Gram positive cocci

Cocci in cluster

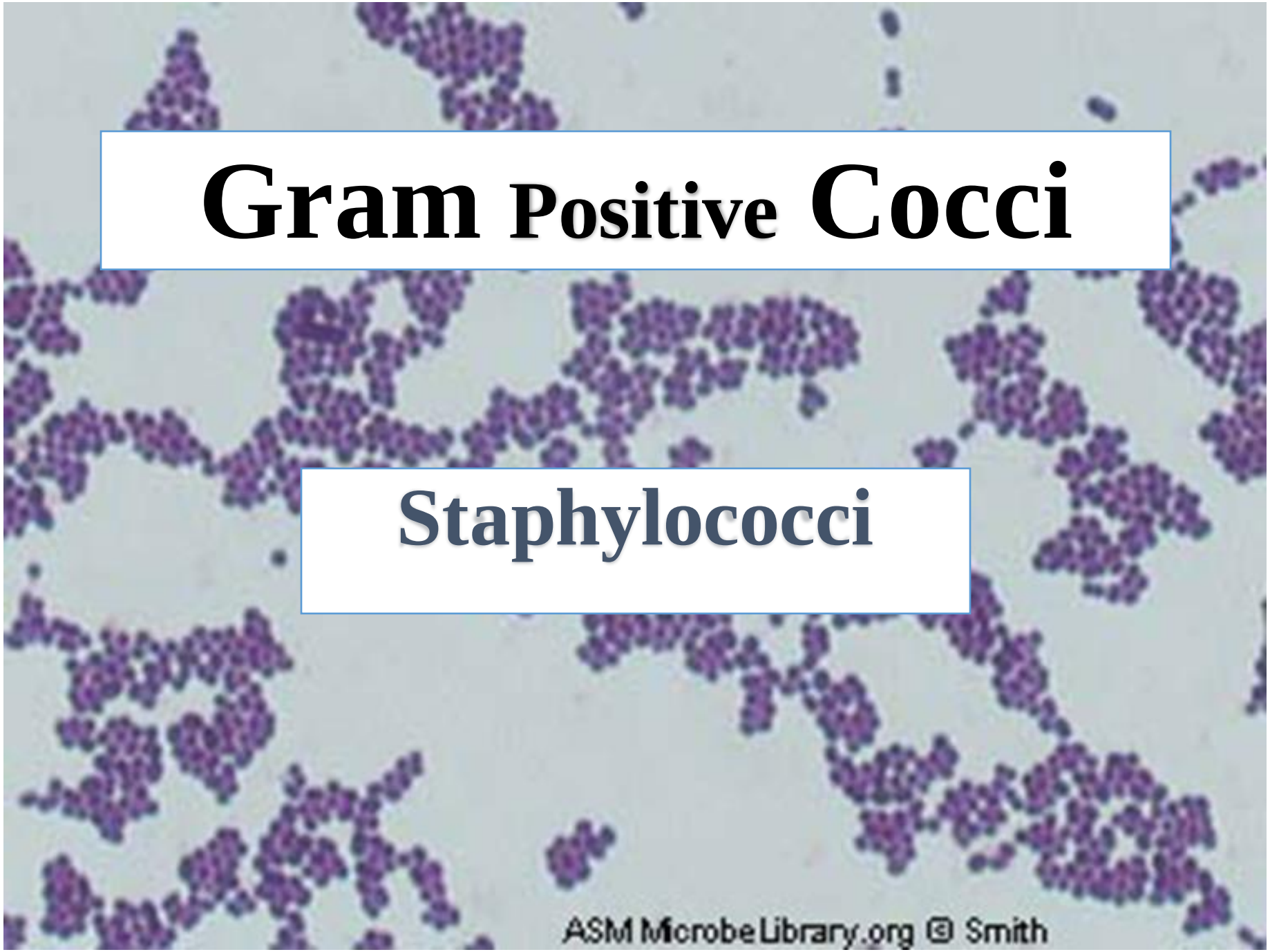
- Staphylococcus sp.

Cocci in pairs or chains

- Streptococcus pneumoniae (Diplococci)
- Streptococci (chains of variable length)

Gram Positive Cocci

Staphylococci



Family: Micrococcaceae

Genus: Micrococcus
Present in air (contaminate open culture media), skin and mucus membrane and not pathogenic.

Genus: Planococcus
Are motile cocci not pathogenic, produce tetrads and yellow brown pigment on nutrient agar (contaminants).

Genus: Staphylococci. More than 40 species associated with man and animals

General characteristics

1- Gram +ve cocci arranged in grape or cluster like but single cocci, diplococcic, tetrads and short chains can be found in liquid culture. (Diagnosis can not depend only on morphology)

2- Are aerobic and facultative anaerobic bacteria

3- One of the hardy bacteria which can withstand heat, dryness and 7.5% NaCl, therefore Mannitol salt agar is a selective media for isolation.

4- They are associated with skin as normal flora and can grow inside and outside of cells but commonly extracellular bacteria.

5- Catalase +ve which differentiate from Streptococci (This is the second test used to confirm the genus after Gram stain morphology)

6- Staphylococcus. sp are divided into pathogenic and non pathogenic species according to coagulase test. Coagulase +ve Staphylococci are mainly *Staph. aureus* (both human and animals) , *Staph. intermedius* (zoonotic bacteria) and Coagulase negative Staphylococci (CNS). Among CNS two species are important, *Staph.epidermidis* (albus) and *Staph.saprophyticus* (citreus)

Classification of Staphylococci

Pigment production
Mainly developed at room temperature after 2 or more days

1- Staph.aureus (pyogenes)-golden yellow

2- Staph.epidermidis (albus)-White

3- Staph.saprophyticus (citreus)-lemon yellow

Coagulase test

Pathogenic species
Staph.aureus

Non pathogenic species
Others
1- Staph.albus
2-Staph.citreus

Staphylococcus aureus

Cultural characteristics

1- Simple bacteria grow on both simple (nutrient) and enriched (blood)media

2- Facultative anaerobic but prefer aerobic conditions.

3- It produces uniform turbidity in liquid media and no pigment is produced.

4- colonies on blood after 24 hrs are pigmented, golden yellow, 2 to 4 mm, circular, convex, smooth, shiny, opaque, with entire edge and emulsifies easily and surrounded by a zone of beta hemolysis (Figure 1).

5- Pigment production occurs optimally at 22°C and only in aerobic culture.

