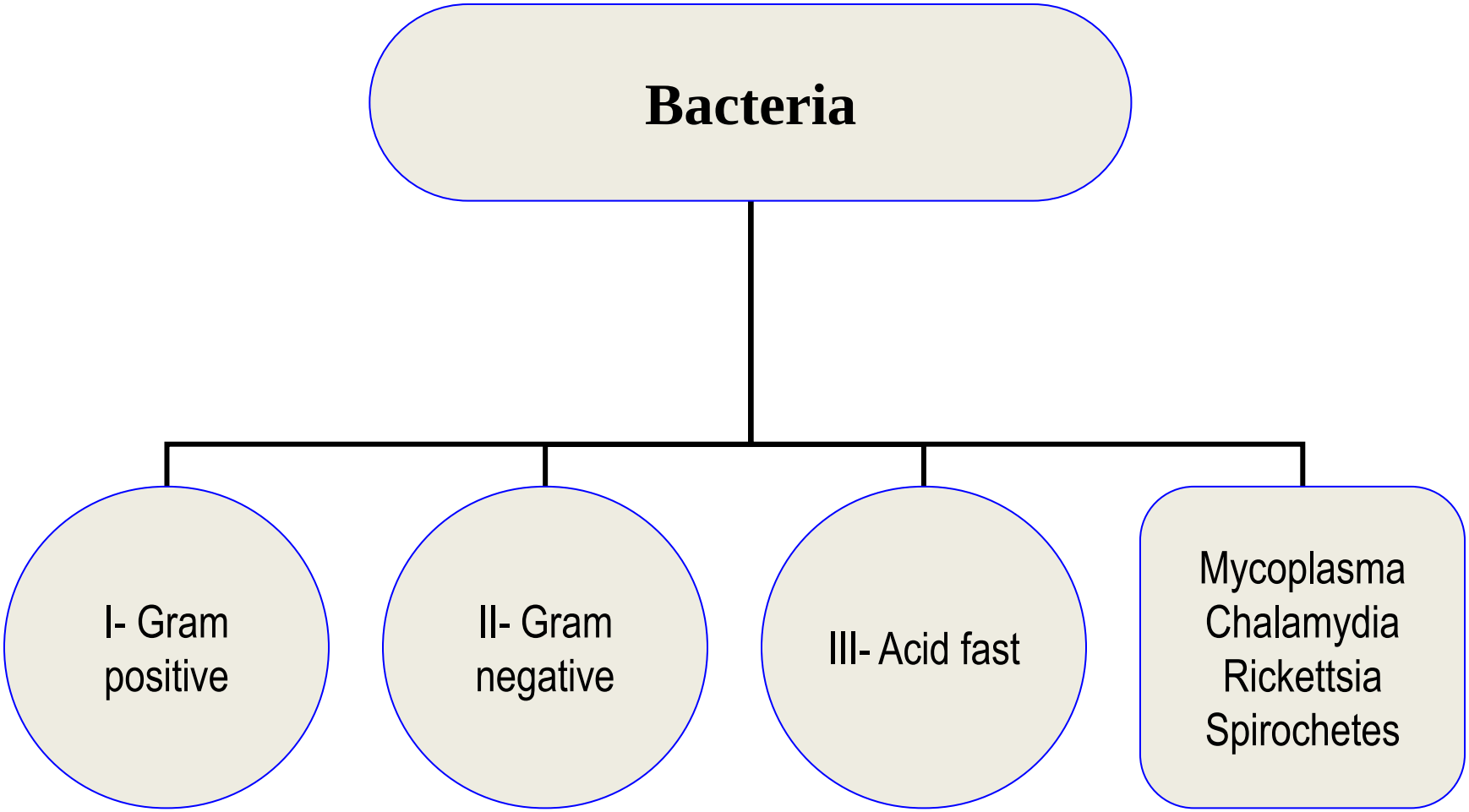
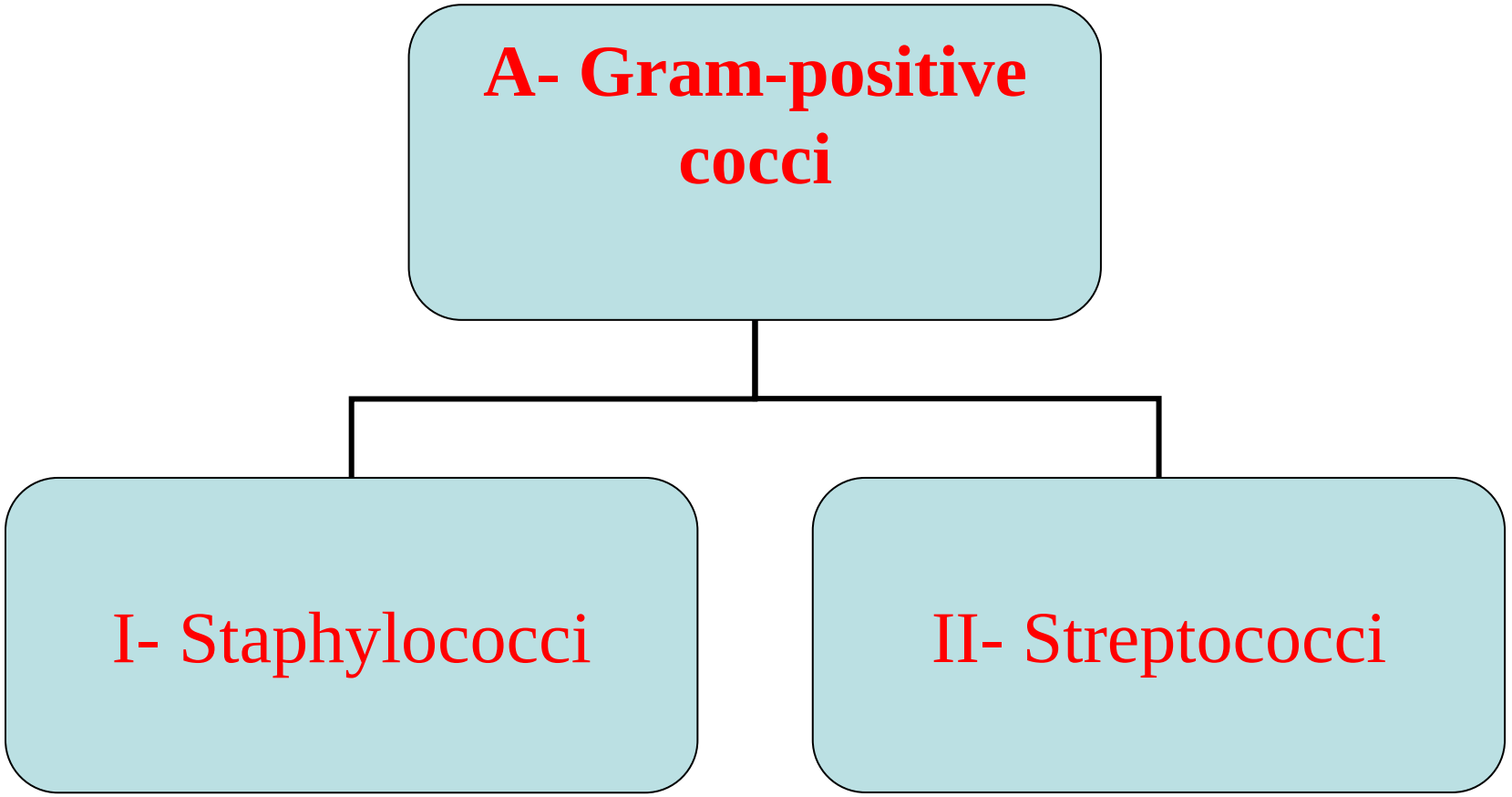
SPECIAL BACTERIOLOGY

Medical Bacteriology



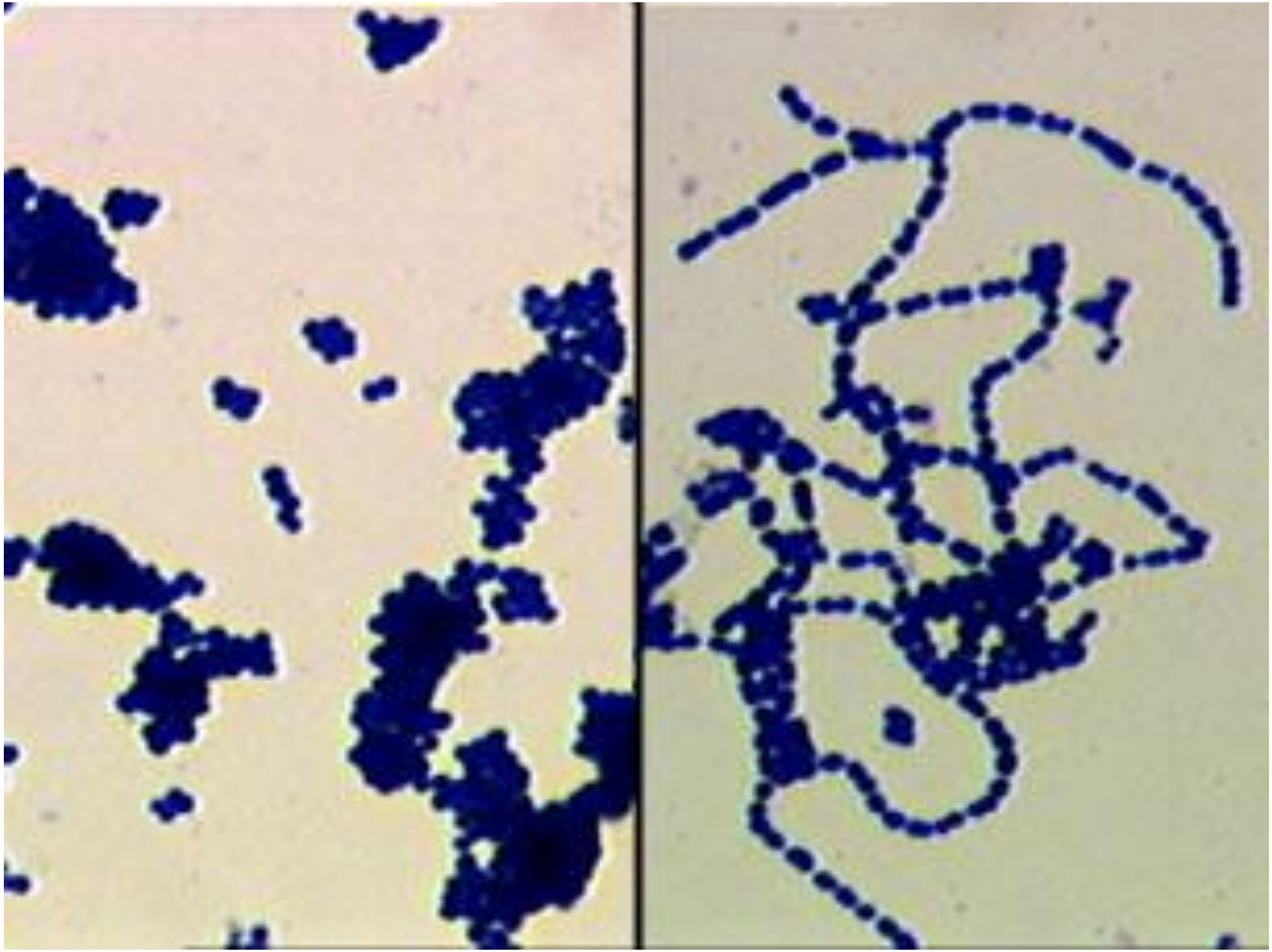
Gram-Positive Cocci



Learning objectives

- To know the classification and imp features of gram positive bacteria
- To recognize the cultural characteristics, virulence factors, pathogenesis and clinical findings of diseases caused by Staphylococcus aureus
- To interpret the lab diagnosis of Staphylococcus aureus

Staphylococcus *Streptococcus*

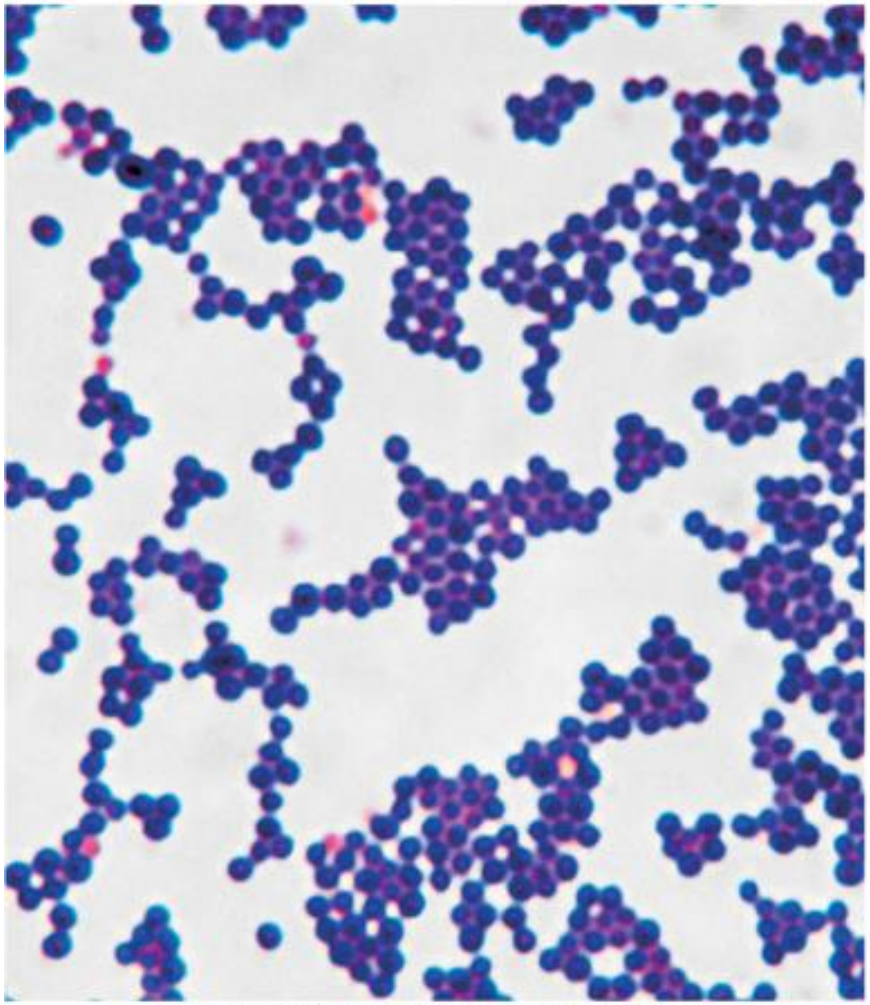


STAPHYLOCOCCUS

Staphylococcus

- Staphyloccocci - derived from Greek “Staphyle” (bunch of grapes)
- Gram positive cocci arranged in clusters
- Hardy organisms surviving many non physiologic conditions
- Include a major human pathogen and skin commensals

Morphology



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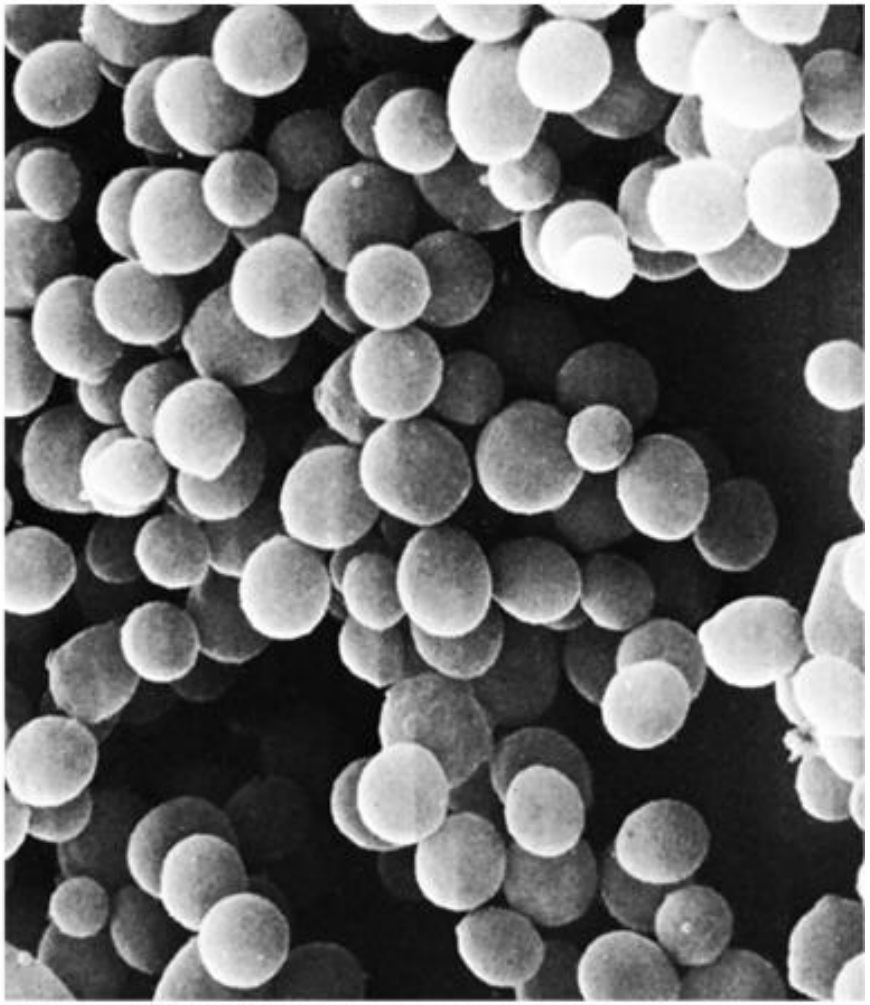

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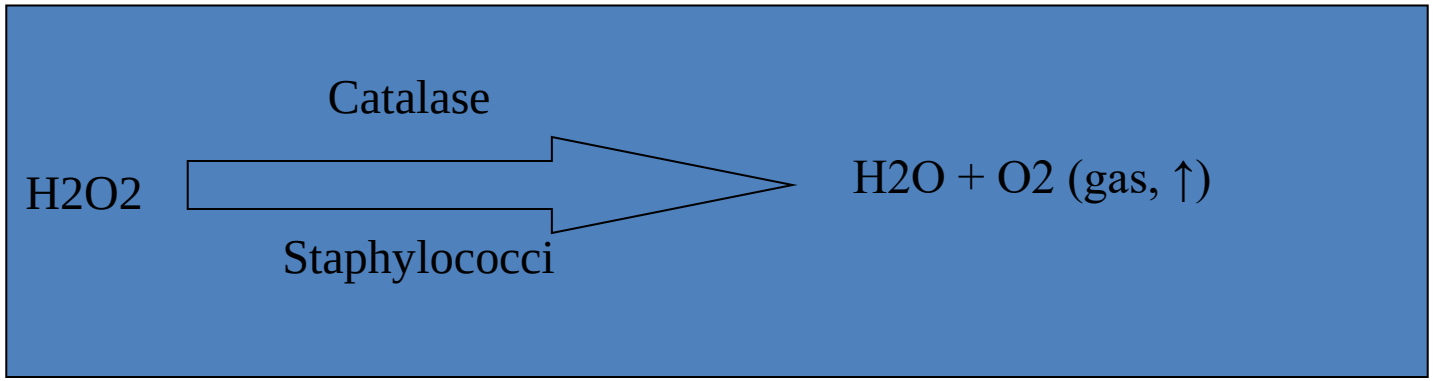
Staphylococcus: General Characteristics

- Non motile
- Non–spore-forming
- Non encapsulated
- Catalase-producing
- Facultative anaerobe

Catalase test

- The catalase test is use to distinguish Streptococci from Staphylococci.



