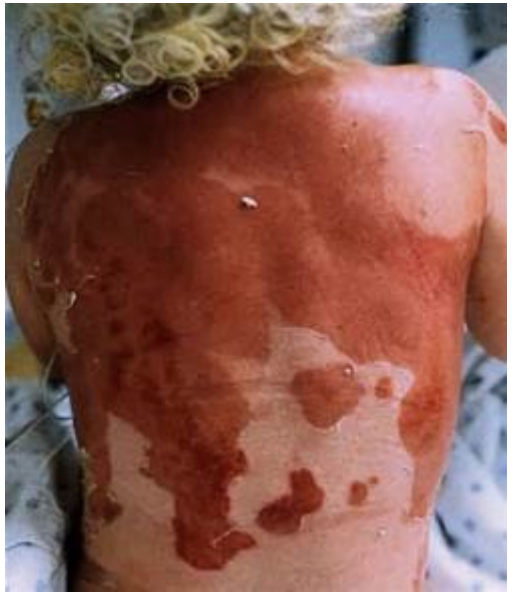


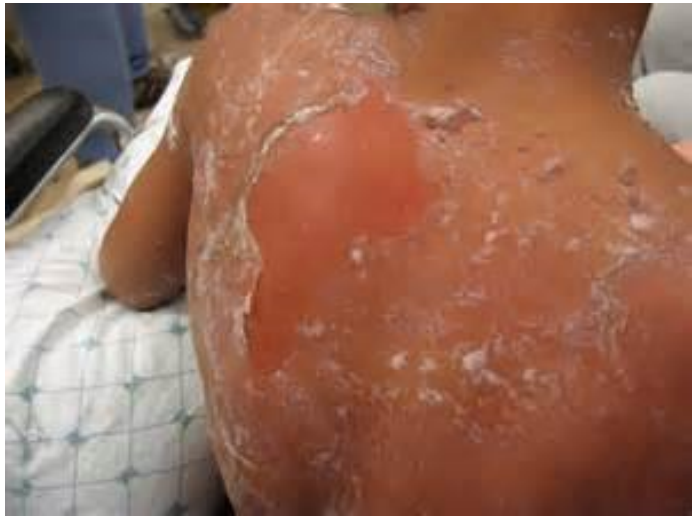
Bullous Impetigo

- **Folliculitis-** mild inflammation of a hair follicle or oil gland
- **Furuncles** (boil)- large, raised, superficial abscesses
- **Carbuncles** (cluster of boils)- larger, more invasive lesions develop from multiple furuncles.
- **Bullous Impetigo**-pustules are large and surrounded by a small zone of erythema. Highly contagious easily spread by direct contact.

Infections caused by S. aur: Scalded Skin Syndrome (SSS)

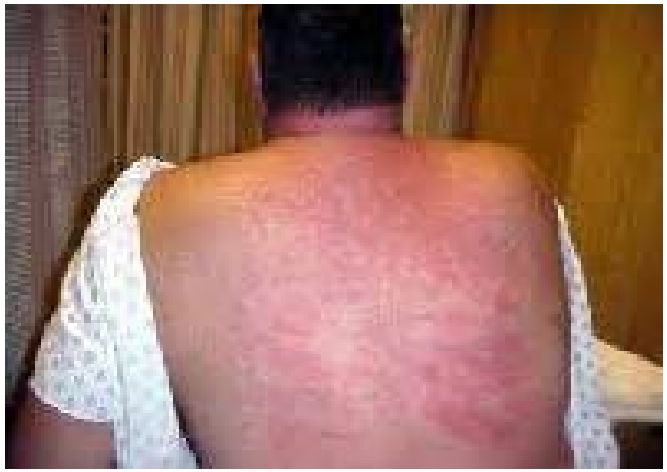
- SSS is bullous exfoliative dermatitis
- Can be anywhere from a few skin lesions to Ritter's disease which is extensive blisters affecting 90% of the body
- The exfoliative/epidermolytic toxin is secreted by the kidneys.

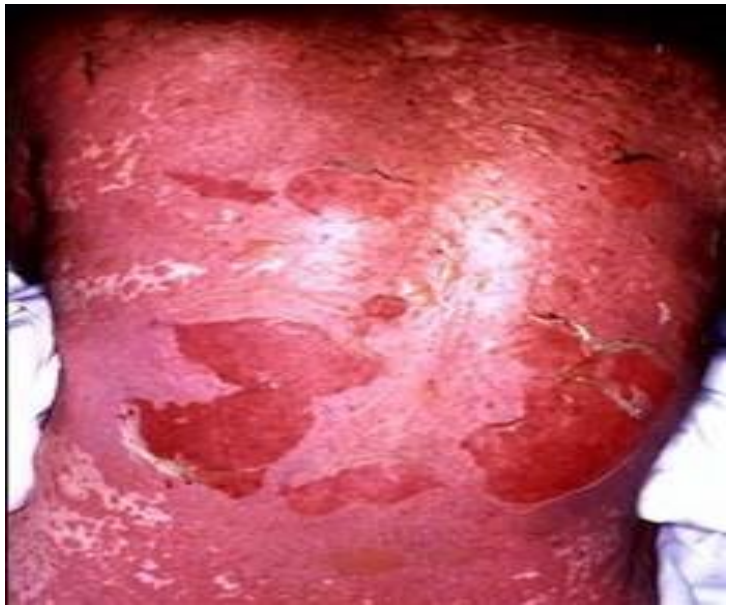




Infections caused by S. aur:
Toxic Epidermal Necrolysis (TEN)

