Staphylococci and Streptococci

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Main Objectives

• Understand the epidemiology of staphylococcal infections

• Understand how staphyloccoci evade the innate immune system
General Characteristics

• Gram positive cocci found in grape like clusters
• **Catalase positive**
• Oxidase negative
• Facultative anaerobes – grow by either aerobic respiration or by fermentation.

• Grow at large temperature range (15-45°C) and salt tolerant (up to 15%)

• Greater than 20 species of *Staphylococcus*
• The only relevant species include:
  - *S. aureus*
  - *S. epidermidis*  
    \[\text{Coagulase Negative}\]
  - *S. saprophyticus*

*S. aureus* – large golden colony; produces a yellow carotenoid pigment that is a virulence factor
*S. epidermidis* – grows as a small white colony.
Epidemiology of *S. aureus*

Successful pathogen because resistant to harsh conditions and high carrier rate

- **20-40% of the population carries in the anterior nares**
  - 20% never carry
  - 30% long term carriers
  - 50% intermittent carrier
- Rapidly acquired after birth
- Carrier rate higher in certain populations:
  - Hospital workers, diabetics, hemodialysis patients and IV drug users

- **Trama** = portal of entry to cause localize or generalized disease.

<table>
<thead>
<tr>
<th>Anterior nares</th>
<th>Skin</th>
<th>Breach in mucus membrane</th>
<th>Dissemination bloodstream &amp; lymphatics</th>
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<td></td>
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<td>(trauma or surgery)</td>
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- **Control**
  - Hand washing – careful/repeated/compulsive
  - Eliminate carriage; recolonization will occur
    - Temporary fix
Epidemiology of *S. aureus* continued

• **Nosocomial:**
  – >60% methicillin resistant (MRSA)
  – High drug resistance

• **Community acquired:**
  – Changing epidemiology – community acquired infections are on the rise
  – More susceptible to β-lactam antibiotics (clindamycin), trimethoprim-sulfate and tetracyclines
  – Traditionally methicillin sensitive
  – Methicillin resistant community isolates (CA-MRSA) first reported in 1990s
  – Increased toxin production
    • Rapidly spread
    • Assoc. w/ skin infections (Athletes with skin infection)
    • Also invasive infections and severe pneumonia
Public Health concerns

Incidence and Cost:
- *S. aureus* is the most abundant cause of bloodstream, skin and soft tissue infections in the US, Canada, Europe, Latin America and the Western Pacific.
- *S. aureus* is the most abundant cause of bacterial infection in the US

Drug resistance:
- ~ all Penicillin resistant (β-lactamase)
- Methicillin Resistance (MRSA; MecA)
  - Nococomial MRSA >60%
  - Community Acquired CA-MRSA >20%
- Vancomycin (VRSA; VanA); reported
METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS (MRSA)

- **80,461** severe MRSA infections per year
- **11,285** deaths from MRSA per year

Staph bacteria are a leading cause of healthcare-associated infections.

VANCOMYCIN-RESISTANT STAPHYLOCOCCUS AUREUS

- **13** cases in 4 states since 2002
- Some Staphylococcus strains are resistant to vancomycin, leaving few or no treatment options.

HAZARD LEVEL:
- **SERIOUS**

This bacteria is serious concern and requires prompt and sustained action to ensure the problem does not grow.

THREAT LEVEL:
- **CONCERNING**

This bacteria is concerning, and careful monitoring and prevention action are needed.
S. aureus Infections
Suppurative “pus-forming” Infections and “toxinoses”

• **Superficial skin lesions**
  – boils, furuncles, carbuncles, stys
  – Impetigo

• **More serious Invasive Disseminated Infections**
  – **Pneumonia**
    • community acquired is associated with influenza
    • hospital acquired is associated with intubation & aspiration
  – **Spreading pyodermas** - all localized skin infections can spread to the soft tissues and cause deeper infection such as cellulitis and even necrotizing fasciitis
  – **Osteomyelitis** - infection of the bone; especially long bones. Characterized by fever and pain.
    • 2° to trauma
    • Can be associated with bacteremia
    • Post implants (knees and hips)
  – **Wound infections** - especially hospital acquired wounds
Hospital Acquired Infections

**Septicemia (SAB)** – one of the most prevalent & hardest to treat
- Local infection that spreads to bloodstream
- Chills, rigor, joint pain, petechia and subconjunctival hemorrhages
- High mortality and high economic burden

