LECTURE I

Introduction to basic microbiology. The pathogenic and opportunistic cocci (genus of Staphylococcus, Streptococcus, Enterococcus and Neisseria) and bacillus (genus of Pseudomonas, Acinetobacter, Proteus and Klebsiella)

Lecture plan:

1. 1. Introduction to special microbiology, clinically important bacteria.

2. Pathogenic and opportunistic cocci:

- Gram-positive cocci: staphylococci, their morpho-biological characteristics, pathogenicity factors, diseases they cause, antibiotic-resistant forms (methicillin-resistant Staphylococcus aureus (MRSA), methicillin-resistant coagulase-negative staphylococcus (MRCNS)), microbiological diagnosis.

- Streptococci, their morpho-biological characteristics, pathogenicity factors, diseases theycause, microbiological diagnosis, specific treatment and prevention

- Enterococci, their morpho-biological characteristics, pathogenicity factors, diseases they cause, antibiotic-resistant forms (vancomycin-resistant enterococcus (VRE)), microbiological diagnosis.

- Gram-negative cocci: meningococci and gonococci, their morpho-biological characteristics, pathogenicity factors, diseases they cause, microbiological diagnosis, specific treatment and prevention.

3. Pathogenic and opportunistic Gram-negative bacteria:

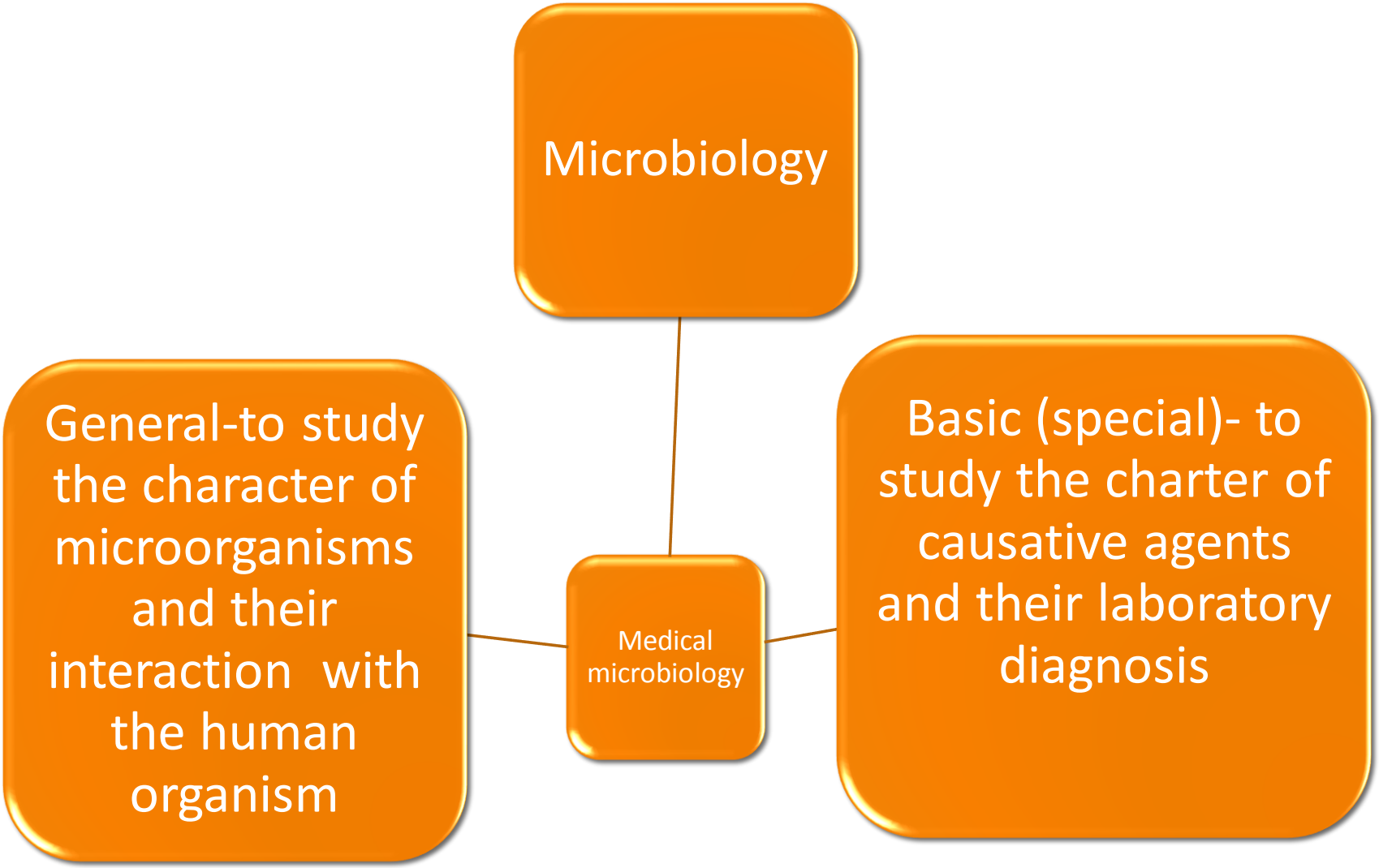
- Pseudomonas genus, morpho-biological characteristics, pathogenicity factors, diseases they cause, antibiotic-resistant forms, microbiological diagnostics.

- Acinetobacter genus, morpho-biological characteristics, pathogenicity factors, diseases they cause, antibiotic-resistant forms, microbiological diagnosis.

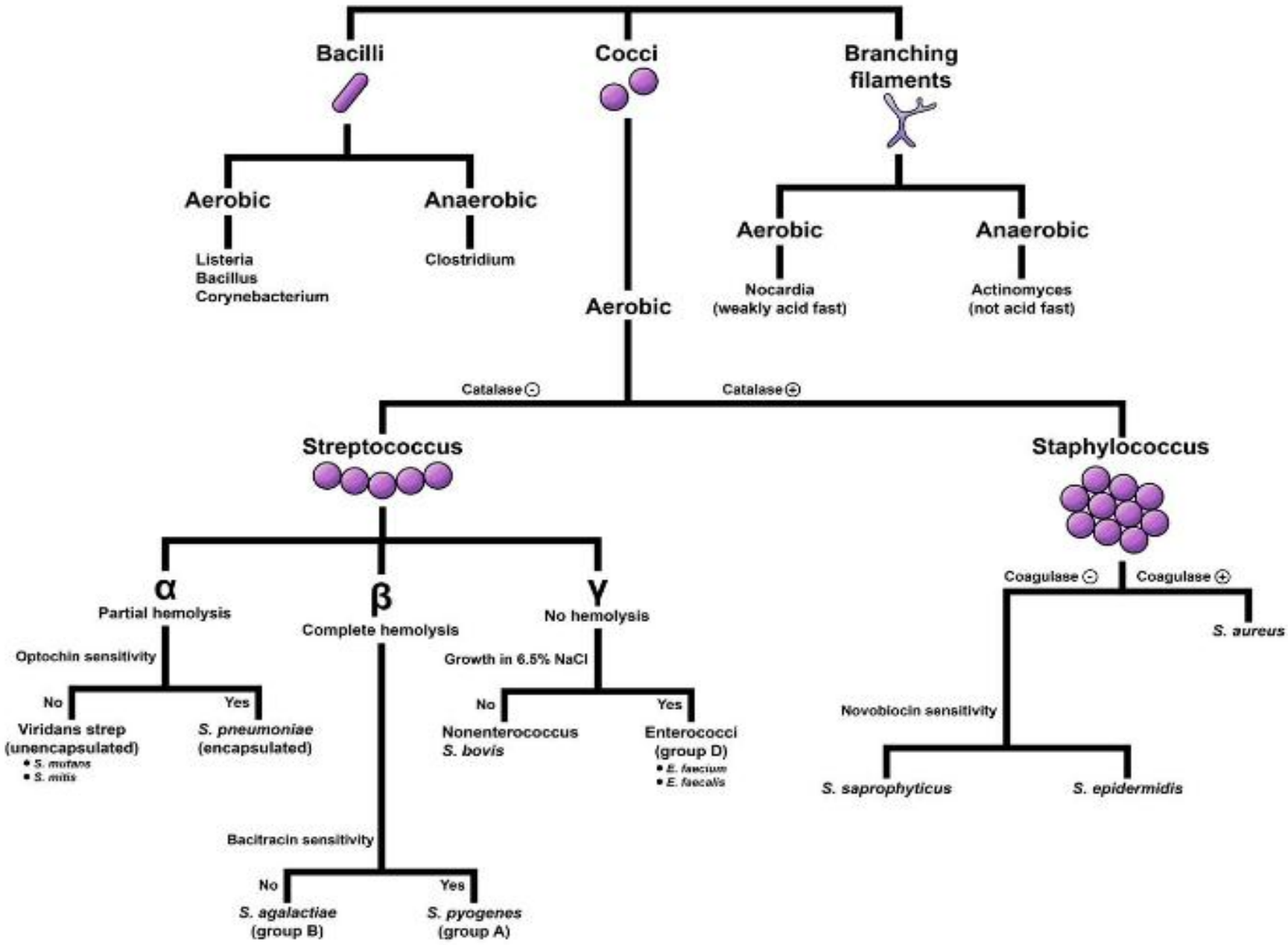
- Proteus genus, morpho-biological characteristics, pathogenicity factors, diseases they cause, antibiotic-resistant forms, microbiological diagnosis.

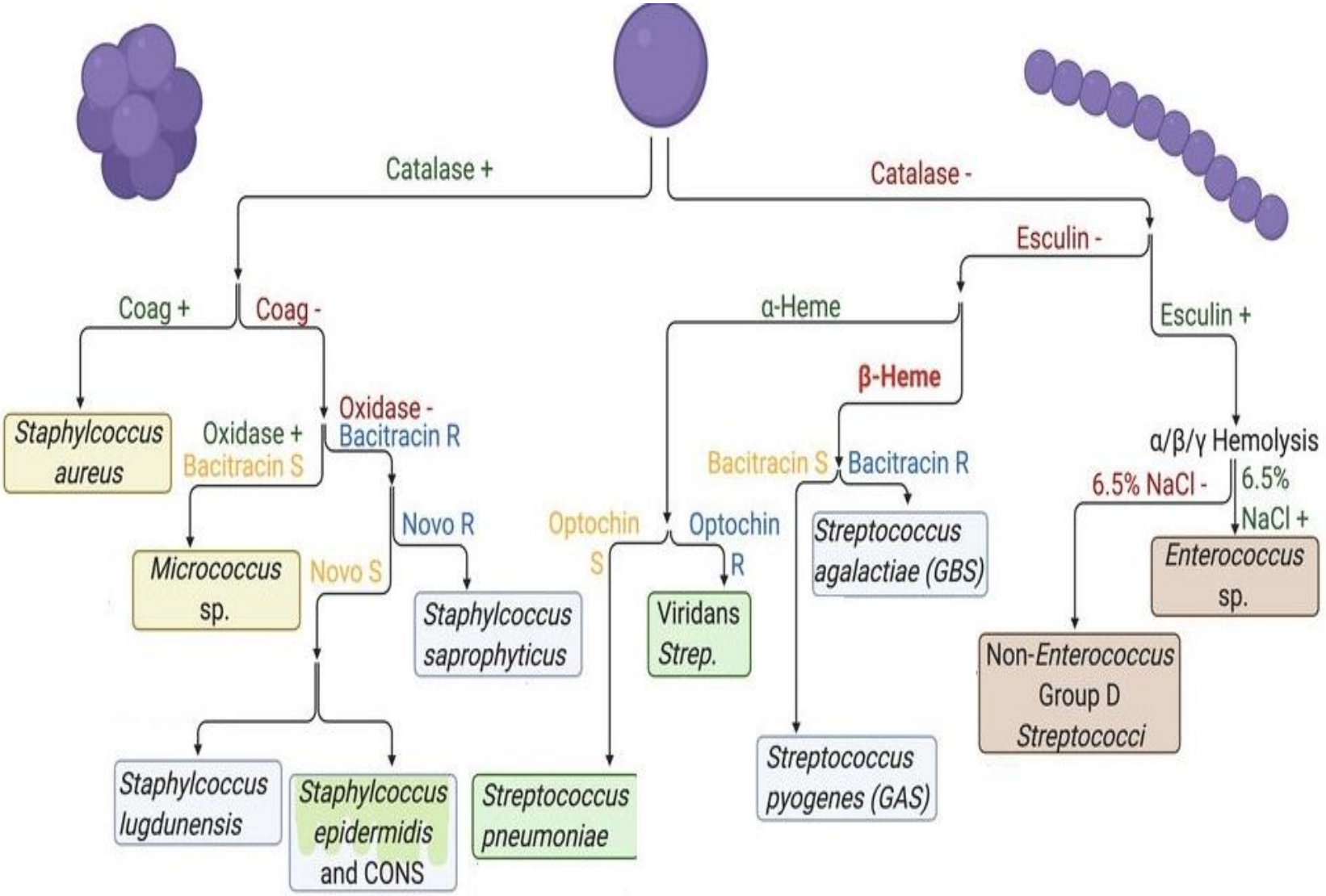
- Klebsiella genus, morpho-biological characteristics, pathogenicity factors, diseases they cause, antibiotic-resistant forms, microbiological diagnosis.

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.


Microbiology
General-to study the character of microorganisms and their interaction with the human organism
Medical microbiology
Basic (special)- to study the charter of causative agents and their laboratory diagnosis

Gram-Positive Bacteria




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
Streptococcus pneumoniae
Viridans Strep.
Bacitracin S
Bacitracin R
Streptococcus agalactiae (GBS)
Streptococcus pyogenes (GAS)
α/β/γ Hemolysis
6.5% NaCl -
6.5% NaCl +
Non-Enterococcus Group D Streptococci
Enterococcus sp.

Staphylococcaceae (taxonomy)

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

- Genus:
  - Abyssicoccus
  - Aliicoccus
  - Auricoccus
  - Corticicoccus
  - Gemella
  - Jeotgalicoccus
  - Macrococcus
  - Nosocomiicoccus
  - Salinicoccus
  - **Staphylococcus**
    - Species: more than 40 (*S.hominis*, *S.albus*, *S.haemolyticus*, *S.simulans*, *S.sciuri*...).
    - Medical important- ***S.aureus, S.epidermidis, S.saprophyticus***.

Schleifer and Bell 2010

culture characteristic

colony morphology on many types of agars:

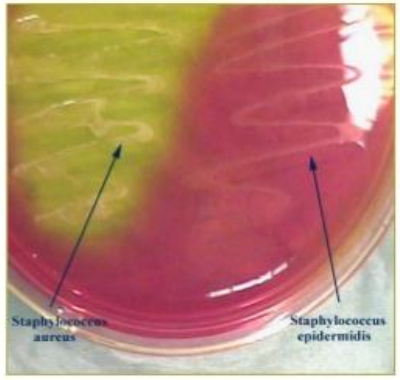
-**On nutrient agar**

Staphylococcus aureus colonies are: large, circular, smooth , shiny surface and are pigmented (golden-yellow).

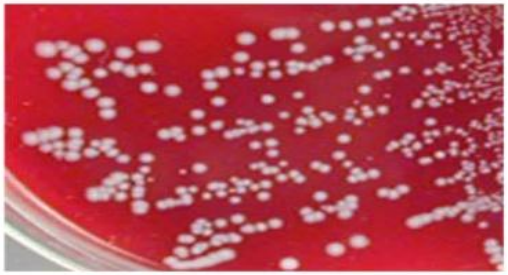
- On Blood agar,
  - golden yellow colonies, surrounded by a clear zone of hemolysis (beta-hemolysis),esp. When incubated in sheep or rabbit blood agar in atmosphere of 20% CO2

- On MacConkey agar,
  - Smaller colonies than those on NA(0.1-0.5 mm) and are pink coloured due to lactose fermentation

- 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



- ***S. Saprophyticus***
  - white-yellow colony
  - no haemolysis of red blood cells



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	+	-	-

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

Properties(....contd)

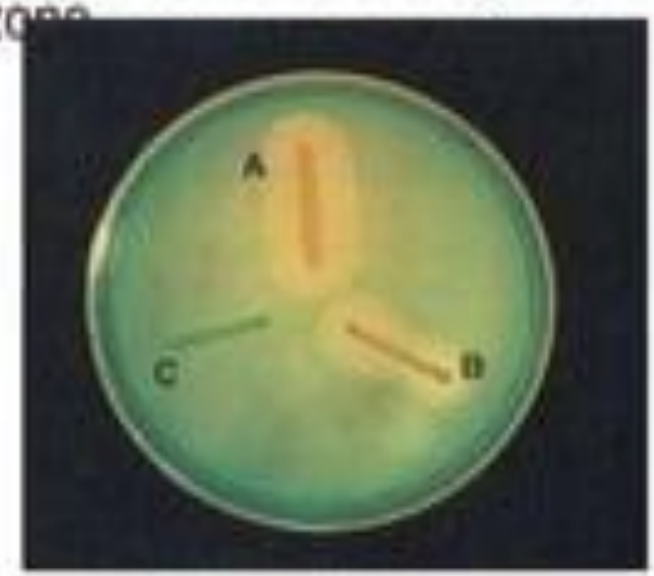
- Indole test= negative
- MR test= positive
- VP test= positive
- Urease test= positive
- Hydrolyse gelatin
- Reduces nitrate to nitrite
- Phospahatase= positive
- DNA-ase test= positive
- Coagulase test= positive

Slide test (clumping factor)

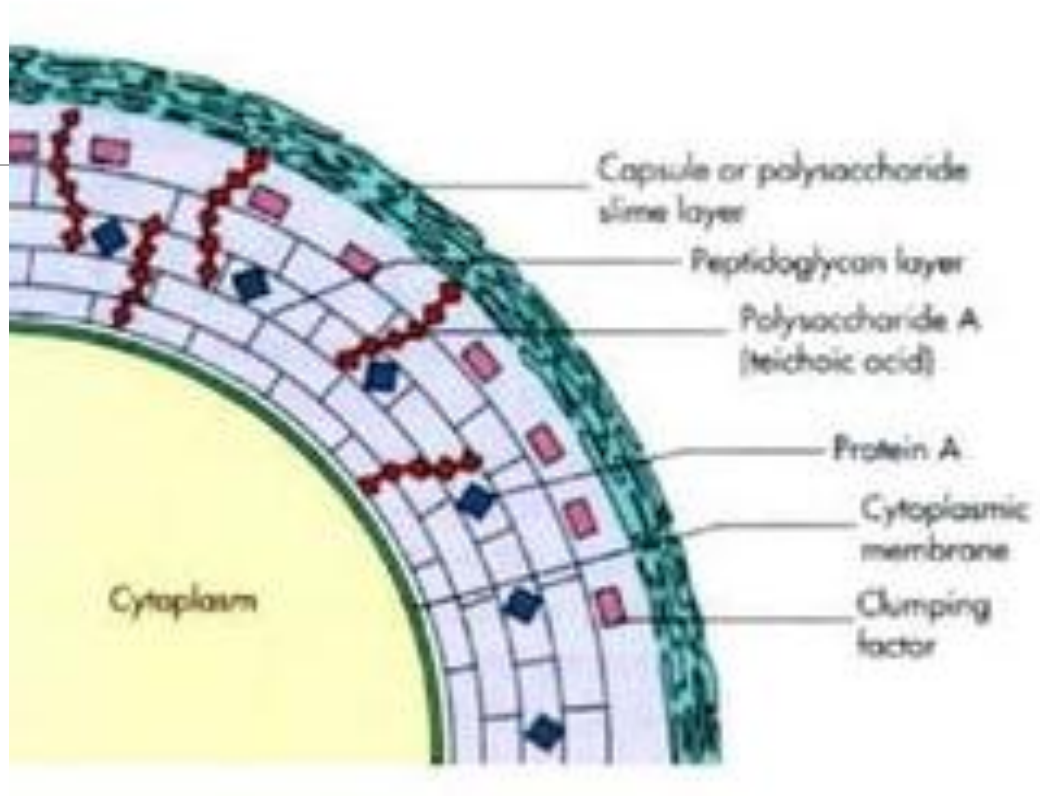
Tube test (free coagulase)

DNA Hydrolysis

- Principle : this test is used to determine the ability of an organism to hydrolyze DNA. Green color of the medium is due to DNA-methyl green complex. If the organism growing on the medium hydrolyzes DNA, the green color fades & the colony is surrounded by a colorless zone.



Antigenic Structure



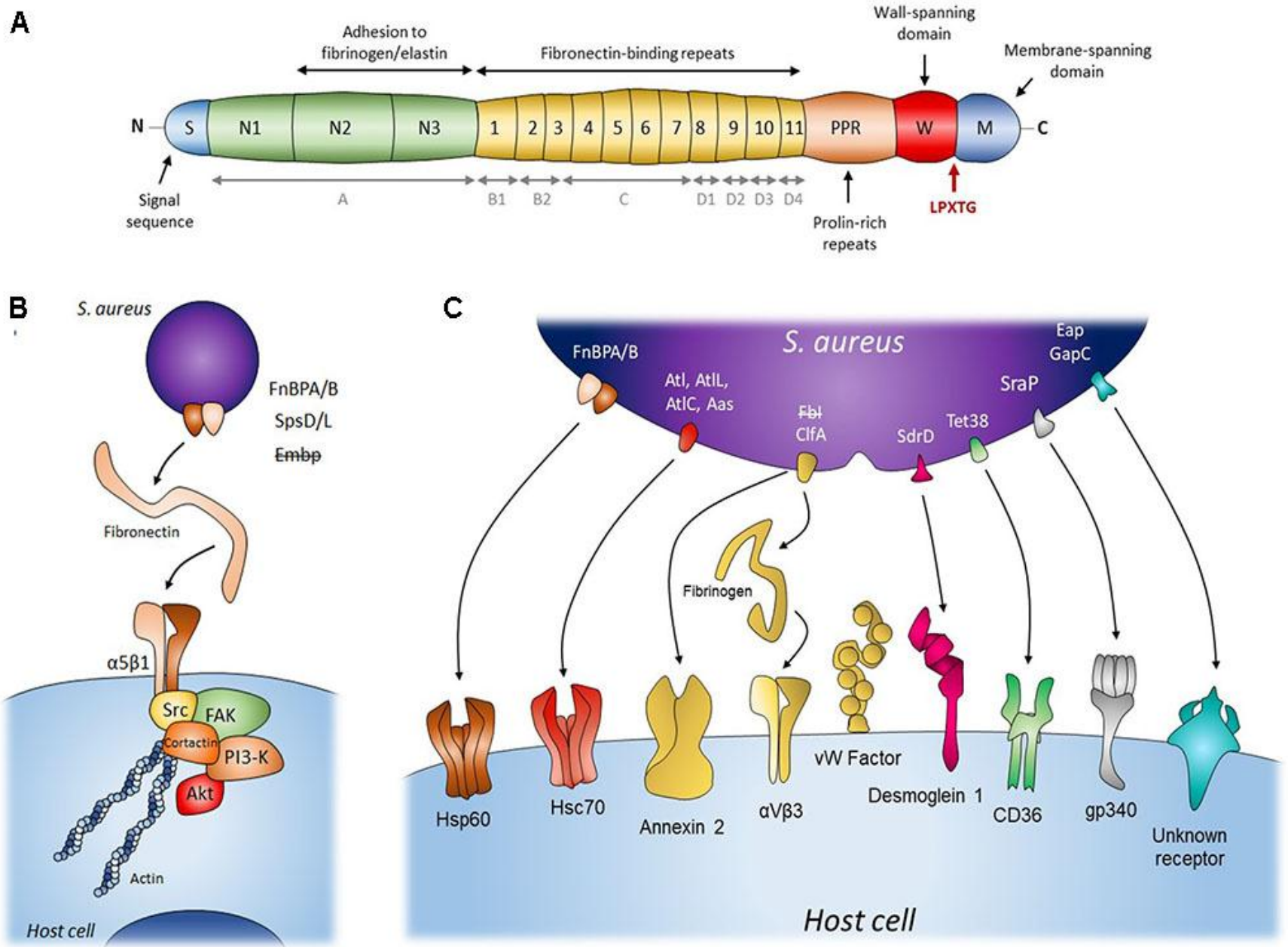
Antigenic structure

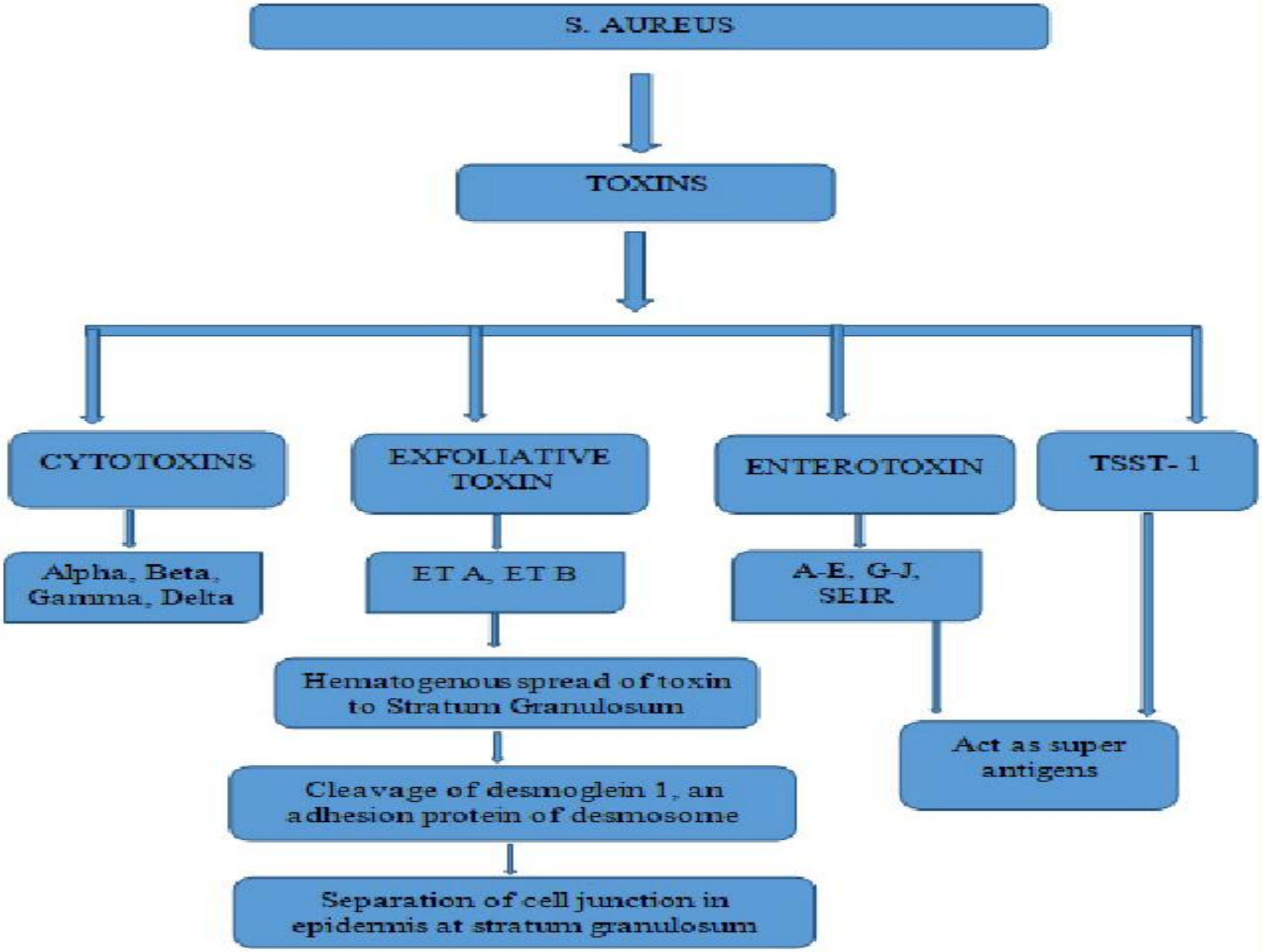
- Capsule
  - Prevents phagocytosis
  - Promotes adherence to cells of prosthetic devices
- Peptidoglycan
  - Acts as endotoxin
  - Chemotactant for neutrophils
  - Stimulates complement and coagulation
- Teichoic acid
  - Adherence to mucosal surface
- Protein A
  - Binds to Fc portion of IgG
  - Phagocytosis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

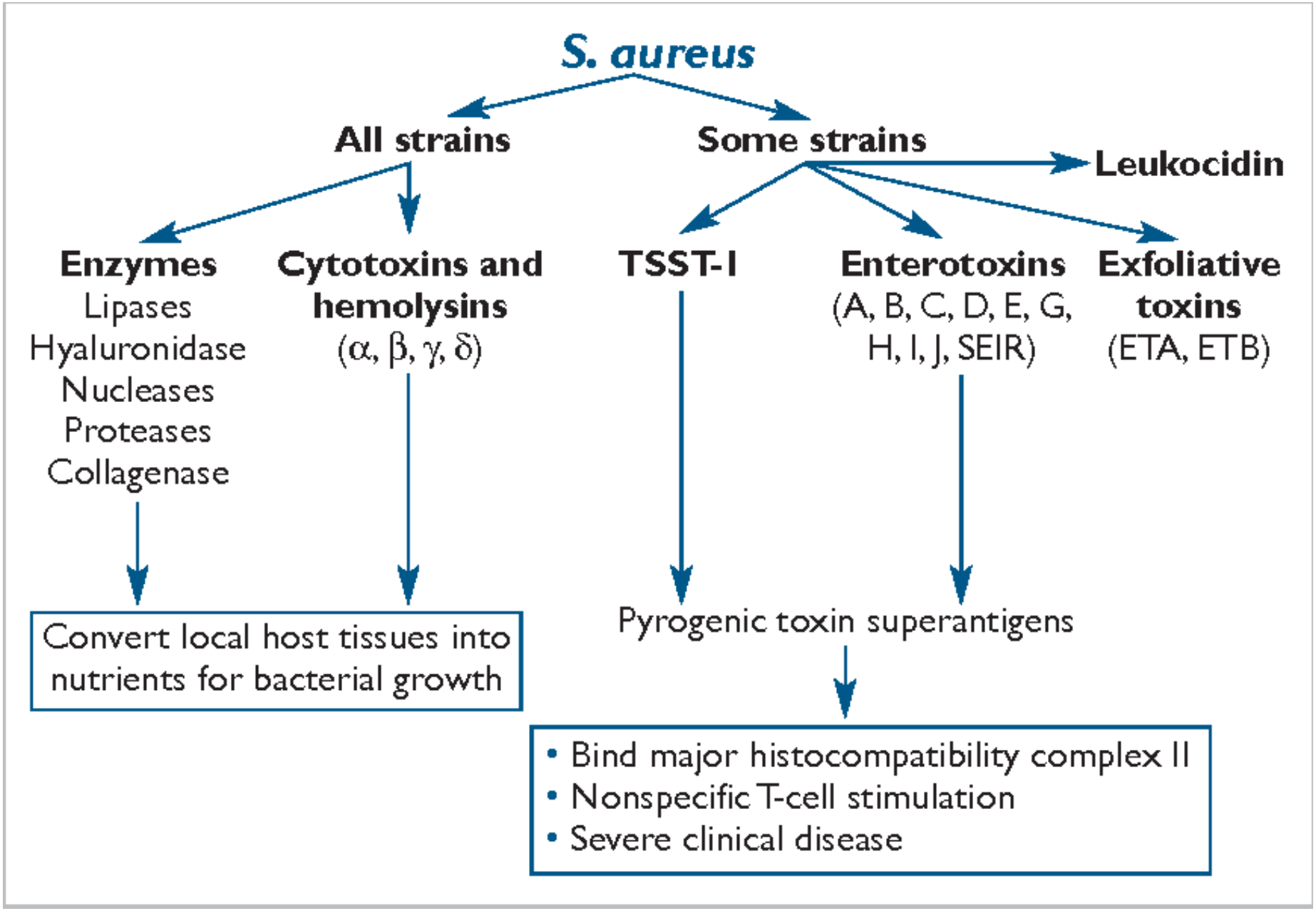
Virulence factors	Biological functions
Cell wall associated polymers and proteins	
Peptidoglycan	Inhibits chemotaxis of inflammatory cells
Capsular polysaccharide	Inhibits phagocytosis and chemotaxis
Teichoic acid	Mediates attachment of staphylococci to mucosal cell
Protein A	Chemotactic, anticomplementary, and antiphagocytic; causes platelet injury; and elicits l
Enzymes	
Coagulase	The enzyme coats the bacterial cells with fibrin, rendering them resistant to opsonizatio
Catalase	Produces nascent oxygen which causes oxidative damage to host tissue
Hyaluronidase	Hydrolyzes hyaluronic acids present in the matrix of the connective tissues, thereby facilitati tissues
Penicillinase	Inactivates penicillins
Nuclease	Hydrolyzes DNA
Lipases	Hydrolyzes lipids
Toxins	
Toxic shock syndrome toxin	Superantigen, stimulates the release of large amount of interleukins (IL-1 and IL-2)
Enterotoxin	Superantigen, acts by producing large amounts of interleukins (IL-1 and IL-2)
Exfoliative toxin	Splits intercellular bridges in the stratum granulosum of epidermis of the skin
Leukocidin toxin	Leukolysin is thermostable and causes lysis of leukocytes
Hemolysin	Causes lysis of erythrocytes