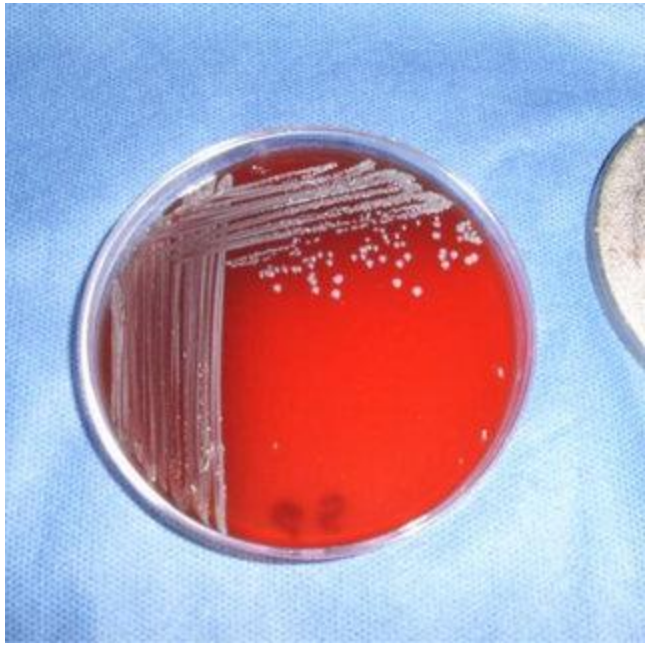
6- Can grow on Mac Conkey agar producing small and pink colonies (lactose fermenter) as shown in figure 2.

Fig.1. Colonies of *Staph.aureus* on blood agar

Fig.2. Colonies of *Staph.aureus* on Mac Conkey agar

Methicillin Resistant Staphylococcus aureus (MRSA)

Appear as white, small and non hemolytic colonies on blood agar similar to CNS. Morphologically similar with MSSA.



Selective media

Mannitol salt agar (7.5% NaCl+ Phenol red pH indicator) is a selective and differential media for Staphylococcus spp.

Can differentiate between mannitol fermenter (yellow colonies) usually Staph.aureus and mannitol non fermenter Staphylococci (non pathogenic species) (Figure 3.)

Baird-Parker agar: Is a selective medium for isolation and enumeration of Staph.aureus from food. Colonies are black in color surrounding a halo as shown in Fig.4

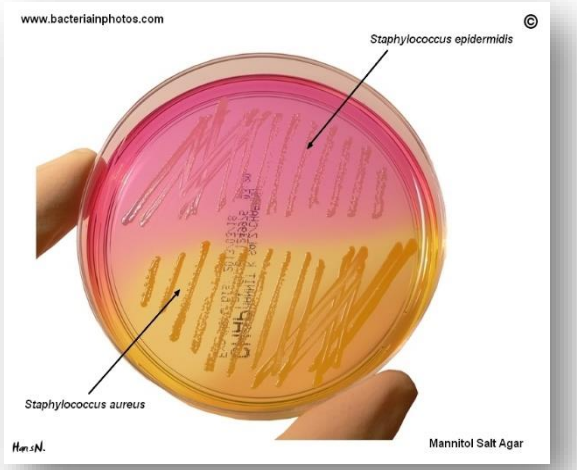


Fig.3. Growth of *Staph.aureus* on mannitol salt agar

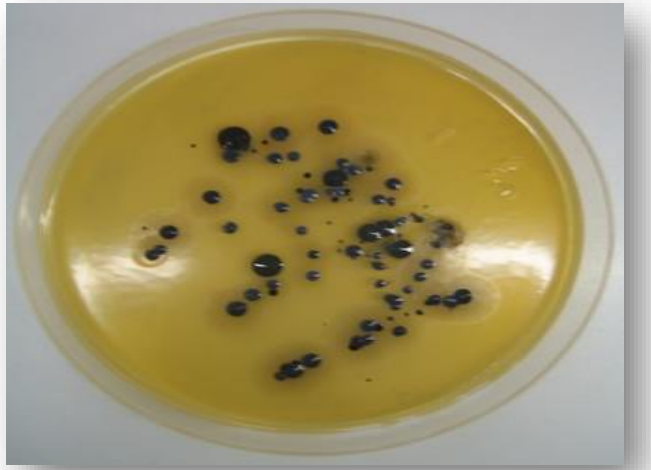
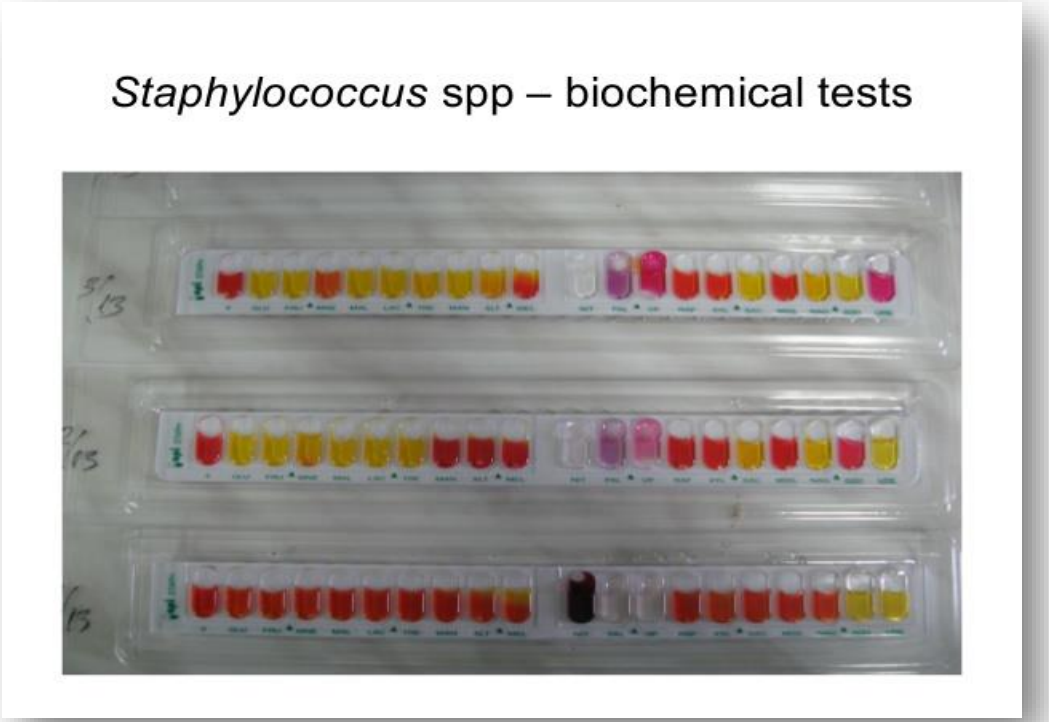


Fig.4. Growth of *Staph.aureus* on Baire-Parker medium

Characteristics of *Staph.aureus*

1. Coagulase positive.
2. Mannitol fermentation.
3. Beta hemolysis.
4. Golden yellow pigment.
5. Liquefies gelatin.
6. Phosphatase is produced.
7. Sensitivity to lysostaphin.
8. Hydrolyses urea.
9. Reduces nitrates to nitrites.
10. Tellurite reduction. Baired Parker media
11. Deoxyribose nuclease enzyme production
12. Produce lipase



API STAPH strips

Biochemical reactions
Staph.aureus produce different enzymes and ferment different sugars which can be done by API STAPH (20 miniaturized tests to differentiates among different Staph.species)

Resistance

Staph. aureus (hardy bacteria) is the most resistant bacteria against the heat and dryness among Gram positive bacteria

It withstands 60°C for 30 minutes.

