12

Microbiology

Doctor 2017 | Medicine | JU

Sheet Slides

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DOCTOR

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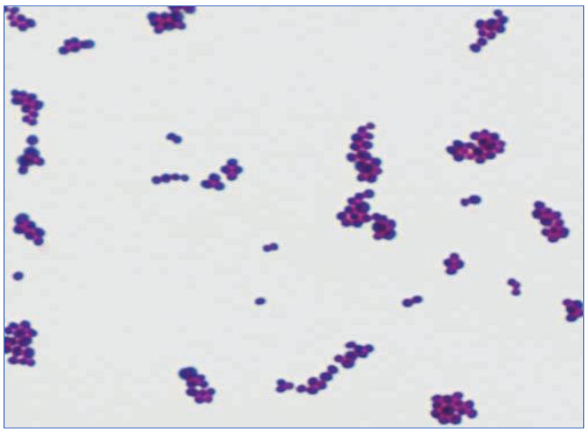
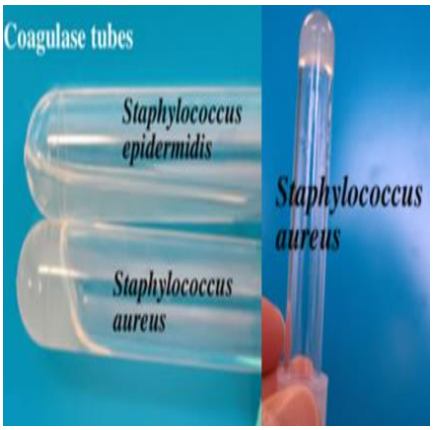
Staphylococci/Structure and Physiology:

- One of the most common and important pathogens. It's so wide spread as we'll find it everywhere. Also, it causes diseases to both humans and animals.

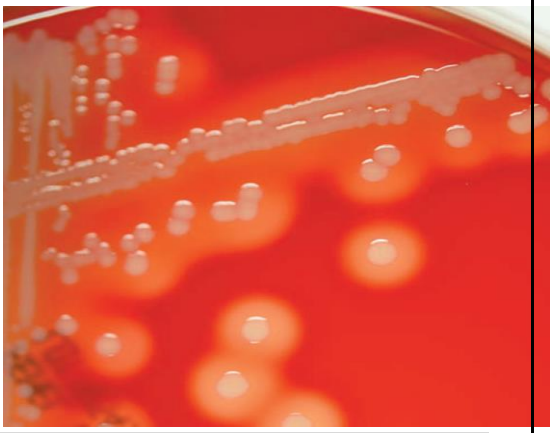


- We have to understand the structure and physiology of it.
- The staphylococci are **gram-positive spherical cells**, about 1 µm in diameter usually arranged in grapelike **irregular clusters, It does not form spores and it is non-motile**(you can't see any flagella)**.** check the following image (S. aureus)



- The four most frequently encountered species of clinical importance are ***Staphylococcus aureus, Staphylococcus epidermidis, Staphylococcus lugdunensis*** **, and** ***Staphylococcus saprophyticus***.
- Staphylococci produces coagulase (like *S. aureus)*, so we call it **coagulase-positive** and it clots plasma in the test we have seen in previous lectures. *(Will be discussed later when we talk about the pathogenesis of S. aureus)*
- Whereas, The **coagulase-negative** staphylococci are normal human microbiota, like *S. epidermidis, S. saprophyticus.* In the test, the plasma remains in the liquid form in this case.

