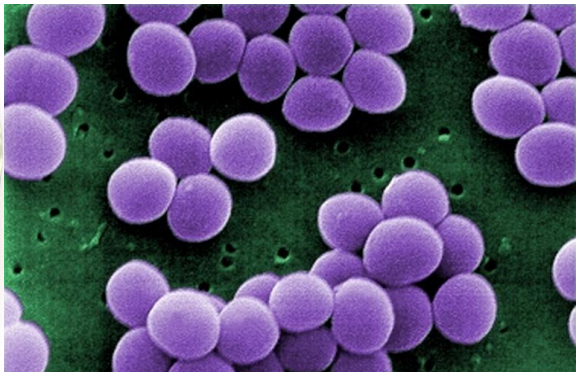
Medical Bacteriology- Lecture: 5

Bacterial Pathogens and Diseases of Humans

Gram Positive Cocci

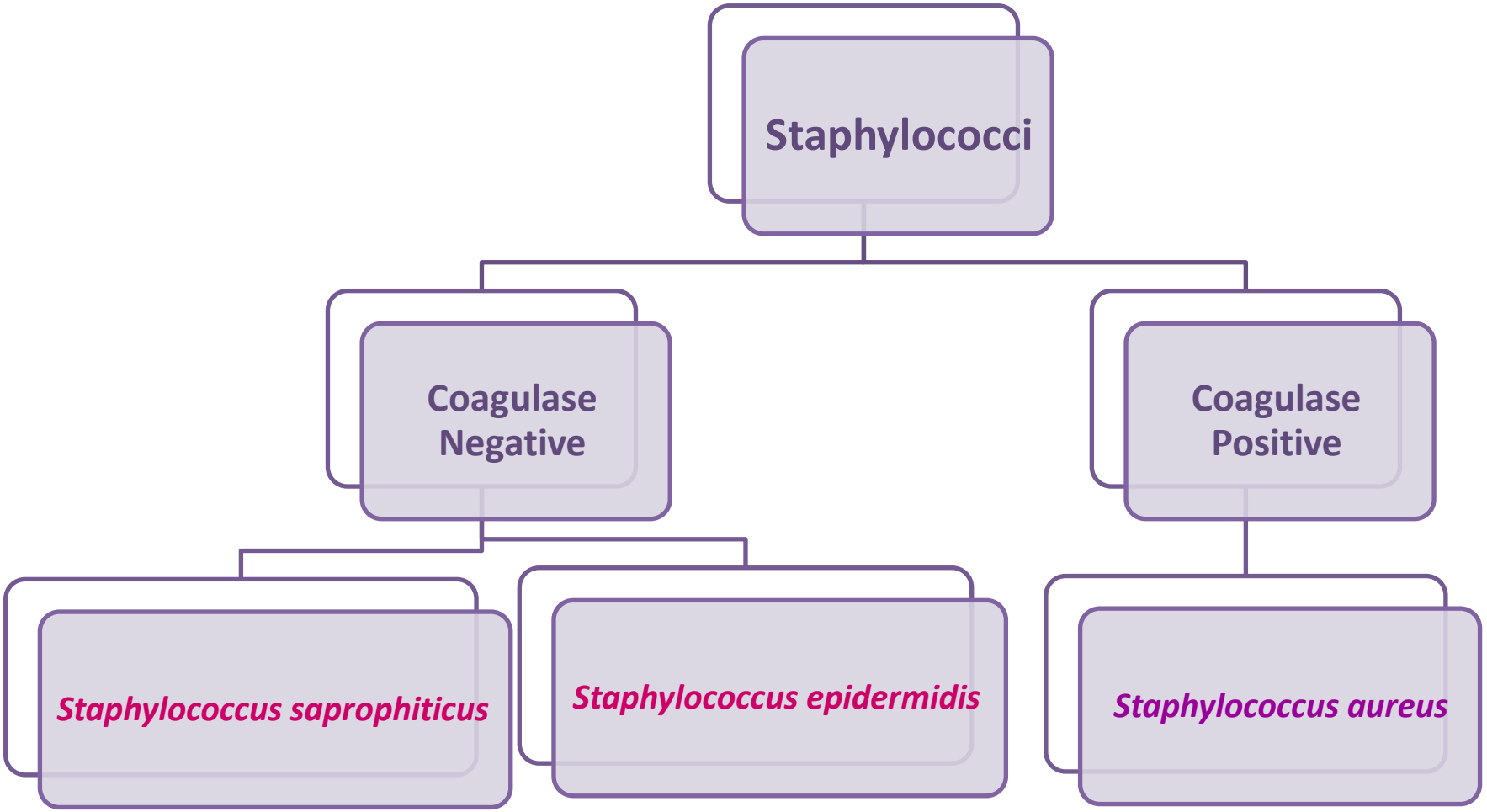
Staphylococci



Micrococcaceae

Staphylococcus: Pathogenic or commensal

***Micrococcus*:** Free-living saprophytes



The Staphylococci (Staph)

General characteristic of Staphylococci

Staphylococci are **Gram-positive- cocci** - **clusters resembling grapes**.

non motile, non spore forming, facultative anaerobes (respiration or fermentation)

catalase-positive & oxidase-negative

Withstands high salt. Grow at **15 % NaCl** concentrations.

Optimum temperature at 37C, can grow at a temperature range (15 to 45 c)

fermentation of glucose produces mainly lactic acid

- ***S. aureus*** colonizes mainly the nasal passages as normal flora, but it may be found in other anatomical locales (skin, oral cavity & gastrointestinal tract). Always considered a **potential pathogen**

- ***S. epidermidis*** is an inhabitant of the skin and mucous membranes, mostly **nonpathogenic** & may play a protective role in humans as normal flora. May be a pathogen in the **hospital environment (nosocomial infections)**.

  S.aureus forms a large **yellow colony** on rich medium; ***S. epidermidis*** has a relatively small **white colony**.

- ***S. aureus*** is often **beta hemolytic** on blood agar; ***S. epidermidis*** is **non hemolytic**.

- All strains of ***S. aureus*** produce **coagulase.** All strains of ***S. epidermidis*** lack this enzyme.

- Without microscope, the **catalase test is an important test in distinguishing streptococci (catalase-negative) from staphylococci**. The test is performed by adding 3% hydrogen peroxide to a colony on an agar plate or slant. Catalase-positive cultures produce O_2 and bubble at once. The test should not be done on blood agar because blood itself contains catalase.

The staphylococci grow in clusters because the cells divide in three perpendicular planes with the sister cells remaining attached to one another following each division. Since the exact point of attachment of sister cells may not be within the divisional plane, and the cells may change position slightly while remaining attached, the result is formation of an irregular cluster of cells.