Crystal violet (1/500000) and brilliant green (1/1000000) are lethal therefore added to Mac Conkey agar to increase its selectivity against growth of Staph.

Staph.aureus produce beta lactamase enzyme (penicillinase) (plasmid carry gene spread mainly by conjugation or transduction). Methicillin Resistant Staph.aureus (MRSA) due to the mutation in the PBPs which can be detected phenotypically using methicillin or cloxacillin disc diffusion method or genetically using primers for *mecA gene* (PCR technique)

Sometimes Staph.aureus becomes tolerant to antibiotics especially when given in incorrect dose so the pathogen will be inhibited but not killed and can produce protoplast.

Antigenic structure

Although more than 30 different antigens are found in Staph.aureus but rarely used in serological test among them teichoic acid can be used to for serological diagnosis of endocarditis caused by Staph.aureus.

Protein A: This is the major and most important protein in the cell wall of Staph.aureus and can serve two functions

1- Antiphagocytic factor (virulence factor) by binding to FC portion of IgG, exerting antiopsonin (Figure 4).

2- It is important in serological tests (coagulation tests) in which soluble antigens are converted into particulate antigen via binding with protein A of special strain of Staph.aureus(Figure 5).

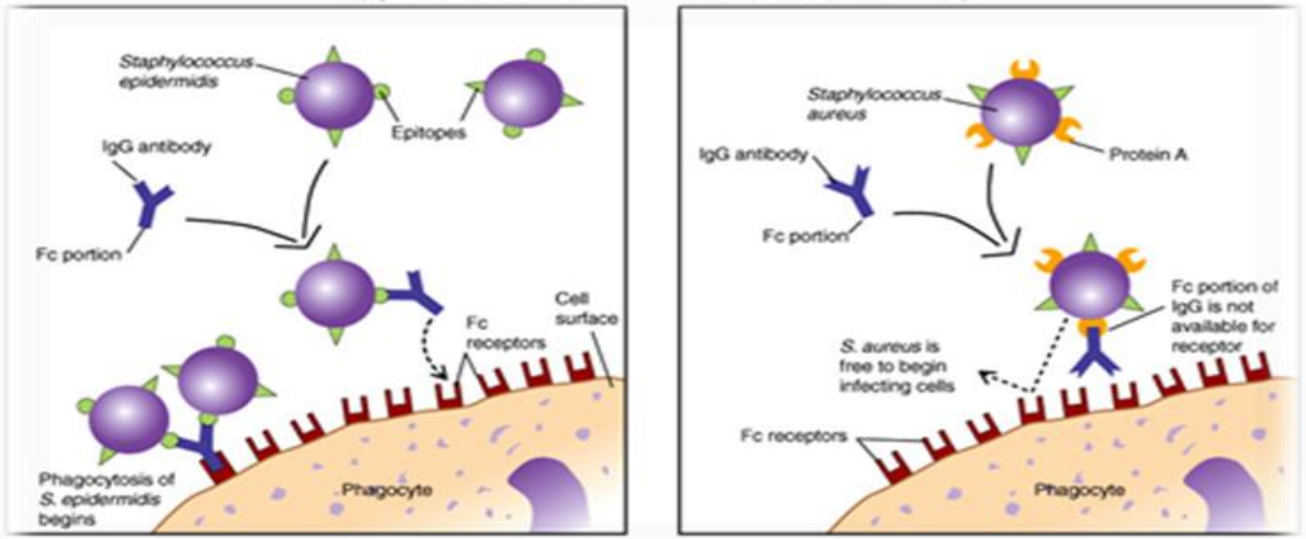


Figure 5. Antiphagocytic effect of protein A

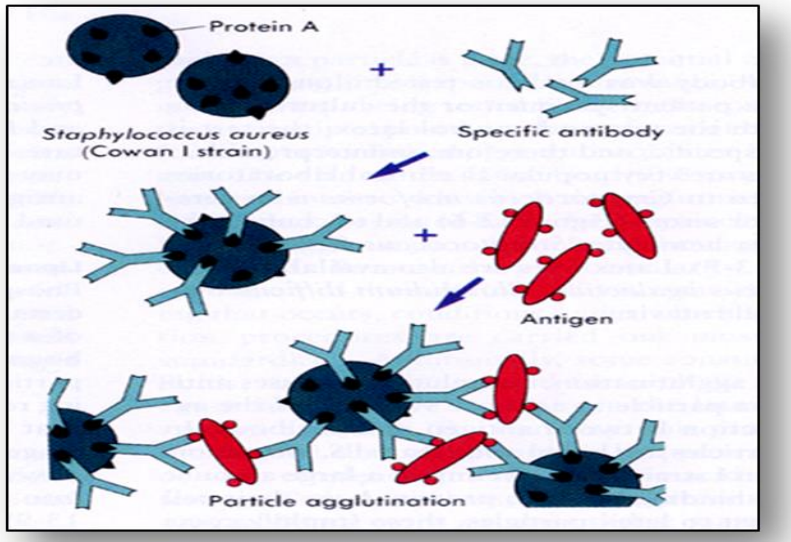


Figure 6. Coagglutination test

Phage typing

It is used to determine the source of infection either from hospital or community. A set of 28 known phages were used to differentiates between community acquired and hospital acquired strains of Staph.aureus as shown in figure 7.