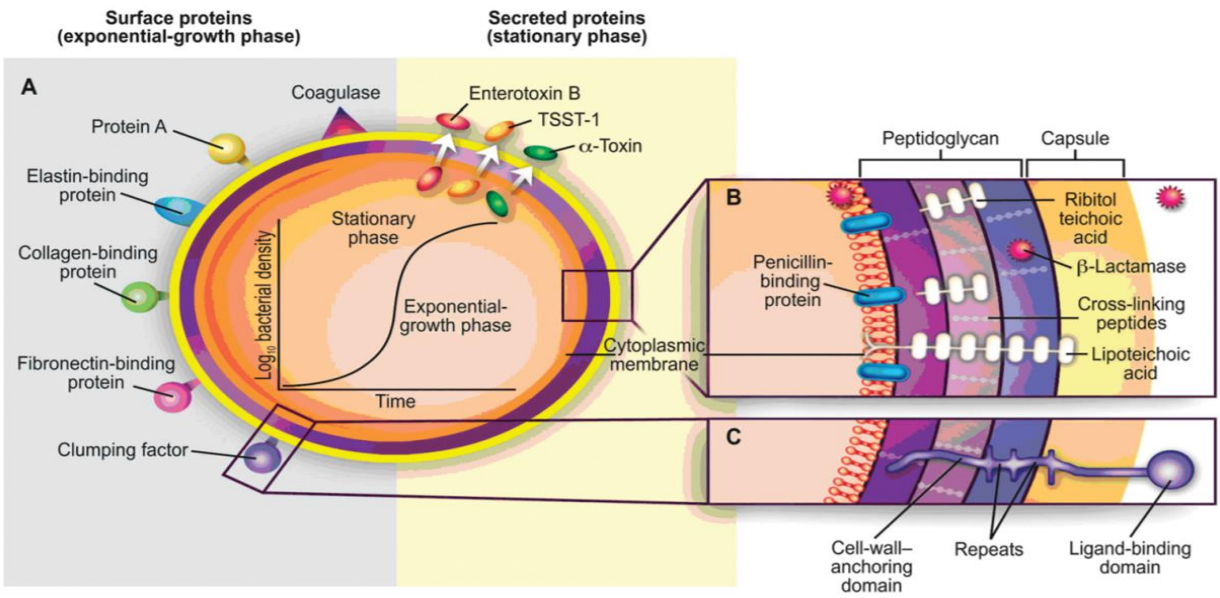
- Now let's talk about colonies, *S. aureus* in normal non-differential nor selective medium(like tryptic soy agar). It forms gray to deep golden colonies (aureus=Golden). So we can differentiate between it and other Staphylococci like *epidermidis*, because they form grayish to whitish colonies. S. aureus can also be found at the start of its growth as white colonies then starts producing the pigment giving the golden pigment of it.



- If you grow *S. aureus* on a blood agar, you will be able to see those white grayish colonies, Also **hemolysis** will be taking place around the colony caused by *S. aureus*. **Also**, these colonies are round,

smooth, raised, glistening(shining), that's because of the polysaccharide layer that surrounds *S. aureus*. **Also**, *S. aureus* has a capsule around it.

- Since that staphylococci is *gram-positive*, it has a cell wall of *thick layer of peptidoglycan.*
- Also, it contain a group of proteins, like *teichoic acid* which help in cross-linking the peptidoglycan and giving strength to cell wall, and can be highly antigenic. So, peptidoglycan can be recognized by immune system and so is lipoic acid.
- **Peptidoglycan in the cell wall activate the immune response (**elicits production of interleukin-1, and opsonic antibodies by monocytes, and it can be a chemoattractant for polymorphonuclear leukocytes, have endotoxin-like activity, and activate complement.(From slides)
- Remember that *Penicillin* prevents transpeptidation of different sheets of peptidoglycan by *penicillin binding protein.*
- There are factors that help Staphylococci adhere to host tissue, like protein A, Elastin-binding protein, collagen binding protein, fibronectin binding protein and clumping factor. Which are referred to as **MSCRAMM (*m*icrobial *s*urface *c*omponents *r*ecognizing *a*dhesive *m*atrix *m*olecules) proteins.** And these are important virulence factors(like clumping factor and protein A).
- *adhesive matrix molecules* like collagen, fibronectin, hyaluronic acid, etc. So adhesion is a big part of pathogenesis of a microbe to attach to host tissue.
- **Clumping factor A** is a fibrinogen-binding protein present on the surface of *S. aureus* that binds to fibrinogen and coats the surface of the bacterial cells with fibrinogen molecules, additionally complicating the recognition process(by forming the fibrin capsule).



