AZERBAIJAN MEDICAL UNIVERSITY
DEPARTMENT OF MEDICAL MICROBIOLOGY and IMMUNOLOGY

Lesson 1.

The main principles, aim and tasks of microbiological diagnostics. Microbiology diagnosis of diseases, caused by Gram positive cocci (staphylococci, streptococci and enterococci)

FACULTY: General Medicine
SUBJECT: Medical microbiology - 2

Discussed questions:

1. The main tasks of special medical microbiology.
2. Importance of selection of pathological material for clinical diagnosis. Collection of pathological material, storage, sending to the laboratory. Disinfection of pathological material left after the examination.
3. Gram-positive cocci, the causative agents of purulent-inflammatory processes
4. Staphylococci, classification, morpho-biological characteristics, pathogenicity factors and antibiotic-resistant forms (methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant coagulase-negative staphylococcus (MRCNS)).
5. Microbiological diagnosis of diseases caused by staphylococci
6. Specific treatment and prevention of staphylococcal infections.
7. Streptococci, their classification, morpho-biological properties, antigenic structure, pathogenicity factors and diseases caused by them. The role of streptococci in the development of rheumatism and glomerulonephritis.
8. Microbiological diagnosis of diseases caused by streptococci.
9. Specific treatment and prevention of streptococcal infections
10. Pneumococci (Streptococcus pneumoniae), morpho-biological properties, antigenic structure, diseases caused by them and signs that distinguish pneumococci from other streptococci.
11. Microbiological diagnosis of pneumococcal infections.
12. Specific treatment and prevention of pneumococcal infections.
13. Enterococci, their morpho-biological characteristics, pathogenicity factors, diseases they cause, antibiotic-resistant forms (vancomycin-resistant enterococcus (VRE)), microbiological diagnosis, specific treatment and prevention
14. Other medically important streptococci (S.agalactiae, S.mutans, S.mitis, etc.)

Purpose of the lesson:

- Introduction to special microbiology for students, clinically important bacteria: pathogenic and opportunistic Gram-positive cocci, especially antibiotic-resistant forms (Staphylococcus (methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant coagulase-negative staphylococcus (MRCNS)), Streptococcus, vancomycin- resistant enterococcus (VRE)), to provide information about their morpho-biological characteristics, pathogenicity, diseases, microbiological diagnostisis, specific treatment and prevention.

➢*Special medical microbiology*

Medical microbiology - studies the characteristics of microorganisms that cause disease in humans and the pathological processes that occur in the organism during these diseases.

Development of laboratory diagnosis, specific prevention and treatment methods of diseases caused by microorganisms are the main tasks of medical microbiology.

- Special microbiology - studies the characteristics of various microorganisms and is divided into the following departments depending on this:

- bacteriology (science of bacteria)

- virology (science of viruses)

- - mycology (science of fungi)

- - protozoology (the science that studies protozoas)

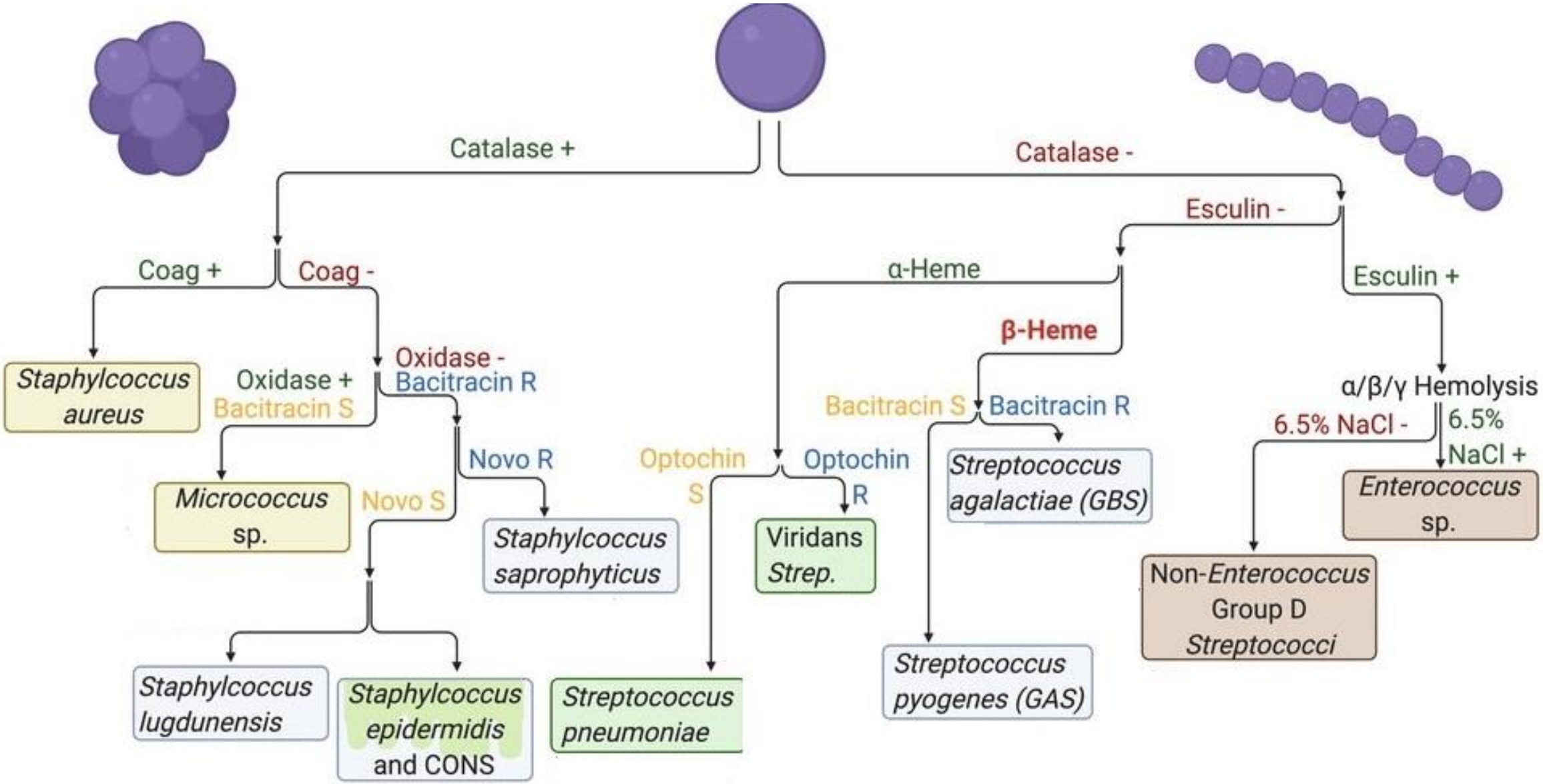
Introduction to Bacteriology
Purulent-inflammatory processes

- Bacteriology is a branch of microbiology that studies the morphology, ecology, genetics, biochemistry of bacteria, as well as the identification, classification and characterization of bacterial species.
- Purulent-inflammatory processes - when the reactivity of the body is weakened, as well as during various injuries and traumas, pyogenic bacteria, mostly Streptococcus, Staphylococcus, Enterococcus, Neisseria gonorrhoeae, Neisseria meningitidis, rarely Pseudomonas, Proteus spp. and E.coli overcome the skin and mucous membrane barriers and cause purulent-inflammatory processes.
- Purulent infection can develop in the form of a disease (furuncle, carbuncle, measles, osteomyelitis, etc.) or as a complication of a wound. Generalization of the purulent process can lead to the development of a general purulent infection, that is, sepsis. Purulent infections are often resistant to antibiotics.

Gram-positive cocci:

- **Aerobic genus**: Micrococcus, Planococcus və Deinococcus
- **Facultative anaerobic genus:** Staphylococcus, Stomatococcus, Streptococcus, Leuconostoc, Pediococcus, Aerococcus and Gemella
- **Anaerobic genus:**

  Peptococcus, *Peptostreptococcus*, *Ruminococcus*, *Coprococcus*, *Sarcina*


Catalase +
Catalase -
Coag +
Coag -
Esculin -
Esculin +
α-Heme
β-Heme
Staphylcoccus aureus
Oxidase +
Bacitracin S
Oxidase -
Bacitracin R
Micrococcus sp.
Novo S
Novo R
Staphylcoccus saprophyticus
Staphylcoccus lugdunensis
Staphylcoccus epidermidis and CONS
Optochin S
Optochin R
Viridans Strep.
Streptococcus pneumoniae
Bacitracin S
Bacitracin R
Streptococcus agalactiae (GBS)
Streptococcus pyogenes (GAS)
α/β/γ Hemolysis
6.5% NaCl -
6.5% NaCl +
Enterococcus sp.
Non-Enterococcus Group D Streptococci

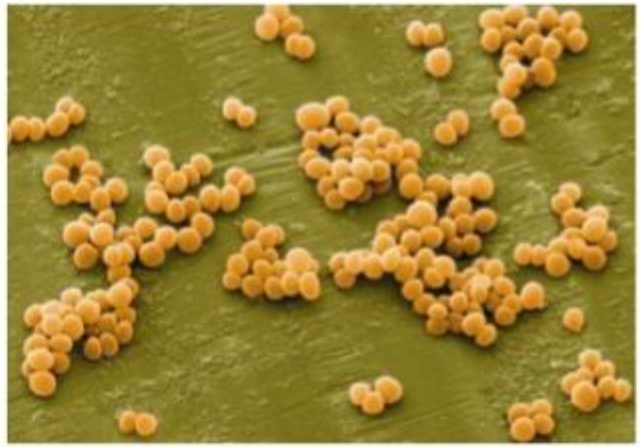
Staphylococcaceae family (new taxonomy)

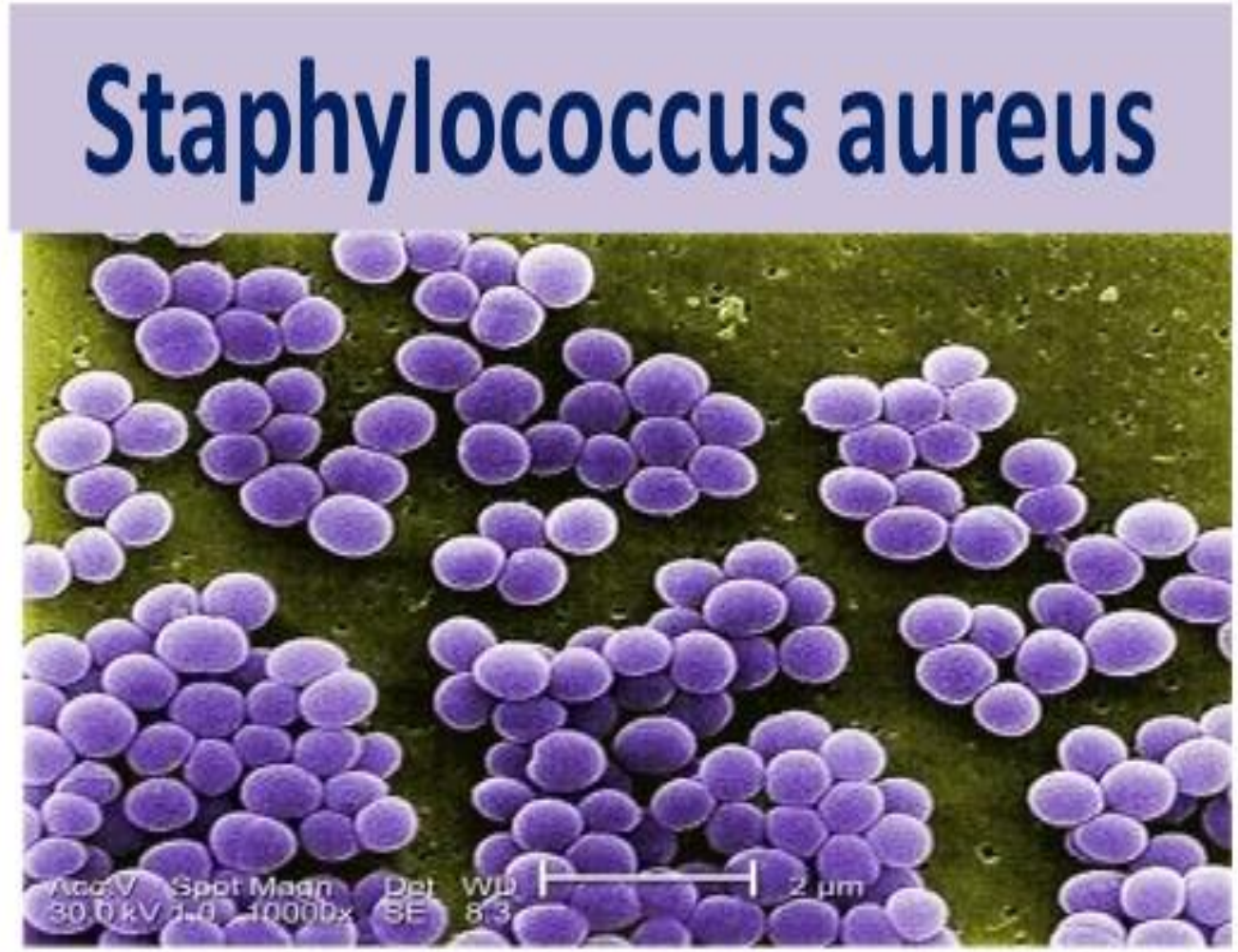
- **Domain: Bacteria**
- **Kingdom: Bacillota**
- **Class: Bacilli**
- **Order: Bacillates**
- **Family: Staphylococcaceae**
- **Genus: Staphylococcus**

- **Genus:**
  - Abyssicoccus
  - Aliicoccus
  - Auricoccus
  - Corticicoccus
  - Gemella
  - Jeotgalicoccus
  - Macrococcus
  - Nosocomiicoccus
  - Salinicoccus
  - **Staphylococcus**
    - **Species**: It consists of about 40 species (S.hominis, S.albus, S.haemolyticus, S.simulans, S.sciuri, etc.). But medically important species are ***S.aureus, S.epidermidis, S.saprophyticus.***

Schleifer and Bell 2010

STAPHYLOCOCCI





INTRODUCTION

- Family: Micrococceae (consists of Gram positive cocci, arranged in tetrads, clusters)
- Genus : Staphylococcus
- Term “staphylococcus” derived from Greek :Staphyle = bunch of grapes and Kokkos = berry, meaning bacteria occurring in grapelike clusters or berry.

S.aureus

- Natural habitat:-Nostril and skin

Morphology:-

- Gram-positive, cocci, 0.5-1.5µm in diameter; occur characteristically in group, also singly and in pairs
- Form irregular grapelike clusters (since divide in 3 planes)
- Non-motile, non- sporing and few strains are capsulated

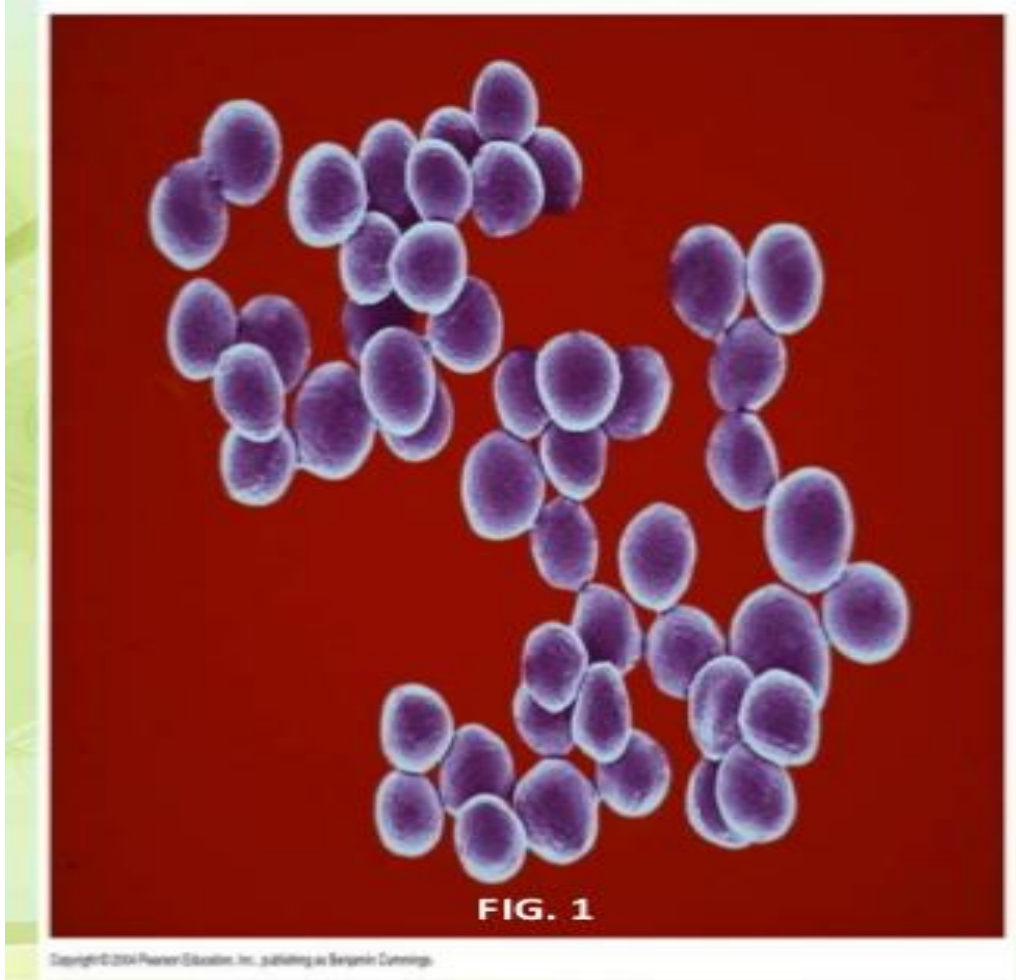
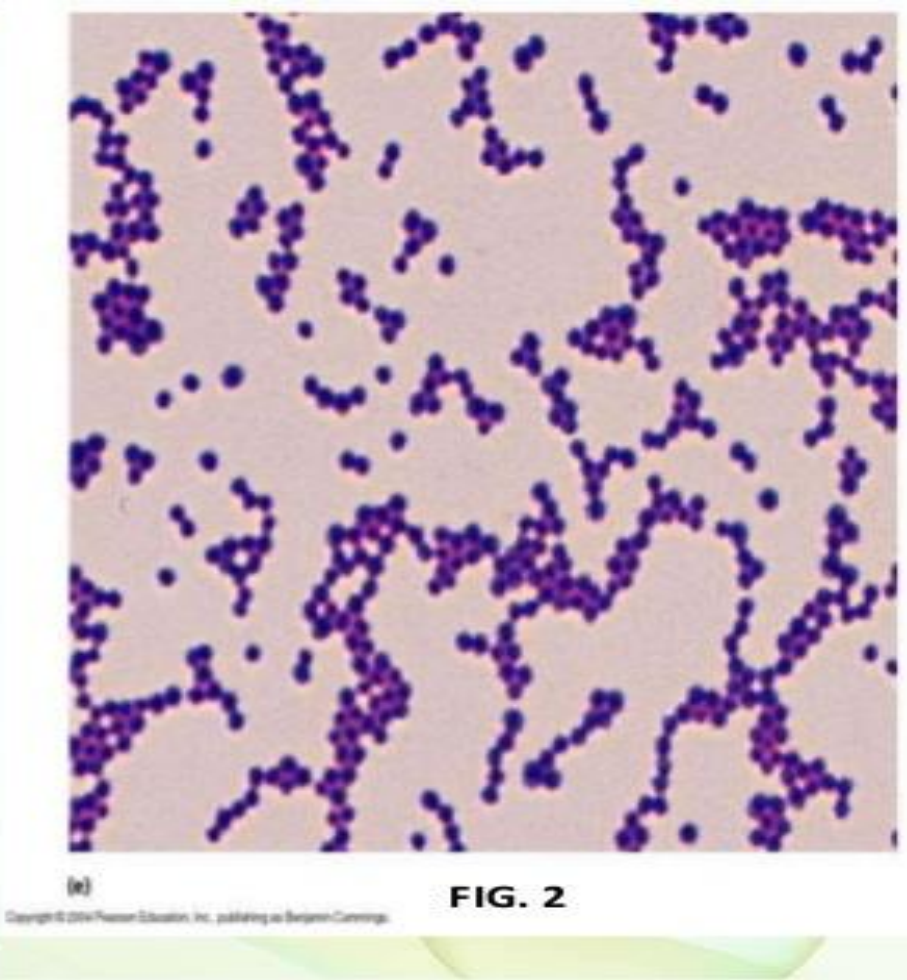


FIG. 1



(a)