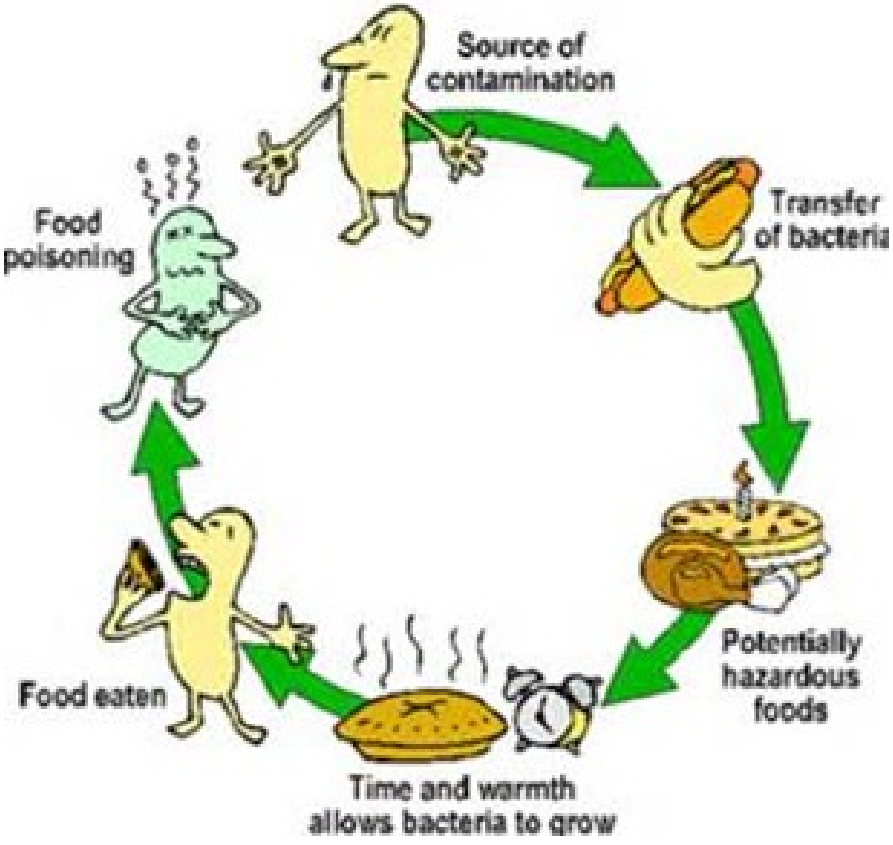
- Symptoms appear to be due to a hypersensitivity reaction.
- Most common cause is drug induced. Some cases linked to infections and vaccines.
- Similar presentation as SSS but treatment is different- treated with steroids and stop suspected drug.





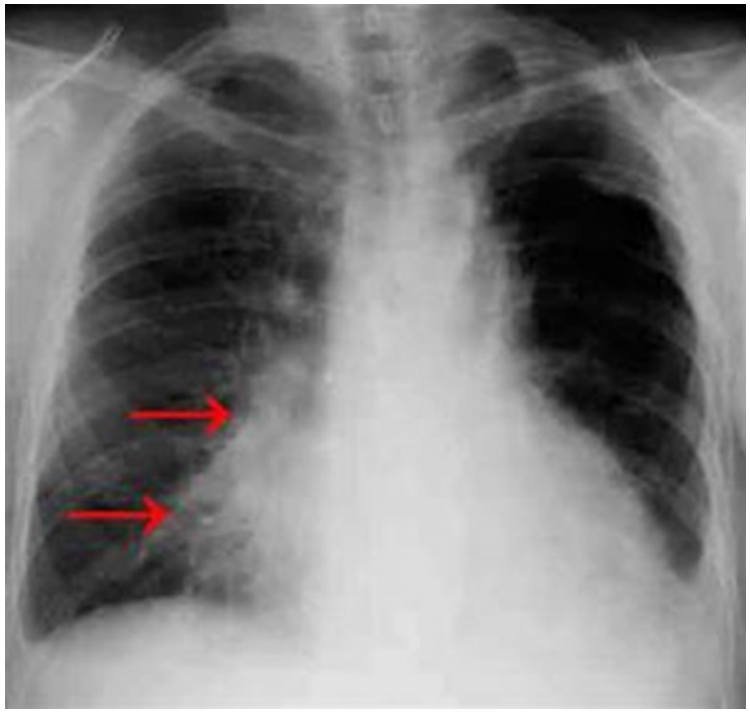
Infections caused by S. aur:

1. Enterotoxins A, D, and B
2. Source is usually infected food handler and food is often improperly stored which allows growth of the organism
3. Food kept at room temp are more commonly associated with food poisoning.
4. The enterotoxins do not cause any detectable odor, change in appearance, or taste in the food.