**Endocarditis** – *S. aureus* is one of the only causes of infective endocarditis in normal heart valves.
- Complication of SAB
- Risks - prosthetic heart valves and intravascular devices
- High Mortality 40-60%

**Risk Factors for SAB:**
- Previous MRSA infection or colonization
- Skin ulcers or cellulitis
- Hospital admission
- Catheters
- Surgical site
- Injection drug use
- Immunosuppressive drugs / conditions
Toxigenic Diseases

*S. aureus* makes up to 24 distinct super antigen toxins & numerous other toxins

**Food Poisoning** – true intoxication that arises from ingestion of Staphylococcal enterotoxins from contaminated foods. Causes vomiting within 2-6 hours.

- Toxin is heat stable
- Emetic and super antigen domains are distinct

**Scalded skin syndrome*** – acute exfoliative dermatitis characterized wrinkling and peeling of the skin.

- Symptoms caused by the “exfolitatin toxin”, a super Ag toxin.
- Organisms may or may not be present at the site.

**Toxic shock syndrome*** – rapid onset of fever, erythematous deep red rash, vomiting, desquamation (palms and soles), hypotension and shock.

- Symptoms and shock are caused by dissemination of the toxic shock syndrome toxin TSST-1; a super antigen toxin
- Commonly initiates with vaginal colonization with a TSST-1 producing strain
  - Colonization often occurs during the menstrual cycle
  - Associated with use of high absorbency tampons
- Also occurs in males and non-menstruating females
  - Associated with prior *S. aureus* infection (cutaneous/subcutaneous) or a “trauma” (childbirth, surgery)
- 5-15% mortality rate

*Usually localized infections with disseminated symptoms due to toxin*
Staphylococcal Immune Evasion & Virulence

**Immune evasion**
- *S. aureus* is a successful pathogen because it is able to evade and subvert the 3 main axes of the innate immune response:
  1. recruitment and activation of inflammatory cells
  2. complement activation
  3. killing by antimicrobial peptides (AMPs) and PMN

Neutrophils are the main enemy for Staphylococci

**Virulence**
- Secretion of toxins
- Super Antigens
Normal Innate Immune Response

- Neutrophils chemotaxis toward the site of infection – leave the blood vessels and move through the vascular endothelium to reach the site of infection

- Activated neutrophils engulf opsonized bacteria and kill using a large repertoire of proteases, lipase, amidases, antimicrobial peptides (AMPs), lysozyme and oxygen radicals

- Resident macrophages sense pathogens and send cytokine signals to recruit neutrophils

- Complement is activated by the invading organism:
  - generates chemo-attractants that recruit neutrophils to the site
  - tag (opsonizes) bacteria for phagocytosis
  - Lyse Gram negative organisms via MAC

- Resident macrophages sense pathogens and send cytokine signals to recruit neutrophils
Resident macrophages sense pathogens and send cytokine signals to recruit neutrophils

- **PAMPS** – *Pathogen Assoc. Molecular Patterns* are structures on bacterial surface that the innate immune system uses to recognize non-self via pathogen recognition receptors (PRRs, i.e TLRs, DC-SIGN, MBL and ficolins)

- Activating TLRs with PAMPS initiates intracellular signaling that ultimately results in NFκB inducing expression of cytokines. The cytokines recruit neutrophils, professional killers, to the site of infection.

<table>
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<th>Receptor</th>
<th>Ligands</th>
<th>Microorganisms Recognized</th>
<th>Notes</th>
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<tbody>
<tr>
<td>TLR-2 (TLR-1, -6) heterodimers</td>
<td>Peptidoglycan, bacterial lipoprotein and lipopeptide, porins, yeast mannan, lipoarabinomannan, glycophostidyl-inositol anchors</td>
<td>Gram-positive bacteria, mycobacteria, <em>Neisseria</em>, yeast, trypanosomes</td>
<td>Carried on macrophages</td>
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<tr>
<td>TLR-3 homodimer</td>
<td>Double-stranded RNA</td>
<td>Viral RNAs</td>
<td></td>
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<tr>
<td>TLR-4 homodimer</td>
<td>Lipopolysaccharide (LPS)</td>
<td>Gram-negative bacteria</td>
<td>Carried on macrophages</td>
</tr>
<tr>
<td>TLR-5 homodimer</td>
<td>Flagellin</td>
<td>Gram-negative bacteria</td>
<td>Carried on intestinal epithelium; interacts directly with ligand</td>
</tr>
<tr>
<td>TLR-9 homodimer</td>
<td>DNA with unmethylated CpG motifs</td>
<td>Bacteria</td>
<td>Intracellular receptor</td>
</tr>
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TLR2 recognizes LTA
Inflammatory mediators / cytokines

