Chapter 15

Gram positive cocci

Staphylococcus

Gram positive cocci

Introduction

There are two important genera of Gram positive cocci: ***Staphylococcus*** and ***Streptococcus***. Both are **non-motile** and **do not form spores**.

Microscopically **staphylococci** appear in **grapelike clusters** and **streptococci** in **chains**.

Biochemically **staphylococci** produce **catalase** which degrades microbicidal H_2O_2 whereas **streptococci** do not.

Their most important representatives are ***Staphylococcus aureus*** and ***Streptococcus pyogenes***.

Staphylococcus

Diseases

-- *Staphylococcus aureus* causes a variety of **suppurative** (pus-forming) infections and **toxinoses** in humans.

-- It causes superficial skin lesions such as **boils**, **styes** and **furuncules**; more serious infections such as **pneumonia**, **mastitis**, **phlebitis**, **meningitis**, and **urinary tract infections.**

-- It also causes deep-seated infections, such as **osteomyelitis** and **endocarditis**.

-- ***S. aureus*** is a major cause of **hospital acquired (nosocomial) infection** of surgical wounds and infections associated with indwelling medical devices.

-- ***S. aureus*** causes **food poisoning** by releasing **enterotoxins** into food, and **toxic shock syndrome** by release of **superantigens** into the blood stream.

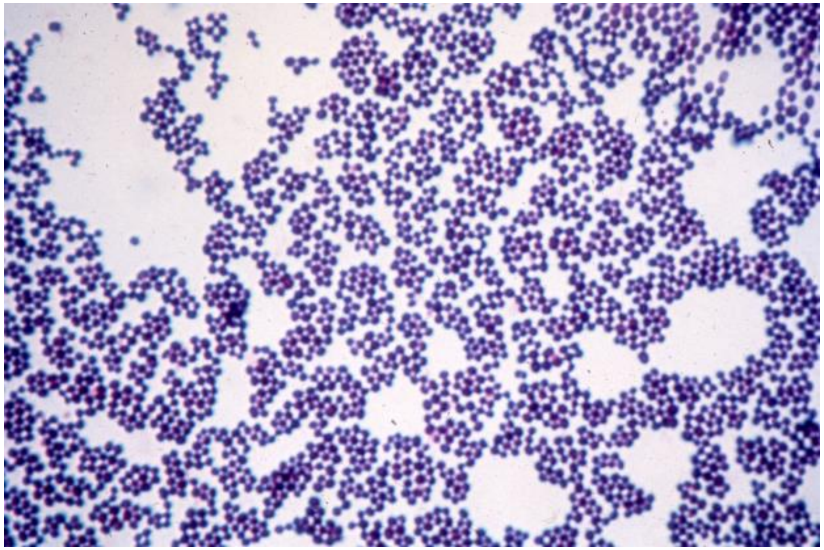
-- **Methicillin-resistant *Staph aureus* (MRSA)** have been entrenched in hospital settings for several decades.

-- **MRSA** strains have recently emerged outside the hospital becoming known as **community associated- MRSA (CA-MRSA)** or **superbug** strains of the organism.

-- These account for the majority of staphylococcal infections seen in clinics.

Important properties

Staphylococci are Gram-positive spherical bacteria that occur in microscopic clusters resembling grapes.



-- Bacteriological culture of the **nose** and **skin** of normal humans invariably yields **staphylococci**.

-- Based on colony appearance, historically there are two species: ***Staphylococcus aureus*** (yellow) and ***Staphylococcus albus*** (white), now called ***Staphylococcus epidermidis***.

-- Another species ***Staphylococcus saprophyticus*** is involved in urinary infections.

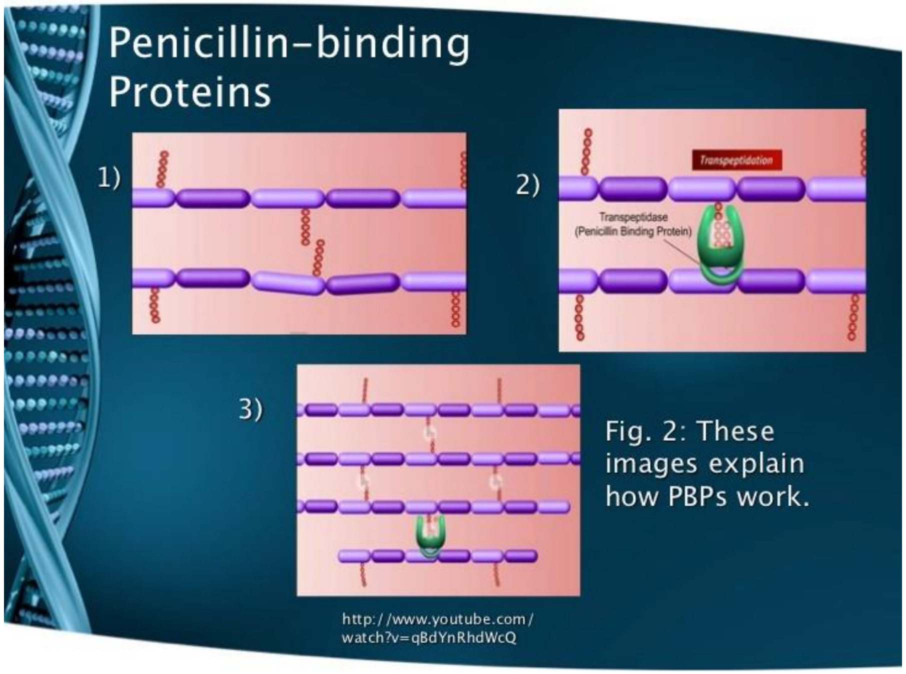
-- *Staphylococcus aureus* produce **yellow** colonies due to a pigment called **staphyloxanthin**.

-- It also **inactivates toxic oxygen species**.