CLASSIFICATION

- **1. Coagulase positive Staphylococci**
- *Staphylococcus aureus*

- **2. Coagulase negative Staphylococci**
- *Staphylococcus epidermidis*
- *Staphylococcus saprophyticus*

Three species of Staphyloccoci have medical importance:

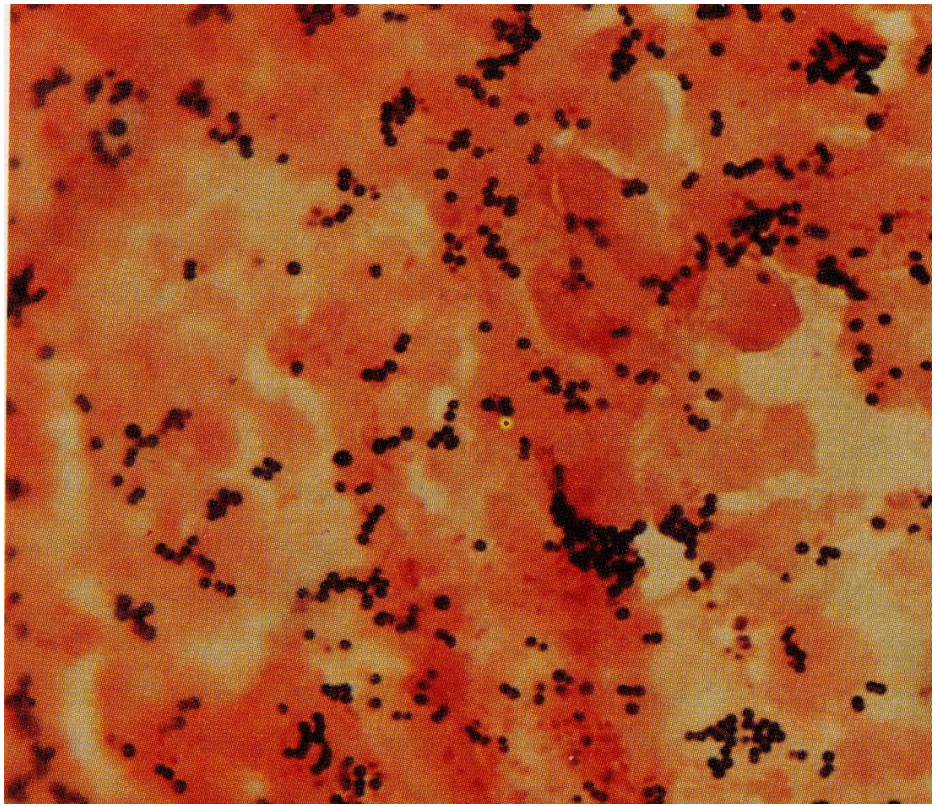
- ***S.aureus***: Pathogenic & commensally found in nose (nares)
- ***S.epidermidis***: non pathogenic & common commensals in nares & skin
- ***S.saprophyticus:*** Cause UTI in female & occasionally commensally found on skin

Staphylococcus aureus

Staphylococcus aureus

MORPHOLOGY:

- These are spherical cocci.
- Approximately 1µm in diameter.
- Arranged characteristically in grape like clusters.
- They are non motile and non sporing.
- A few strains possess capsules.



Staphylococcus aureus

- Facultative anaerobe
- Withstands high salt, extremes in pH, and high temperatures.

CULTURE

Media used :-

Non selective media: Nutrient agar,

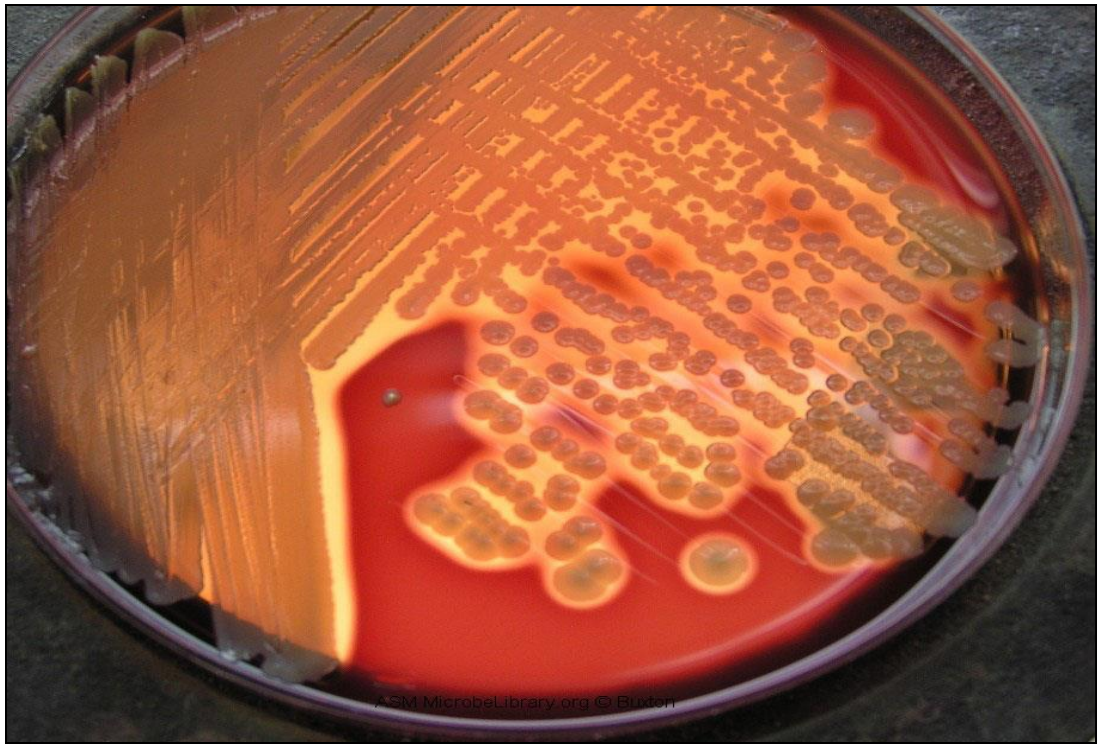
Blood agar.

Selective media: Manitol Salt agar.

Cultural Characteristics

On nutrient agar- The colonies are large, circular, convex, smooth, shiny, opaque and easily emulsifiable. Most strains produce golden yellow pigments.

ii) On blood agar- Most strains produce β-haemolytic colonies. Colony morphology: buttery looking, cream or white colored



Staphylococcus aureus

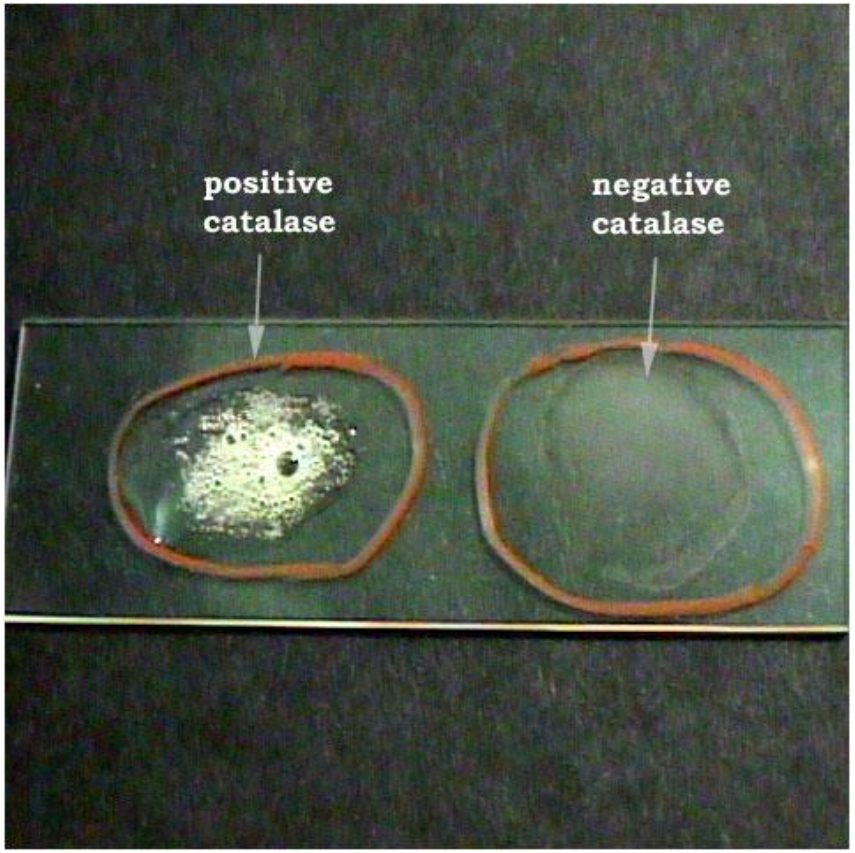
Biochemical reactions

- ***Catalase test – positive***
- ***Production of Coagulase Positive***
- ***Production of Dnase***
- **Ferment Mannitol**

Catalase Test: Interpretation

– Presence of bubbles
  - Positive
  - Staphylococci

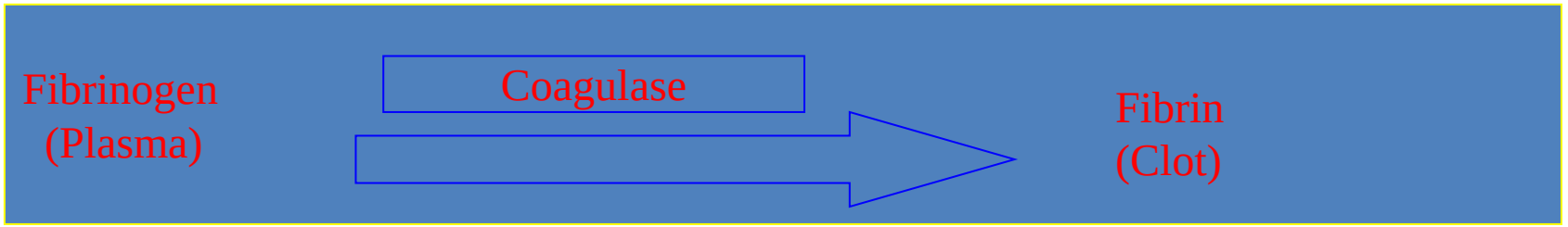
– Absence of bubbles
  - Negative
  - Streptococci

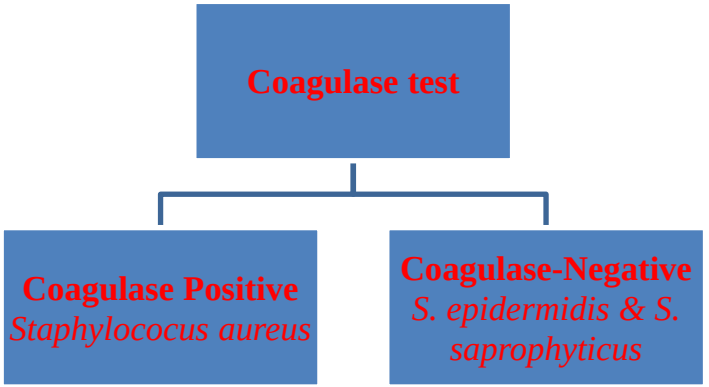


Coagulase Test

Principle:

- This test used to differentiate between *S. aureus* (CPS) & other *Staphylococcus* species (CNS)

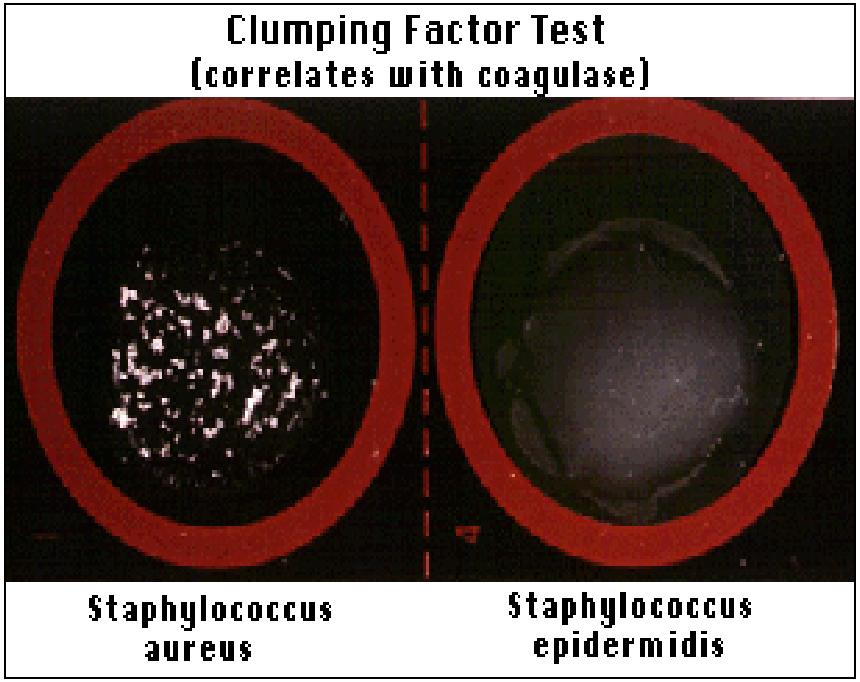


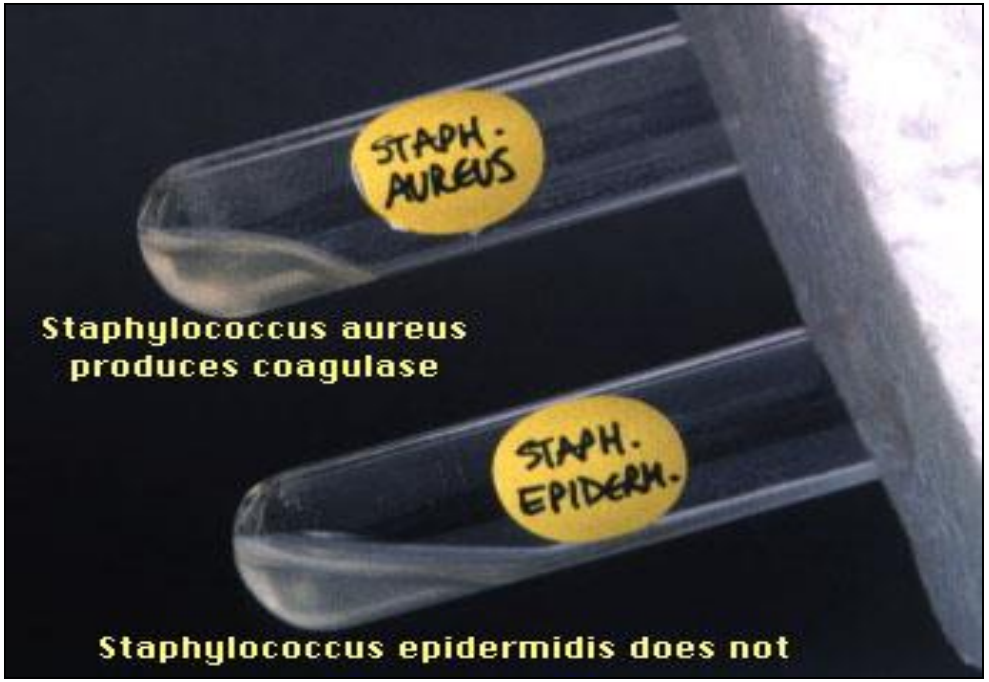


2) Coagulase test-

i) Slide coagulase test- Positive.

ii) Tube coagulase test- Positive.