- **IL-1β**
  - Activates vascular endothelium
  - Activates lymphocytes
  - Local tissue destruction
  - Systemic effects
    - Fever
    - IL-6
    - Fever, acute-phase proteins ↑

- **IL-6**
  - Activates lymphocytes
  - Increases antibody production
  - Fever, acute-phase proteins ↑

- **TNF-α**
  - Activates vascular endothelium
  - Increases vascular permeability
  - Fever
  - Shock

- **IL-12**
  - Activates natural killer (NK) cells
  - CD4+ cells
  - TH1 cells

- **CXCL8**
  - Chemotactic factor
  - Recruits neutrophils, basophils, and T-cells
Activation of Complement - 3 main functions

1. **Complement opsonizes microbes for phagocytosis**
   - deposition of C3b/ iC3b
   - C3b interacts with CR1 on phagocyte
   - iC3b interacts with CR3 on phagocyte

2. **Generate chemoattractants**
   - generation of C3 and C5 convertases release the potent chemoattractants C3a and C5a

3. **Formation of the membrane attack complex (MAC)**
   - C3 and C5 convertases also allow for the formation of MAC which is important for direct lysis of Gram negative organisms

Note: iC3b is an inactive form of C3b
- Inactivated by complement regulatory protein
- Serves as an opsonin but can not be incorporated into convertases and lead to the generation C3a / C5a
Migration of neutrophil to the site of infection

**Neutrophil recruitment:** by cytokines, complement and the invading microbes.
- For *S. aureus* the major signals are
  - C5a
  - fMetLeuPhe - formylated peptides released from bacteria.
- These molecules interactions with C5aR and FPR; G-protein receptors that signal neutrophils to move to the site of infection.

**Neutrophil rolling:** required for migration into the adjacent tissues:
- **P-selectin** – upregulated on endothelial cells during inflammation
- **PSGL-1** on neutrophils binds to P-selectin to initiate neutrophil rolling.

**Neutrophil transmigration:**
- **MAC-1** and **LFA-1** on neutrophils interact with **ICAM-1** on endothelial cells to begin transmigration
  - MAC-1 (CD11b/CD18)
  - LFA-1 (CD11a/CD18 ; leukocyte function associated Ag)
  - ICAM-1 (Intracellular adhesion molecule 1)

\[ \beta_2 \text{ integrins} \]
Activated neutrophils phagocytose opsonized bacteria:
- C3b/iC3b
- Ab

Mechanisms of neutrophil killing:
- Engulf opsonized bacteria
- Fusion of phagosome and lysosome
- Oxidative burst / Oxygen radicals
- Proteases, lipases, amidases
- Antimicrobial peptides (AMPs)
- Lysozyme
- Lactoferrin
- Nitrosative stress
- Low pH
Evasion of Recruitment of Inflammatory Cells

**Chemotaxis** - C5a and formylated peptides signal neutrophils, via the G-protein receptors C5aR and FPR, to move to the site of infection

- fMet-Leu-Phe is a powerful chemo-attractant for neutrophils
- fMet is unique to bacteria and contained in peptides generated from bacterial proteins
- C5aR – C5a receptor
- FPR - formylated peptide receptor

**CHIPS (chemotaxis inhibitory protein of S. aureus)**

- binds to C5aR and FPR
- blocks chemotactic signals (C5a and formylated peptides) from reaching the neutrophils

Note: there is also a staph protein called FLIPr (FLRP-1 inhibitory protein) that binds to a 2nd low affinity receptor for formylated peptides on neutrophils called FLRP-1 (Formyl peptide receptor like protein)
Inhibition of Neutrophil Migration

Neutrophil Rolling:
- neutrophils leave the vasculature by rolling on endothelial cells.
- PSGL-1 (P-selectin glycoprotein ligand -1) on neutrophils binds to P-selectin on activated endothelial cells.
- P-selectin is upregulated during inflammation

SSL-5 (Staph SuperAg like protein 5):
- binds to PSGL-1 on neutrophils
- blocks the interaction of PSGL-1 with P-selectin on endothelial cells.
- SSL-5 - structurally similar to Super Ags toxins, BUT does not possess Super-Ag activity
  - lacks the regions that bind to MHC class II and the TCR

Neutrophil transmigration:
ICAM-1 on the endothelium interacts with β2 integrins (MAC-1 and LFA-1) on neutrophils

Eap (extracellular adherence protein):
binds to ICAM-1 and interferes with extravascularization of neutrophils
- Disrupts β2-integrin and urokinase receptor mediated leukocyte adhesion, in vitro
- In mice, Eap+ bacteria elicit fewer neutrophils that Eap− bacteria
Evasion of Complement / You Can’t Tag Me!