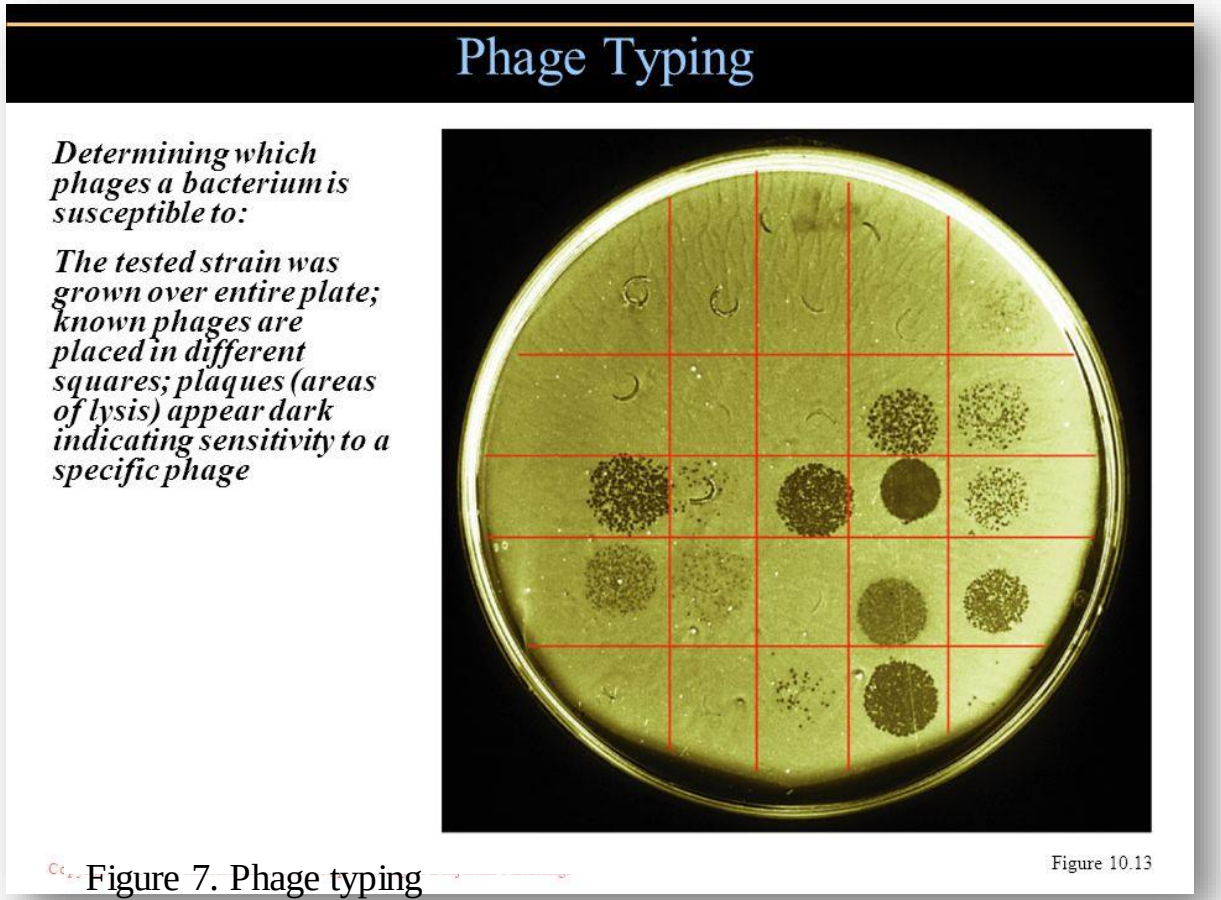


Figure 7. Phage typing

Enzymes produced

1- Coagulase (2 types with 2 methods)

A- Free coagulase (detected by tube method)

- It is filterable
- Heat labile
- Antiphagocytic (virulence factor)
- Antigenic with different types
- With coagulase reactive factor (CRF) in the plasma converts fibrinogen into fibrin clot (Figure 8)

Procedure

0.1 ml of overnight growth+0.9 ml of 1/10 diluted rabbit or human plasma or 0.1 ml bacteria + 0.5 ml plasma and incubate at 37 C for 4 hrs (Figure 9).