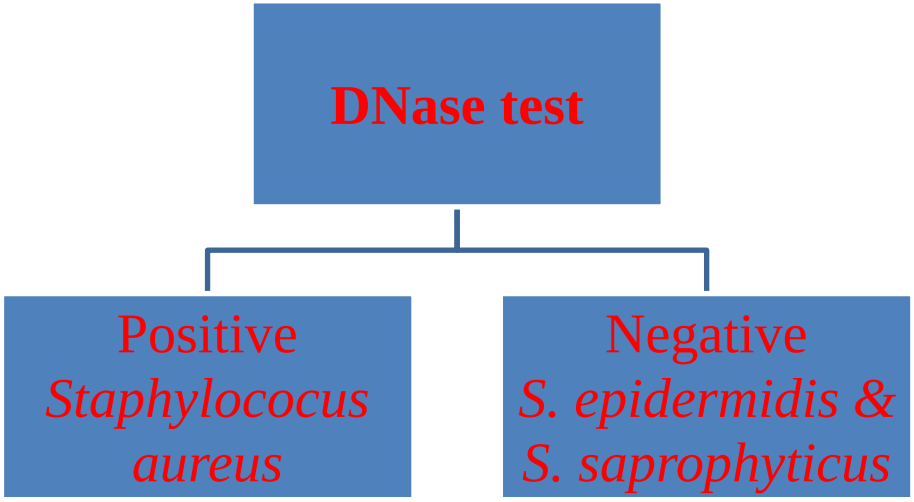




SLIDE COAGULASE TEST

TUBE COAGULASE TEST

Deoxyribonuclease (DNAase) test

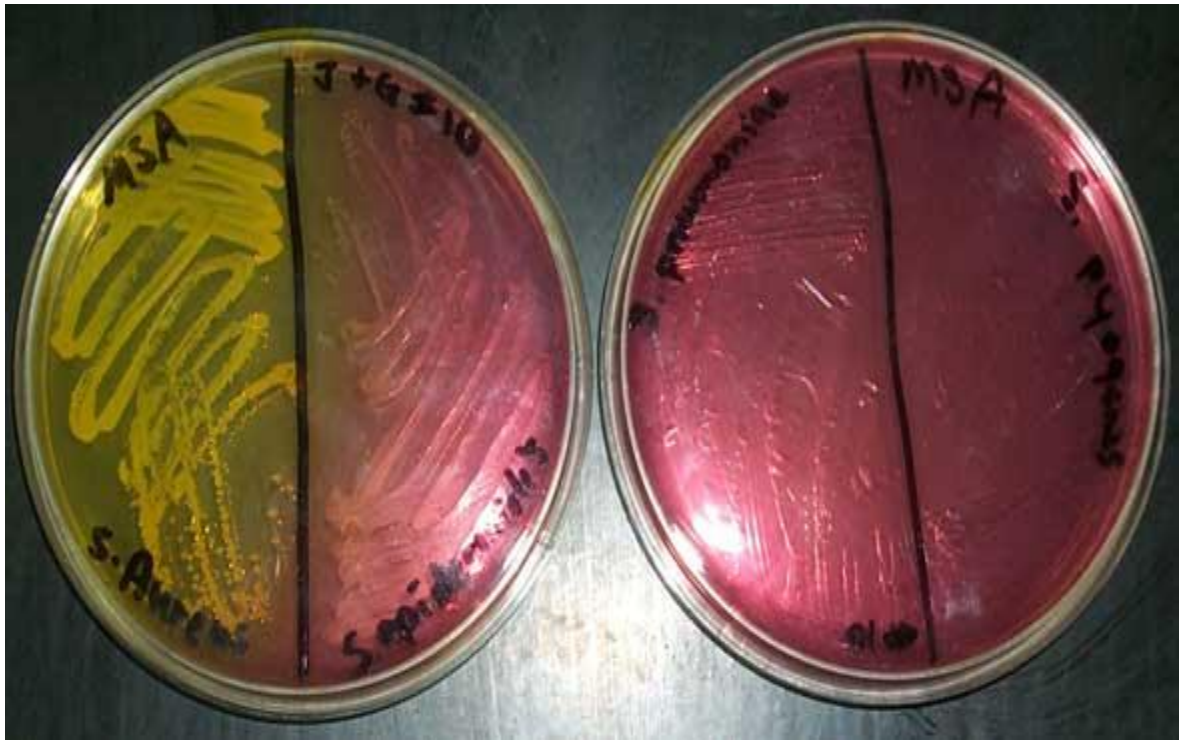


- Principle:
  - DNA is insoluble in acid
  - DNA is hydrolyzed into oligonucleotides by the action of DNase
  - Nucleotides soluble in acid

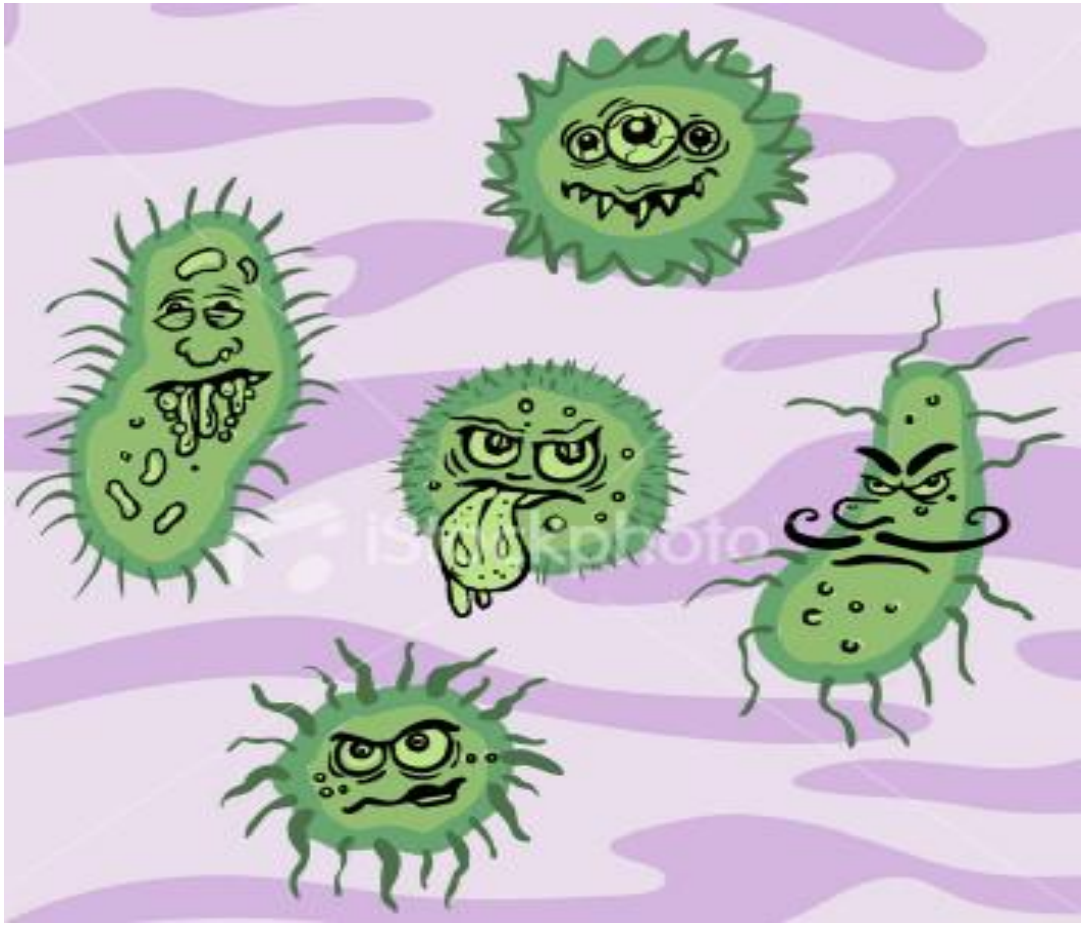
Manitol fermentation

- **MSA** (Manitol Salt Agar)is selective differential medium for staphylococci
  - It contains: NaCl (7.5%), Mannitol, & Phenol Red
  - The cause of selectivity due to presence of high salt concentration
  - The cause of differential is because it contains mannitol (sugar) and phenol red (pH indicators turns yellow in acidic pH and turns red in alkaline pH).

- *Staphylococcus aureus* is capable of using sugar mannitol as a food source and will produce acidic by products of fermentation that will lower the pH of the media.



Never underestimate the power of bacteria….



Transmission

- Humans are the reservoir for staphylococci.
- The **nose is the main site of colonization of *S. aureus.***
- Approximately 30% of people are colonized at any one time.
- People who are chronic carriers of *S. aureus* in their nose have an increased risk of skin infections caused by *S. aureus* .

- The skin of hospital personnel and patients is a common site of *S. aureus* colonization.

- Approximately 5% of women have *S.aureus* in their vagina, which predisposes them to toxic shock syndrome.

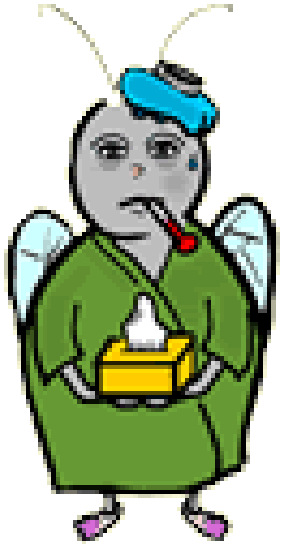
- Shedding from human lesions

- Fomites such as towels and clothing contaminated by these lesions are additional sources of infection.

Hand washing decreases transmission.

- **Colonization** means that the staph is present in or on the body but is not causing illness.

- **Infection** means that the staph is present and is causing illness.



PATHOGENICITY

Source of infection:

1- Exogenous: patients or carriers

2- Endogenous: From colonized site

Virulence factors:

These include

A- Cell wall associated factors

B- Extracellular factors

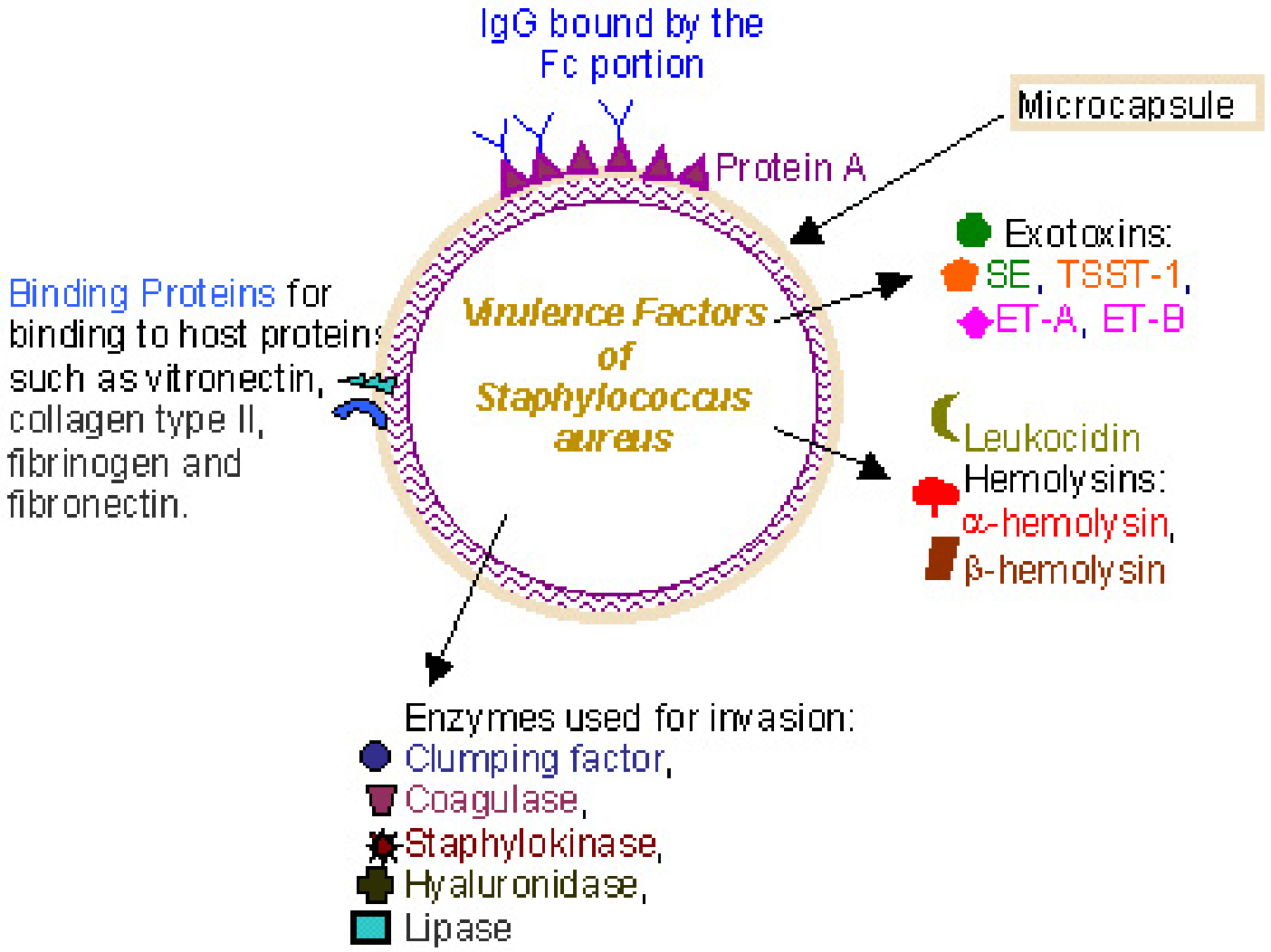
- Enzymes
- Toxins

Cell wall associated factors

- **S. aureus has several important cell wall components and antigens:**

  1. Cell wall polysaccharide
  2. Teichoic acid
  3-Lipotechoic acid
  4- Protein A
  5- Peptidoglycan layer
  6- Clumping factor (bound coagulase)

Virulence factors of Staphylococcus aureus



Virulence Factors; (con't)

- **Polysaccharide capsule:**
- It is an important virulence factor.
- There are 11 serotypes based on the antigenicity of the capsular polysaccharide, but types 5 and 8 cause 85% of infections.
- Some strains of *S. aureus* are coated with a small amount of polysaccharide capsule, called a microcapsule.
- The capsule is poorly immunogenic, which has made producing an effective vaccine difficult.

Virulence Factors; (con't)

- **Teichoic acids**

- They mediate adherence of the staphylococci to mucosal cells.

- **Lipoteichoic acids** play a role in the induction of septic shock by inducing cytokines such as interleukin-1 (IL-1) and tumor necrosis factor (TNF) from macrophages.

Virulence Factors cont

- **Protein A:**
  - Found in cell wall
  - Binds to F_c part of IgG
  - Blocks phagocytosis

Virulence Factors; (con't)

- **Peptidoglycan**
- *S. aureus* has peptidoglycan which has endotoxin-like properties (i.e., it can stimulate macrophages to produce cytokines and can activate the complement and coagulation cascades).
- This explains the ability of *S. aureus* to cause the clinical findings of septic shock yet not possess endotoxin.

Virulence Factors: Extracellular Enzymes

- **Hyaluronidase:**
  - Hydrolyzes hyaluronic acid in connective tissue allowing spread of infection

- **Staphylokinase:**
  - Fibrinolysin which allows spread of infection

- **Coagulase:**
- It acts by clotting the plasma.
- It walls off the infected site, thereby retarding the migration of neutrophils and antibiotics into the site.

Virulence Factors: Extracellular Enzymes (con't)

- **DNase:**
  - Degrades DNA
- **Lipase:**
  - Allows colonization by acting on lipids present on the surface of the skin.
  - **Proteases**
  - **Catalase**
- **Beta-lactamase:**
  - Cuts the beta lactam wall of certain antibiotics

Virulence Factors: Extracellular TOXINS

- Several important toxins are produced by *S. aureus.*
- The three clinically important are enterotoxin, toxic shock syndrome toxin and exfoliatin.

Toxins (cont.)

- **Enterotoxin:**
  It has six immunologic types, types A–F.
- It acts as a super antigen within the gastrointestinal tract to stimulate the release of large amounts of IL-1 and IL-2 from macrophages and helper T cells.

Toxins (cont.)

- It causes food poisoning characterized by prominent vomiting and watery, non bloody diarrhea.
- It is heat-resistant .
- It is resistant to stomach acid and to enzymes in the stomach and jejunum.

Toxins (cont.)

- **Toxic shock syndrome toxin (TSST)**
- TSST is a super antigen and causes toxic shock by stimulating the release of large amounts of IL-1, IL-2, and TNF.
- Causes toxic shock, especially in tampon-using menstruating women.

Toxic shock syndrome toxin (TSST)

- Toxic shock also occurs in patients with nasal packing used to stop bleeding from the nose.
- It is produced locally by *S. aureus* in the vagina, nose, or other infected site.

Toxins (cont.)

- **Exfoliatin:**
  It is “epidermolytic”, acts as a protease that cleaves desmoglein in desmosomes, leading to the separation of the epidermis at the granular cell layer.
- It causes scalded skin syndrome.

Toxins (cont.)

- Several exotoxins can kill leucocytes (leukocidins) and cause necrosis of tissues in vivo. Of these, the two most important are **alpha toxin and P-V toxin**.

  Alpha toxin causes marked necrosis of skin and hemolysis.

Toxins (cont.)

- **P-V (Panton-Valentine**) leukocidin kills cells especially white blood cells by damaging cell membrane.
- The importance of P-V leukocidin as a virulence factor is indicated by the severe skin and soft tissue infection caused by MRSA strains that produce this leukocidin.
- A severe necrotizing pneumonia is also caused by strains of *Staph. aureus* that produce P-V leukocidin.
- Approximately 2% of clinical isolates of *Staph. aureus* produce P-V leukocidin.

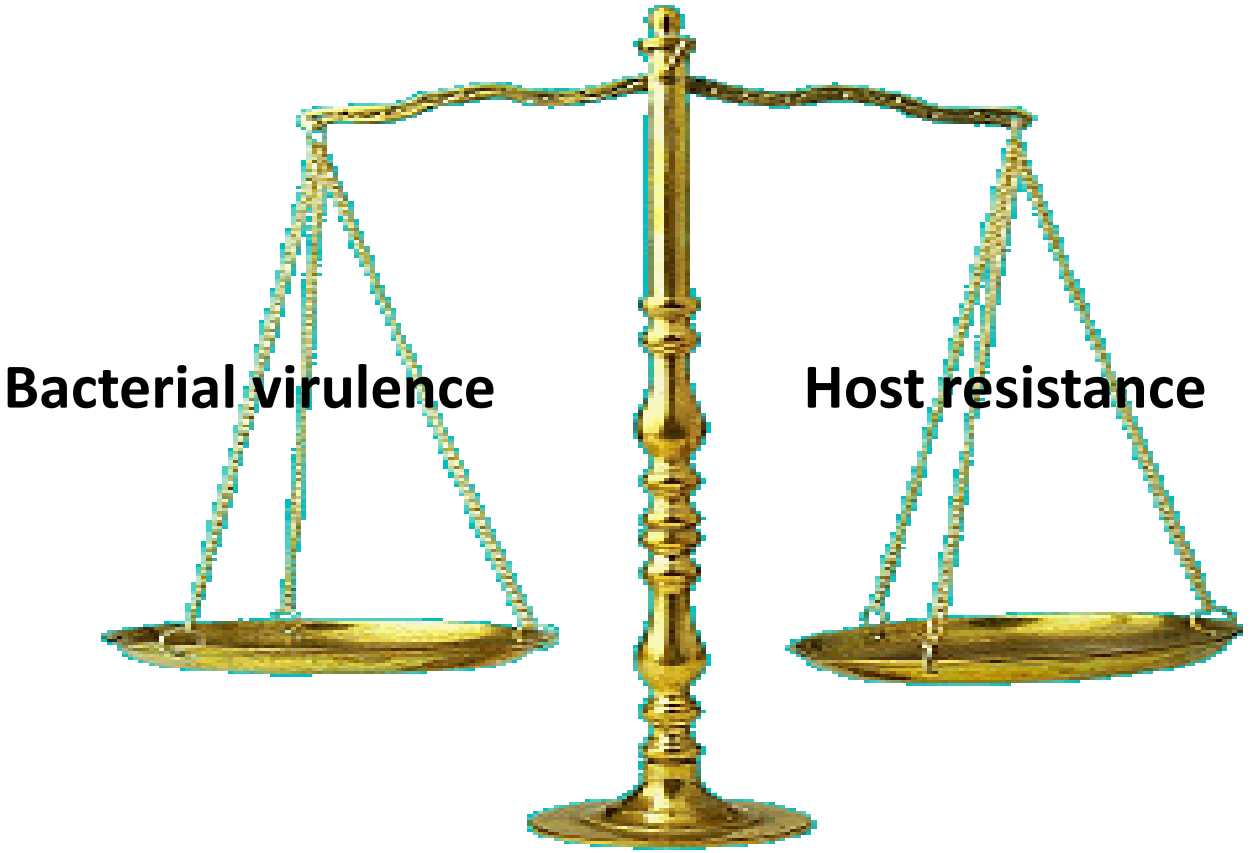
Predisposing factors

- Reduced humoral immunity, including low levels of antibody, complement or neutrophils.