Inhibiting complement Activation

**SCIN – Staphlococcal complement inhibitor**
- Stabilizes C3 convertases (CP, LP and AP) and prevents cleavage of C3 → C3b + C3a
- Prevents the formation of C5 convertases and thus C5a and MAC.
- Blocks opsonization (C3b) and chemotaxis (C3a and C5a)

**Efb – Fibrinogn binding protein**
Binds to C3 and prevents deposition of C3b onto bacteria.
- Blocks all convertases that contain C3 (AP C3 convertase & CP,AP & LP C5 convertases)
- Blocks opsonization

**Sak - staphylokinase**
- Staphylococci bind to plasminogen via several surface plasminogen receptors
- Converts plasminogen → plasmin
- Plasmin (serine protease) cleaves surface bound C3b/iC3b and IgG (removes entire Fc)
- Removal of opsonins reduces phagocytosis by neutrophils

**Sbi**
- Activates complement in the fluid phase and thus creates a futile situation
- Consumes complement
- Decreases opsonization.

**Protein A**
- Binds Fc of IgG
- Blocks Fc mediated phagocytosis
- Blocks C1q mediated complement activation.
Evasion of Complement / You Can’t Tag Me!

Inhibiting complement Activation / Complement regulatory proteins:

**Factor H and Factor I**
- host regulatory proteins that inactivate C3b → iC3b.
- iC3b - opsonin and binds CR3 on neutrophils
- iC3b cannot be used in a C3 convertase or to form C5 convertases /MAC.

**Staphlococci bind and activate factor H and Factor I, independently:**
- Factor I - **Clumping factor A** *may be the* ligand
- Factor H – SdrE *may be* the receptor
  - a tripartate complex of Ecb, FH, & C3b *may also* be involved
  - **Ecb** - extracellular complement binding protein
  - **SdrE** - Serine Asparagine rich fibrinogen binding protein

**C4BP – C4b-binding protein**
- classical pathway regulator
- C4BP binds to staphylococcal **SdrE** and an allelic variant, **Bbp** (bone sialoprotein-binding protein)
- Inhibits antibody-initiated complement-mediated opsonization
- Decreases deposition of C3b
Evasion of killing by Neutrophils

Resistance to phagocytosis:
Efficient phagocytosis requires opsonization with complement or Ab
- **Antiphagocytic capsule** - interfere with access to deposited C3b and Ab
- **Antiphagocytic proteins**
  - **Clumping factor A** – fibrinogen binding protein that coats the surface with fibrinogen
  - **Protein A, Sak, Efb** – reduce opsinization

Evasion of oxygen species attack:
**Catalase** – inactivates $\text{H}_2\text{O}_2$ and free radicals
**Caretenoid pigments** - scavenge free $\text{O}_2$ radicals & increase resistance to oxidative killing
  - Pigment mutants are less pathogenic in mice
  - pigment enhanced *S. pyogenes*
**SOD** – super oxide dismutase
**IsdA** – scavenges Fe needed to generate reactive $\text{O}_2$ intermediates

Resistance inside the neutrophil:
- **IsdA** – Fe regulated protein binds to lactoferrin & neutralizes the serine protease
- **OatA** - acetylates PG & provides resistance to lysozyme
- **leukotoxin** - escapes endosome
- **NO inducible lactate dehydrogenase** – prevents NO damage to cytochromes by removing reducing equivalents by converting pyruvate $\rightarrow$ lactate
- **methionine sulphoxide reductases** – repairs oxidized sulphur residues on Met ($\text{O}_2$ damage)
Combating AMPs

*Staphylococci* detect AMPs & up-regulate AMP-defense systems

- AMPs - major defense on skin & in neutrophils
- *S. epidermidis* lives on skin & has effective systems to inactivate them.

