17

Microbiology

Doctor 2018 | Medicine | JU

Sheet ○ Slides

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Gram-positive cocci

-There are two medically important genera of Gram-positive cocci: Staphylococcus and Streptococcus.

-Both Staphylococci and streptococci are nonmotile and do not form

spores.

- How can we differentiate them from each other?

**Both of them have the same size, shape, and gram stain (gram positive)

1)Cluster shape: under the microscope:

Staphylococci appear in grapelike clusters ➔ it likes to maintain a nest, that's why they are bunched up together, and they have enzymes to break what surrounds them.

whereas streptococci grow in chains and try to invade as much as they can.

2)Biochemical test (enzymatic distinguishing): staphylococci produce catalase which converts hydrogen peroxide (H_2O_2) into water and oxygen.

the catalase test differentiates staphylococci which is positive from the streptococci which are negative.

Staphylococcus

- Staphylococci are facultative anaerobes, catalase enzyme gives them ability to live within and without oxygen.

If they want to live with oxygen, they'll have to deal with ROS.

-they are usually part of Normal flora.

- Neutrophils have H_2O_2 that can kill bacteria, unless it has catalase that will survive it. So, catalase is also important pathogenic and virulence factor.

-Species of staphylococcus: aureus, epidermidis, and saprophyticus.

- However, Staphylococcus aureus in pathologic state causes forming of:

1-**abscesses and various pyogenic (pus producing) infection** such as: (endocarditis, septic arthritis, and osteomyelitis) this is due to its ability as a facultative anaerobe (likes to nest, cause local destruction)

2- food poisoning (due to production of a toxin)

3- scalded skin syndrome and toxic shock syndrome (also due to production of two exotoxins).

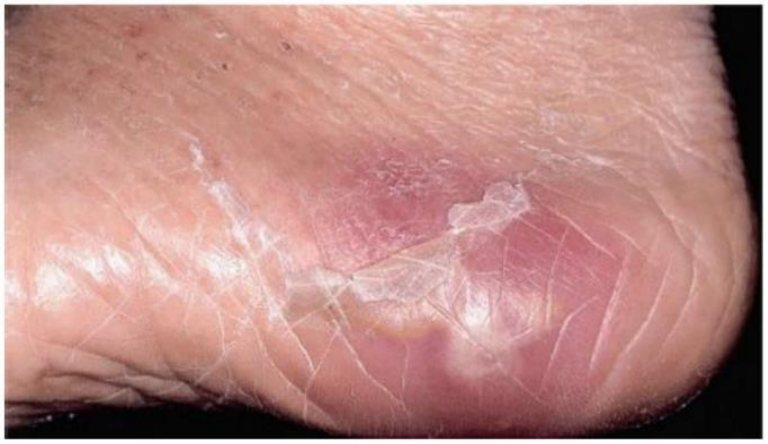
4- one of the most common causes of hospital-acquired pneumonia, septicemia, and surgical-wound infections.

5-It is **an important cause of skin infections**, such as folliculitis cellulitis, and impetigo.

6-It is the most common cause of bacterial conjunctivitis. (Eye rubbing)

- How does abscess seem? (signs)

It is usually erythematous and there is:



Pain, tenderness, and a pus (visible collection of fluid, typically white or yellow) under the skin in the affected area.

- why are abscess mostly found in the outside of the skin?

It happens in both ways (outside in the skin or inside the body), but it favors the least pressure area (in the skin), so it doesn't go frequently to the blood vessels because there is high pressure there.

But in some cases, it can reach the blood especially in toxin mediated infections (**sepsis**).

- **Staphylococcal scalded skin syndrome (SSSS)**

O_o: is a serious skin infection caused by exotoxins produced by bacterium staphylococcus aureus that destroys desmoglein (thus it destroys desmosomes).

This exfoliative toxin causes the outer layers of skin (epidermis) to blister and peel as if they've been doused with a hot liquid for a long time. It happens very quickly and all over the skin and all over the same time.