FIG. 2

GRAM STAIN

Classification

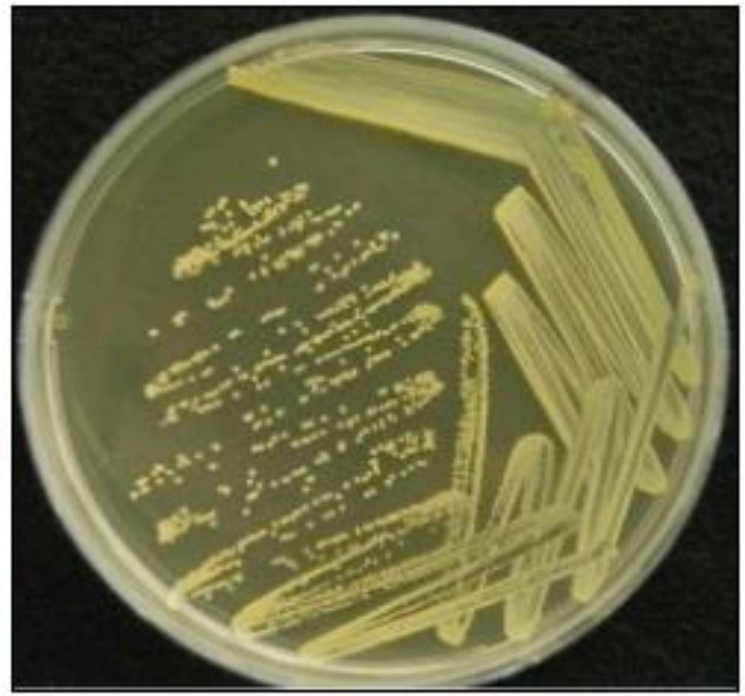
- Based on pigment production:
  - *S.aureus* :-golden-yellow pigmented colonies
  - *S.albus* :- white colonies
  - *S.citrus* :-lemon yellow colonies

S. albus , S. aureus , S. citrus on Nutrient Agar

- Based on pathogenecity:
  - Pathogenic:- includes only one i.e., *S.aureus*
  - Non-pathogenic:- includes *S.epidermidis, S.saprophyticus, S.albus, S. citrus, S.hominis,etc.*
- Based on coagulase production:
  - Coagulase positive: *S. aureus*
  - Coagulase negative: *S. epidermidis, S. saprophyticus*

Culture

- Aerobes and facultative anaerobes
- Opt. Temp. For growth= 37°C
- Opt. pH for growth= 7.5
- On Nutrient agar,
  - golden yellow and opaque colonies with smooth glistening surface, 1-2 mm in diameter (max. pigment production@22 °C)



culture characteristic

colony morphology on many types of agars:

-**On nutrient agar**

Staphylococcus aureus

colonies are:

large, circular, smooth ,

shiny surface and are

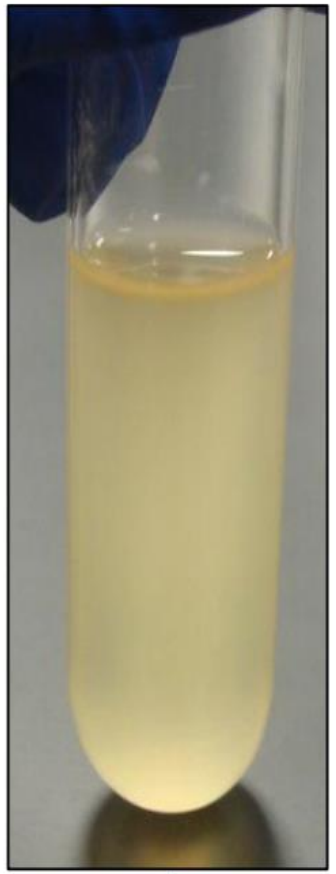
pigmented (golden-yellow).

Staphylococcus aureus
Cultural characteristics:

In a liquid nutrient media, it forms a sedimentary diffuse turbidity.

They develop better in sugary media.

Egg yolk-salt or milk-salt agars are selective nutrient media for them.



Staphylococcus aureus
liquid nutrient media

Staphylococcus aureus

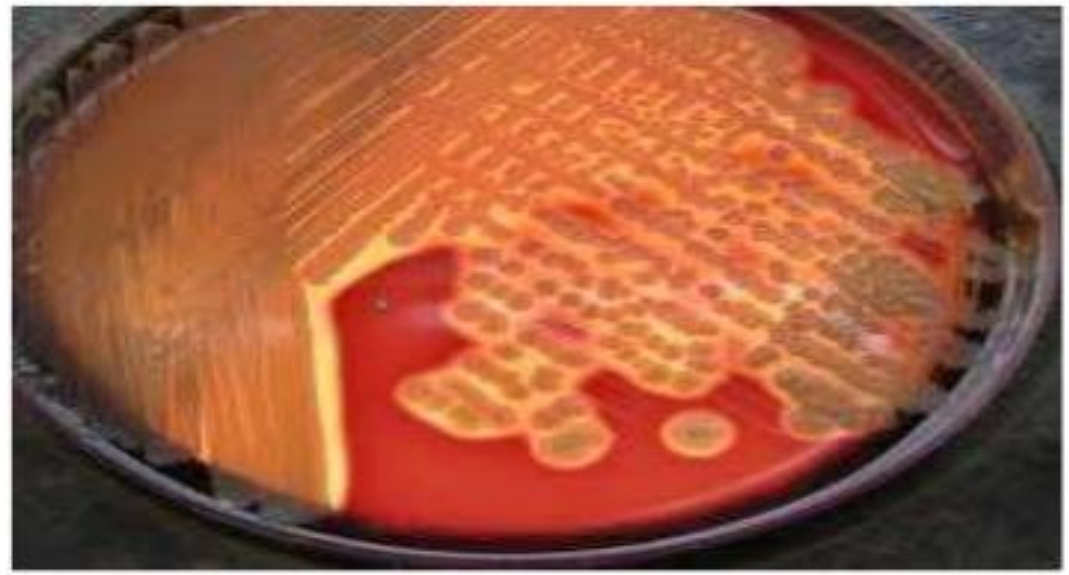
(lecithinase test in egg yolk-salt agar)

***Lecithinase test* -** due to the action of lecithinase enzyme, a crown-shaped blurred border is formed around S.aureus colonies.

-**On blood agar**

- ***S. aureus***

β-hemolysis /clear zone around the colonies.



- ***S.epidermidis***

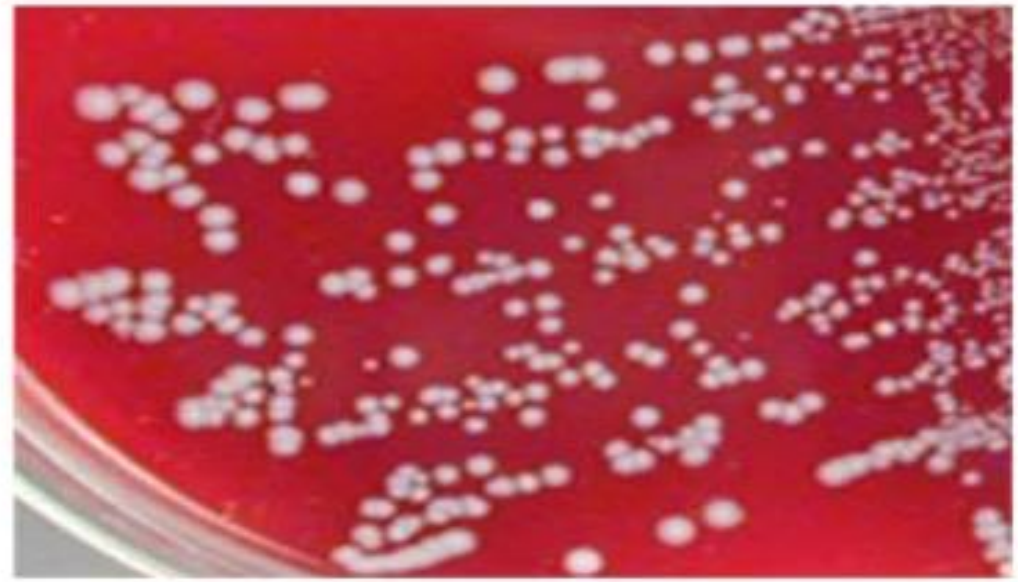
-White-creamy colonies

-no hemolysis of red blood cells.

- ***S. Saprophyticus***

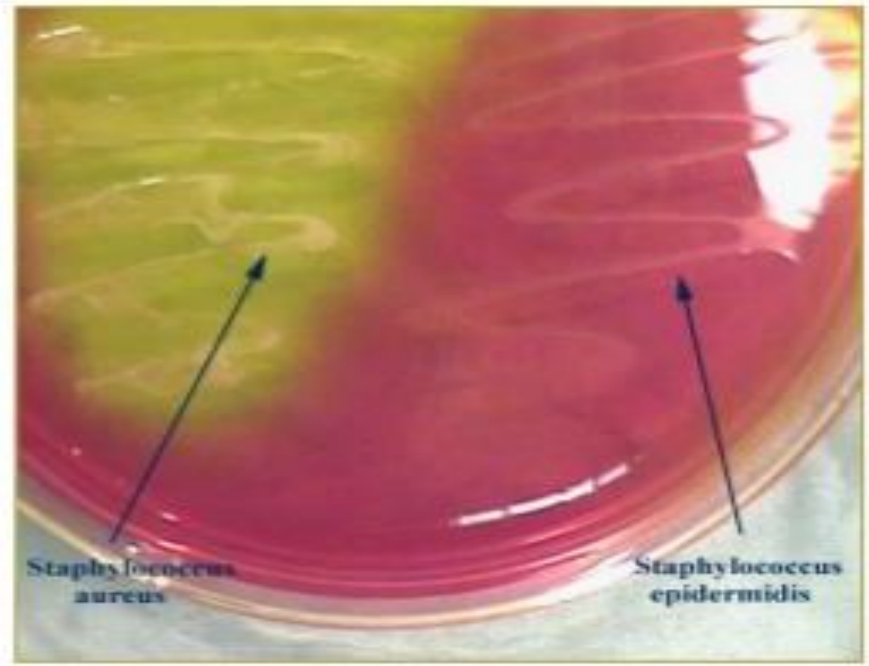
  - white-yellow colony

  -no haemolysis of red blood cells



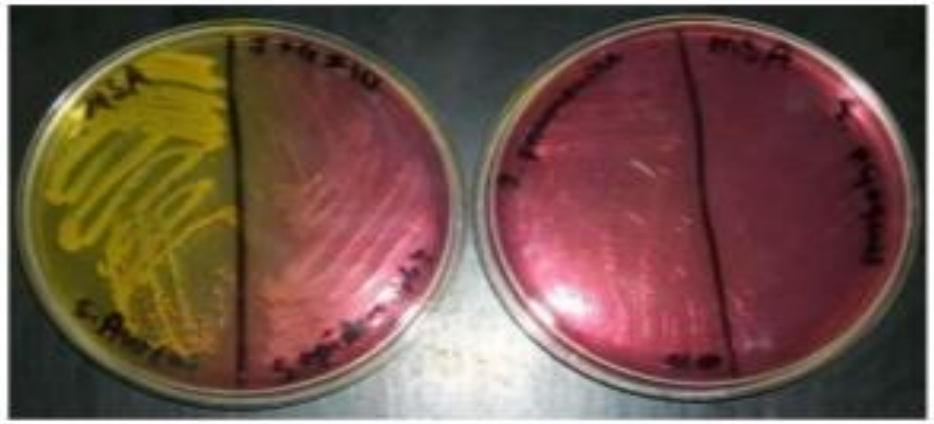
Culture (….contd)

- On Mannitol salt agar,
  - *S.aureus* ferments mannitol and appear as yellow colonies
  - MSA is a useful selective medium for recovering *S.aureus* from faecal specimens, when investigating food poisoning



Biochemical Properties

- Catalase positive; oxidase negative
- Ferment glucose, lactose, maltose, sucrose and mannitol, with production of acid but no gas
- Mannitol fermentation carries diagnosis significance



Biochemical Properties(....contd)

- Indole test= negative
- MR test= positive
- VP test= positive
- Urease test= positive
- Hydrolyse gelatin
- Reduces nitrate to nitrite
- Phospahatase= positive
- DNA-ase test= positive
- Coagulase test= positive

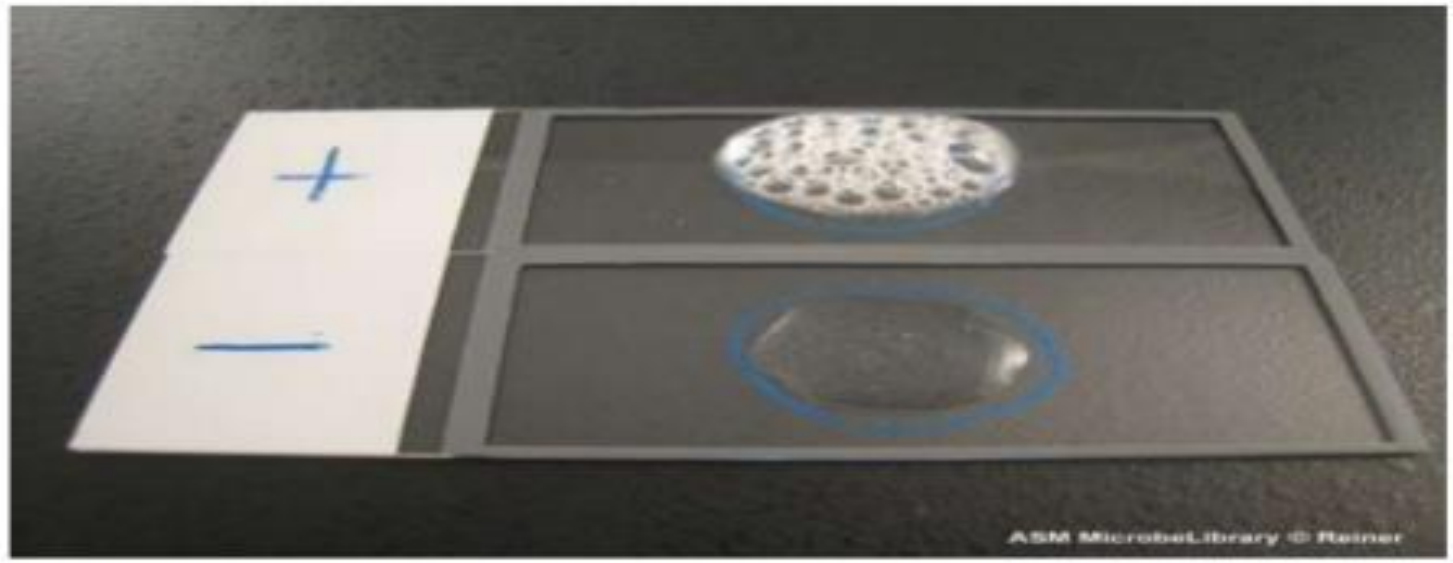
Slide test (clumping factor)

Tube test (free coagulase)

Biochemical tests

- **Catalase test:**

- Is used to differentiate between staphylococci (catalase +ve) and streptococci(catalase –ve).



- **Coagulase test**

is used to differentiate *Staphylococcus aureus* from coagulase-negative staphylococci.

fibrinogen —(coagulase)→ fibrin

(clot formation)

Staphylococcus aureus

Coagulase negative staphylococci, e.g. *S. epidermidis*

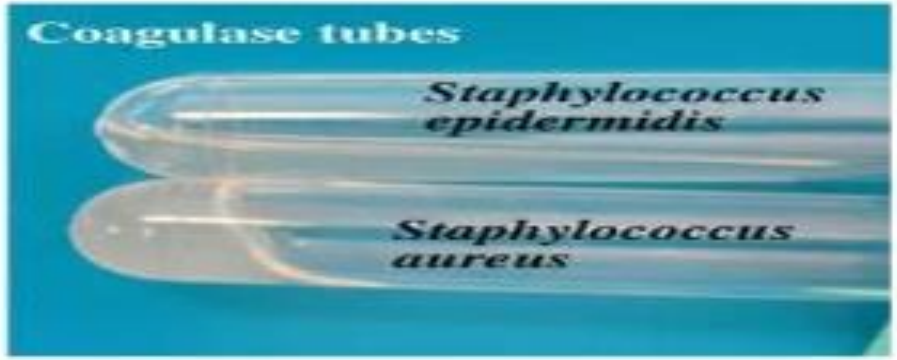
slide coagulase test

- Procedure
  - Place a drop of sterile water on a slide and emulsify a colony
  - Add a drop of rabbit plasma to the suspension
  - Observe
    - Agglutination = Positive (*S. aureus*)
    - No agglutination= Negative (other *staphylococcci)*

tube Coagulase Test

- Causes a clot to form when bacterial cells are incubated with plasma.

- **Procedure**
  - Inoculate rabbit plasma with organism and incubate at 35-37 ^{0} C
  - Observe at 30 minutes for the presence of a clot
  - Continue for up to 24 hours, if needed.



Biochemical characteristics of staphylococci (differential signs)

Properties	*S.aureus*	*S.epidermidis*	*S.saprophyticus*
ß-hemolytic activity	+	-	-
Nitrate reduction	+	+	-
Degradation of mannitol under anaerobic conditions	+	-	-
Degradation of mannitol under aerobic conditions	+	-	+
Coagulase	+	-	-
Hyaluronidase	+	±	-
Fibrinolysis	±	±	-
Alkaline phosphatase	+	+	-
DNA-aza	+	-	-

Staphylococcus aureus

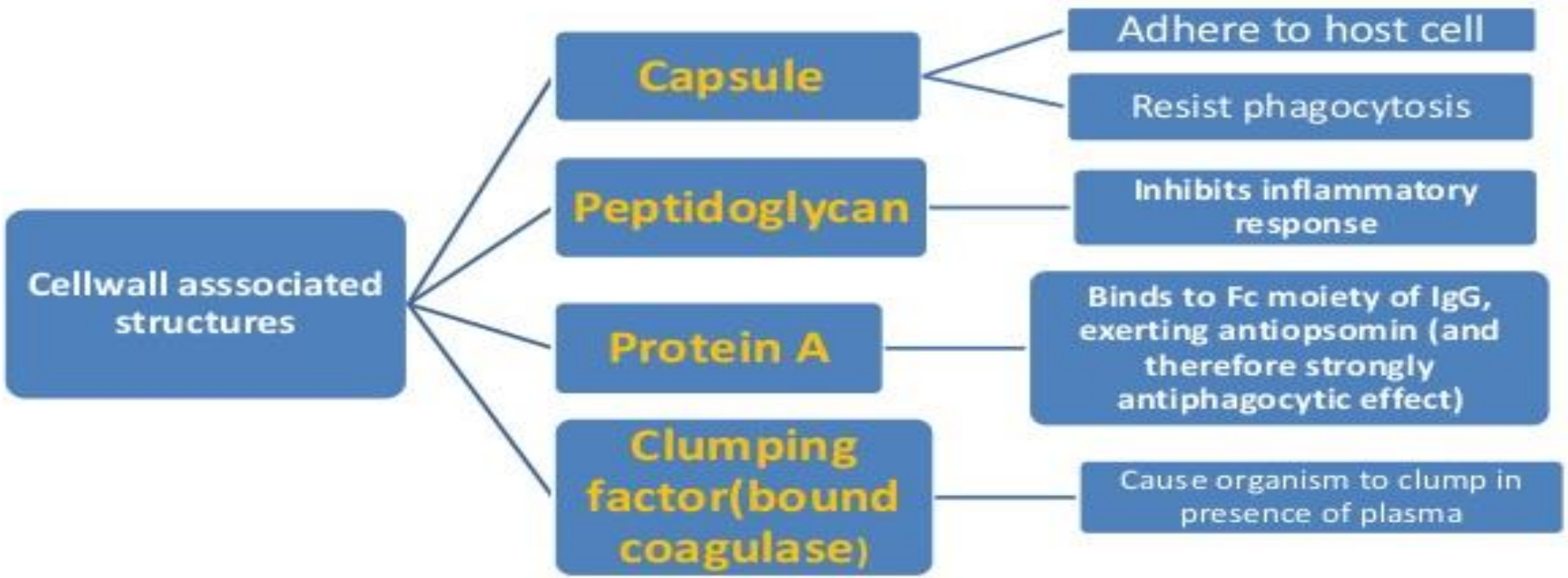
Antigenic structure

- Capsule
  - Prevents phagocytosis
  - Promotes adherence to cells of prosthetic devices
- Peptidoglycan
  - Acts as endotoxin
  - Chemotactant for neutrophils
  - Stimulates complement and coagulation
- Teichoic acid
  - Adherence to mucosal surface
- Protein A
  - Binds to Fc portion of IgG
  - Phagocytosis is reduced