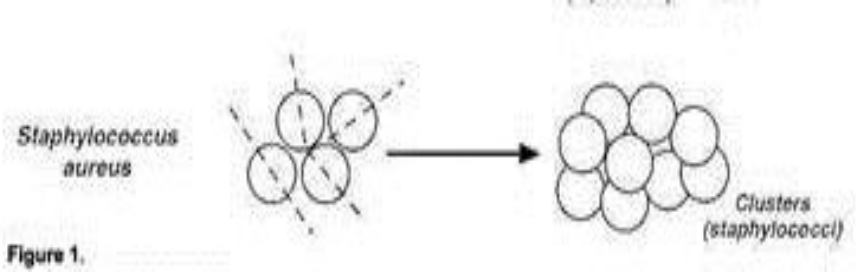
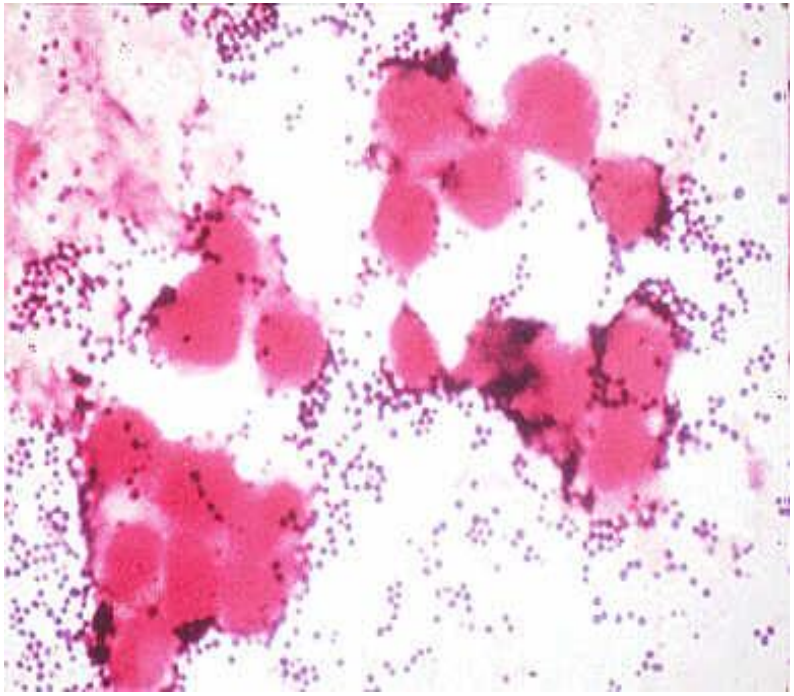


Figure 1.

Important phenotypic characteristics of *S. aureus*

Gram-positive, cluster-forming coccus
ferments mannitol (distinguishes from *S. epidermidis* & *S. saprophyticus)*
catalase positive
coagulase positive
golden yellow colony on media
normal flora of humans found on nasal passages, skin and mucous membranes
pathogen of humans, causes a wide range of suppurative infections, as well as food poisoning and toxic shock syndrome



Gram stain of *S. aureus* in pustular exudate

***S. aureus* on Tryptic Soy Agar. (yellow pigment).**

Pathogenesis of *S. aureus* infections

- *S. aureus* causes a variety of **suppurative (pus-forming)** infections and **toxigenesis** in humans.
- It causes:
- **superficial skin lesions** such as **boils** and **furuncules,** Abscess **or** more serious skin infections such as **impetigo (**bubble-like swellings that can break and peel away; most common in newborns)
- **or** other skin lesions, **Staphylococcal scalded skin syndrome (SSSS)** or Ritter's disease (relatively rare); (toxin induces bright red flush, blisters, then desquamation of the epidermis)
- **more serious infections** such as **pneumonia (**infections in the lung), **meningitis**, skeletal muscle, Kidney or **urinary tract infections**
- **deep-seated infections**, such as **osteomyelitis** (Localized infection of the bone), **endocarditis** (heart)-
- *S. aureus* is a major cause of **hospital acquired (nosocomial) infection** of surgical wounds and infections associated with medical devices.
- *S. aureus* causes **food poisoning** by releasing heat stable **enterotoxins** into food (even at 4C), and it can produce **toxic shock syndrome** (toxemia leading to shock and organ failure) by release of superantigens into the blood stream
- **Serious consequences of staphylococcal infections (**Systematic infections) occur when the bacteria invade the blood stream. A resulting **septicemia** may be rapidly fatal or **bacteremia**.

S. aureus expresses many potential virulence factors

- **(1) surface proteins** promote colonization of host tissues such as **laminin** and **fibronectin.** Most strains express a fibrin/fibrinogen binding protein (**clumping factor**) which promotes blood clots.
- **(2) invasins** promote bacterial spread in tissues (**leukocidin**, **kinases**, **hyaluronidase**
- **(3)** DNase- digests DNA
- **(4)** Lipases – digest oils; enhances colonization on skin
- **(5) surface factors** inhibit phagocytic engulfment (**capsule**, **Protein A**)
- **(6) biochemical properties** enhance their survival in phagocytes

carotenoids; (Staphyloxanthin; carotenoid pigment which responsible for golden colonies, and it has an antioxidant action that helps bacteria to evade reactive oxygen by the host immune system.