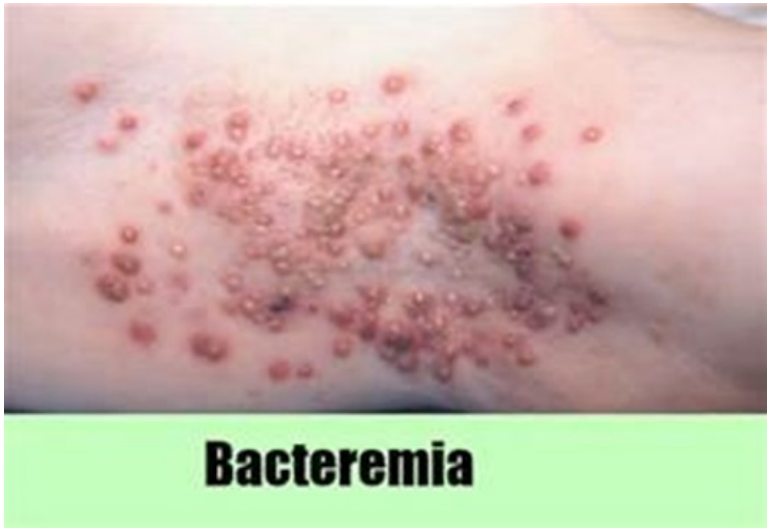
Infections caused by S. aur: Staphylococcal pneumonia

- Can occur secondary to influenza viral infection
- Rarely occurs but when it does it occurs mostly in infants and immunocompromised adults with high mortality rates.



Infections caused by S. aur: Staphylococcal bacteremia

- Leads to secondary pneumonia and endocarditis among iv drug users
- Organisms gain entrance to the bloodstream via contaminated needles or from lesions on the skin.



Infections caused by S. aur: Staphylococcal osteomyelitis (Septic arthritis)

- Occurs secondary to bacteremia
- S. aureus is present in a wound and gains entrance to the blood where the bacteria may lodge in the shaft of the long bones and establish an infection.
- S. aureus may or not be recovered from an aspirated joint fluid

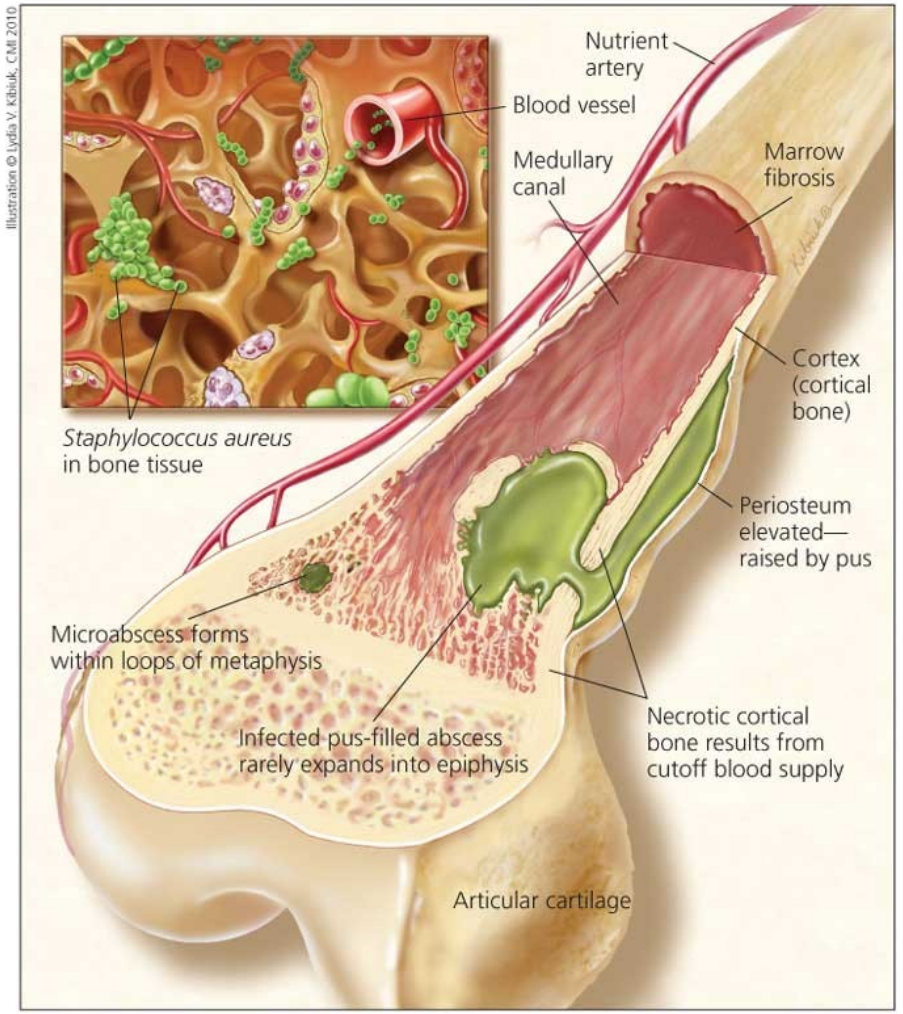


Figure 1 – *This diagram shows hematogenous osteomyelitis of a tubular bone in a child.*