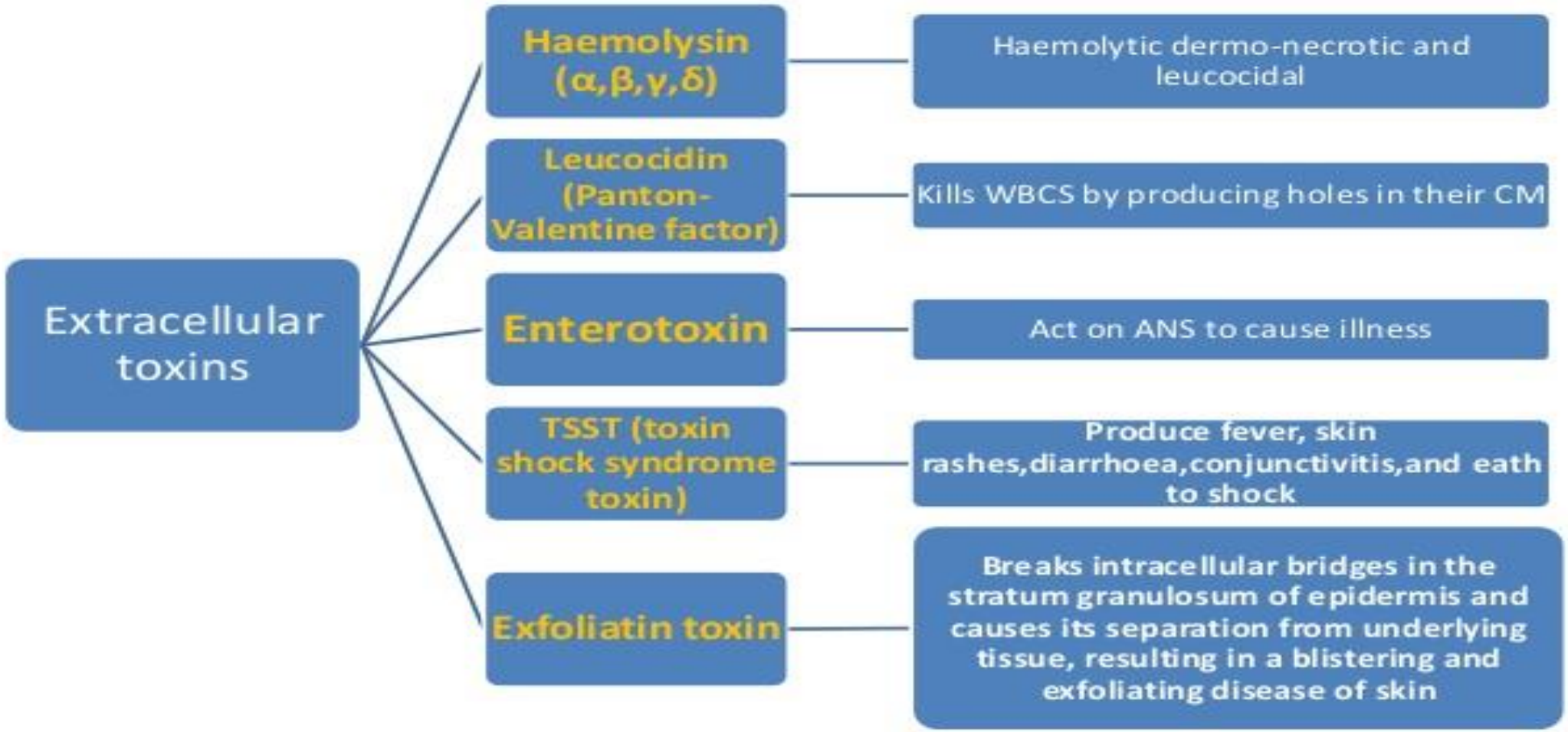
Virulence Factors

Cellwall associated structures	Extracellular toxins	Coagulase
• Peptidoglycan • Capsule • proteinA • Clumping factor (bound coagulase)	• Haemolysin • Leukocidin • Enterotoxin • TSST • Exfoliatin toxin	• staphylokinase • DNAase • Phosphatase • lipase • Phospholipase • hyaluronidase • serokinase • protease

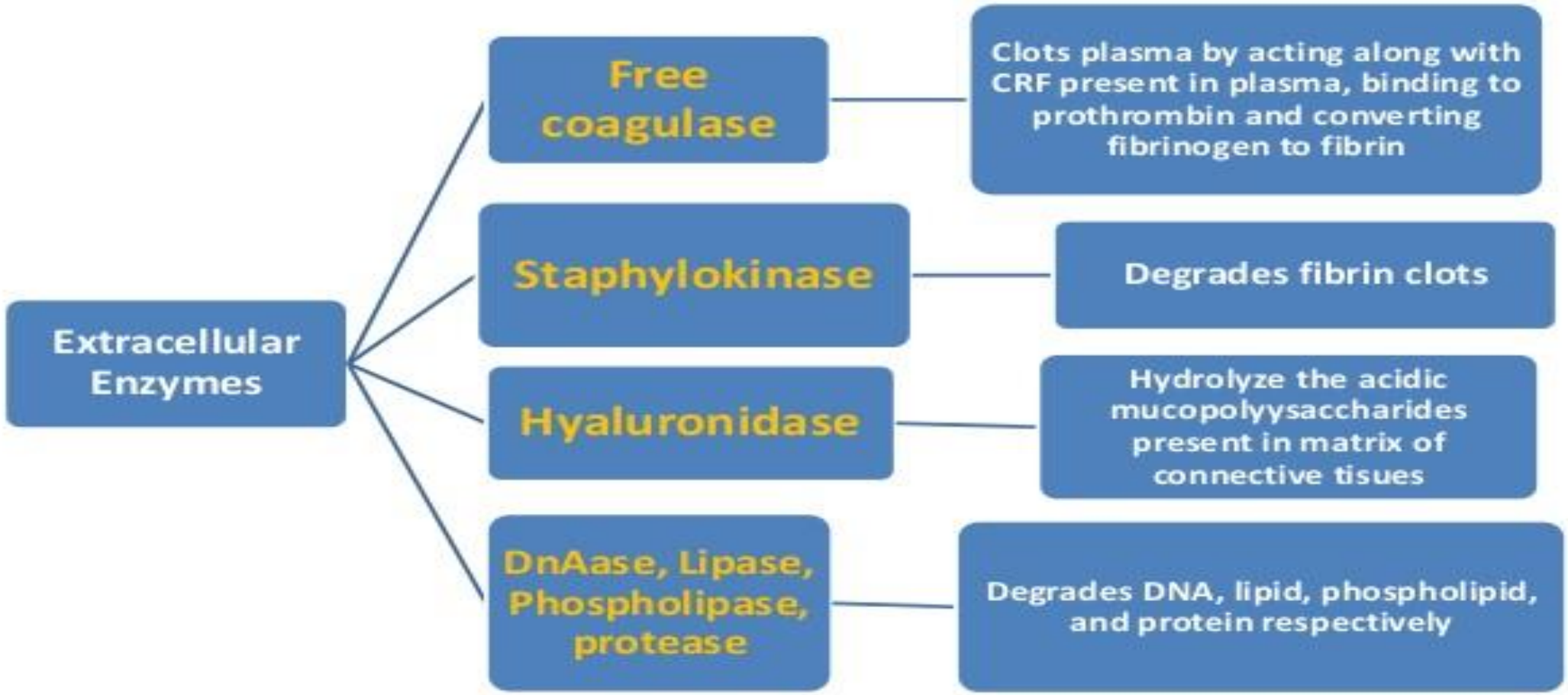
Virulence Factors(contd....)



Virulence Factors(contd....)



Virulence Factors(....contd)

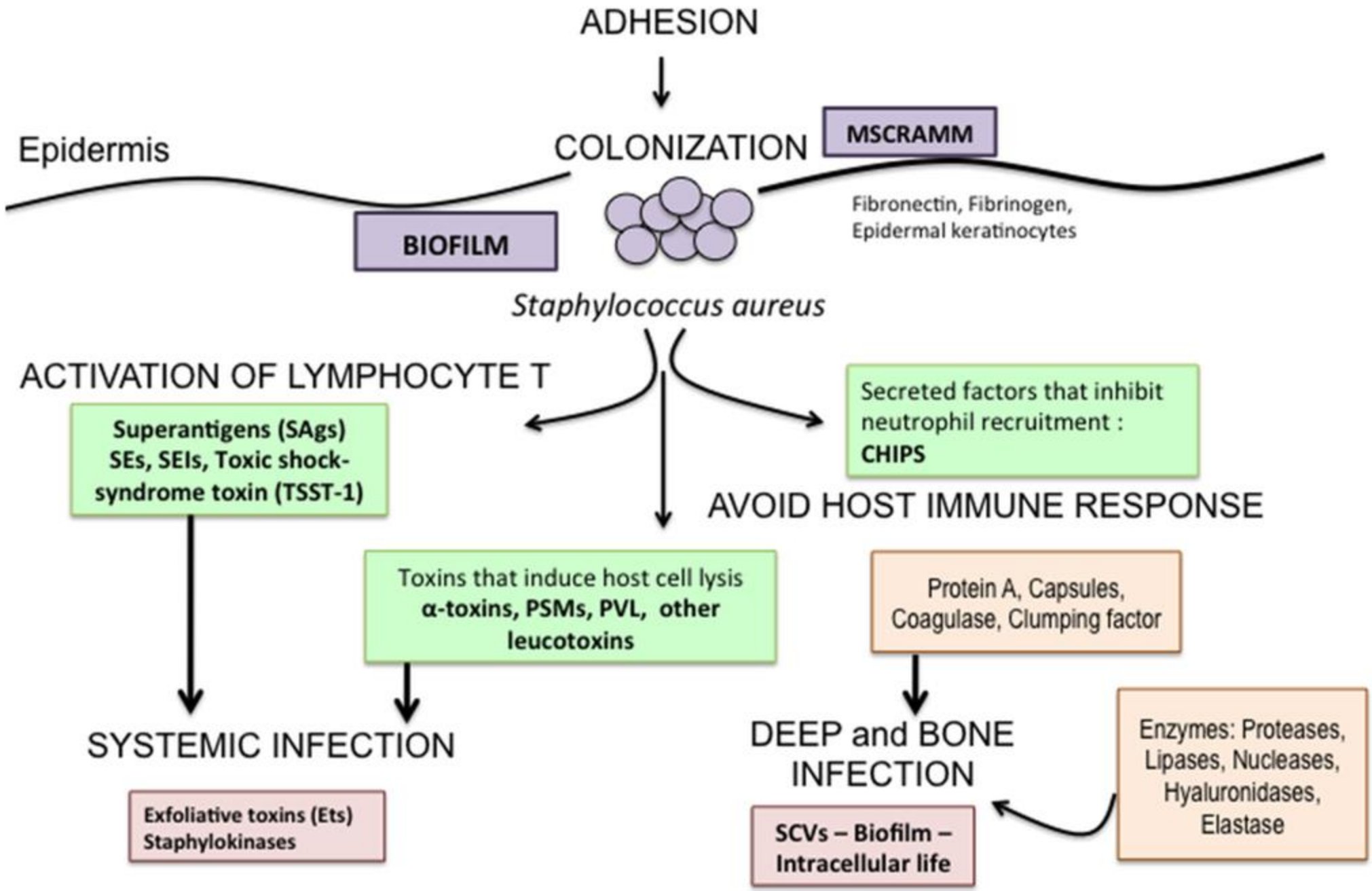


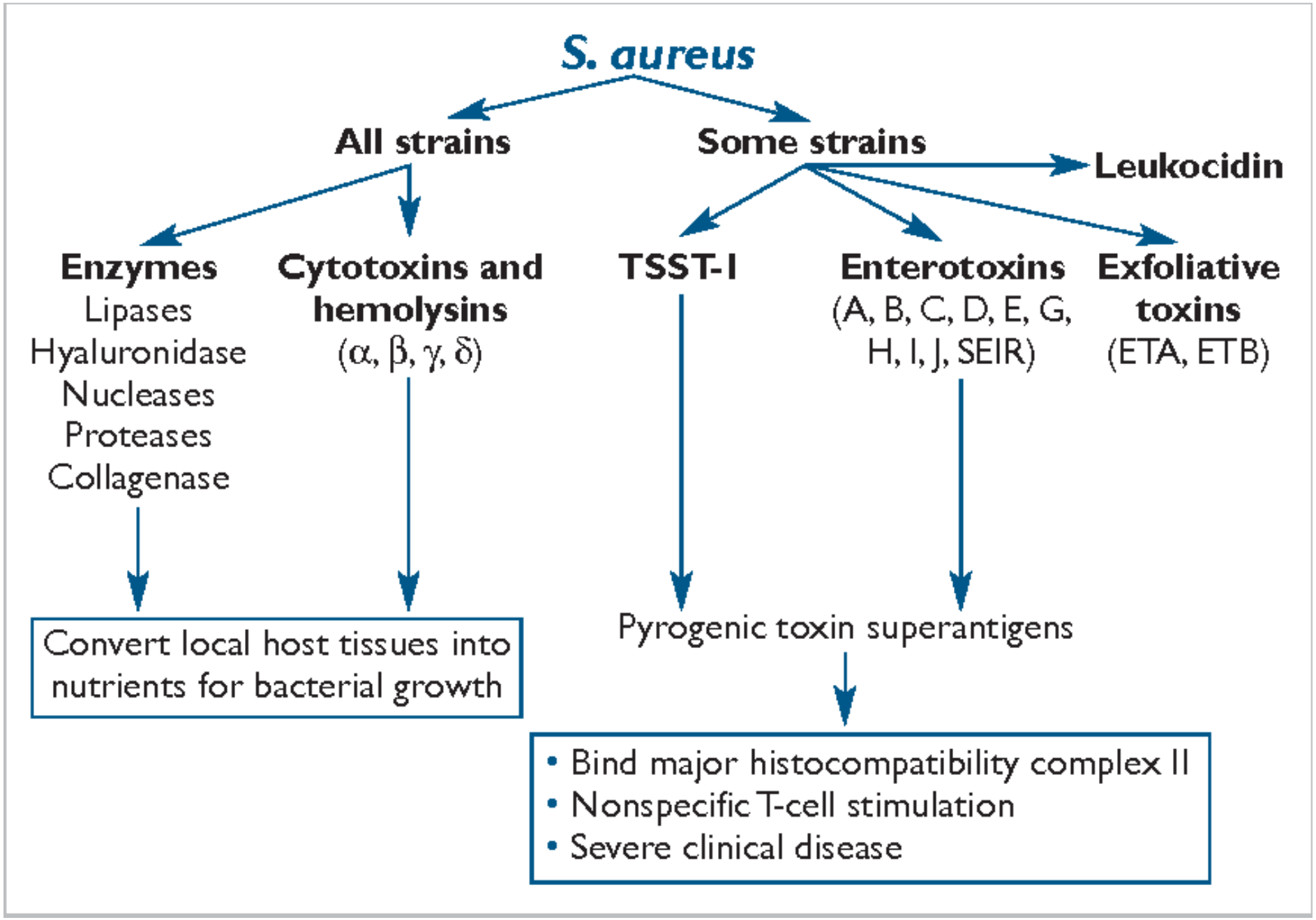
Resistance to external environmental factors

- Staphylococci are quite resistant to environmental factors. Direct sunlight destroys them in just a few hours. They are resistant to drying and heating - 20-30 minutes at 70-80ºC, during 10 minutes at 150ºC. they are destroyed. They are sensitive to the effects of antiseptic and disinfectant preparations. During 15-20 min in 3% phenol solution. they are destroyed.
- Staphylococci have a paradoxical sensitivity to aniline dyes - crystal violet (1:500000) and brilliant green (1:1000000). Brilliant green is used in the treatment of pyodermas.

Pathogenesis

- Adhere to damaged skin, mucosa or tissue surfaces
  - At these sites, they evade defence mechanisms of the host, colonize and cause tissue damage
- *S.aureus* produces disease by
  - Multiplying in tissues
  - Liberating toxins,
  - Stimulating inflammation


ADHESION
COLONIZATION
MSCRAMM
Epidermis
BIOFILM
Fibronectin, Fibrinogen,
Epidermal keratinocytes
Staphylococcus aureus
ACTIVATION OF LYMPHOCYTE T
Superantigens (SAgs)
SEs, SEIs, Toxic shock-syndrome toxin (TSST-1)
Secreted factors that inhibit neutrophil recruitment :
CHIPS
AVOID HOST IMMUNE RESPONSE
Toxins that induce host cell lysis
α-toxins, PSMs, PVL, other leucotoxins
Protein A, Capsules,
Coagulase, Clumping factor
SYSTEMIC INFECTION
Exfoliative toxins (Ets)
Staphylokinases
DEEP and BONE INFECTION
SCVs – Biofilm – Intracellular life
Enzymes: Proteases, Lipases, Nucleases, Hyaluronidases, Elastase


S. aureus
All strains
Some strains
Leukocidin
Enzymes
Lipases
Hyaluronidase
Nucleases
Proteases
Collagenase
Cytotoxins and hemolysins
(α, β, γ, δ)
TSST-1
Enterotoxins
(A, B, C, D, E, G, H, I, J, SEIR)
Exfoliative toxins
(ETA, ETB)
Convert local host tissues into nutrients for bacterial growth
Pyrogenic toxin superantigens
• Bind major histocompatibility complex II
• Nonspecific T-cell stimulation
• Severe clinical disease

PATHOGENICITY

Source of infection:

A) Exogenous: patients or carriers

B) Endogenous: From colonized site

Mode of transmission:

A) Contact: direct or indirect(through fomites)

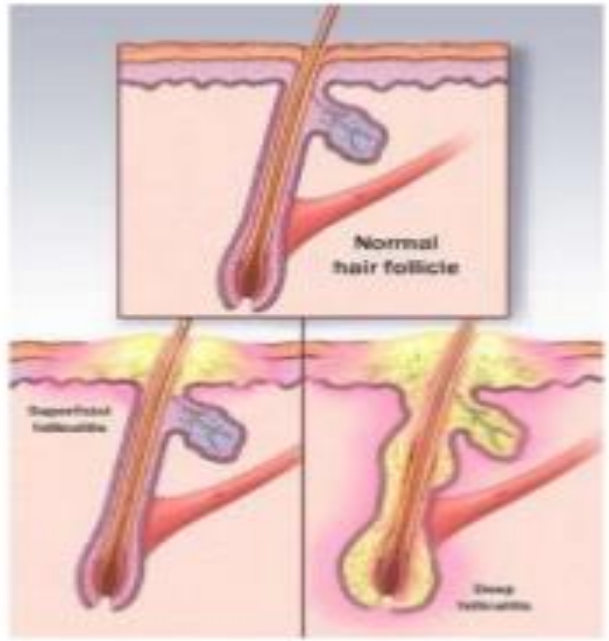
B) Inhalation of infected air borne droplets

Clinical Syndromes

1. **Cutaneous infections**
   - Folliculitis
   - Boils/furuncles
   - Carbuncle
   - Impetigo
   - Wound infections
2. **Deep infections**
   - Osteomyelitis
   - Periostitis
   - endocarditis
3. **Exfoliative diseases**
4. **Toxin shock syndrome**
5. **Staphylococcal food intoxication**

1) Cutaneous Infections

- **Folliculitis**: It is inflammation of the hair follicles.
- A small red bump or pimple develops at infection sites of hair follicle.



•**Sty**: A sty is folliculitis affecting one or more hair follicles on the edge of the upper or lower eyelid.

Cutaneous Infections(contd....)

- **Furuncle/boils**: Furuncle is deep seated infection, originating from folliculitis,(if infection extends from follicle to neighbour tissue)
- Causes redness, swelling, severe pain
- Commonly found on the neck, armpit and groin regions

- **Carbuncle**: Carbuncle is an aggregation of infected furuncles. Carbuncles may form large abscesses.
- It is a large area of redness, swelling and pain, punctuated by several sites of drainage pus.

Cutaneous Infections(contd....)

- Impetigo: a very superficial skin infection common in children, usually produces blisters or sores on the face, neck, hands, and diaper area.
- It is characterized by watery bristles, which become pustules and then honey coloured crust

impetigo with vesicles, pustules, and sharply demarcated regions of honey-colored crusts.