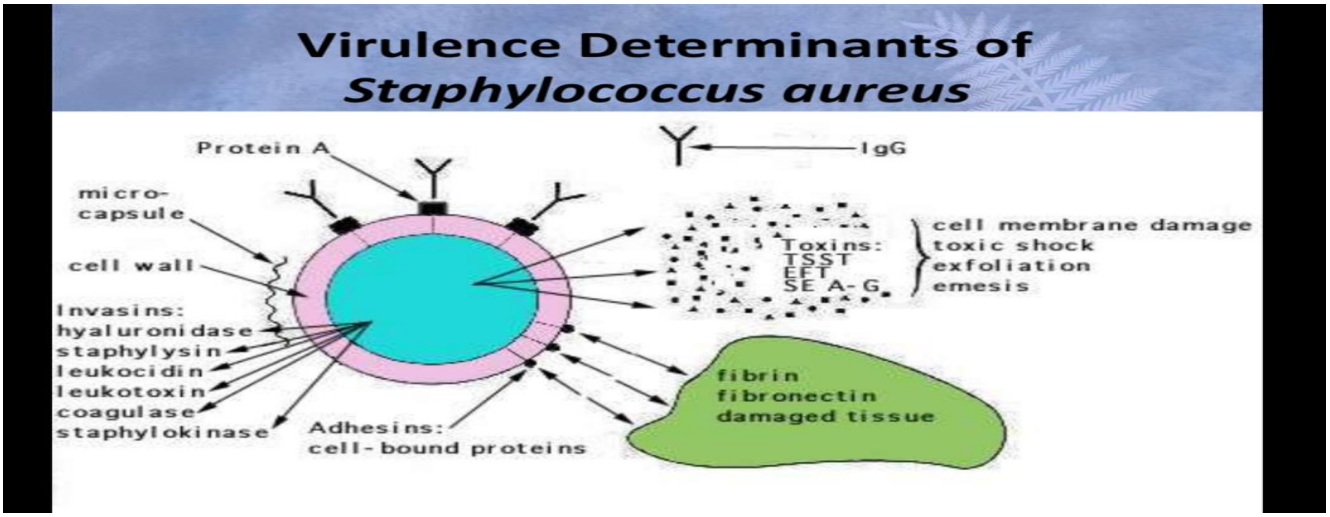
Staphylococci/ Enzymes and Toxins :

- **Coagulase** binds to *prothrombin*; together they form a clot in plasma and become enzymatically active and initiate fibrin polymerization.
- Staphylococci produce **catalase**, which converts hydrogen peroxide into water and oxygen. The catalase test differentiates the staphylococci, which are positive, from the streptococci, which are negative, ***HOW??!***
- **Ans:** Since catalase is used in identification reactions, so you use a catalase reaction if there is a gram positive cocci. So what happen is, you add a bit of streptococci and a bit of staphylococci, and we have hydrogen peroxide. *If we see the formation of Oxygen and water in the form of bubbles, that means it's catalase positive, so we can use that as an indicator that we have staphylococci(catalase positive)*,and so on differentiate it from streptococci(catalase negative).
- Other enzymes produced by staphylococci :
  1. *Hyaluronidase* (which helps in spreading infection by breaking down hyaluronic acid) (spreading factor).
  2. *Staphylokinase* (similar to streptokinase, which dissolves clots and used in medicine) results in fibrinolysis but act much more slowly than streptokinase.
  3. *proteases* 4. *lipases*
  5. β-*lactamase*, breaks down antibiotics that are under group of β-lactam.

Now let's talk about **TOXINS** :

- Toxins are important in the pathogenesis of the bacterial infection
- *S. aureus* produces many toxins, including five **cytolytic or membrane-damaging toxins** (alpha, beta, delta, gamma, and Panton-Valentine [P-V] leukocidin). two **exfoliative toxins** (A and B), numerous **enterotoxins** (A to E, G to X, plus multiple variants), and **toxic shock syndrome toxin-1** (TSST- 1).
- First, *cytolytic or membrane-damaging toxins* target certain lipids within the host cells, they can target RBCs, WBCs and endothelial cells. When they target certain lipids within the membrane and form pore within it and then it leads to death of target cell by *S. aureus.*
- These toxins are found either on plasmids or phages, etc. And they are transferred by Horizontal gene transfer.