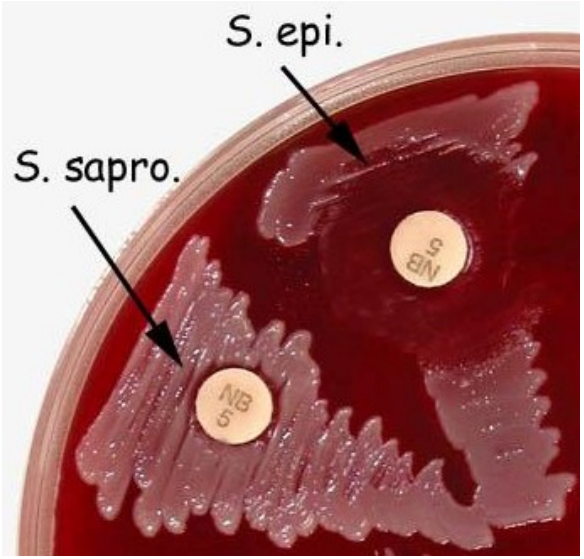
Coagulase Negative Staph

Coagulase Negative Staphylococcus: *Staphylococcus epidermidis*

- After 18-24 hours incubation on SBA, colonies are usually small to medium size, nonhemolytic, gray-to-white colonies.
  - Note: some can be weakly hemolytic.
- Predominantly hospital acquired infection

Coagulase Negative Staphylococcus: Staphylococcus saprophyticus

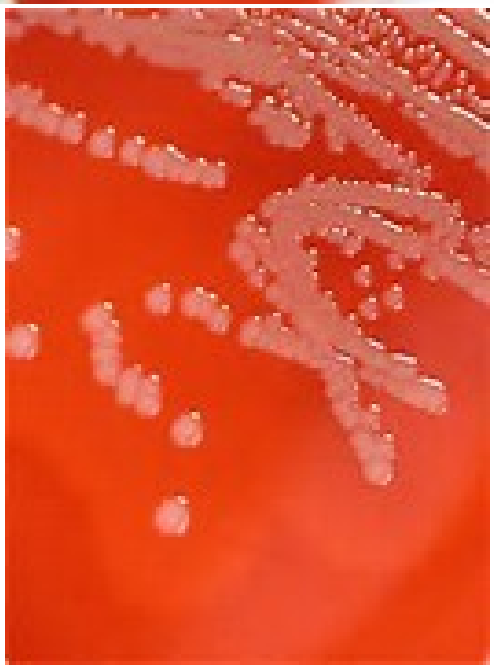
- After 18-24 hours incubation on SBA, colonies are slightly larger than S. epidermidis and about 50% of the strains produce a yellow pigment.
- Associated with UTIs in young women
- Species adheres to the epithelial cells lining the urogenital tract



S. Sapro is novobiocin **resistant** where other CNS (S. epi) are novobiocin **sensitive**

Coagulase Negative Staphylococcus: *Staphylococcus lugdunensis*

- After 18-24 hours incubation on SBA, colonies are often hemolytic and medium sized.
- Positive clumping factor, negative tube coagulase tests
- Known to contain the *mec*A gene which encodes oxacillin resistance
- Endocarditis by S. lugdunensis is particularly aggressive, requires valve replacement, and has a high mortality rate.



Coagulase Negative Staphylococcus: Staphylococcus haemolyticus

- After 18-24 hours incubation on SBA, colonies are medium sized with moderate to weak hemolysis. Can produce variable pigments
- Has been known to be vancomycin resistant in some isolates.

Other Coagulase Negative Staphylococcus

- Most species of CNS are normal flora in humans and animals.
- CNS are often considered contaminants, they can become a problem because the develop resistance to multiple antibiotics, they can form biofilm on implanted medical devices, and they can spread nosocomially due to medical procedures.
- S. warneri, S. capitis, S. simulans, S. hominis, and S. schleiferi are opportunistic pathogens causing endocarditis, septicemia, and wound infections.

Micrococcus Spp.