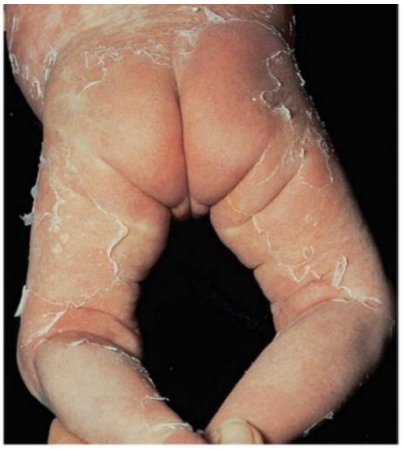


FIGURE 15–2 Scalded skin syndrome. Note widespread areas of "rolled up" desquamated skin in infant. Caused by an exotoxin produced by *Staphylococcus aureus*. (Used with permission from Wolff K, Johnson R (eds): *Fitzpatrick's Color Atlas & Synopsis of*

Folliculitis (where the hair follicle being infected), **Cellulitis, and **impetigo** ➔➔➔ these will be discussed in more details next semester.

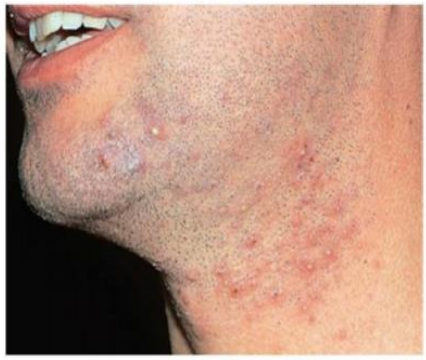


FIGURE 15–3 Folliculitis. Note the multiple, small pustules on the chin and neck. *Staphylococcus aureus* is the most common cause of folliculitis. (Reproduced

other types of staph:

1) Staphylococcus epidermidis: *present in the epidermis*.

It causes endocarditis and prosthetic joint infections.

*Although it is epidermidis, it doesn't cause skin infection as aureus.

*It is the first enemy when foreign bodies are inserted to the body.

(So, it is Specialized in foreign bodies infections)

2) Staphylococcus saprophyticus: *present in female genital tract.*

It causes urinary tract infections. **(So, it is Specialized in urinary tract infections)**

As we said before the staph aureus can be specialized in a lot of things; It has all the enzymes, toxins and capacities. It is specialized in antimicrobial

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* **S. aureus is by far the most important (the chief).**

**S. aureus is distinguished from the others primarily by coagulase production and mannitol test.

-Coagulase is an enzyme that causes plasma to clot by activating prothrombin to form thrombin.

-Thrombin then catalyzes the activation of fibrinogen to form the fibrin clot.

- S. epidermidis and S. saprophyticus are often referred to as coagulase-negative staphylococci (CONS).

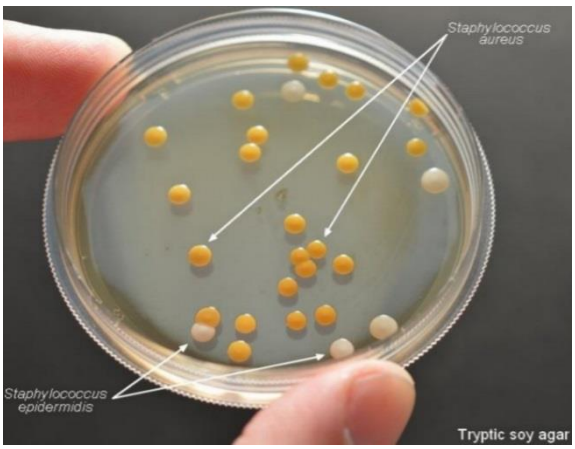
Species	Coagulase Production	Typical Hemolysis	Important Features[1]	Typical Disease
S. aureus	+	β (Plus can use mannitol sugar)	Protein A on surface	Abscess, food poisoning, toxic shock syndrome
S. epidermidis	–	None	Sensitive to novobiocin	Infection of prosthetic heart valves and hips; common member of skin flora
S. saprophyticus	–	None	Resistant to novobiocin	Urinary tract

As shown in the table:

-S. aureus is beta hemolytic (complete lysis of RBCs in the blood agar)

Staphylococcus aureus is able to ferment mannitol but others (coagulase negative Staphylococcus) are not. So, if particular specimen contains S. aureus, it ferments mannitol. (in Mannitol Salt Agar (MSA))

-S. aureus produces a carotenoid pigment called **staphyloxanthin**, which imparts a golden color to its colonies that's why it is called aureus. Other types appear in white color at same agar.