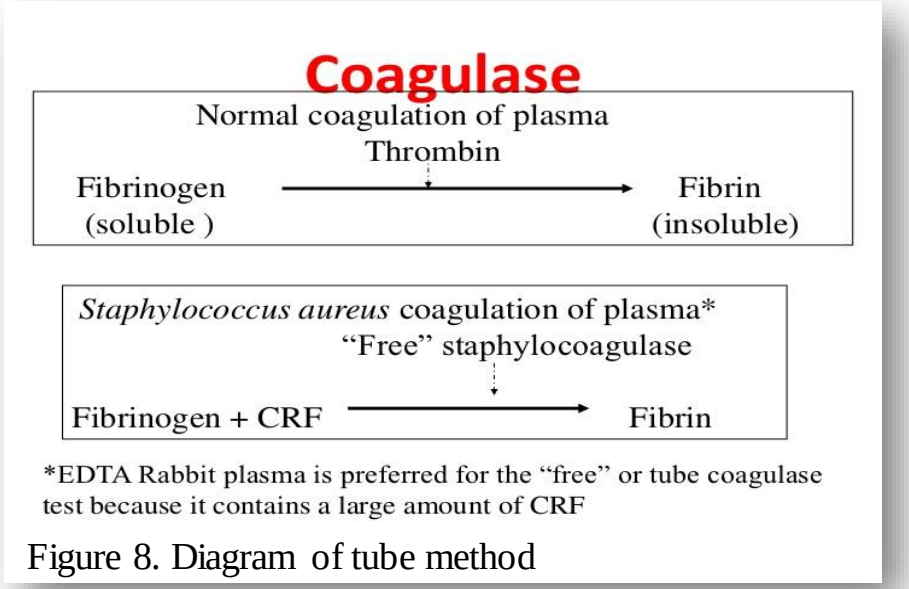


Figure 8. Diagram of tube method

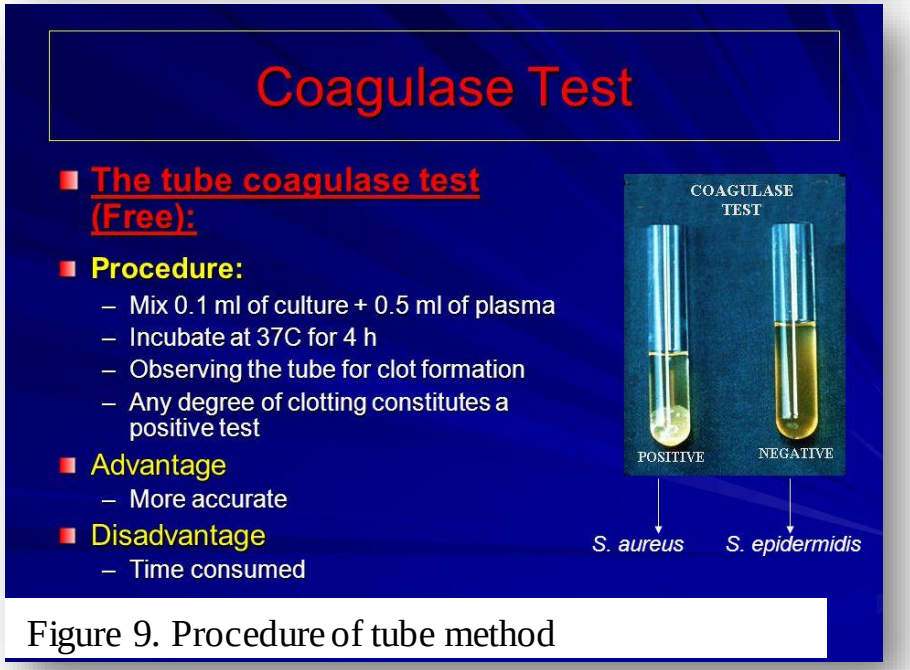


Figure 9. Procedure of tube method

B- Bound or clumping factor or fibrinogen binding protein(slide method)

- Heat stable protein
- It doesn't require coagulase reacting factor (CRF)
- fibrinogen is not converted into fibrin
- Antigenically homogenous
- Is a component of cell wall liberated upon lysis of bacteria
- Not associated with pathogenicity
- Binds directly with fibrinogen and clumping bacteria

Procedure

-Make two separate drops of distilled water

-Emulsify bacteria with two drops of NS or D.W to obtain milky suspension

- Add a drop of plasma to one suspension and left other as control.
- Rotate and observe the clump formation during 10 seconds (Figure 10).

Advantage

Rapid

Disadvantages

Not accurate because 5% of Staph.aureus can give false negative results and false positive results due to the autoagglutination .

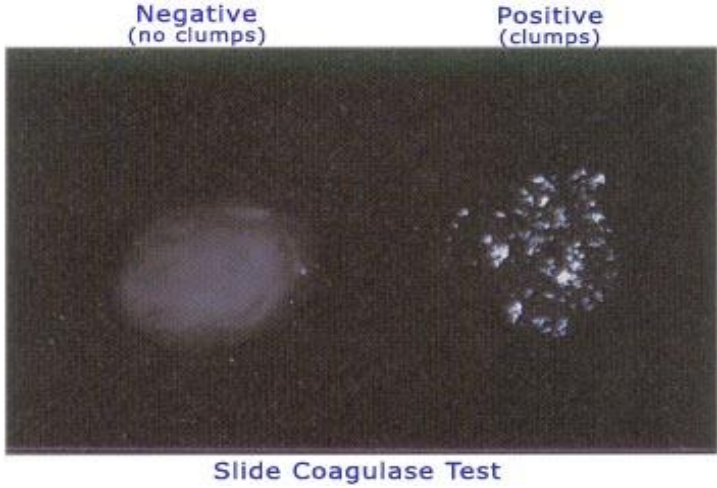


Figure 10. Slide coagulase test

2-Catalase enzyme : Use it to detoxify H2O2 produced by phagocytic cells and prevent intracellular killing by oxygen burst mechanism (antiphagocytic action)

Principle of test: Catalase enzyme degrades hydrogen peroxide and immediately releases oxygen as bubbles or effervescence. The importance of this enzyme in bacteria life is as a detoxifying agent.

Procedure
Plate culture
Place a drop of 3% of H_2O_2 on colonies of nutrient agar.
Immediate or prompt effervescence indicates catalase positive.
Culture media containing blood are unsuitable for the test as blood contains catalase. (Figure 11)
Slide method
Place a drop of 3% of H_2O_2on the surface of clean slide. Transfer bacteria from colony and mix with a drop of 3% of H_2O_2. (Figure 12)
Capillary tube method
Transfer small quantity of bacteria by one end of capillary tube and put another end of capillary tube in 3% of H_2O_2. H_2O_2 will move upward and releases air bubbles inside the tube.

Fig.11. Catalase test by culture plate method.

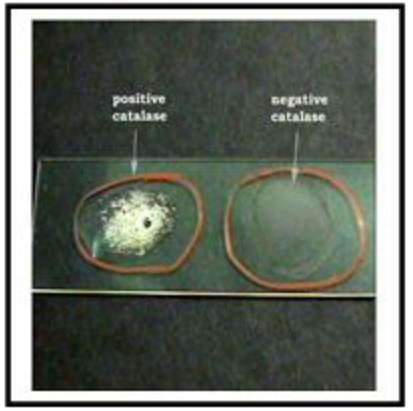


Fig.12. Catalase test by slide method

Staphylokinase or fibrinolysin: Dissolve clot and helps bacteria to spread in the tissue

Hyaluronidase (spreading factors)
Breakdown hyaluronic acid (intercellular glue) and spread in the tissue especially in skin infections.

Lipase: Digest lipids and allowing the bacteria to grow on the skin surface as well as in the sebaceous skin glands.

Beta Lactamase destroy beta Lactam drugs like penicillins and cephalosporins

Toxin produced

Generally 2 kinds of toxins
1- Cytolytic toxins like **hemolysins** (α,β and γ) can lyse RBCs, leukocytes ,skeletal muscles, heart muscle and renal tissues. **Leukocidin** (panton-Valentine) lyses WBCs.

2- Pyrogenic toxin act as superantigens
Pyrogenic because most of them can induce fever and superantigen because can stimulate a large number of T lymphocytes directly and release a storm of inflammatory cytokines

Toxic shock syndrome toxin-1 responsible for TSS in menstruating women using tampons

Exfoliative toxins or epidermolytic toxins cause desquamation of epidermis in scalded skin syndrome mainly in infants and young children

Enterotoxins : More than 6 types (A-F) are produced and responsible for food poisoning

Epidemiology

Source of infection is the man (clinically infected or carrier)

Carriage is mainly in the anterior nares, and the rate of carriage in the general population is about 30% to more than 50% in hospitals.