Micrococcus

- Micrococcus is found in the environment and normal skin flora.
- Can be confused with Staphyloccoci.
- Opportunistic infection.
- Staphylococci ferment glucose while micrococci do not ferment glucose

Test	Staphylococci	Micrococcus
Gram stain	GPC CL	GPC CL
Hemolytic	+/-	-
Coagulase Test	+/-	-
Catalase	+	+
Modified oxidase	**Negative**	**Positive**
Pigment on SBA	**None**	**Very Yellow**
Lysostaphin test	**Susceptible**	**Resistant**
Bacitracin	**Resistant**	**Sensitive**
Furazolidone	**Sensitive**	**Resistant**

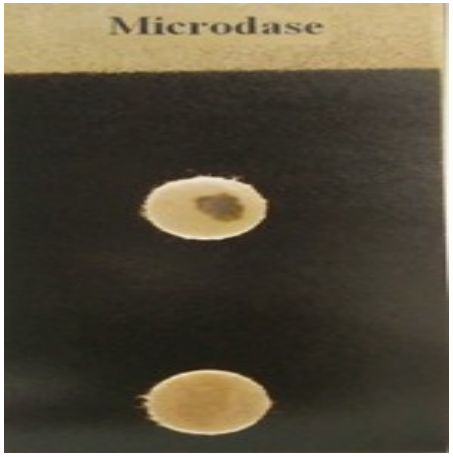
Micrococcus spp

Clearing

Showing positive reaction **(lysostaphin susceptible--Staph).**

No Clearing

Showing negative reaction **(lysostaphin resistant--Micrococcus).**



Modified oxidase test (Microdase Disks) is a paper disk impregnated with oxidase reagent in dimethyl sulfoxide (DMSO). In the presence of atmospheric oxygen, the oxidase enzyme reacts with the oxidase reagent and cytochrome C to form the colored compound, indophenol. Blue-purple color is positive.

Rothia mucilaginosa (formerly Stomatococcus)

- Found in the normal flora of the respiratory tract
- Referred to as the "sticky staph"
- Weakly catalase positive
- Microdase negative

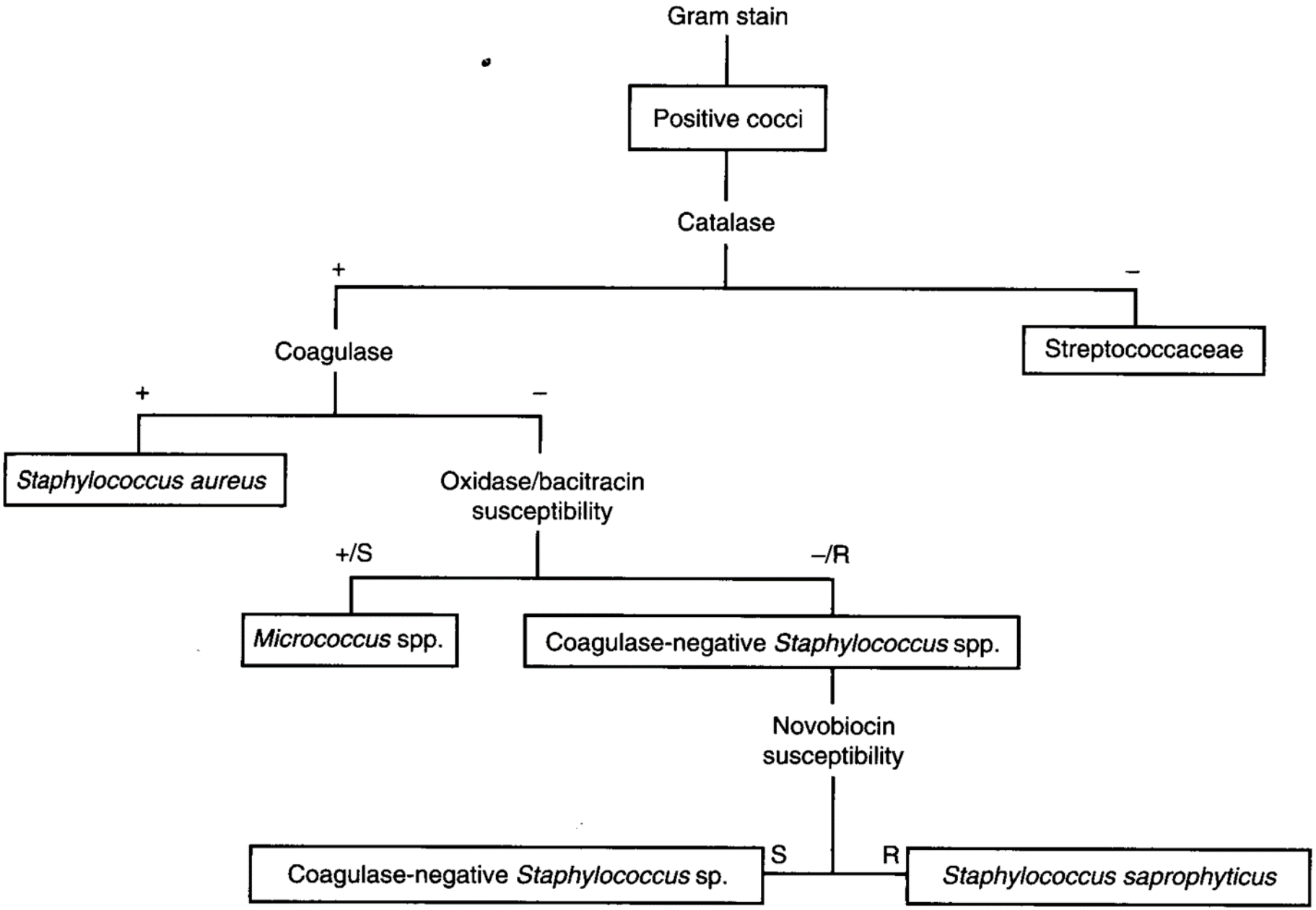
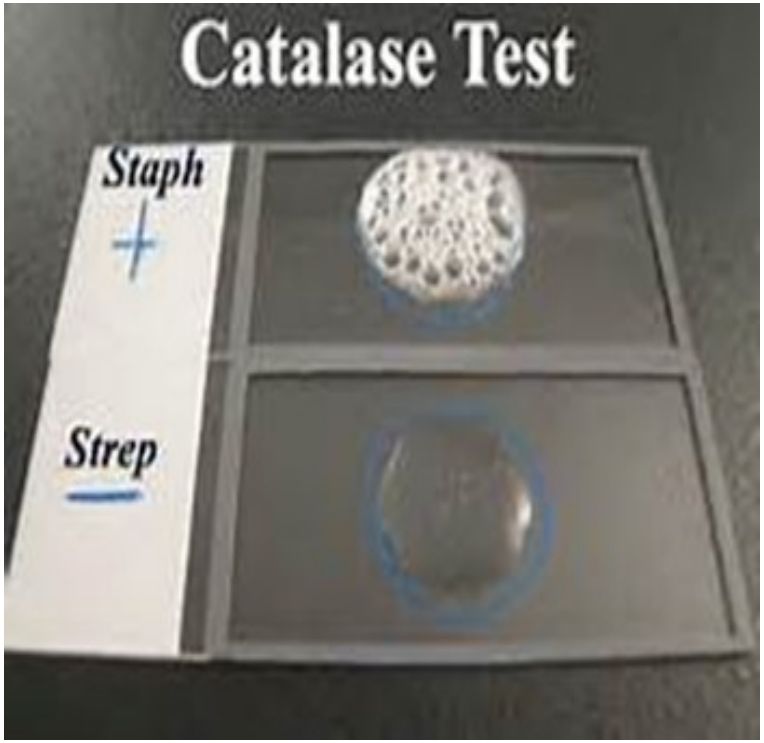