- Diabetes and intravenous drug use.

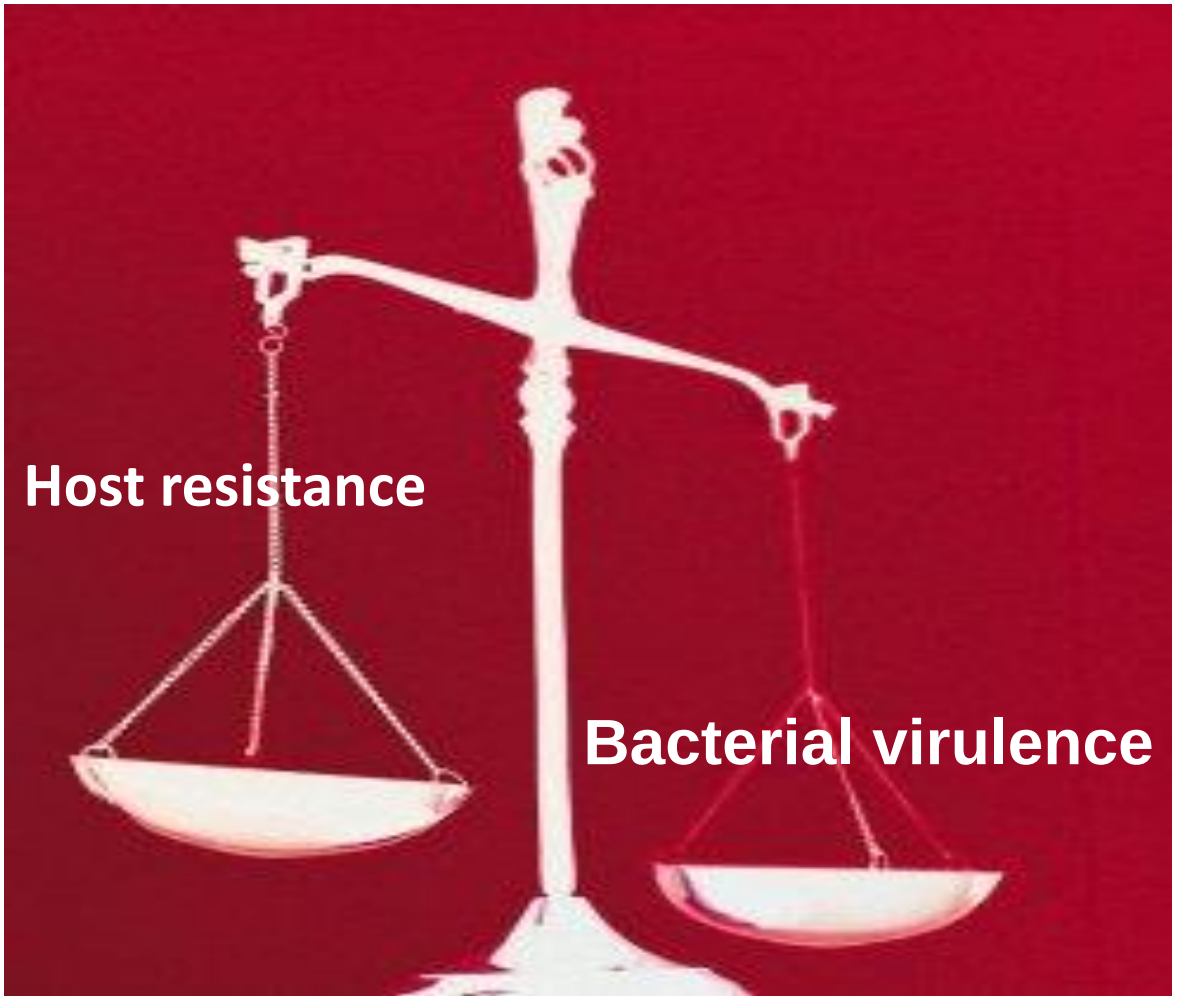
- Patients with chronic granulomatous disease (CGD), a disease characterized by a defect in the ability of neutrophils to kill bacteria.

No Disease



Disease

- Trauma
- Underlying diseases
- Burns
- Surgery
- Immune suppression
- Catheterization
- Intubation
- Antibiotics
- Cytotoxic Drug



- Capsule
- Toxin
- Pili
- Invasive
- Intracellular

Clinical findings

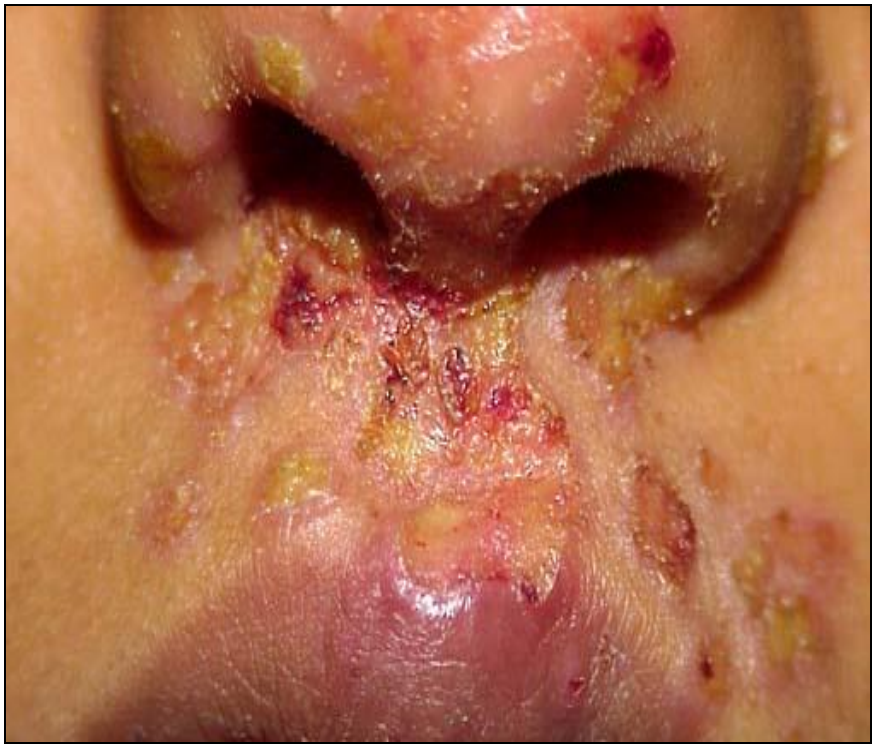
Diseases produced by Staphylococcus aureus are studied under 2 groups:

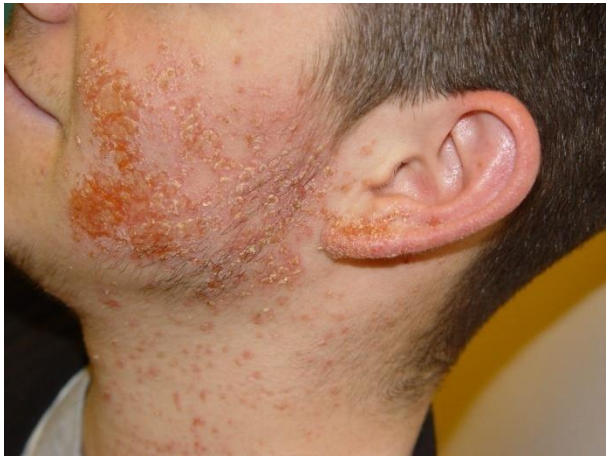
A- Infections

B- Intoxications

Skin and soft tissue

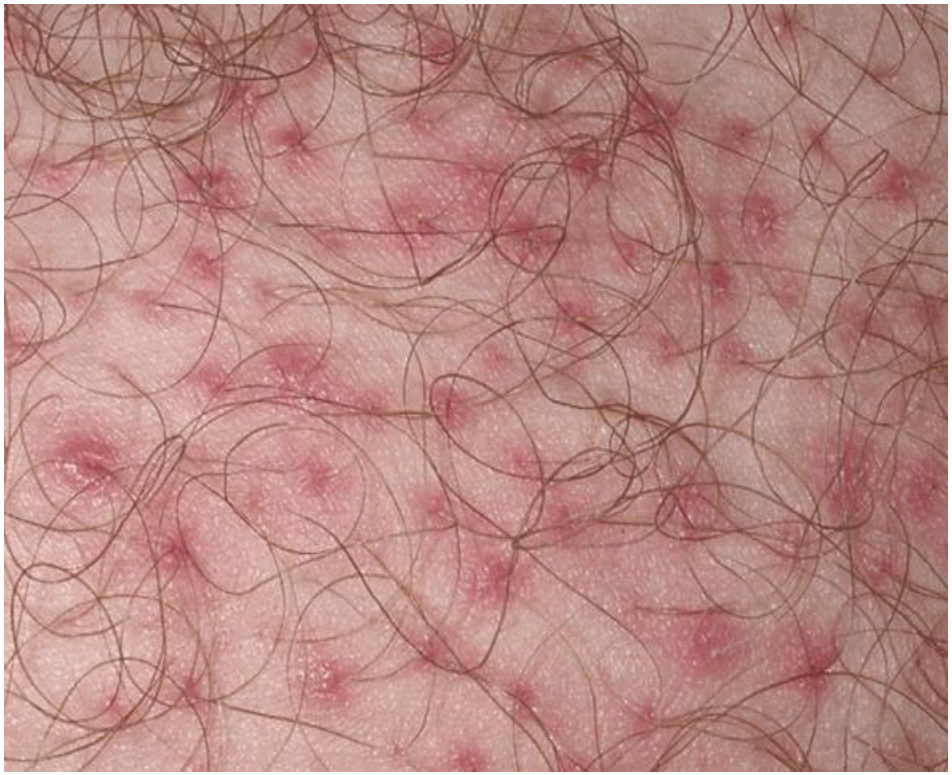
- **Impetigo** ;Superficial bacterial infection of the skin. Thin vesicles with honey colored crusting.

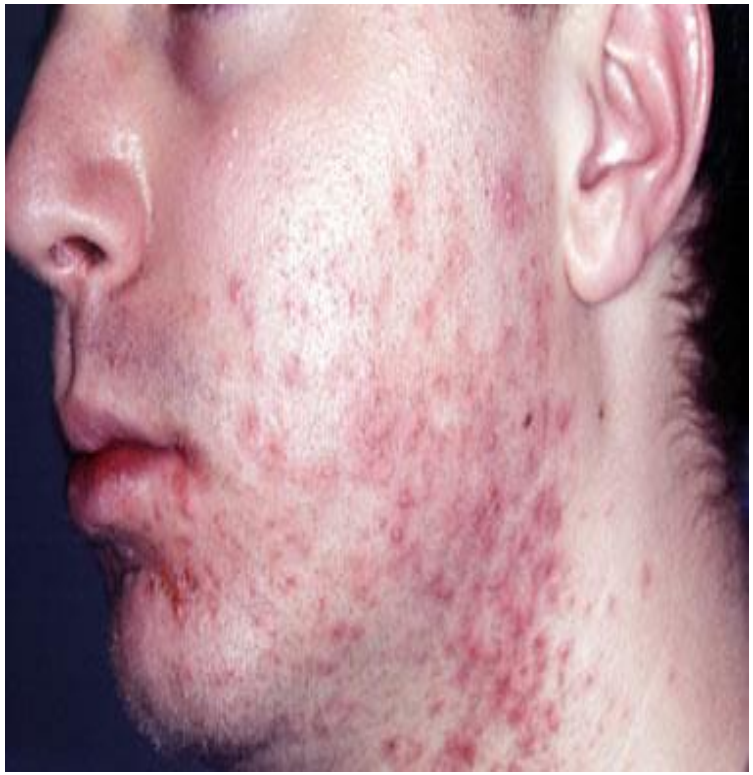




Skin and soft tissue

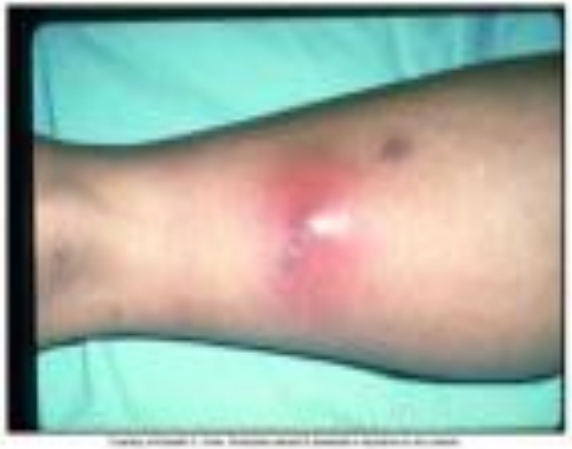
- **Folliculitis**- is Superficial or deep infection of the hair follicle

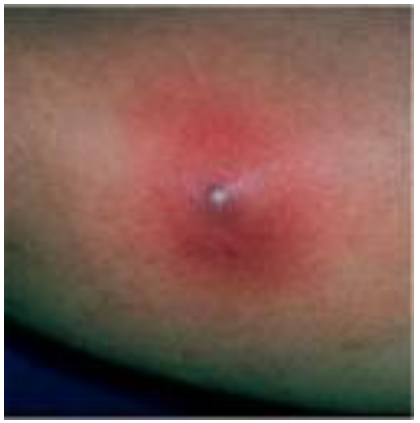




Skin and soft tissue

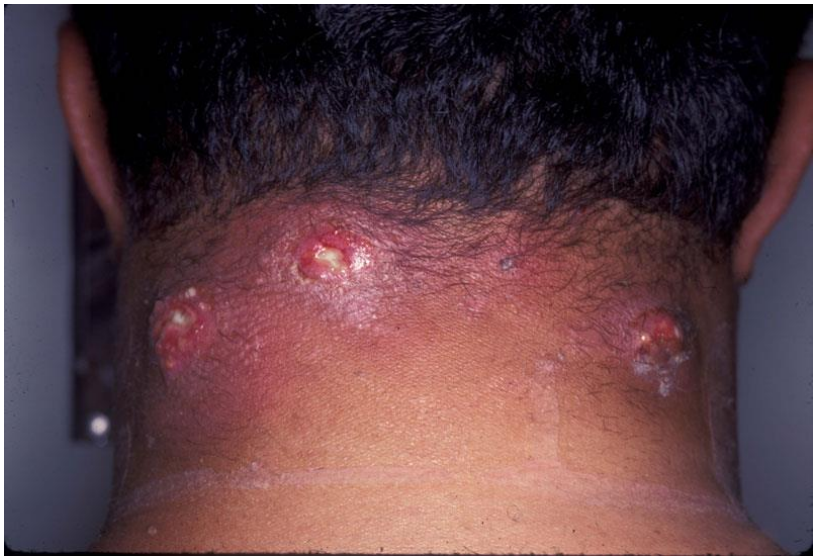
- **Furuncle (Boil)** -Deep extension of superficial folliculitis into the dermis and subcutaneous tissue

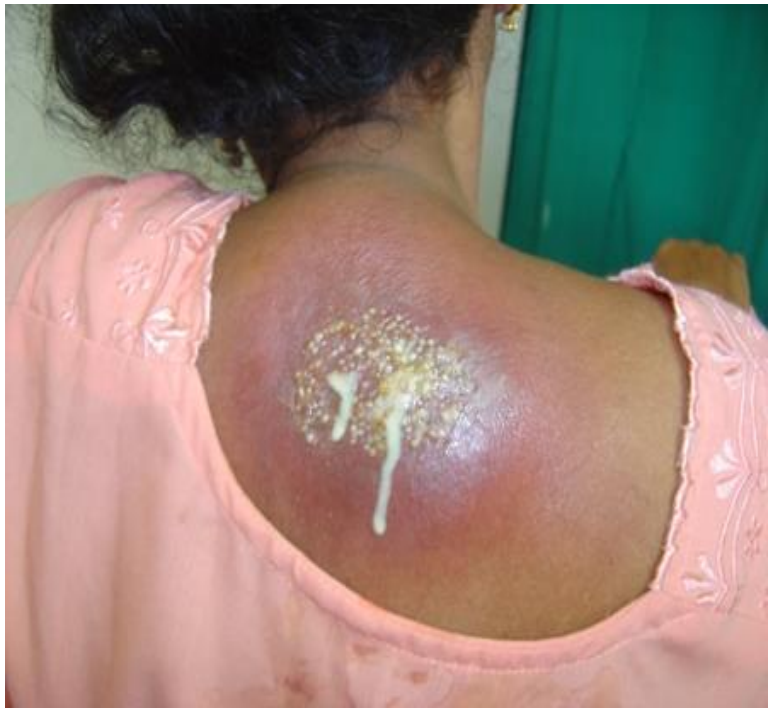




Skin and soft tissue

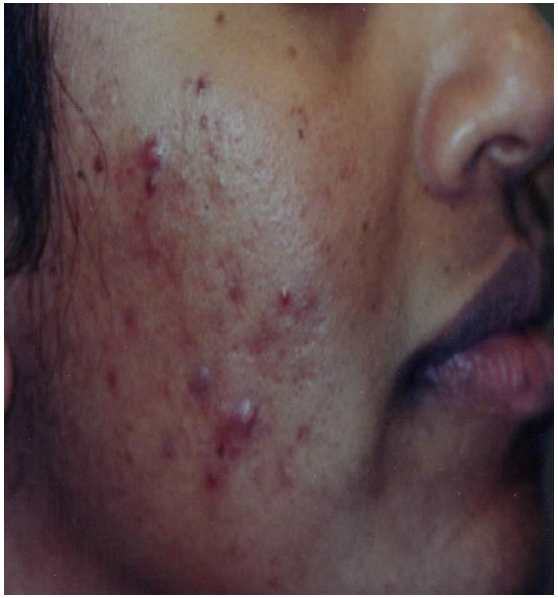
- **Carbuncle** -Large deep abscess that is a progression of a furuncle