2) Deep Infections

- **Osteomyelitis**: inflammation of bone
- Bacteria can get to the bone
  - Via bloodstream
  - Following an injury

  Clinical features: pain, swelling, deformity, defective healing, in some case pus flow,

  Diagnosis: X-ray, MRI, bone aspirates

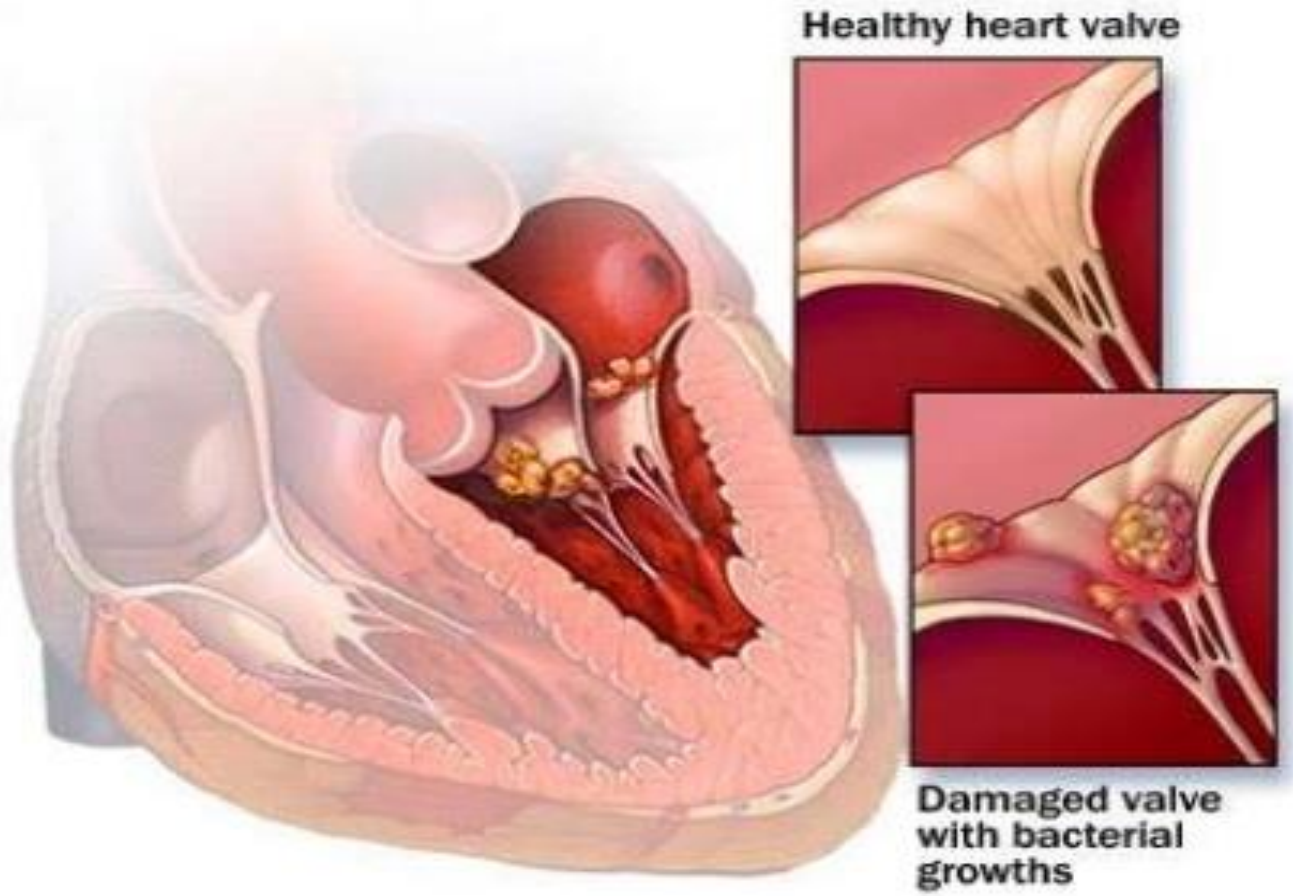
Deep Infections(contd....)

- **Periostitis**: inflammation of periosteum
- Clinical features: fever, localised pain, leucocytosis
- Diagnosis: needle aspiration of subperiosteal fluid

Deep Infections(....contd)

- **Endocarditis**: It is an inflammation of the inner layer of the heart, the endocardium

- Endocarditis occurs when bacteria enter bloodstream, travel to heart, and lodge on abnormal heart valves or damaged heart tissue.



3)Exfoliative Disease

- (Exfoliate= scaling off tissues in layers)
- Also known as **'Staphylococcal skin scalded syndrome'**

- previously called dermatitis exfoliativa, pemphigus neonatorum, Lyell's disease and Ritter's disease

- Epidermal toxin produced by *S.aureus* at skin and is carried by bloodstream to epidermis , where it causes a split in a cellular layer i.e., this toxin separates outer layer of epidermis from underlying tissue

4) Toxic Shock Syndrome

- Caused when Toxin shock syndrome toxin (TSST) liberated by S.aureus enters bloodstream
- It is a multisystem illness, characterized by:

High Fever

Headache

Vomiting

Diarrhoea

Conjunctival reddening

Hypotension

Skin rashes

Kidney failure

5) Staphylococcal Food Poisoning

- Caused when consuming food in which S.aureus has multiplied and formed endotoxin
- Symptoms:
  - Nausea
  - Vomiting
  - Severe abdominal cramp
  - Diarrhoea
  - Sweating
  - Headache,etc.

- **Respiratory**
  - Tonsilitis
  - Pharyngitis
  - Sinusitis
  - Otitis
  - Bronchopneumonia
  - Lung abscess
  - empyema

- **Endovascular**
  - Bacteremia
  - Septicemia
  - Pyemia
  - Endocarditis

- **Urinary**
  - Urinary tract infection

- **Central nervous system**
  - Abscess
  - Meningitis
  - Intracranial thrombophlebitis

LABORATORY DIAGNOSIS:

Specimen collection

- **Pus** from pyogenic lesions.
- **blood** from septicaemia.
- **Cerebrospinal fluid** from meningitis.
- **sputum** from respiratory infection
- suspected **food, vomit or faeces** from food poisoning.
- Mid-stream **urine** in urinary tract infection.
- Anterior **nasal swab** from suspected carriers.

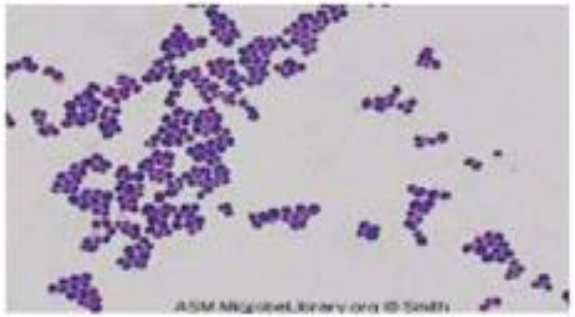
Laboratory diagnosis

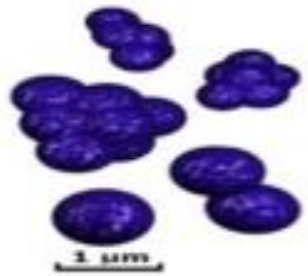
microscopic examination

1-Gram stain → gram positive.

2-Morphology → cocci (spherical).

3-Arrengment → single cell or pairs or in short chain but appear predominantly in grape-like clusters.





Laboratory Diagnosis (contd....)

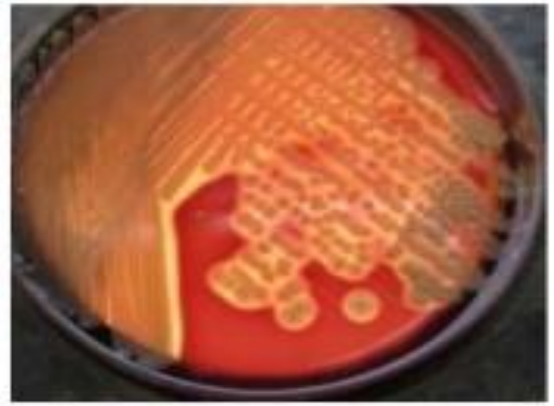
B. Bacteriological Investigation:

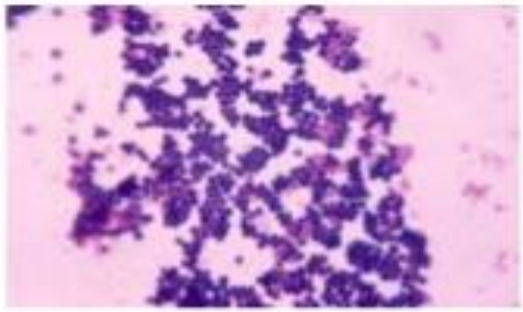
- Specimens:
  - Pus: from wound or abscess or burns]
  - Nasal Swab: from suspected carrier
  - Food: to diagnose staphylococcal intoxication
  - Blood: to diagnose endocarditis and bacteremia
  - Sputum: to diagnose lower respiratory tract infection

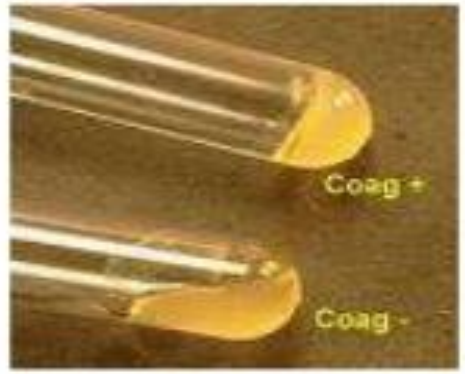
Laboratory Diagnosis (contd....)

- **Culture and isolation:**
  - Specimens are cultured on BA plate and are incubated @ 37 °C for 24 hours
  - After incubation, BA plate is observed for significant bacterial growth (> 2mm in diameter)
  - Then, Gram-staining is performed of the isolated organisms
  - Then, subcultured on NA plate for further biochemical tests

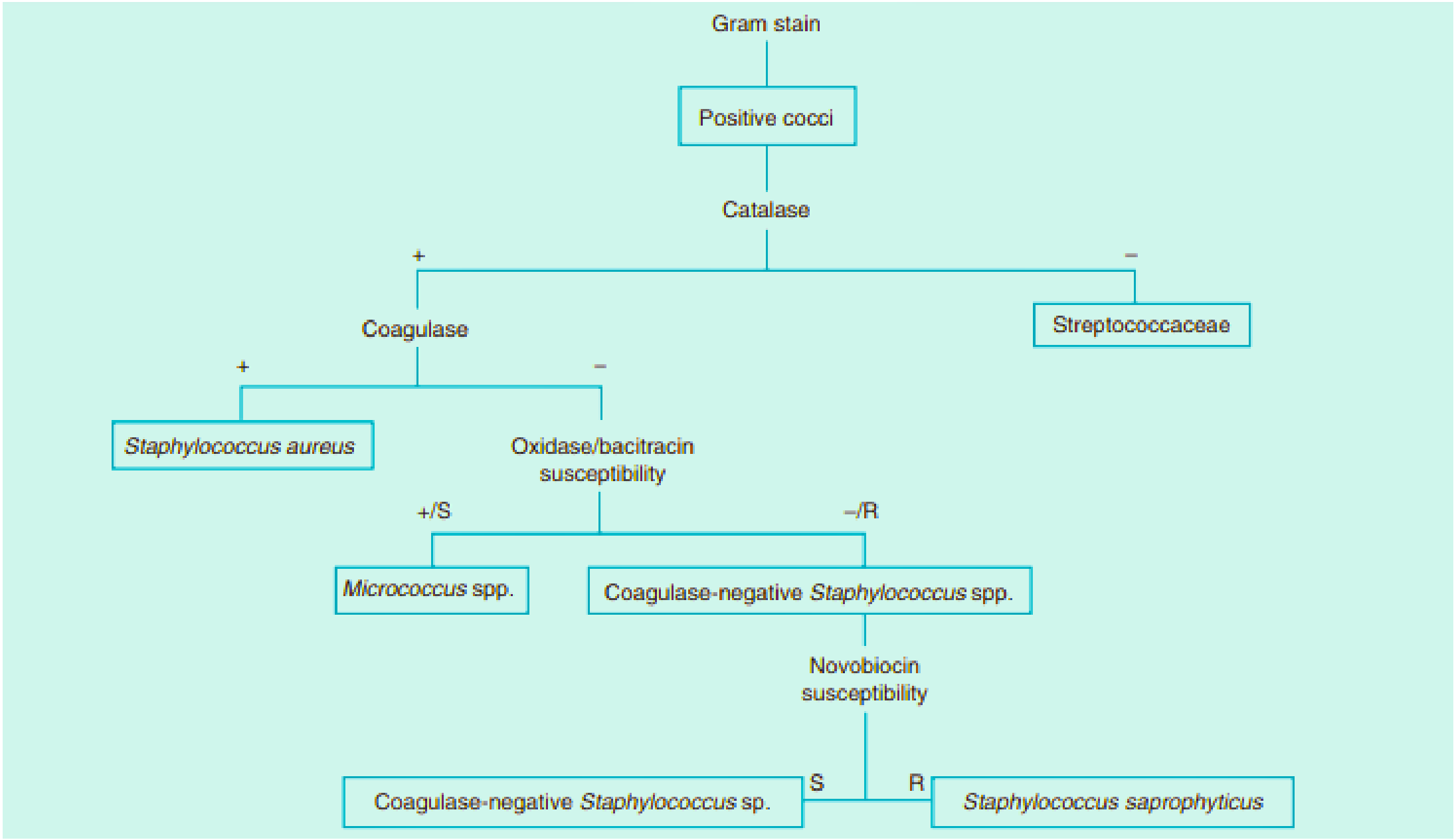
- **Tube coagulase test:**
  - i. Mix 0.5ml of human plasma with 0.1ml of an overnight broth culture of S.aures
  - ii. Incubate the mix in a water bath @ 37°C for 3-6 hours
  - Result: plasma clots and doesn't flow if the tube is inverted



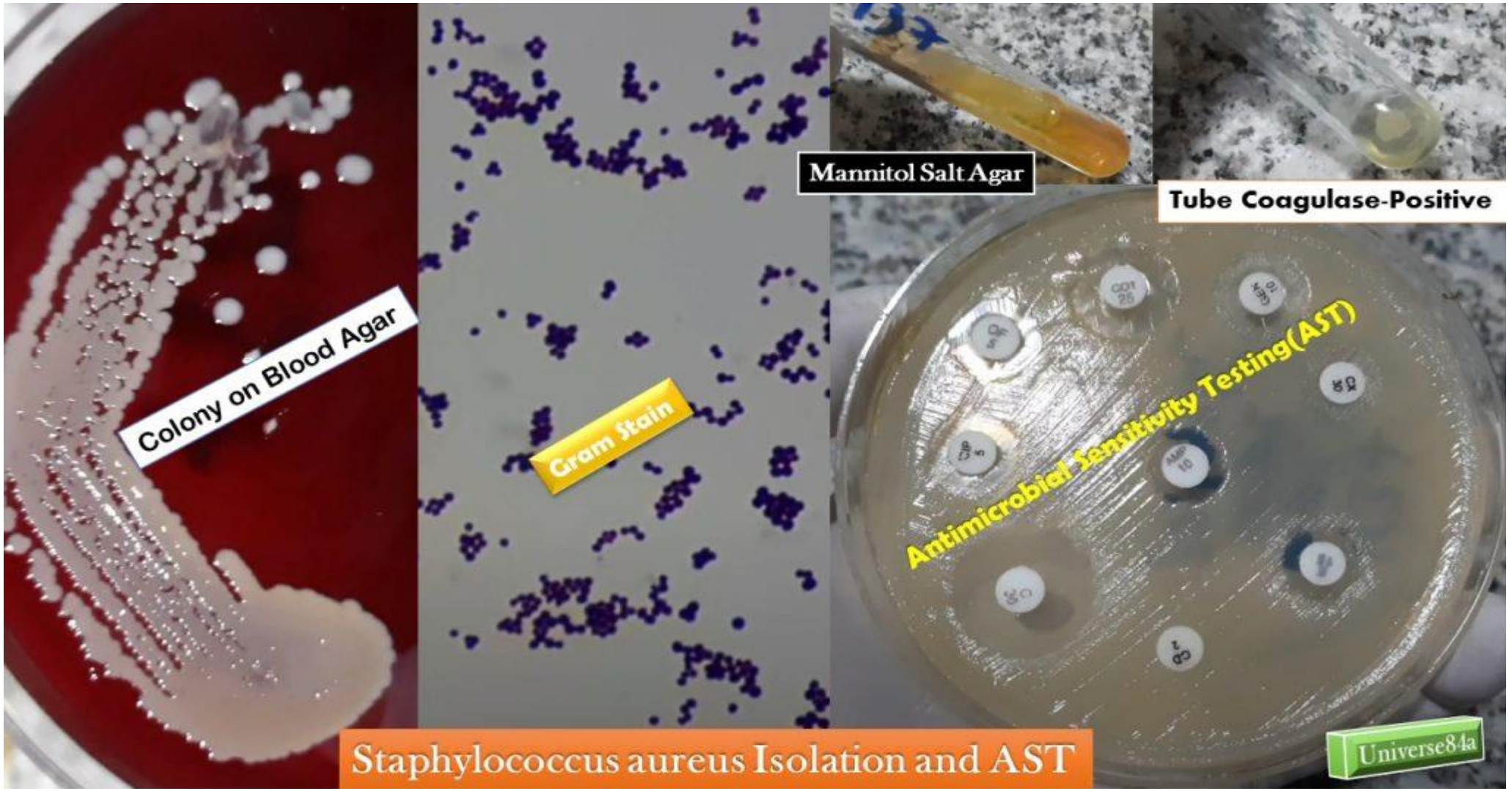




IDENTIFICATION ALGORITHM OF STAPHILOCOCCI



Microbiological diagnosis



Automated identification and antibiotic susceptibility systems:

- **Rapidec Staph (Bio-Merieux-Vitek)** is a kit used to differentiate S.aureus, S.epidermidis and S.saprophyticus and gives results within 2 hours and consists of 4 domes.
- **API STAPH-IDENT** is a kit for the identification of 17 types and subspecies of staphylococci with some additional tests that allow the study of 10 biochemical tests.
- **API STAPH (Biomeriuex-Vitek)** is a strip that gives results within 18-24 hours for the identification of micrococci and staphylococci.ID 32 STAPH (Bio Merieux) – with this strip consisting of 32 domes, identification of micrococci is carried out for 24 hours.
- **MINITEK GRAM POSITIVE PANEL (BD Microbiology System)** is a system consisting of dry discs impregnated with various test substrates, used for the identification of micrococci, staphylococci and streptococci.

Biomerieux VITEK-2 - bacteriological analyzer

- Vitek-2 Compact analyzer is an automatic system
- Microorganisms are being identified
- Sensitivity to antimicrobial drugs is determined (within 1 day)
- It consists of plastic cards with 64 depressions
- The time to get the result is 6-8 hours

Biomerieux VİTEK-2

Species identification (ID) cards

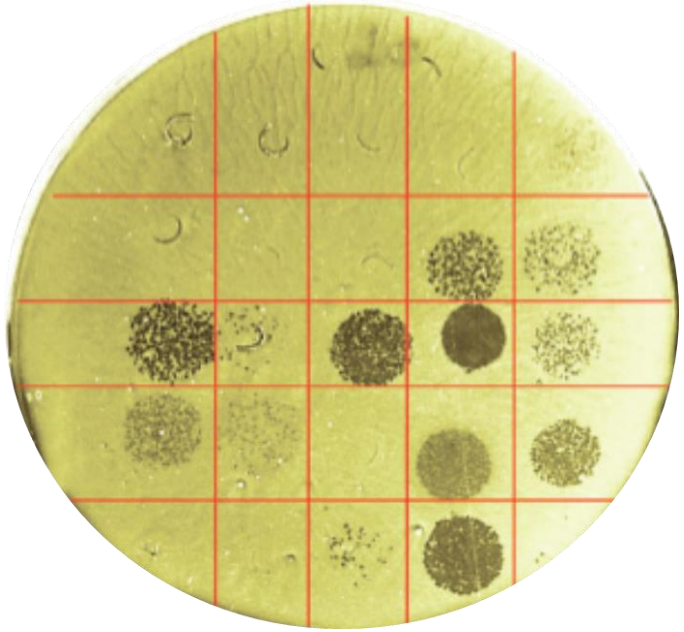
Antimicrobial susceptibility test - AST (strips)

Bacteriophage typing

- *S.aureus* shows specific sensitivity to a group of bacteriophages, using which it is possible to type them.
- Typing of *S.aureus* with bacteriophages is important in terms of monitoring the epidemiology of nosocomial infections.