Contaminated hands are the main mean of transportation

Clinical findings

Although this is a true pathogen but significant host compromise is required to ensure infections like
1- Presence of foreign bodies like stitch infections
2- Secondary bacterial infection after viral infections
3- Diabetes mellitus
4- Break in skin defenses like burns and trauma

Infections of Staph.aureus mainly due to
1- Invasion and colonization of the host tissue aided by secretion of various extracellular substances which facilitate invasion followed by multiplication and production of various toxins and enzymes that overcome host defense mechanisms. This leads to pyogenic infections.
2- Toxin mediated infections or intoxication.
3- Combination of both invasion and intoxication

Routes of infection
1- Direct and indirect contact
2- Inhalation

A- Localized Staphylococcal infections

These infections usually remain localized at the site of entry by normal host defenses in the form of small, superficial abscesses involving hair follicles (folliculitis), sweat and sebaceous glands and eye lash follicles (styes) (Figure 13). Furuncle or boil: abscess around hair follicles. Carbuncle: cluster of furuncles. Impetigo (purulent dermatitis) –small vesicles on face or legs, arms or trunk- rupture-thin yellow fluid-dry into a honey colored crusts

Cellulitis is acute spreading infection of both epidermis and dermis and characterized by redness, edema and tenderness (painful) (Fig.14). Wound infections like post operative and post traumatic infections.

Fig.13. Localized Staphylococcal skin infections.

Fig.14. Cellulitis.

B- Deep, localized infections

- Osteomyelitis and septic arthritis
- Acute endocarditis
- Septicemia
- Pneumonia
- Urinary tract infection
- Otitis media
- Wound infections
- Burn infections
- Toxic shock syndrome:

Is a severe systemic involvement following a local infection with a staphylococcal strain producing Toxic Shock Syndrome Toxin -1 (TSST-1). TSS was first associated with menstruated women who used tampon contaminated with toxigenic strains of this bacteria. The condition also occurs in women who are not menstruating and in men, and can arise as a result of infection by toxin producing strains of *S. aureus* at any site. An erythematous rash which goes on to desquamation, particularly involving the palms and soles, is characteristic, along with multiple organ involvement and shock (Figure 15). Fever, hypotension.

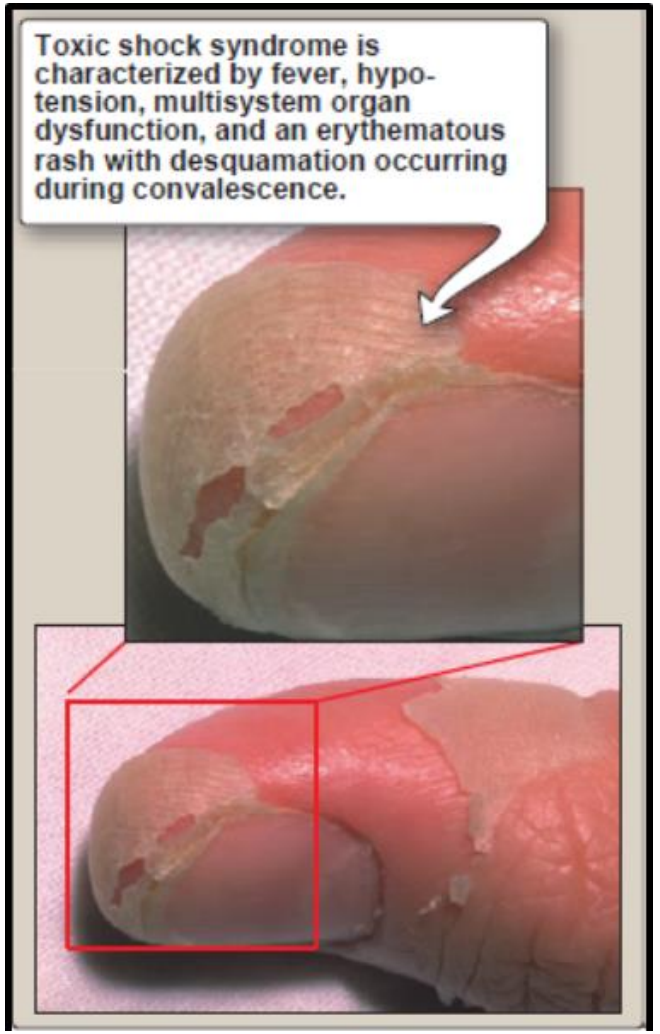


Fig.15. Toxic shock syndrome

Scalded skin syndrome

Due to exfoliatin producing strains of *S. aureus* and occurs mainly in the newborn and young children. The initial infected lesion may be minor but the toxin spreads to involve the whole body, with the formation of bullae which slough off to leave raw areas like burns and heal without scar formation. (Figure 16)

Staphylococcal gastroenteritis

Due to the ingestion of contaminated food with enterotoxin producing Staph.aureus (50%) strains mainly by contaminated hands of food handlers in the restaurants. This type of food poising is due to the ingestion of preformed toxin (intoxication) which characterized by short incubation period less than 6 hours with nausea, vomiting and diarrhea. No fever and self limited. Enterotoxin is heat stable and cause no change in the food.

Coagulase negative Staphylococcal infections

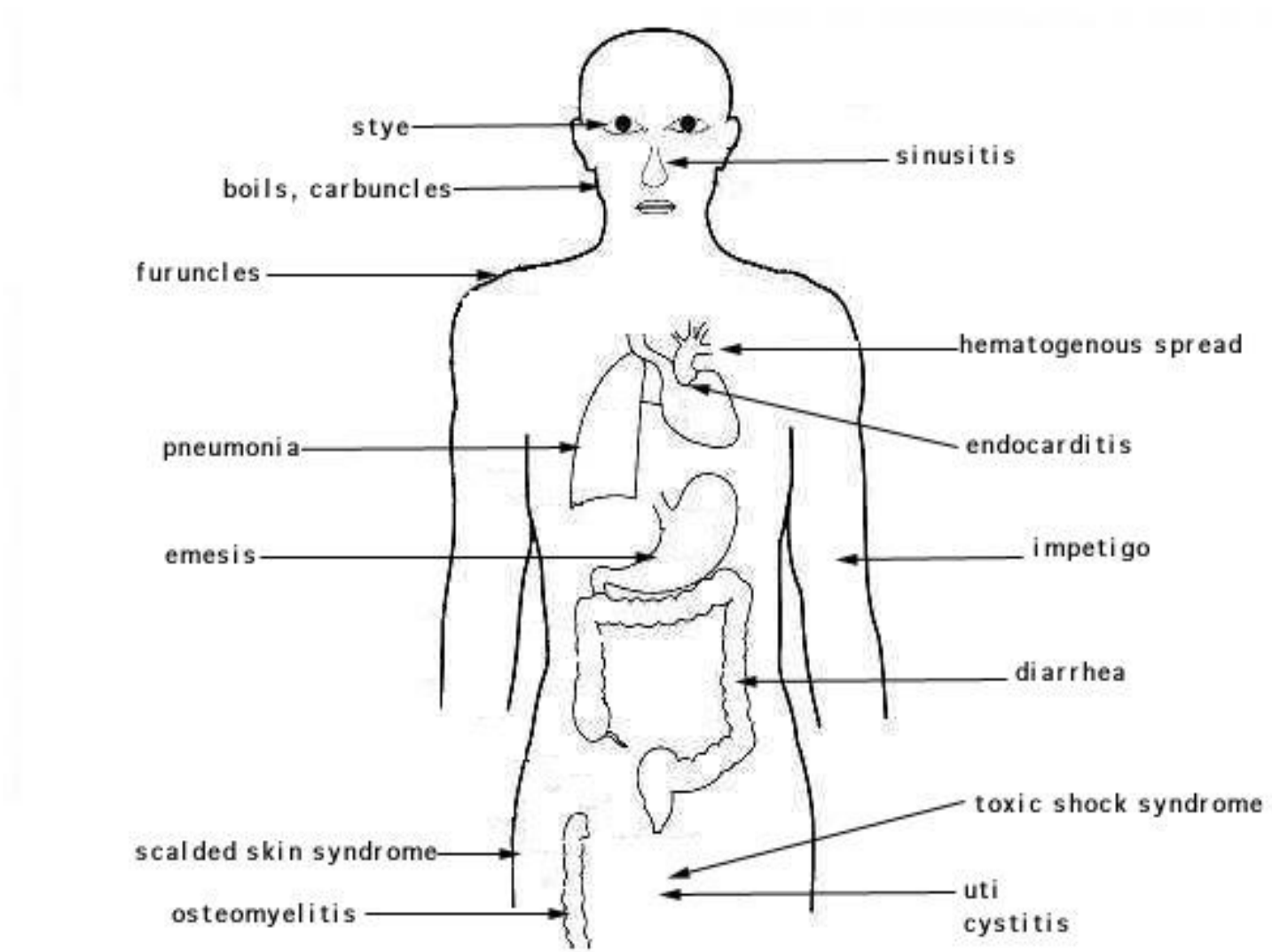
Staph.epidermidis (albus) mainly associated with indwelling catheter infection or hospital infection.