**AMP Sensing (Aps) System**
(ApsS, ApsX and ApsR)
- **ApsS (His kinase)** – short negatively charged extracellular loop binds to cationic AMPs
- **ApsS** - transduces a signal to ApsR via ApsX
- **ApsR (response regulator)** - triggers expression of AMP resistance mechanisms (e.g. MrpF, Dlt and VraFG)

**AMP Defense Systems**

Decreases the negative charge of the surface to curtail interaction with cationic AMPs:

- D-alanation of teichoic acids by Dlt system
- Lysylation of phospholipids by MprF

**Removes of AMPs from the cytoplasmic membrane**

- VraFG is an ABC transporter that functions as a AMP exporter

*Staphylococci* detect AMPs & up-regulate AMP-defense systems

- The SepA and Aurolysin metalloproteinase proteases and Sak (staphylokinase) - inactivate non- cationic AMPs such as dermicidin and cathelicidin LL-37
**Toxins / Damage**

*Staphylococcus aureus* secretes many leukotoxins:

- Cytotoxic to leukocytes
- Kill neutrophils that are attempting to engulf & kill bacteria
- Contribute to abscess formation and

**α-toxin**

- Forms β-barrel pores in membrane of target cell.
- α-toxin is secreted as a monomer and assembles into heptomers in membrane to form a 14 stranded β-barrel pore.
- Assists in spread of super antigen toxins such as TSST-1

**Bi-component leukotoxins:**

- Composed of 2 components that are secreted separately and assemble into hexameric or heptomeric oligamers in the membrane of leukocytes
- γ-toxin/γ-hemolysin
- Panton-Valentine leukocidin (PVL)
  - Associated with severe skin diseases but is only found in 1-2% of strains
  - Found in *new CA-MRSA* strains that are associated with severe pneumonia
- Leukocidin F/D
- Leukocidin M/F-(PV-like)

**SSL-7 - secreted super Ag like 7**

- Binds to IgA and blocks recognition of the IgA receptor in neutrophils (FcαRI)
Super Antigen / Pyrogenic Toxins

**General Features:**
- Secreted by all human pathogenic *S. aureus* (24 distinct toxins) & *S. pyogenes* (11 distinct toxins)
- pyrogenic
- Resistant to: heat (> 1 hour boiling)
  - desiccation (>1 year on a dried out petri plate)
  - acid (survives stomach acid)
- Most are encoded on variable genetic elements – phage & pathogenicity islands
- Neutralizing Abs directed against super Ags are effective therapeutics
- All over-stimulate the immune system

**Concentration and penetration are important to clinical picture:**
- complex regulation – generally increased in early stationary phase
- delivery enhanced by cytolsins:
  - $\alpha$-toxin for Staph
  - SLO (streptolysin O) for Strep

*At least 25 illnesses are associated with super Ags*
*Super Ags contribute to all serious illnesses caused by staph and strep*
**Super Antigen / Pyrogenic Toxins**

**Enterotoxins (SE’s; A, B\textsubscript{n}, C\textsubscript{n}, D, E, G)**
- emetic when ingested and Super Ag toxins when secreted systemically
  - Concentration and penetration are too low when ingested for Super Ag activity
- responsible for staphylococcal food poisoning.
- stimulate neural receptors in the G.I. tract → pain, vomiting, & diarrhea within 6 hours of ingestion.

**Enterotoxin like toxins (SE-I; H, I, J-X)**
- not emetic

**TSST-1**
- Not emetic
- pyrogenic (fever causing) due to IL-1 induction
- causes erythoderma (red skin)

**Exfoliative or epidermolytic toxin (ETs)**
- Associated with Staphlococcal Scalded Skin Syndrome (SSSS)
- Low mitogenic activity
- Contain a serine proteases that recognizes and hydrolyzes desmosomal proteins in the skin
Super Antigens – how do they work?

Super antigens simultaneously interact with the Vβ domain of the T-cell receptor (TCR) and MHC class II molecules on APCs

- potent polyclonal activation T-cell population
- 2-20% of all T-cells activated (normally ~ 0.001%)
- response is not specific to any particular epitope

Crosslinking the TCR & MHC class II:
- massive cytokine storm
- fever, hypotension
- vascular injury causes capillary leak (vasoactive mediators (TNFα and β))
- extravasation of plasma and proteins (decreased blood volume & pressure)
- May synergize with LPS / TNF production – increase lethality of LPS by $10^6$
- Activates the coagulation cascade
- DIC $\rightarrow$ multiple organ failure and death
Why have super Ags??