FIGURE 14-8 Schema for the identification of staphylococcal species. NOTE: Other *Staphylococcus* spp. that are coagulase positive besides *S. aureus* include *S. schleiferi* and *S. lugdunensis* (which can be slide-test positive), *S. intermedius,* and *S. hyicus* (tube positive and slide positive).

Biochemical Testing for Staphylococci Identification

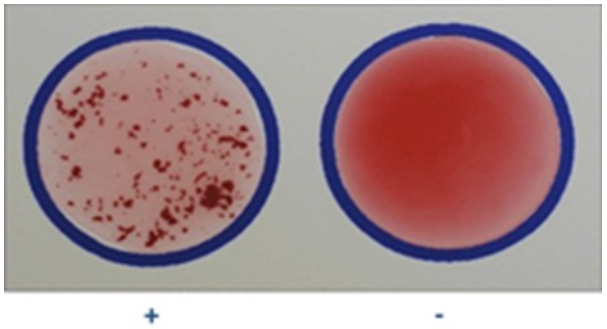
ID Methods: Catalase

- All staphs are catalase positive.
- Catalase Test – enzyme catalase produced by the bacteria breaks down hydrogen peroxide into water and O2.
- Used mostly for gram positive organisms.
- Can't do directly on a blood plate because RBCs contain catalase.



ID Methods: Staphyloslide Test

- Rapid agglutination test-gives results in less than 1 minute.
- Used to differentiate the staphylococcal species.
- Test uses latex sensitized with fibrogen and IgG to detect clumping factor and protein A which are biochemically characteristic of S. aureus
- PASTOREXTM STAPH-PLUS is designed to detect:
  - The fibrinogen affinity factor (aka bound coagulase or “clumping factor”)
  - Protein A
  - Capsular polysaccharides (used by MRSA to mask clumping factor and protein A)
  - Culture isolates of S. aureus are mixed with the latex reagent on a slide



Agglutination present within 30 seconds=S. aureus
No agglutination= CNS .

Note: Some strains of S. Saprophyticus, S. sciuri, S. lugdunensis, and Micrococcus can produce a positive Staphyloslide result but are tube coagulase negative

ID Methods: Tube Coagulase Test

- Used to differentiate the Staphylococcal species by detecting staphylocoagulase (free coagulase).
- Staphylocoagulase is an extracellular molecule that causes a clot to form when bacterial cells are incubated with plasma.