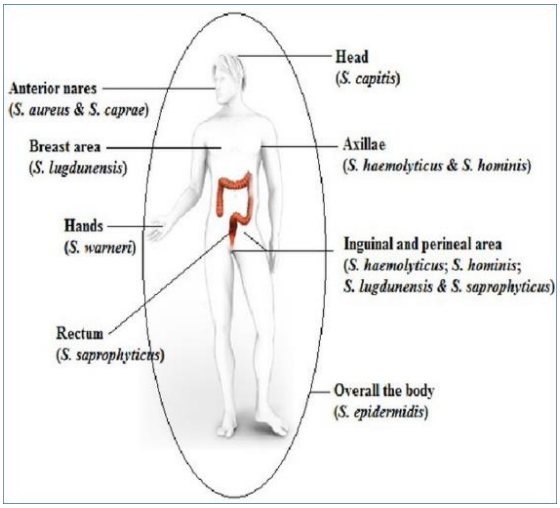
- Exfoliative toxin A, the enterotoxins, and TSST-1 belong to a class of polypeptides known as **superantigens.**
- **Superantigens are a class of antigens(released by S. aureus) that cause non-specific activation of T-cells resulting massive cytokine release, WHY??**
- because it results in less efficient and less specific exaggerated immune response that harms the host.
- Note: We know that antigen is recognized by immune system and elicit an immune response. Whereas, superantigens elicit a more massive immune response than normal antigens.
- So, some toxins targets cell membrane and cause cell damage. Some other toxins



cause exfoliation and toxic shocks.

- Note : *enterotoxins* that target the intestine or gut, causes diarrhea or emesis.

Staphylococci/ Epidemiology:



- Now we have to indicate where do find staphylococci.
- Remember that we separated staphylococci into coagulase positive(S. aureus) and coagulase negative(staph that is part of microbiota of all people/S. epidermidis).
- Staphylococci, particularly ***S epidermidis,*** are members of the normal microbiota of the human skin and respiratory and gastrointestinal tracts.
- the cause of Nasal carriage and presence of ***S aureus*** on hands, it will be easily transmitted between people (occurs in 20–50% of humans), with a **higher incidence** reported for **hospitalized patients**, **medical personnel, *WHY?*** **(**Because in medical

profession, there is a lot of contact between patients/doctors, that helps transmission of this bacteria**)**. Also, there are people with eczematous skin.

- Staphylococci can also be found in ***fomites*** (a general word for anything that can carry pathogens), like clothes, bedlinens, etc. So S. aureus is found in almost everywhere in our bodies, but also it can be found on clothing or any non-living material.
- We have what's called MRSA (Methicillin-resistant Staphylococcus aureus), which is a S. aureus that is resistant to methicillin. Check the following timeline of how this MRSA evolved:
  I. S. aureus was discovered more than a 100years ago.
  II. Then antibiotics were discovered (like penicillin, in 1920s), so we started treating S. aureus successfully with penicillin
  III. Within 10years, the staph became resistant to penicillin. (ONE of the reasons, is that staphs are producing β-lactamase, that can break down penicillin).
  IV. Since that time, we started using another antibiotic called *methicillin*.
  V. Within 10years, first case of MRSA was diagnosed. Then, it started spreading rapidly in susceptible hospitalized patients in the beginnings of 1980s, *dramatically* changing the therapy available for preventing and treating staphylococcal infections.
  VI. MRSA began as a hospital-acquired infection, but has become **community-acquired** as well as **livestock-acquired**.
  VII. years later, we could use ***vancomycin*** to treat this MRSA. But quiet recently, within the past 10/20years a new resistant type of S. aureus, that through horizontal gene transfer. This type is called VERSA(vancomycin-resistant S. aureus) appeared. *(Go back to sheet 8)*