- This pigment is not just for color, bacteria don't have aesthetics, the protein staphyloxanthin enhances pathogenicity of the organism. Staphyloxanthin counteracts the killing effect of superoxide and other reactive oxygen species within neutrophils (now Staph. aureus can escape hydrogen peroxide with catalase and against superoxide with staphyloxanthin).

-More pathogenic bacteria ⟶ Higher ability to escape defenses bodies ⟶ has longer life ⟶ causes more destruction

S. epidermidis does not synthesize this pigment, so its virulence is significantly less that that of S. aureus.

-Hemolysis of red cells by hemolysins-enzymes- produced by S. aureus, this is how the bacteria acquires its iron required for growth, by destroying RBCs and taking their iron. IT IS aggressive.

-S. aureus and our bodies fight for iron. When you have an infection, most of acute phases protein come to area to capture iron and bring it back to the liver, so bacteria try to get it before this happens, HOW?

-Iron is recovered by bacteria and utilized in the synthesis of cytochrome enzymes used to produce energy.

-More than 90% of S. aureus strains contain plasmids that encode β lactamase, the enzyme that degrades many (but not all) antibiotics such as penicillin (*so we can't use penicillin G*). Some strains contain 'ESBL' enzyme which resists almost all antibiotics.

A β-lactamase–resistant penicillin was manufactured. (ويا فرحة ما تمت) ➔ the bacteria was capable to get over it.

• Some strains of S. aureus are resistant to the β-lactamase–resistant penicillins, such as methicillin and nafcillin, this was due to the change of the structure of penicillin-binding protein (PBP) in their cell wall.

Penicillin-binding proteins (PBPs): are bacterial proteins that bind to penicillin and other antibiotics of the β-lactam class. Penicillin-binding proteins are generally enzymes involved in peptidoglycan biosynthesis, having essential roles in bacterial cell wall biosynthesis and without it there is no peptidoglycan layer and the cell wall will be destroyed. PBPs bind β-lactam antibiotics because their chemical structure is similar to that of the sugar–amino acid backbone that forms peptidoglycan.

• Genes on the bacterial chromosome called mecA genes encode these altered PBPs.

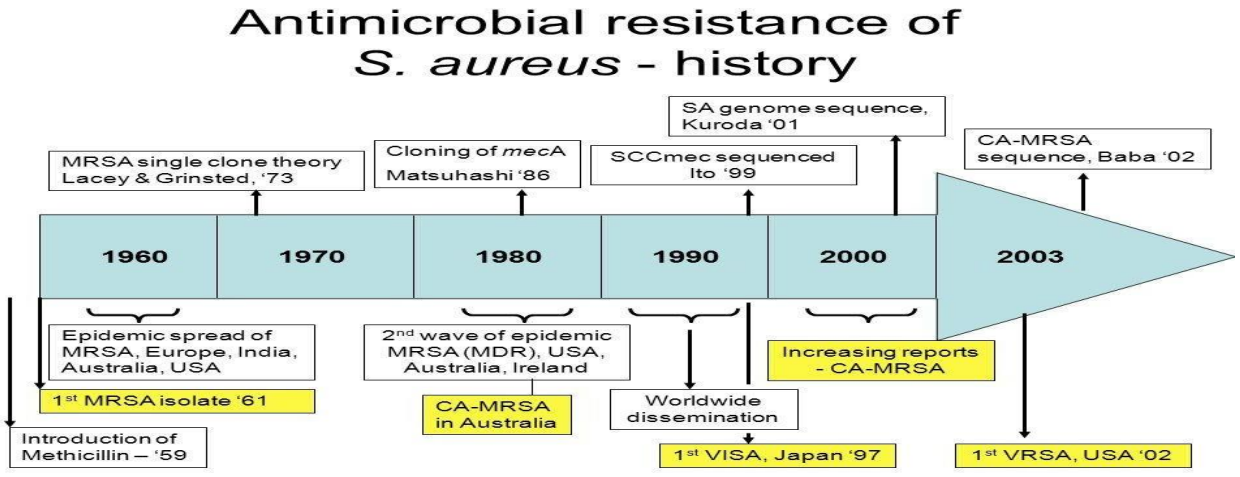
• These strains are commonly known as methicillin-resistant S. aureus (MRSA).