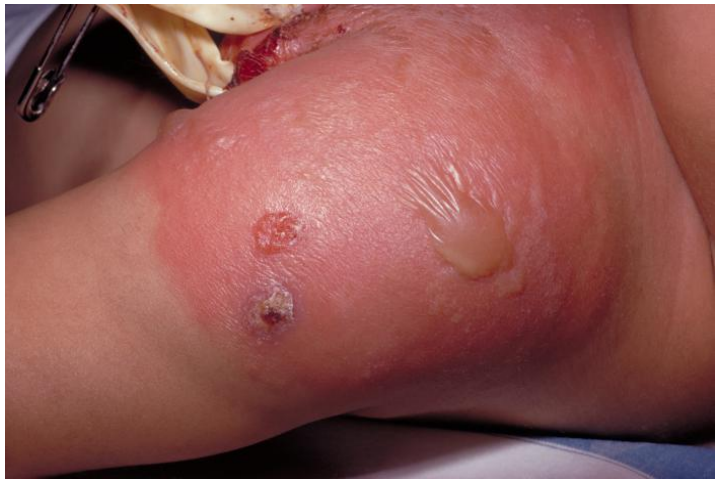


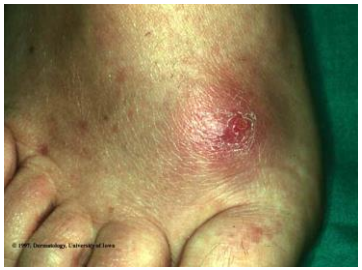
Skin and soft tissue

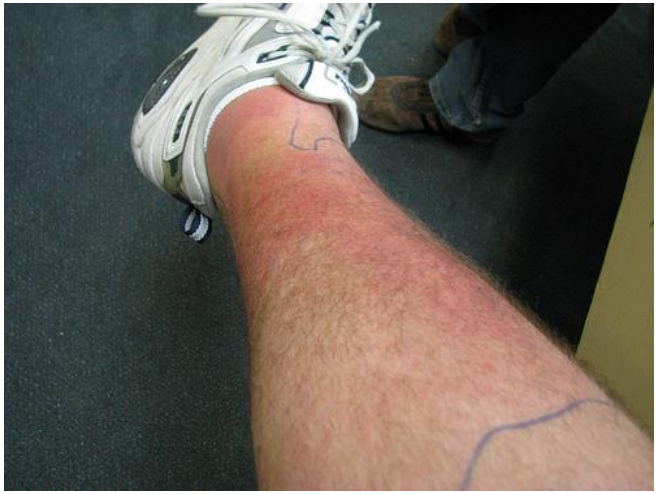
- **Acne**- Obstruction of sebaceous follicles (oil glands)

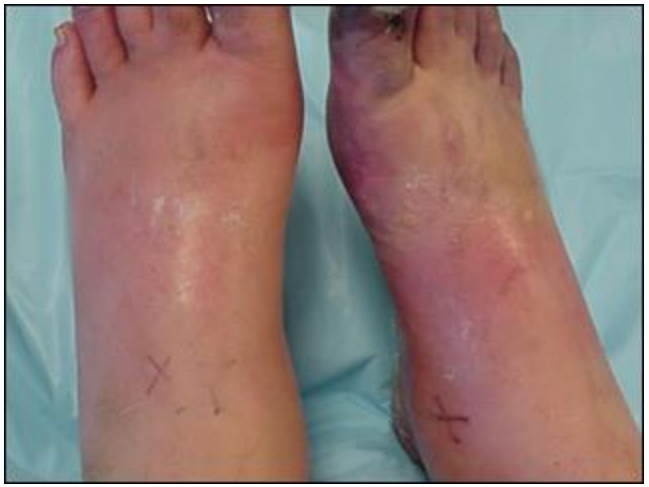


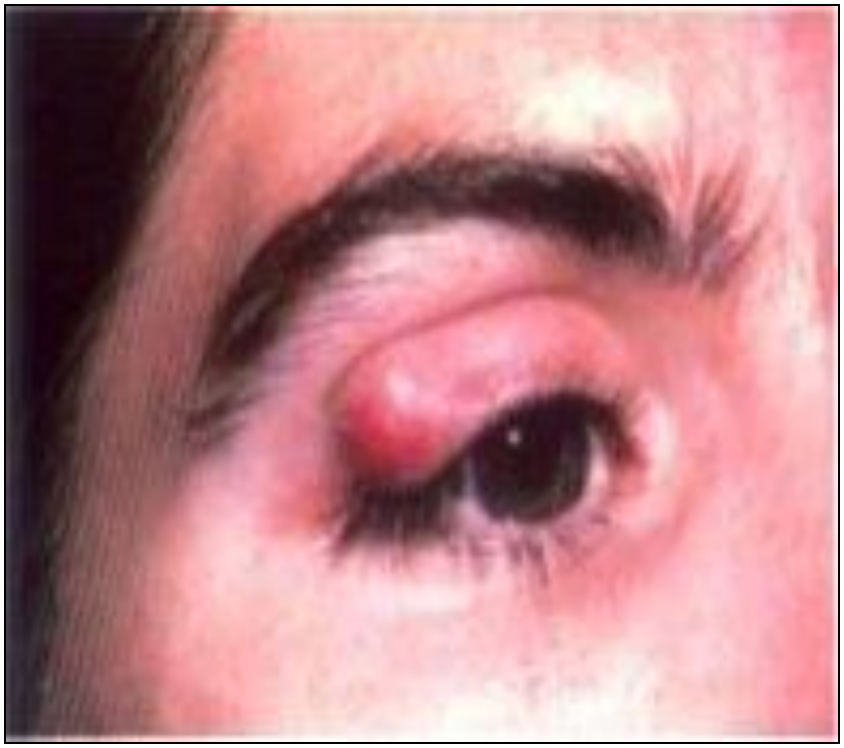
Skin and soft tissue Cellulitis



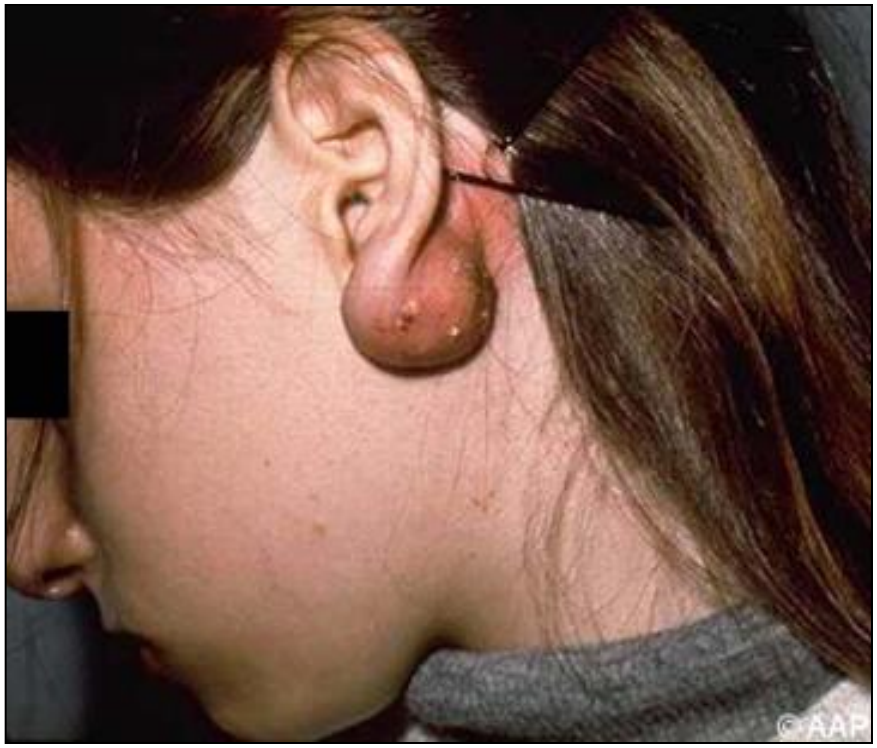






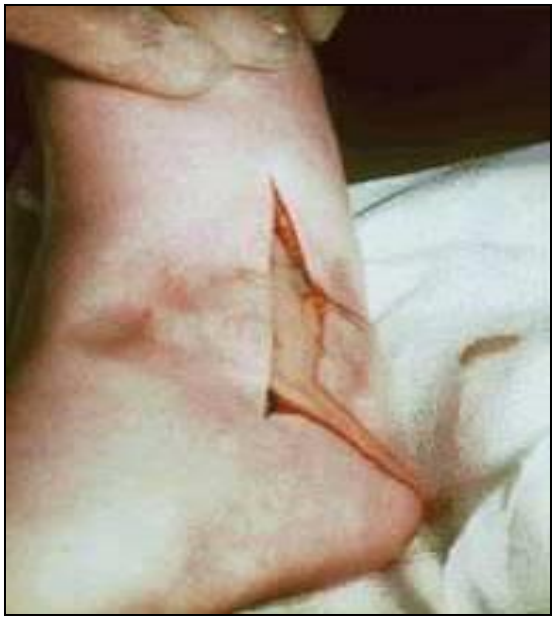


Styes



Abscess

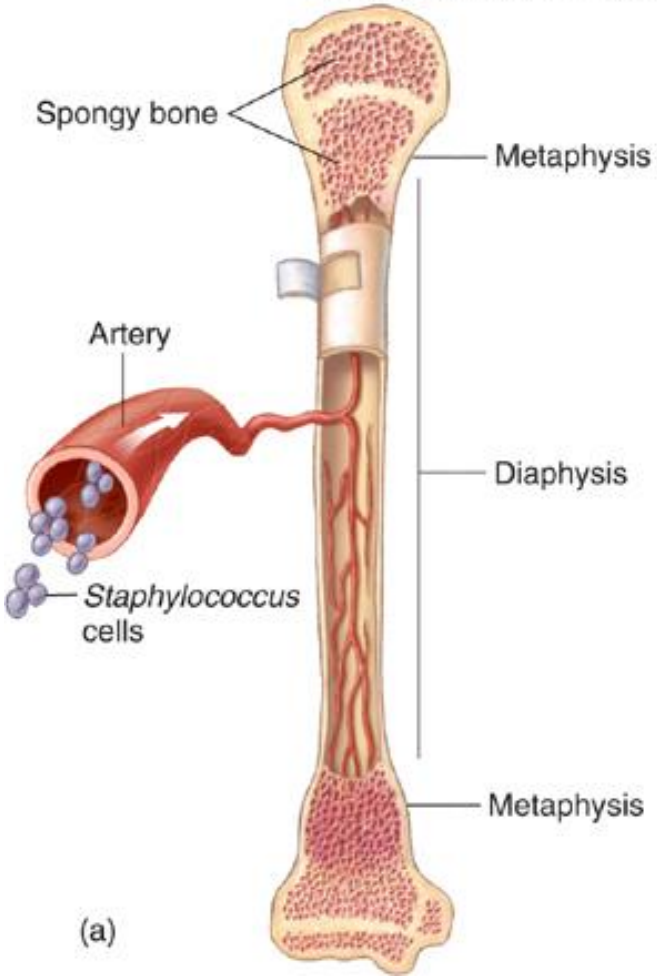
2- **Musculoskeletal**: Osteomyelitis, arthritis, bursitis, pyomyositis.

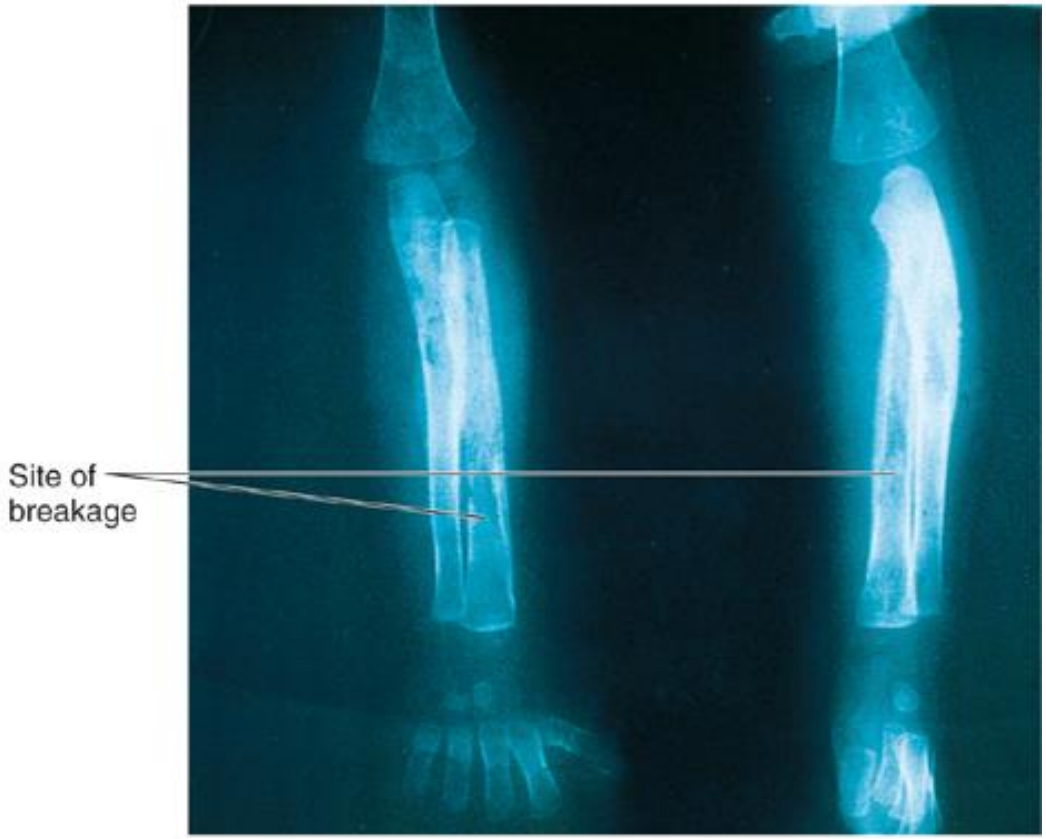


osteomyelitis

3- Respiratory: Tonsillitis, pharyngitis, sinusitis, otitis, bronchopneumonia, lung abscess, empyema,

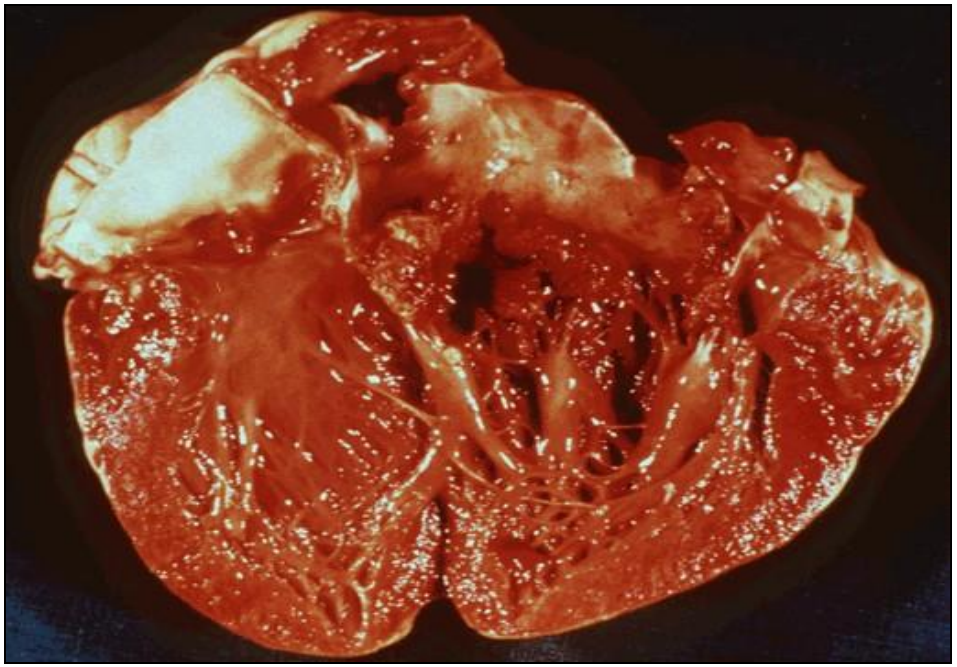
Staphylococcal osteomyelitis in a long bone





4- Central nervous system: Abscess, meningitis, intracranial thrombophlebitis.

5- Endovascular: Bacteremia, septicemia, pyemia, endocarditis.



Endocarditis

Systemic infections

- **7-Endocarditis** may occur on normal or prosthetic heart valves, especially right-sided endocarditis (tricuspid valve) in intravenous drug users.

- **8- Postsurgical wound infections.**

- 6- **Urinary:** Urinary tract infection.

B- **INTOXICATIOINS:**

The disease is caused by the bacterial exotoxins, which are produced either in the infected host or preformed in vitro.

There are 3 types-

1. **Food poisoning**
2. **Toxic shock syndrome**
3. **Staphylococcal scalded skin syndrome**

ENTEROTOXIN

- 1- Food poisoning (gastroenteritis) by ingestion of pre formed enterotoxin which has a short incubation period (1–8 hours).
- The toxin acts directly on the autonomic nervous system to cause the illness, rather than gut mucosa.

- The common food items responsible are - milk and milk products, meat, fish and ice cream.

- Clinical symptoms- nausea, vomiting and non bloody diarrhoea.

Staphylococcal Toxic shock syndrome (STSS):

- STSS is associated with infection of mucosal or sequestered sites by TSST(formerly known as enterotoxin type F) producing S.aureus.

- It is fatal multisystem disease presenting with fever, hypotension, myalgia, vomiting, diarrhoea, mucosal hyperemia and erythematous rash which desquamates subsequently.

2 Types of STSS known:

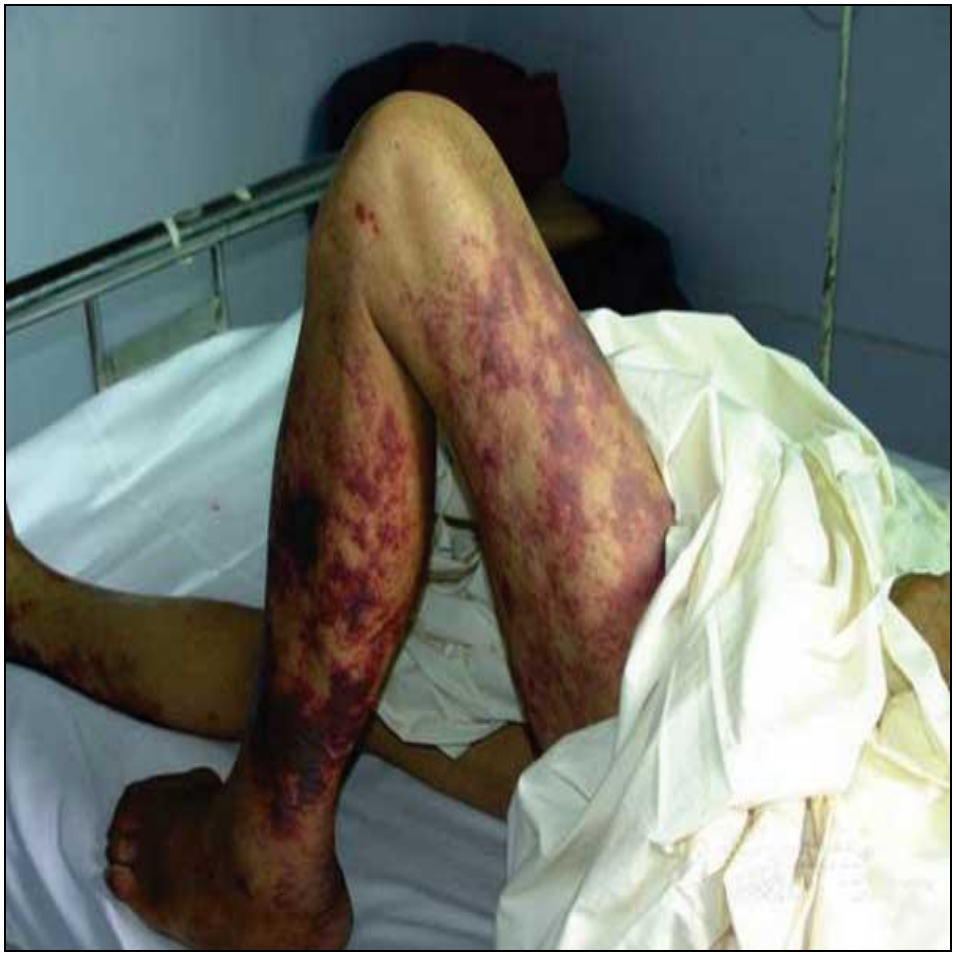
i- Menstrual associated STSS: Here colonization of S.aureus occurs in the vagina of menstruating woman who uses highly absorbent vaginal tampons.