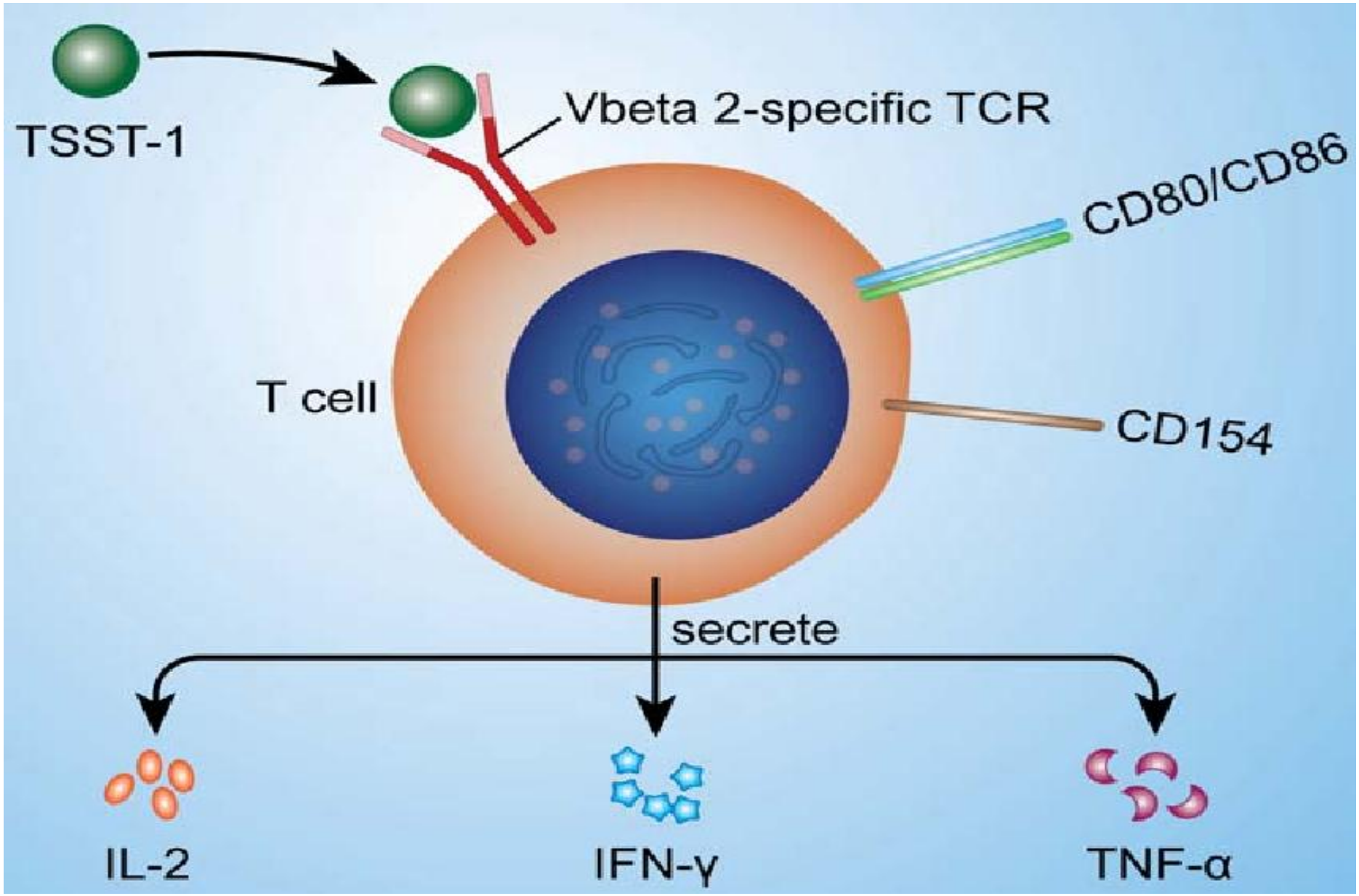
Staphylococcal mechanisms of adherence to and internalization into host cells.

(A) Schematic diagram of structural organization of FnBP from S. aureus. Gray items (A, B1, B2, C, D1, D2, D3, D4) represent an alternative nomenclature to the fibronectin-binding repeats. (B) The Fibronectin α5β1 integrin pathway for adherence and internalization of S. aureus (FnBP A/B), S. pseudintermedius (SpsD/L) and S. epidermidis (Embp). This internalization pathway was hypothesized for S. epidermidis (Embp) but refuted. (C) Staphylococcal secondary mechanisms involved in adherence to and internalization into host cells. Bacterial adhesins presented in the figure's panel are FnBP A/B, adhesion/autolysin family (Atl), fibrinogen binding adhesion (Fbl and ClfA), sdrD, Tet38, SraP, Eap, and GapC. The internalization pathway including Fbl, fibrinogen and host receptor was hypothesized for S. lugdunensis but refuted. Please refer to Table 1 for adhesin/Staphylococccus species concordance.


A
Adhesion to fibrinogen/elastin
Fibronectin-binding repeats
Wall-spanning domain
Membrane-spanning domain
N
S
N1
N2
N3
1
2
3
4
5
6
7
8
9
10
11
PPR
W
M
C
Signal sequence
A
B1
B2
C
D1
D2
D3
D4
Prolin-rich repeats
LPXTG
B
S. aureus
FnBPA/B
SpsD/L
Embp
Fibronectin
α5β1
Src
FAK
Cortactin
PI3-K
Akt
Actin
Host cell
C
S. aureus
FnBPA/B
Atl, AtlL, AtlC, Aas
Fbl
ClfA
SdrD
Tet38
SraP
Eap
GapC
Fibrinogen
Hsp60
Hsc70
Annexin 2
αVβ3
vW Factor
Desmoglein 1
CD36
gp340
Unknown receptor
Host cell


S. AUREUS
TOXINS
CYTOTOXINS
EXFOLIATIVE TOXIN
ENTEROTOXIN
TSST- 1
Alpha, Beta, Gamma, Delta
ET A, ET B
A-E, G-J, SEIR
Hematogenous spread of toxin to Stratum Granulosum
Act as super antigens
Cleavage of desmoglein 1, an adhesion protein of desmosome
Separation of cell junction in epidermis at stratum granulosum


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


TSST-1
Vbeta 2-specific TCR
CD80/CD86
T cell
CD154
secrete
IL-2
IFN-γ
TNF-α

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.

Epidemiology

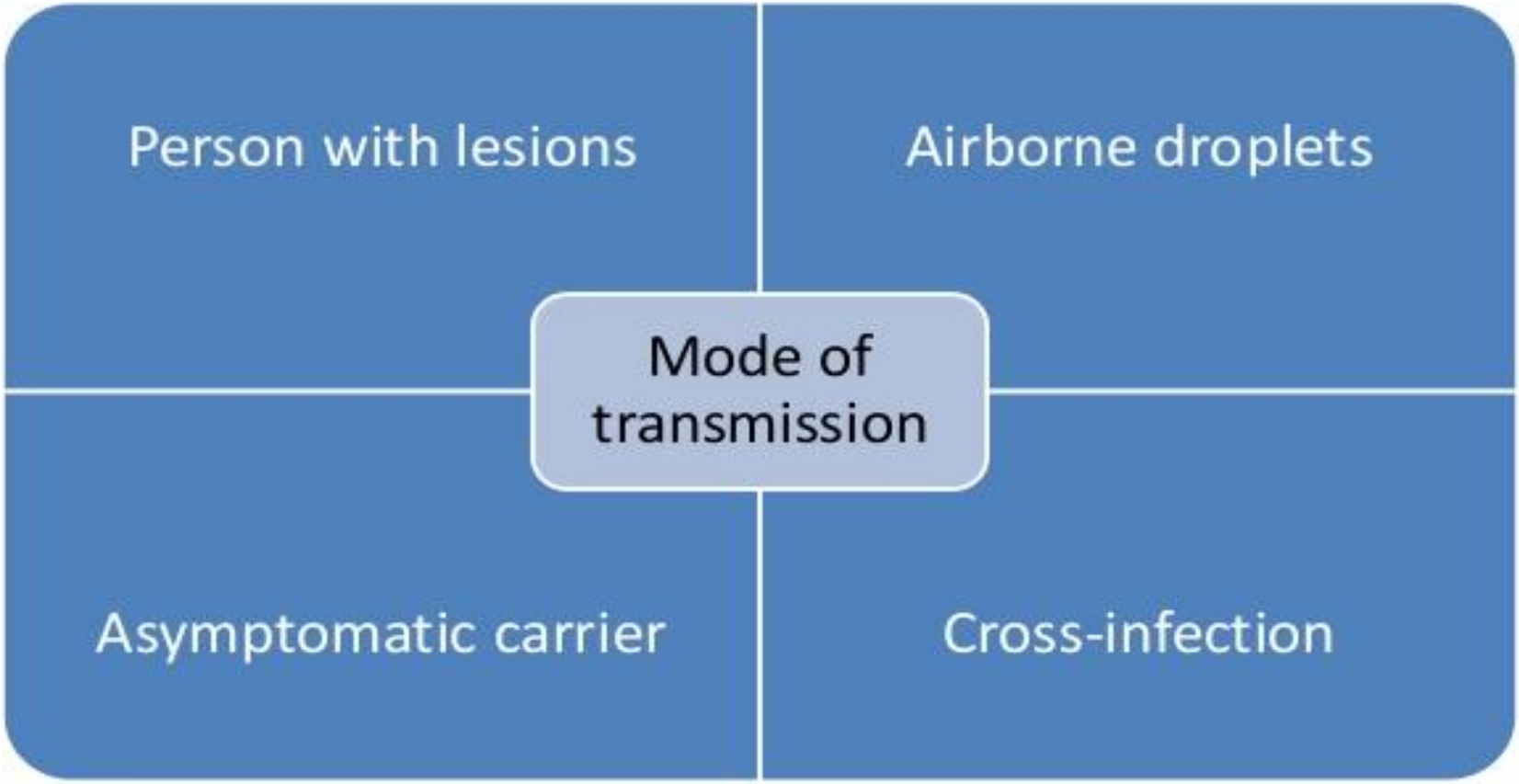
Staphylococci can permanently (coagulase-negative strains) or transiently (*S. aureus*) colonize various areas of the human body, with the anterior nasopharynx as the most common colonization site for *S. aureus* in older children and adults (30% of healthy adults.)

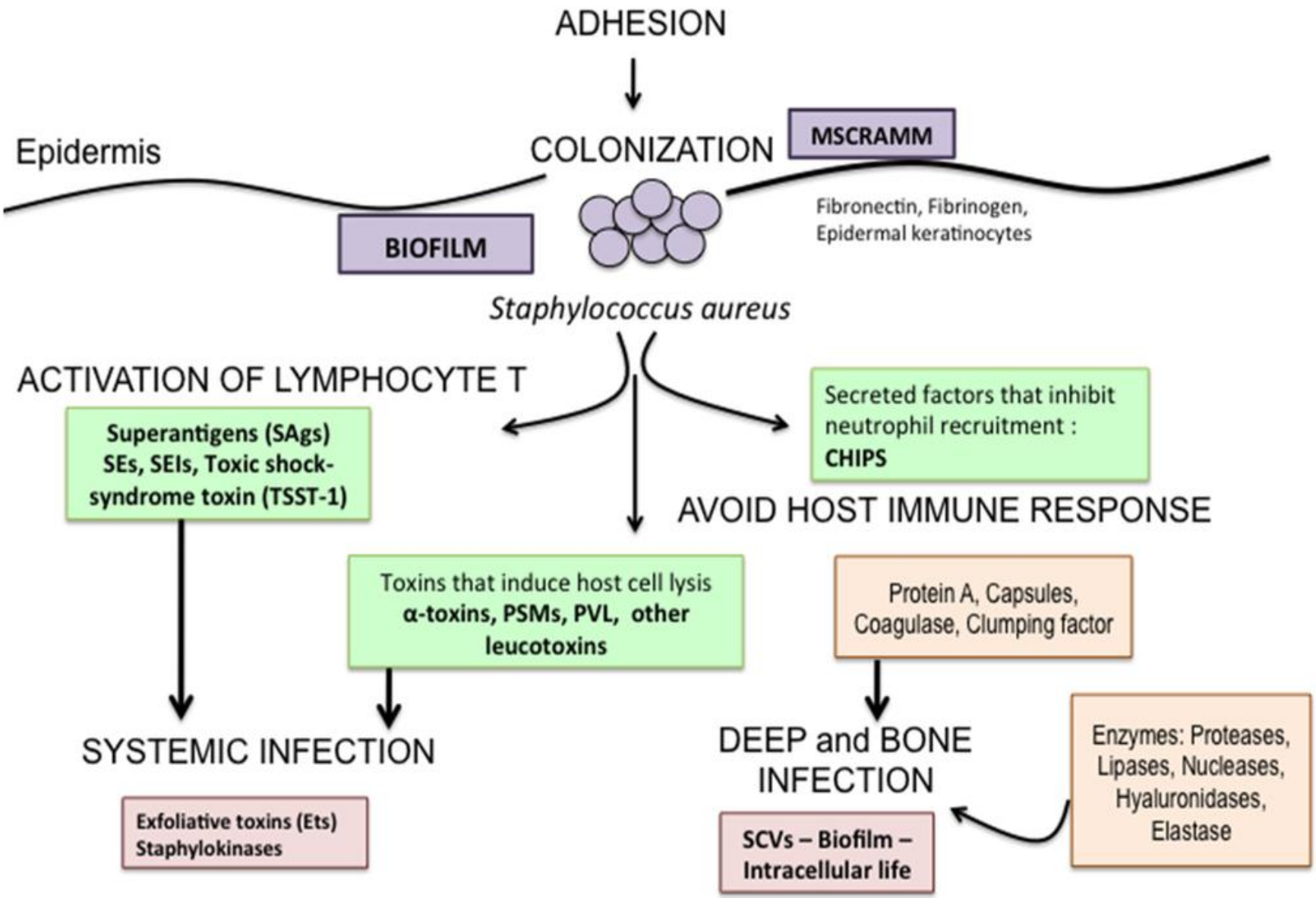
Nasopharyngeal or skin carriers of *S. aureus* are responsible for many hospital infections.

S. aureus can be transmitted through direct personal contact or contact with contaminated fomites.

Areas at highest risk for severe infections: new born nursery, ICU, operating rooms and cancer chemotherapy wards.

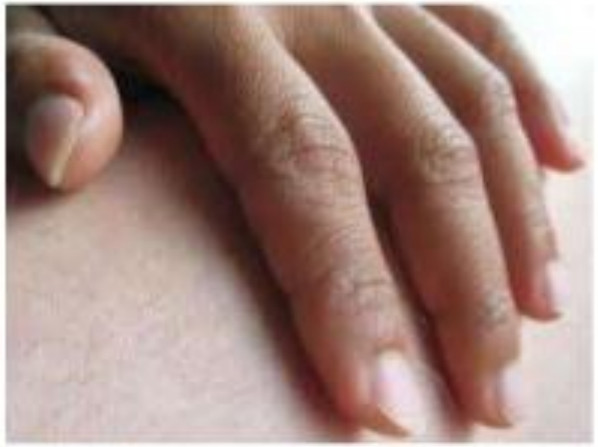
Mode Of Transmission



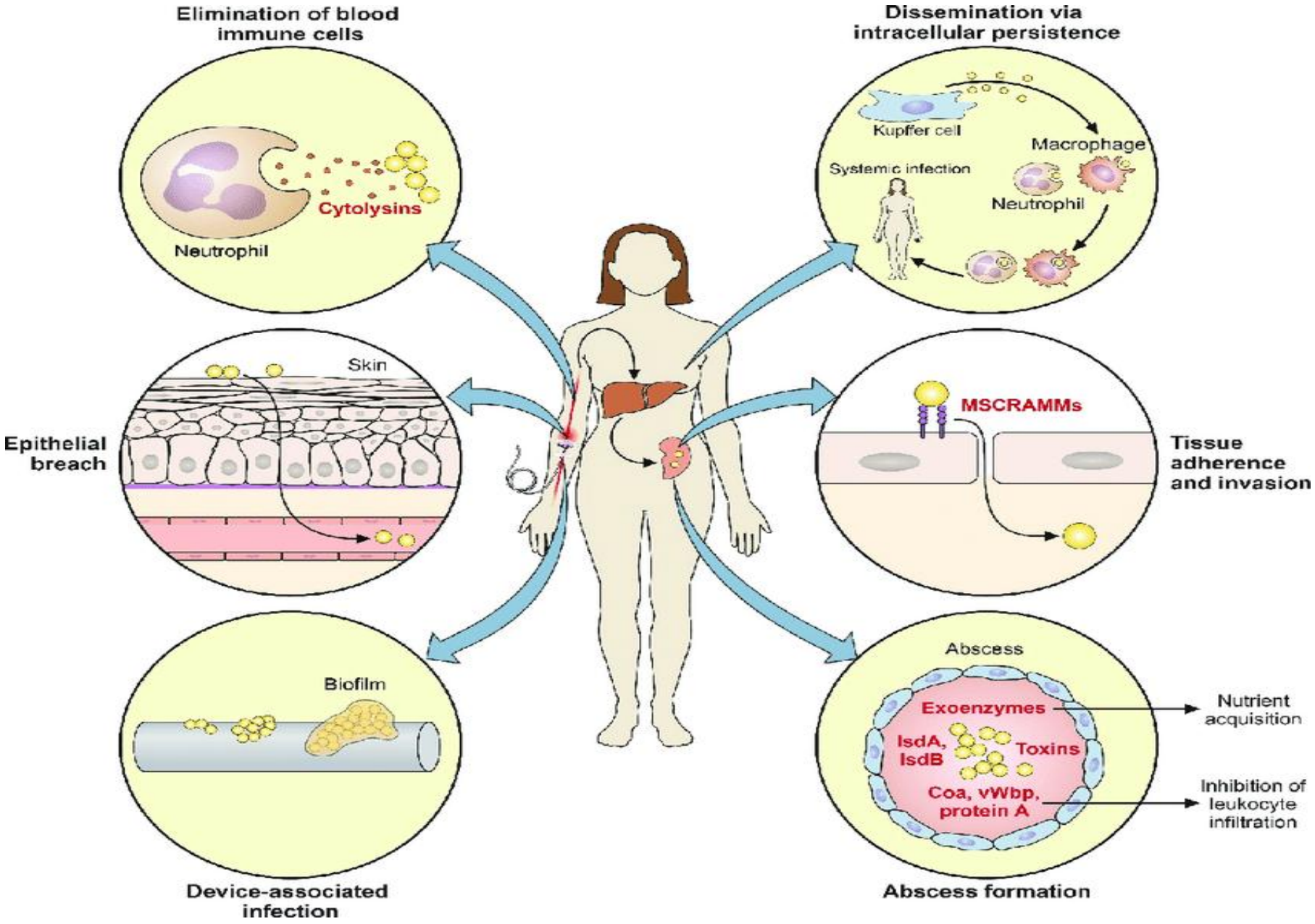

ADHESION
COLONIZATION
MSCRAMM
Epidermis
BIOFILM
Fibronectin, Fibrinogen,
Epidermal keratinocytes
Staphylococcus aureus
ACTIVATION OF LYMPHOCYTE T
Superantigens (SAgs)
SEs, SEIs, Toxic shock-syndrome toxin (TSST-1)
Secreted factors that inhibit neutrophil recruitment :
CHIPS
AVOID HOST IMMUNE RESPONSE
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

Origins of *S. aureus*

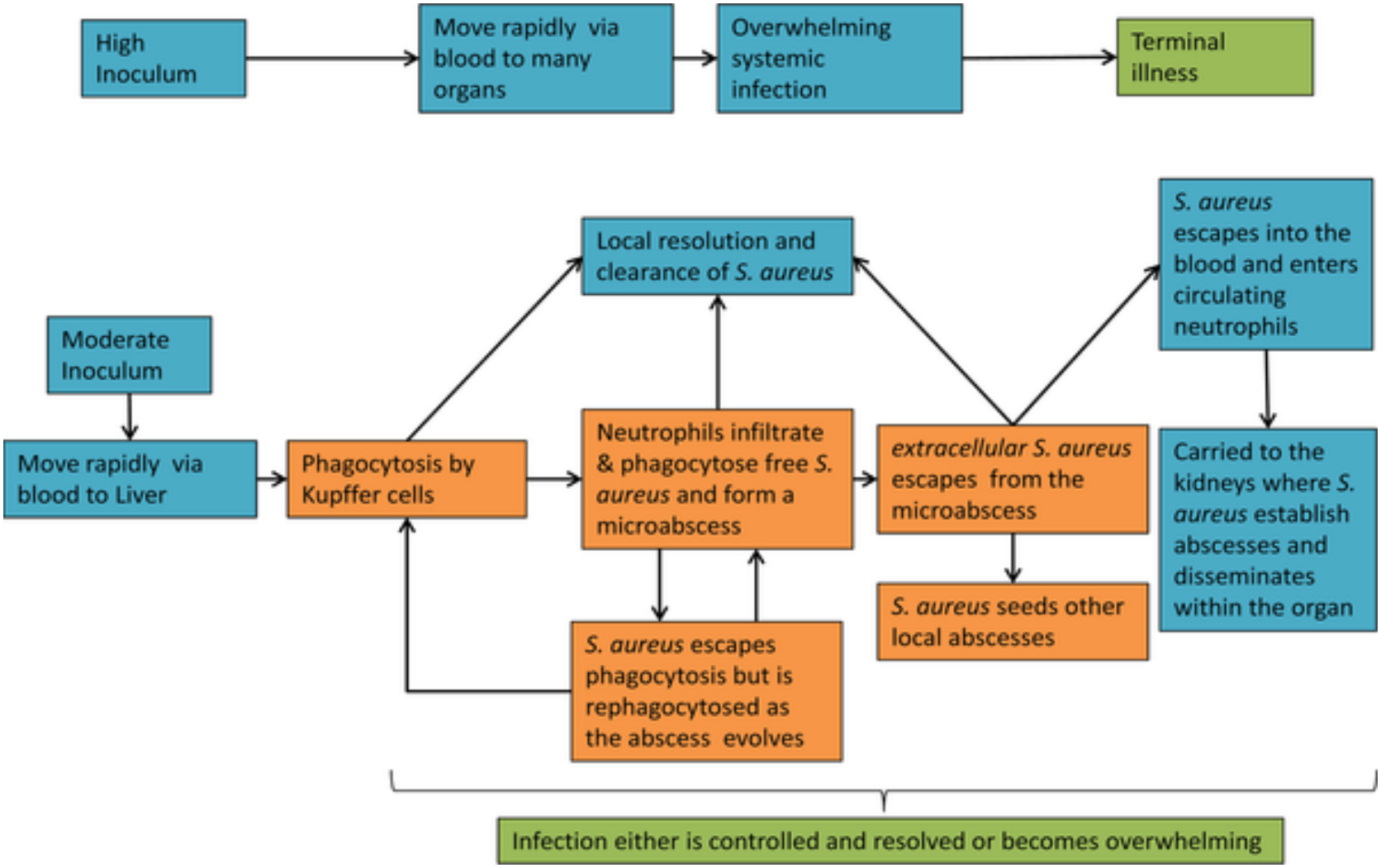
- Primary reservoirs are humans and animals



- Carried by up to 50% of humans (in nostrils, on skin or hair)


Elimination of blood immune cells
Cytolysins
Neutrophil
Dissemination via intracellular persistence
Kupffer cell
Macrophage
Systemic infection
Neutrophil
Skin
Epithelial breach
MSCRAMMs
Tissue adherence and invasion
Biofilm
Device-associated infection
Abscess
Exoenzymes
IsdA, IsdB
Toxins
Coa, vWbp, protein A
Nutrient acquisition
Inhibition of leukocyte infiltration
Abscess formation

S.aureus pathogenesis



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

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

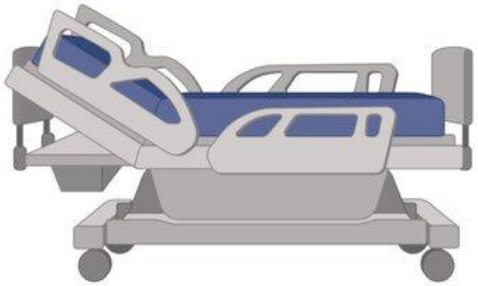
Carriage

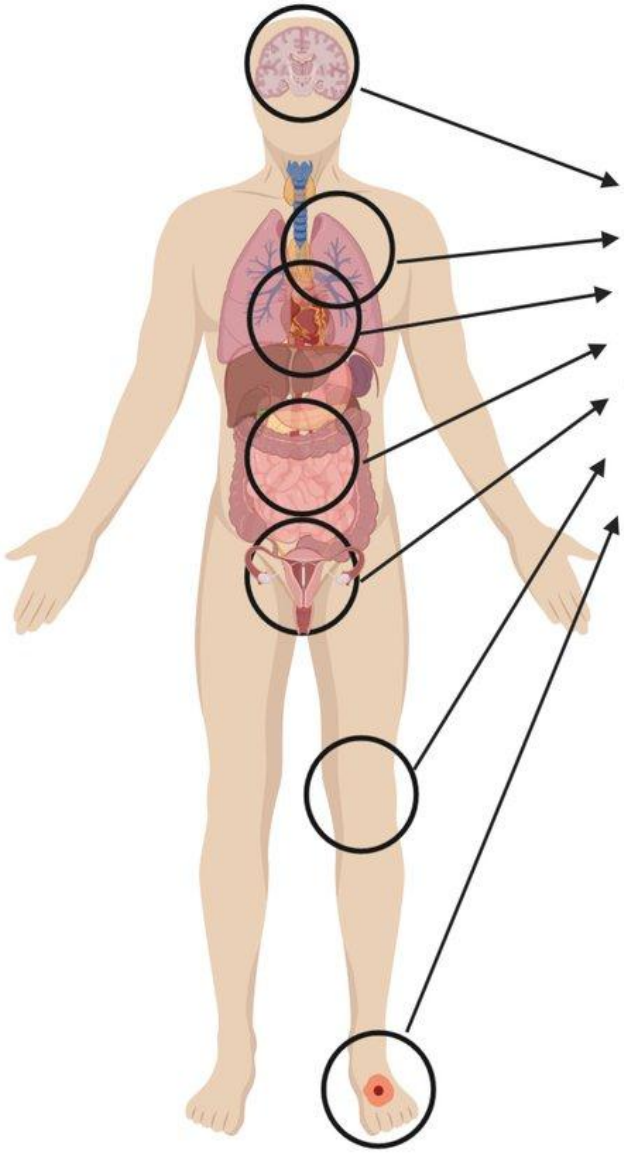
1 in 3 people

Incidence

323,700 cases

10,600 deaths in US





Diseases

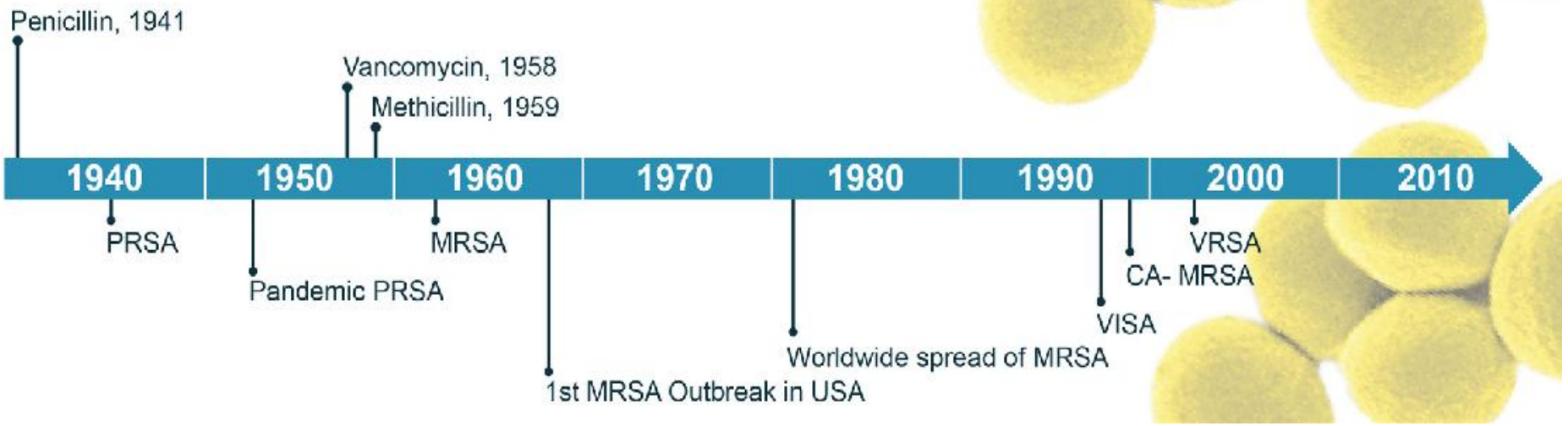
Mild to severe

- Meningitis/ Brain Abscess
- Pneumonia
- Endocarditis
- Food poisoning
- Toxic shock syndrome
- Osteomyelitis
- Skin and soft tissue

Antibiotic resistance

MRSA, VISA, VRSA

Staphylococcus aureus Drug Resistance and Epidemics



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.