-- *Staphylococcus aureus* usually **ferments mannitol** and **hemolyzes RBCs** whereas other two species do not. Hemolysis provides **iron** for growth.

-- More than **90%** ***Staphylococcus aureus*** strains contain **plasmids** that **encode β-lactamases**.

-- Some strains are even resistant to β-lactamase resistant penicillins such as **methicillin** and **nafcillin** by making changes in PBPs.



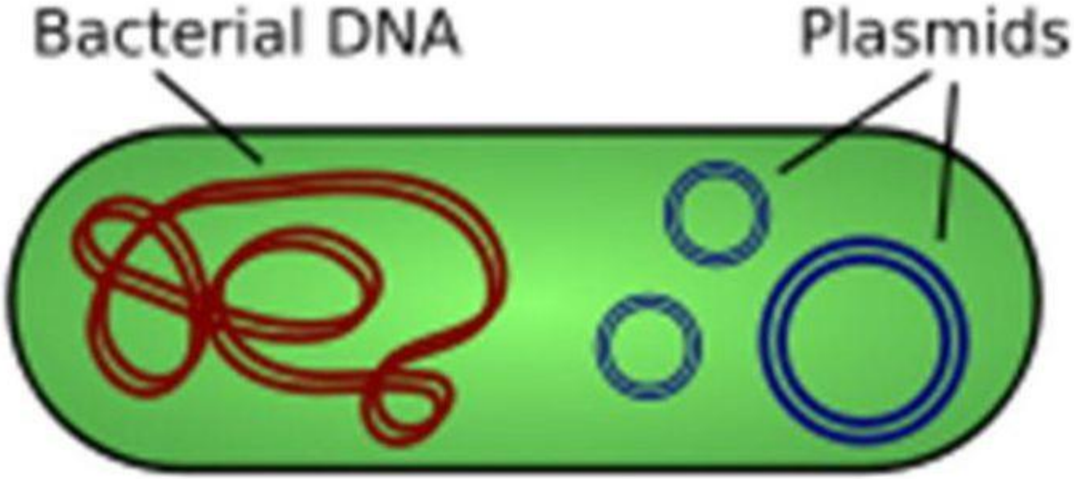
-- Relevant gene, ***mecA***, is located on chromosome.

-- These strains are called **methicillin** or **nafcillin** resistant ***Staphylococcus aureus*** (**MRSA** and **NRSA**) and constitute for 50% of ***Staphylococcus aureus*** causing hospital infections in USA.

-- Strains with intermediate and full resistance to vancomycin (**VISA** and **VRSA**) have been detected.

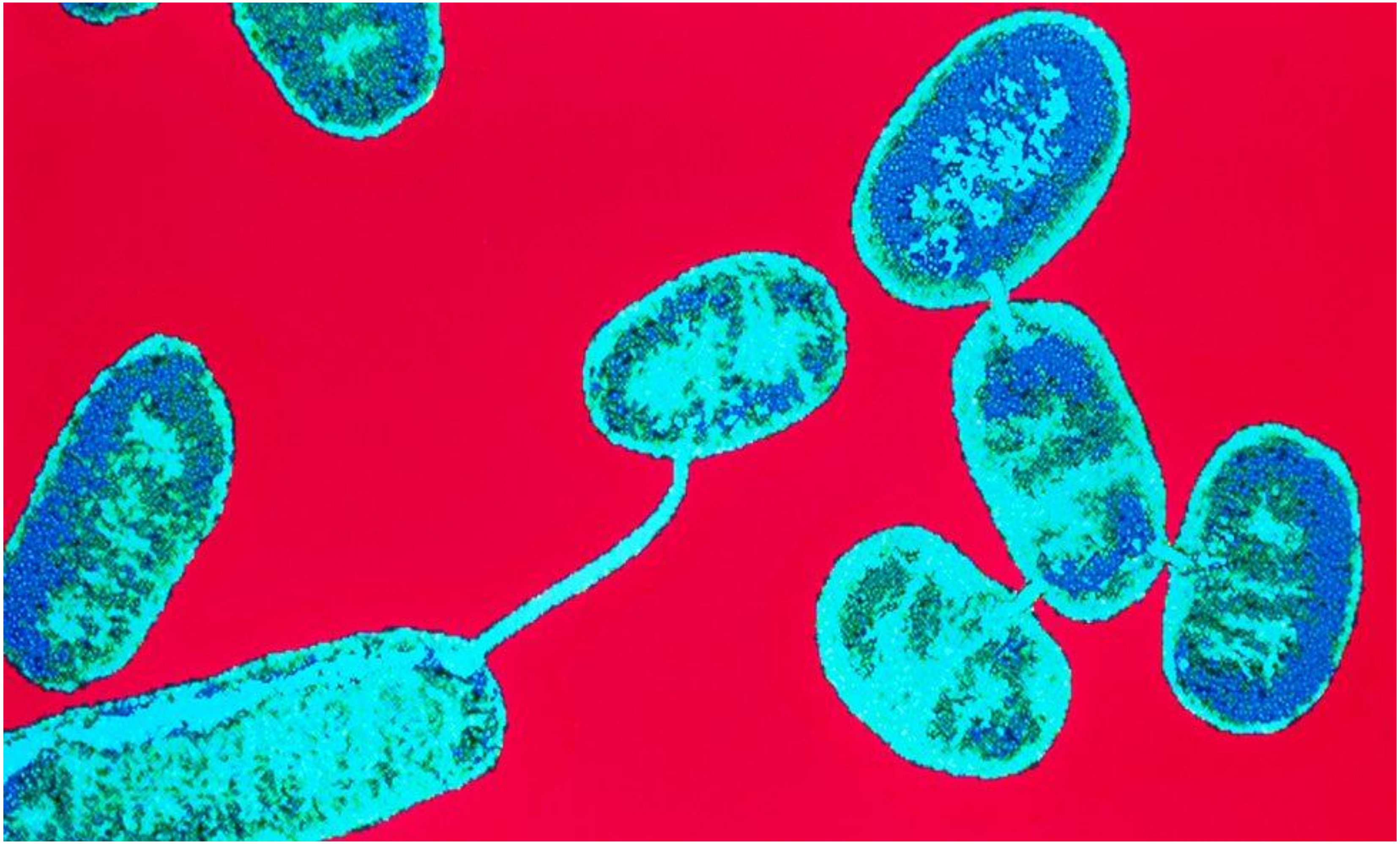
-- The relevant genes are located on a **transposon** and encode enzymes that substitute **D-lactate** for **D-alanine**.