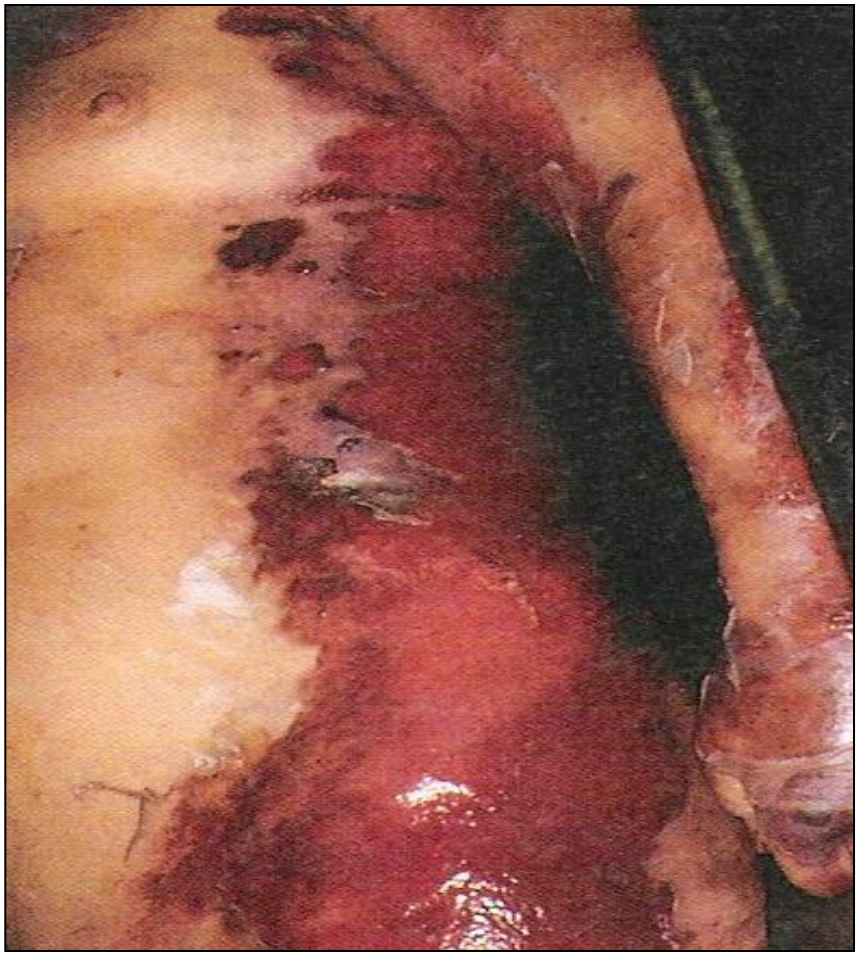
ii- Non menstrual associated STSS: Here colonization of S.aureus occurs in other sites like surgical wound.

Staphylococcal scalded skin syndrome (SSSS):

- Exfoliative toxin produced by S.aureus is responsible for this.

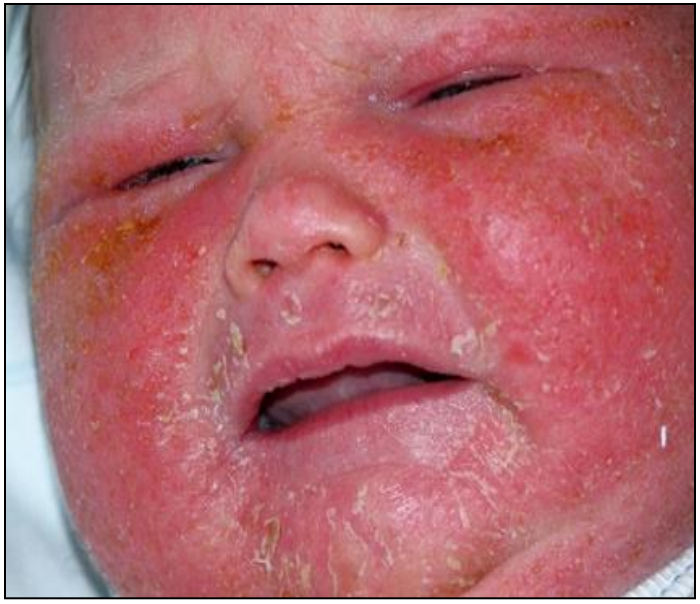
- It is a skin disease in which outer layer of epidermis gets separated from the underlying tissues.



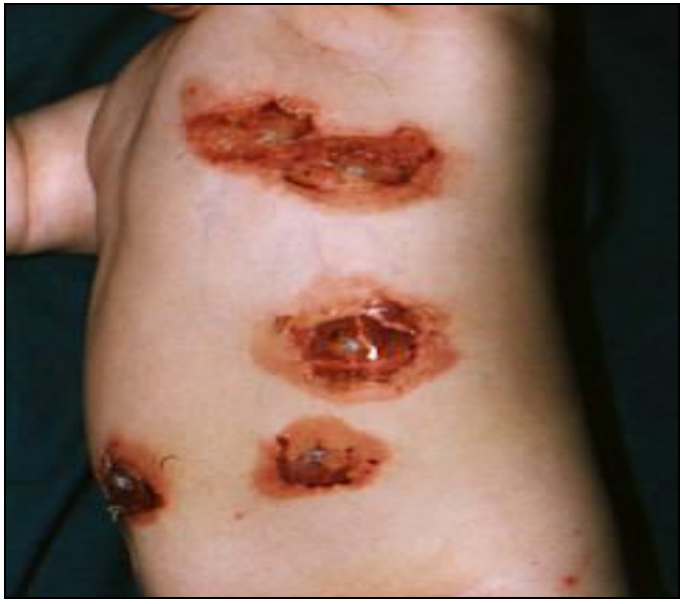


Types of SSSS:

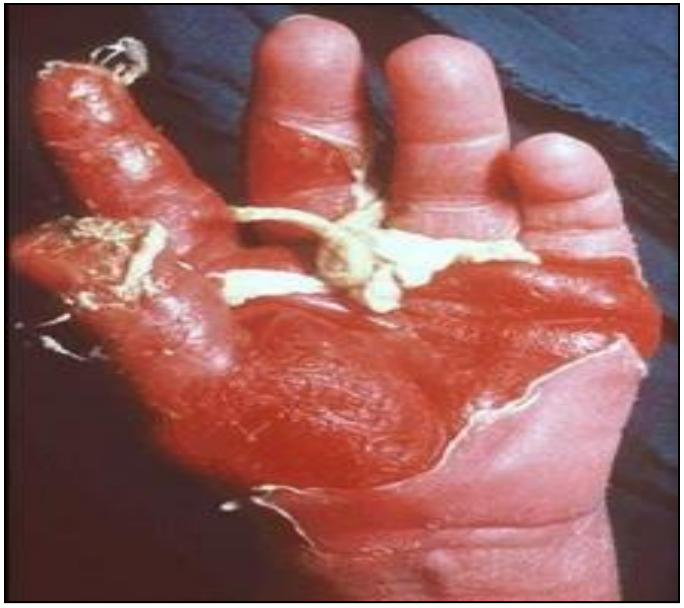
	Severe form	Milder form
In new born	- Ritter's disease	- Pemphigus neonatorum
In older patients	- Toxic epidermal necrolysis	- Bullous impetigo



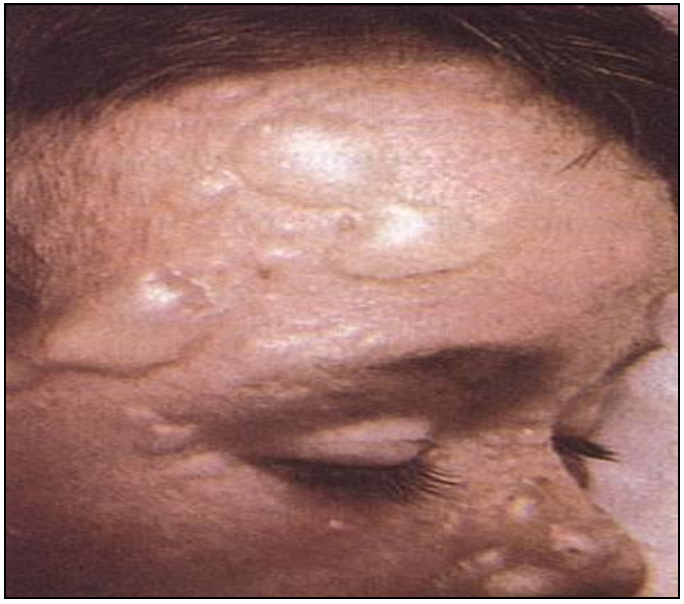
Ritter’s disease



Pemphigus neonatorum



Toxic epidermal necrolysis



Bullous impetigo

Staphylococcus aureus : Kawasaki Syndrome

- Kawasaki syndrome (KS) is a disease of unknown etiology.
- Caused by the super antigens of *Staph. aureus* (and *Str. pyogenes*).
- KS is a vasculitis involving small and medium-size arteries, especially the coronary arteries.

Staphylococcus aureus : Kawasaki Syndrome

- KS is much more common in children of Asian ancestry.
- Certain major histocompatibility complex (MHC) alleles may predispose to the disease.
- There is no definitive diagnostic laboratory test for KS.
- Effective therapy consists of high-dose immune globulins (IVIG)

Coagulase Negative Staphylococci(CoNS)

Coagulase Negative Staphylococci (CoNS):

Two species of coagulase negative Staphylococci can cause human infections-

1. ***Staphylococcus epidermidis***

2. ***Staphylococcus saprophyticus***

***S. epidermidis*:**

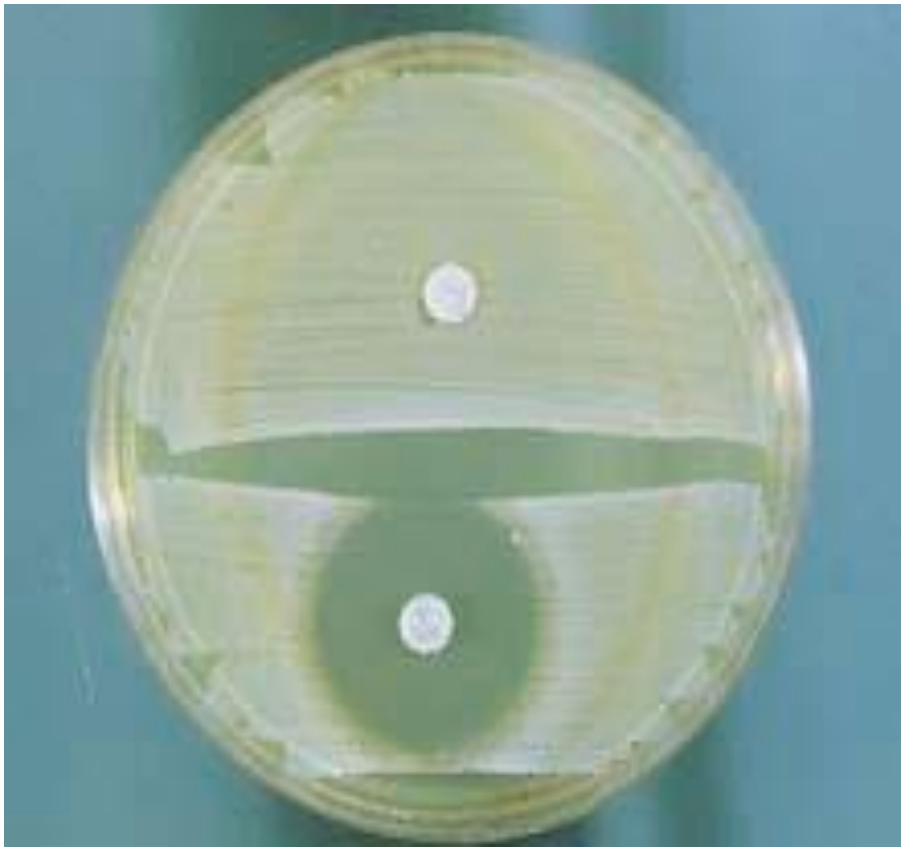
- It is a common cause of stitch abscesses.
- *S epidermidis* infections are almost always hospital acquired.
- It has predilection for growth on implanted foreign bodies such as artificial valves, shunts, intravascular catheters and prosthetic appliances leading to bacteraemia.
- Endocarditis may be caused, particularly in drug addicts.

***S.saprophyticus*:**

- It causes urinary tract infections, mostly in sexually active young women.
- The infections are almost always community acquired.
- Men are infected much less often.
- It is one of the few frequently isolated CoNS that is **resistant** to **Novobiocin**.

Novobiocin Susceptibility Test

- Test to differentiate between coagulase-negative staphylococci , *S.saprophyticus* in urine sample
  - *S. saprophyticus* is resistant
  - Other CoNS are susceptible to novobiocin



Distinguishing features of the major species of staphylococcus

Characters	S.aureus	S.epidermidis	S.saprophyticus
Coagulase	+	-	-
Novobiocin sensitivity	Sensitive	Sensitive	Resistant
Acid from mannitol fermentation anaerobically	+	-	-
Phosphatase	+	+	-

LAB DIAGNOSIS:

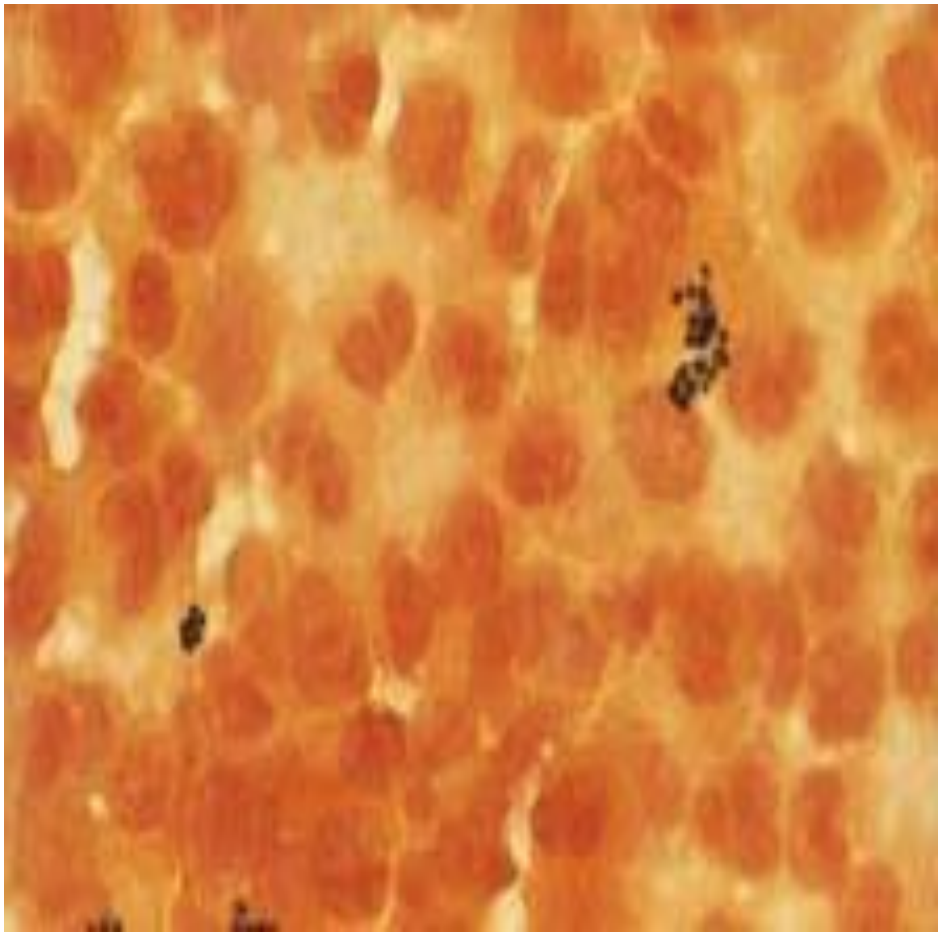
Specimens collection: Depends on the type of infection.

- Suppurative lesion- Pus,
- Respiratory infection- Sputum,
- Bacteremia & septicemia- Blood,
- Food poisoning- Feces, vomit & the remains of suspected food,
- For the detection of carriers- Nasal swab.