catalase production

- **(7) immunological disguises** (**Protein A**, **coagulase**)
- **(8) membrane-damaging toxins** lyse eucaryotic cell membranes
- **hemolysin;s (**α, β, γ, δ) lysis red blood cells
- **leukocidin; (lysis neutrophils and macrophages)**
- **(9) exotoxins** damage host tissues or provoke symptoms of disease

Staphylococcal enterotoxins; (**SEA-G)**; food poising (nausea, vomiting, diarrhea)

- **Toxic shock syndrome toxin** (**TSST**); induces fever, vomiting, shock, systemic organ damage

Exfolioative toxins (**ETs); responsible for Staphylococcal scalded skin syndrome (SSSS);** separates the epidermis from the dermis**.**

Panton-Valentine Leukocidin (PVL) creates pores in the membranes of infected cells. It is associated with severe necrotizing pneumonia in children

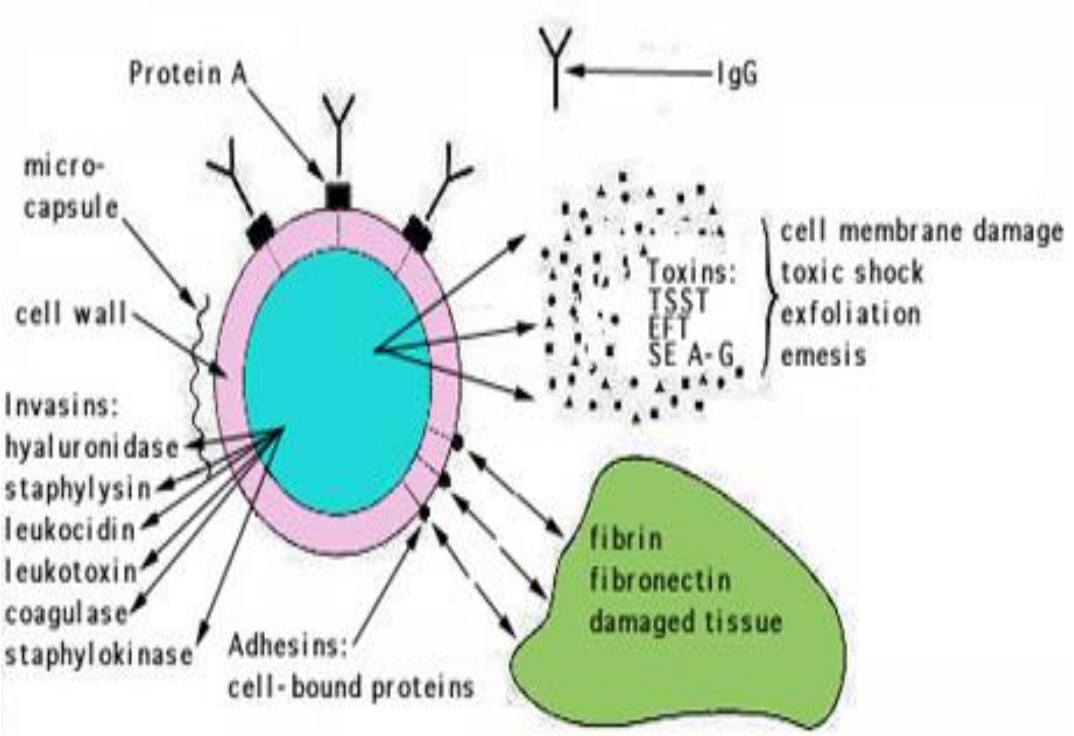
- **(10**) inherent & acquired **resistance to antimicrobial agents** (Penicillinase- inactivates penicillin)

Human staphylococcal infections are frequent, but usually remain localized at the portal of entry by the normal host defenses.