What are Plasmids?



- Plasmids are circular pieces of bacterial DNA that often contain genes not related to basic life functions
- Often contain antibiotic resistance genes
- Humans often cut open plasmids…attach a desired gene…reinsert the plasmid to the bacteria
- When complete, the bacteria will contain a new gene (instructions) to create a desired protein such as insulin.

CONJUGATION



Dr Linda Stannard, UCT/SPL 26th Sept 2014 iWonder BBC News

Transposon mediated resistance

-- Transposons are self directed elements that code for **transposases**.

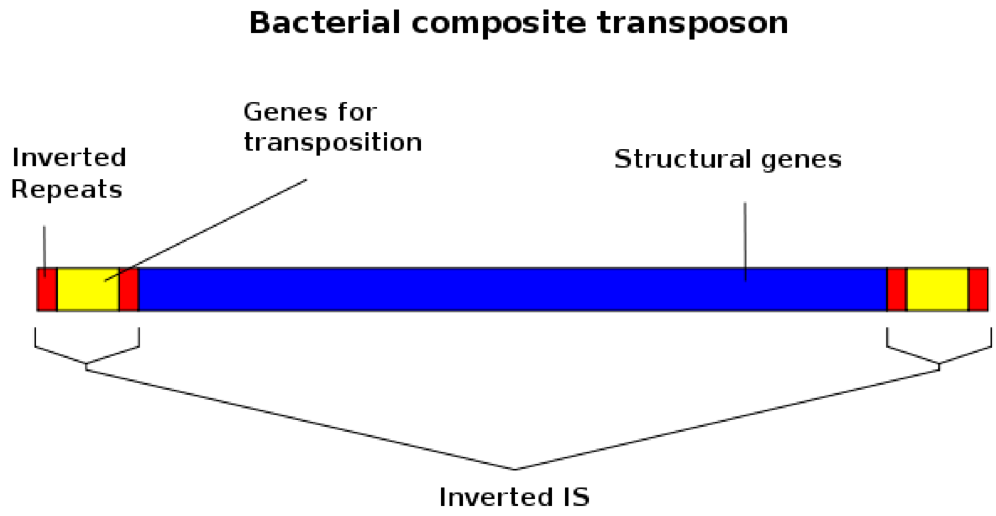
-- **Transposases** are enzymes that bind to the ends of a transposon and **catalyze its movement** to another part of the genome by a **cut and paste mechanism** or a **replicative transposition mechanism**.

-- A typical drug resistance transposon is composed of **three genes flanked on both sides by shorter DNA segments**, usually a series of **inverted repeats** that mediate the interaction of the transposon and larger DNA.

Transposon

The three genes code for :

1. **transposase** which controls excision and reintegration
2. **repressor** that controls synthesis of transposase and
3. **drug resistance** genes.



Staphylococcus aureus has several important **cell wall components**, **excretions** and **antigens**:

Coagulase

Coagulase is an **extracellular protein** which binds to **prothrombin** in the host to form a complex called **staphylothrombin**.

The **protease activity** characteristic of thrombin is activated in the complex, resulting in the conversion of **fibrinogen** to **fibrin**.

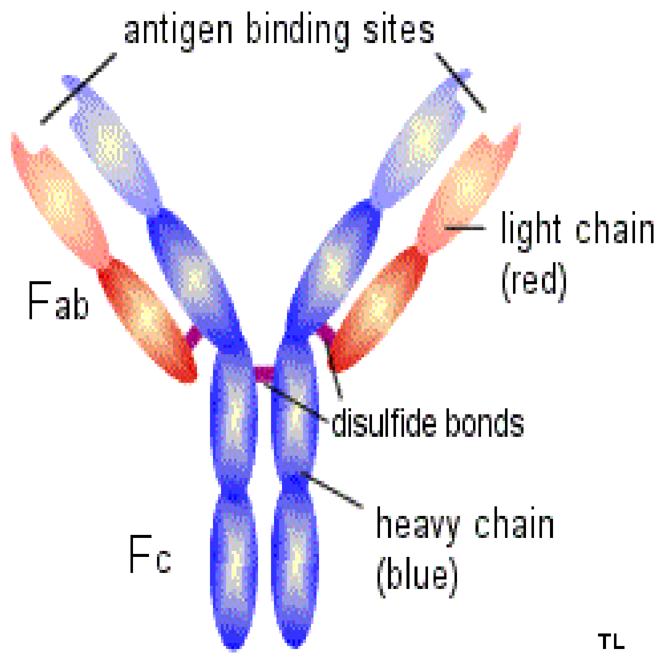
Coagulase is a **traditional marker** for identifying ***S. aureus*** in the clinical microbiology laboratory.

Protein A

Protein A is a surface protein of ***S. aureus*** which binds **IgG** molecules by their **Fc** region.

In serum, the bacteria will bind IgG molecules in the **wrong orientation** on their surface, which disrupts **opsonization** and **phagocytosis**.

Mutants of ***S. aureus*** **lacking protein A** are more efficiently phagocytosed in vitro, and mutants in infection models have **diminished virulence**.



Capsular Polysaccharide

-- It is an important **virulence factor**.

-- There are **11** serotypes but **majority** of clinical isolates of ***S. aureus*** express either **serotype 5 or 8**.

-- This has been 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.

-- The capsule is **poorly immunogenic** so an effective vaccine production is difficult.