Laboratory Diagnosis: Direct Smear Examination

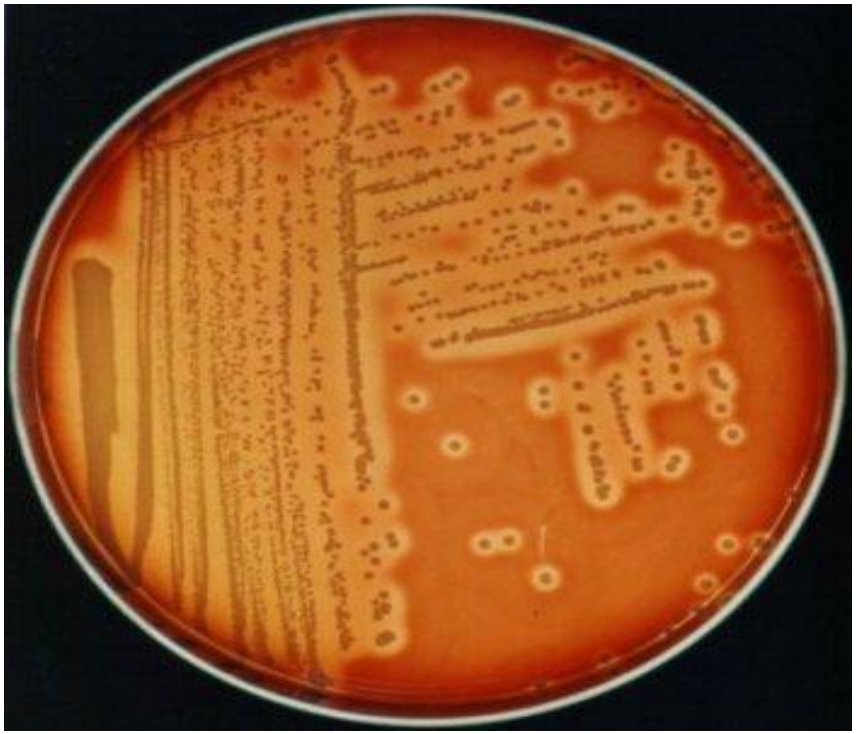
Microscopic Examination

- Gram-positive cocci
- pairs and clusters
- Numerous polymorphonuclear cells (PMNs)



Laboratory Diagnosis: Cultural Characteristics-

- ***Staphylococcus aureus***
  - Colony morphology
    - Smooth, butyrous, white to yellow, creamy
    - *S. aureus* may produce hemolysis on blood agar



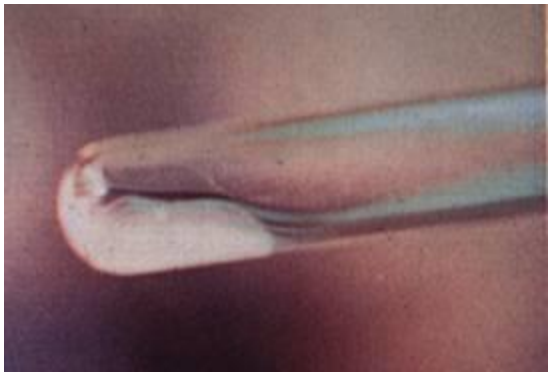
S. aureus

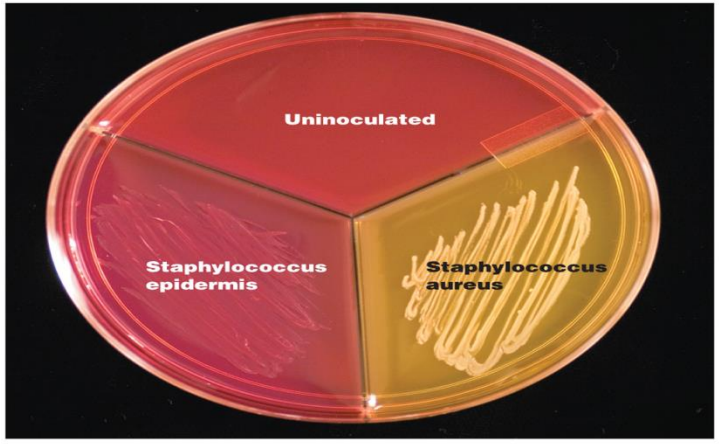
Laboratory Diagnosis: Cultural Characteristics

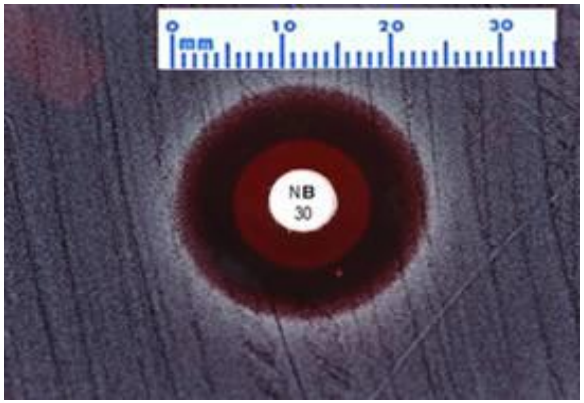
- **S. epidermidis**
  - Smooth, creamy, white
  - Small-to medium-sized, usually non-hemolytic
- ***S. saprophyticus***
  - Smooth, creamy, may produce a yellow pigment

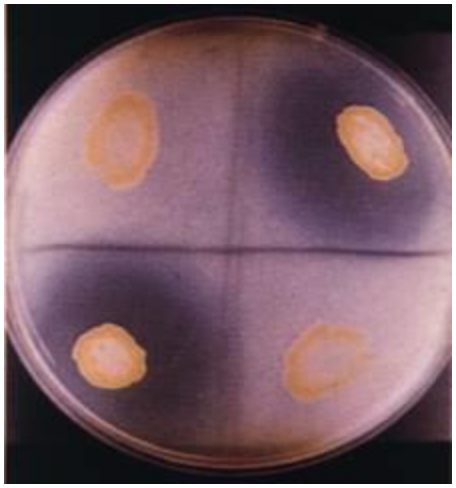
Biochemical Reactions*(S. aureus)*

- Coagulase +
- Mannitol salt +
- DNase +
- Novobiocin S



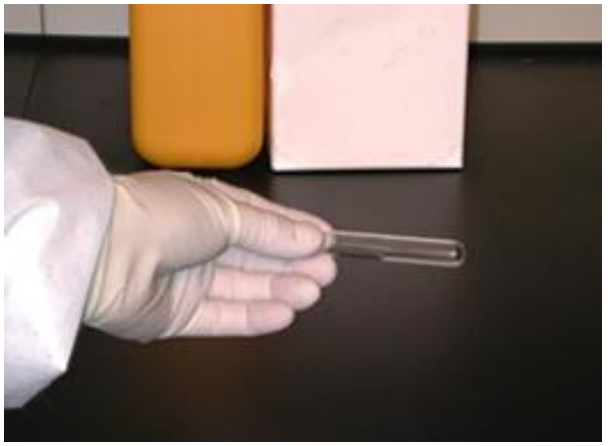


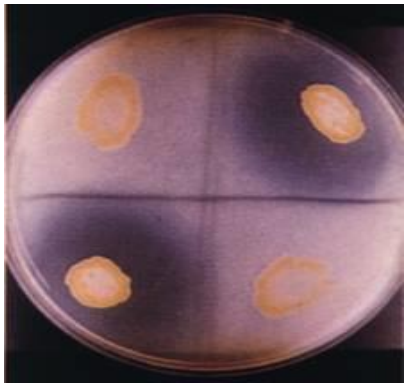


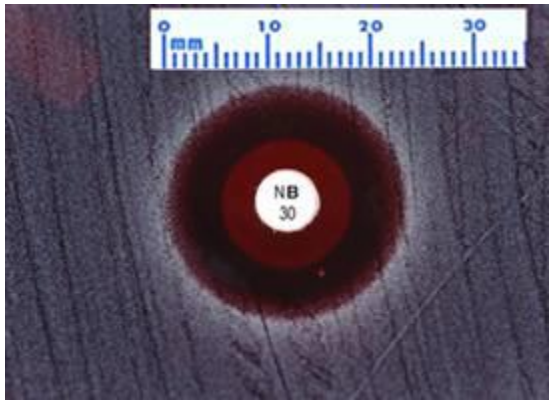


Biochemical Reactions (S.epidermidis)

- **Coagulase -ve**
  - **Mannitol salt -ve**
  - **DNase –ve**
  - **Novobiocin “S”**

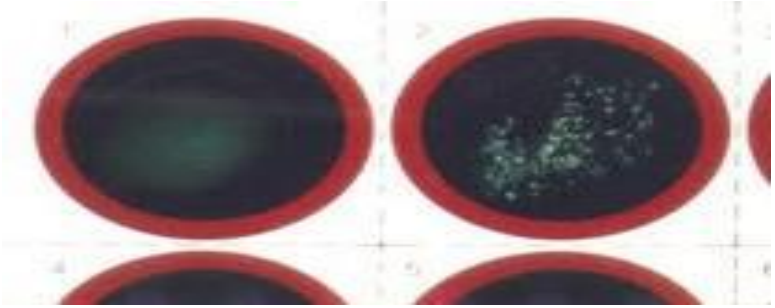






Identification Tests: Rapid Coagulase Test

- Latex Agglutination Assays
  - Detects cell-bound "clumping factor," protein A or a combination of both
  - Procedure
    - Varies depending on kit type
    - Positive reaction demonstrated by agglutination



III) Antibiotic sensitivity tests is done as a guide to treatment.

IV) Bacteriophage typing is done for epidemiological purposes.

V) Serological tests are not useful.

Phage typing

- For epidemiological purposes, *S. aureus* can be subdivided into subgroups based on the susceptibility of the clinical isolate to lysis by a variety of bacteriophages.
- A person carrying *S. aureus* of the same phage group as that which caused the outbreak may be the source of the infections.

TREATMENT

- In the USA, 90% or more of *S. aureus* strains are resistant to penicillin G. Most of these strains produce beta lactamase. Such organisms can be treated with beta lactamase- resistant penicillins e.g nafcillin or cloxacillin, some cepha-losporins, or vancomycin.

TREATMENT

- Approximately 20% of *S. aureus* strains are methicillin resistant or nafcillin-resistant by virtue of altered penicillin binding proteins. These resistant strains of *S. aureus* are abbreviated MRSA or NRSA. Such organisms can produce out breaks of disease, especially in hospitals.

What is MRSA?

MRSA

- **MRSA stands for *Methicillin-Resistant Staphylococcus aureus.***

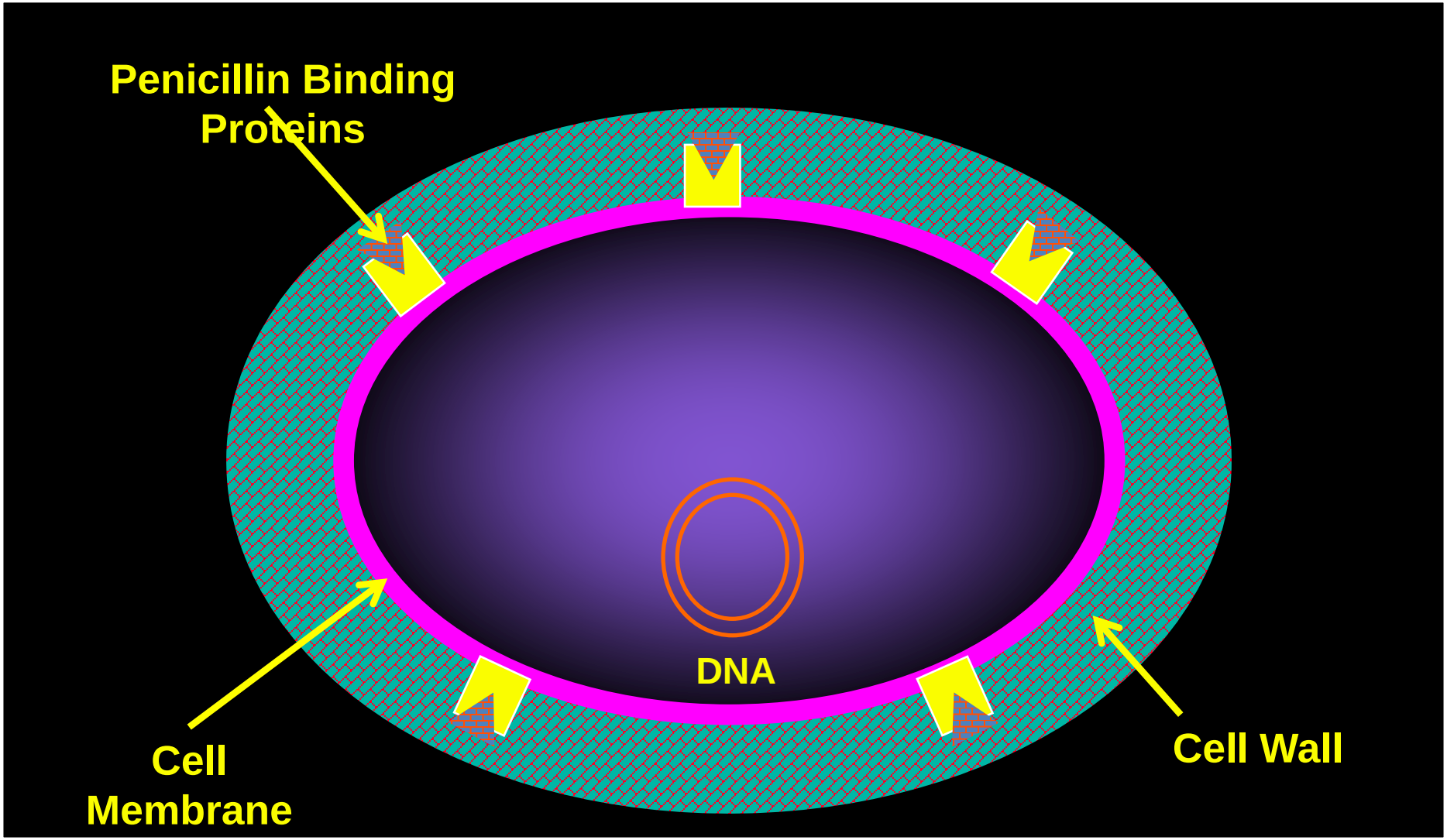
MRSA

- **Strains of S.aureus that are oxacillin and methicillin resistant, termed methicillin-resistant Staph aureus.**

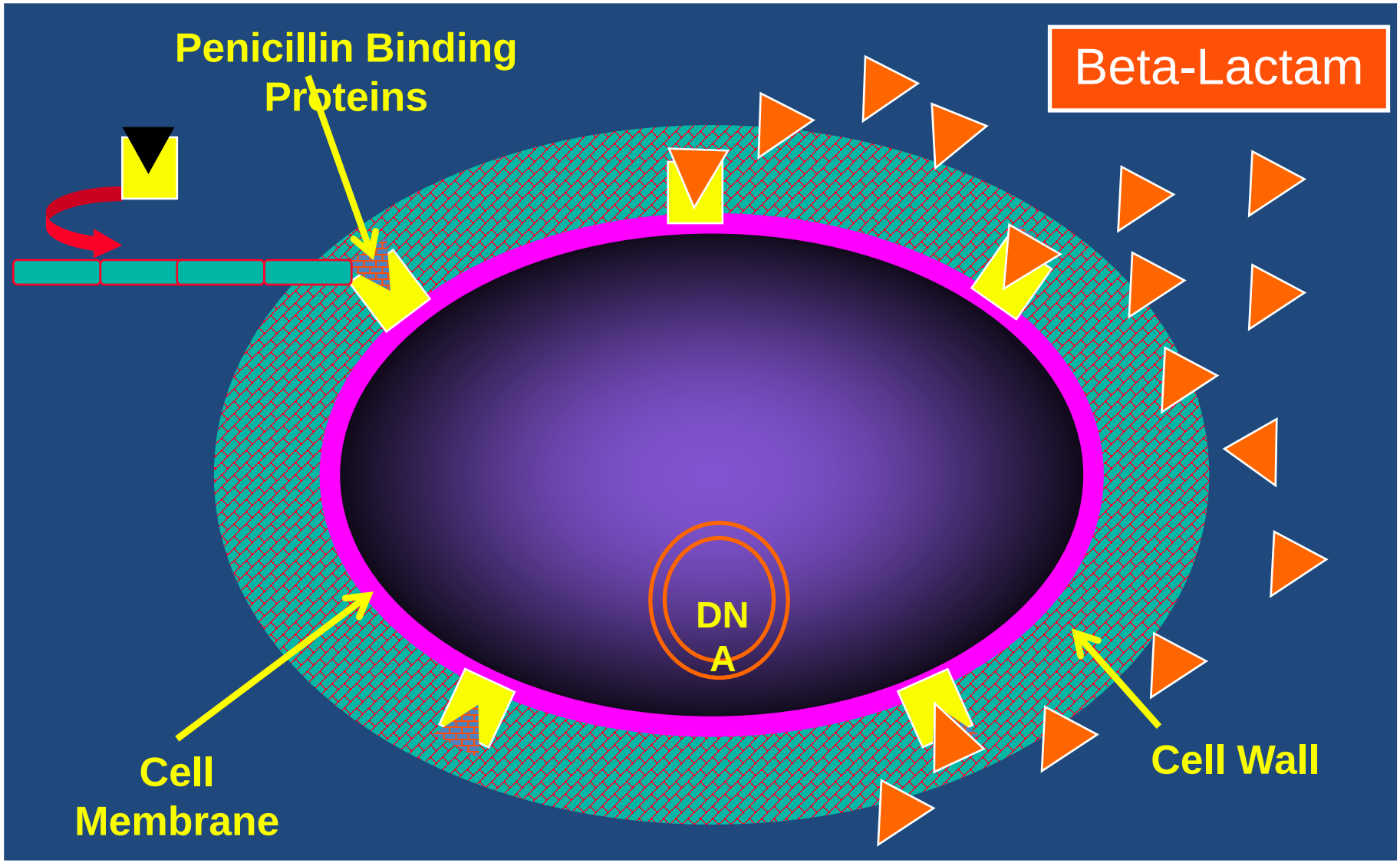
- **Methicillin-resistant *S. aureus* (MRSA) have acquired a gene mec A encoding PBP2a giving them resistance to methicillin.**

- **MRSA, are resistant to all beta-lactam agents, including cephalosporins and carbapenems.**

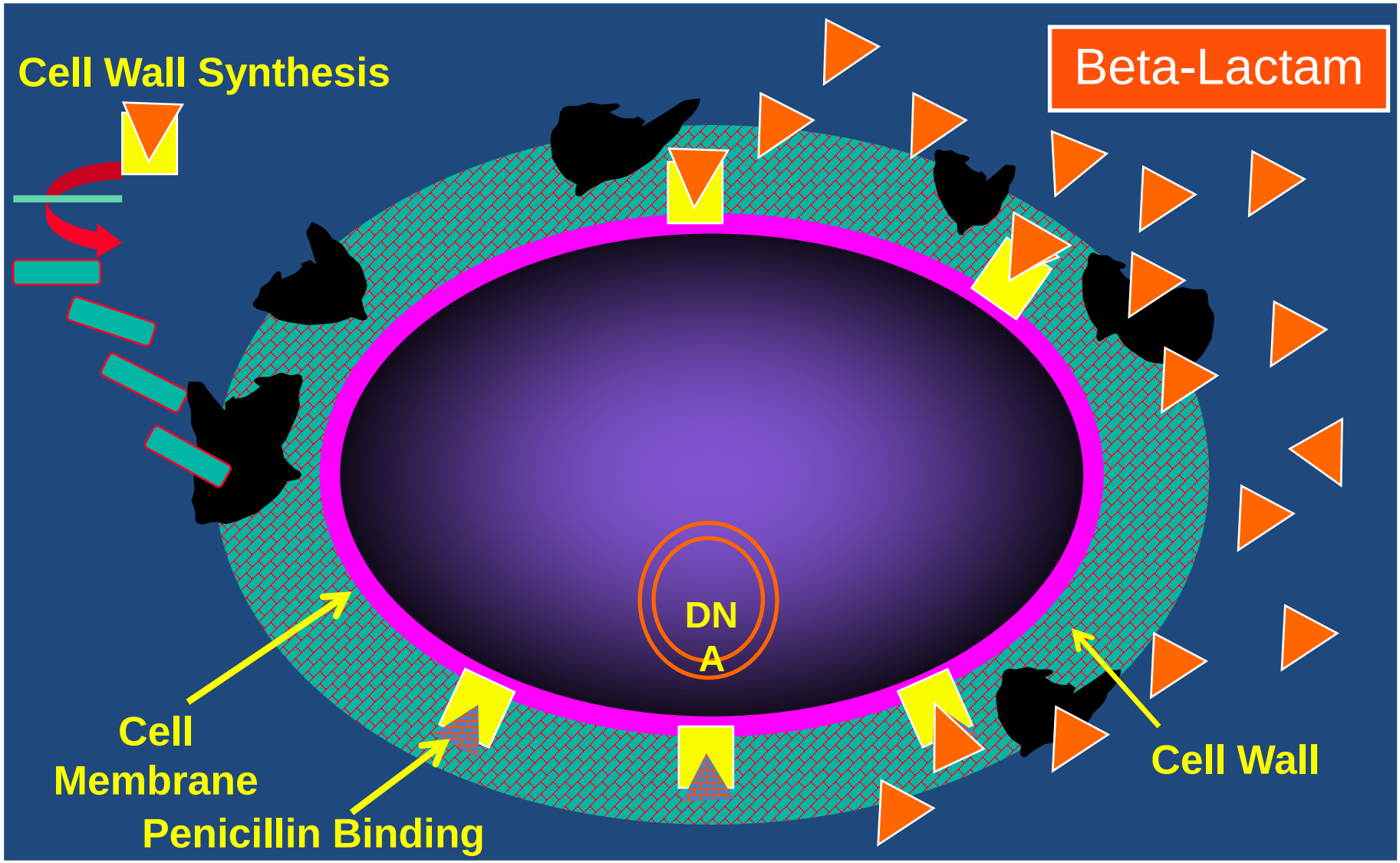
Structure of Gram-Positive Bacteria



Beta-Lactams: Mechanism of Action



Beta-Lactam: Mechanism of Action



Vancomycin resistance

- Vancomycin has been the drug of choice for the treatment of MRSA infections, however, the strains of *Staphylococcus aureus* with intermediate susceptibility of vancomycin (VISA) and vancomycin resistant Staphylococcus aureus (VRSA) emerged.