Staph.saprohyticus (citreus)

Common cause of honey moon cystitis in newly married girls

Fig.16. Staphylococcal scalded skin syndrom

Staphylococcal disease summary



Laboratory diagnosis

Specimen: Surface swabs, pus, blood, sputum, cerebrospinal fluid, Mid stream urine, synovial fluid

Smear: Gram positive cocci in clusters, singly or in pairs. (non reliable)

Isolation
Culture on blood agar and Mannitol salt agar at 37 C for 24 hrs

Colony morphology
Colonies on blood are 2-3 mm in diameters, convex, round with entire age and opaque. Golden pigmentation may be or not and usually with beta hemolysis.
On mannitol salt agar ferment mannitol with yellow color of the media.

Gram stain
Gram positive cocci in cluster

Catalase positive

Coagulase positive

Antibiotic sensitivity or PCR to detect mRSA

Laboratory diagnosis of food poisoning

Specimens: Vomit or Stool

Methods
Direct detection of enterotoxins by ELISA technique or isolation on MSA then identification.

Laboratory diagnosis of TSS

Specimens: Tampons, vaginal swab or serum

Methods
Direct detection of TSST-1 from serum by ELISA technique or isolation (vaginal or tampons) on MSA then identification.

Coagulase positive

Novobiocin test : This test is useful to differentiate *S. saprophyticus* (resistant) from *S. aureus and S.albus* (sensitive)

Species	Frequency of disease	Coagulase	Color of colonies	Mannitol fermentation	Novobiocin resistance
S. aureus	Common	+	Golden yellow	+	–
S. epidermidis	Common	–	White	–	–
S. saprophyticus	Occasional	–	Variable	–	+

Note

Sometimes genus micrococcus can contaminate blood agar and morphologically similar to Staphylococcus, therefore there is a test called lysostaphin test : Staphylococci is sensitive while micrococcus spp are resistant

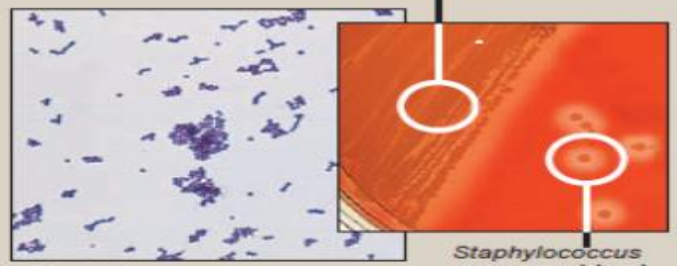
Treatment

Most minor infections do not need antibiotics.
Abscesses must be drained.
About 95% of staphylococci are resistant to penicillin (β-lactamase production), therefore β- lactamase-stable penicillins such as cloxacillin are the mainstay of treatment. With the emergence of cloxacillin resistance (MRSA), other agents have come into use. Vancomycin is most often used in this regard, but in certain circumstances fusidic acid or clindamycin may be useful. Best choose for effective treatment is by sensitivity test. For Vancomycin resistant Stpah.aureus (VRSA) linezolid and daptomycin or Streptogramins (Quinopristin-dalfopristin) I.V use.

Prevention

1- HAND WASHING is a simple but effective way of preventing the spread of these organisms.
2- Avoidance of sharing personnel items.
3- Shared exercised equipment should be disinfected between users.
4- Patients who are infected with cloxacillin resistant strains (MRSA) should have additional precautions instituted (contact precautions) to prevent spread of these organisms to other patients.
5- Treatment of the carrier state (for both cloxacillin sensitive and cloxacillin resistant strains) in patients who have repeated infections may be indicated. Nasal creams and antibacterial soaps have been used with varying degrees of success.
6- There is no effective vaccine against S. aureus.

Staphylococcus species



Staphylococcus aureus cultured from a wound infection

Staphylococcus aureus on blood agar surrounded by zone of beta hemolysis.