Super Ags prevent the development of a normal immune response:

- interfere with a systemic immune response from a local infection
- Ag specific T-cells fail to respond to presented Ags in MHC class II leading to anergy, immunosuppression and lack of a memory response

Anti-staphylococcal immunity relies on Ab and C’ opsonization of bacteria to allow for killing by phagocytic cells – both are blocked by super-Ags:

- Massive TNF production suppresses infiltration of phagocytic cells
- IFN-γ produced by overactive T-cells suppresses Ab generation
  - no anti-TSST-1 Ab following TSS / no anti-toxin neutralizing Ab
Vaccine Strategies

In Trials:
- Phase I - capsular polysaccharide serotypes 5 and 8, MSCRAMM protein (binds to matrix proteins) and clumping factor A
- CflA – binds fibrinogen
  - A fibronectin binding protein based vaccine is effective against bovine mastitis

Failures
- capsular polysaccharide-based vaccine known as StaphVAX, showed promise in an initial phase 3 trial, but was found to be ineffective in a confirmatory trial
- ClfA and SdrG base preparation also made it into phase 3, but failed out

Theurapeutics
- Need for new antibiotics
- Need for novel therapeutics
- IVIG – IV immunoglobulin is effective against TSS
  - Anti-super Ag antibodies are present and neutralize the effects of toxin
  - Cocktail of Humanized mAbs against super-Ags may be effective
  - Recombinant VB-TCR molecules may block super-Ags
Will not get likely have time for the *S. epidermidis* / biofilm slides, they are here for your information only.
Epidemiology of *S. epidermidis*

- Commensal bacteria of the skin of mammals
- Most prevalent Staphylococcal species in humans (60-90% of all staph)
  - Healthy people carry 10-24 different *S. epidermidis* strains at any one time
  - Highly transmissible
- Relatively low virulence; pathogenic factors are associated with persistence rather than overt virulence.
- Highly drug resistant (>80% Methicillin Resistant)
  - Also resistant to penicillins, macrolides, licosamides, tetracyclines, chloramphenicol, trimethoprim, aminoglycosides
  - May serve as a reservoir of antibiotic resistance genes for *S. aureus*
  - Biofilm formation increases resistance to antibiotics
- Essentially all *S. epidermidis* infections are nosocomial and associated with indwelling foreign objects
  - Must remove foreign body to effectively treat
- Control = Prevent infection by proper sterilization of equipment, patients and health care personnel.
  - Not realistic or wise to eradicate a ubiquitous commensal
**S. epidermidis Infections**

All are nosocomial and associated with indwelling objects; especially in neonates and immunocompromised

**Indwelling objects**
- Intravenous catheters – *S. epidermidis* is the #1 infecting organism
- Prosthetic joints, pacemaker wires & power pacs, breast implants, dialysis equip, CSF fluid shunts

**Endocarditis**
- *S. epidermidis* is the 2\(^{nd}\) most common cause of infected cardiac prosthetic valves

**Bacteremia**
- Source is indwelling catheter
- High morbidity and mortality in immunocompromised patients

**Vascular graft rejection**
- Occurs days to months later
- Requires removal of the graft

**Ocular infections**
- Following surgery or associated with contact lenses

**Neonatal bacteremia in the ICU**
- >75% of all neonatal bacteremia are *S. epidermidis*
- Infections are associated with indwelling catheters and mechanical ventilators

**Infections with *S. epidermidis* can occur days to months later. This is in contrast to nosocomial infections with *S. aureus* which occur within days on the surgery or insertion of an indwelling catheter.**
This 61-year-old man received a pacemaker at another institution for complete atrioventricular block 9 months before the photograph shown in the Figure was taken. The patient considered follow-up unnecessary, and he was not concerned when the device began to show through the skin. Only when it fell out, 2 weeks before admission, did he notify his physician. No systemic reaction occurred, and the pacemaker functioned normally. The patient prevented the device from catching in his clothing by holding it against his chest wall with a gauze flat and adhesive tape. Explantation and replacement of a new unit under the pectoral major muscle on the opposite side was accomplished without incident. Cultures of the wound were positive for Staphylococcus epidermidis. At follow-up 6 months later, the wounds had healed and there was no recurrence of infection at either site.
S. epidermidis Virulence