❑New Antibiotic Options

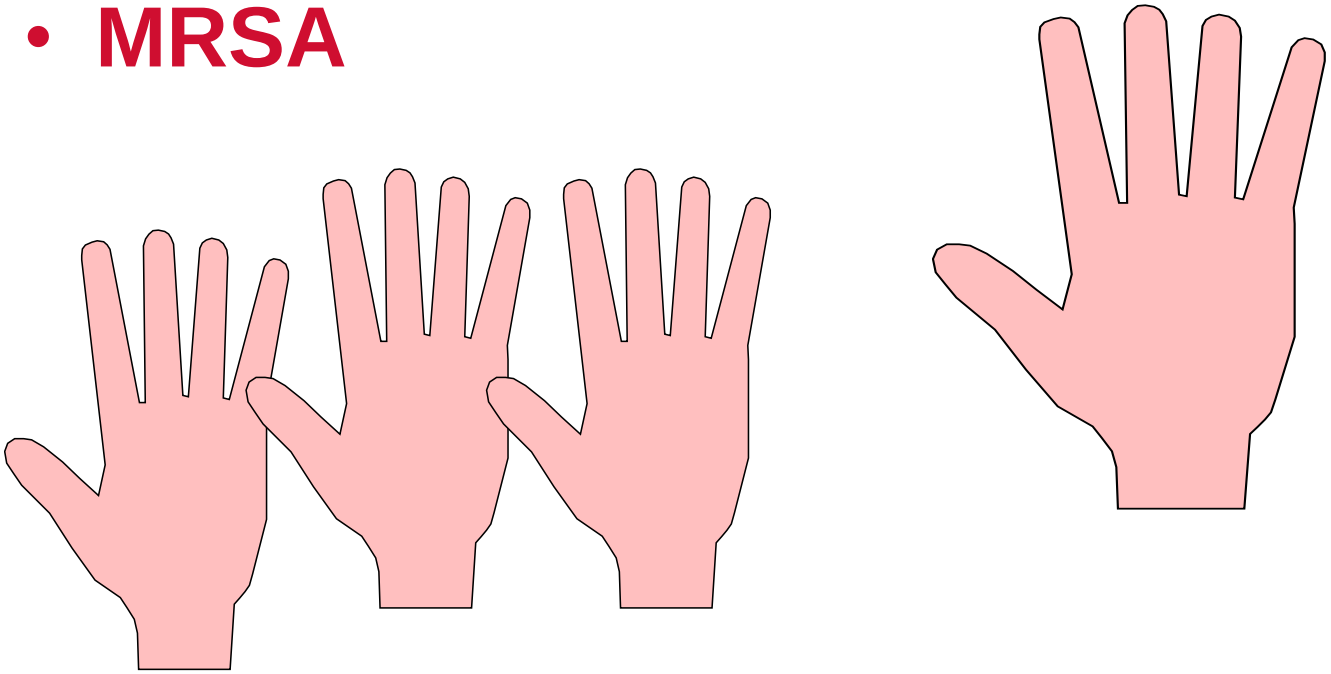
- ❑Linezolid
- ❑Quinopristin/Dalfopristin

HOW is the MRSA spread?

MRSA is transmitted primarily by contact with a person who has an infection or is colonized with the bacteria.

SPREAD OF INFECTONS

- **The hands are the most important**
- **vehicle of transmission of**
- **MRSA**



Why are MRSA important?

1. Hospital –acquired infections. MRSA are common nosocomial pathogens around the world.
2. The treatment is very difficult. Vancomycin often is the only drug of choice for severe infections.
3. MRSA with reduced susceptibility to glycopeptides. Since 1996 has been identified in Europe, Asia and United States. That increases the possibility some strains became fully resistant to glycopeptides.
4. MRSA are easily transmissible between patients.

Control of MRSA in Hospitals

- **Handwashing.** Health care workers should wash their hands before and after contact with all patients, even when gloves are worn.
- A written protocol detailing proper hand wash technique should be available for reference.
- **Isolation** and treatment is necessary for infected patients .
- **Screening of patients for MRSA** at the nose, throat and perineum.

PREVENTION:

- Isolation & treatment of MRSA patients.
- Detection of carriers among hospital staff, their isolation & treatment.
- Avoid indiscriminate usage of antibiotics.

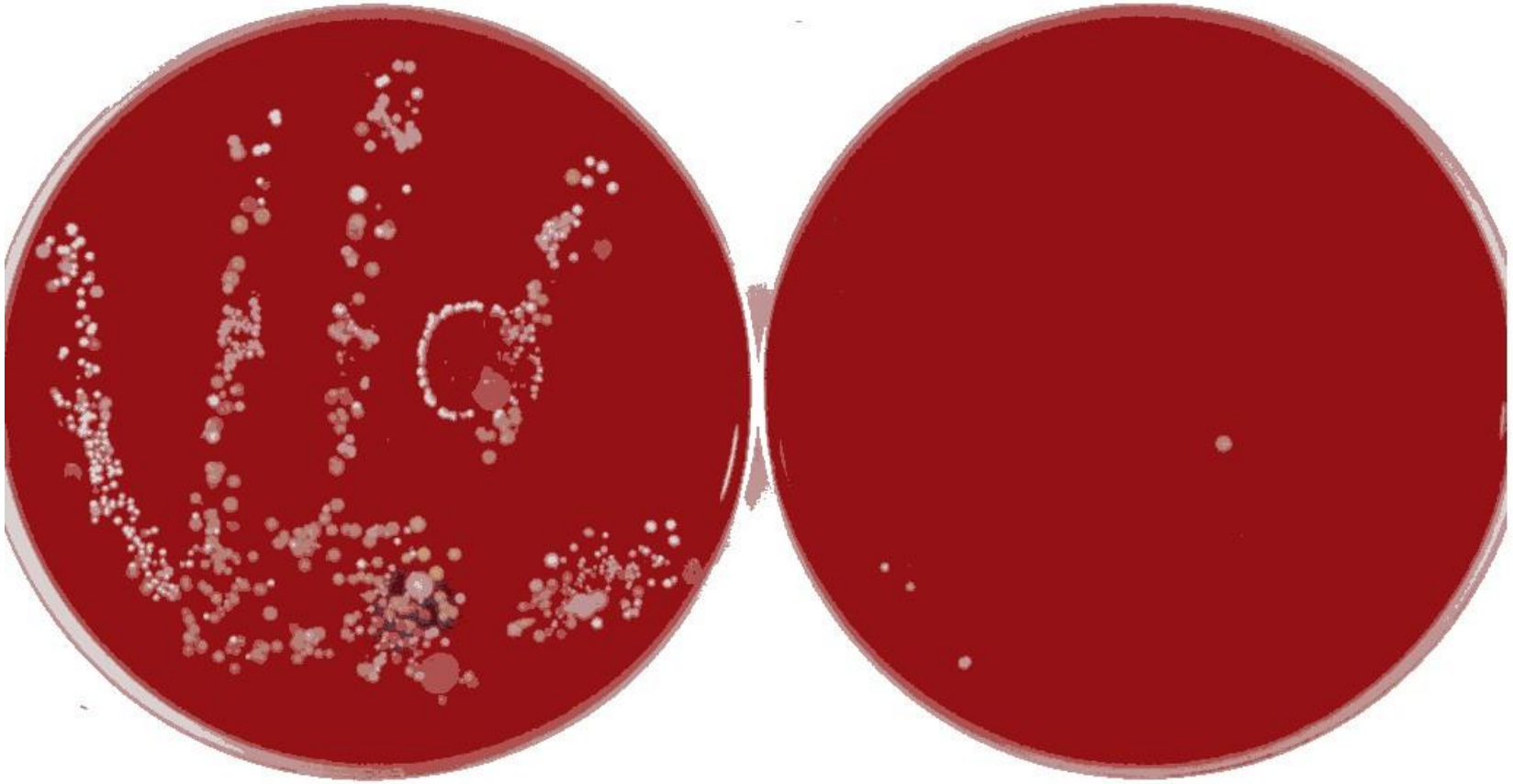
- Mupirocin is very effective as a topical antibiotics in skin infections caused by *S. aureus.* It has also been used to reduce nasal carriage of the organism in hospital personnel and in patients with recurrent staphylo-coccal infections.

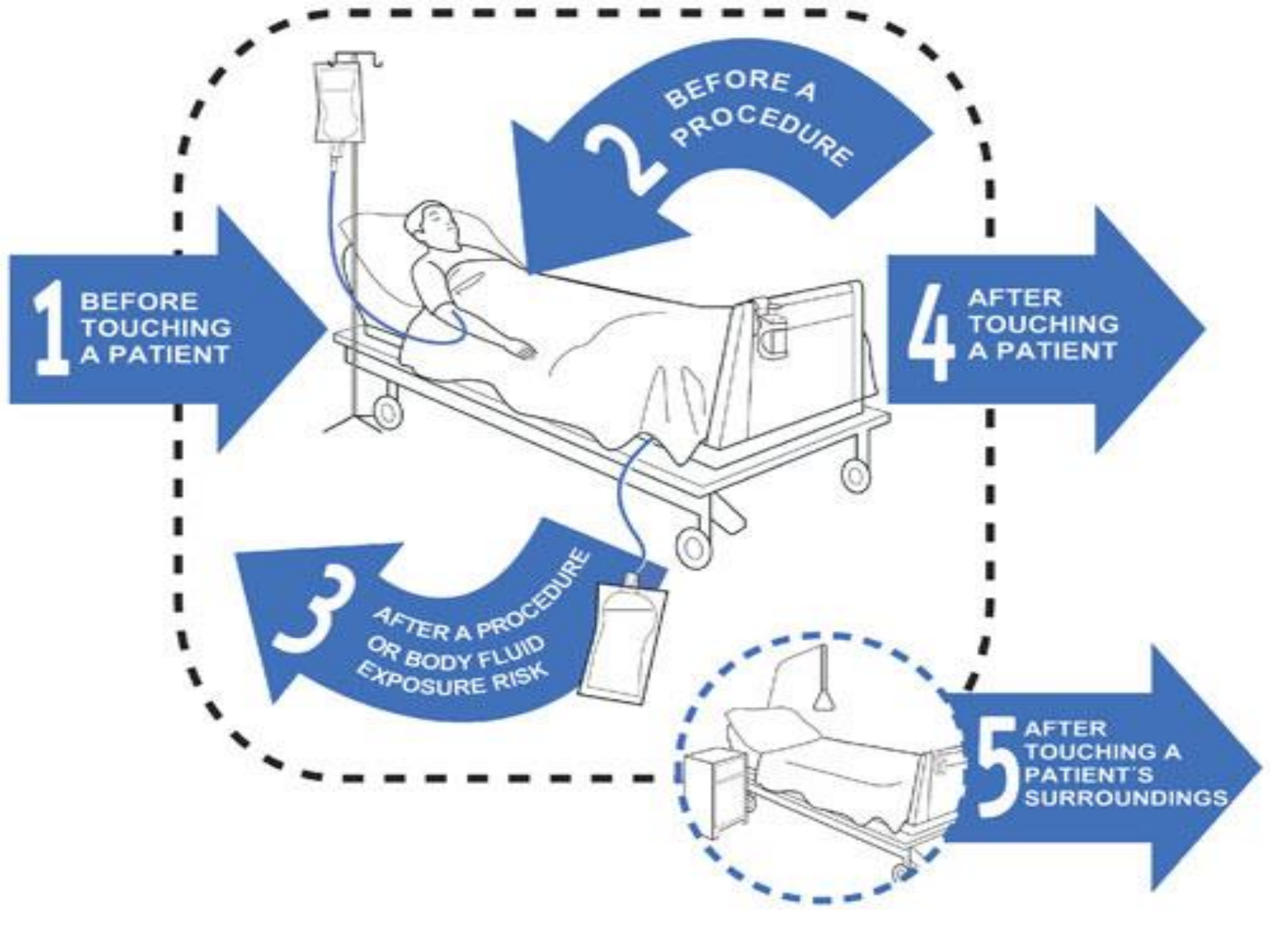
‘MRSA is NOT stronger than hospital hygiene…….. if you control MRSA, you control all the other organisms as well

MRSA is the best indicator of hospital hygiene

Potentially harmful microorganisms

Handwashing can remove Germs!




1
BEFORE TOUCHING A PATIENT
2
BEFORE A PROCEDURE
3
AFTER A PROCEDURE OR BODY FLUID EXPOSURE RISK
4
AFTER TOUCHING A PATIENT
5
AFTER TOUCHING A PATIENT'S SURROUNDINGS

Palm to palm

Between fingers

Back of hands

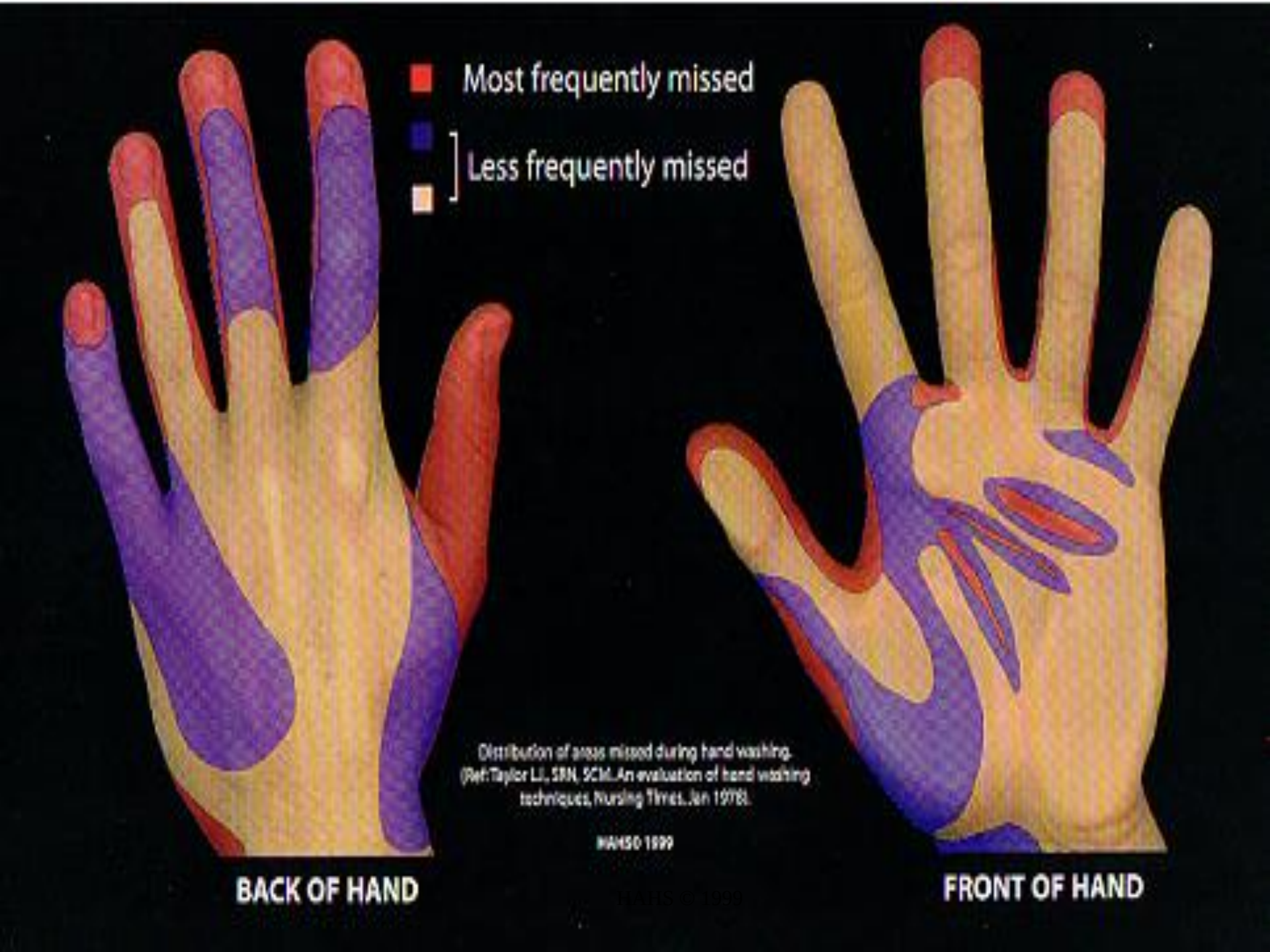
Base of thumbs

Back of fingers

Fingernails

Wrists

Rinse and wipe dry



Distribution of areas missed during hand washing.
(Ref:Taylor LJ., SRN, SCM..An evaluation of hand washing techniques, Nursing Times..Jun 1978).

HAHSO 1999


It's a fact Jack.
Hand washing is the best way to prevent infectious disease.
TEXAS
Department of State Health Services

Wash your hands!!!