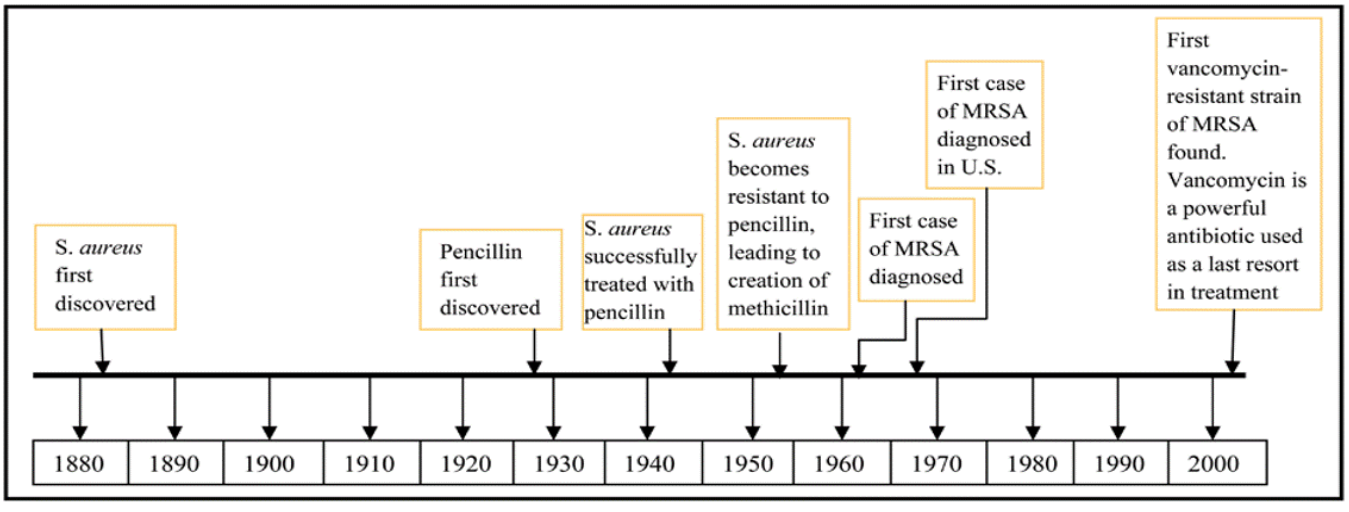
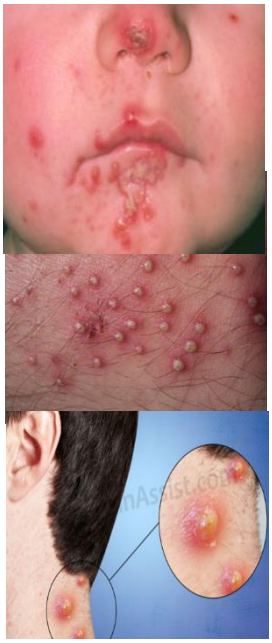


Fig. 1. Timeline of MRSA.

- So we conclude that VERSA is an important pathogen in the hospital, and nowadays coming from community and even livestock.
- People such as (elderly, diabetics, HIV/AIDS)are more susceptible to groups of MRSA, ***WHY ?? (***Because they have a compromised immune systems**)**. Also, hospitalized patients and children are some of the susceptible groups to MRSA, ***WHY?? (***Because there is a lot of contact taking place**)**.

Staphylococci/ Clinical correlations:



1. A localized staphylococcal infection appears as a **"pimple," hair follicle infection, or abscess**.

Remember that S. aureus is the most common skin pathogen. We have many forms of skin staphylococcal infection :