Coagulase positive Staph
S. aureus (PYR-)
S. intermedius (PYR +) **

- Found as a pathogen in animals
- May be found in wound infections from dog bites

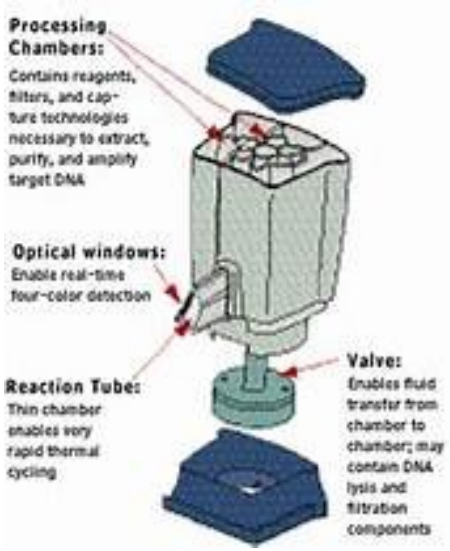
S. delphini
S. lutrae
some strains of S. hyicus **
** = can be found in animals too

S. Aureus coagulase troubleshooting

- *S. aureus* produces 2 forms of coagulase: bound and free
- This enzyme is able to clot rabbit plasma with EDTA (not citrated plasma) is usually used
- Clumping factor converts fibrinogen to fibrin which precipitates onto the cell surface causing agglutination (slide coagulase test).
- Free coagulase is an extracellular enzyme that only clots plasma in a tube
- Not all Staph aureus produce bound coagulase but they all do have free coagulase
- If Staph aureus is suspected, a negative slide test must be confirmed with a tube coagulase test.
- Any degree of clotting in a 3-4 hour period is regarded as a positive result.
- Many weak enzyme-producing strains will coagulate the plasma only after 24 hours of incubation
- ***Some stains of *S. aureus* produce staphlokinase which may lyse clots. If the tubes are positive at 4 hours and left to incubate for another 20 hours, false-negative results may occur***

Automated/Molecular Methods

- There are many automated and rapid id biochemical systems available but their accuracy varies.
- Real time polymerase chain reaction (PCR) is available to rapidly ID MRSA and MSSA.
  - Some hospitals have standard screening programs for admissions which is used to decrease the spread of nosocomial MRSA infections among inpatients.
  - Surgical patients are screened to see if they are carriers of MSSA or MRSA.
- MOLDI-TOF is a mass spectrometer that is available to ID staphs accurately and rapidly



Susceptibilities

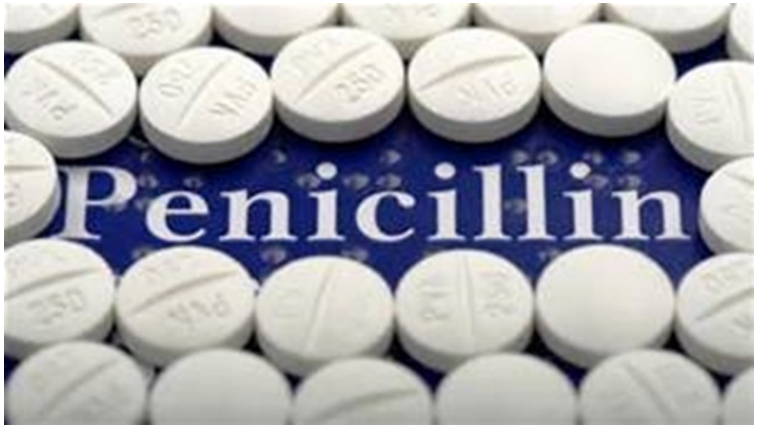
- Testing of coagulase negative staph is dependent on determination if it is a pathogen or contaminant
- S. saprophyticus in urines does not require routine testing because isolates are typically sensitive to antibiotics commonly used to treat UTIs.
- S. aureus and S. lugdunensis usually requires testing

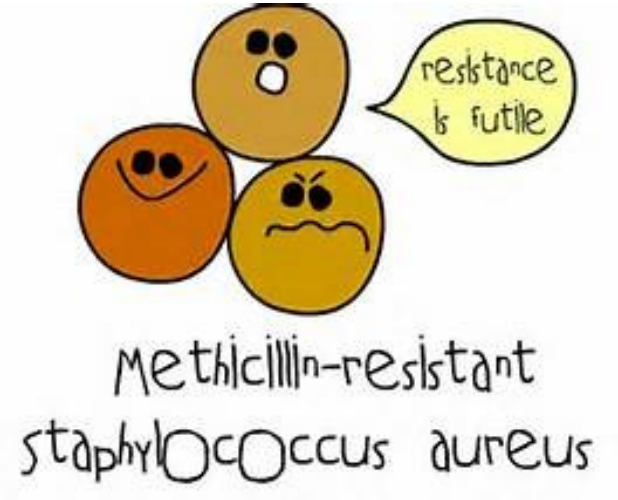
Penicillin

- Is a β-lactam drug—these drugs work by blocking the division of bacteria.

- Staph produce β-lactamases (penicillinases) which break down the β-lactam ring of the penicillin rendering the drug ineffective.