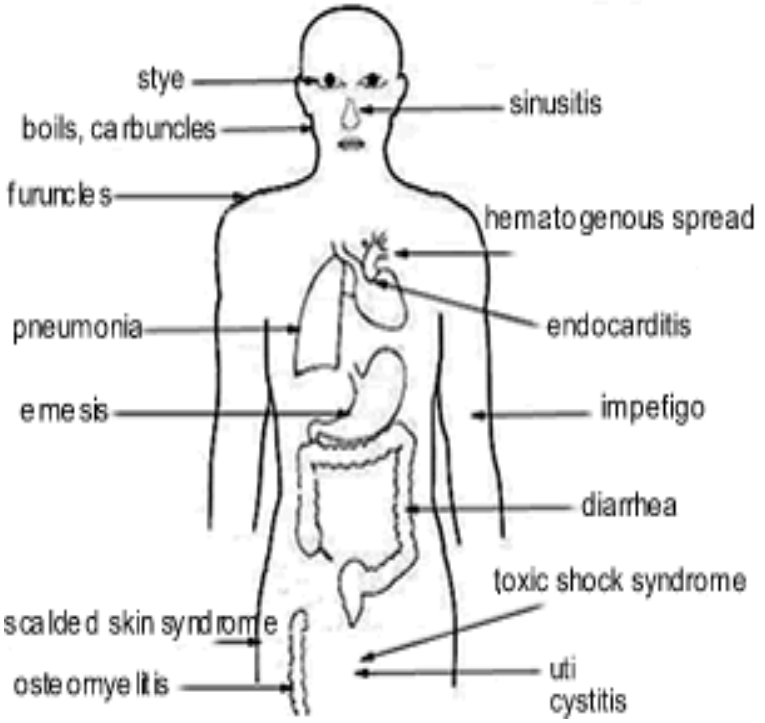
The portal may be a **hair follicle**, by the **skin** which may be a minute needle-stick or a surgical wound.

Foreign bodies, including sutures, are readily colonized by staphylococci, which may make infections difficult to control. Another portal of entry is the **respiratory tract**. Staphylococcal pneumonia is a frequent complication of influenza.

The localized host response to staphylococcal infection is inflammation, characterized by an elevated **temperature** at the site, **swelling**, the accumulation of **pus**, and **necrosis of tissue**. Around the inflamed area, a fibrin clot may form, walling off the bacteria and leukocytes as a characteristic pus-filled boil or abscess.



Virulence determinants of *S. aureus*



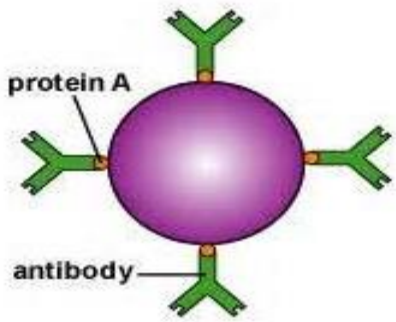
Sites of infection and diseases caused by *S. aureus*

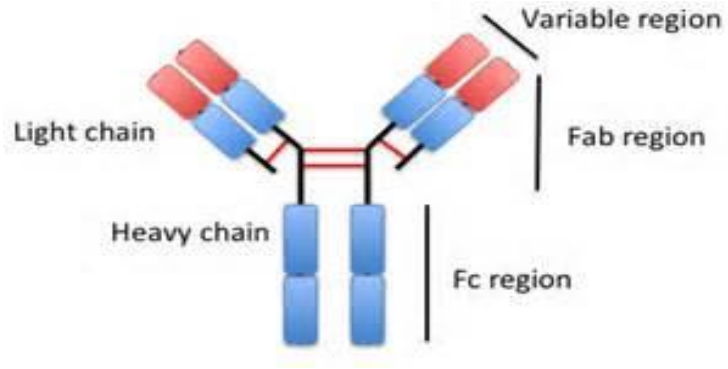
Avoidance of Host Defenses

S. aureus expresses a number of factors that have the potential to interfere with host defense mechanisms. This includes both structural and soluble elements of the bacterium.