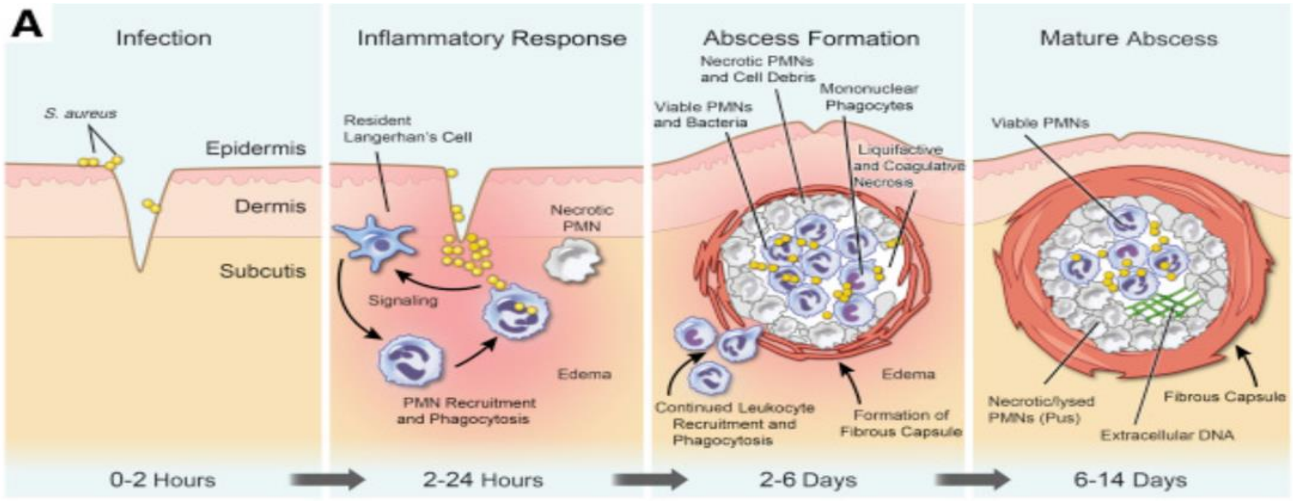
- Impetigo : it's a localized cutaneous infection, characterized by pus filling vesicle with redness behind them, and usually happens in children.
- Folliculitis : if impetigo involved hair follicles.(hair forms it)
- Furuncles or boils : if abscess were large, painful, pus filled cutaneous nodules.
- Carbuncles : if they coalescence (merge) of furuncles, with extension into subcutaneous tissues (forming a bigger region) and evidence of systemic disease (fever, chills, bacteraemia), ***WHY??*** Because Carbuncles are quiet big they cause systemic diseases, so bacteria is not only localized in skin region but it even invade to the plot and cause those systemic diseases, in the case of carbuncles.

How is abscess formed?!

a. S. aureus enters deep layers of the skin, most commonly because of a compromised integrity of the skin.

Note: skin is an important physical barrier in the body, so it must remain as a healthy intact skin. When the integrity is compromised either by a break in the skin, or by some diseases like *Atopic Dermatitis "= Atopic Eczema"* **(**An inflammatory disease that takes place in the skin, so continuous inflammation compromises the integrity of the skin**)**, The skin loses it's barrier function and so on it can be affected by S. aureus.

b. Then S. aureus will enter through the skin and will contact immune cells like PMN (neutrophils), which will be sensed by resident cells (E.g: langerhan cells, which works on antigen presentation mostly).
c. So they take part of the bacteria and antigen, which will be presented on the surface, which results recruitment of immune cells (PMN and phagocytosis/Inflammatory mediators) because they could recognize the antigen found on the surface.
d. Recruitment of more cells to the site of action, AND if the cell didn't clear S. aureus within days, then it can replicate and form a fibrin clot (capsule), which within it, you will find dead and alive immune cells trapped inside the capsule. Also, a lot of necrosis will be taking place.



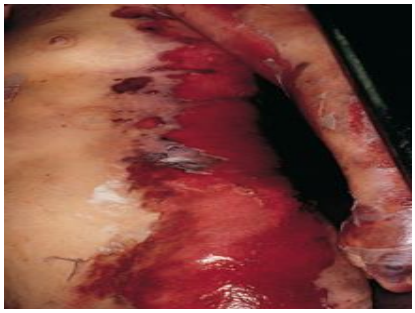
e. In conclusion, we can see that *(macroscopically)* as the *pus*, **(**which represents the debris of WBCs that are dying within fibrous capsule**)**, is found on the surface of abscess will raise a little bit, So it must be cleared or treated with antibiotics.