Transmission

-- **Nose** is the main site of colonization being present in about **30%** of general population. Such people have increased risk of skin infections.

-- **Skin** is also a common site of colonization.

-- **Hand contact** is an important mode of transmission. It also colonizes vagina of 5% women which predisposes them to **toxic shock syndrome.**

-- **Lesions** and **fomites** contaminated with material from these lesions may also spread staphylococcal infection.

-- **Staphylococcal disease** is favored by **heavily contaminated environment** and a **compromised immune system**, esp. humoral immunity.

-- **Diabetes**, **chronic granulomatous disease** (CGD; defective neutrophils) and **intravenous drug use** are also favorable factors.

-- ***Staphylococcus epidermidis*** is found on **human skin** and can enter **bloodstream** at the site of iv catheters.

-- ***Staphylococcus saprophyticus*** is found primarily on the **mucosa** of genital tract of young women and can ascend to **urinary bladder** to cause infection.

Pathogenesis

Staphylococcus aureus

-- It can cause disease both by producing **toxins** and by inducing **pyogenic** inflammation.

-- Typical lesion is **abscess** which undergo **central necrosis** and usually drain to the outside (e.g., furuncles and boils) but organisms may disseminate via the blood stream as well.

-- Foreign bodies, such as **sutures** and **iv catheters** are important predisposing factors.

Several important **toxins** and **enzymes** are produced:

Enterotoxins

-- ***S. aureus*** secretes two types of toxins with **superantigen** activity: **enterotoxins**, of which there are six antigenic types (named **SE-A, B, C, D, E and G**), and **toxic shock syndrome toxin** (**TSST-1**).

-- **Enterotoxins** cause **diarrhea** and **vomiting** when ingested and are responsible for staphylococcal **food poisoning**.

Toxic shock syndrome toxin

-- **TSST-1** is expressed **systemically** and is the cause of **toxic shock syndrome (TSS)**.

-- When expressed systemically, **enterotoxins** can also cause **toxic shock syndrome (TSS)**.

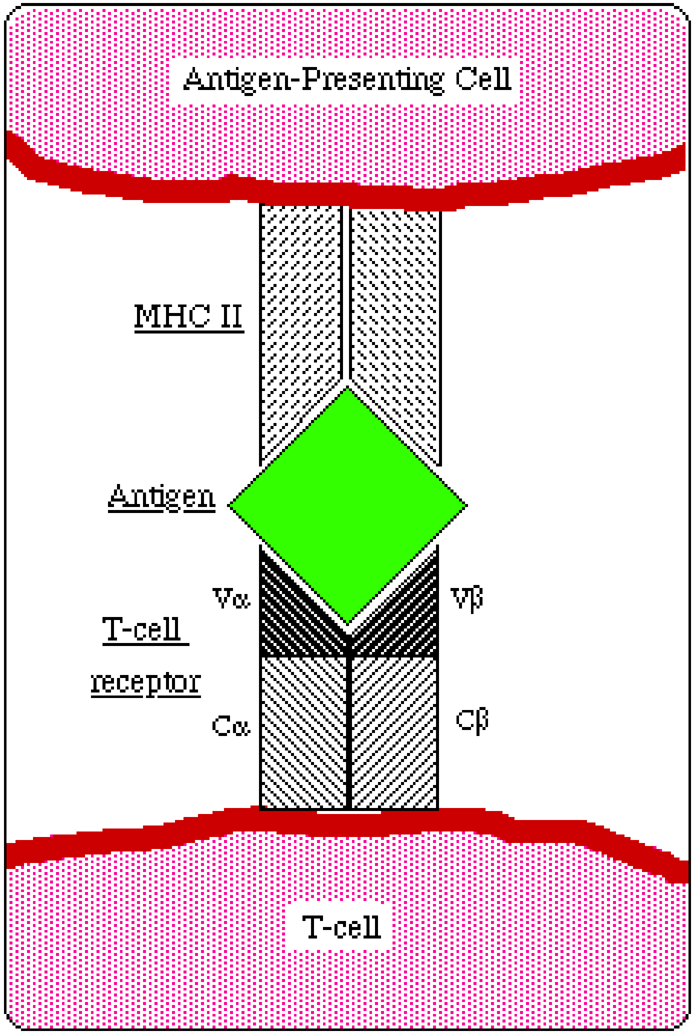
-- **TSST-1** is responsible for **75% of TSS**.

-- **TSS** can occur as a **sequel** to any staphylococcal infection if an enterotoxin or TSST-1 is released systemically, and the host lacks appropriate neutralizing antibodies.

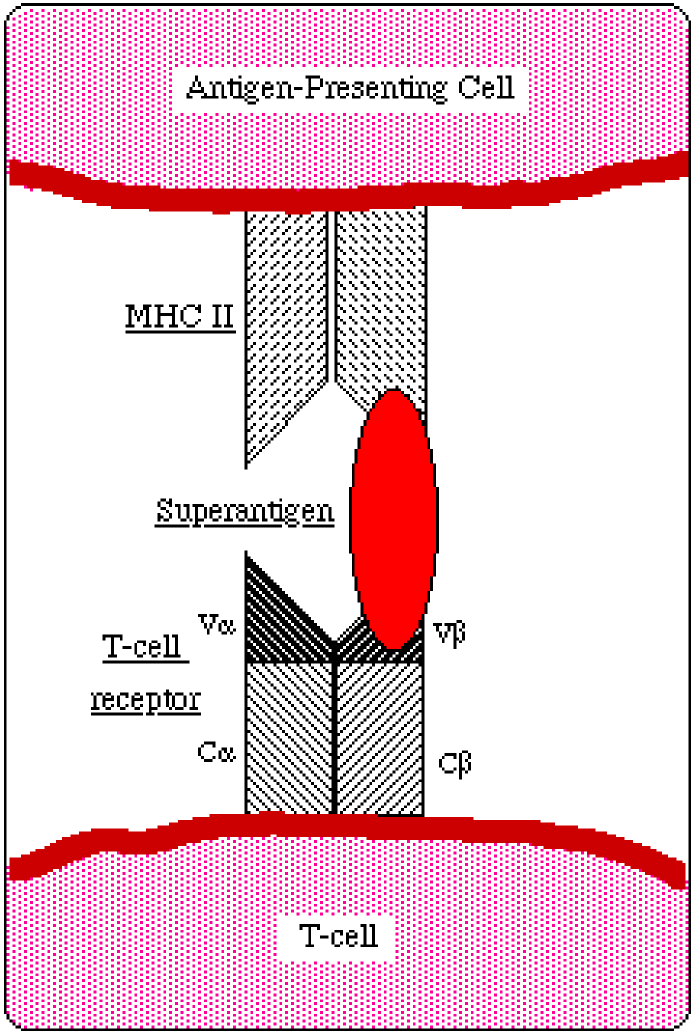
-- **Superantigens** stimulate T cells **non-specifically** without normal antigenic recognition.