Standard staphylococcal phages and the most common S. aureus phagotypes and models

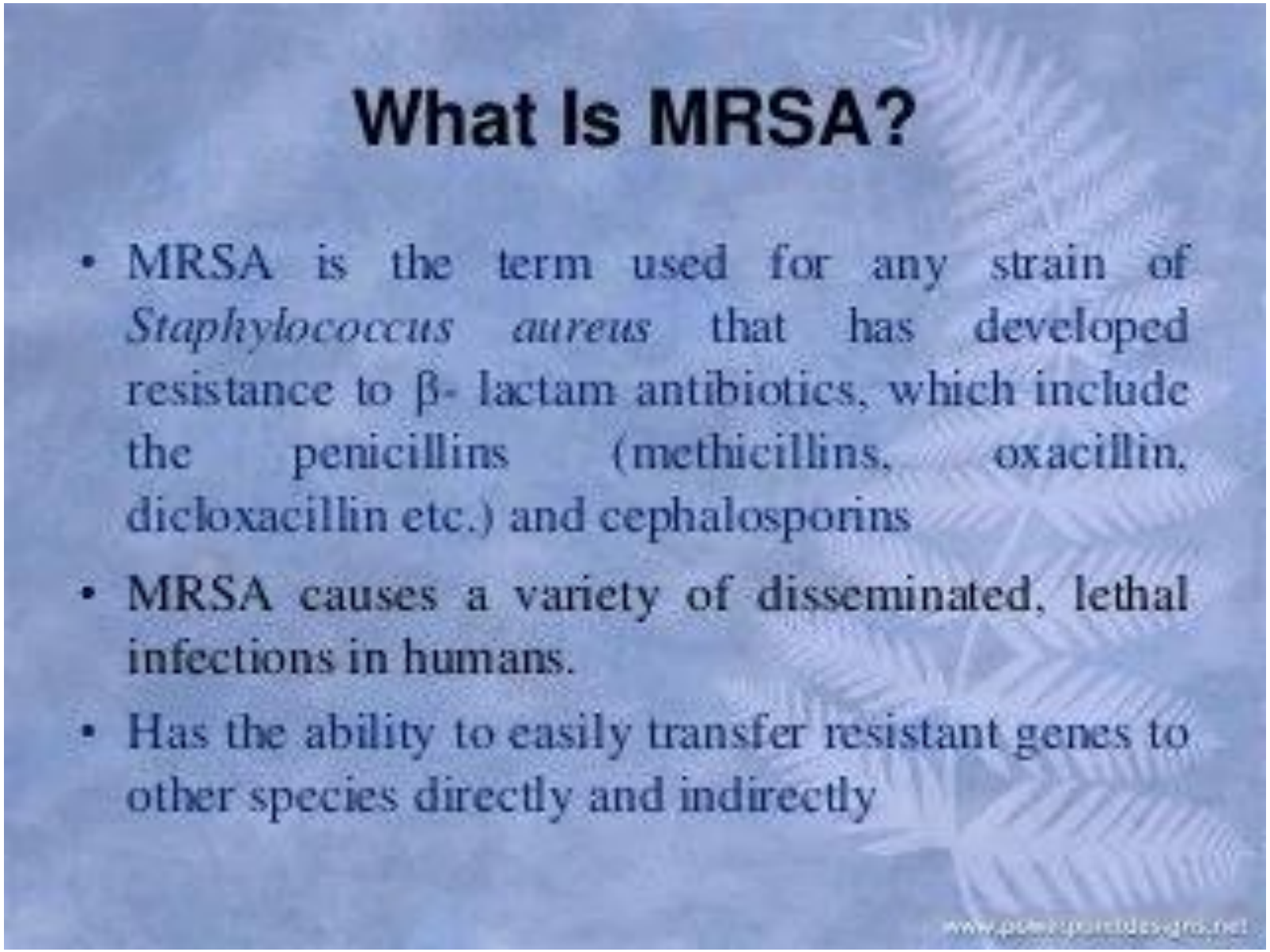
Phage group	Phages	Phagotypes
I	29, 52, 52A,79, 80	29, 52/52A, 52/52A/80/81, 80
II	3A, 3B, 3C, 55, 71	3A/3B/3C, 3C/55
III	6, 7, 42E, 47, 53, 54, 75, 77, 83A, 84, 85	6/7/47/53/75/77
IV	42D	
Unclassified	81, 87, 93, 94, 96, 187	



Treatment and prevention:

- In specific treatment and prevention, **staphylococcal anatoxin** and **antistaphylococcal immunoglobulin** are used.

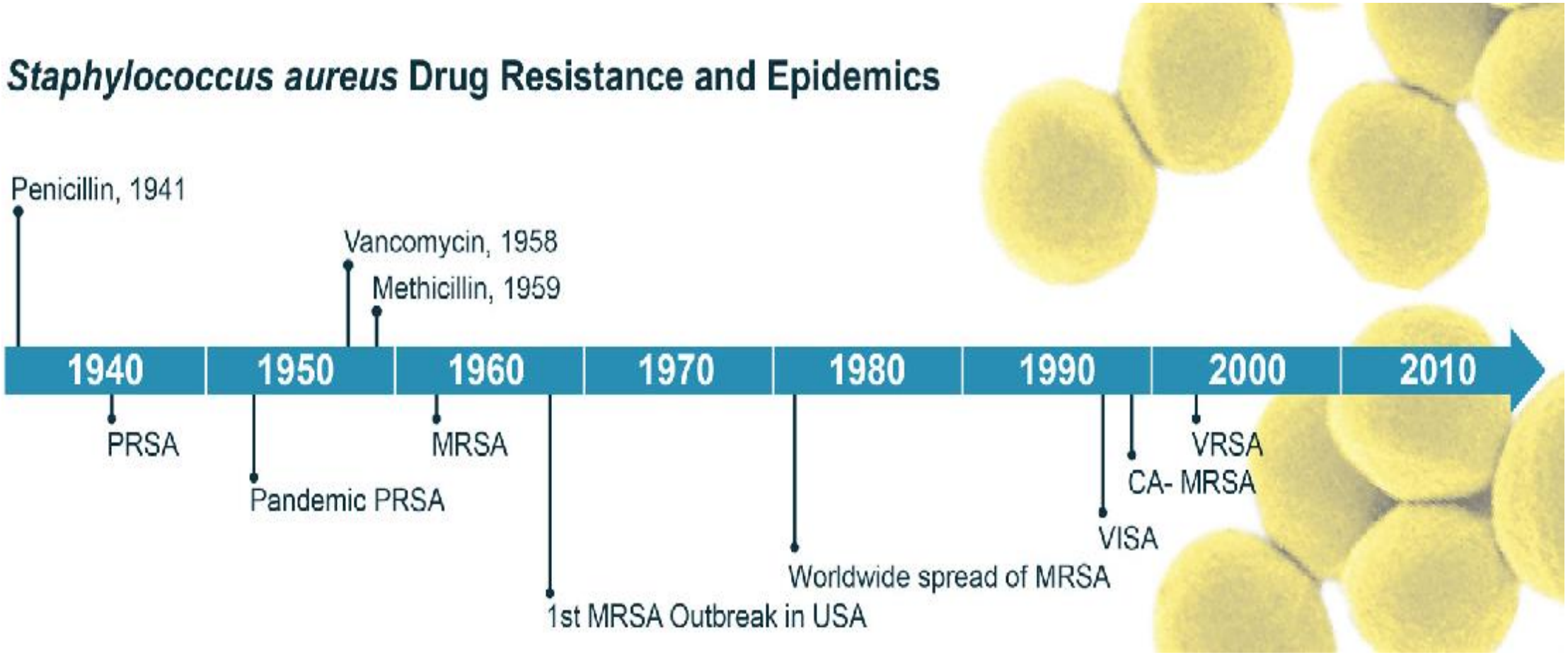
Methicillin-resistant Staphylococcus aureus (MRSA),



MRSA - methicillin-resistant Staphylococcus aureus

- MRSA stands for methicillin-resistant Staphylococcus aureus, a type of bacteria resistant to several antibiotics.
- In hospitals, MRSA can cause serious problems (bloodstream infections (sepsis, bacteremia), pneumonia, surgical infections).
- Methicillin resistance of S. aureus strains is associated with gene mutations in them, which encode the synthesis of penicillin-binding proteins (PBPs) that cannot combine with beta-lactam antibiotics. For this reason, methicillin-resistant S. aureus (MRSA) strains are resistant to all beta-lactam antibiotics.
- This resistance is related to the Mec A gene.

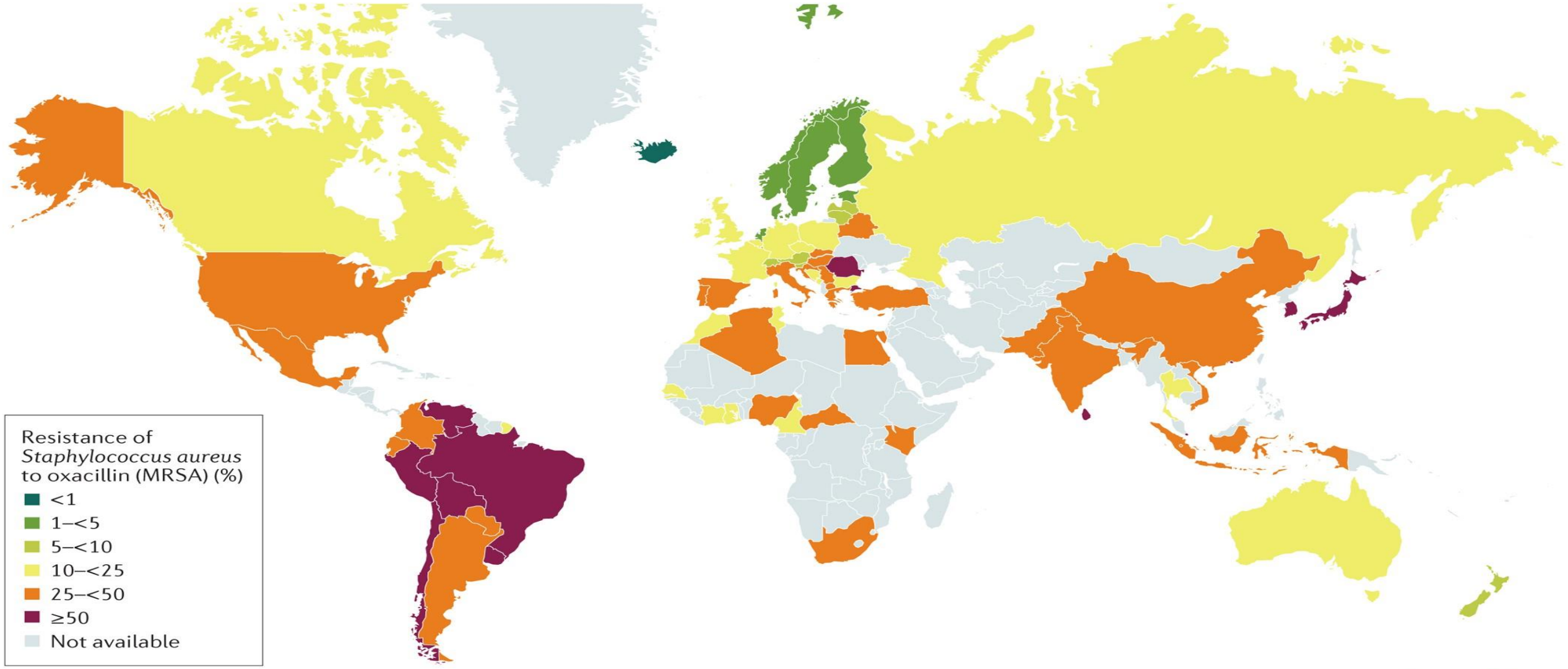
Staphylococcus aureus – drug resistance



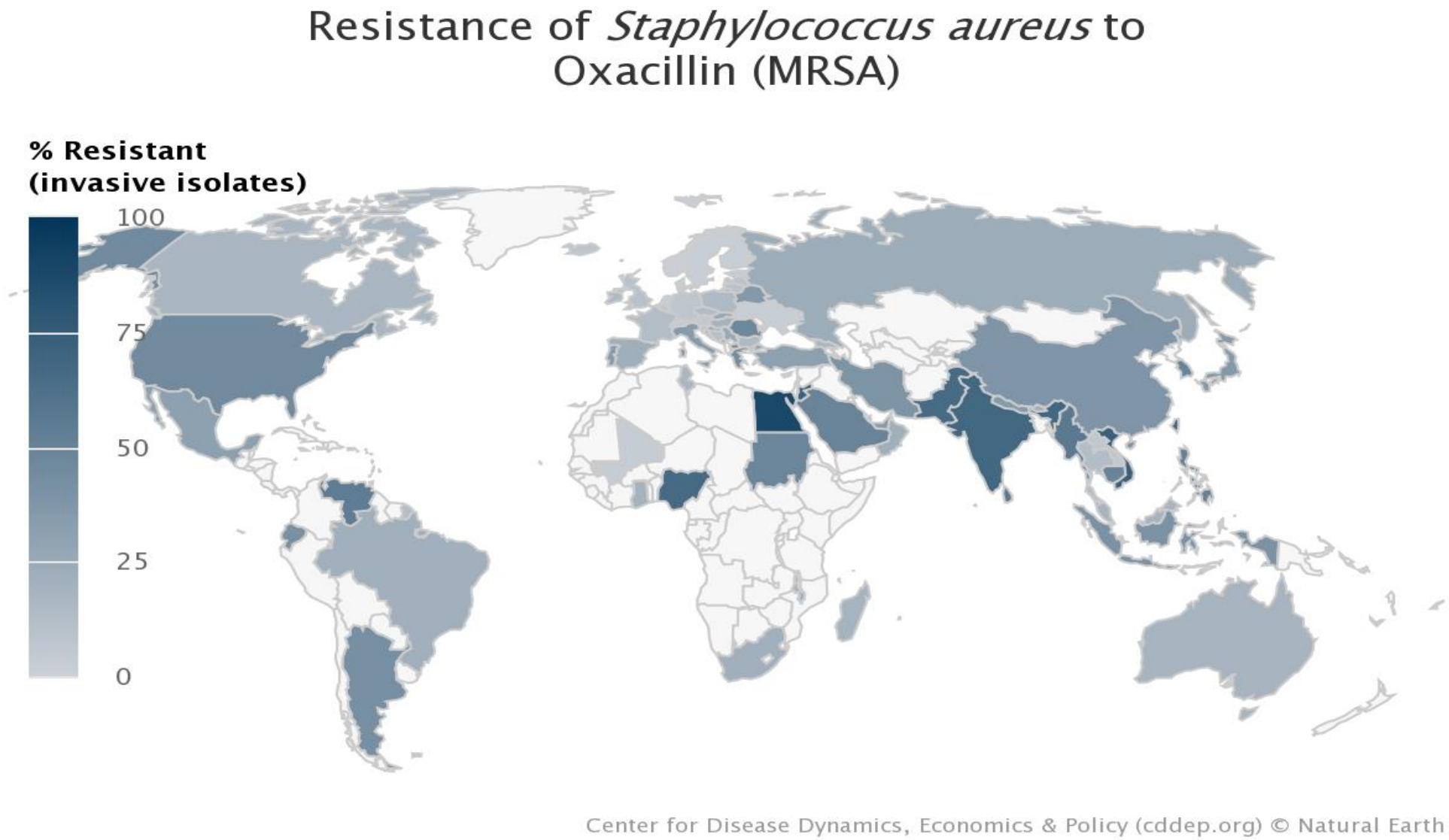
Drug resistance in staphylococci

- In methicillin-resistant staphylococcal infections (usually severe infections), glycopeptide antibiotics (vancomycin, teicoplanin) are mainly used.
- If sensitivity to quinolone, co-trimoxazole, gentamicin, etc. is found in other infections, it may be preferred.

Methicilllin-resistant *Staphylococcus aureus* (MRSA) – **epidemiology (2018)**



Methicilllin-resistant *Staphylococcus aureus* (MRSA) – **epidemiology (2022)**



Wound infections caused by the MRSA strain

Other forms of resistance in Staphylococci

- In recent years, glycopeptide-resistant *S.aureus* strains have also been reported.
- *S.aureus* can normally be found on the skin and in the nose.
- Nasal carriage of *S.aureus* can cause recurrent skin infections, and the surgical site poses a risk for infections.

STREPTOCOCCUS

Streptococcaceae (taxonomy)

- (Domain): Bacteriae
- (Kingdom): Bacillota
- (Class): Bacilli
- (Order): Lactobacillales
- (Family): Streptococcaceae
- (Genus): Streptococcus

- (Genus): Streptococcus
  - Floricoccus
  - Lactococcus
  - Lactovum
  - Okadaella
  - **Streptococcus**
    - (Species):

      S.pygenes,

      S.pneumoniae,

      S.viridans,

      S.agalactiae etc.

Classification of Streptococci

- Brown`s classification
- Lancefield grouping
- Griffith typing

Streptococcus

α-hemolytic
green,
partial hemolysis

- **pneumoniae**
  optochin sensitive,
  bile solube,
  capsule –>
  quellung +
- **Viridans**
  mutans, sanguis
  optochin resistent,
  not bile soluble,
  no capsule

β-hemolytic
clear,
complete hemolysis

- **pyogenes**
  Group A,
  badtradn sensitive
- **agalactiae**
  Group B,
  badtradn resistant

γ-hemolytic
no hemolysis

- **Enterococcus**
  E. faecalis,
  E. faecium

Classification of streptococci

- Classification based on antigen structure (Lensfield classification):
- according to polysaccharide C antigen in the cell wall of aerobic streptococci is divided 20 serogroups - A, B, C, D, E, F, G, H, K, L, M, N, O, P, Q, R, S, T, U,V.
- serogroup A beta-hemolytic streptococci - *Streptococcus pyogenes* are more important in human pathology.

Classification of streptococci

- **Alpha-hemolytic**
- Pneumococci
  - Viridans group: alpha-hemolytic

- **Beta-hemolytic**
  - Group A
  - Group B
  - Group C
  - Group D (enterococci)
  - Group F **streptococci**
  - Group G **streptococci**
  - Group H **streptococci**

Overview of the Medically Important Gram Positive Cocci

Family, Genus, species	Characteristics	Clinical manifestations
Staphylococcaceae	Cocci in cluster; catalase-positive	
Staphylococcus aureus	Coagulase +ve, yellow-pigmented colonies	Pyogenic infections, toxicoses
S. epidermidis	Coagulase -ve, whitish colonies, normal flora	Foreign body infections
Streptococcaceae	**Cocci in chains and in pairs, catalase-negative**	
Streptococcus pyogenes	Cocci in chains, Lancefield group A, β - hemolysis	Tonsillitis, scarlet fever, skin infections
S. pneumoniae	Diplococci, α-hemolysis	Pneumonia, otitis media, sinusitis
S. agalactiae	Chain-forming cocci, group antigen B, β-hemolysis	Meningitis/sepsis in neonates
S. viridans	Cocci in chains, α-hemolysis	Endocarditis, dental caries
Enterococcaceae	In chains & pairs, α, β, or γ-hemolysis, group antigen D, catalase -ve	Flora of intestines of humans and animals
Enterococcus faecalis Enterococcus faecium	Aesculin-positive, growth in 6.5% NaCl, pH 9.6	Opportunistic infections