- **Gram-positive**, staining darkly
- Round cocci tending to occur in bunches like grapes
- True facultative anaerobic organisms
- Cultured on enriched media containing broth and/or blood

Staphylococcus aureus

- Skin infections
- Septicemia
- Osteomyelitis
- Necrotizing pneumonia
- Endocarditis
- Toxic shock syndrome
- Food poisoning

PENICILLINS[1] — 1 Oxacillin; 1 Nafcillin

CEPHALOSPORINS
TETRACYCLINES
AMINOGLYCOSIDES
MACROLIDES
FLUOROQUINOLONES

OTHER — 2 Vancomycin[2]

OTHER — 3 Quinupristin-dalfopristin[3]; 3 Linezolid[3]; 3 Daptomycin[3]

[1] Most isolates resistant to penicillin G
[2] Used in methicillin-resistant isolates
[3] Used in vancomycin-resistant isolates

Staphylococcus epidermidis

- Infections of catheters and heart valves

PENICILLINS[1] — 1 Oxacillin; 1 Nafcillin

CEPHALOSPORINS
TETRACYCLINES
AMINOGLYCOSIDES
MACROLIDES
FLUOROQUINOLONES

OTHER — 2 Vancomycin[2]

[1] Most isolates resistant to penicillin G
[2] Used in methicillin-resistant isolates

Staphylococcus saprophyticus

- Cystitis in women

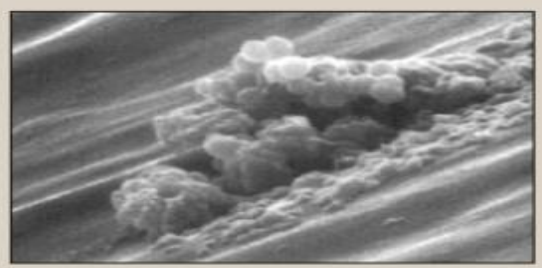
PENICILLINS — 1 Penicillin G

CEPHALOSPORINS
TETRACYCLINES
AMINOGLYCOSIDES
MACROLIDES
FLUOROQUINOLONES
OTHER

Folliculitis caused by *Staphylococcus aureus*

Carbuncle caused by *Staphylococcus aureus*

Furuncle caused by *Staphylococcus aureus*



Scanning electron micrograph of cardiac pacemaker lead colonized by *S. aureus*

Staphylococcal scalded skin syndrome

Superficial impetigo

Challenge your understanding

Which of the following statements regarding the role of **protein A** in the pathogenesis of infections caused by *S aureus* is correct?
(A) It is responsible for the rash in toxic shock syndrome.
(B) It converts hydrogen peroxide into water and oxygen.
(C) It is a potent enterotoxin.
(D) It is directly responsible for lysis of neutrophils.
(E) It is a bacterial surface protein that binds to the Fc portion of IgG1.

Which of the following staphylococcal organisms produces coagulase and has been implicated in infections following a dog bite?
(A) *Staphylococcus intermedius*
(B) *Staphylococcus epidermidis*
(C) *Staphylococcus saprophyticus*
(D) *Staphylococcus hominis*
(E) *Staphylococcus hemolyticus*

Over a period of 3 weeks, a total of five newborns in the hospital nursery developed *S aureus* infections with *S aureus* bacteremia. The isolates all had the same colony morphology and hemolytic properties and identical antimicrobial susceptibility patterns, suggesting that they were the same. (Later molecular methods showed the isolates were identical.) Which of the following should be done now?
(A) Prophylactic treatment of all newborns with intravenous vancomycin
(B) Protective isolation of all newborns
(C) Closing the nursery and referring pregnant women to another hospital
(D) Hiring all new staff for the hospital nursery
(E) Culture using mannitol salt agar of the anterior nares of the physicians, nurses, and others who cared for the infected babies.

Problem

A 35- year- old women suffered from third degree of **burning**. After two days of hospital admission, she developed burn infection characterized by copious purulent discharge. **Wound swab** on blood agar revealed heavy growth of golden yellow, medium sized, beta hemolytic colonies and yellow coloration on mannitol salt agar. Gram stained smears from colonies revealed Gram positive cocci in cluster. Antimicrobial susceptibility test showed resistant to many beta lactam drugs like ampicillin and cloxacillin.

Questions

1-What is your first diagnosis of this isolate?
2-Why this pathogen causes this infection?
3-What make you to think for your first diagnosis?
4-Name some major virulence factors of this pathogen?
5-What are the mechanisms of resistance of this pathogen?
6-How this pathogen can cause different infections?
7-Is this pathogen is zoonotic? If yes how it transmits to human and from which animals?
8-Does human carrier occur with pathogen? If yes where in the body and in whom carrier state is dangerous? What will cause if such carriers spread it?
9-Mention three localized skin infections and three deep systemic infections of this pathogen?
10-Is Gram stain and colony morphologies enough for final diagnosis of this pathogen? If No. What other tests do you need for final identification?
11- Is yellow discoloration of MSA is due to pigment production or pH changing?
12- What is the drug of choice for this pathogen?
13- Mentions two virulence factors that prevent phagocytosis, one is the structure of this pathogen, while other enzyme.
14- Name two selective media of this pathogen, one for isolation from clinical samples, while other for isolation and numeration of this pathogen from food samples.
15- Why natural penicillins are not effective against this pathogen?
16- How to determine the source of infection?
17-What will happen if this pathogen produce toxic shock syndrome toxin 1?

Case #4

A 25-year-old newly married women suffered from lower abdominal pain. GUE revealed Pus cell: ++++/HPF, RBCs: +/HPF, Albumin: Negative. Urine culture showed pure and heavy growth ($1x10^5$ CFU/ml) of Gram Positive cocci in cluster, Catalase: Positive and Coagulase test: Negative.

Q1- What is the most likely causative agent of this case?

Q2- Which part of the urinary tract is infected?

Q3- What is the source of infection?

Q4- How to confirm your diagnosis based on phenotypic method?

Case #5

You eat at one of the public restaurants and after 4 hours you developed nausea, vomiting and diarrhea. GSE showed no pus and RBCs.

Q1)- What do you developed?

Q2)- Is your case is due to the ingestion of bacterial contaminated food or toxin contaminated food?

Q3) Do you need to take antibiotics?

Q4) What is the main treatment for you?

Q5) How to make laboratory diagnosis?

Q6) What is best method used for preventing food poisoning?

Q7) Is heating is enough to prevent such kind of food poisoning?

What will happen in patient with cystitis that caused by methicillin sensitive Staph.aureus (MSSA)receiving premature course of antibiotics like cloxacillin?

What is the role of special strain of Staph. aureus for serological diagnosis of other bacterial infections?

Answer by True and False

1- Among Gram positive bacteria, Staph.aureus is the most resistant against heat and dryness.

2- Staph.aureus is facultative anaerobic bacteria but pigmentation is better under aerobic conditions

3- Pasteurization at 60 C for 30 min. kills Staph.aureus.

4- Gram stained morphology is enough for determining genus Staphylococcus.

5- Resistance to natural penicillins is due to production of beta lactamase enzymes

Although Staph. aureus is facultative anaerobic bacteria but prefer aerobic condition for their growth. Why?

What is the importance of catalase enzyme in the pathogenicity of Staph.aureus?

If there are two strains of Staph.aureus, one is beta lactamase producer and the other is MRSA so which one will spread drug resistance among bacteria more rapidly and why?

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