MRSA - methicillin-resistant Staphylococcus aureus

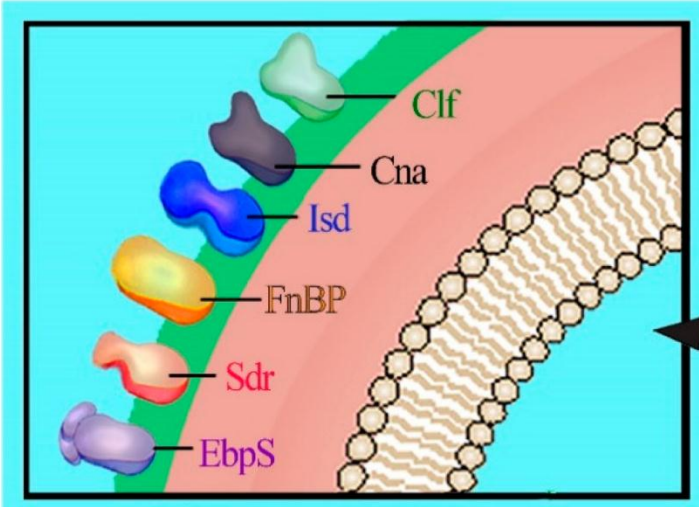
In hospitals, MRSA can cause serious problems (bloodstream infections (sepsis, bacteremia), pneumonia, surgical infections). **Mupirosin for carrier**

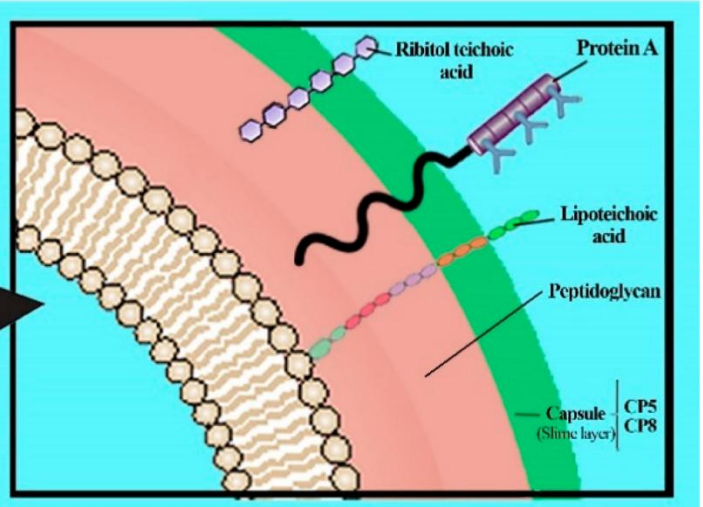
Methicillin resistance of S. aureus strains is associated with gene mutations in them, which encode the synthesis of penicillin-binding proteins (PBPs) that cannot combine with beta-lactam antibiotics. For this reason, methicillin-resistant S. aureus (MRSA) strains are resistant to all beta-lactam antibiotics.

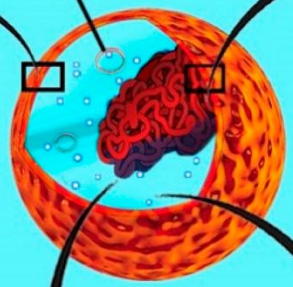
This resistance is related to the Mec A gene.

MRSA

- Most strains of *S.aureus*, even those acquired in community, are penicillin resistant
  - Resistance is attributable to beta-lactamase production due to genes located on extrachromosomal plasmids.
- Some are resistant to the newer beta-lactamase resistant semisynthetic penicillins, such as methicillin, oxacillin, nafcillin.
  - Resistance is due to presence of unusual penicillin-binding protein(PBP)in the cellwall of resistant strains
- Infection with MRSA is likely to be more severe and require longer hospitalization, with incumbent increased costs than infection with a methicillin susceptible strain.







MSCRAMMs (Adhesions)

1. Clf { ClfA, ClfB } Clumping factor
2. Cna: Collagen-binding protein
3. Isd: Iron surface determinant
4. FnBP { FnBPA, FnBPB } (Fibronectin -binding protein)
5. Sdr (SdrE): Serine-Aspartate repeat
6. EbpS

Toxins

1. Membrane-damaging toxins (Pore-forming toxin)
   - Hemolysin: α-Toxin, β-Toxin, γ-Toxin, δ-Toxin
   - P-V Leukocidine
2. Exfoliative toxin (Epidermolytic toxin): ETA, ETB, ETD
3. Enterotoxin (A-E , G-X)
4. Toxic shock syndrome toxin-1 (Menstruation-associated toxin)

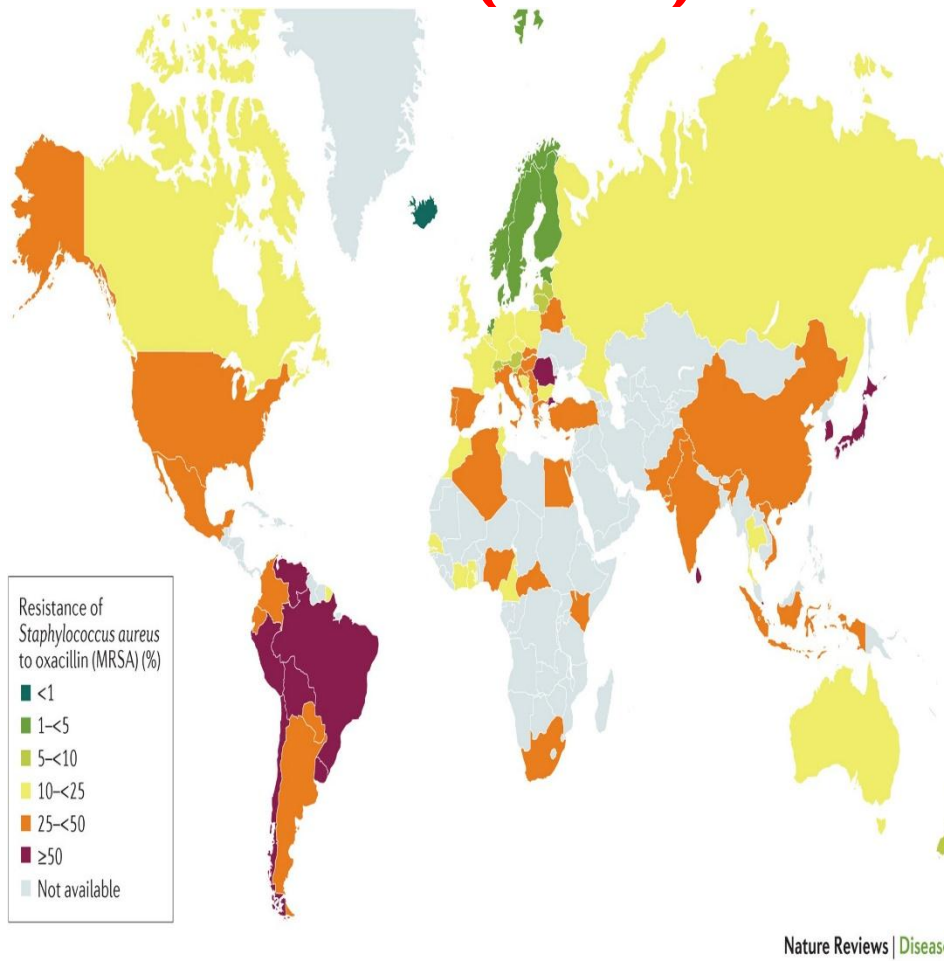
Superantigens

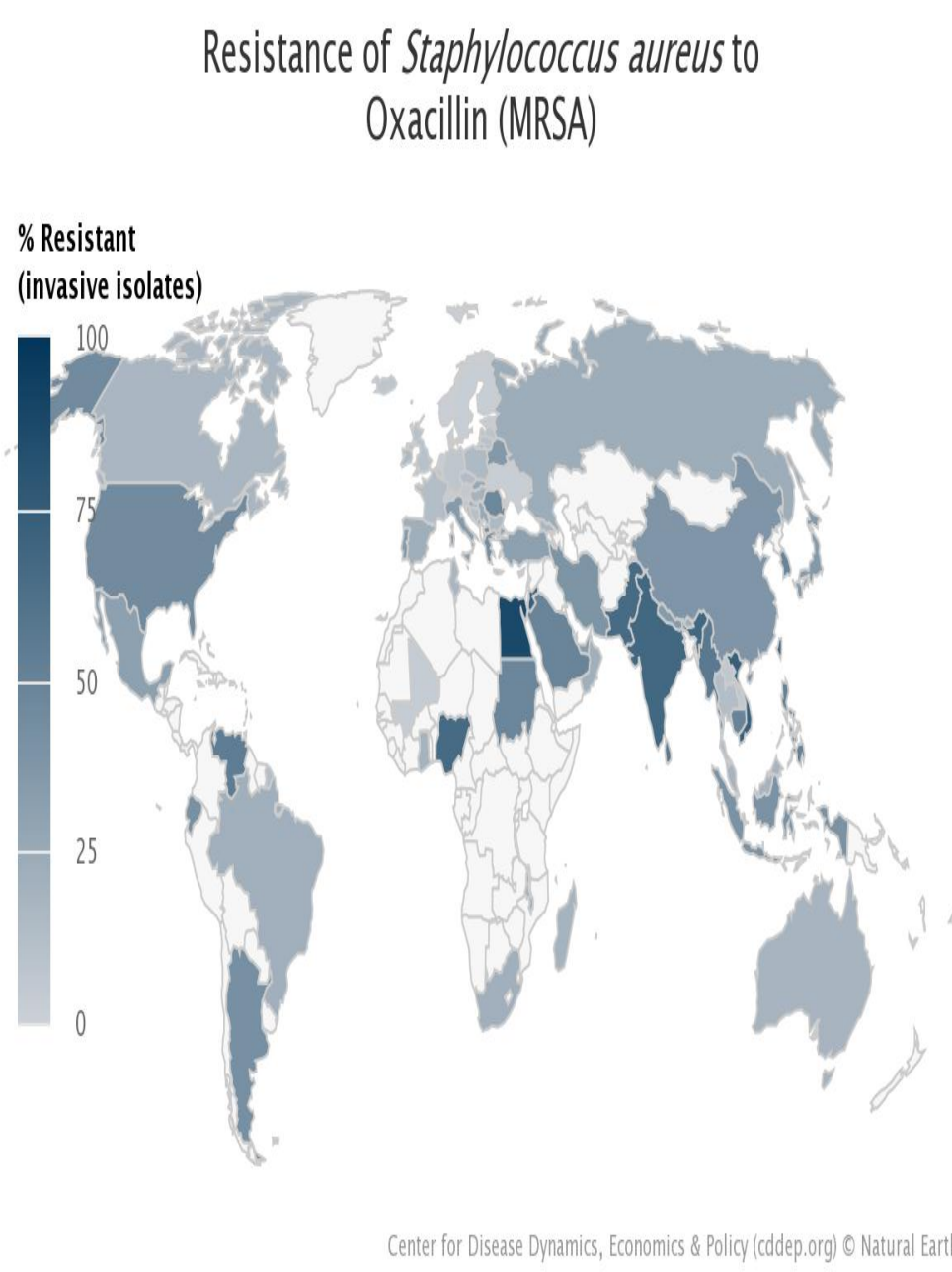
Enzymes

1. Spreading factor (invasion factor)
2. Survival factor

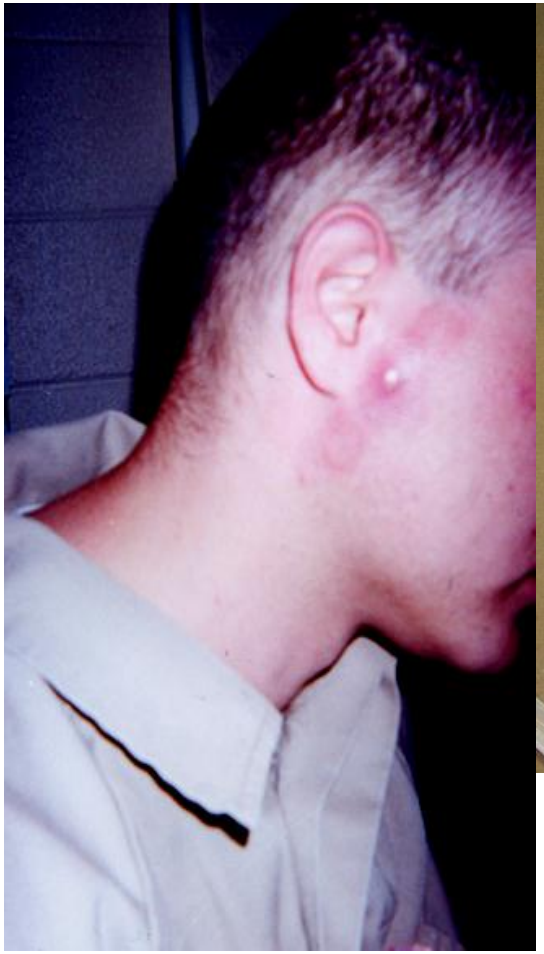
Coagulase, Catalase, Hyaluronidase, Staphylokinase (fibrinolysin), Lipase (SAL1, SAL2), Nuclease, Penicillinase, FAME, Protease (SSPA, SSPB, AUR)

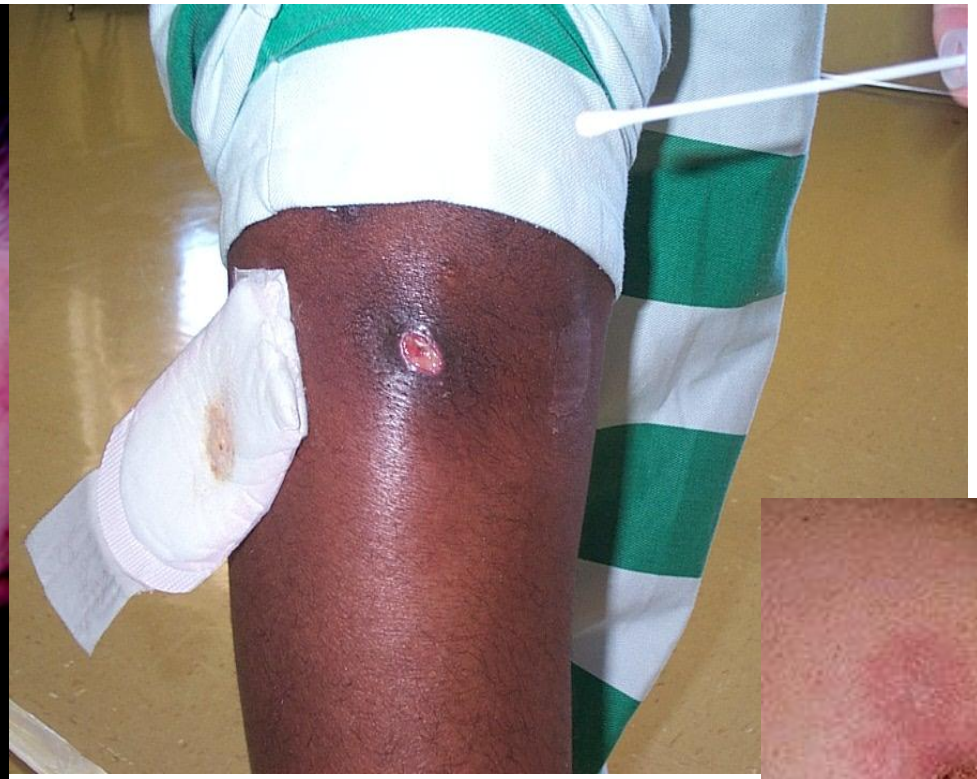
Methicilllin-resistant *Staphylococcus aureus* (MRSA) – **epidemiolog (2018)**

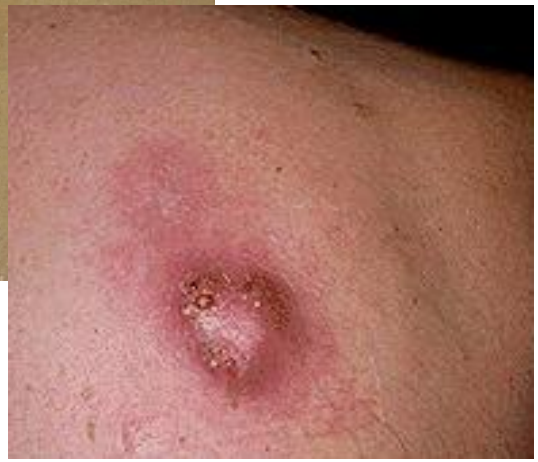




Wound infections caused by the MRSA strain







Other forms of resistance in Staphylococci

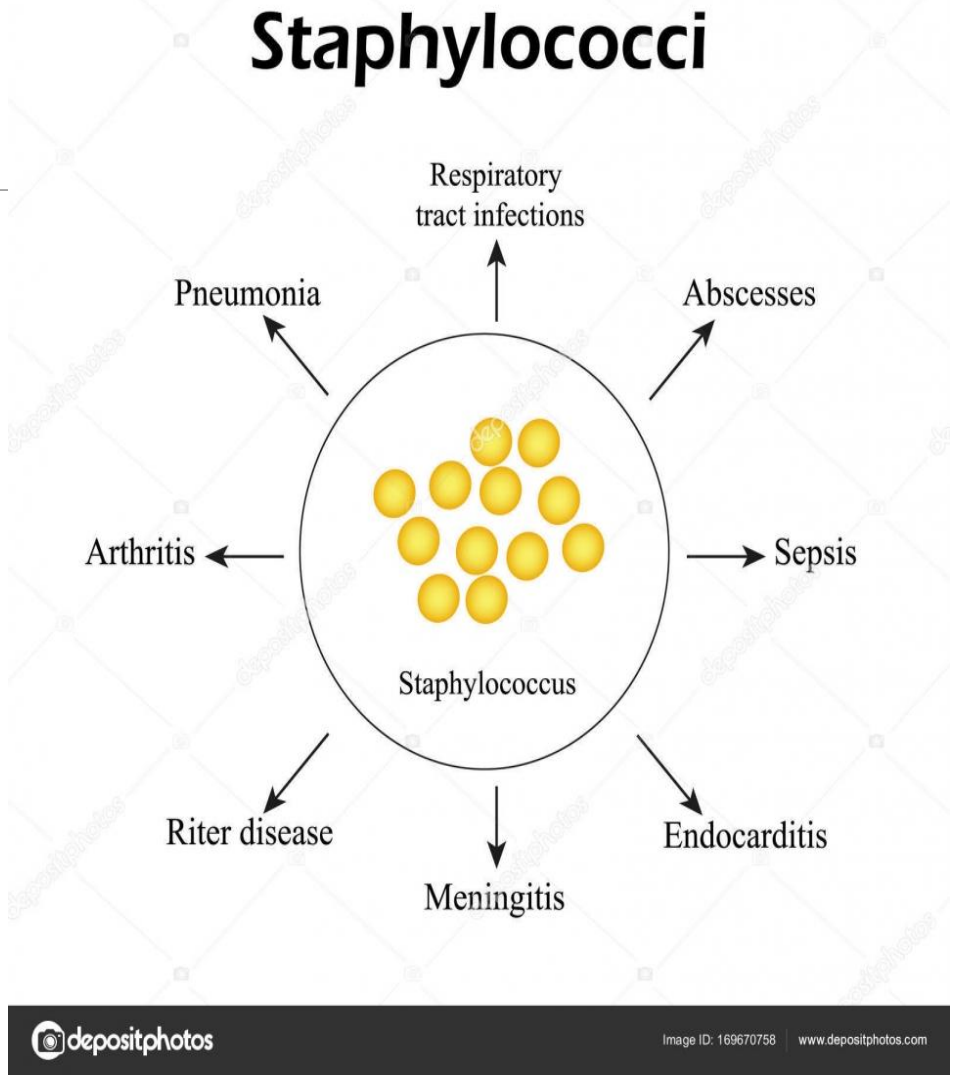
In recent years, glycopeptide-resistant *S.aureus* strains have also been reported.

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.

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**



- **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

Clinical Syndromes

1. **Cutaneous infections**
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   - endocarditis
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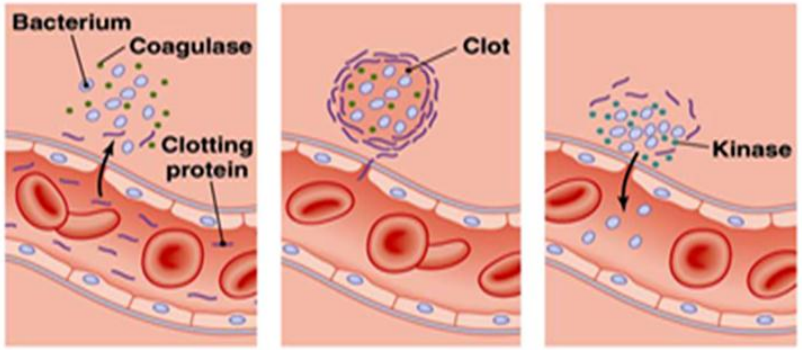
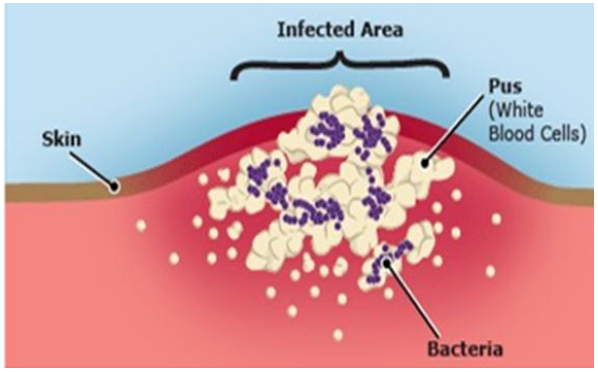
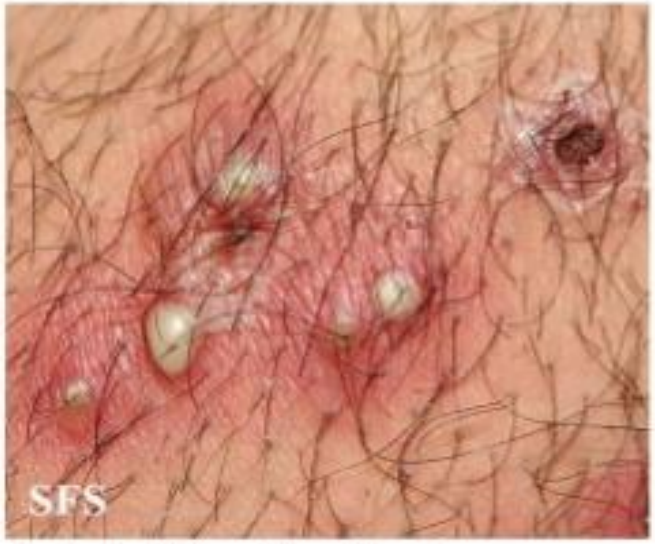
Koaqulaza

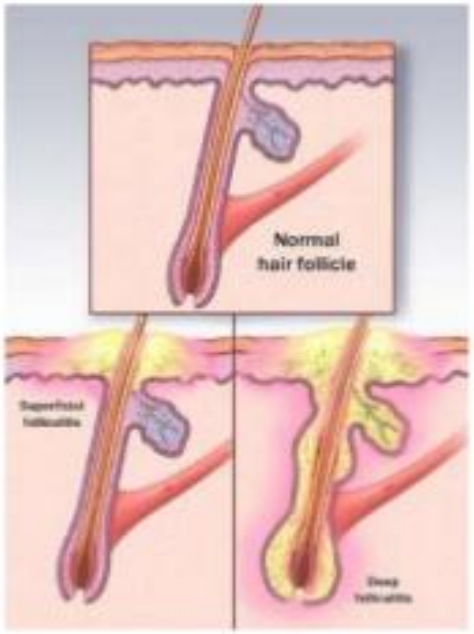




1) Cutaneous Infections

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

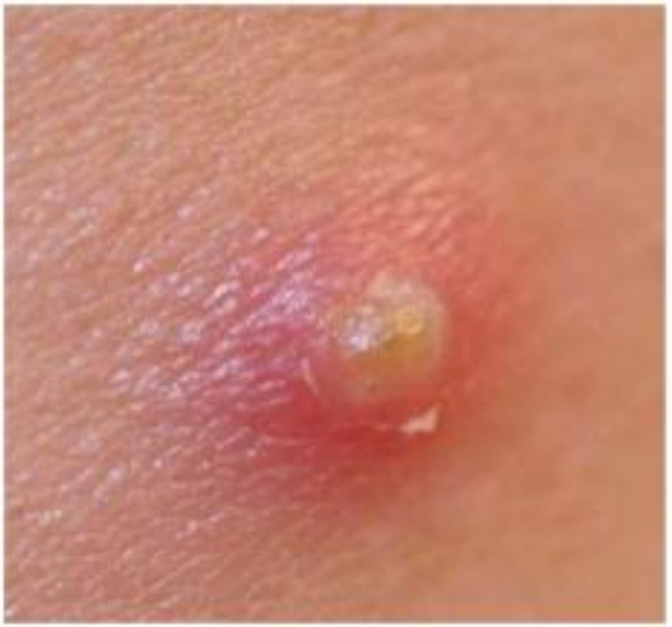




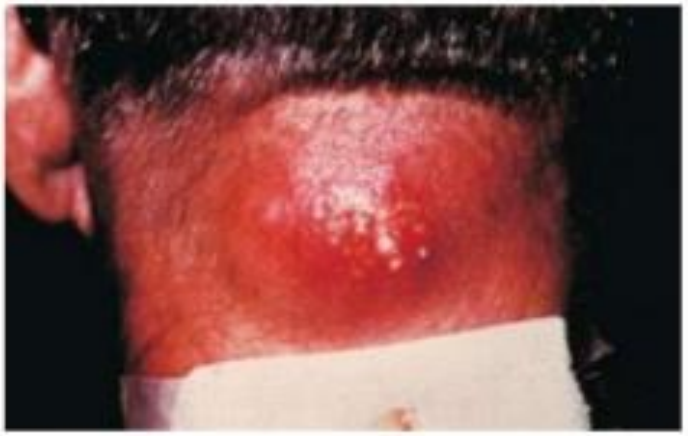
•**Sty**: A sty is folliculitis affecting one or more hair follicles on the edge of the upper or lower eyelid.

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

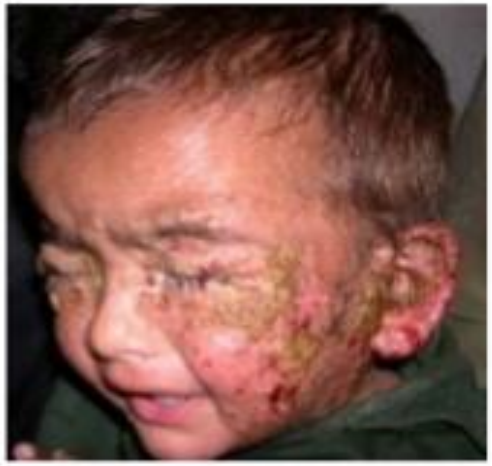


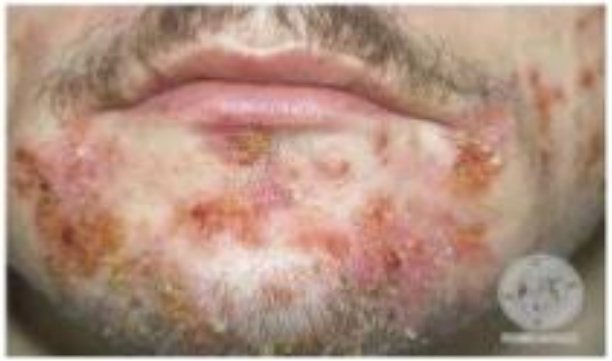
- **Carbuncle**: Carbuncle is an aggregation of infected furuncles. Carbuncles may form large abscesses.
- It is a large area of redness, swelling and pain, punctuated by several sites of drainage pus.

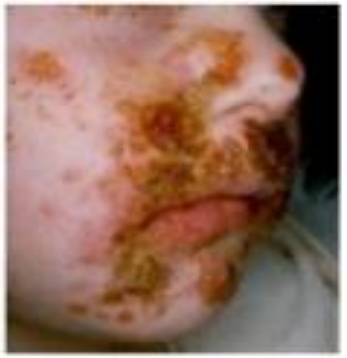


Cutaneous Infections(contd....)

- 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







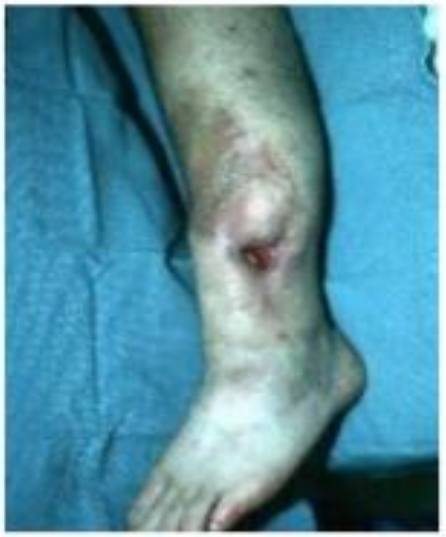
impetigo with vesicles, pustules, and sharply demarcated regions of honey-colored crusts.

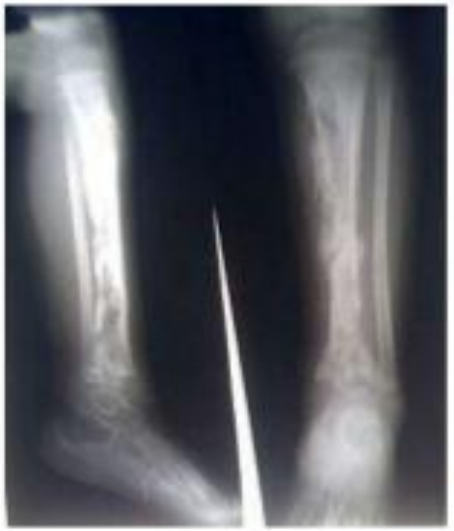
2) Deep Infections

- **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,

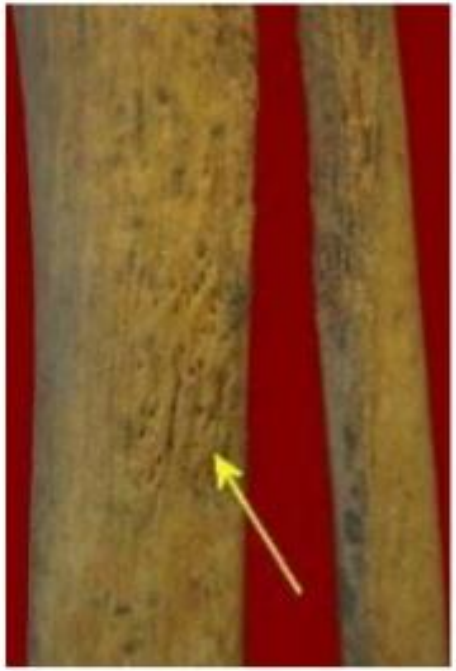
  Diagnosis: X-ray, MRI, bone aspirates

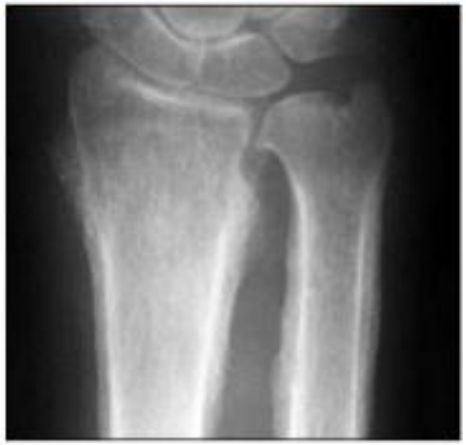




Deep Infections(contd....)