MRSA currently accounts for more than 50% of *S. aureus* strains isolated from hospital patients in the United States.

Strains of *S. aureus* with **intermediate** resistance to **vancomycin** **(VISA)** and with **full** resistance to **vancomycin** **(VRSA)** have also been detected. Vancomycin is the next stero-drug going to be used for penicillin resistance strains.

Vancomycin doesn't work on the same penicillin binding protein, rather it works on the target that will sit on the penicillin binding protein and changes 2 amino acids that are about to work on the PBP. The vancomycin resistance genes are in a transposon on a plasmid and encode the enzymes that substitute **D-lactate for D-alanine** in the peptidoglycan layer. The cassette of genes that encodes vancomycin resistance in *S. aureus* is the same as the cassette that provides vancomycin resistance in enterococci.

Remember// transposon is a way of horizontal gene transfer element.



Looking at one pattern, when examining resistance over 1 or 2 decades it becomes a considerable percentage like 10-20% & we should be afraid for the next decade from its increasing even more.

Important cell wall components and antigens in S. aureus:

(1) Protein A:

Protein A (which is a major protein in the cell wall) is an important virulence factor, it inactivates complement activation by binding to the Fc portion of IgG at the complement-binding site.

Consequently, no C3b is produced, and the opsonization and phagocytosis of the organisms are greatly reduced. Even if the bacteria was captured it has mechanisms that allows it to escape the neutrophilic destruction.

Protein A is used in certain tests in the clinical laboratory because it binds to IgG and forms a "coagglutinate" with antigen–antibody complexes. The coagulase-negative staphylococci do not produce protein A.

(2) Teichoic acids:

Teichoic acids, which are polymers of ribitol phosphate, promote two main functions: 1- the adherence of the staphylococci to mucosal surfaces.

2-working similar to endotoxins, Lipoteichoic acids play a role in the induction of septic shock by inducing cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) from macrophages.

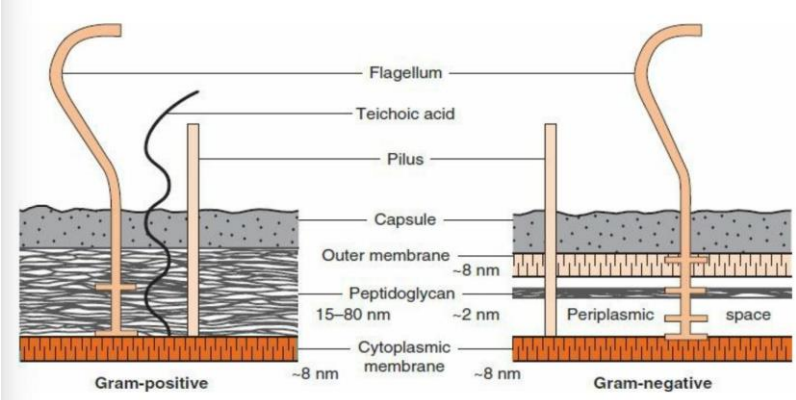


FIGURE 2–4 Cell walls of gram-positive and gram-negative bacteria. Note that the peptidoglycan in gram-positive bacteria is much thicker than in gram-negative bacteria. Note also that only gram-negative bacteria have an outer membrane containing endotoxin (lipopolysaccharide [LPS]) and have a periplasmic space where β-lactamases are found. Several important gram-positive bacteria, such as staphylococci and streptococci, have teichoic acids. (Reproduced with permission from Ingraham JL, Maaløe O, Neidhardt FC. *Growth of the Bacterial Cell*. Sinauer Associates; 1983.)