-- Up to **1 in 5** T cells may be activated, whereas only **1 in 10,000** are stimulated during a usual antigen presentation.

-- **Cytokines** are released in large amounts, causing the symptoms of TSS.



Normal Antigen Presentation



Superantigen

Exfoliatin toxin (ET)

-- The **exfoliatin toxin**, associated with **scalded skin syndrome**, causes **separation within the epidermis**, between the living layers and the superficial dead layers.

-- Healing occurs with little scarring although the risks of **fluid loss** and **secondary infections** are increased.

Exotoxins

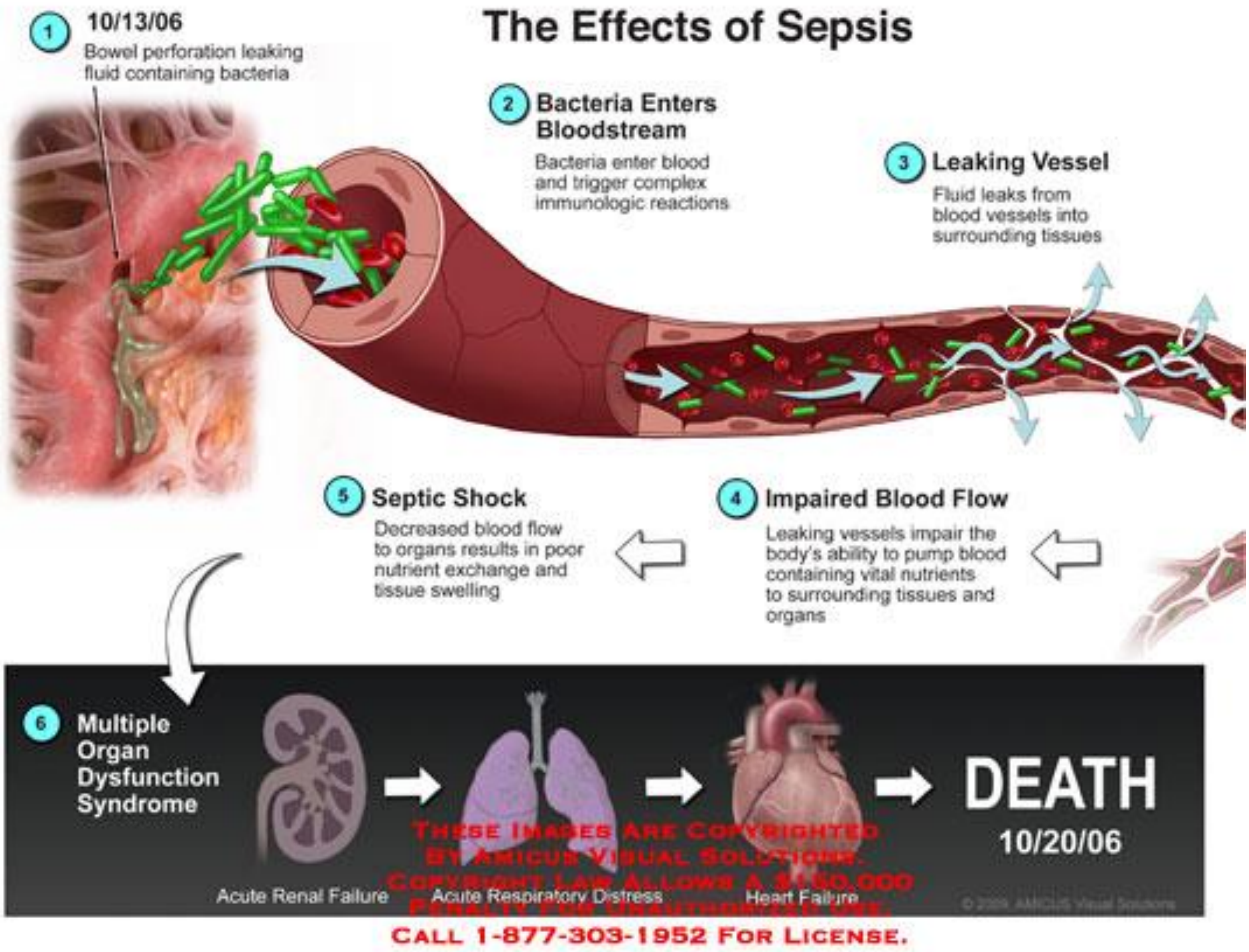
S. aureus can express several different types of **protein toxins**.

alpha toxin (alpha-hemolysin)

-- Systemic release of alpha toxin causes **septic shock**.

-- In humans, **platelets** and **monocytes** are particularly **sensitive** to alpha toxin.

-- Susceptible cells have a **specific receptor** for alpha toxin which allows the toxin to bind causing **small pores** through which monovalent cations can pass.