Group A betahemolytic Streptococci

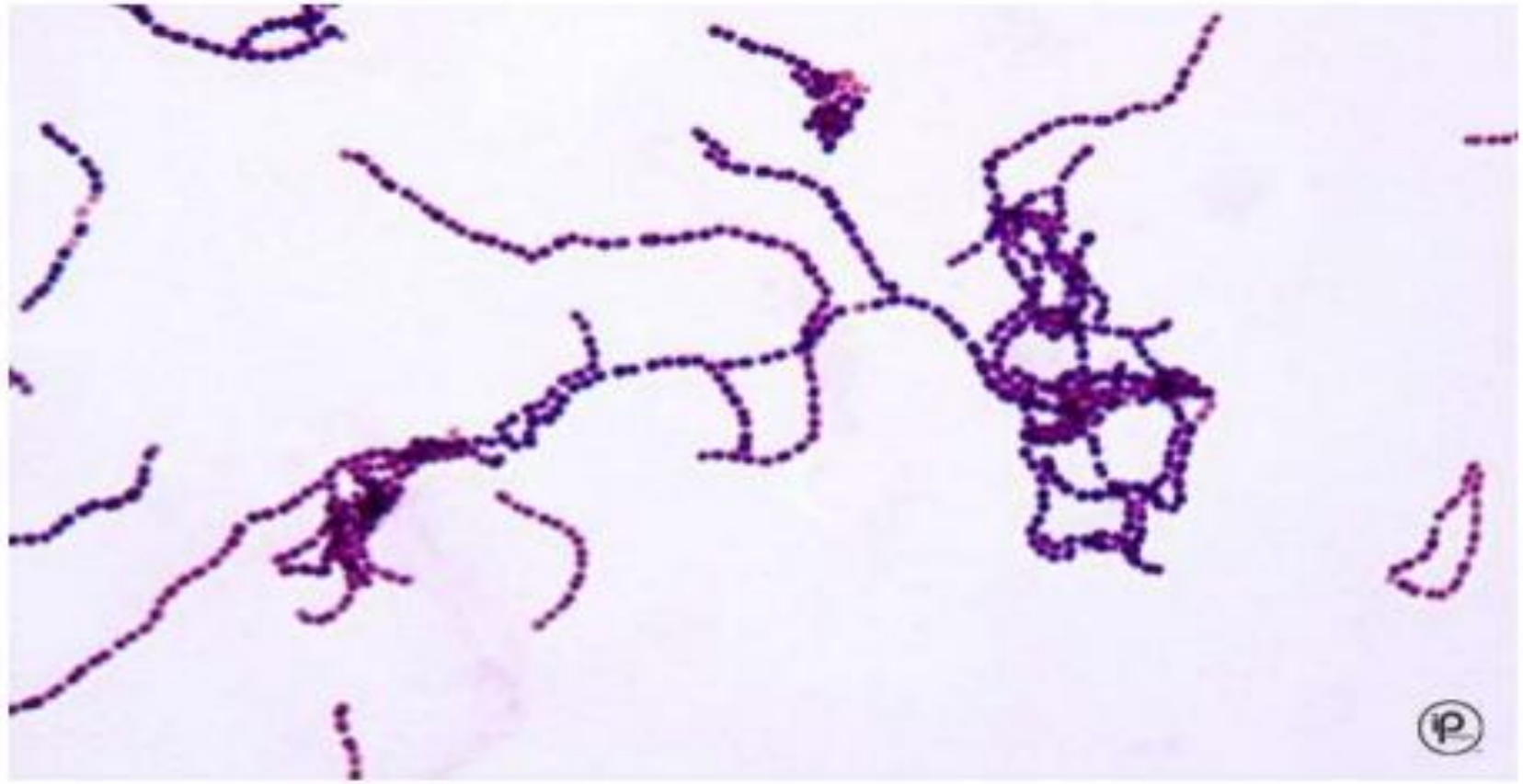
Streptococcus pyogenes

Streptococcus pyogenes

Morphology

- Ovoid to spherical in shape
- Gram-positive cocci arranged in chains or pairs
- Chain formation is due to the cocci dividing in one plane only and the daughter cells failing to separate completely
- Chains are longer in liquid than in solid media
- Non motile and non-sporing
- Capsulated (hyaluronic acid; non-immunogenic)
- Group A b-hemolytic streptococci

Gram positive cocci in chains



Cultural characteristics

- Aerobes and facultative anaerobes
- Optimum temperature: 37C
- Growth occurs only in media containing fermentable carbohydrates or enriched with blood or serum

i. Blood agar:

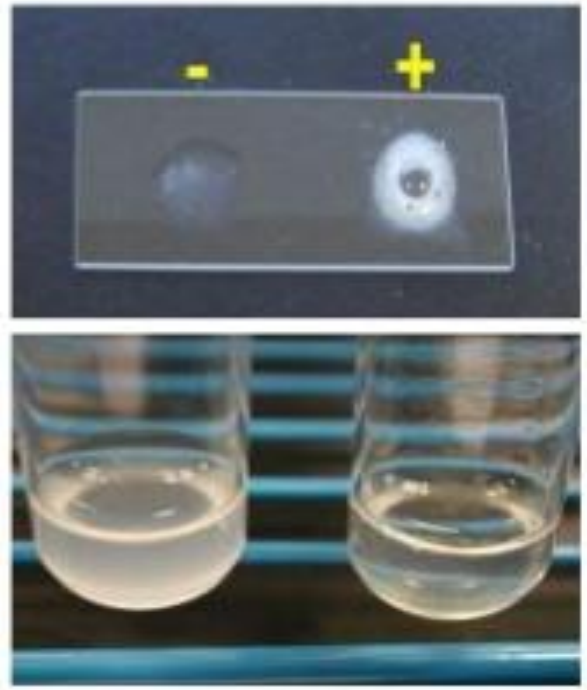
- Small (0.5-1mm), circular, semi-transparent colonies
- Produce wide zone of β- hemolysis
- Growth and hemolysis are promoted by 5-10% CO2
- Virulent strains, on fresh isolation form lesions, produce a 'matt' (finely granular) colony while avirulent strains form 'glossy' colonies
- Mucoid colonies are formed by strains that produce large capsules

ii. Liquid media:

- Glucose or serum broth
- Growth occurs as a granular turbidity with a powdery deposit
- No pellicle is formed

Biochemical reactions

- Catalase negative
- Bile insoluble
- Ferments sugars producing acid but no gas
- PYR test positive



Bile insoluble

- Hydrolyse pyrrolidonyl-beta-napthylamide (PYR) due to presence of peptidase, the resulting napthylamide produces a red colour upon the addition of 0.01% cinnamaldehyde reagent
- Faliure to ferment ribose

Biochemical properties of streptococci:

Properties	*S.pyogenes*
Hemolytic activity	**beta-hemolysis**
Catalase	**-**
Glucose	**+**
Lactose	**+**
Sucrose	**+**
Maltose	**+**
Mannitol	**+**
Inulin	
Rotting of milk	**+**
Gelatin hydrolysis	**-**
Indol	**-**

Streptococcus pyogenes - antigen structure

- Species antigen - located in the cytoplasm, contains a nucleoprotein.
- Group-specific antigen - a polysaccharide located in the cell wall.
- Type-specific antigen (M-, T-, R-) - located in the outer layer of the cell wall, contains protein. M-antigen forms protrusions (fimbriae) on the surface of the cell wall of S. pyogenes, so it is sometimes called fimbrial protein. Antibodies formed against it provide long-term resistance against re-infection with streptococci.

Antigenic structure

- Structural antigens – Cell wall antigens
- Toxins
- Enzymes

Capsular hyaluronic acid:

- Non antigenic as **hyaluronic acid** is identical to that found in human connective tissue and hence bacteria can disguise themselves with an immunological self substance
- Has weak anti-phagocytic activity but protects streptococci against immunological attacks

Antigenic structure

A. **Cell wall:**

1. Outer layer: Protein and lipoteichoic acid
2. Middle layer: Group specific carbohydrate
3. Inner layer: Peptidoglycan (mucoprotein)

- Responsible for cell wall rigidity
- Enhances non specific resistance (pyrogenic and thrombolytic activity)

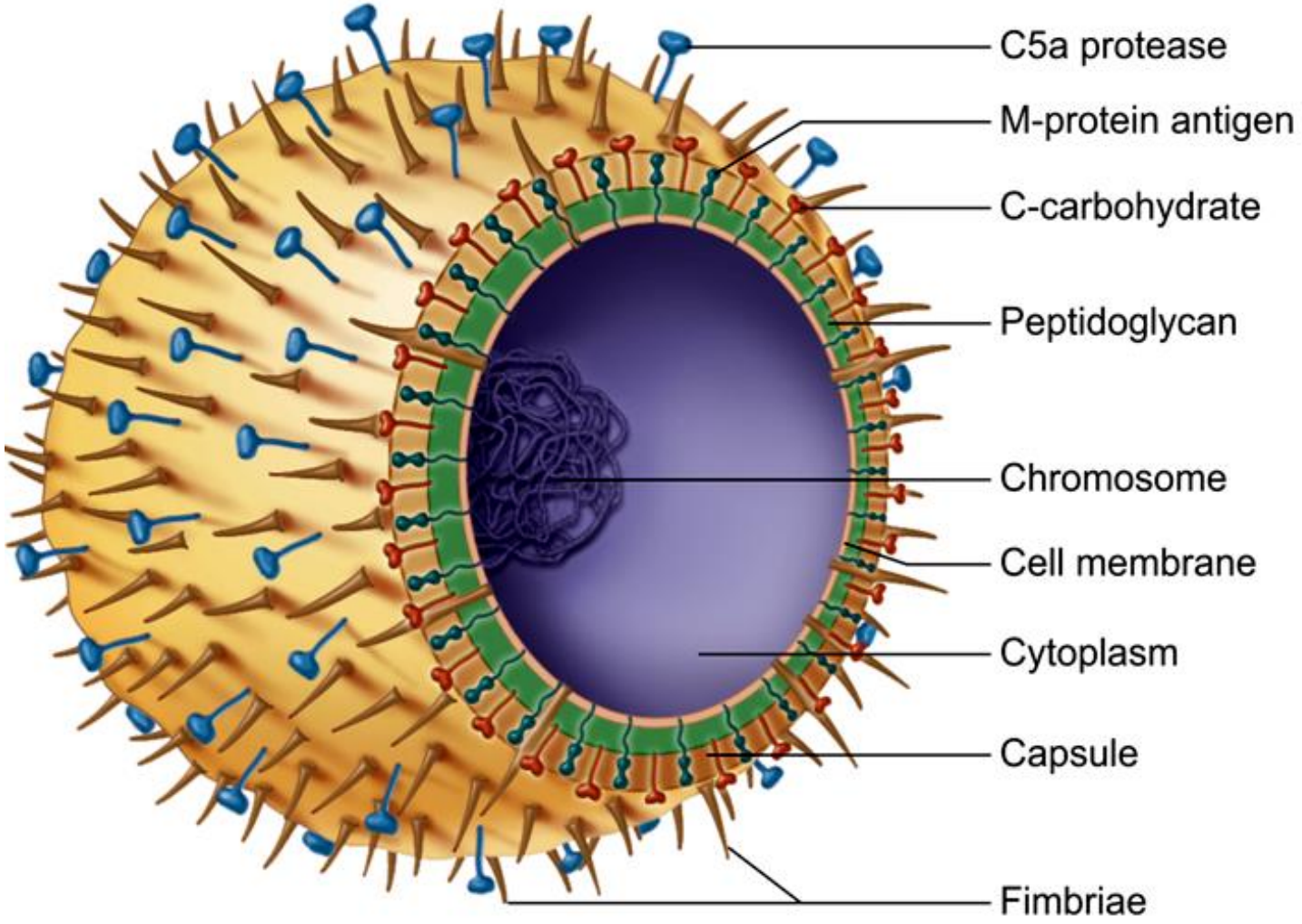
Toxins and enzymes

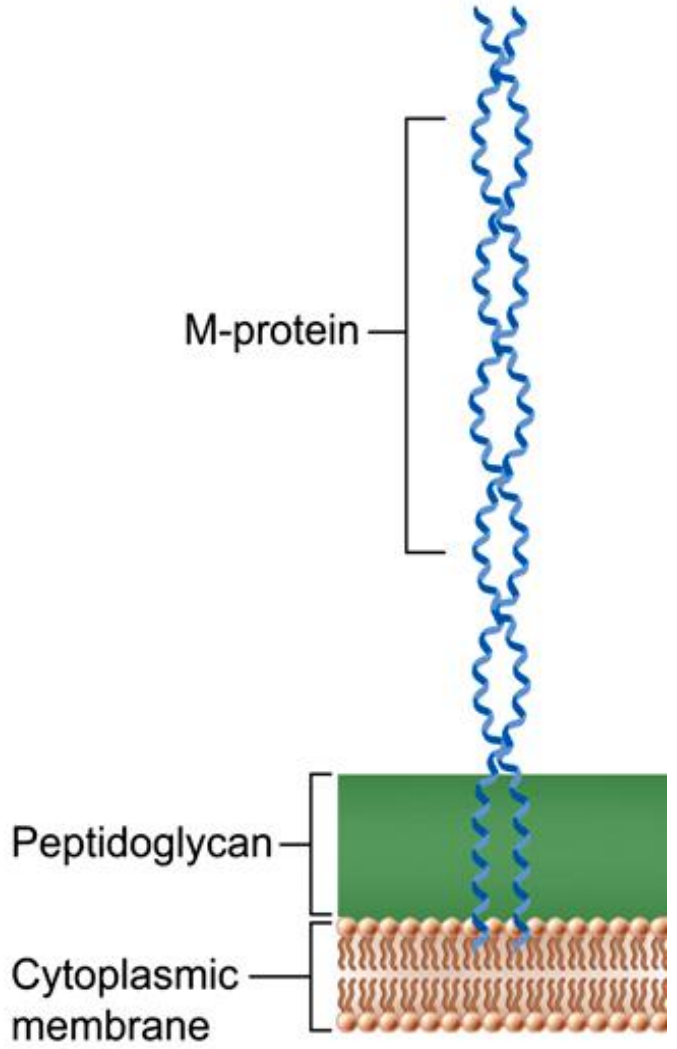
1. **Hemolysins (Streptolysins)**
2. **Erythrogenic toxin**
3. **Streptokinase (Fibrinolysin)**
4. **Deoxyribonucleases (Streptodornase, DNAase)**
5. **Hyaluronidase**

Hemolysins (Streptolysins)

- Produce complete disruption of RBC
- Contribute to tissue invasion and destruction
- There are two types of Streptolysins
  - **Streptolysin O**
  - **Streptolysin S**

Streptococcus pyogenes
(localization of M-protein and other pathogenicity factors)





Resistance to external environmental factors

- Streptococci are quite resistant to environmental factors. During 30 min at 60ºC. they are destroyed.They are stored in dried pus and sputum for months.
- The usual concentrations of disinfectant solutions kills them during 15-20 min.

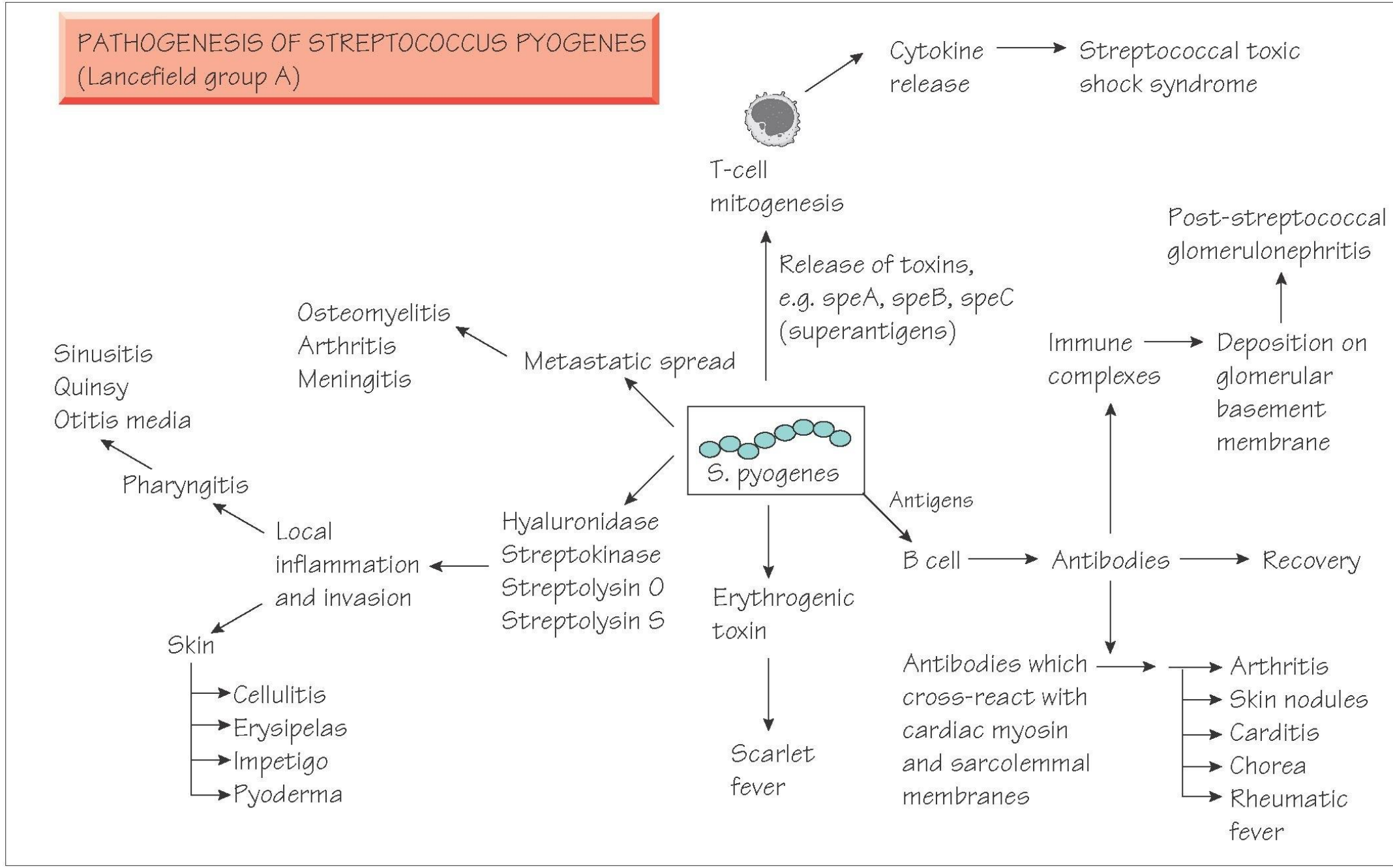
Pathogenicity

- Produces pyrogenic infection with a tendency to spread locally, along lymphatics and through blood stream
- Disease caused can be:
  - **Suppurative** or
  - **Non suppurative**

- Suppurative diseases:
1. Respiratory infections
2. Skin and soft tissue infections
3. Genital infections

- Non suppurative sequelae:
1. Acute rheumatic fever
2. Acute glomerulonephritis

PATHOGENESIS OF STREPTOCOCCUS PYOGENES
(Lancefield group A)



Streptococci - non-purulent infections:

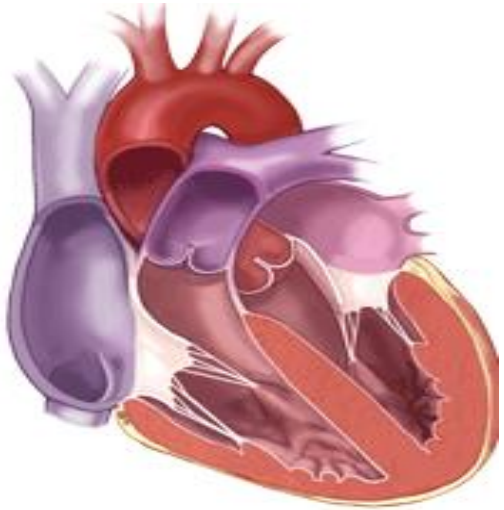
Erysipelas is a phlegmon-like acute non-purulent inflammatory disease of the skin. Signs of inflammation, such as redness and swelling on the skin, are very vivid and differ sharply from healthy skin. Inflammation (redness) on the surface of the skin tends to spread, its borders are indented-protruding, reminiscent of "flame".

Streptococci - non-purulent infections:

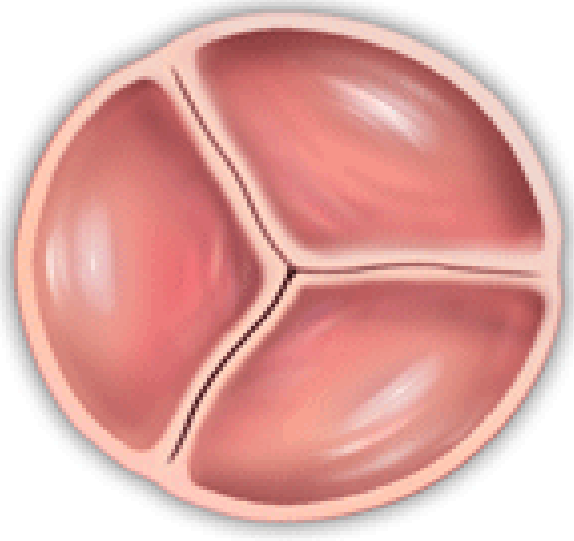
Scarlet fever - transmitted by airborne droplets, accompanied by three main symptoms - angina, fever and rash. A unique feature of scarlet fever-causing streptococci is their secretion of a pyrogenic (scarlatinous) toxin. The mechanism of action of this toxin is related to its superantigen property.