(3) Polysaccharide capsule:

It is adherent to the cell membrane and is presented above the cell wall; it gives resistance against phagocytosis. It's an important virulence factor. There are 11 serotypes based on the antigenicity of the capsular polysaccharide that are important for the diagnosis, but types 5 and 8 cause 85% of infections. Some strains of *S. aureus* are coated with a small amount of polysaccharide capsule, called a microcapsule. The capsule is poorly immunogenic, which has made producing an effective vaccine difficult which is a plus for the S. aureus & negative for us.

(4) **Surface receptors:** for specific staphylococcal bacteriophages permit the "phage typing" of strains for epidemiologic purposes. Teichoic acids make up part of these receptors.

(5**) The peptidoglycan of *S. aureus:*** has endotoxin-like properties (i.e., it can stimulate macrophages to produce cytokines and can activate the complement and coagulation cascades). This explains the ability of *S. aureus* to cause the clinical findings of septic shock yet not possess endotoxin.

Transmission

Humans are the reservoir for staphylococci. Health workers are exposed to accept and donate bacteria frequently thus they need to be disinfected (decolonized) repeatedly; especially when dealing with an immune compromised patient. Regular people encountering patients in hospital who might be prone to illness (newborn, immune compromised) are also given decolonization with nose antiseptic cream.

Decolonization of the body is done by putting a tube in the nose (**nose antiseptics**) because nose is the main colonization site of colonization of *S. aureus*.

The skin, especially of hospital personnel and patients, is also a common site of *S. aureus* colonization. Hand contact is an important mode of transmission. Therefore, handwashing is important to reduce transmission.

S. aureus is also present in the vagina flora of approximately 5% of women, this predisposes them to toxins that are inserted to the uterine blood resulting in toxic shock syndrome with limited symptoms (uterine colonization); making it harder for the doctor to reveal the main site of infection.

Additional sources of staphylococcal infection are shedding from human lesions and sharing clothing articles with infected people (such as towels and clothing contaminated).

More things that make Staphylococcal infection easier:

-Contaminated environment ⟶ more S. aureus diseases.

-Compromised immunity: such as reduced humoral immunity

-Chronic granulomatous disease (CGD), a disease characterized by a defect in the ability of neutrophils to kill bacteria

-Intravenous catheters can help in S. epidermidis to enter from skin to bloodstream.

Pathogenesis:

• Staphylococcus aureus: causes disease in two main mechanisms,

1) toxin mediated and 2) by inducing pyogenic inflammation (local tissue damage).

The typical lesion of S. aureus infection is an abscess. How can abscess be made?

S. aureus has degradative enzymes for each component of our tissues. **Proteases, Lipases, Nucleases, Hyaluronidases,** that degrade **proteins, lipids, nucleic acids, ECM components** respectively**.** It utilizes coagulase to stop blood (and immunity cells) from coming to the tissue. It also has a fibrinolysin (called staphylokinase) to use when needed! This whole process contributes to the liquefactive necrosis that we call abscess.

NOTE: Staphylokinase had been used clinically for a long time to break down clots before TPA (Tissue Plasminogen Activator) drugs were found.

Now let's talk about toxins ☠

KEEP IN MIND:
Not all S. aureus have all toxins, it depends for each bacterium on what it has seen in its life!
Some of them have already faced a bacteriophage so they obtained a certain toxin, and so on.

1) Enterotoxin: It's a super antigen, which activates macrophages and T cells indiscriminately (with no specificity), releasing large amounts of IL-1 and IL-2. Luckily, this toxin is ingested only, so it only affects GI tract.

The normal scenario of this infection: A worker in 'Shawerma' restaurant can be colonized with enterotoxin producing bacteria. This worker doesn't stick to hygienic rules and while working on your meal, this enterotoxin is transferred directly to it and unfortunately, this cut meat isn't heated properly so the toxins remain there.. Bon Appetit :) You'll have in couple of hours, thanks to IL-1 and other mediators, many symptoms which are characteristic of this infection such as: very prominent vomiting and non-bloody diarrhea. Fortunately, it's self-limited so you're going to be ok in the next day. This might look simple to you, but dehydration can be fatal for babies, old people, or non-healthy individuals.