The Effects of Sepsis
1 10/13/06
Bowel perforation leaking fluid containing bacteria
2 Bacteria Enters Bloodstream
Bacteria enter blood and trigger complex immunologic reactions
3 Leaking Vessel
Fluid leaks from blood vessels into surrounding tissues
4 Impaired Blood Flow
Leaking vessels impair the body's ability to pump blood containing vital nutrients to surrounding tissues and organs
5 Septic Shock
Decreased blood flow to organs results in poor nutrient exchange and tissue swelling
6 Multiple Organ Dysfunction Syndrome
Acute Renal Failure
Acute Respiratory Distress
Heart Failure
DEATH
10/20/06

PV Leukocidin

-- It specifically acts on **polymorphonuclear leukocytes**. It forms **pores** in the affected membrane.

-- Leukocidin is **hemolytic**, but less so than alpha hemolysin.

-- Only **2%** of all of ***S. aureus*** isolates express leukocidin, but nearly **90%** of the strains isolated from severe **dermonecrotic lesions** express this toxin, which suggests that it is an important factor in necrotizing skin infections.

-- There are several other enzymes which include **coagulase**, **fibrinolysin**, **hyaluronidase**, **proteases**, **nucleases**, and **lipases**. **Staphylokinase** is a fibrinolysin that can **lyse thrombi**.

Clinical findings

S. aureus: pyogenic diseases

1. **Skin infections** are very common.

   These include **impetigo**, **furuncles, carbuncles, paronychia, cellulitis, folliculitis, hydradenitis supparativa, conjunctivitis, eyelid infections, mastitis,** and **lymphangitis**.

   Severe **necrotizing** and **soft tissue** infections are caused by **MRSA strains** that produce **leukocidin**. These are typically community acquired.

   These **CA-MRSA** are different from **HA-MRSA**.

2. **Septicemia** can originate from **any localized lesion** especially wound infections.

   Clinical features of sepsis are similar to that caused by some Gram negative bacteria.

3. **Endocarditis** may occur on normal or prosthetic heart valves.

4. **Osteomyelitis** and **arthritis** may arise either by hematogenous spread from a distant infected focus or be introduced locally at a wound site.

5. It is the most common cause of **postsurgical wound infections.**

6. **Pneumonia** may occur in **postoperative patients** or following viral respiratory infection, esp. influenza.

   It often leads to **empyema** or **lung abscess**.

   In many hospitals, it is the most common cause of nosocomial pneumonia, esp. in ventilator associated pneumonia in ICUs.

   CA-MRSA causes a severe necrotizing pneumonia.

7. **Conjunctivitis** typically presents with unilateral burning eye pain, hyperemia of conjunctiva, and a purulent discharge.

   Transmission is from hands. It is the most common cause of conjunctivitis.

8. If **bacteremia** occurs, abscesses can form in any organ. These are called **metastatic abscesses**.

Abscess

Carbuncle, a multiheaded abscess

Folliculitis: multiple, small pustules

Impetigo: vesicular lesions covered with“honey-colored” crust erythema.

S. aureus: toxin mediated diseases

1. **Food poisoning (gastroenteritis)** is caused by **ingestion of enterotoxin** which is **preformed** in foods and has a short incubation period.

   Vomiting is typically more prominent than diarrhea.

2. **TSS** is characterized by **fever, hypotension, a sunburn like rash** that goes on to **desquamate** and involvement of three or more of following organs:

   liver, kidney, GIT, CNS, muscle or blood.

3. **Scalded skin syndrome** is characterized by fever, large bullae, erythromatous, macular rash.

Large areas of skin **slough, serous fluid exudes** and **electrolyte imbalance** can occur. Hair and nails can be lost.

Recovery occurs in 7-10 days. It is most common in young children.

S. epidermidis

-- *S. epidermidis* infections are almost always **hospital acquired** whereas those of *S. saprophyticus* are usually **community acquired**.

-- *S. epidermidis* can cause **metastatic infections** especially at the site of implants such as iv catheters, prosthetic heart valves, prosthetic joints, vascular grafts.

-- It is also a major cause of **sepsis in neonates**, and of **peritonitis** in patients with renal failure. It also causes CSF shunt infections.

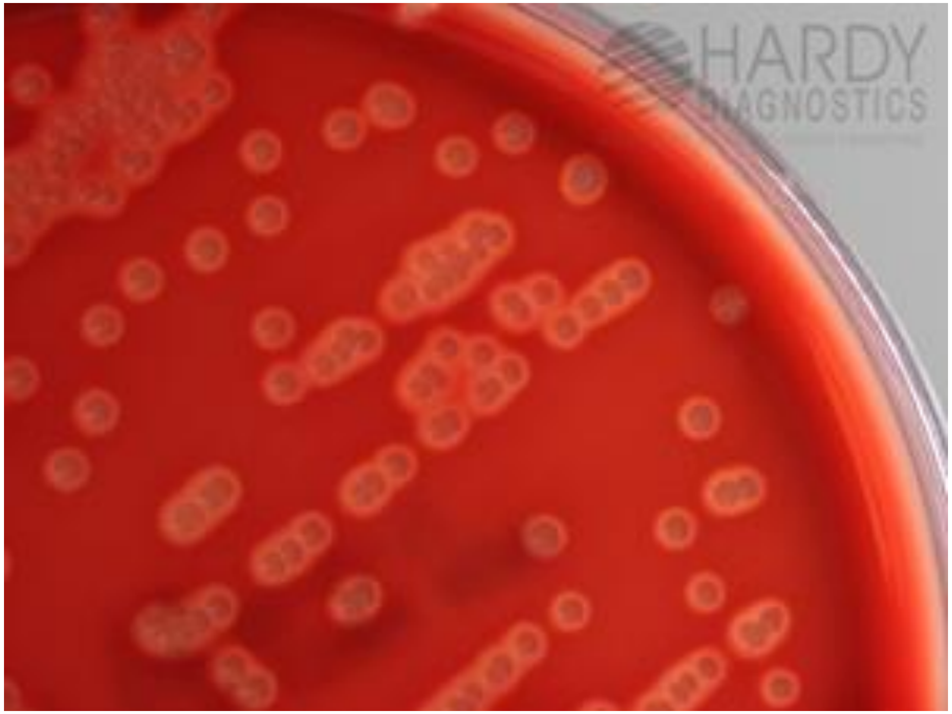
S. saprophyticus

S. saprophyticus causes **UTI**. It is second most common pathogen in community acquired UTI in young women after *E.coli*.