Capsular Polysaccharide: The majority of clinical isolates of *S aureus* express a surface polysaccharide. called a **microcapsule** because it can be visualized only by electron microscopy unlike the true capsules of some bacteria which are readily visualized by light microscopy. *S. aureus* strains isolated from infections express high levels of the polysaccharide but rapidly lose the ability when cultured in the laboratory. The function of the capsule in virulence is not entirely clear. It does impede phagocytosis in the absence of complement, it has a role in colonization of damaged heart valves, perhaps by masking adhesins.

Protein A: is a surface protein of *S. aureus* which binds IgG molecules by their Fc region. In serum, the bacteria bind IgG in the wrong orientation on their surface, which disrupts opsonization and phagocytosis.





Leukocidin: a toxin that specifically acts on polymorphonuclear leukocytes, and damage its membrane.

Exotoxins: *S. aureus* can express several different types of toxins which are responsible for symptoms during infections. Those which damage the membranes of cells. Some will lyse erythrocytes, causing hemolysis. alpha toxin causes septic shock, enterotoxins and TSST-1 are superantigens that may cause toxic shock and evade immune system..

Summary- Possible virulence determinants expressed in the pathogenesis of *S. aureus* infections (further reading)

boils & pimples (folliculitis)

Colonization: cell-bound (protein) adhesins
Invasion: **Invasins**: staphylokinase
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
Resistance to phagocytosis: coagulase, leukocidin
Resistance to immune responses: coagulase
Toxigenesis: cytotoxic toxins (hemolysins and leukocidin)

pneumonia

Colonization: cell-bound (protein) adhesins
Invasion: **Invasins**: staphylokinase, hyaluronidase
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
Resistance to phagocytosis: coagulase, leukocidin, hemolysins, carotenoids, superoxide dismutase, catalase, growth at low pH
Resistance to immune responses: coagulase, antigenic variation
Toxigenesis: Cytotoxic toxins (hemolysins and leukocidin)

food poisoning (emesis or vomiting)

Toxigenesis: Enterotoxins A-G

septicemia (invasion of the bloodstream)

Invasion: **Invasins**: staphylokinase, hyaluronidase
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
Resistance to phagocytosis: coagulase, protein A, leukocidin, hemolysins, carotenoids, superoxide dismutase, catalase, growth at low pH
Resistance to immune responses: coagulase, protein A, antigenic variation
Toxigenesis: cytotoxic toxins (hemolysins and leukocidin)

Con. Possible virulence determinants expressed in the pathogenesis of *S. aureus* infections (further reading)

Osteomyelitis (invasion of bone)

Colonization: cell-bound (protein) adhesins
Invasion: **Invasins**: staphylokinase, hyaluronidase
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
Resistance to phagocytosis: coagulase, protein A, leukocidin, hemolysins, carotenoids, superoxide dismutase, catalase, growth at low pH
Resistance to immune responses: coagulase, protein A, antigenic variation
Toxigenesis: cytotoxic toxins (hemolysins and leukocidin)

toxic shock syndrome

Colonization: cell-bound (protein) adhesins
Resistance to immune responses: coagulase, antigenic variation
Toxigenesis: TSST toxin, Enterotoxins A-G

surgical wound infections

Colonization: cell-bound (protein) adhesins
Invasion: **Invasins**: staphylokinase, hyaluronidase
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
Resistance to phagocytosis: coagulase, protein A, leukocidin, hemolysins, carotenoids, superoxide dismutase, catalase, growth at low pH
Resistance to immune responses: coagulase, protein A, antigenic variation
Toxigenesis: cytotoxic toxins (hemolysins and leukocidin)

scalded skin syndrome

Colonization: cell-bound (protein) adhesins
Invasion: **Invasins**: staphylokinase, hyaluronidase
Other extracellular enzymes (proteases, lipases, nucleases, collagenase, elastase. etc.)
Resistance to phagocytosis: coagulase, leukocidin, hemolysins
Resistance to immune responses: coagulase, antigenic variation
Toxigenesis: Exfoliatin toxin