How the vomiting happen?
It is caused by cytokines that stimulate the enteric nervous system and induce it to activate the vomiting center in the

NOTE: ***Enterotoxin is resistant to mild heat, stomach's acid and enzymes and jejunum's enzymes.***

2) Toxic shock syndrome toxin (TSST): The story here is different. You are bleeding (from your skin, nose, or even vagina) so you put cloth, tampons, etc... To stop the bleeding. Unlucky for you, TSST (*which is a super antigen*) is produced from S. aureus in these sites, so the toxin finds its way to the bloodstream, stimulate the release of large amounts of IL-1, IL-2, and TNF, causing a toxic shock (hypotension). The bacteria don't necessarily exist in the blood, just having a contact with blood is enough to do its work. (You can do bacteria culture, have a negative result, and still have toxic shock syndrome)

NOTE: ***Not all S. aureus carry this toxin, only about 5% to 25% of isolates of S. aureus carry the gene for TSST, and even so, toxic shock will occur only in people who do not have antibody against TSST.***

3)Exfoliatin: It is the cause of "scalded skin" syndrome in young children that we've mentioned before.

Other toxins** include ***alpha toxin and ***P-V leucocidin***. The cytotoxic effect of both these toxins is by poking holes in target cells (pore formation). Alpha toxin targets skin and RBCs mainly, whereas P-V leukocidin targets mainly WBC. (Prominent necrosis is observed. E.g: necrotizing pneumonia and necrotizing severe skin infections).

NOTE: ***-Unfortunately, community strains are the ones that mostly have these two enzymes.***

-The gene encoding P-V leukocidin is located on a lysogenic phage.

How do they work? The toxin itself, is made of two subunits that literally assemble in the cell membrane of target cells and form a pore that destabilized the balance across membrane and cause the cells to leak out their content.

Clinical Findings:

A) Pyogenic Diseases caused by S. aureus:

1-Skin infections: such as Impetigo**,** cellulitis, and folliculitis.

*Severe necrotizing skin and soft tissue infections are caused by MRSA (that produce P-V leukocidin).

*hospital-acquired MRSA causes approximately 50% of all nosocomial S. aureus infections.

2-Septicemia (sepsis):

Can originate from any localized lesion, especially wound infection, or as a result of intravenous drug abuse (introducing skin Staph aureus into the vein without reducing the bioburden with antiseptics).

1/3 of people who develop sepsis DIE! (usually ICU patients).

Sepsis is characterized by three symptoms (Low BP, High Respiratory Rate, Fever + altered mental status).

Sepsis caused by S. aureus has clinical features similar to those of sepsis caused by certain gram-negative bacteria, such as Neisseria meningitidis.

NOTE: ***50% of sepsis cases are caused by gram-positive bacteria, the other half is caused by gram-negative bacteria.***

3- Endocarditis:

May occur on **normal or prosthetic heart valves**, especially right sided endocarditis (tricuspid valve) in intravenous drug users (the pressure in the right side of the heart is less, which allows the bacteria to settle). Patients are most susceptible during dental surgeries. When bleeding occurs, many bacteria enter the bloodstream and travel all the way to the heart.

Coagulase positive Staph aureus, Streptococcus viridans and coagulase negative Staph are the most common causes of infective endocarditis, all are mouth/skin flora.

NOTE: ***Prosthetic valve endocarditis is often caused by S. epidermidis.***

4- Osteomyelitis:

(infection of the bone, it is rare, but very serious infection) and septic arthritis (infection of the joints). Both may be caused by spread of Staph through the blood or by local wounds sites. S. aureus is a very common cause of these diseases, especially in children. It used to be a purely surgical disease pre antibiotic era.

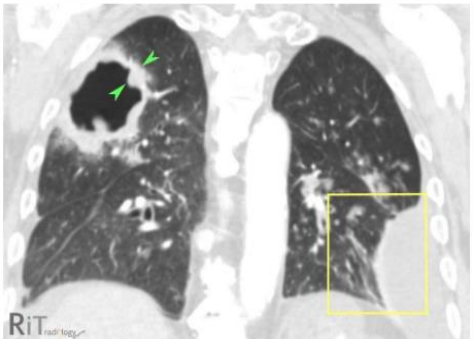
5- S. aureus is the most common cause of **postsurgical wound infections** which are an important cause of morbidity and mortality in hospitals.