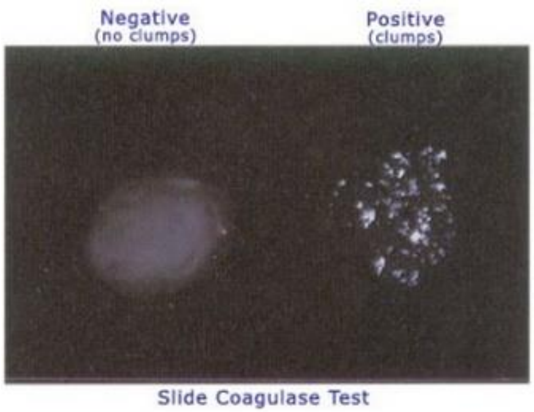
Laboratory diagnosis

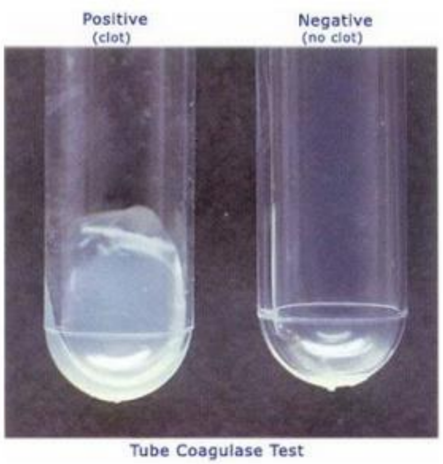
-- **Smears** from staphylococcal lesions reveal **Gram positive cocci** in **grape like clusters**.

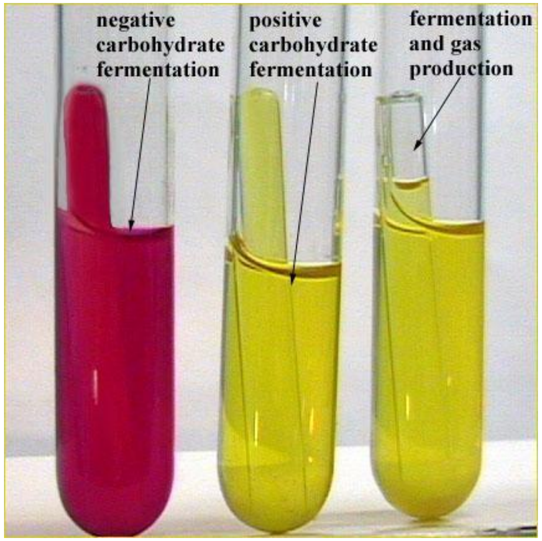
-- ***Staph. aureus*** typically yields **golden-yellow** colonies that are usually **β-hemolytic.**



-- *Staph. aureus* are **coagulase** and **mannitol positive**.







-- Cultures of **coagulase negative Staphylococci** yield **white, non-hemolytic colonies**.

-- They can be further differentiated by **sensitivity to novobiocin**. ***Staph. epidermidis*** is **sensitive**, ***Staph. saprophyticus*** is **resistant**.