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

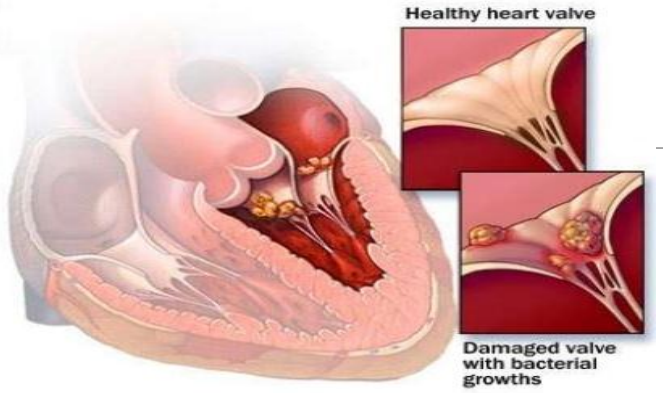


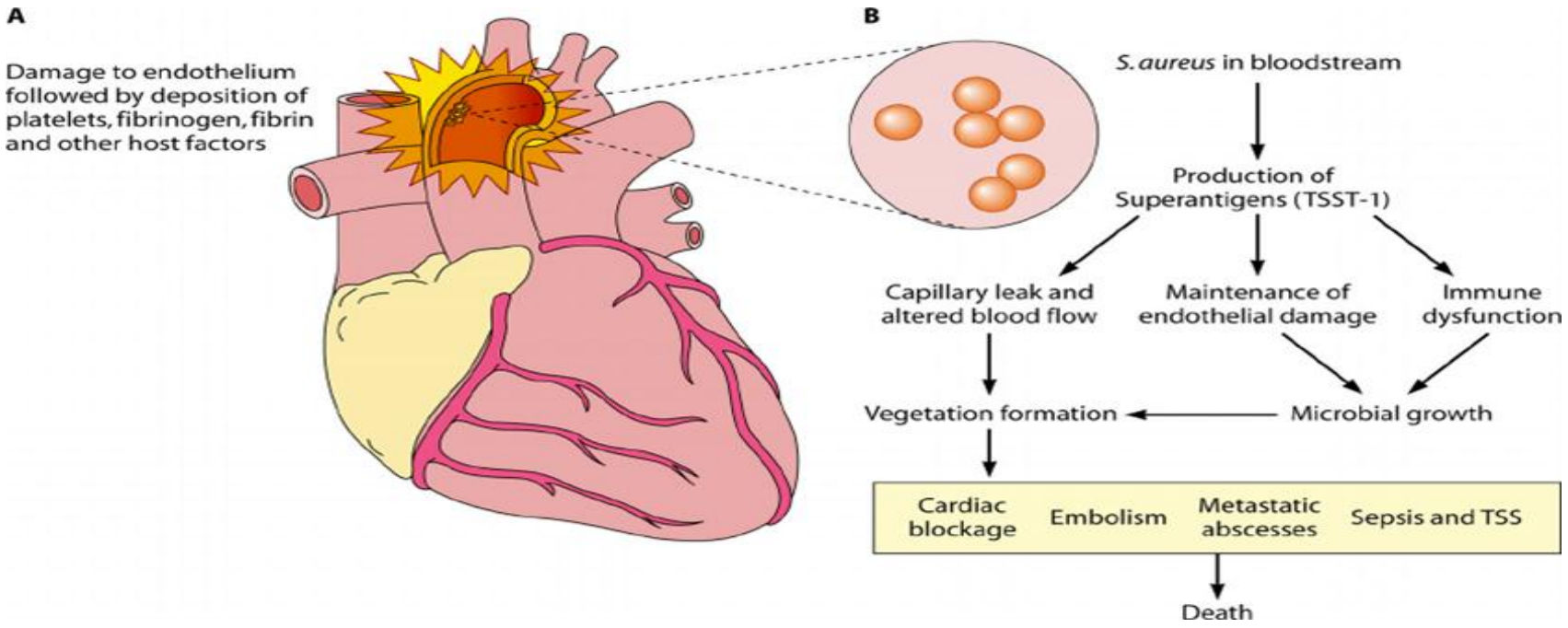


Deep Infections(....contd)

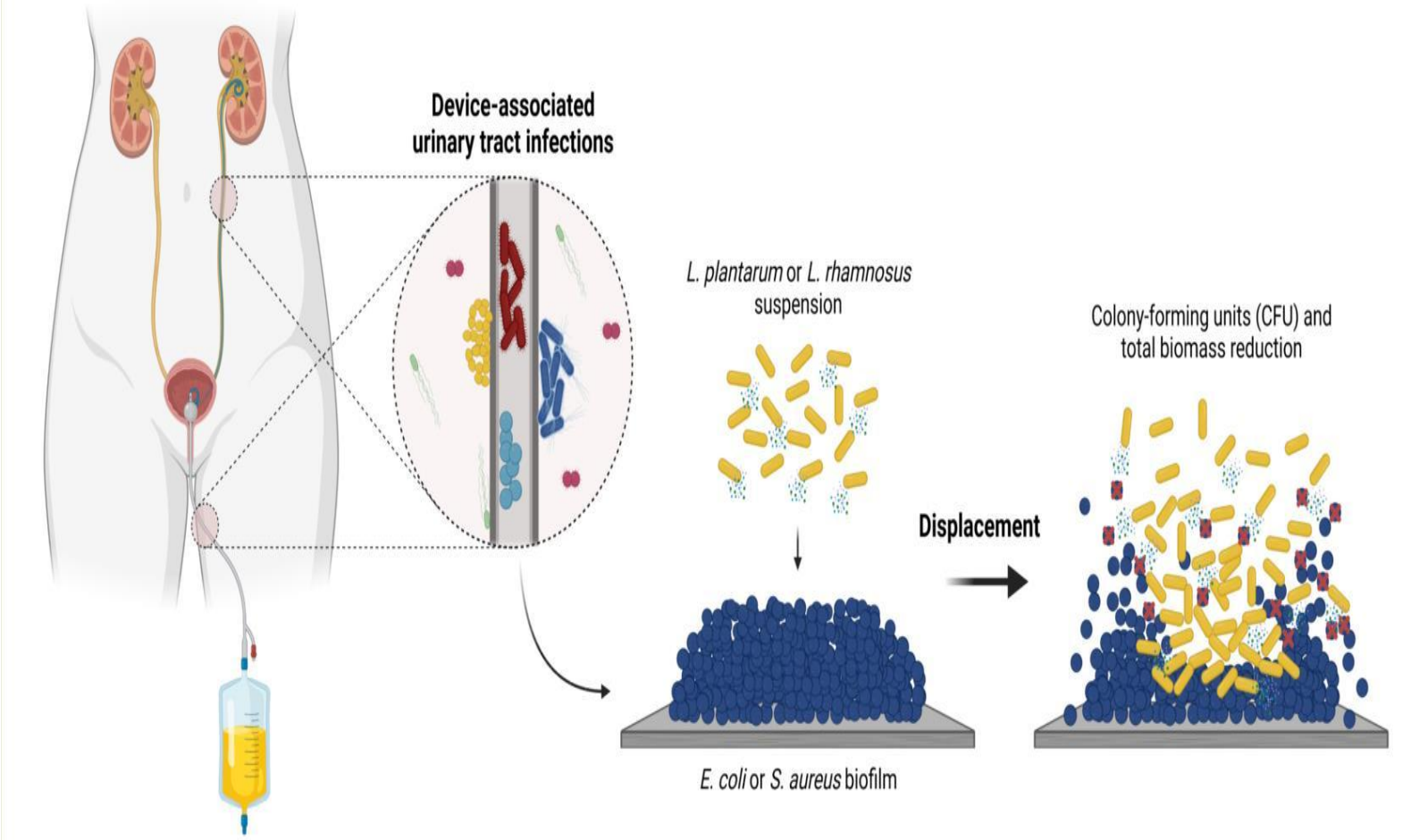
- **Endocarditis**: It is an inflammation of the inner layer of the heart, the endocardium

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





URİNARY TRACT DİSEASES

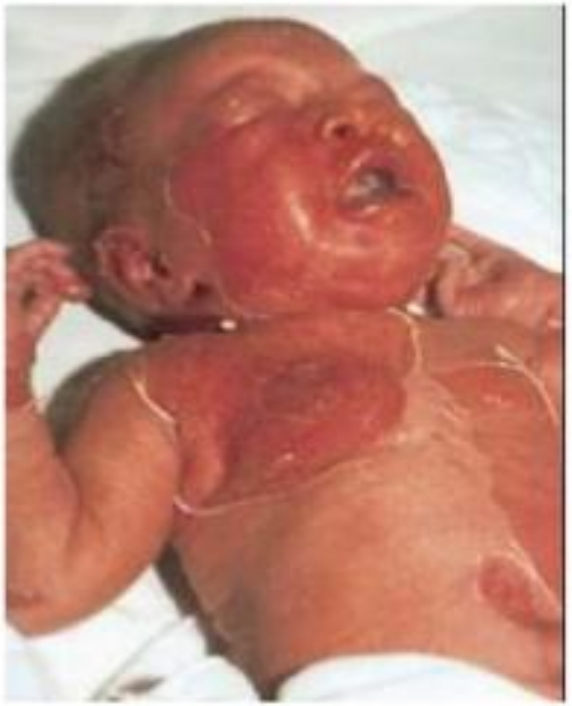


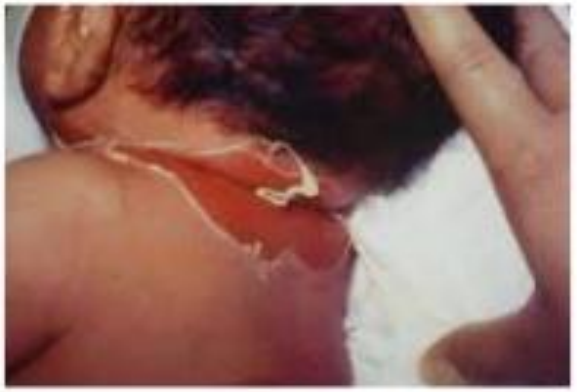
3)Exfoliative Disease

- (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

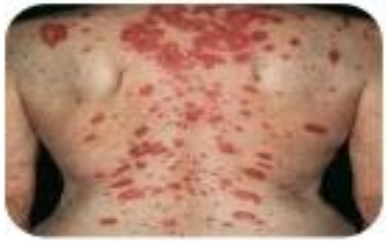
Headache

Vomiting

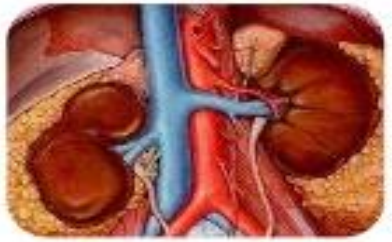
Diarrhoea

Conjunctival reddening

Hypotension



Skin rashes

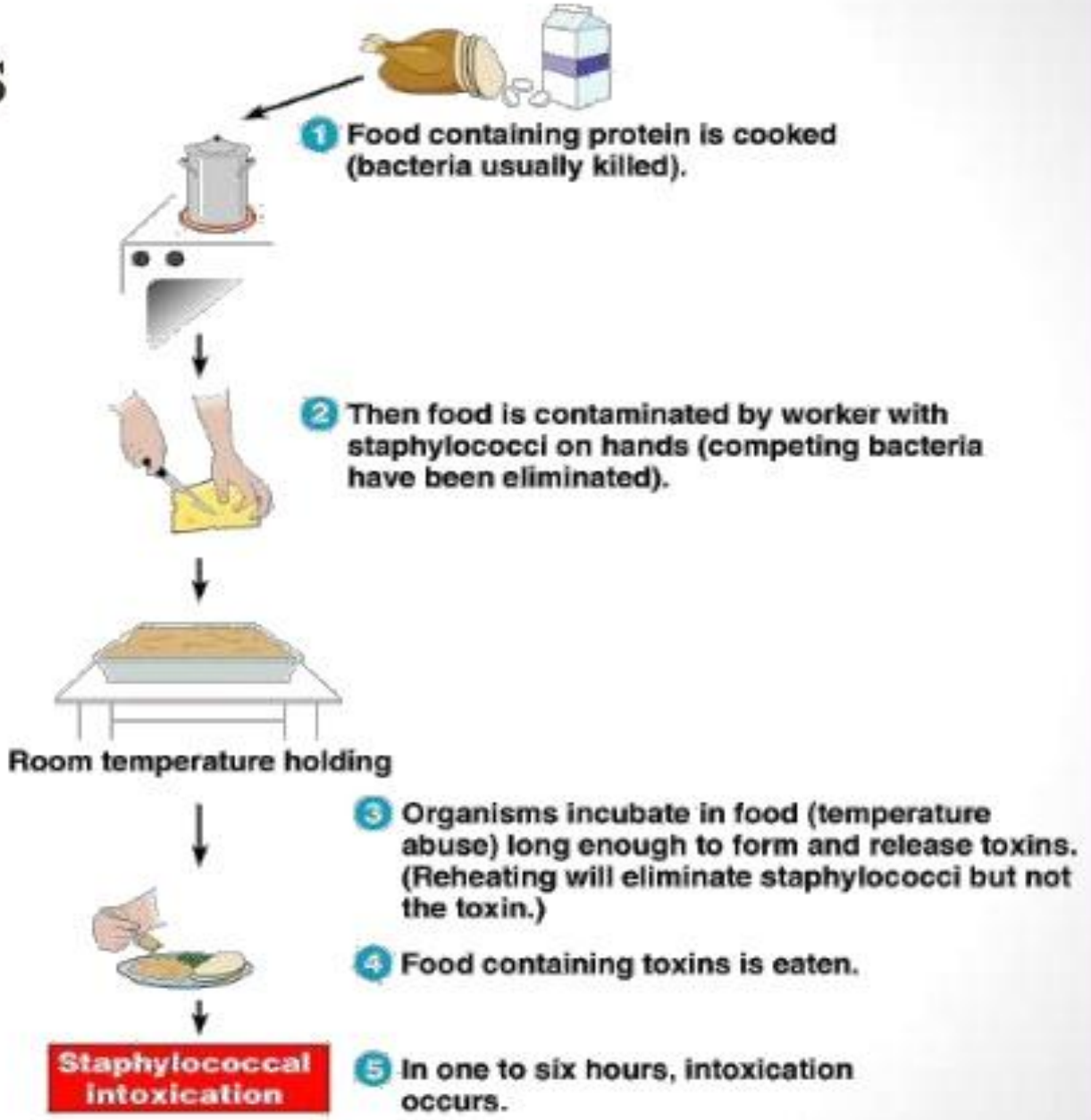


Kidney failure

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.

Causes


1 Food containing protein is cooked (bacteria usually killed).
2 Then food is contaminated by worker with staphylococci on hands (competing bacteria have been eliminated).
Room temperature holding
3 Organisms incubate in food (temperature abuse) long enough to form and release toxins. (Reheating will eliminate staphylococci but not the toxin.)
4 Food containing toxins is eaten.
Staphylococcal intoxication
5 In one to six hours, intoxication occurs.

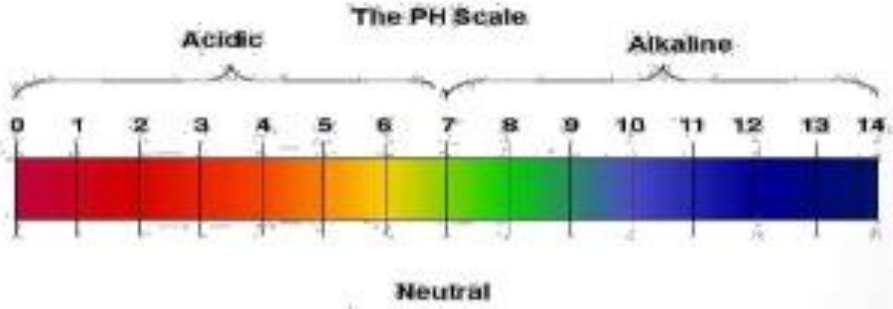
WHAT IS STAPHYLOCOCCAL FOOD POISONING??

- Food-borne intoxication
- Often abrupt and severe in onset
- Occurs usually 2-8 hours after eating
- Severe nausea
- Caused by **staphylococcal enterotoxins ,** protein exotoxins released during growth of ***Staphylococcus aureus***

Properties of Staphylococcal Enterotoxins

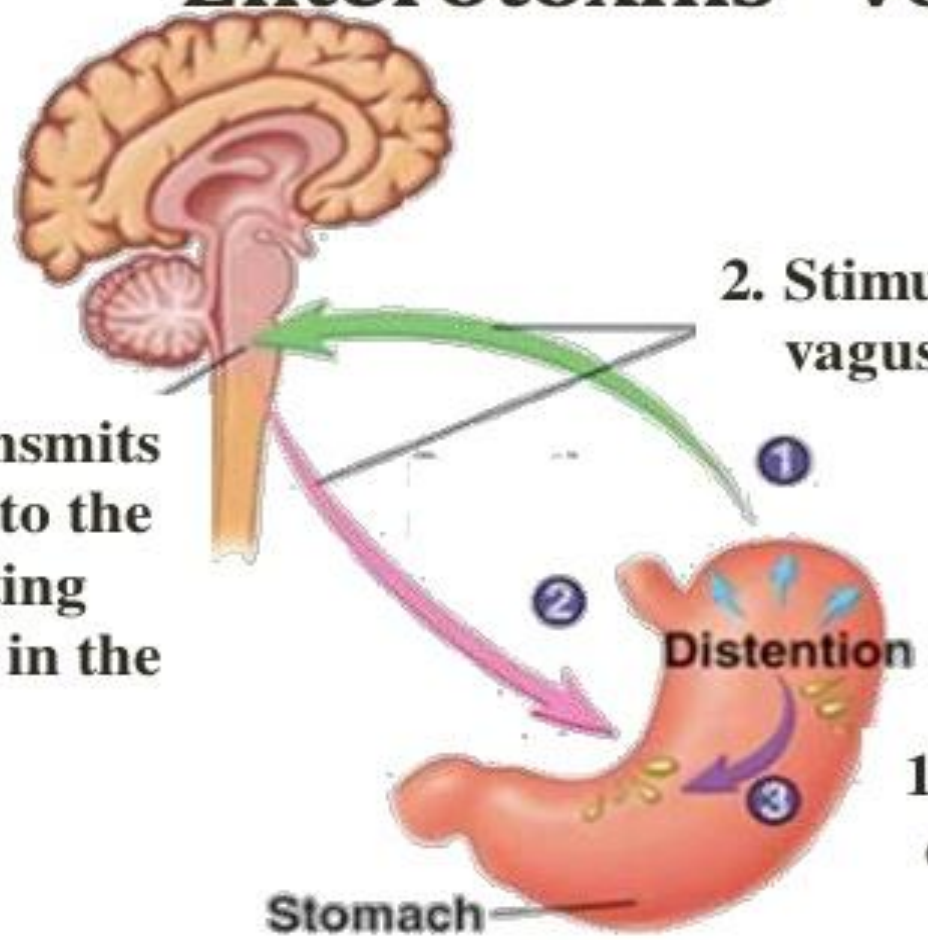
- Resistant to heat treatment

- Stable over a wide pH range



- Resistant to gastrointestinal and other proteases

Mechanism of Staphylococcal Enterotoxins - vomit



Mechanism of Staphylococcal Enterotoxins – Immune response

Staphylococcal penetrate the gut lining

Activate local and systemic immune response

Release inflammatory mediators

Vomit

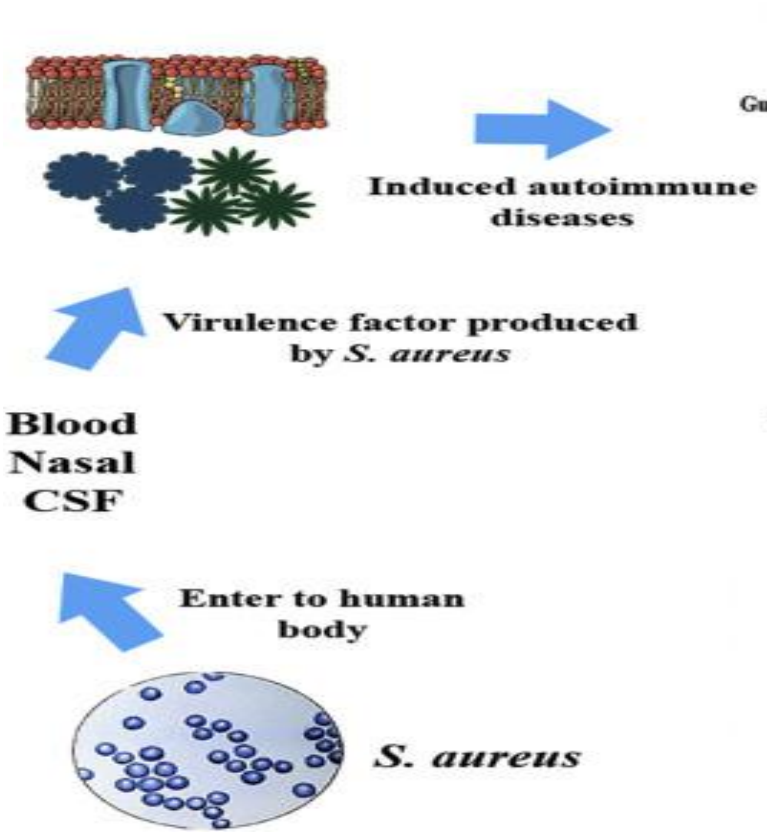
Mechanism of Staphylococcal Enterotoxins - Diarhea

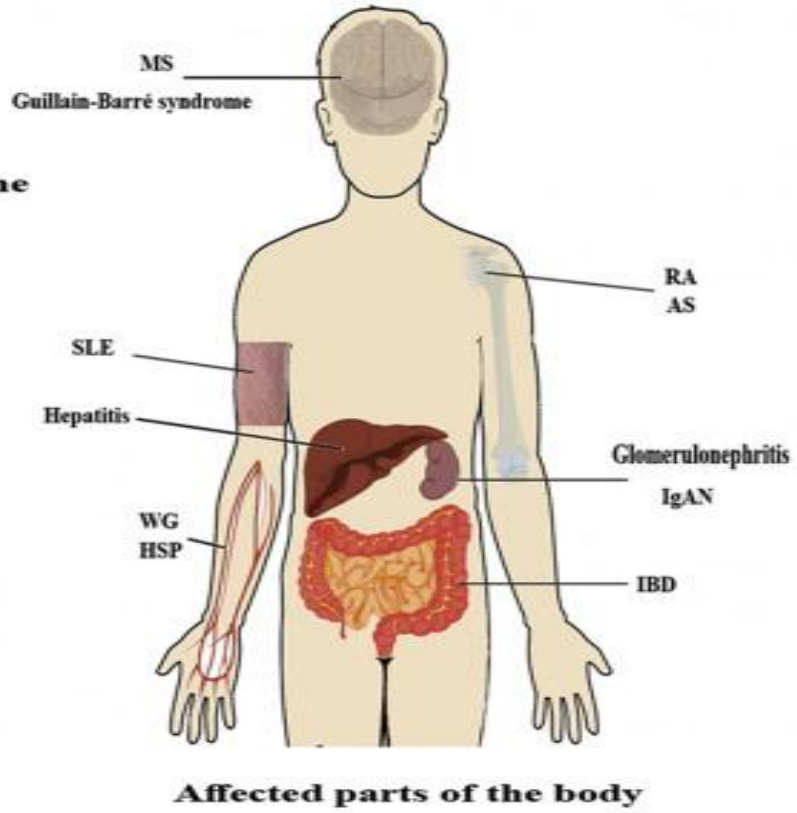
Staphylocaccal Enterotoxins disturb the activity in the large intestine

Water can't be reabsorption in large intestine

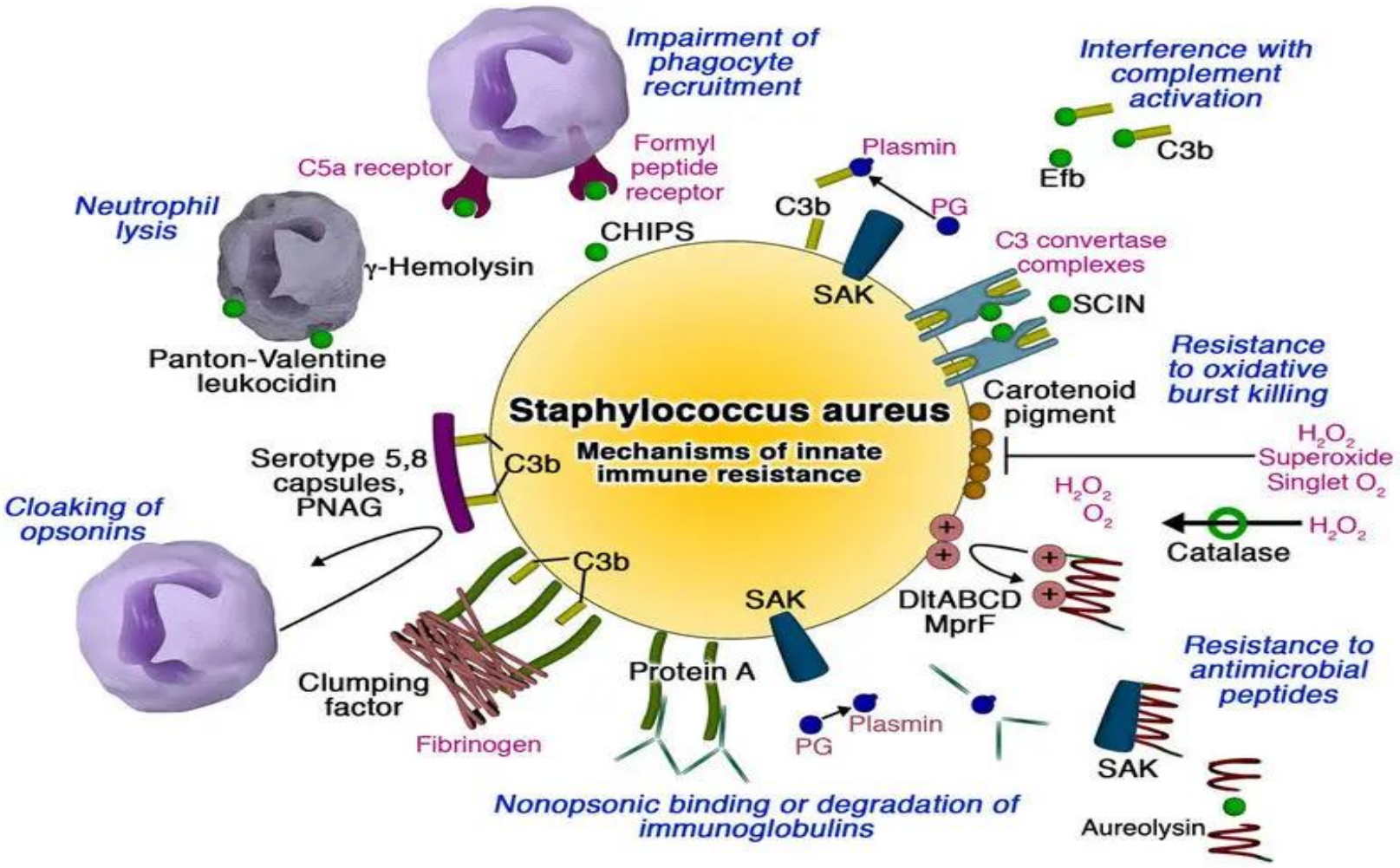
Diarrhea

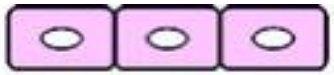
S.aureus in autoimmune diseases





İMMUNİTY


Impairment of phagocyte recruitment
Formyl peptide receptor
C5a receptor
Neutrophil lysis
γ-Hemolysin
Panton-Valentine leukocidin
CHIPS
Plasmin
PG
C3b
SAK
Interference with complement activation
C3b
Efb
C3 convertase complexes
SCIN
Resistance to oxidative burst killing
Carotenoid pigment
H_2O_2
Superoxide
Singlet O_2
H_2O_2
O_2
H_2O_2
Catalase
DltABCD
MprF
Staphylococcus aureus
Mechanisms of innate immune resistance
Serotype 5,8 capsules, PNAG
C3b
Cloaking of opsonins
C3b
Clumping factor
Fibrinogen
SAK
Protein A
Plasmin
PG
Resistance to antimicrobial peptides
SAK
Aureolysin
Nonopsonic binding or degradation of immunoglobulins



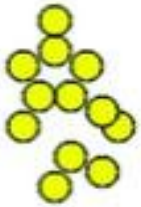
Sequestration within epithelial cells
(MSCRAMM)

Evasion of neutrophil killing

- *Blockade of neutrophil recruitment*
  (CHIP, Eap)
- *Inhibition of antimicrobial peptide killing*
  (staphylokinase, aureolysin,
  alteration of bacterial surface charges)
- *Neutralization of ROS*
  (catalase, SOD, pigment)
- *Neutrophil cytolysis*
  (PSMs and other toxins)

S. aureus

Evasion of opsonophagocytosis
(capsule, clumping factor A, protein A,
multiple inactivators of complement)



Iron acquisition
(Isd, aureochelin, staphyloferrin)

B cell depletion
(Protein A)

Inactivation of T cell functions
(TSST, Eap, Enterotoxins)

Diagnosis

Microscopy useful for pyogenic infections but not blood infections or toxin-mediated infections

Staphylococci grow rapidly when cultured on nonselective media

Selective media (e.g., chromogenic agar, mannitol-salt agar) can be used to recover *Staphylococcus aureus* in contaminated specimens

Nucleic acid amplification tests are useful for screening patients for carriage of methicillinsensitive *S. aureus* (MSSA) and MRSA

S. aureus is identified by biochemical tests (e.g., coagulase), molecular probes, or mass spectrometry

TABLE 23-6 Various specimens collected in staphylococcal infections

Specimen	Condition
Pus	Suppurative lesions and osteomyelitis
Sputum	Respiratory infections
Blood	Bacteremia
Feces and vomitus	Food poisoning
Urine	Urinary tract infections
Nasal and perineal swab	Suspected carriers

LABORATORY DIAGNOSIS

- Sample collection and Transportation
- Direct smear Microscopy
- Culture
- Biochemicals
- Typing of Staphylococcus aureus
- Antibiotic Sensitivity Testing (AST)

Laboratory Diagnosis

A. Haematological Investigation:

1. TLC (Total leukocyte count):

Normal: 4000-10000 cells/mm³

In case of infection: > 10000 cells/mm³

2. DLC (Differential leukocyte count):

Normal neutrophil : 80%

In case of infection: > 80%

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.

Antimicrobial susceptibility

- MRSA can be due to
  - Production of penicillin-binding protein 2a (PBP2a) encoded by *mecA* gene
  - Production of beta-lactamase

- Resistance due to *mecA* can be detected via **cefoxitin disk** diffusion or dilution methods.

- Resistance due to beta-lactamase production can be detected via the use of beta-lactamase inhibitor such as **clavulanic acid** which would result in an increase in zone size (disk diffusion method).