• Associated with the ability to form **biofilms** on foreign bodies

• **Do NOT produce exotoxins** like *S. aureus*

• Biofilm – multi-cellular, surface-attached, agglomerations of microorganisms

• Architecture and physiology make them resistant to antibiotics and host defenses
  – Cells are not actively growing and therefore some antibiotics do not work.
4 stages of Biofilm formation
(Adhesion, Aggregation, Maturation and Dispersal)

**Initial Adhesion** - mediated by surface hydrophobicity
- Teichoic acids
- AltE - abundant surface protein / contribute to surface hydrophobicity
- Bap/Bhp - adhesin/autolysis / contributes to surface hydrophobicity

**Secondary Adhesion** – mediated by Staphylococcal MSCRAMMS (microbial surface components recognizing adhesive matrix molecules) binding to host matrix proteins (fibronectin, collagen)

- SdrG/Fbe – binds fibrinogen
- SdrF – binds collagen
- AltE - binds fibrinogen, fibronectin & vitronectin
- Aae - binds fibrinogen, fibronectin & vitronectin
- Eph – binds elastin

**SdrG** – stretches through the peptidoglycan (PG) via its Ser/Asp repeat domain and binds fibrinogen via the A domain. The B domain binds Ca+2. Anchored to PG via LPXTG domain

**AltE** - non covalently anchored to teichoic acid. Bifunctional adhesin / autolysin.
Contributes to biofilm via hydrophobicity and binding to matrix proteins
Biofilm Aggregation

**Exopolysaccharides - poly-N-acetylglucosamine homopolymer (PNAG/PIA)**
- Surrounds and connects cells in the biofilm
- Crucial for *in vitro* biofilm formation; but NOT essential *in vivo* (some clinical strains assoc. with biofilms lack the *ica* cluster involved in PNAG/PIA synthesis)
- Expression is regulated but by *icdR*; not controlled by Agr system

**Teichoic acids and poly γ-glutamic acid (PGA)**
- Negatively charged, bind to PNAG/PIA on adjacent cells

**Extracellular DNA** - from lysed cells. DNA is very viscous making dissemination more difficult.

**Aap protein** - Aap has a fibril like structure and is covalently attached to peptidoglycan. Aap requires photolytic activation and Zn+ for polymerization of G5 domains to form fibrils.
- Interactions between the G5 domains of adjacent Aap molecules on different cells in the presence of Zn+, may connect cells in a biofilm
- May be important in PNAG/PIA negative strains
Biofilm Maturation and Dispersal

**Maturation:**
- Poorly understood
- Global changes in gene expression are involved
- Quorum sensing (by agr) controls expression of detergent like compounds and proteolytic activity in the exposed layers that leads to dissemination and detachment

**Dispersal:**
- Poorly understood
- Needed to disseminate the infection
- Controlled by quorum sensing Agr locus
  - In *agr* negative strains the biofilm gets thicker *in vitro*
- Enzymatic degradation of exopolymers and a disruption of non-covalent interactions by detergent-like molecules may be involved:
  - Proteases
  - Phenol soluble modulins (PSMS) = short amphipathic detergent like molecules that may disrupt electrostatic and hydrophobic interactions
  - Expression of both of these is controlled by agr
Staphylococcus aureus uses a two-component response system (TCRS) to mediate quorum sensing (QS). The regulation of QS involves the production of an autoinducer and an increase in its concentration, expression of RNAIII and the subsequent regulation of QS genes. S. aureus produces an autoinducing peptide (AIP) that accumulates extracellularly and activates the TCRS. The TCRS involves signal recognition by a histidine kinase (AgrC) (1), followed by histidine phosphorylation (2) and phosphotransfer to a response regulator (AgrA) (3), which then binds to the RNAIII transcript that encodes a small RNA that functions to modulate gene expression of S. aureus genes (4).
Most “Virulence Factors” of *S. epidermidis* have Roles In Commensal Lifestyle

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<tr>
<th>Pathogenesis</th>
<th>Biofilm proteins (Aap, Bhp)</th>
<th>Colonization</th>
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<tr>
<td>Mechanical resistance (biofilm)</td>
<td>PNAG/PIA</td>
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<td>Immune evasion, AMPs, immunoglobulins, complement and phagocytosis</td>
<td>Protease (SepA)</td>
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<td>Immune evasion and AMPs</td>
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<td>Adhesion to tissue</td>
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