6- Pneumonia:

Infection of the lung which can occur in patients after surgery or following viral respiratory infection, especially influenza (bacteria superinfection). Staphylococcal pneumonia often leads to empyema (collection of pus in pleural cavity) or lung abscess (collection of pus inside the lung). In many hospitals, it is the most common cause of nosocomial pneumonia in general, especially **of ventilator-associated pneumonia**.

7- Conjunctivitis; typically presents with **unilateral** burning eye pain, hyperemia of the conjunctiva, and a **purulent discharge**. The organism is transmitted to the eye by contaminated fingers. S. aureus is the most common cause overall, but Streptococcus pneumoniae and Haemophilus influenzae are more common in children.

8- Abscesses can occur in any organ when S. aureus circulates in the bloodstream (bacteremia). These abscesses are often called "metastatic abscesses" because they occur by the spread of bacteria from the original site of infection, often in the skin.

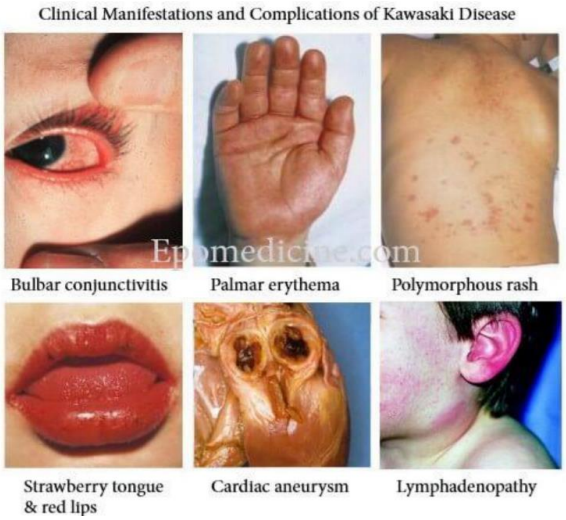


***This figure shows an abscess in the lung caused by S. aureus (green arrow) and empyema (yellow square)**

We've talked about toxin-mediated and local damage diseases of S.aureus. Ther'es one disease that is thought to be immunogenic and it's called Kawasaki Disease.

Kawasaki disease (KD) is a type of vasculitis (inflammation of the blood vessels), that usually targets small and medium-sized blood vessels, especially coronary arteries of the heart. It is the most common cause **of acquired (and not congenital-genetic)** heart disease in children in the United States. The main cause of KD has been unknown for a long time. However, it was discovered recently that certain staphylococci strains produce certain proteins which cause an abnormal immunogenic reaction that affects blood vessels.

Clinically, KD is characterized by **a high fever** of at least 5 days' duration; **bilateral nonpurulent (without pus) conjunctivitis**; lesions of the **lips and oral mucosa** (e.g., strawberry tongue, edema of the lips, and erythema of the oropharynx); cervical lymphadenopathy; a **diffuse** erythematous, maculopapular **rash**; and **erythema and edema of the hands and feet that often ends with desquamation.**



We've talked a lot about S. aureus, what about other species? S. epidermidis and S. saprophyticus?

***S. epidermidis infections are almost always hospital-acquired, whereas S. saprophyticus infections are almost always community-acquired.**

S. epidermidis is part of the normal human flora on the skin and mucous membranes but can enter the bloodstream (bacteremia) and cause **metastatic infections**, especially at the site of implants. It commonly infects **intravenous catheters** and **prosthetic implants** (e.g., prosthetic heart valves [endocarditis], vascular grafts, and prosthetic joints [arthritis or osteomyelitis]). It is also a major cause of sepsis in neonates and of peritonitis in patients with renal failure who are undergoing peritoneal dialysis through an indwelling catheter. It is the most common bacterium to cause cerebrospinal fluid shunt infections.

*Strains of S. epidermidis that produce a glycocalyx are more likely to adhere to prosthetic implant materials and therefore are more likely to infect these implants than strains that do not produce a glycocalyx.

S. saprophyticus causes urinary tract infections, particularly in sexually active young women. It is second to Escherichia coli as a cause of community acquired urinary tract infections in young women.