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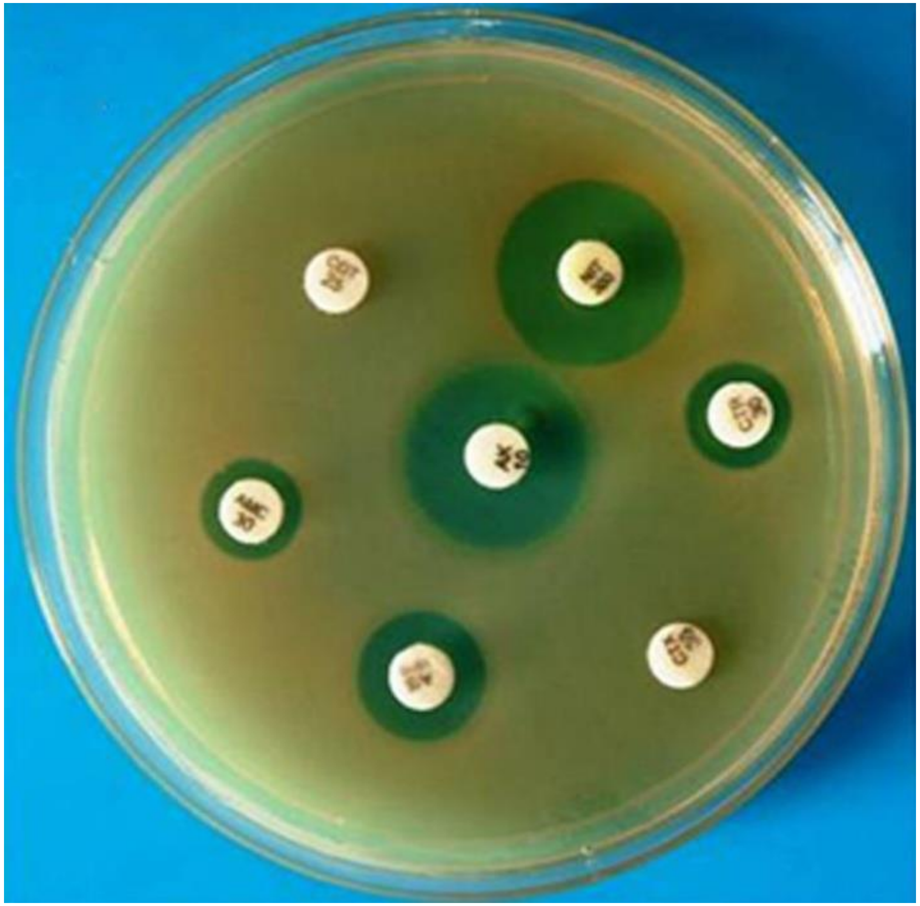
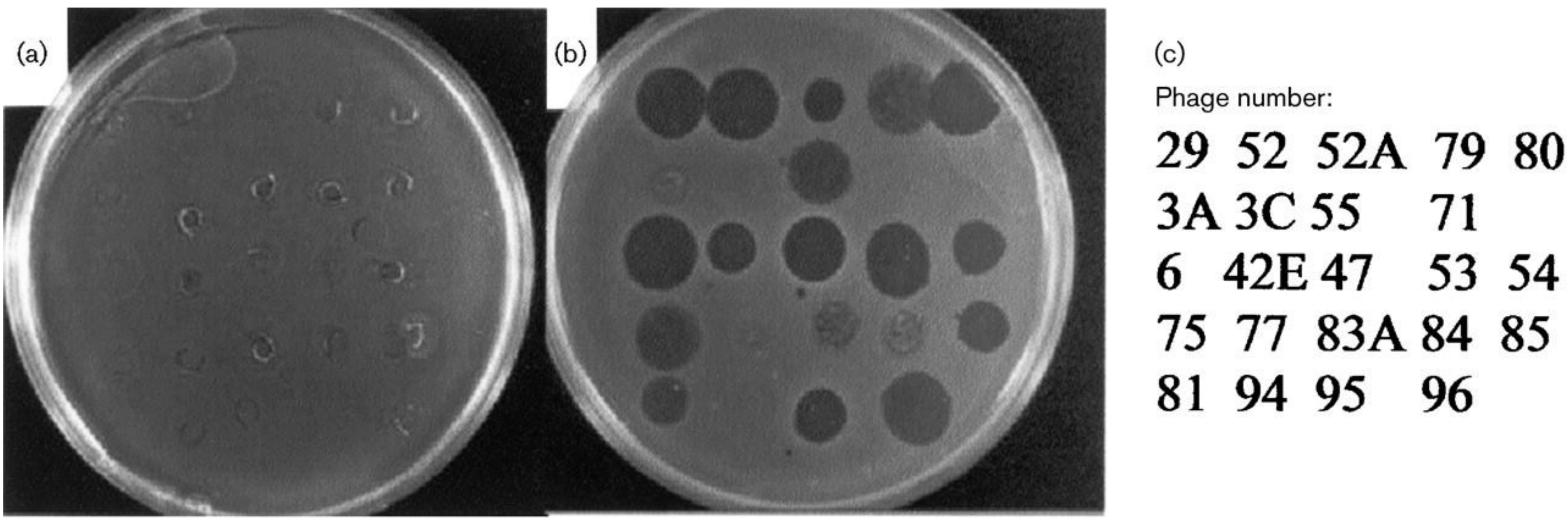


Epidemiology

-- For epidemiology, *Staph. aureus* can be divided into subgroups based on the **susceptibility of the clinical isolate to lys**is by a variety of **bacteriophages**.

-- A person carrying the **same phage group** as that which caused an outbreak may be the cause.



Treatment

-- Most strains of ***Staph. aureus*** are **resistant** to **penicillin G** as they **produce β-lactamases**.

-- β-lactamase resistant penicillins such as **nafcillin** or **cloxacillin,** some **cephalopsorins** or a **combination** of a β-lactamase sensitive penicillin (e.g. **amoxicillin**) and a β –lactamase inhibitor (e.g. **clavulanic acid**) may be used.

-- Nearly 20% strains are **MRSA** or **NRSA** due to **altered PBP**. **MRSA** are **resistant** to almost all β-lactam drugs.

-- These strains can be a cause of **large outbreaks** especially in **hospitals**.

-- They can be treated with **vancomycin,** sometimes supplemented by **gentamicin. Daptomycin** may also be used.

-- **Trimethoprim-sulfamethoxazole** or **clindamycin** may be used for **non-life threatening** infections.

-- **VISA** and **VRSA** strains which are usually also methicillin and nafcillin resistant are very difficult to treat. **Daptomycin** or **quinupristin-dlafopristin** may be used.

-- **Treatment** of **TSS** involves **correction of the shock** by using fluids, pressor drugs, and inotropic drugs.

-- Pooled serum globulins which contain **antibodies against TSST** may also be useful.

-- **Tolerant strains** which **inactivate drugs** that stop inhibitors of autolytic enzymes are treated with a combination of drugs.

-- **Drainage** is most important for **abscess treatment** and antimicrobials may not be needed in most cases.

-- ***Staph.epidermidis*** are **highly resistant** to antimicrobials but are **sensitive** to β-lactamase resistant drugs such a **methicillin** and **nafcillin.**

-- These are called **methicillin sensitive** ***Staph.epidermidis*** (**MSSE**). Some strains can be **MRSE**. Drug of choice is **vancomycin** with added rifampin or an aminoglycoside.

-- Removal of catheter or other devices is often necessary.

-- ***Staph. saprophyticus*** **UTI** can be treated with **trimethoprim-sulfamethoxazole** or a fluoroquinolone, e.g., **ciprofloxacin**.

Prevention

-- There is **no vaccine** for staphylococcal diseases.

-- Cleanliness, frequent hand washing and effective management of lesions

-- help in control.

-- Persistent **colonization of nose** can be reduced by intranasal **mupirocin** or oral antimicrobials such as **ciprofloxacin** or **trimethoprim-sulfamethoxazole**, but is difficult to eliminate totally.

-- **Shedders** may have to be removed from high risk areas.

-- **Cefazolin** is often used preoperatively.


Thank You!!