2. Toxin mediated Diseases :

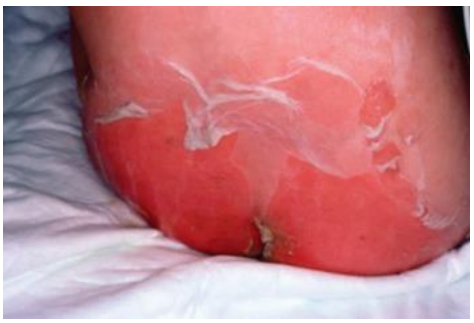
a) **Food poisoning :**

- One of the most important toxins is enterotoxin, which causes foodborne illness, HOW ??
- Ans : By Food poisoning, so S. aureus infect food, (usually cured meats because they are highly salt and S. aureus can survive this environment), and after a while of it's replication it will start producing enterotoxins. So, by eating this food directly, you will be intoxicated by S. aureus toxins. OR, you can heat the food and clear S. aureus but most probably, heat won't kill the toxin.

- Note: Enterotoxins produced by S. aureus is considered as heat stable bacterial toxin (can survive heating at 100o for several minutes), whereas Exotoxins are heat labile.
- Symptoms of enterotoxins include : violent nausea, vomiting, abdominal cramping and diarrhea, which can be observed within (1-8) hours (short incubation period).
- There is a rapid convalescence (recovery) from intoxication, so there is no need for using antibiotics.



b) Staphylococci scalded skin syndrome :



- Is a condition which predominantly affects **infants** and younger **children** and causes a spectrum of skin lesions.
- It's represented by that epidermidis or inner layer of the skin can easily be sloughed off.
- Caused by **exfoliative toxins** of S. aureus
- Disseminated desquamation of epithelium in infants; blisters with no organism or leukocytes.*(From slides)*

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c) Toxic shock syndrome :

- Caused by **toxic shock syndrome toxin-1** (TSST- 1) (A superantigen).
- Multi system intoxication characterized initially by fever, hypotension, and a diffuse macular erythematous rash.
- It used to be more common because of the epidemic broke out in 1980s, that's because of the use of hyper absorbent tampons. So, in this case S. aureus, which was found in certain types of tampons, could grow in them quiet easily and then invade the vagina/vaginal canal, and then form a focus of infection from which S. aureus starts releasing its toxins, causing ***toxic shock syndrome.***
- This disease is much less common nowadays after banning those tampons from the markets.

Note: In Toxic shock syndrome and scalded skin syndrome, we have a region where S. aureus is infecting, so we have a focus of infection, then S. aureus sends its toxins causing these systemic effects.

Note: In the case of food poisoning, S. aureus releases its toxins in food, then it will be ingested. Even in microgram quantities, you will have some symptoms of this intoxication.

Note: S. aureus can cause other several diseases like :

Osteomyelitis (osteo=bone, litis=infection), Due to S. aureus presence near blood it can cause **septic shock**, **pneumonia** (if it reaches lungs), and they can infect **blood vessels or joints.**

Note: So S. aureus doesn't only infect skin/epidermis but also it can reach circulation and effect other organs.