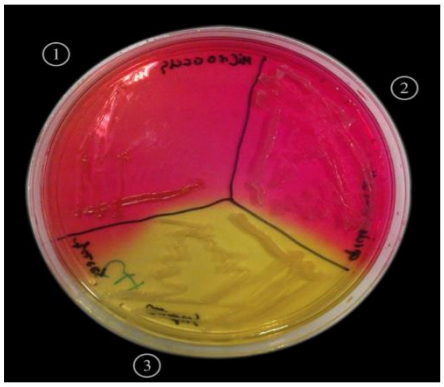
Laboratory Diagnosis:

To distinguish between the two coagulase-negative staphylococci we depend on their reaction to the antibiotic novobiocin where:

→ S. epidermidis is sensitive to novobiocin.

→ S. saprophyticus is resistant to novobiocin.

Remember: S. aureus ferments mannitol and turns the media yellow.



Organism	Type of Pathogenesis	Typical Disease	Predisposing Factor	Mode of Prevention
S. aureus	1. Toxigenic (superantigen)	Toxic shock syndrome	Vaginal or nasal tampons	Reduce time of tampon use
		Food poisoning	Improper food storage	Refrigerate food
	2. Pyogenic (abscess)			
	a. Local	Skin infection (e.g., impetigo, surgical-wound infections)	Poor skin hygiene; failure to follow aseptic procedures	Cleanliness; handwashing; reduce nasal carriage
	b. Disseminated	Sepsis, endocarditis[1]	IV drug use	Reduce IV drug use
S. epidermidis	Pyogenic	Infections of intravenous catheter sites and prosthetic devices	Failure to follow aseptic procedures or remove IV catheters promptly	Handwashing; remove IV catheters promptly
S. saprophyticus	Pyogenic	Urinary tract infection	Sexual activity	

***This table summarizes what we've said about diseases.**

Treatment:

In the US, 90% or more of S. aureus strains are resistant to penicillin G (**so don't give it**). Most of these strains produce β-lactamase. They can be treated with β-lactamase–resistant penicillins **(e.g., nafcillin or cloxacillin),** some cephalosporins, or vancomycin. Treatment with a combination of a β-lactamase–sensitive penicillin (e.g., amoxicillin) and a β-lactamase inhibitor (e.g., **clavulanic acid**) is also useful.

Approximately 20% of S. aureus strains are methicillin-resistant (MRSA) or nafcillin resistant (NRSA). Such organisms can produce sizable outbreaks of disease, especially in hospitals. In this case of these organisms, the drug of choice is **vancomycin**, to which **gentamicin** is sometimes added. **Daptomycin** can be used as well.

Trimethoprim-sulfamethoxazole or **clindamycin** can be used to treat non-life-threatening infections caused by these organisms.

Note that MRSA strains are resistant to almost all β-lactam drugs, except Ceftaroline fosamil.

Quinupristin-dalfopristin(Synercid)-streptogramins is another useful choice. (Linezolid) :)

***The treatment of toxic shock syndrome involves correction of the shock by using fluids and increasing BP, administration of Methicillin/nafcillin, and removal of the tampon or the blocking material.**

*Mupirocin is very effective as a topical antibiotic in skin infections caused by S. aureus. This is what we usually used to decolonize the nasal carriage of the organism in hospital personnel and in patients (especially those with recurrent staphylococcal infections).

A topical skin antiseptic, such as chlorhexidine, can be added to mupirocin (especially for hospital staff known to have staph).

Some strains of staphylococci exhibit tolerance (i.e., they can be inhibited by antibiotics but are not killed). Tolerance happens when the ratio of minimum bactericidal concentration [MBC] to minimum inhibitory concentration [MIC] is very high.) Tolerant organisms should be treated with drug combinations.

***The only treatment for abscess is drainage.**

***Similar to S. aureus, Some S. epidermidis strains are methicillin/nafcillin resistant (MRSE) also due to an altered penicillin-binding protein, in this case the drug of choice is vancomycin, to which either rifampin or an aminoglycoside can be added.**

Removal of the catheter or other device is often necessary. S. saprophyticus urinary tract infections can be treated with trimethoprimsulfamethoxazole or a quinolone (ciprofloxacin).

***There is no vaccine against staphylococci. :(**