- Use nafcillin or oxacillin to treat—these are penicillinase-resistant penicillin

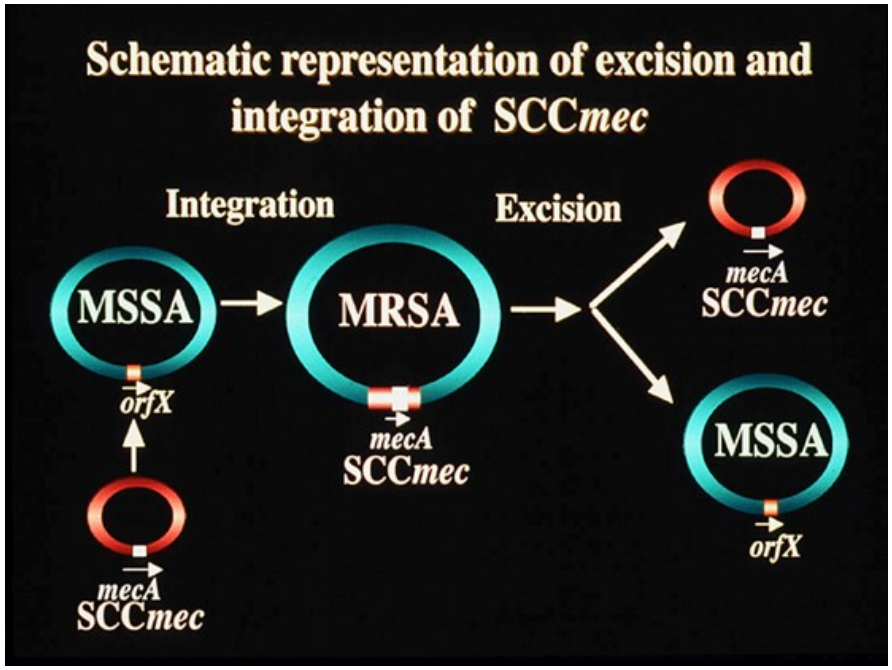




- Staphs resistant to nafcillin or oxacillin are termed methicillin resistant
- Different types of Methicillin-Resistant S. aureus (MRSA)
  - Community-associated MRSA (CA-MRSA)
  - Health care-associated community-onset MRSA (HACO-MRSA)
  - Hospital-associated MRSA (HA-MRSA)
- Vancomycin is treatment of choice

mecA gene & staph aureus

- Cefoxitin is used to detect methicillin resistance.
- mecA gene is carried on a mobile cassette known as SCCmec.
- The gene codes for an unique penicillin-binding protein (PBP) called PBP2a or PBP2', that binds methicillin and thereby promotes bacterial survival by preventing the antibiotic from inhibiting cell wall synthesis

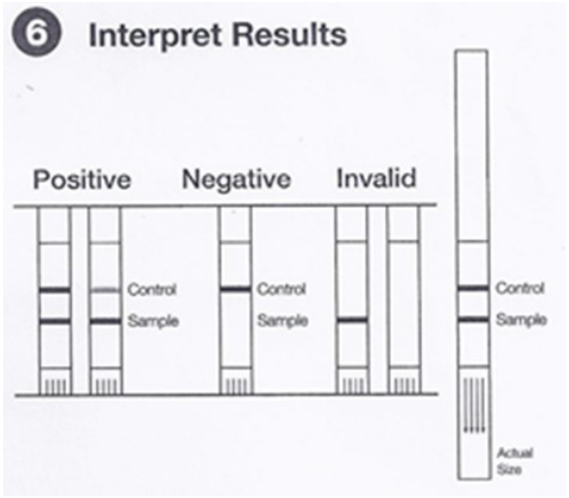


Testing for MRSA

- Agar plates
- Oxacillin-salt agar plates
- Chromogenic selective differential media

Chromogenic agar

- PBP2a testing
- Bench top test used to test S. aureus colonies for the mecA gene.
- Able to give the doctors presumptive MRSA or MSSA result before susceptibilities are ready.



Vancomycin

- Drug of choice for MRSAs
- Vancomycin-intermediate S. aureus (VISA)
  - Difficult to detect so incidences are underreported.
- Vancomycin-resistant S. aureus (VRSA)
  - Isolated from patients undergoing long-term vancomycin treatment.
  - If detected, these isolated need to be confirmed by a reference lab.
- CLSI recommends using vancomycin agar plates to screen for VISA and VRSA

Macrolide Resistance

- Erythromycin and clindamycin are frequently used to treat staph skin infections.
- Erythromycin and clindamycin results are usually the same
- Modified double disk diffusion method test (D-zone test) to check for inducible clindamycin resistance when CC is resistant from E.
  - Inducible clindamycin resistance is detected in vitro only when bacteria are also exposed to erythromycin
  - This can be detected by disk diffusion by placing the erythromycin disk near the clindamycin disk.
  - The bacteria will grow around the erythromycin disk and in the area where the 2 drugs overlap.
  - A zone of inhibition is observed around the clindamycin furthest away from erythromycin disk. The clindamycin zone is flattened look like a letter “D”.