Methods of antimicrobial susceptibility testing

Phenotypic methods

Constrained by bacterial growth time

- Dilution — 48 h
- Diffusion — 48 h
- Gradient test — 48 h
- Chromogenic media — 24 h
- Automated devices — > 20 h

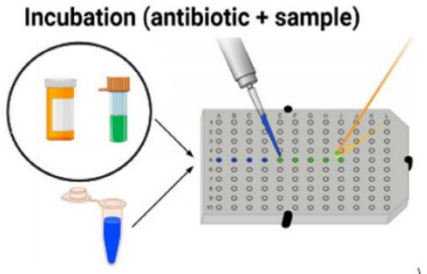
Molecular-based methods

- PCR (Gene 1, Gene 2 ··· Gene N) — several h
- qPCR — several h
- Cycle sequencing — 24 - 48 h
- NGS — several days

** estimated time depend on a sample analysed (clinical specimen vs. isolated bacterial culture)*

Mass spectrometry

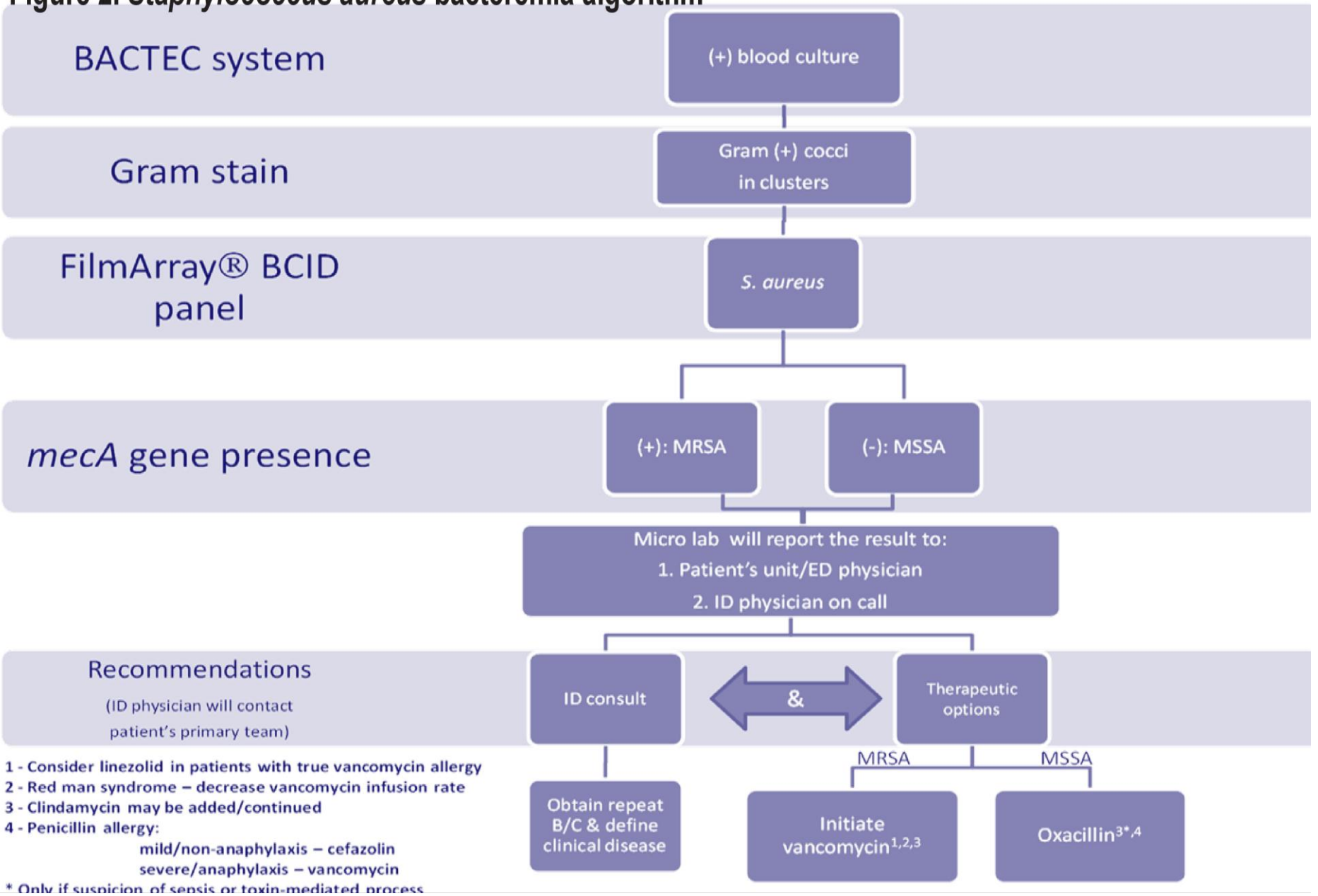
MALDI–TOF MS



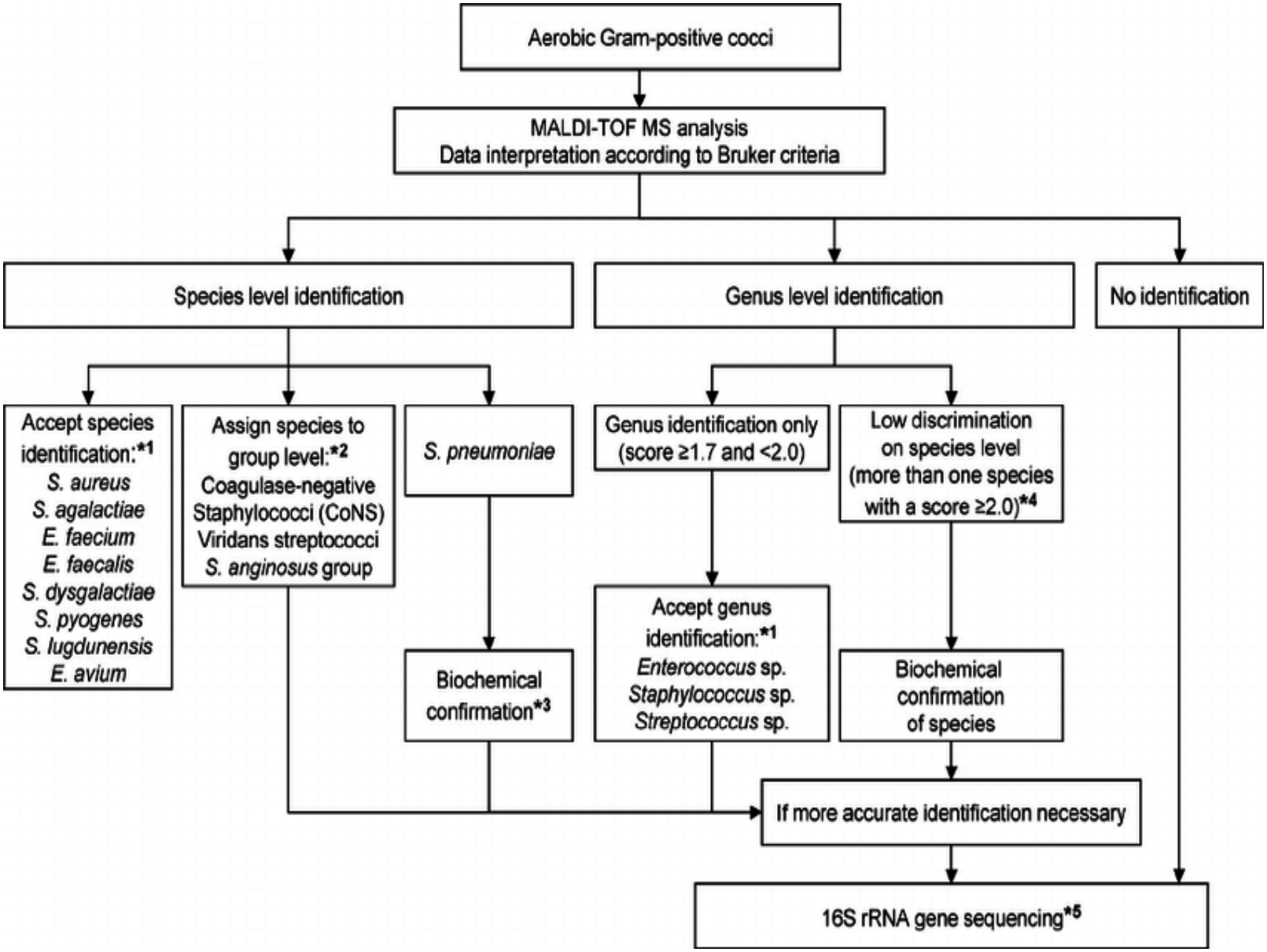
several h

** estimated time depend on a sample analysed (clinical specimen vs. isolated bacterial culture)*

Figure 2. *Staphylococcus aureus* bacteremia algorithm



1 - Consider linezolid in patients with true vancomycin allergy
2 - Red man syndrome – decrease vancomycin infusion rate
3 - Clindamycin may be added/continued
4 - Penicillin allergy:
mild/non-anaphylaxis – cefazolin
severe/anaphylaxis – vancomycin
* Only if suspicion of sepsis or toxin-mediated process


Aerobic Gram-positive cocci
MALDI-TOF MS analysis
Data interpretation according to Bruker criteria
Species level identification
Genus level identification
No identification
Accept species identification:*1
S. aureus
S. agalactiae
E. faecium
E. faecalis
S. dysgalactiae
S. pyogenes
S. lugdunensis
E. avium
Assign species to group level:*2
Coagulase-negative Staphylococci (CoNS)
Viridans streptococci
S. anginosus group
S. pneumoniae
Biochemical confirmation*3
Genus identification only (score ≥1.7 and <2.0)
Low discrimination on species level (more than one species with a score ≥2.0)*4
Accept genus identification:*1
Enterococcus sp.
Staphylococcus sp.
Streptococcus sp.
Biochemical confirmation of species
If more accurate identification necessary
16S rRNA gene sequencing*5

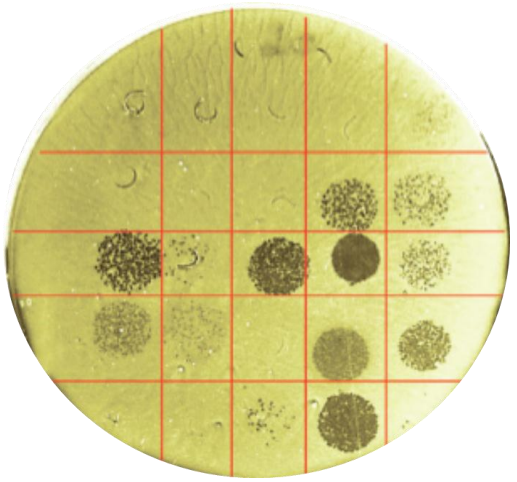
Bacteriophage typing

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**	



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

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

Streptococcaceae taxonomy

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

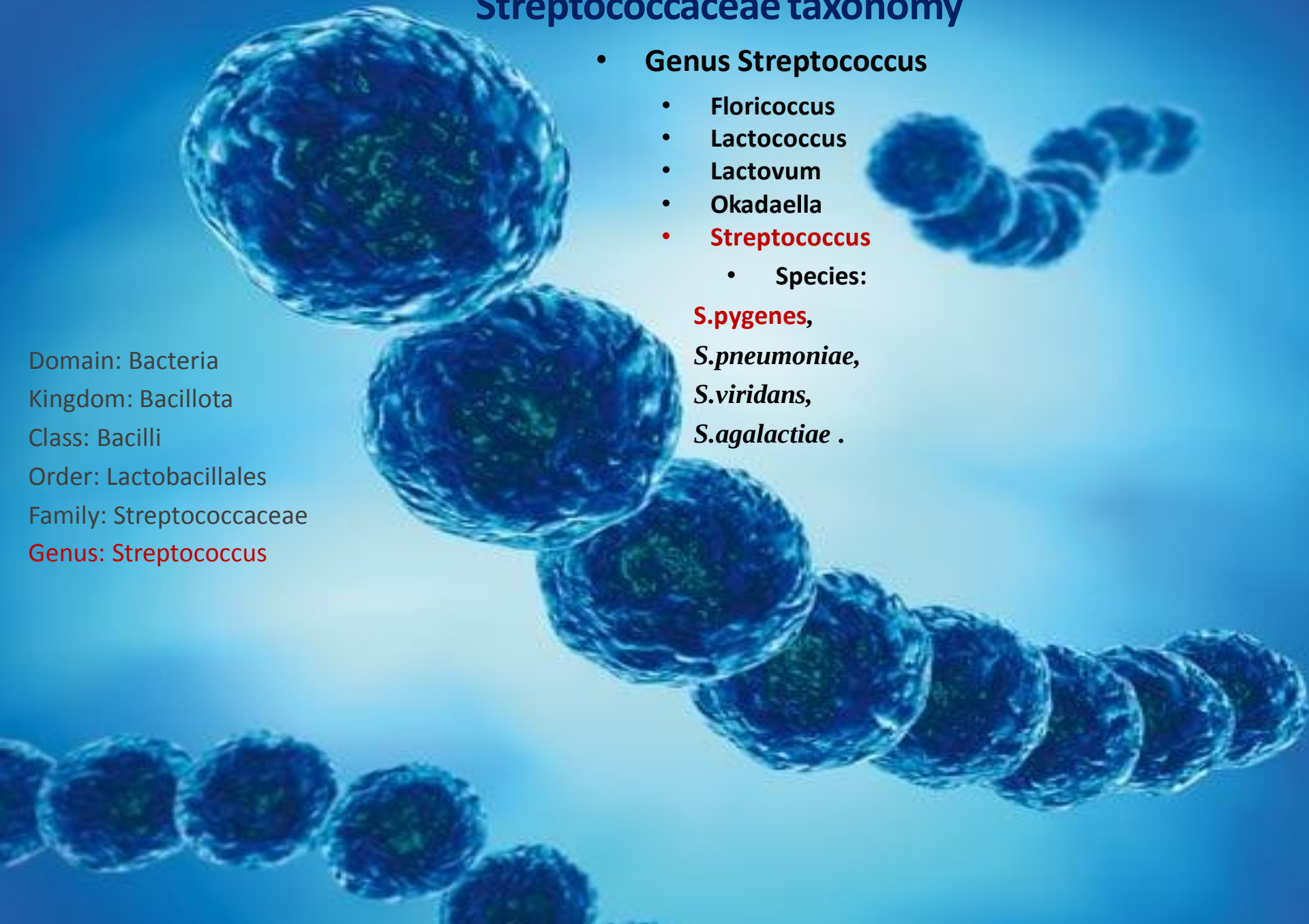
S.pygenes,

S.pneumoniae,

S.viridans,

S.agalactiae .

Domain: Bacteria
Kingdom: Bacillota
Class: Bacilli
Order: Lactobacillales
Family: Streptococcaceae
Genus: Streptococcus



Classification of Streptococci

- Brown`s classification
- Lancefield grouping
- Griffith typing

Classification based on antigen structure (Lensfield classification):

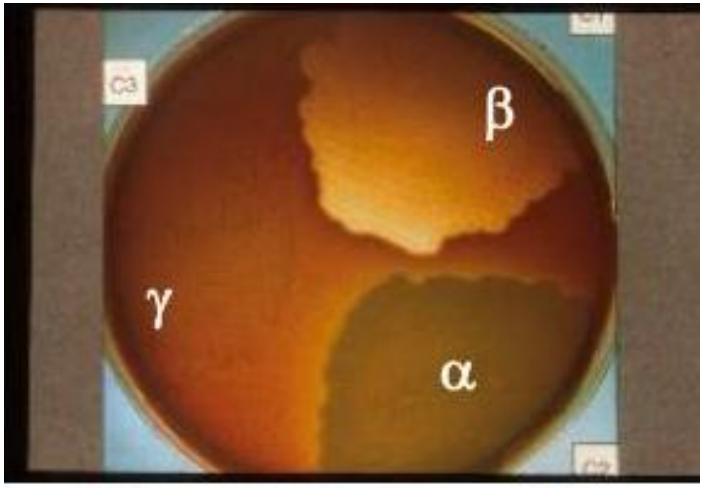
according to **polysaccharide** C antigen in the **cell wall** of aerobic streptococci is divided 20 **serogroups** - A, B, C, D, E, F, G, H, K, L, M, N, O, P, Q, R, S, T, U,V.

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**



Hemolytic Reactions

- Hemolysis
  - beta
  - alpha
  - gamma
- Lancefield Groups
  - *(A-T- β hemolytic)*
  - group-specific cell wall polysaccharide
- Species
  - phenotypic biochemical reactions

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*

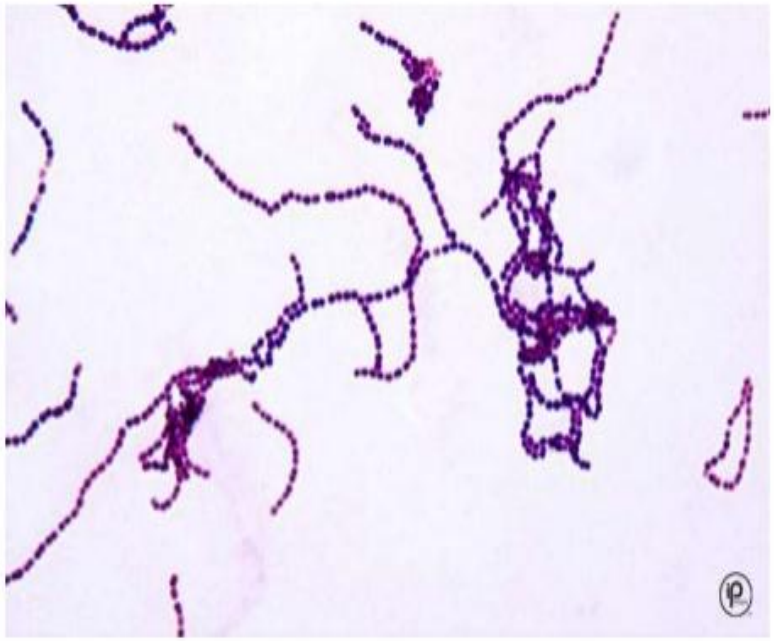
age

Moises Dor

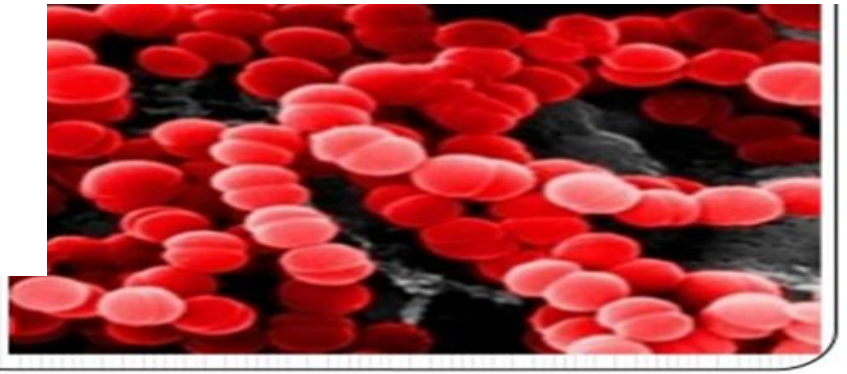
Group A betahemolytic Streptococci

Streptococcus pyogenes

Morphology

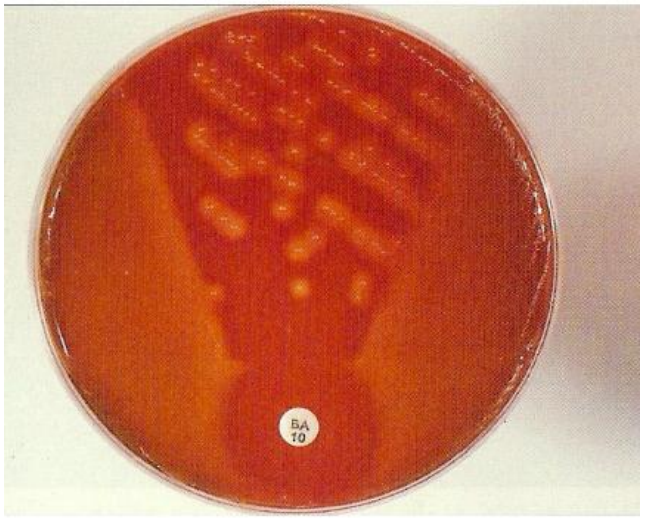


- Ovoid to spherical in shape
- Gram-positive cocci arranged in chains or pairs
- Chain formation is due to the cocci dividing in one plane only and the daughter cells failing to separate completely
- Chains are longer in liquid than in solid media
- Non motile and non-sporing
- Capsulated (hyaluronic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



Cultural characteristics

Streptococcus pyogenes
on blood agar



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

i. Blood agar:

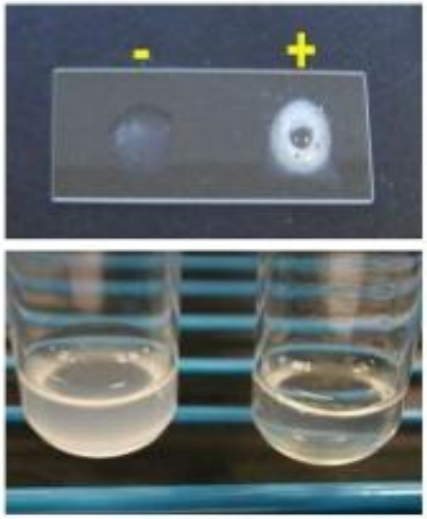
- Small (0.5-1mm), circular, semi-transparent colonies
- Produce wide zone of β- hemolysis
- Growth and hemolysis are promoted by 5-10% CO2
- Virulent strains, on fresh isolation form lesions, produce a 'matt' (finely granular) colony while avirulent strains form 'glossy' colonies
- Mucoid colonies are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

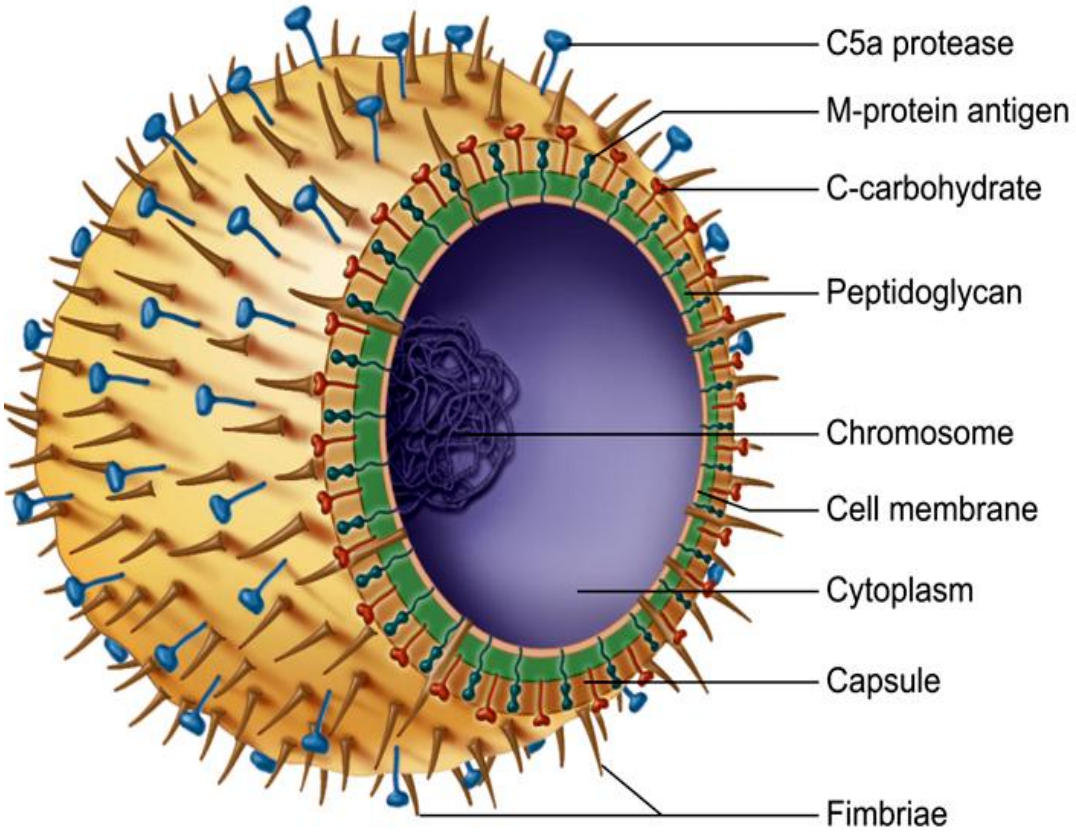
Streptococcus pyogenes - antigen structure

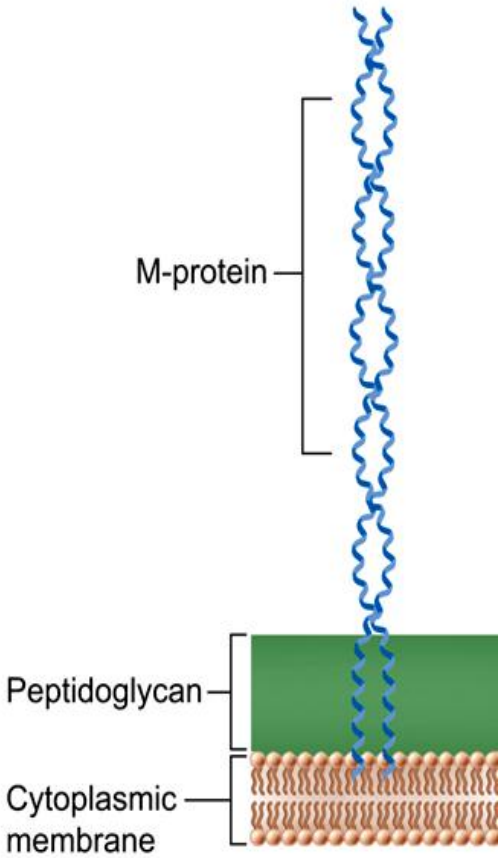
Species antigen - located in the cytoplasm, contains a nucleoprotein.

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.

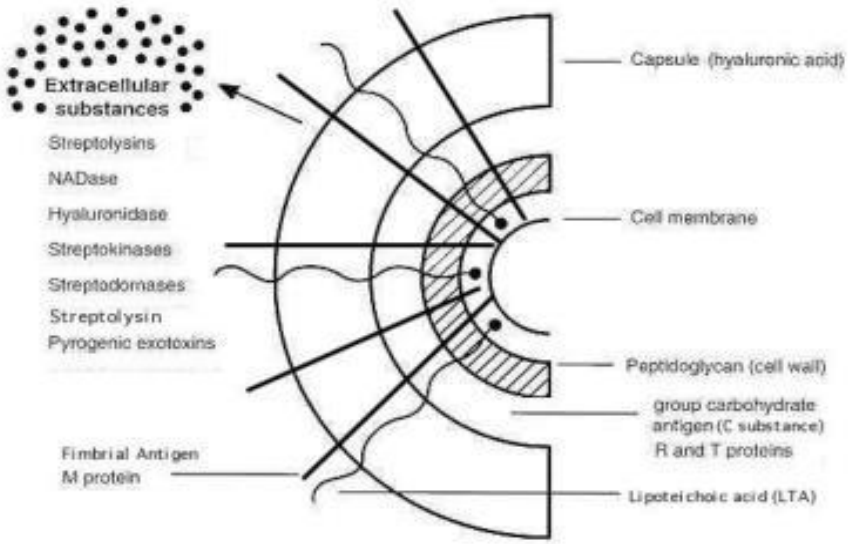
Streptococcus pyogenes





Antigenic Structure & Virulence Factors of *S. pyogenes*

- Hyaluronic acid capsule - antiphagocytic
- Hyaluronidase - tissue penetration
- Group specific cell wall antigen distinguishes from B,C,D,F,G, etc.
- Beta hemolytic



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)

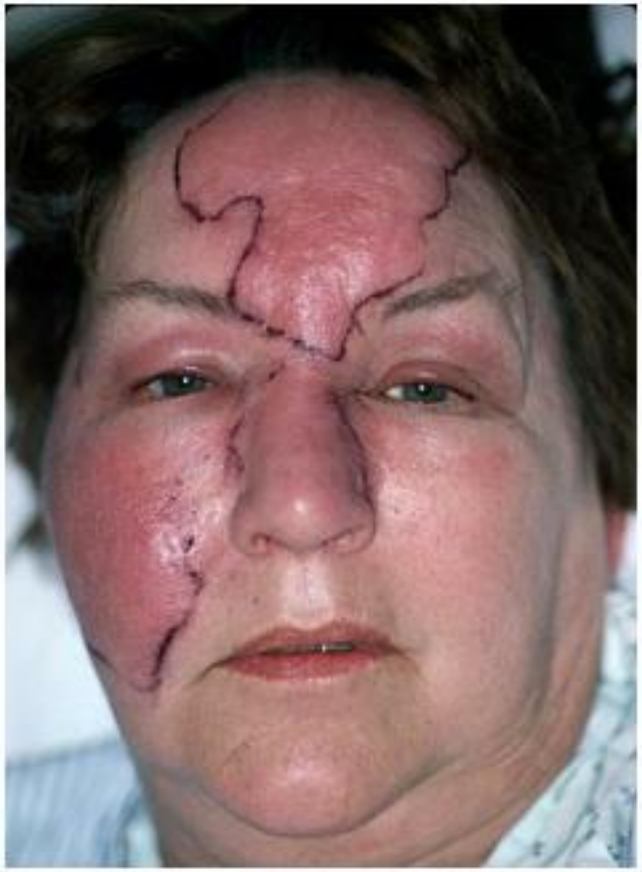
- Responsible for cell wall rigidity
- Enhances non specific resistance (pyrogenic and thrombolytic activity)

Antigenic Structure & Virulence Factors of *S. pyogenes*

- **M Protein**
  - Virulence factor present on pilus with teichoic acid
  - Organisms lacking it are readily opsonized and phagocytized
  - Binds fibrinogen, fibrin & degradation products forming dense coating on the organism's surface, blocking complement
  - Antibody against M protein is an important protective mechanism, but repeated infections with strains possessing one of over 80 different serotypes can occur
  - Autoantibody target-Acute Rheumatic Fever

Antigenic Structure & Virulence Factors of *S. pyogenes*

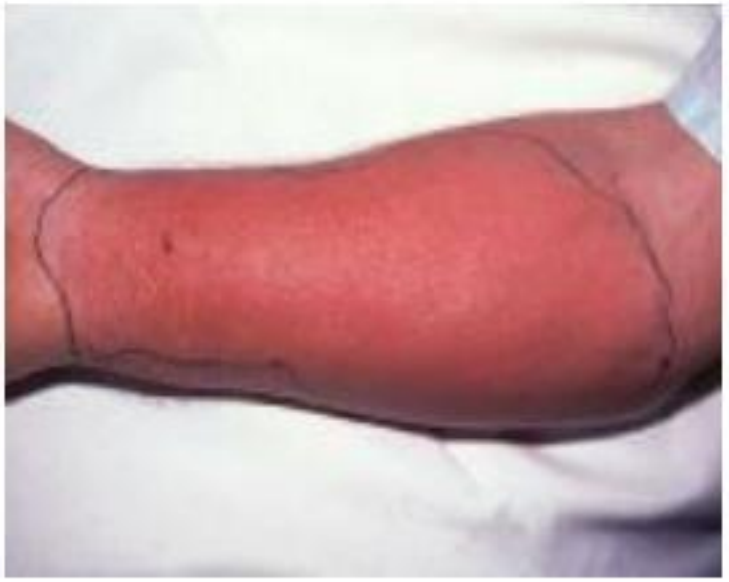
- Protein F - facilitates attachment by binding fibronectin
- Protein G - binds Fc portion of antibody
- Diphosphopyridine nucleotidase (DPNase) – enzyme kills WBCs
- C5a peptidase