Streptococci - non-purulent infections:

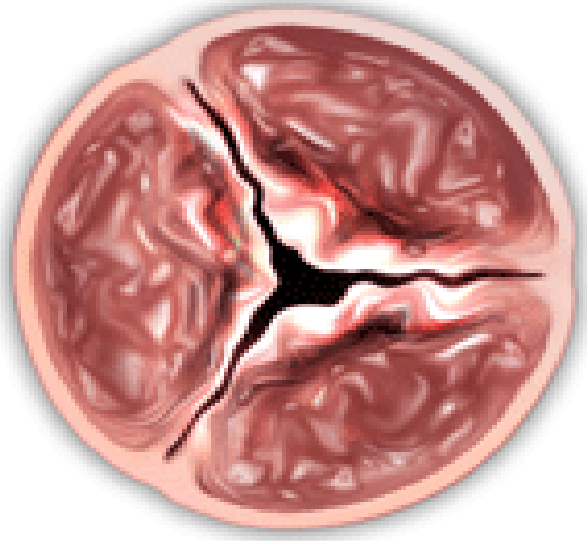
The first manifestation of **Rheumatic fever (acute rheumatic fever)** manifests itself as an acute inflammation of the joints - aseptic polyarthritis, followed by pancarditis (inflammation of all membranes of the heart - endocardium, myocardium and pericardium). Endocarditis, as a chronic inflammatory process, deforms the heart valves and disrupts its functions, causing stenosis and insufficiency of the mitral and aortic valves. Some antigens of streptococci (M-protein) cross-react with cardiac tissue, causing the formation of autoantibodies and sensitized T-lymphocytes against cardiac antigens.



Normal heart beats



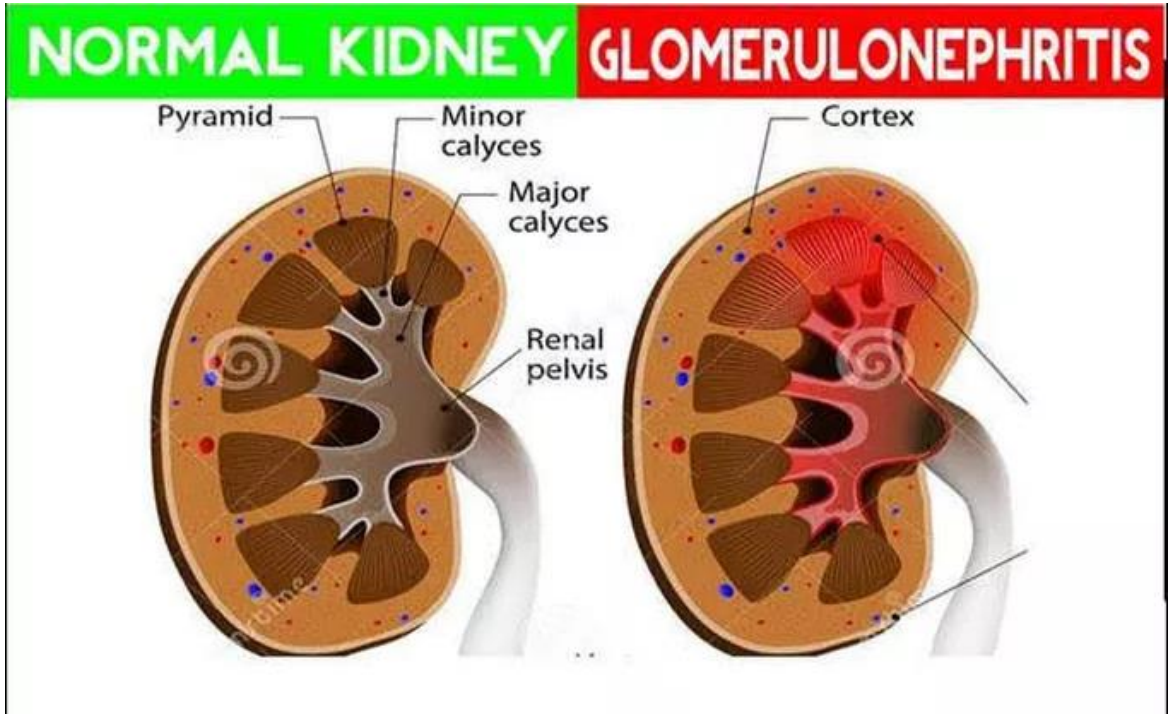
Normal heart valves



Defective heart valves

Streptococci - non-purulent infections:

- ***Glomerulonephritis*** - mainly develops after pyodermas of streptococcal origin and is caused by nephritogenic strains of different M-serotypes (M1, M12, M49, M59) of S. pyogenes. Deposition of streptococci+antibody complex (immune complexes) and activation of complement in the walls of the capillaries of kidney glomeruli cause acute inflammation.



Laboratory Diagnosis

a. Throat swab culture: Detection of group A antigen
b. Specific nucleic acid based test
c. Elevation of anti hyaluronidase antibodies(strong evidence)

1. **Specimen:**

- Throat swab, pus swab or exudates are collected.

2. **Microscopy:**

- Gram-staining of pus can be examined
- Presence of Gram-positive cocci in chains can be indication.

3. Culture:

- Swab from the affected area is collected and are either plated immediately or sent to laboratory in Pike's medium.

- The specimen should be plated on blood agar and incubated at 37°C anaerobically or under 5-10% CO_2 ,as hemolysis develops better.

Streptoccocus

Gram positive cocci in chains, catalase negative

Hemolysis

α
Partial hemolysis

Optochin sensitivity

No
- Viridans strep (unencapsulated)
  - *S. mutans*
  - *S. mitis*

Yes
- *S. pneumoniae* (encapsulated)

β
Complete hemolysis

Bacitracin sensitivity

No
- Group B strep
  - *S. agalactiae*

Yes
- Group A strep
  - *S. pyogenes*

γ
No hemolysis

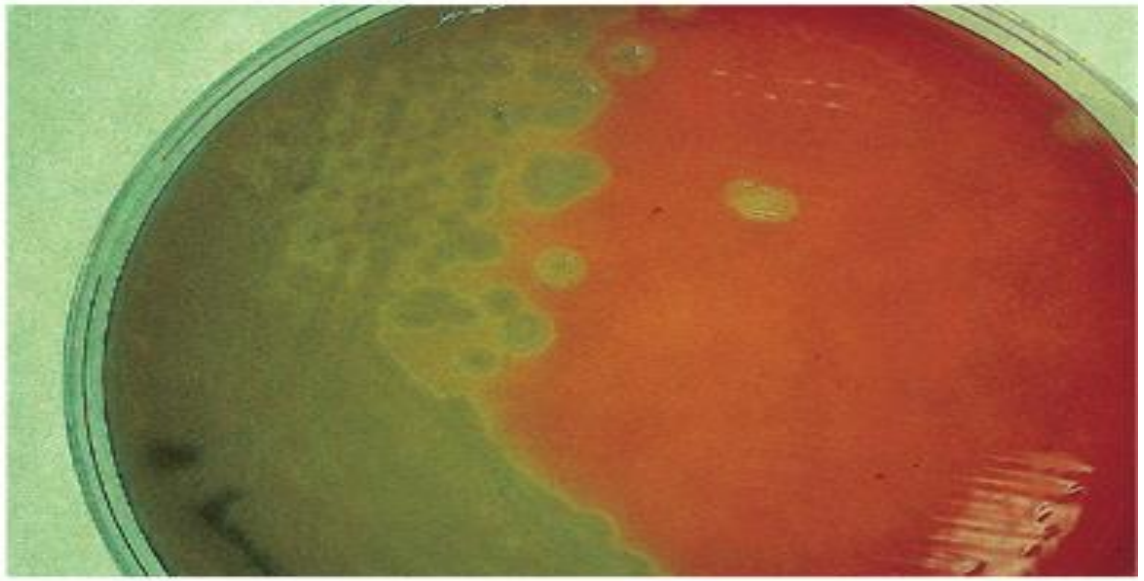
Growth in 6.5% NaCl

No
- *S. bovis*

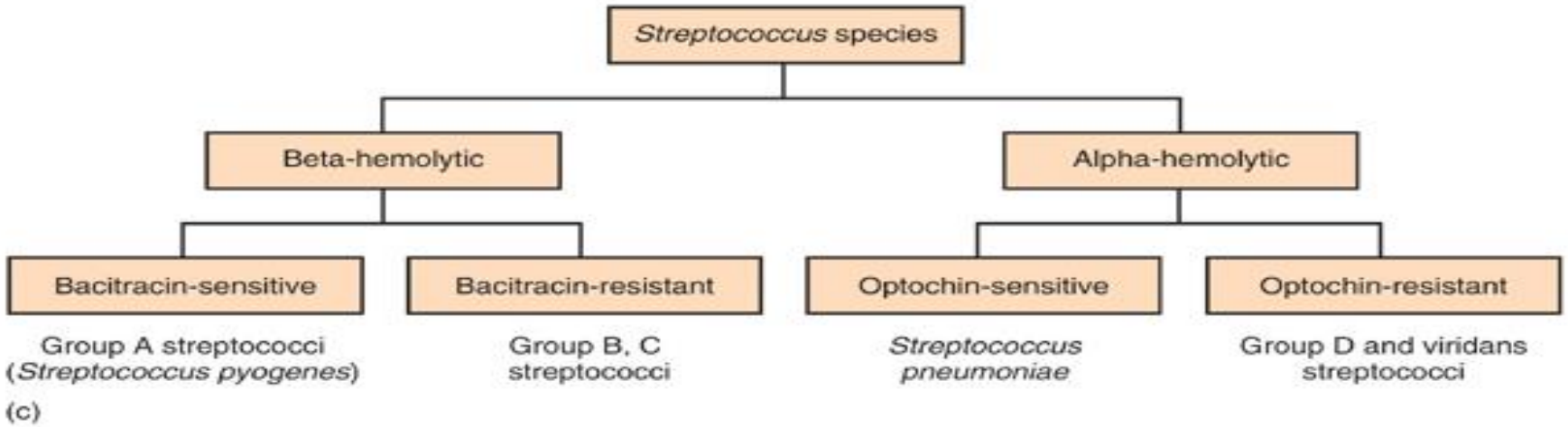
Yes
- Enterococci
  - *S. faecium*
  - *S. faecalis*

Hemolysis on blood agar

(a)



(b)



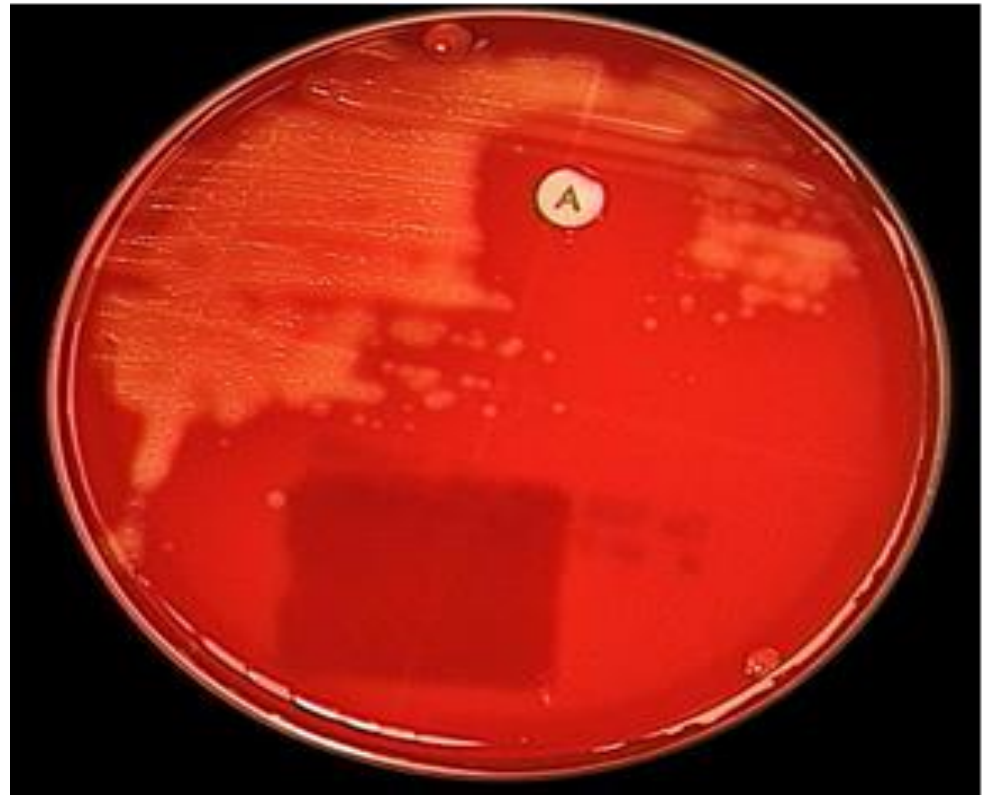
(c)

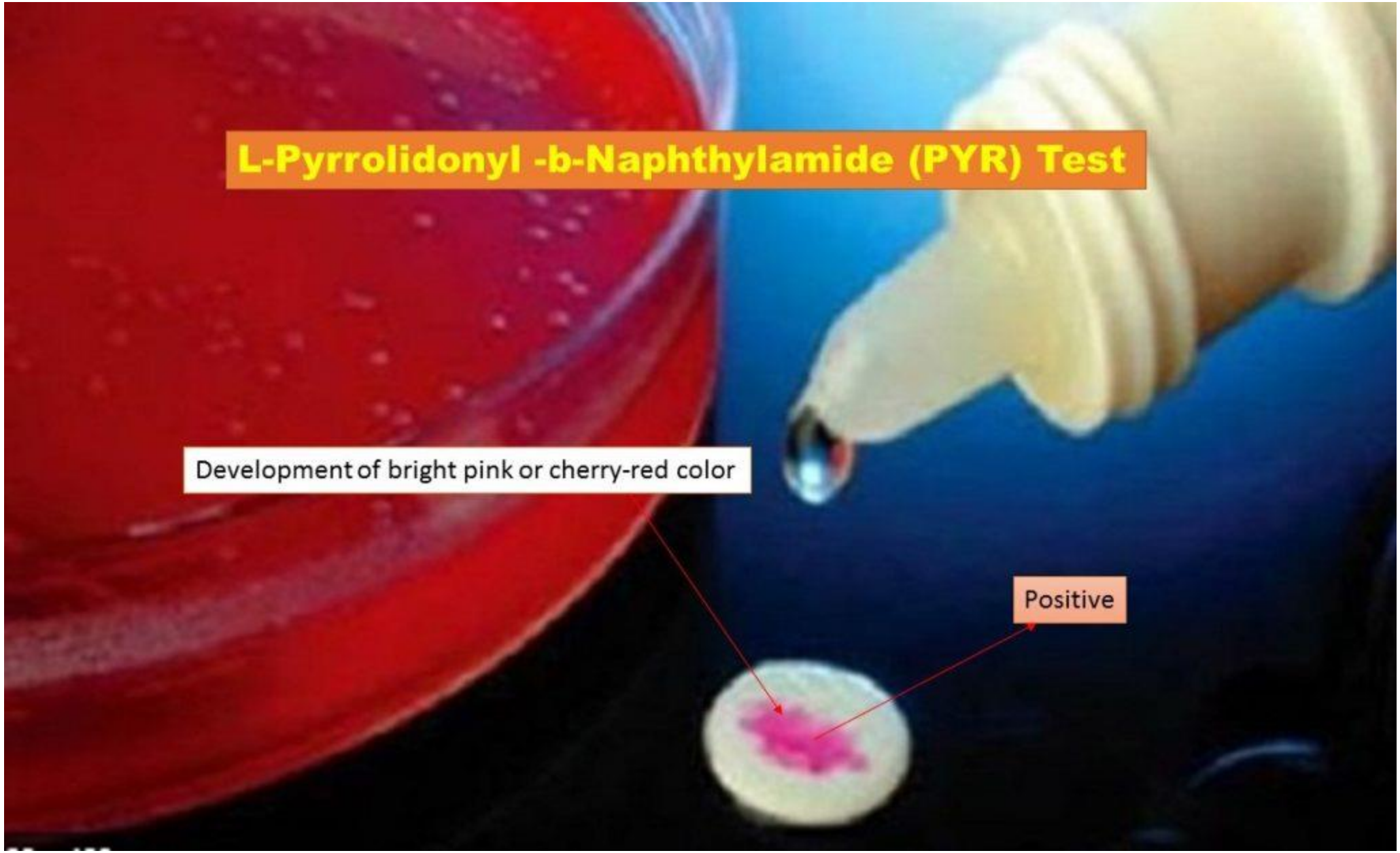
S. pyogenes blood agar culture (beta-hemolysis)

4. Identification:

- Rapid diagnostic test kits are available for the detection of streptococcal group A antigen from throat swab

- **Bacitracin sensitivity:**
- Based on Maxted's observation that they are more sensitive to bacitracin than other streptococci
- A filter paper disc of 0.04U is applied on the surface of an inoculated blood agar
- After incubation, a wide zone of inhibition is seen with *S.pyrogenes* but not with other streptococci




L-Pyrrolidonyl -b-Naphthylamide (PYR) Test
Development of bright pink or cherry-red color
Positive

5. Serology:

a) **Antistreptolysin O titration**

Standard test ASO titres higher than 200 are indicative of prior streptococcal infection. High levels are usually found in acute rheumatic fever but in glomerulonephritis, times are often low

b) **Antideoxyribonuclease B (anti-DNAase B):**

Commonly used

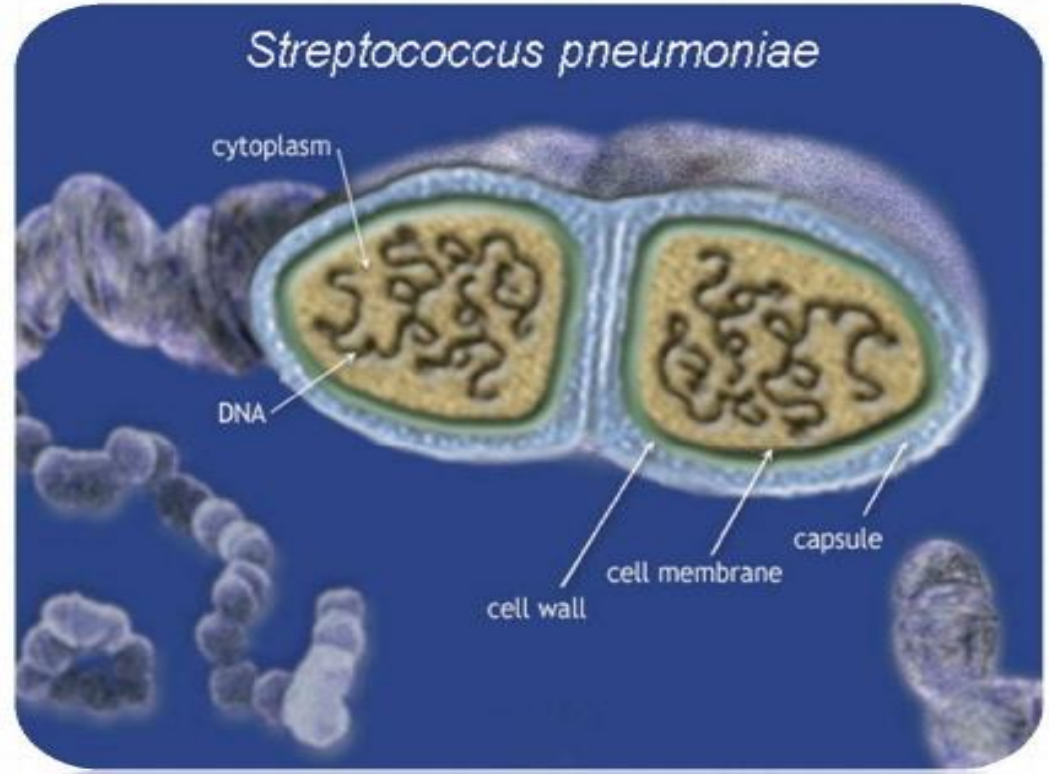
Titres higher than 300 are taken

c) **Streptozyme test:**

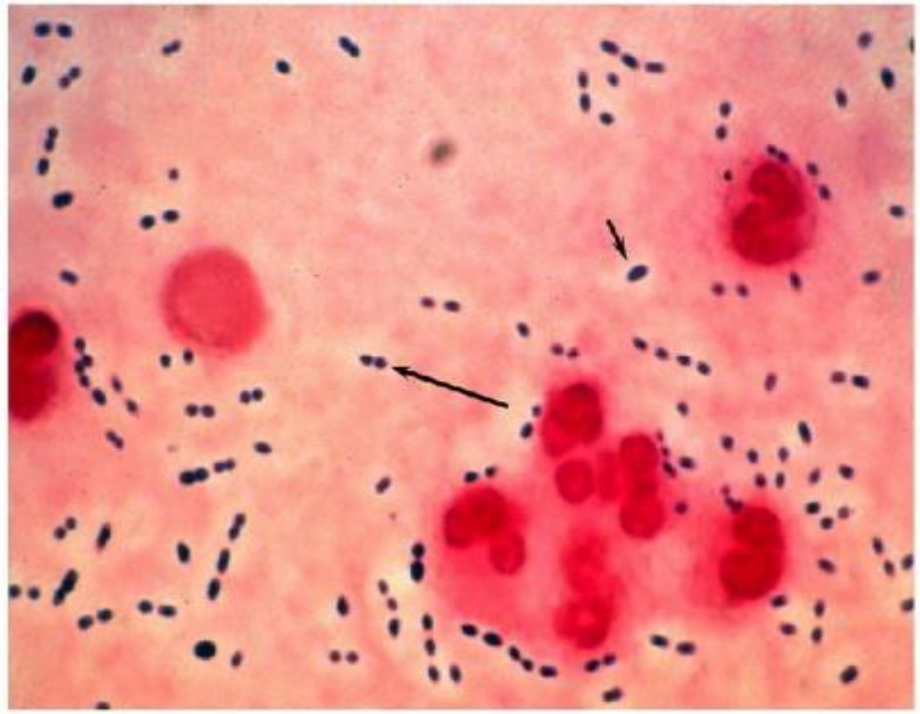
- A passive slide hemagglutination test using erthyrocytes sensitised with a crude preparation of streptococci
- It is a convenient, sensitive and specific screening test.