** septic shock can be caused by many organisms that reach the circulation.

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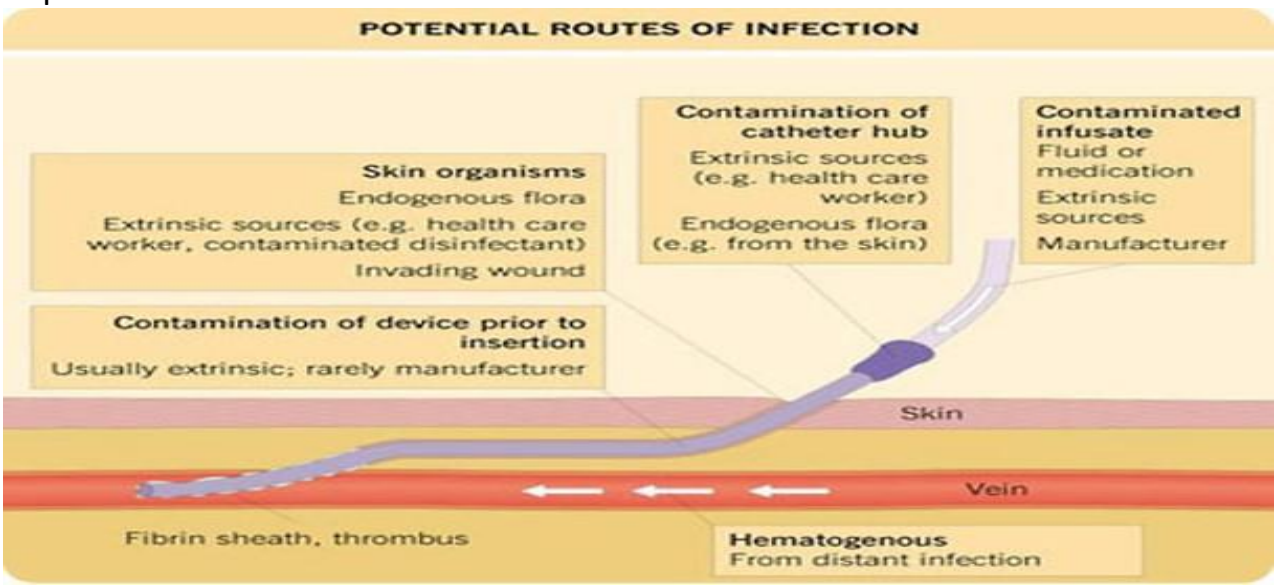
3. Coagulase Negative :

- E.g.: microbiota like *S epidermidis*. *(Remember coagulase (+) is S. aureus)*
- Microbiota only cause a disease when it's introduced into a new environment.
- It's important to understand, because many manipulation in medicine will involve breaking the integrity of the skin and getting into deeper tissue/circulation. So if microbiota enters circulation it causes infections.
- More than 50% of all infections of **catheters and shunts** are caused by **coagulase-negative staphylococci**.
- These infections have become a major medical problem because long-dwelling catheters and shunts are used commonly for the medical management of critically ill patients.



- So as in the picture, in site of insertion of catheter you'll observe infection on the sides, that's because you are introducing bacteria into deeper layers in the skin and sometimes to the circulation. So, this S. epidermidis can come from the surface of the skin , and sometimes from the health care worker hand that if he touches the catheter or shunts with his naked hands and contaminate it with S. epidermidis.
- This bacteria (E.g.: S. epidermidis) is commonly found on the outside of the skin.
- ***S. epidermidis*** infections are difficult to cure because they occur in **prosthetic devices** where the bacteria can sequester themselves in a **biofilm.** So the patient will come back suffering from infections.
- staphylococci are a major cause of **endocarditis of artificial valves.**
- Other coagulase-negative infections :

1) Wound infection:

- When there is a major wound, S. epidermidis will be introduced into deeper layers causing infection.
- Characterized by erythema and pus at the site of traumatic or surgical wound; infections with foreign bodies can be caused by S. aureus and coagulase-negative staphylococci.

2) Urinary tract infections (From slides) : dysuria and pyuria in young sexually active women (S. saprophyticus, in patients with urinary catheters (other coagulase-negative staphylococci), or following seeding of the urinary tract by bacteremia(S. aureus).

Coagulase-Negative *Staphylococcus* Species

Wound infections: Characterized by erythema and pus at the site of a traumatic or surgical wound; infections with foreign bodies can be caused by *S. aureus* and coagulase-negative staphylococci

Urinary tract infections: Dysuria and pyuria in young sexually active women (*S. saprophyticus*), in patients with urinary catheters (other coagulase-negative staphylococci), or following seeding of the urinary tract by bacteremia (*S. aureus*)

Catheter and shunt infections: Chronic inflammatory response to bacteria coating a catheter or shunt (most commonly with coagulase-negative staphylococci)

Prosthetic device infections: Chronic infection of device characterized by localized pain and mechanical failure of the device (most commonly with coagulase-negative staphylococci)