Erysipelas

Antigenic Structure & Virulence Factors of *S. pyogenes*

- Erythrogenic Toxin "Scarlet Fever"
- Streptokinases
  - transform plasminogen to plasmin
  - digest fibrin
- DNAase
  - depolymerizes DNA
    antibody used to follow pyoderma
- Hemolysins "Streptolysins"
  - Important immunogens
  - Antibody against streptolysin O used to follow course of pyoderma
  - Streptolysin S - β hemolysis



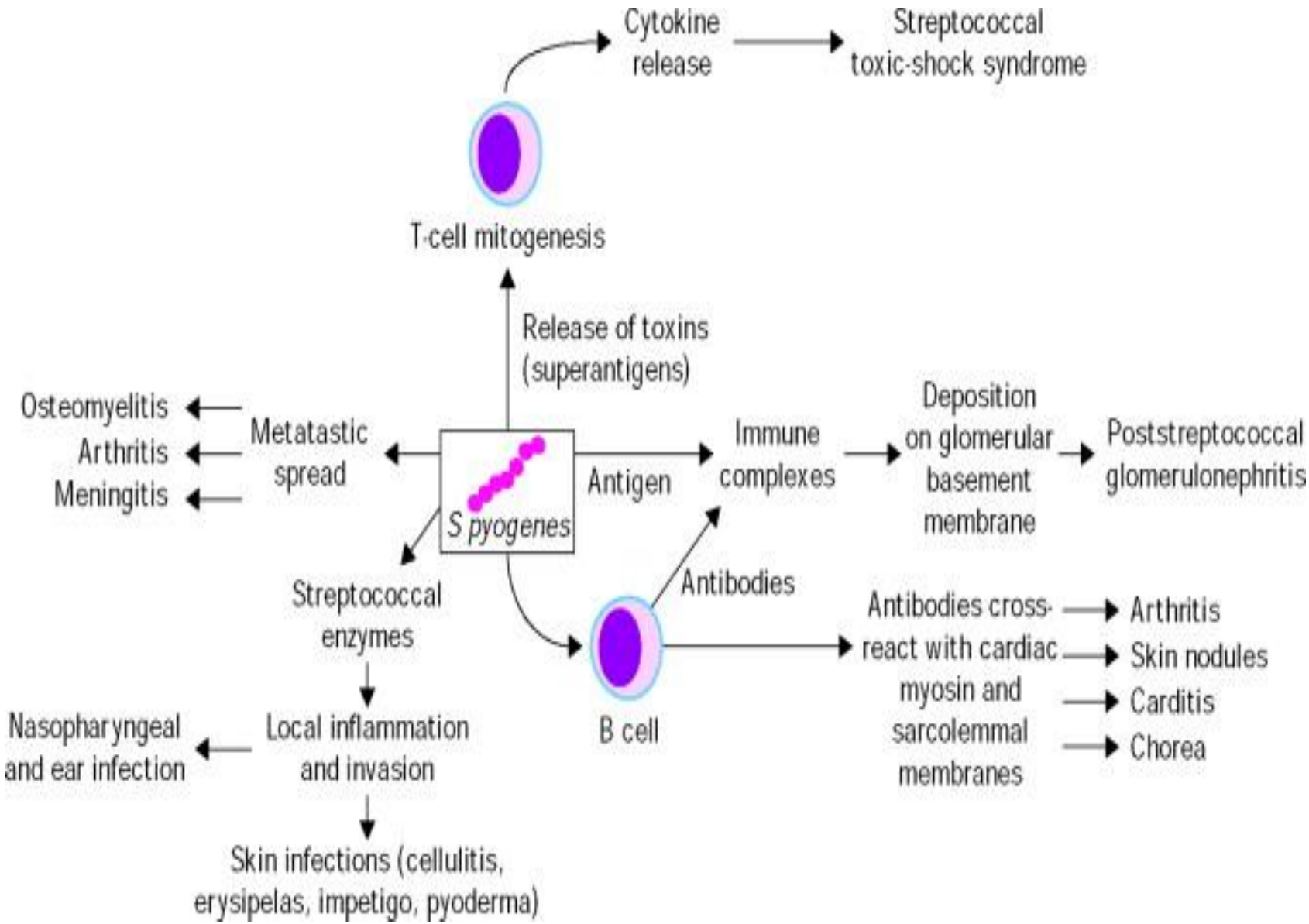
Strep. cellulitis

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**


Cytokine release
Streptococcal toxic-shock syndrome
T-cell mitogenesis
Release of toxins (superantigens)
Osteomyelitis
Arthritis
Meningitis
Metatastic spread
S pyogenes
Antigen
Immune complexes
Deposition on glomerular basement membrane
Poststreptococcal glomerulonephritis
Streptococcal enzymes
Antibodies
Antibodies cross-react with cardiac myosin and sarcolemmal membranes
Arthritis
Skin nodules
Carditis
Chorea
B cell
Nasopharyngeal and ear infection
Local inflammation and invasion
Skin infections (cellulitis, erysipelas, impetigo, pyoderma)

Streptococcus Habitat

- Skin, mucous membranes, respiratory tract and GI/GU tracts, depending on species
- 20% of children may carry GAS in their pharynx during winter months.
- *S. pneumoniae* is commonly isolated from the respiratory tract of asymptomatic carriers.
- Enterococci in gut flora are are important pathogens in hospitals where they are selected by high antibiotic usage.
- Organisms spread by droplets, direct contact and fomites.

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

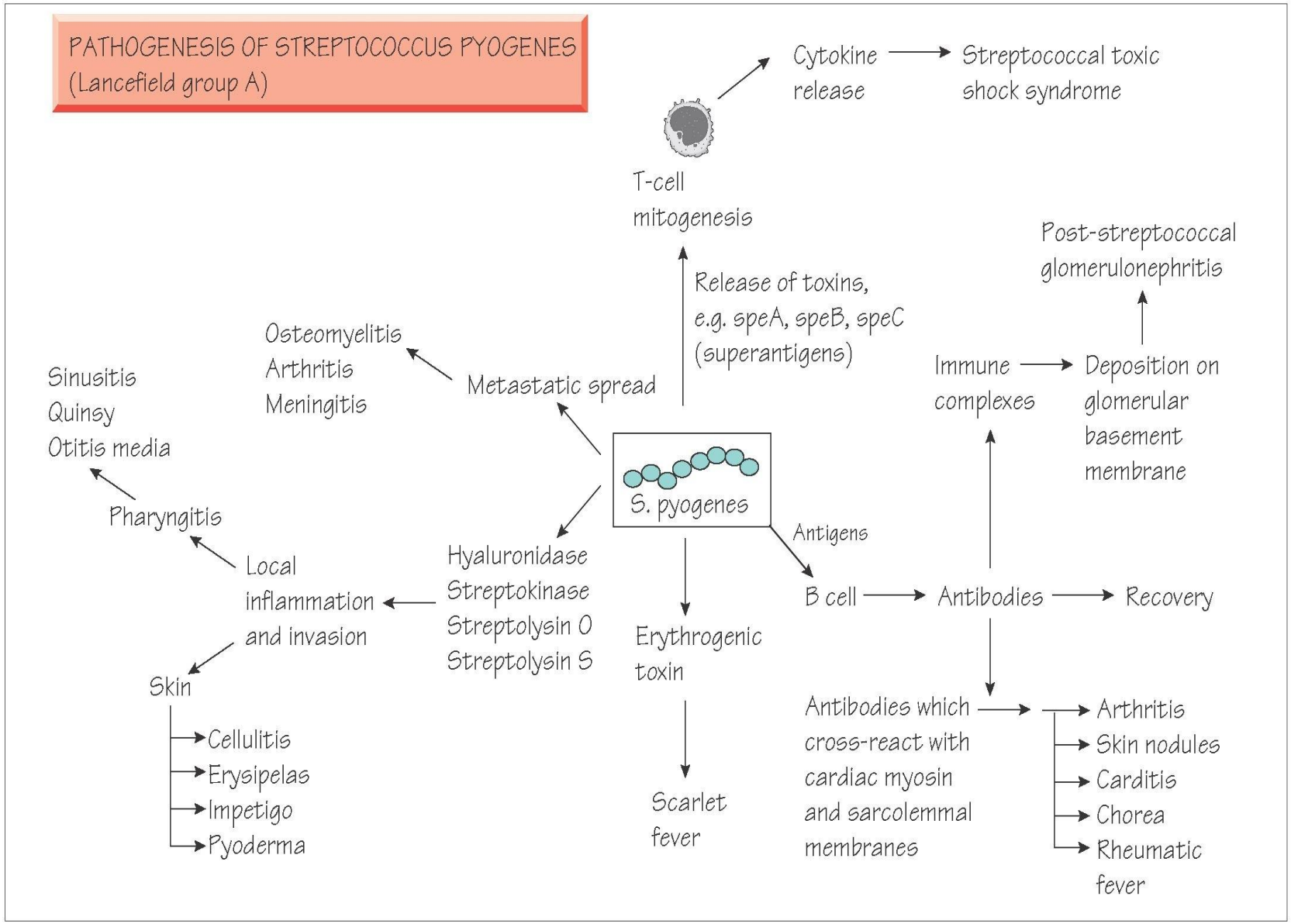
- Produces pyrogenic infection with a tendency to spread locally, along lymphatics and through blood stream
- Disease caused can be:
  - **Suppurative** or
  - **Non suppurative**

- Suppurative diseases:

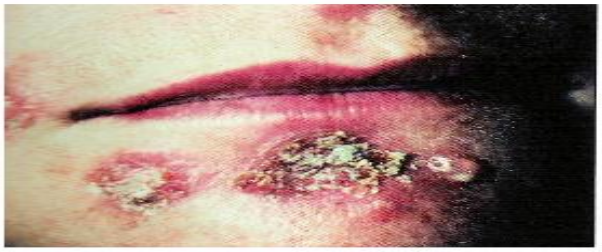
1. Respiratory infections
2. Skin and soft tissue infections
3. Genital infections

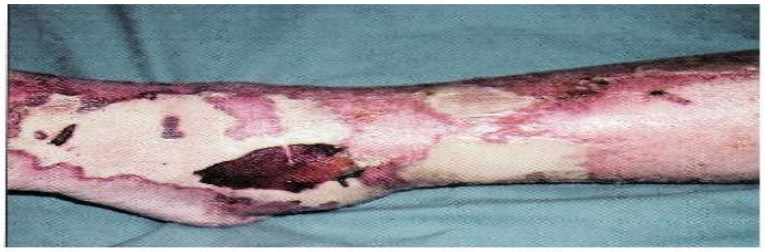
- Non suppurative sequelae:

1. Acute rheumatic fever
2. Acute glomerulonephritis


PATHOGENESIS OF STREPTOCOCCUS PYOGENES
(Lancefield group A)
Cytokine release
Streptococcal toxic shock syndrome
T-cell mitogenesis
Release of toxins, e.g. speA, speB, speC (superantigens)
Post-streptococcal glomerulonephritis
Immune complexes
Deposition on glomerular basement membrane
Osteomyelitis
Arthritis
Meningitis
Metastatic spread
Sinusitis
Quinsy
Otitis media
S. pyogenes
Antigens
Pharyngitis
Local inflammation and invasion
Hyaluronidase
Streptokinase
Streptolysin O
Streptolysin S
Erythrogenic toxin
B cell
Antibodies
Recovery
Skin
Cellulitis
Erysipelas
Impetigo
Pyoderma
Scarlet fever
Antibodies which cross-react with cardiac myosin and sarcolemmal membranes
Arthritis
Skin nodules
Carditis
Chorea
Rheumatic fever

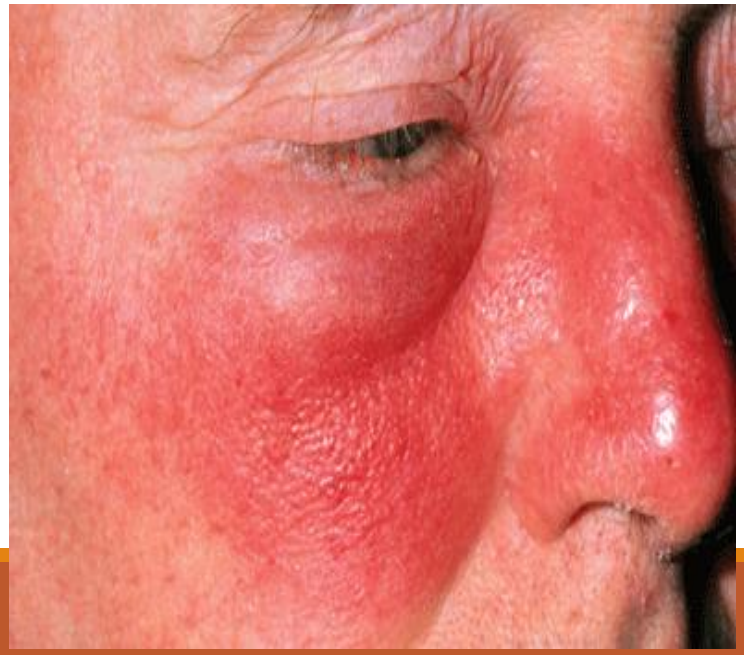
Erysipeloid

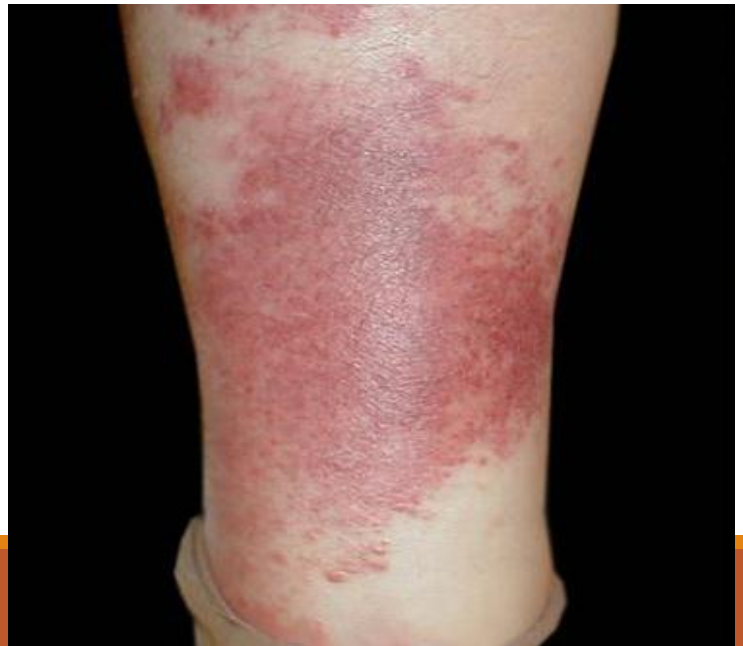




Streptococci - non-purulent infections:

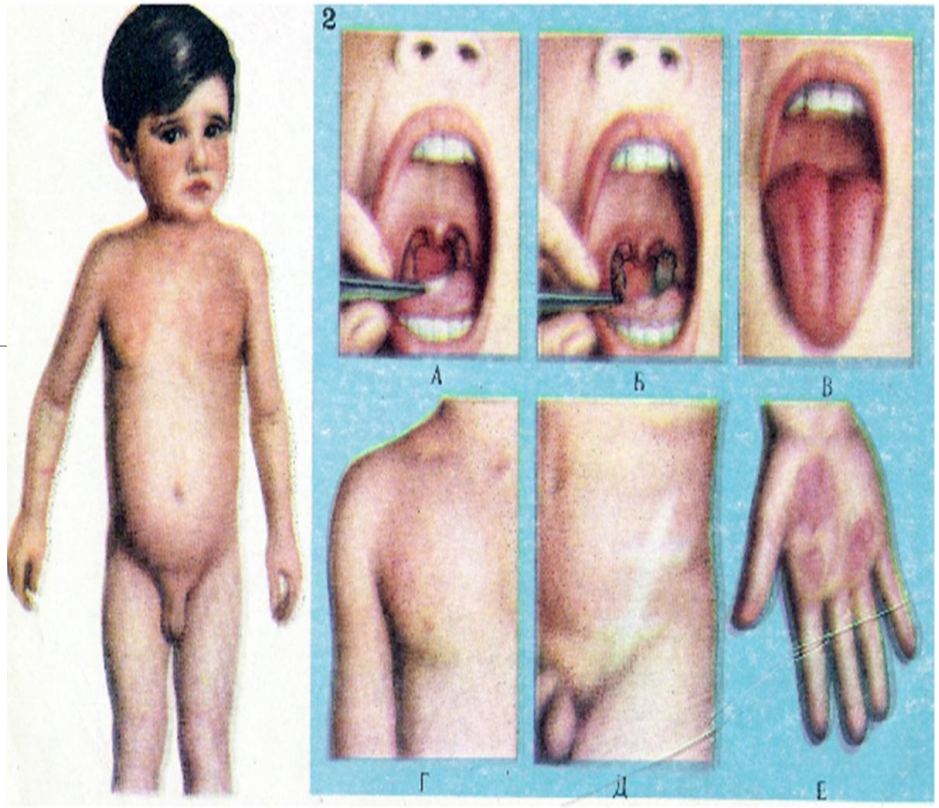
Erysipelas is a phlegmon-like acute non-purulent inflammatory disease of the skin. Signs of inflammation, such as redness and swelling on the skin, are very vivid and differ sharply from healthy skin. Inflammation (redness) on the surface of the skin tends to spread, its borders are indented-protruding,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

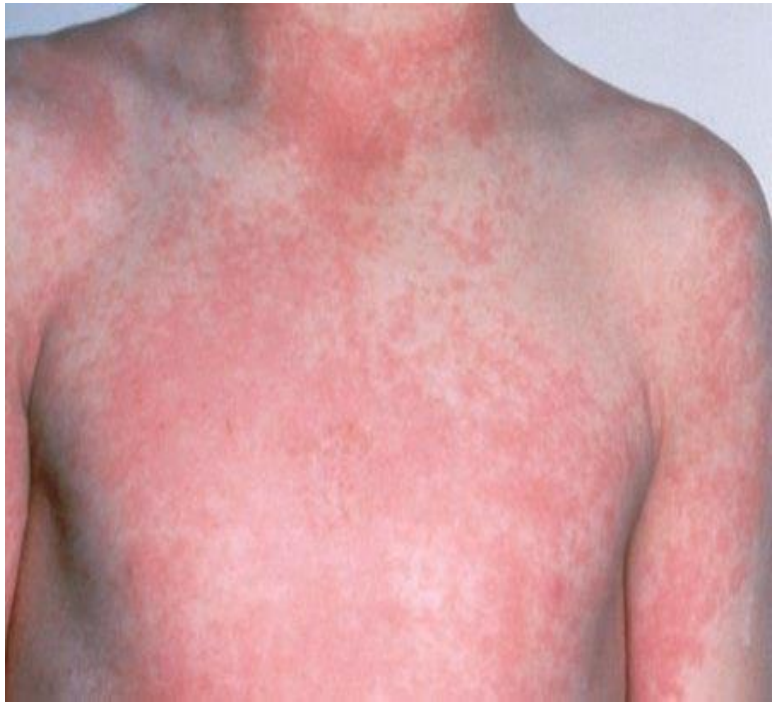


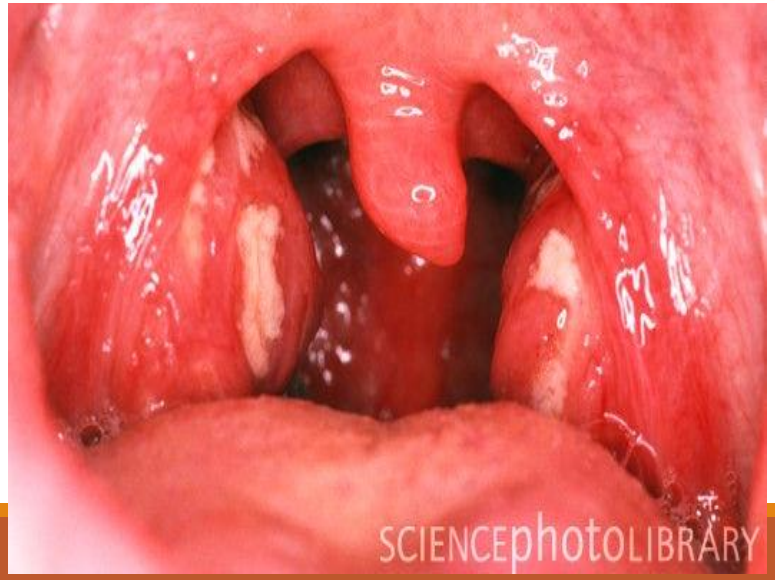


Streptococci - non-purulent infections:

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.

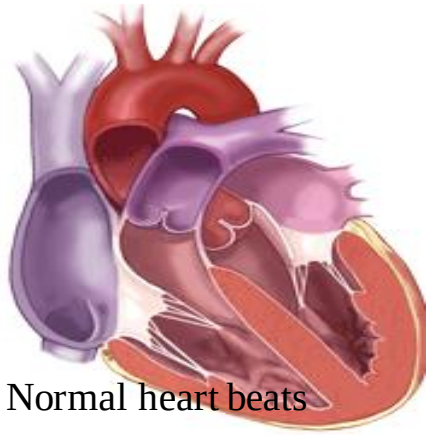




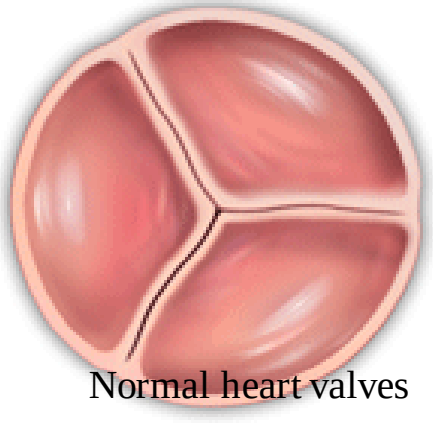


Streptococci - non-purulent infections:

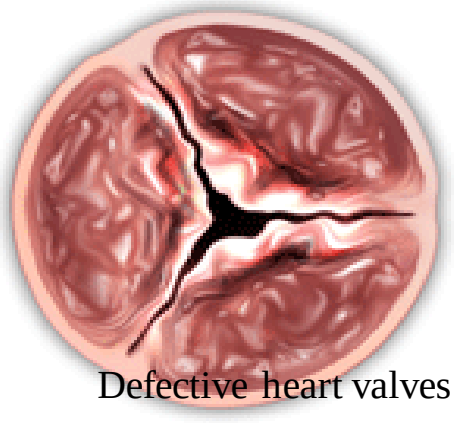
The first manifestation of **Rheumatic fever (acute rheumatic fever)** manifests itself as an acute inflammation of the joints - aseptic polyarthritis, followed by pancarditis (inflammation of all membranes of the heart - endocardium, myocardium and pericardium). Endocarditis, as a chronic inflammatory 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



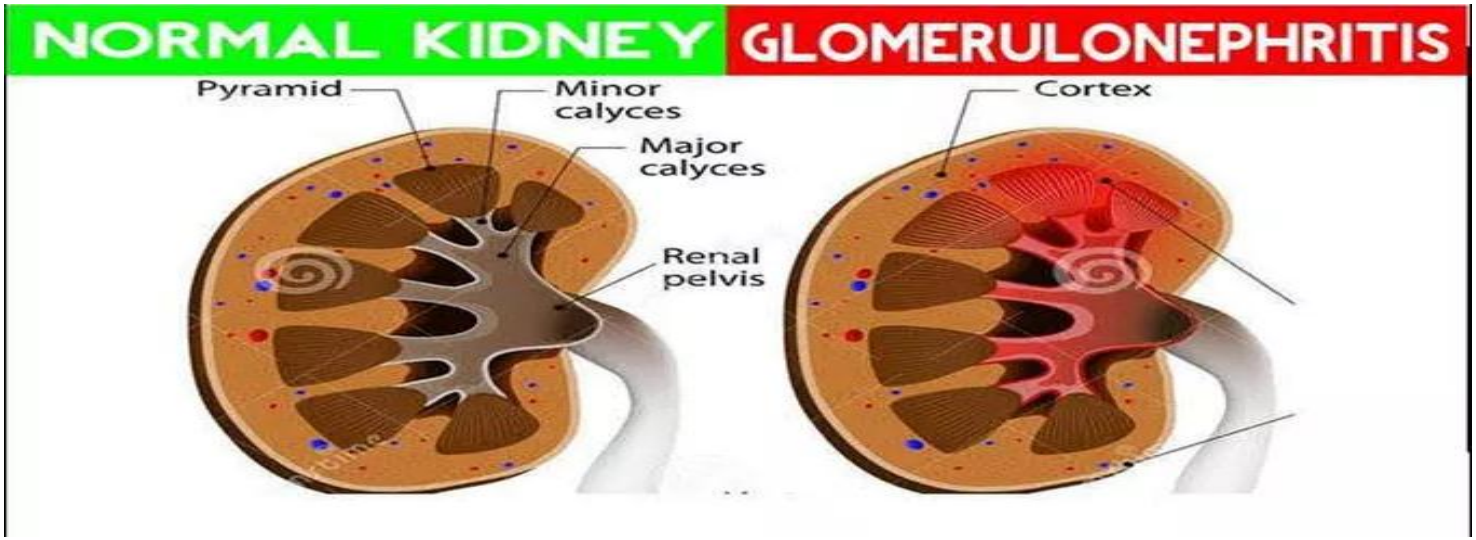
Normal heart valves



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:**

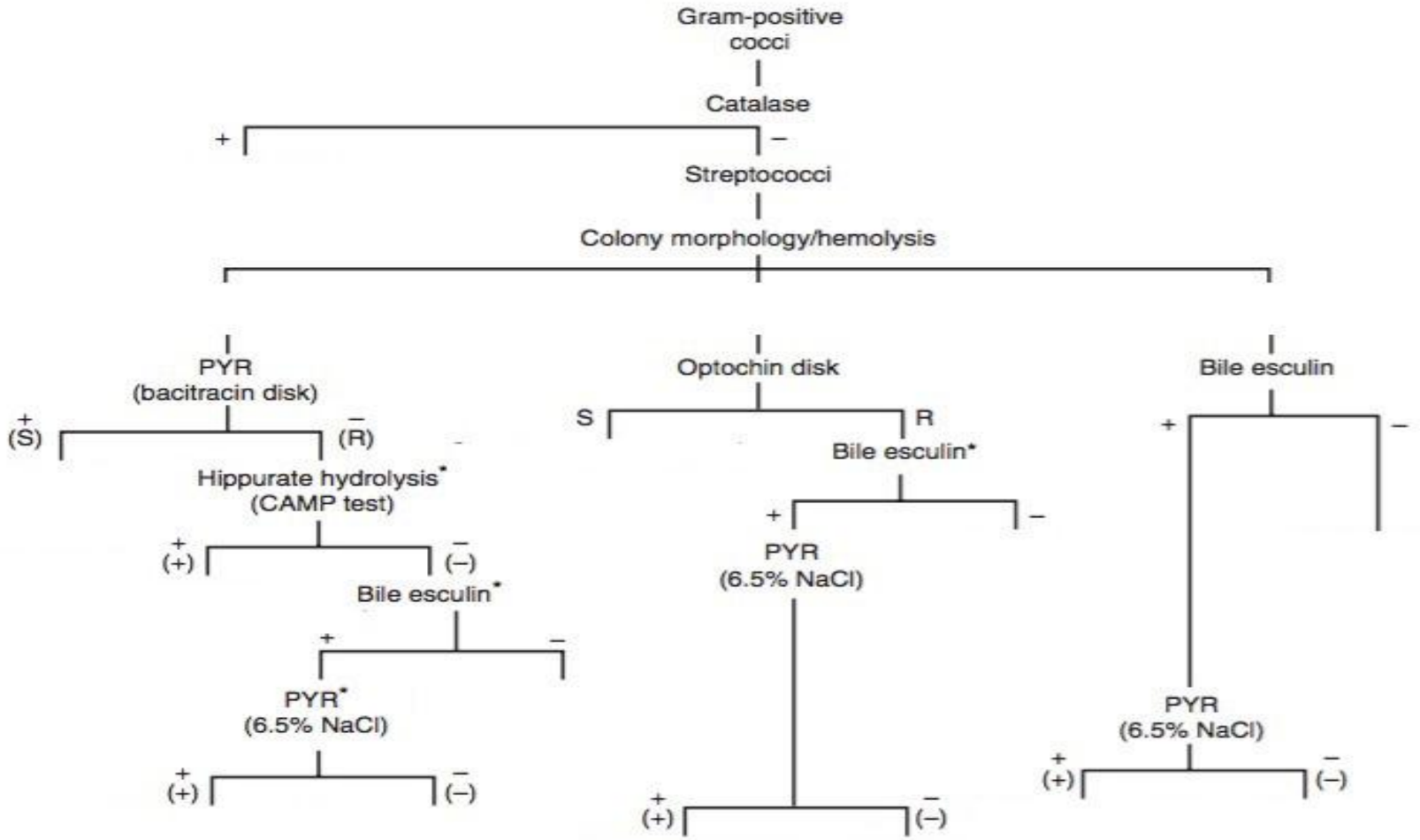
- Throat swab, pus swab or exudates are collected.

2. **Microscopy:**

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

3. **Culture:**

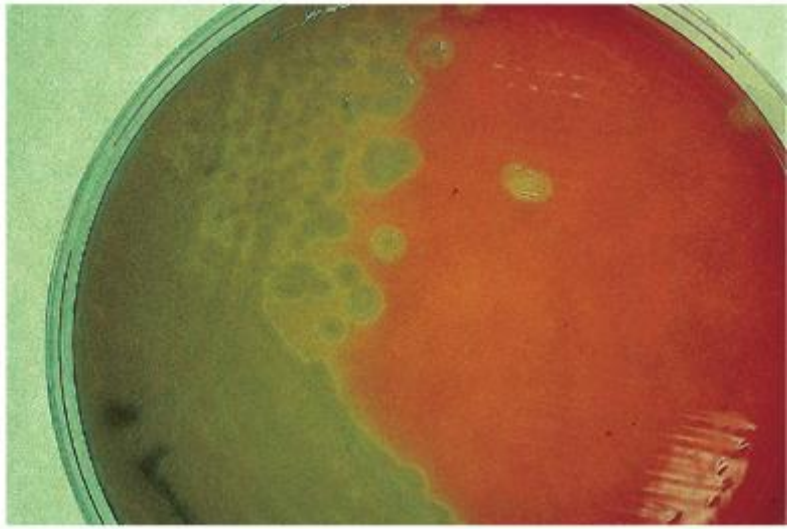
- Swab from the affected area is collected and are either plated immediately or sent to laboratory in Pike's medium.
- The specimen should be plated on blood agar and incubated at 37°C anaerobically or under 5-10% CO_2 ,as hemolysis develops better.