Pathogenic of Coagulase Negative Staphylococci

S. epidermidis and S. saprophiticus

- Coagulase-negative staphylococci (CoNS) are part of the normal flora of human skin and mucous membranes
- frequently involved in nosocomial and opportunistic infections
- relatively low virulence, less frequently found as pathogens
- but are increasingly recognized as agents of clinically significant infection of the bloodstream and other sites, occasionally associated with endocarditis, prosthetic joint infection, wound infections, endocarditis, bacteremia, Urinary tract infections (UTI).

In contrast to *S. aureus*, little is known about mechanisms of pathogenesis of *S. epidermidis* infections.

Adherence is obviously a crucial step in the initiation of foreign body infections.

Bacteria-plastic interactions are probably important in colonization of catheters, and a polysaccharide adhesion has been identified.

A characteristic of many pathogenic strains of *S. epidermidis* is the production of **biofilm**. a significant determinant of **virulence** for these bacteria.

S. saprophyticus is a leading cause of **cystitis in young women**. And shares of urinary tract infection.

Host Defense against Staphylococcal Infections

- **Phagocytosis** is the major mechanism for staphylococcal infection.
- **Antibodies** are produced which neutralize toxins and promote opsonization. However, the bacterial capsule and protein A may interfere with phagocytosis. Biofilm growth is also impervious to phagocytes. Staphylococci may be difficult to kill after phagocytic engulfment because they produce catalase which neutralize oxygen and superoxide, which are primary phagocytic killing mechanisms within the phagolysosome.

Treatment

Hospital strains of *S. aureus* are usually resistant to a different antibiotics- A few strains are resistant to all clinically antibiotics except vancomycin, & vancomycin-resistant *S. aureus* strains (VRSA) are increasingly.

Hospital acquired infection is often caused by antibiotic resistant strains (e.g. MRSA) and can be treated with vancomycin or an alternative.

The term **MRSA** refers to **Methicillin resistant *S. aureus*** and related beta-lactam antibiotics (e.g. penicillin, oxacillin, amoxacillin). Methicillin resistance is widespread and most methicillin-resistant strains are also multiply drug-resistant. Some MRSA are resistant to vancomycin (VRSA).

The infections have been treated with combination therapy using sulfa drugs and or rifampin.

(CoNS-) produce an enzyme called **beta lactamase** that makes them resistant to methicillin and oxacillin. Vancomycin is the most common antibiotic used to treat infections caused by CoNS-; if they not resistant. Rifampin and gentamicin may be added to prevent antibiotic resistance.

Vaccines

No vaccine is generally available that stimulates active immunity against staphylococcal infections in humans

Differentiation between Coagulase Positive Staphylococci and Coagulae Negative Staphylococci

	S. aureus	*S. epidermidis*	*S. saprophiticus*
Hemolytic	+ (most strains) Beta haemolytic	-	-
Manitol Fermintation	+	-	-
Cagulase reaction	+	-	-
Pigment production	Usually golden	Usually white	Usually white
DNase production	+	-	-
Sensitivity to novobiocin	Sensitive	Sensitive	Resistant

Review Questions

- What do you know about the Staphylococci?
- What is the Important phenotypic characteristics of *S. aureus*
- What do you know about *S. aureus* infections
- What is the beneficial effect to add 15% NaCl to Staphylococcal cultural media?
- *S. aureus* expresses many potential virulence factors, explain?
- Give three examples of *S. aureus* portal entry?
- What is the role of *S. aureus* protein A and Capslue in avoidance host defences?
- Write the full worlds of the following abbreviations: MRSA, CoNS– VRSA- TSST- ETs- SE- PVL?
- You studied Three Species under the Genus *Staphylococcus*. What are they? And how you differentiate between them؟
- How can we treatment infections caused by pathogenic Staphylococci ?
- What do you know about coagulase negative Staphylococci?