Streptococcus pneumoniae

- **Streptococcus pneumoniae cells are Gram-positive, lancet-shaped cocci (elongated cocci with a slightly pointed outer curvature). Usually, they are seen as pairs of cocci (diplococci), but they may also occur singly and in short chains. When cultured on blood agar, they are alpha hemolytic**



Streptococcus pneumoniae



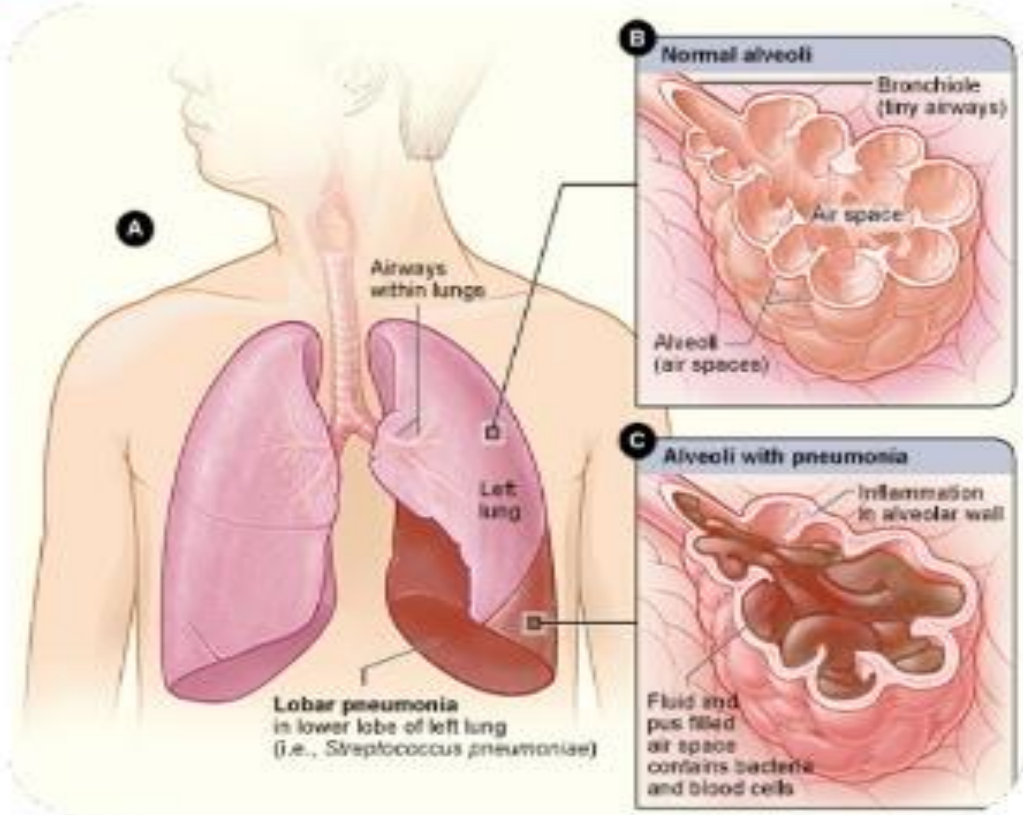
- Individual cells are between 0.5 and 1.25 micrometers in diameter. They do not form spores, and they are nonmotile. Like other streptococci, they lack catalase and ferment glucose to lactic acid

Pneumococcal Disease Clinical Syndromes

A. Pneumonia

B. Bacteremia

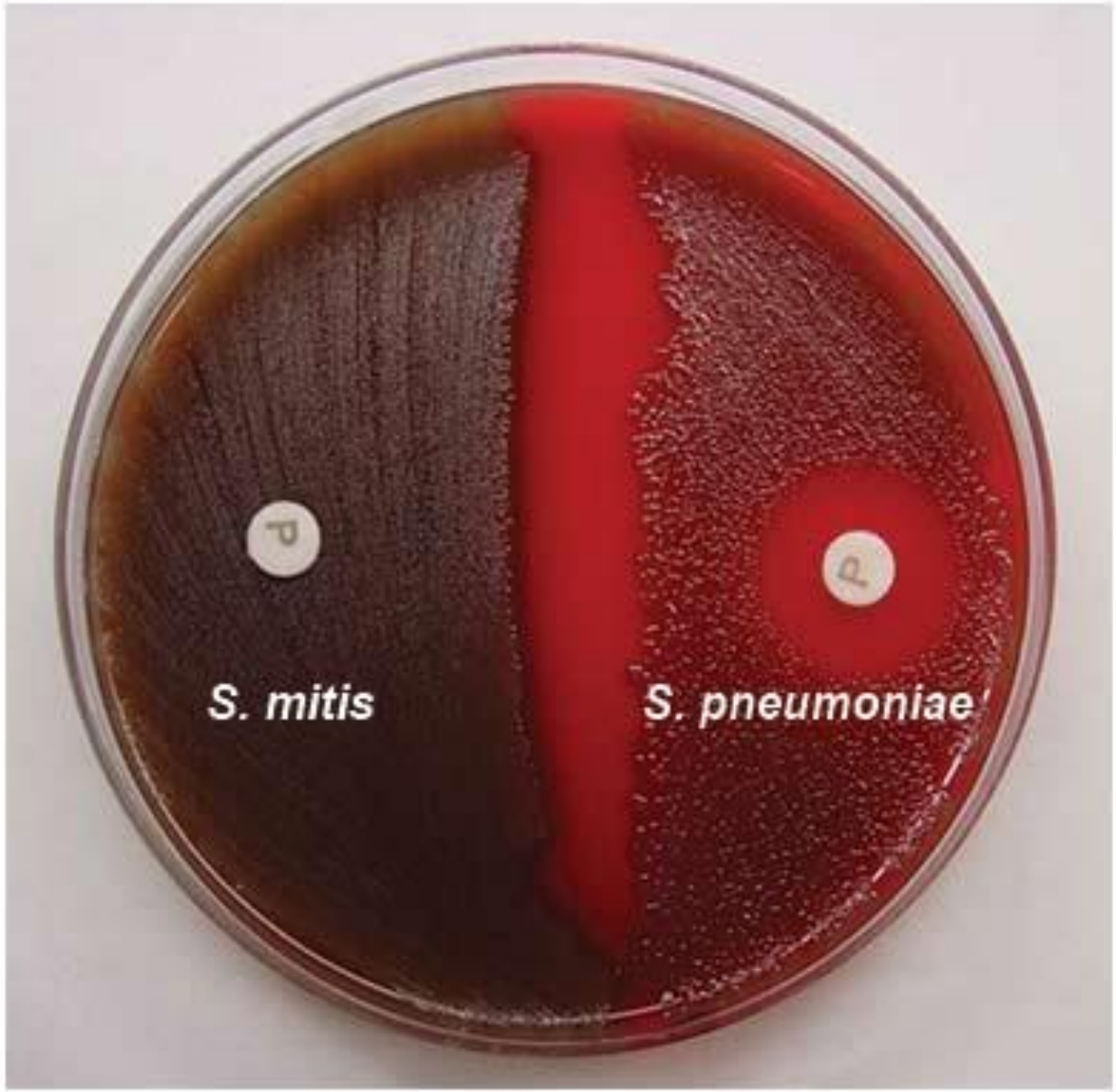
C. Meningitis



Streptococcus pneumoniae

E. Laboratory Diagnosis

1. Direct examination of Sputum
   - Gram-stain (PRESUMPTIVE DIAGNOSIS)
1. Culture
   - Appearance of α-hemolytic colonies that are bile soluble & optochin sensitive & positive Quellung reaction: (if typing sera is available - simplest, most rapid & accurate)



Left Side

S. mitis

Resistant to optochin

Right Side

S. pneumoniae

Susceptible to optochin

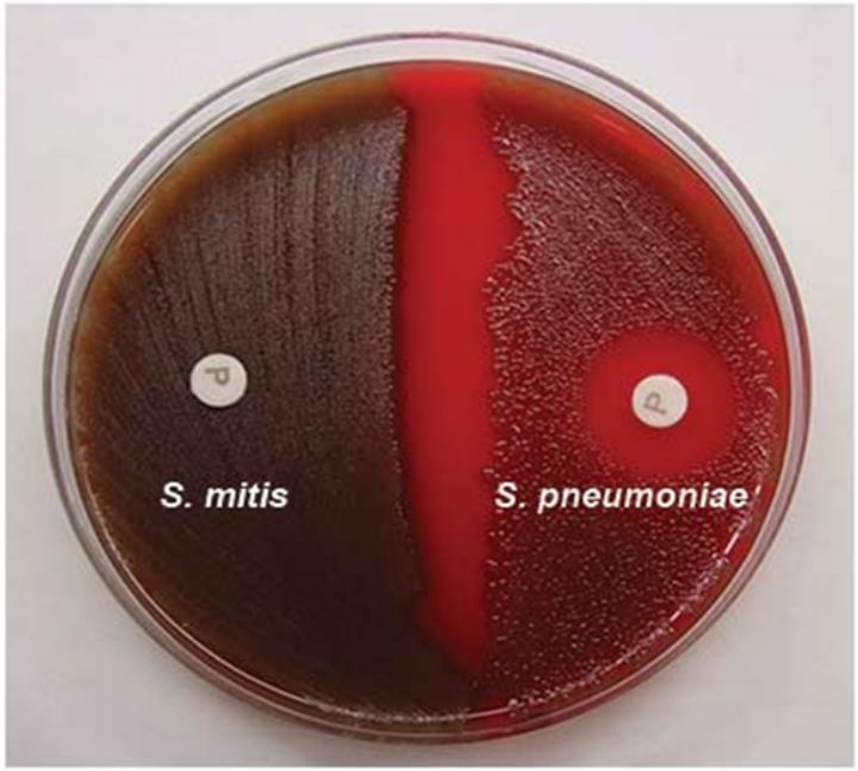
Streptococcus pneumoniae

E. Laboratory Diagnosis

3. Serologic Diagnosis
   i. Detection of pneumococcal antibodies
      - radioimmunoassay
   i. Detection of capsular polysaccharide
      - counterimmunoelectrophoresis

Signs that distinguish pneumococci from other streptococci:

- pneumococcal colonies are not confluent
- produces alpha hemolysis
- breaks down inulin to form acid
- is lysed by bile and bile acids
- has a high sensitivity to optoxin



OPTOXİN testi

S.mitis - davamlı

S.pneumoniae - həssas

Treatment, prevention and control

DRUGS USED:

- For streptococcal pharyngitis: Oral penicillin V or amoxicillin
- Oral cephalosporin or macrolides can be used for penicillin sensitive patients
- For severe, systemic infection: Combined use of intravenous penicillin with protein synthesis inhibiting antibiotics(clindamycin) is recommended
- *Streptococcal pyogenes* have developed resistance over tetracyclines and sulfonamides, newer macrolides
- Antimicrobial drugs has no effect on glomerulonephritis and rheumatic fever

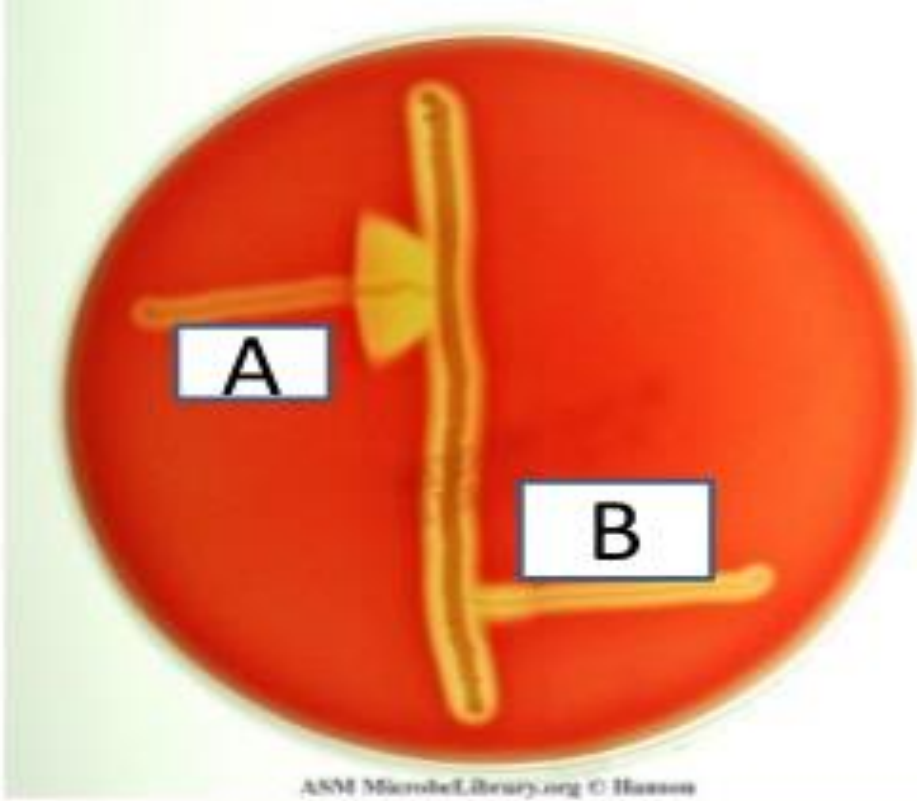
Group B beta hemolytic Streptococci

Streptococcus agalactiae

- Neonatal infection
- Most common cause of neonatal meningitis
- Source – from the maternal vagina during birth
- GBS – puerperal sepsis, pneumonia
- Diagnostic markers – Hippurate hydrolysis, CAMP test

CAMP test

- Christie, Atkins and Munch-Peterson
- When S.agalactiae is inoculated perpendicular to a streak of S.aureus grown on blood agar → an accentuated zone of hemolysis occurs

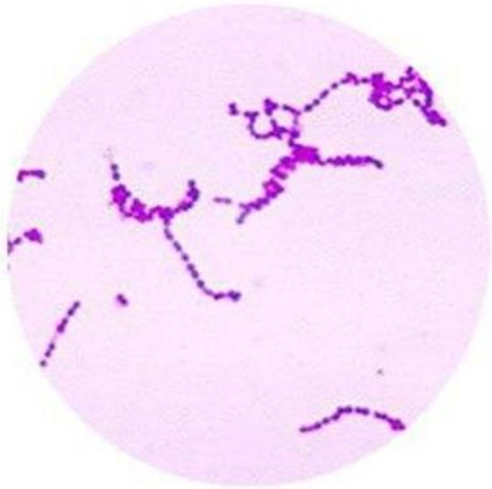


Name of the test: CAMP test
Example A: Positive - Strept agalactiae (Arrow shaped)
Example B: Negative - Strept pyogens
Principle : Strept agalactiae produce CAMP factor (a diffusible extracelluar protein) that synergistically acts with the beta-lysin of *Staphylococcus aureus* and enhances the lysis of red blood cells.

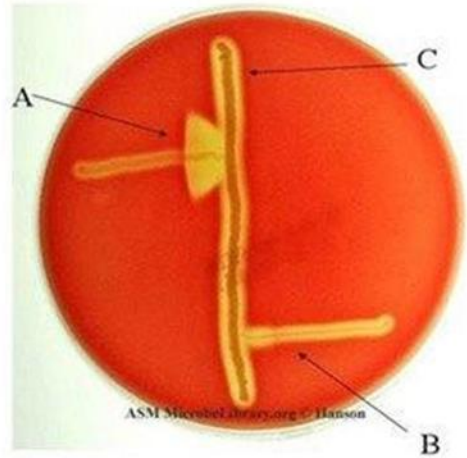
microbeonline

Streptococcus agalactiae (GBS)

β-hemolysis



GPC in chains



CAMP test +ve

Hippurate hydrolysis +ve

Viridans group

- Streptococci normally resident in the mouth and upper respiratory tract
- Alpha lysis on blood agar
- Cannot be categorised under lancefield antigenic groups
- Types:

1. S.mitis
2. S.mutans
3. S.salivarius
4. S.sanguis

- Causes dental caries
- Tooth extraction – seeding into blood stream - endocarditis – hence give prophylactic antibiotics

DENTAL CARIES

--Progressive bacterial damage to teeth exposed to saliva.

--one of the most major causes of all diseases and major cause of tooth loss.

--ultimate effect-to breakdown enamel and dentin and open a path for bacteria to reach pulp.

Consequences-inflammation of pulp and periapical tissues.

AETIOLOGY

- Four major factors involved in etiology:-
- Cariogenic bacteria
- Bacterial plaque
- Susceptible tooth surface
- Fermentable bacterial substrate (sugar)

Bacteriology of Dental Caries

- Major organisms responsible for caries are:-
- Strep mutans
- Lactobacilli
- Other strains of streptocooci

Enterococcus

- Gram positive cocci, non motile, non sporing
- Catalase Negative
- Previously classified as group D streptococci
- Natural inhabitants of GIT
- Distinct features
  - Ability to grow at 10°C and 45°C
  - Ability to grow in 6.5%NaCl
  - Ability to grow at 9.6pH
  - Ability to hydrolyze esculin in 40% bile
  - Ability to process pyrrolidonyl arylamidase (PYR)

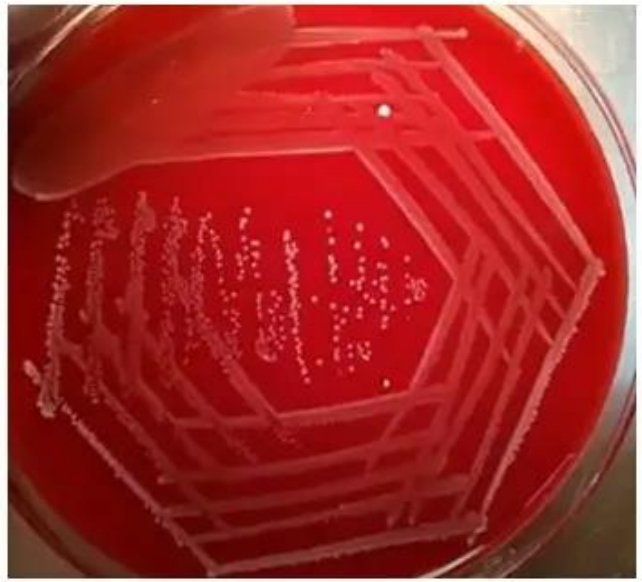
Introduction

- Evolved from intestinal commensal to 2nd most common nosocomial pathogen.
- Common species
  - Entrococcus fecalis
  - Enterococcus fecium
  - E *gallinarum*
  - E *casseliflavus*
  - E *durans*
  - E *avium*
  - E *hirae*

Enterococcal Infections

- Group D cell wall antigen
- *Enterococcus faecalis; Enterococcus faecium*
- GI tract of humans and animals
- UTI most common; wound infections; bacteremia; endocarditis
- Most infections from endogenous source
- Prolonged hospitalization and broad-spectrum antibiotics increase risk
- Antibiotic resistance (VRE)

Enterococcus Identification



Pin point colony

↓

Gram Stain: Gram positive cocci in chains

↓

Catalase test: Negative

↓

Streptococci

↓

Bile Esculin Test: Positive

↓

Group D

↓

Growth in 6.5% NaCl (may also grow @ 10, 45 and even 60°C)

↓

Enterococcus

↓

Pyruvate Fermentation Test

↓

Positive: *Enterococcus faecalis*

Negative: *Enterococcus faecium*

Vancomycin-Resistant Enterococci

- (VRE), are bacterial strains of the genus *Enterococcus* that are resistant to the antibiotic vancomycin. Enterococci are gram-positive coccoid-shaped bacteria found in the digestive tract of some humans. To become VRE, vancomycin-sensitive enterococci typically obtain new DNA in the form of plasmids or transposons which encode genes that confer vancomycin resistance.

Vancomycin-resistant enterococcus (VRE) – epidemiology (2022)