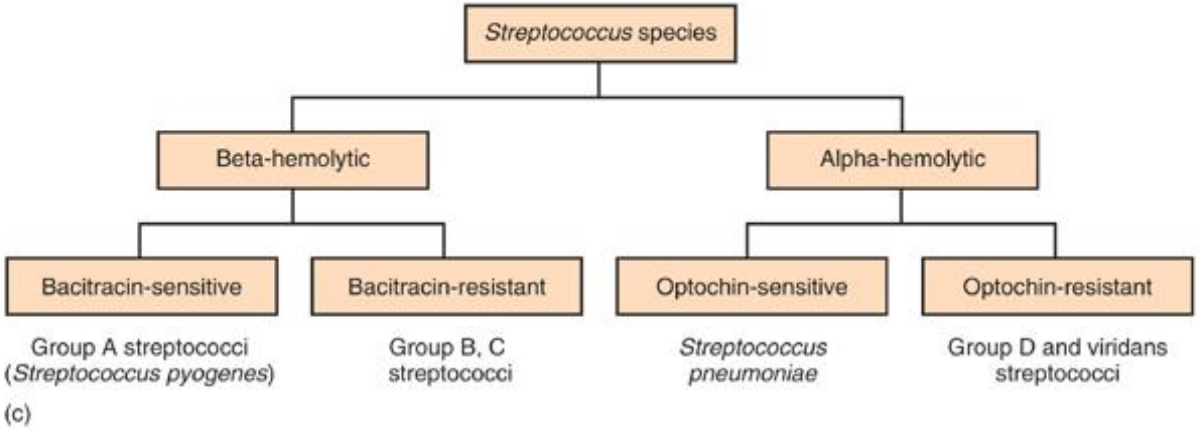
*Perform additional tests if isolate is from nonrespiratory source.

FIGURE 15-15 Schematic diagram for the presumptive identification of gram-positive cocci. *R,* Resistant; *S,* susceptible.

(a) © Kathy Park Talaro



(b) © L.M. Pope and D.R. Grote/Biological Photo Service



(c)

Hemolysis on blood agar

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

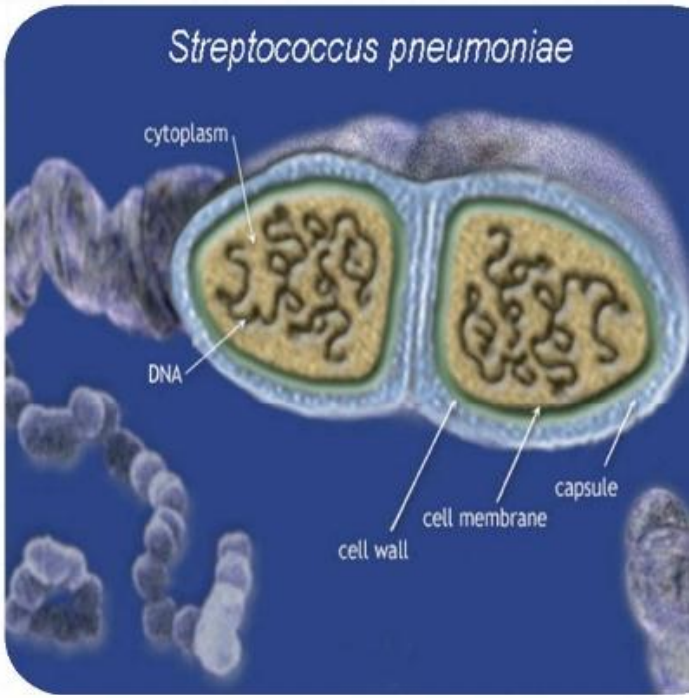
Titres higher than 300 are taken

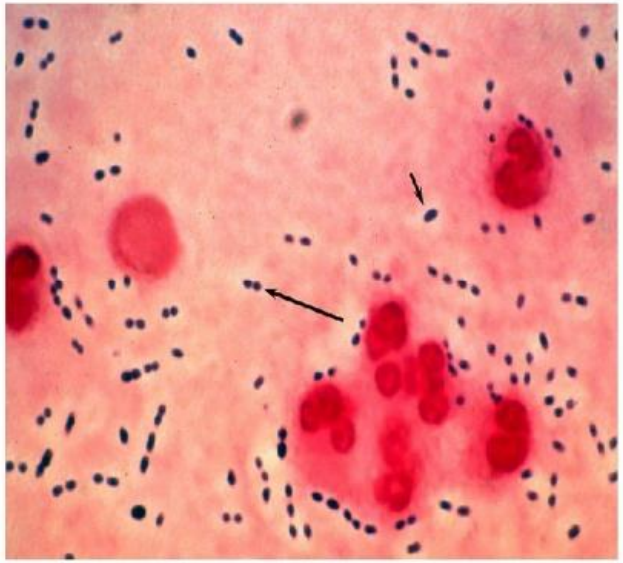
c) **Streptozyme test:**

- 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

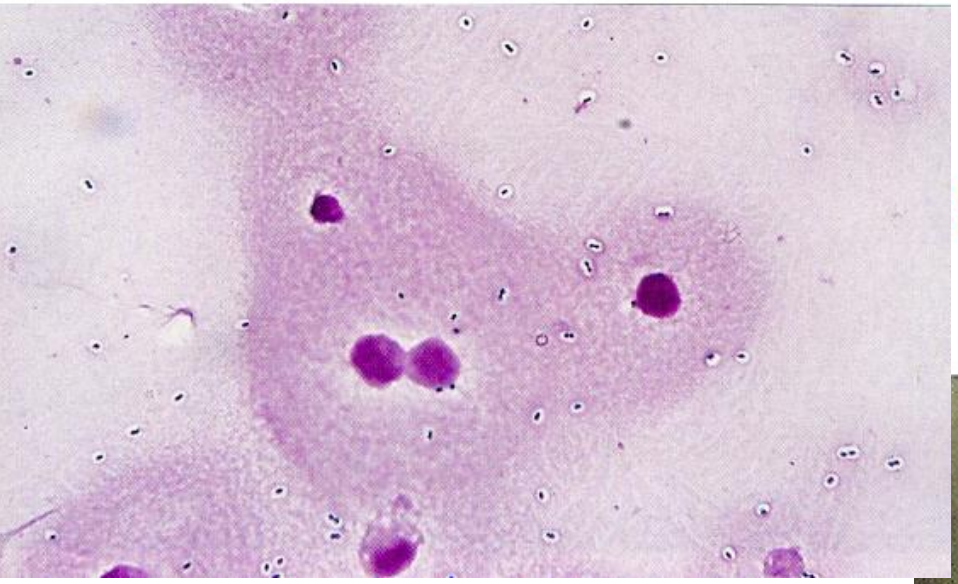
- **Streptococcus pneumoniae cells are Gram-positive, lancet-shaped cocci (elongated cocci with a slightly pointed outer curvature). Usually, they are seen as pairs of cocci (diplococci), but they may also occur singly and in short chains. When cultured on blood agar, they are alpha hemolytic**



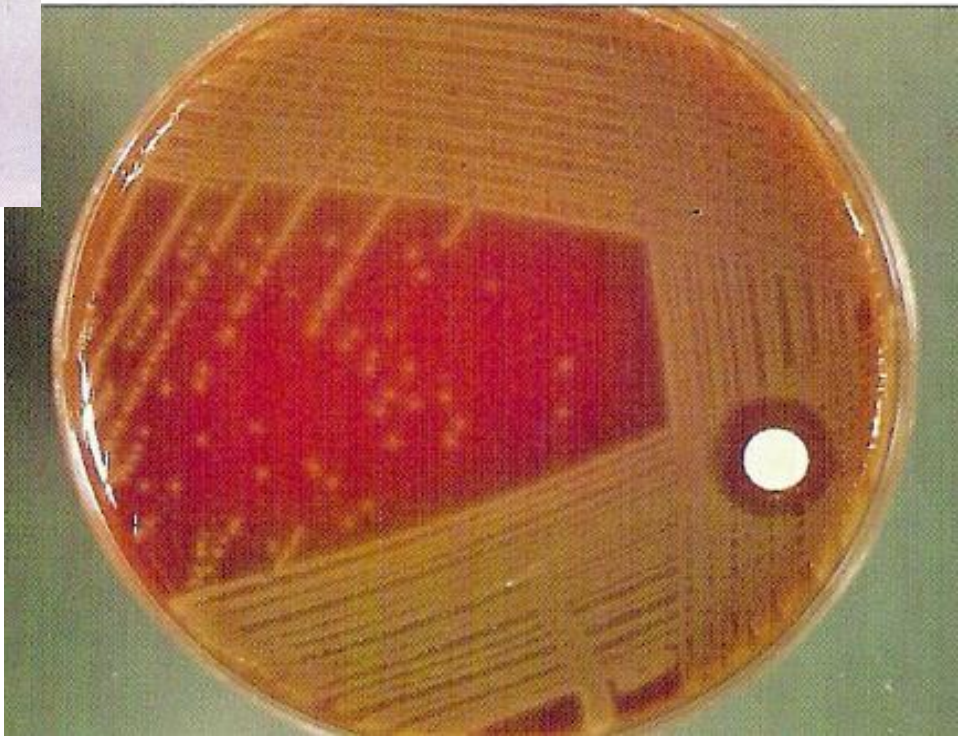


- **Individual cells are between 0.5 and 1.25 micrometers in diameter. They do not form spores, and they are nonmotile. Like other streptococci, they lack catalase and ferment glucose to lactic acid**

Streptococcus pneumoniae

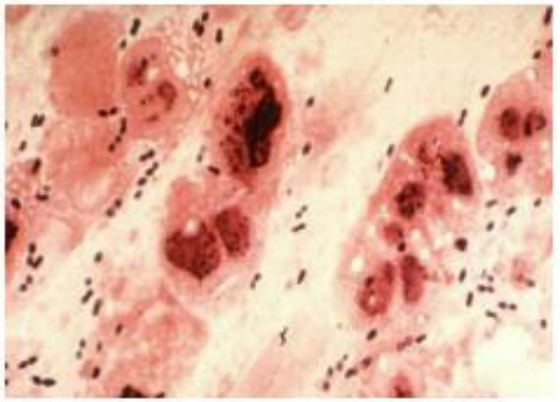


Streptococcus pneumoniae
on blood agar

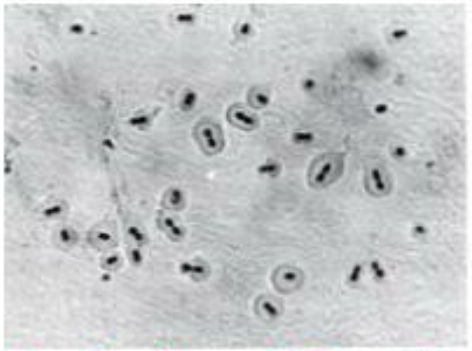


S. pneumoniae Virulence Factors

- Antiphagocytic capsule – immunogen
- PspA: inhibits opsonization
- Autolysin – release cell components
- Pneumolysin
  - Cytotoxic – inhibit cilia, wbcs
  - lyses RBCs
  - activates classic complement path.
  - stimulates cytokines → tissue damage & purulent inflammation
- Hydrogen peroxide - tissue damage
- Surface protein adhesins
- Neuraminidase
- IgA protease
- Peptidoglycan
  - activate alternate complement
  - cytokine release
- Transformation– antibiotic resistance
- Intracellular invasion



sputum - pneumonia



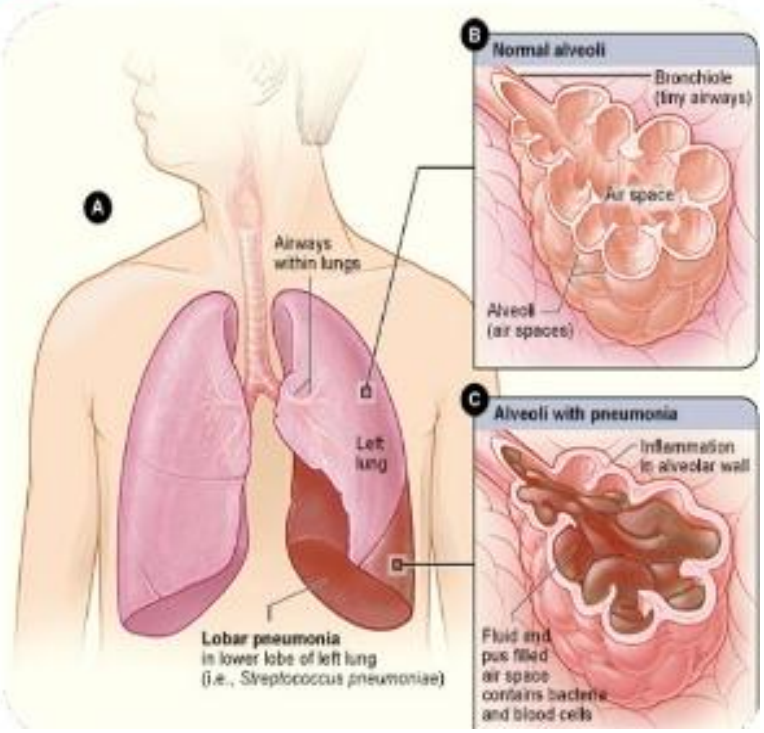
Capsule Quellung Reaction

Pneumococcal Disease Clinical Syndromes

A. Pneumonia

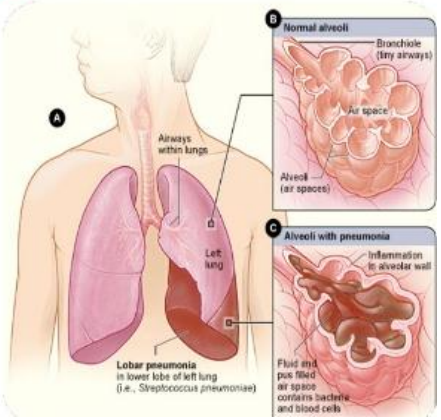
B. Bacteremia

C. Meningitis



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)

Treatment, prevention and control

DRUGS USED:

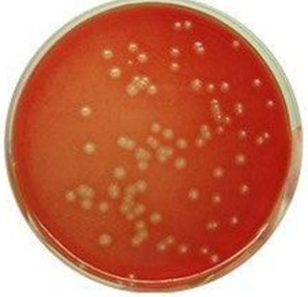
- For streptococcal pharyngitis: Oral penicillin V or amoxicillin
- Oral cephalosporin or macrolides can be used for penicillin sensitive patients
- For severe, systemic infection: Combined use of intravenous penicillin with protein synthesis inhibiting antibiotics(clindamycin) is recommended
- *Streptococcal 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

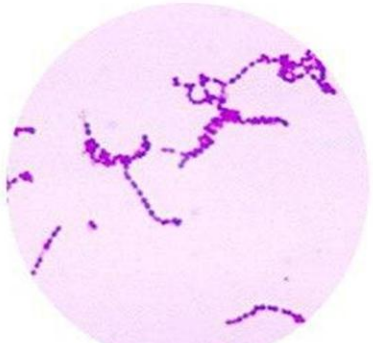
Streptococcus agalactiae

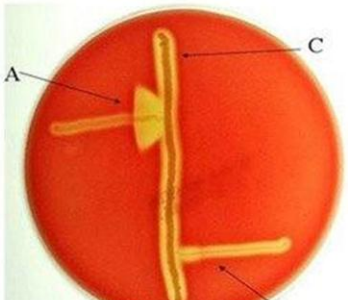
- Neonatal infection
- Most common cause of neonatal meningitis
- Source – from the maternal vagina during birth
- GBS – puerperal sepsis, pneumonia
- Diagnostic markers – Hippurate hydrolysis, CAMP test

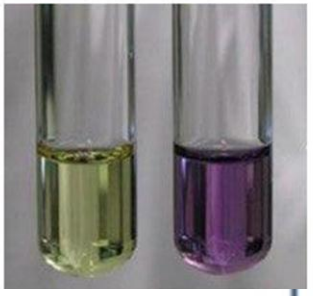
Streptococcus agalactiae (GBS)



β-hemolysis







+ve

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.

- 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

Streptococcus agalactiae

Characteristics

- **Gram** - Positive
- **Shape** – Diplococcus
- **Motility** – None
- **Oxygen Requirements** - Facultative anaerobe
- **Oxidase** – Negative
- **Catalase** – Negative
- **Hemolysis** – Beta
- **Bacitracin** - Resistant
- **Hippurate** – Positive
- **Pyrrolidonyl** - Negative
- **Virulence Factors**
  - CAMP Factor
    - Causes hemolysis
    - Creates synergistic hemolysis when plated with *S. aureus*
  - Polysaccharide capsule
    - Prevents phagocytosis

Transmission

- Ascending
- During vaginal birth

Diagnosis

- Blood cultures
- Lumbar punctures

Symptoms

- Neonatal sepsis and meningitis
  - All women are screened prior to labor because it can be a vaginal normal flora and spread to the neonate at birth
  - Most common cause of neonatal sepsis
  - Mortality rate of 16%
- Infant pneumonia
- Neonatal septic arthritis
  - Particularly of the wrist

Epidemiology and Risk Groups

- Worldwide
- Part of normal flora of vagina, anus, mouth, gastrointestinal tract
- Neonates who had a vaginal birth
- Premature neonates

Treatment

- ✓ Prophylactic IV penicillin can be given to GBS+ mom's during labor
- ✓ Treat neonate with sepsis with ampicillin+gentamycin until cultures confirm GBS, then you can switch to penicillin

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.

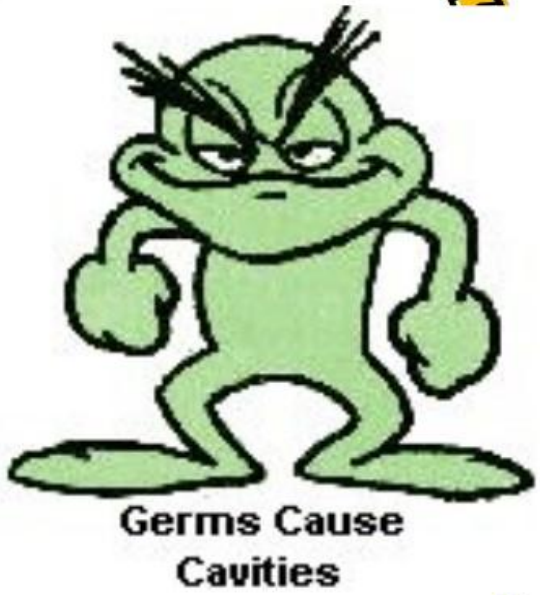
Consequences-inflammation of pulp and periapical tissues.

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 streptococci



Enterococcal Infections

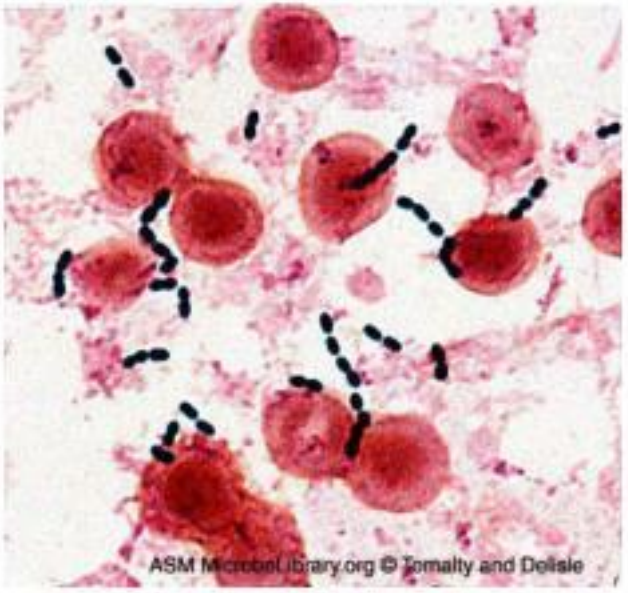
- Group D cell wall antigen
- *Enterococcus faecalis; Enterococcus faecium*
- GI tract of humans and animals
- UTI most common; wound infections; bacteremia; endocarditis
- Most infections from endogenous source
- Prolonged hospitalization and broad-spectrum antibiotics increase risk
- Antibiotic resistance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

- 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*

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)

Enterococcus



- At least 12 species
- Usually non-hemolytic
- *E. faecalis* most common
- Distinguish from streptococci by:
  - esculin hydrolysis
  - growth in 6.5% NaCl
  - PYR hydrolysis (Group A β strep. are +)
- Enteric flora
- Opportunist - nosocomial pathogen
- Intrinsic antimicrobial resistance
- *E. faecium* - vancomycin-resistance
- Abscesses, urinary tract, endocarditis, abdominal/pelvic, bacteremia, wound infections

Enterococcus spp.

Characteristics

- **Gram** - Positive
- **Shape** – Cocci, in chains
- **Motility** – None
- **Oxygen Requirements** - Facultative anaerobe
- **Oxidase** – Negative
- **Catalase** – Negative
- **Pyrrolidonyl Arylamidase** – Positive
- **Hemolysis** – Gamma
- Formerly part of Group D Streptococcus
- Likes high salt
- Likes bile salts

Transmission

- Self-inoculation
- Fecal-oral

Diagnosis

- Laboratory culture and biochemical tests

Symptoms

- Gastroenteritis
- Urinary Tract Infection
  - Especially in those with catheters
- Endocarditis
- Biliary Tract Infection
- Vancomycin-Resistant Enterococcus (VRE)
  - Common cause of nosocomial, multidrug-resistant disease

Epidemiology and Risk Groups

- Worldwide
- Found in the normal flora of the colon
- Hospitalized patients

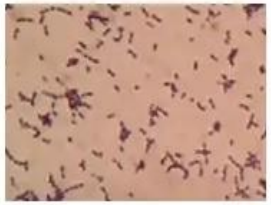
Treatment

- ✓ Ampicillin, when it's susceptible (but often physicians don't even see these infections)
- ✓ Treat VRE with linezolid or tigecycline
- ✓ Treat UTIs with nitrofurantoin

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*

Streptococcus		
Streptococcus pneumoniae	• "MOPS" • Meningitis • Otitis media • Pneumonia • Sinusitis	• Penicillins • 3rd generation cephalosporins

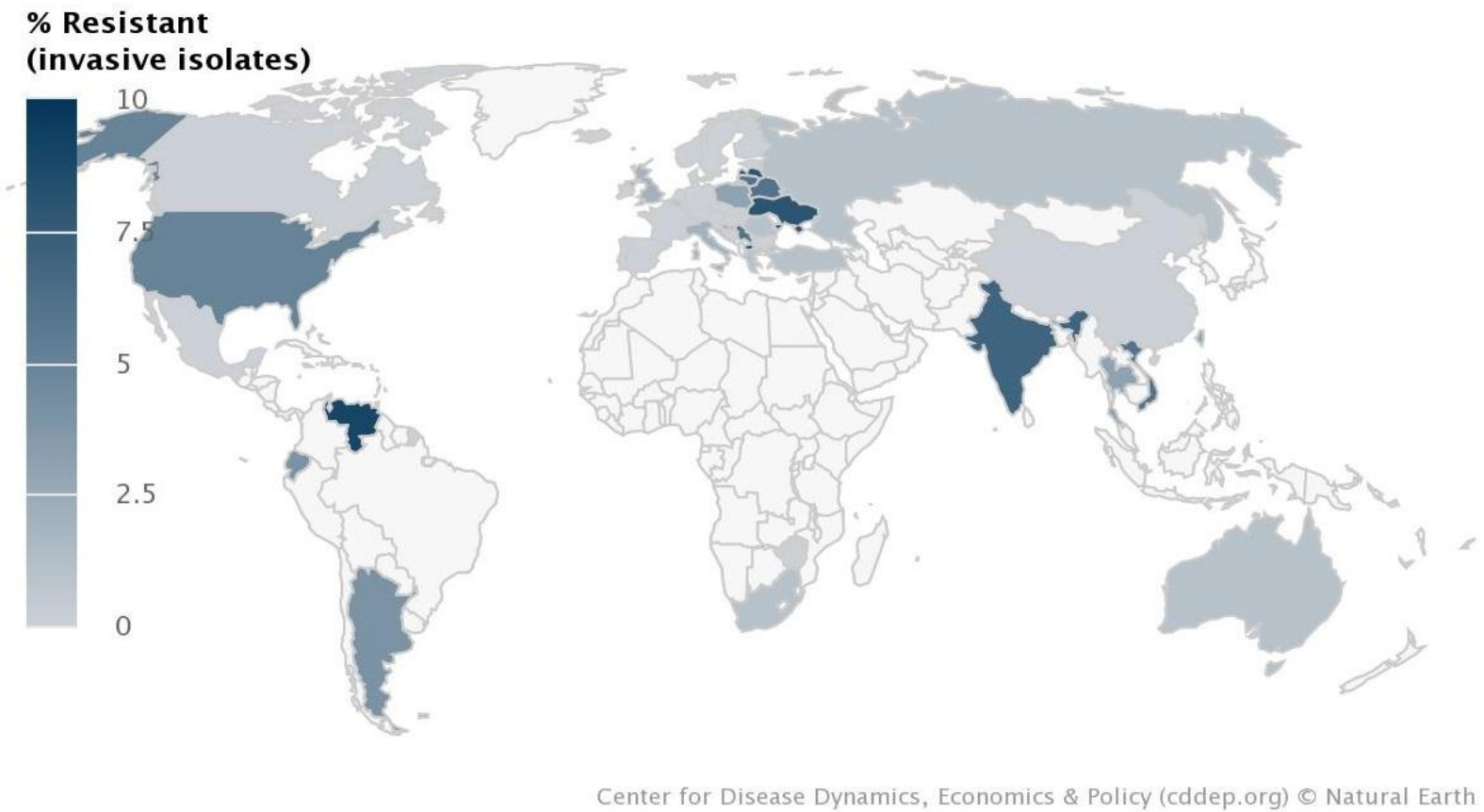
Streptococcus pyogenes (Group A)	• Pyogenic infections
	  ◦ pharyngitis
	  ◦ cellulitis
	  ◦ impetigo
	  ◦ erysipelas
	• Toxigenic infections
	  ◦ scarlet fever
	  ◦ necrotizing fasciitis
	• Immunologic infections
	  ◦ glomerulonephritis
	  ◦ rheumatic fever
		• Penicillins

Streptococcus agalactiae (Group B)	• Normal vaginal flora • Neonatal septicemia • Neonatal meningitis	• Ampicillin
Streptococcus viridans	• Normal throat flora • Dental caries • Endocarditis	• Penicillin G
Streptococcus bovis (Group D)	• Normal gut flora • Bacteremia • Endocarditis (subacute) • Associated with colon cancer	• Penicillin or ceftriaxone
Enterococci		
Enterococci faecalis (Group D)	• Normal gut flora • Endocarditis (subacute) • Urinary tract infection • Biliary tract infections	• Some strains are vancomycin-resistant (VRE) • Linezolid and streptogramins • Penicillins, vancomycin, or daptomycin • Often resistant to penicillin G

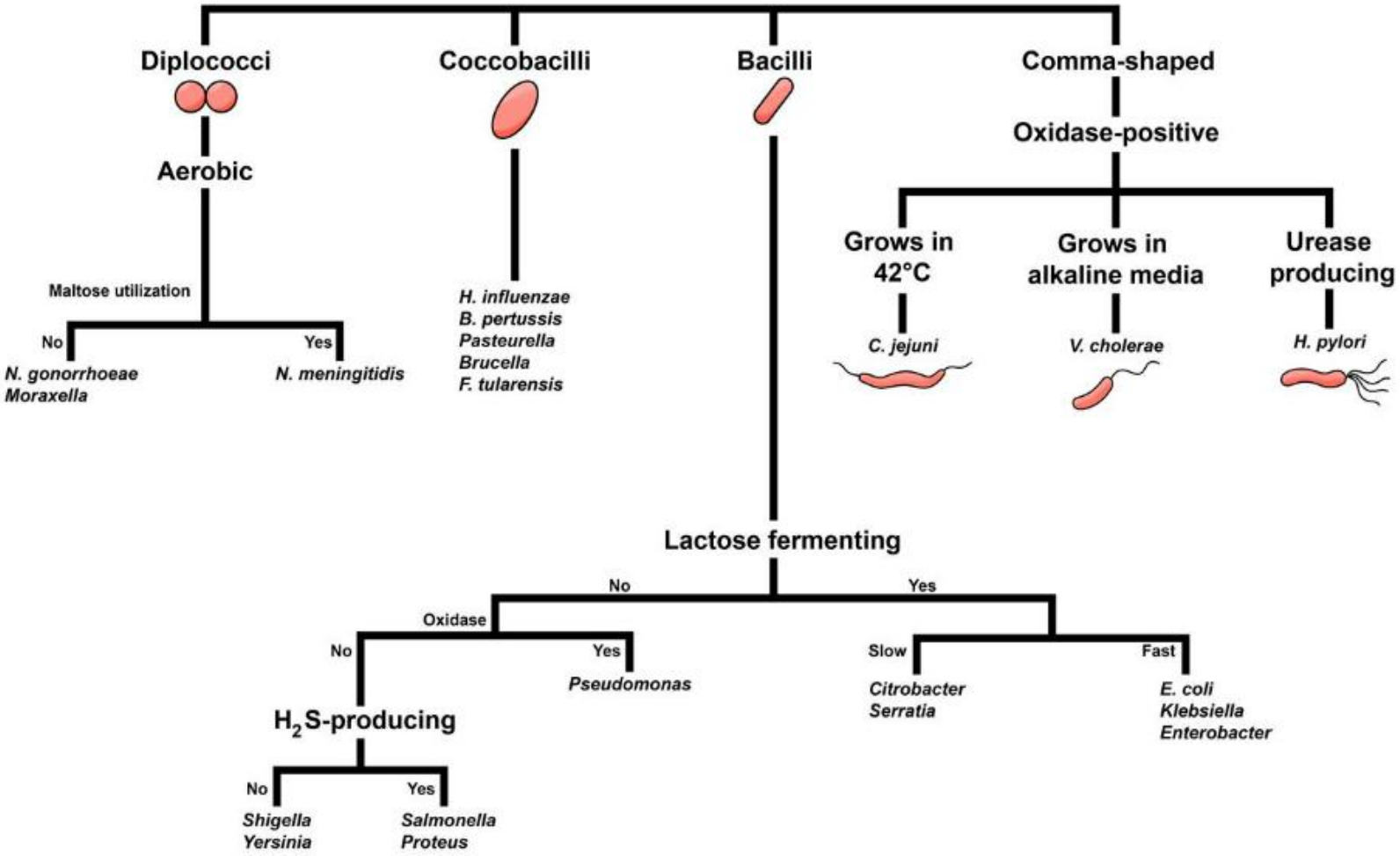
Vancomycin-Resistant Enterococci

- (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)



Gram-Negative Bacteria



Neisseria

Aerobic Gram-negative cocci

- Family - *Neisseriaceae*
- Genus - ***Neisseria***
- Species – ***Neisseria meningitidis, Neisseria gonorrooeae,*** *N.flava, N.subflava, N.perflava, N.sicca, N.mucosa* etc.

Neisseria gonorrhoeae

Neisseria meningitidis

Other species normally colonize mucosal surfaces of oropharynx and nasopharynx and occasionally anogenital mucosal membranes

- Aerobic
- Gram-negative cocci often arranged in pairs (diplococci) with adjacent sides flattened (like coffe beans)
- Oxidase positive
- Most catalase positive
- Nonmotile
- Acid from oxidation of carbohydrates, not from fermentation

Differential Characteristics of Commonly Isolated Neisseria spp.

Characteristic	*N. gonorrhoeae*	*N. meningitidis*	*N. lactamica*	*N. sicca*	*N. mucosa*	*N. flavescens*
Growth on:						
CHOC, BA (22°C)	0	0	V	+	+	+
MTM, ML (35°C)	+	+	+	0	0	0
Nutrient agar (35°C)	0	V	+	+	+	+
Acid from:						
Glucose	+	+	+	+	+	0
Maltose	0	+	+	+	+	0
Lactose	0	0	+	0	0	0
Sucrose	0	0	0	+	+	0
Fructose	0	0	0	+	+	0
Nitrate reduction	0	0	0	0	+	0

Neisseria Associated Diseases

Organism	Diseases
N. gonorrhoeae	Urethritis, cervicitis, salpingitis, pelvic inflammatory disease, proctitis, bacteremia, arthritis, conjunctivitis, pharyngitis
(ophthalmia neonatorum)	
N. meningitidis	Meningitis, meningoencephalitis, bacteremia, pneumonia, arthritis, urethritis
Other *Neisseria* species	Opportunistic infections

General Overview of Neisseria gonorrhoeae (gonococcus)

- Readily transmitted by sexual contact
- Gram-negative diplococci flattened along the adjoining side
- Fastidious, capnophilic and susceptible to cool temperatures, drying and fatty acids
  - Requires complex media pre-warmed to 35-37C
  - Soluble starch added to neutralize fatty acid toxicity
  - Grow best in moist atmosphere supplemented with CO_2
- Produce acid from glucose, but not from other sugars

Neisseria gonorrhoeae

- Gonorrhea (Greek, "flow of seed") is attributed to Galen (130 A.D.), who is said to have believed that urethral exudate in males with gonorrhea was semen.
- In 1879, Neisseria gonorrhoeae was demonstrated by Neisser in stained smears of urethral, vaginal, and conjunctival exudates, making gonococcus 2nd identified bacterial pathogen following discovery of Bacillus anthracis.
- First cultured in vitro by Leistikow in 1882
- Effective antimicrobial therapy in form of sulfonamides was first applied in 1930s.

MORPHOLOGY

Capsulated Gram negative diplococci

0.5 – 1 µm

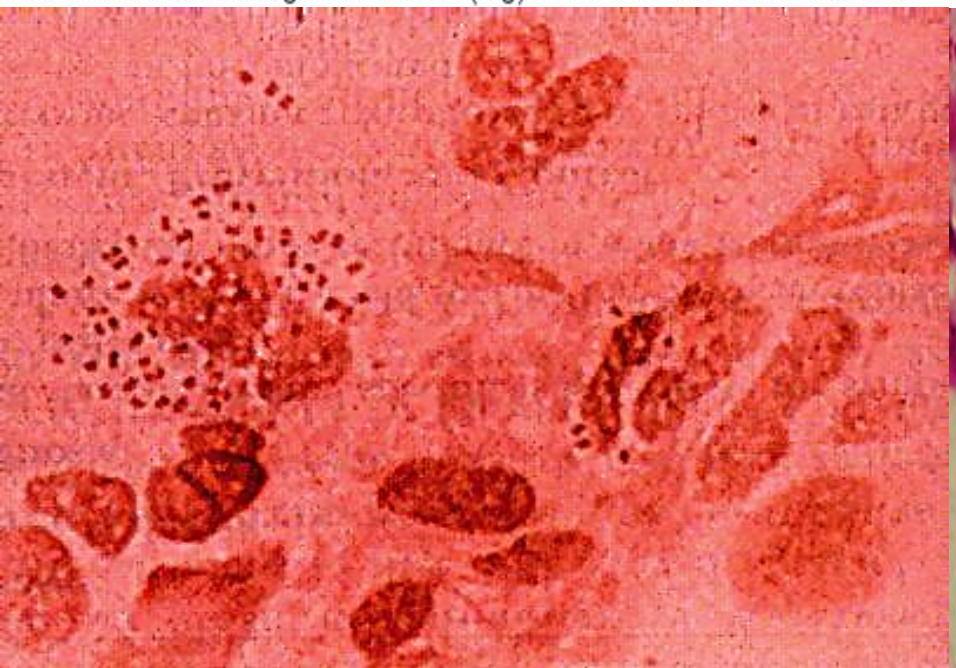
Kidney shaped, flat sides adjacent

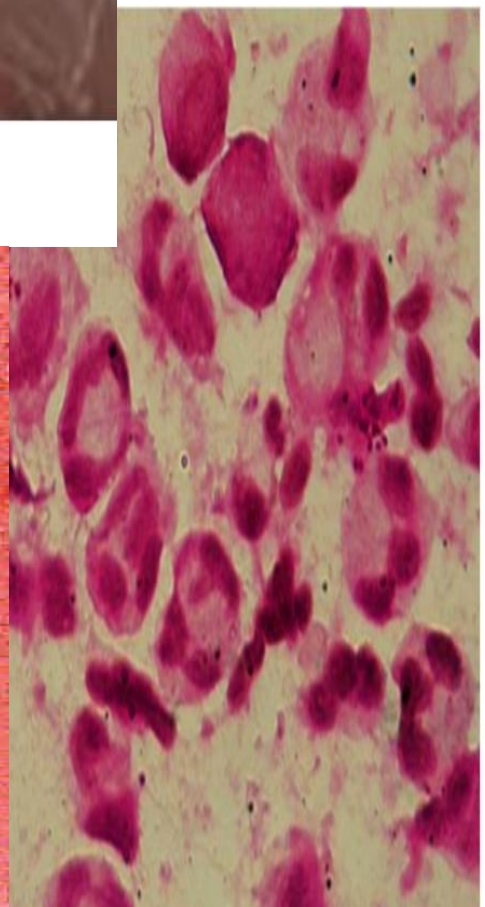
Intracellular, usually

Non-motile

- Non spore-forming

Culture of Neisseria gonorrhoeae (Ng) from a male urethral swab.





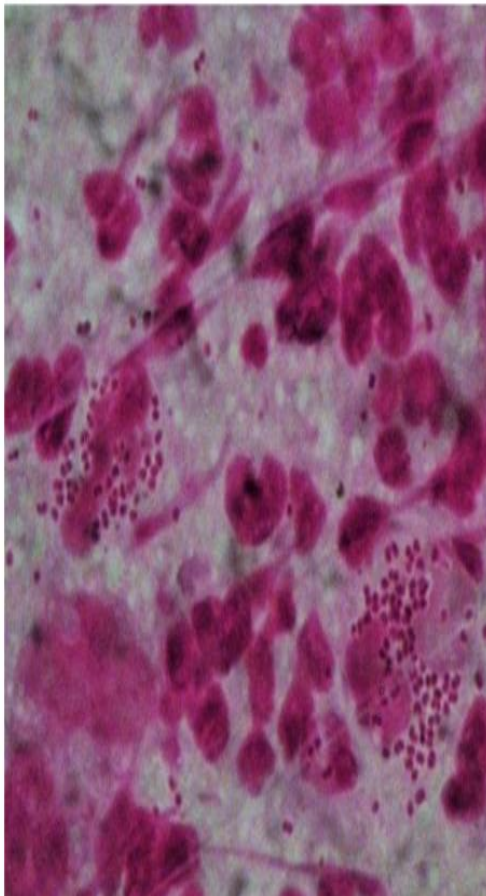


TABLE 26-2 Virulence factors of *Neisseria gonorrhoeae*

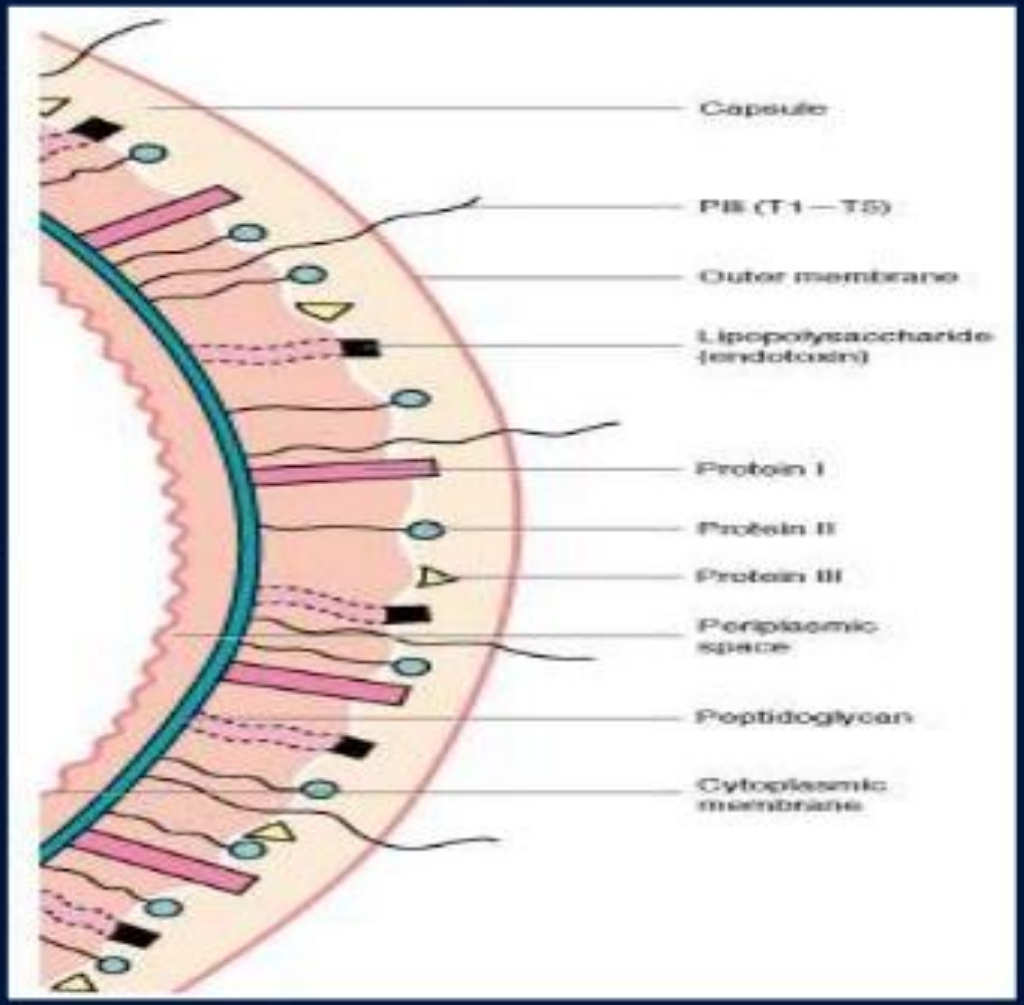
Virulence factors	Biological functions
Capsule	Prevents phagocytosis
Pili	Mediate attachment of gonococci to nonciliated epithelial cell; prevent ingestion and killing of gonococci by neutrophils
Por proteins	Confer resistance to serum killing of gonococci by preventing fusion of phagolysome in neutrophils
Opa proteins	Mediate bacterial adherence to each other, and to the eukaryotic cells
Rmp proteins	Produce antibodies that block serum bactericidal activity against gonococci
Lipo-oligosaccharide (LOS)	Possesses endotoxic activity of the bacteria
IgA protease	Destroys IgA immunoglobulin
Beta-lactamase	Degrades beta-lactam rings in the penicillin
Plasmids	Plasmid-borne virulence determinants are associated with antimicrobial resistance

Epidemiology of Gonorrhea

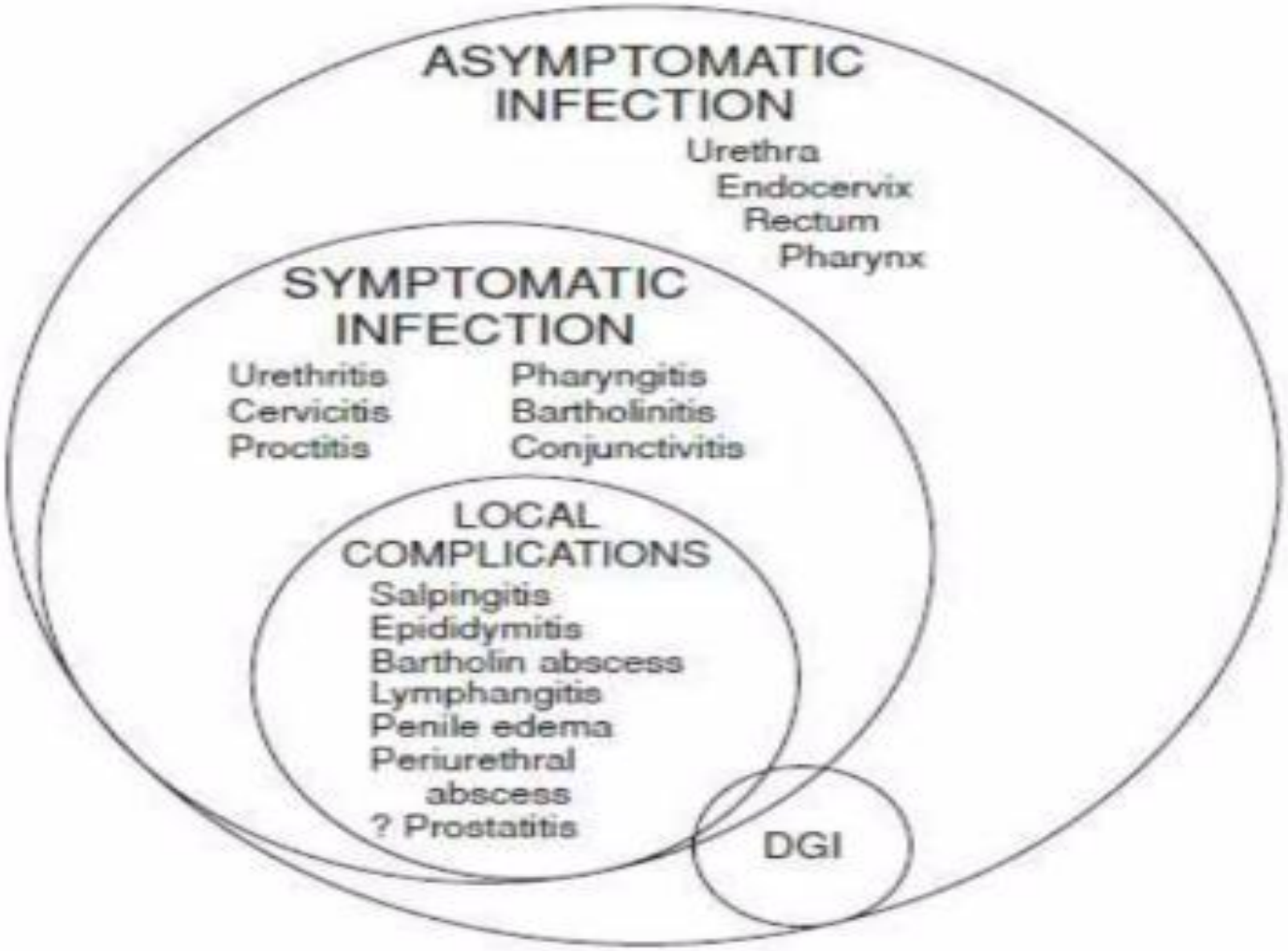
- Seriously underreported sexually-transmitted disease
  - ✓350,000 reported cases in USA in 1998
  - ✓Down from 700,00 cases in 1990
- Found only in humans with strikingly different epidemiological presentations for females and males
- Asymptomatic carriage is major reservoir
- Transmission primarily by sexual contact
- Lack of protective immunity and therefore reinfection, partly due to antigenic diversity of strains
- Higher risk of disseminated disease in patients with late complement deficiencies

VIRULENCE FACTORS

- **Fimbrae** (common pili)-
- **Lipooligosaccharide**:
- **Capsule**
- **Cell membrane proteins**
- **IgA protease-**



CLINICAL MANIFESTATIONS



Gonorrhea

Females	Males
50% risk of infection after single exposure	20% risk of infection after single exposure
Asymptomatic infections frequently not diagnosed	Most initially symptomatic (95% acute)
Major reservoir is asymptomatic carriage in females	Major reservoir is asymptomatic carriage in females
Genital infection primary site is cervix (cervicitis), but vagina, urethra, rectum can be colonized	Genital infection generally restricted to urethra (urethritis) with purulent discharge and dysuria
Ascending infections in 10-20% including salpingitis, tubo-ovarian abscesses, pelvic inflammatory disease (PID) , chronic infections can lead to sterility	Rare complications may include epididymitis, prostatitis, and periurethral abscesses
Disseminated infections more common, including septicemia, infection of skin and joints (1-3%)	Disseminated infections are very rare
Can infect infant at delivery (conjunctivitis, opthalmia neonatorum)	More common in homosexual/bisexual men than in heterosexual populatiuon

Differences Between Men & Women with Gonorrhea

IN MEN:

- Urethritis; Epididymitis
- Most infections among men are acute and symptomatic with purulent discharge & dysuria (painful urination) after 2-5 day incubation period
- Male host seeks treatment early preventing serious sequelae, but not soon enough to prevent transmission to other sex partners
- The two bacterial agents primarily responsible for urethritis among men are *N. gonorrhoeae* and *Chlamydia trachomatis*

Differences Between Men & Women with Gonorrhea (cont.)

IN WOMEN:

- Cervicitis; Vaginitis; Pelvic Inflammatory Disease (PID); Disseminated Gonococcal Infection (DGI)
- Women often asymptomatic or have atypical indications (subtle, unrecognized S/S); Often untreated until PID complications develop
- Pelvic Inflammatory Disease (PID)
  - May also be asymptomatic, but difficult diagnosis accounts for many false negatives
  - Can cause scarring of fallopian tubes leading to infertility or ectopic pregnancy

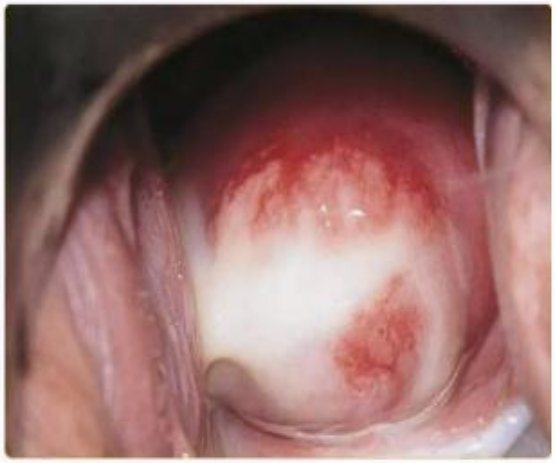
Differences Between Men & Women with Gonorrhea (cont.)

IN WOMEN (cont.) :

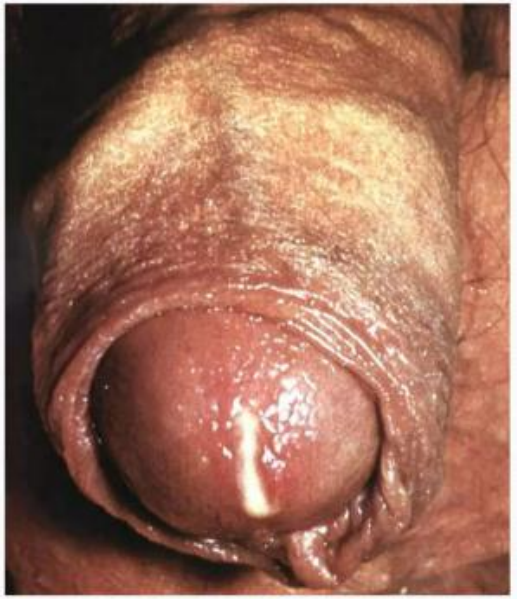
- Disseminated Gonococcal Infection (DGI):
  - Result of gonococcal bacteremia
  - Often skin lesions
  - Petechiae (small, purplish, hemorrhagic spots)
  - Pustules on extremities
  - Arthralgias (pain in joints)
  - Tenosynovitis (inflammation of tendon sheath)
  - Septic arthritis
  - Occasional complications: Hepatitis; Rarely endocarditis or meningitis

UROGENITAL INFECTION IN WOMEN

- Primary site:- endocervical canal
- Urethral colonization :- 70–90% of infected women, but is uncommon in absence of endocervical infection.
- Infection of Bartholin's gland ducts is also common.
- IP:- variable but usually 10 days



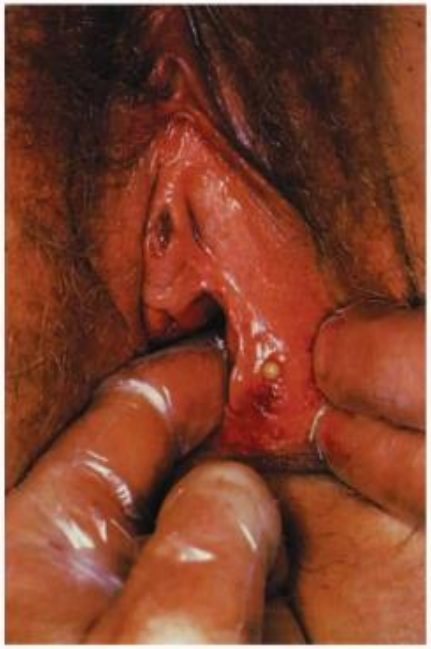
- Variable degrees of edema and erythema of the urethral meatus commonly accompany gonococcal urethritis.



RECTAL INFECTION

- Rectal mucosa is infected in 35–50% of women with gonococcal cervicitis. Only rectum is involved in 5% women.
- 40% in homosexual men.
- Symptoms range from minimal anal pruritus, painless mucopurulent discharge (often manifested only by a coating of stools with exudate), or scant rectal bleeding, to symptoms of overt proctitis, including severe rectal pain, tenesmus, and constipation.

- Most common symptoms are those of most lower genital tract infections in women:-
  - increased vaginal discharge, dysuria, intermenstrual uterine bleeding, and menorrhagia.
- Purulent exudate occasionally may be expressed from urethra or Bartholin's gland duct.



PHARYNGEAL INFECTION

- 3–7% of heterosexual men, 10–20% of heterosexual women, and 10–25% of homosexually active men.
- acute pharyngitis or tonsillitis and occasionally is associated with fever or cervical lymphadenopathy.
- >90% are asymptomatic

INFECTION OF OTHER SITES

- Gonococcal conjunctivitis is rare.
- Primary cutaneous infection i.e. localized ulcer of genitals, perineum, proximal lower extremities, or finger is rare.

COMPLICATED GONOCOCCAL INFECTIONS

- LOCAL COMPLICATIONS IN MEN:-
  - Epididymitis:-present in upto 20%. most common causes of acute epididymitis in patients under age 35 are *C. trachomatis, N. gonorrhoeae*
  - Penile lymphangitis:- penile edema ("bull-headed clap")
  - Post-inflammatory urethral strictures
  - Periurethral abscesses
- LOCAL COMPLICATIONS IN WOMEN:-
  - PID:- most common of all complications of gonorrhea, as well as the most important in terms of public-health impact
  - 10–20% of those with acute gonococcal infection.

Pathogenesis *of Neisseria gonorrhoeae*

- Fimbriated cells attach to intact mucus membrane epithelium
- Capacity to invade intact mucus membranes or skin with abrasions
  - Adherence to mucosal epithelium
  - Penetration into and multiplication before passing through mucosal epithelial cells
  - Establish infection in the sub-epithelial layer
- Most common sites of inoculation:
  - Cervix (cervicitis) or vagina in the female
  - Urethra (urethritis) or penis in the male

Gonococcal Virulence Factors

- Antiphagocytic capsule-like negative surface charge
- Only fimbriated (piliated) cells (formerly known as colony types T1 & T2) are virulent
- Outer membrane proteins (formerly Proteins I, II, & III)
  - Por (porin protein) prevents phagolysosome fusion following phagocytosis and thereby promotes intracellular survival
  - Opa (opacity protein) mediates firm attachment to epithelial cells and subsequent invasion into cells
  - Rmp (reduction-modifiable protein) protects other surface antigens from bactericidal antibodies (Por protein, LOS)
- Acquisition of iron mediated through Tbp 1 and Tbp 2 (transferrin-binding proteins), Lbp (lactoferrin binding protein) & Hbp (hemoglobin-binding protein)

Gonococcal Virulence Factors (cont.)

- Llipooligosaccharide (LOS) (Lipid A plus core polysaccharide but no O-somatic antigen polysaccharide side chain) has endotoxin activity
- IgA_1 protease
- Acquisition in last two decades of two types of antibiotic resistance:
  - Plasmid-encoded beta-lactamase production
  - Chromosomally-mediated changes in cellular permeability inhibit entry of penicillins, tetracycline, erythromycin, aminoglycosides

Laboratory Characterization

- Small, gram-negative diplococci in presence of polymorphonuclear leukocytes (PMN's) seen microscopically in purulent urethral discharge
- Susceptible to drying and cooling, so immediate culture of specimen onto pre-warmed selective (e.g., modified Thayer-Martin, Martin-Lewis agars) and non-selective media (chocolate blood agar) with moist atmosphere containing 5% carbon dioxide
- Some strains inhibited by vancomycin (in many selective agars) and toxic substances like fatty acids and trace metals in protein hydrolysates and agar found in nonselective media
- Five morphologically distinct colony types (formerly T1 through T5) that can undergo phase transition are no longer considered to be a useful distinction

- Oxidase reaction:- aids to identify gonococci from mixed culture
- A drop of tetra methyl-p-phenylene diamine hydrochloride is poured over suspected colonies, which turn pink and then dark blue
- *Nonculture diagnostic techniques:-*
- Nucleic acid amplification tests (NAATs):- polymerase chain reaction (PCR), transcription-mediated amplification (TMA), and other nucleic acid amplification technologies.
- More sensitive than culture for gonorrhea diagnosis and specificities are nearly as high as for culture.

- Immunologic or biochemical detection of gonococcal antigens or metabolic products, including surface proteins, endotoxin and oxidase or other enzymes also has been investigated in past but currently seem less promising than nucleic acid detection.
- Fluorescein-conjugated antibodies detection give positive results 24 hours before conventional culture technique.

Assay (Company)	*Ng* Targets	Cleared Specimen Types
Abbott RealTime CT/NG (Abbott)	Opa gene	Women: urine, swabs (vaginal, endocervical) Men: urine, urethral swab
cobas CT/NG (Roche)	Two different targets in the DR 9 region	Women: urine, swabs (vaginal, endocervical) Men: urine
APTIMA Combo 2 Assay (Hologic)	16S-rRNA	urine swabs (vaginal, endocervical, urethral, rectal, pharyngeal)
BD MAX GC BD MAX CT/GC BD MAX CT/GC/TV	OpcA gene	urine (20-60mL of first morning urine recommended), swabs (vaginal endocervical)
BD ProbeTec Neisseria gonorrhoeae (GC) Qx Amplified DNA Assay	Pilin-gene inverting protein homologue	Women: urine, swabs (vaginal, endocervical) Men: urine, urethral swab
BDProbeTec ET Chlamydia trachomatis and Neisseria gonorrhoeae Amplified DNA Assays	Pilin-gene inverting protein homologue	Women: urine, endocervical swab Men: urine, urethral swab
Xpert CT/NG (Cepheid)	Two distinct chromosomal targets	urine swabs (vaginal, endocervical, rectal, pharyngeal)
binx io CT/NG Assay (binx health)	Not specified	vaginal swabs

Notes: As of December 2019.

SEROLOGICAL DIAGNOSIS

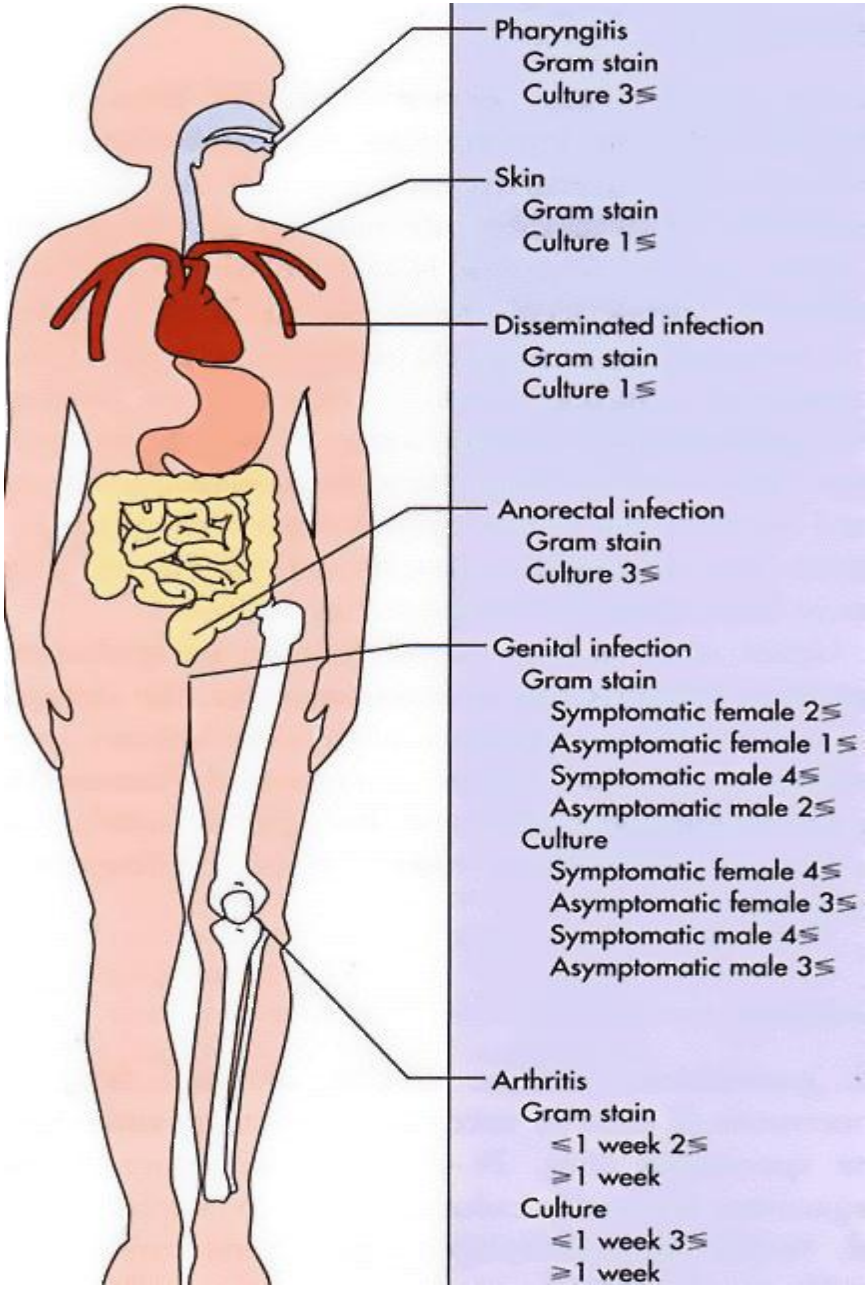
- complement fixation, immunoprecipitation, bacterial lysis, immunofluorescence, hemagglutination, latex agglutination, enzyme-linked immunoabsorbance, and other techniques.
- sensitivities of about 70% and specificities of about 80%.
- Rapid carbohydrate utilization test(RCUT):-used to detect β lactamase production by Neisseria species.
- Detected by change in colour of phenol red pH indicator from red to yellow.

Prevention & Treatment

- Penicillin no longer drug of choice due to:
  - Continuing rise in the MIC
  - Plasmid-encoded beta-lactamase production
  - Chromosomally-mediated resistance
- Uncomplicated infxn: ceftriaxone, cefixime or fluoroquinolone
- Combined with doxycycline or azithromycin for dual infections with *Chlamydia*
- Chemoprophylaxis of newborns against opthalmia neonatorum with 1% silver nitrate, 1% tetracycline, or 0.5% erythromycin eye ointments
- Treatment of newborns with opthalmia neonatorum with ceftriaxone
- Measures to limit epidemic include education, aggressive detection, and follow-up screening of sexual partners, use of condoms or spermicides with nonoxynol 9

Analytic Performance of Different Laboratory Detection Methods for Nesseria gonorrhoeae

NOTE: Importance of Sensitivity vs. Specificity for any Diagnostic Test

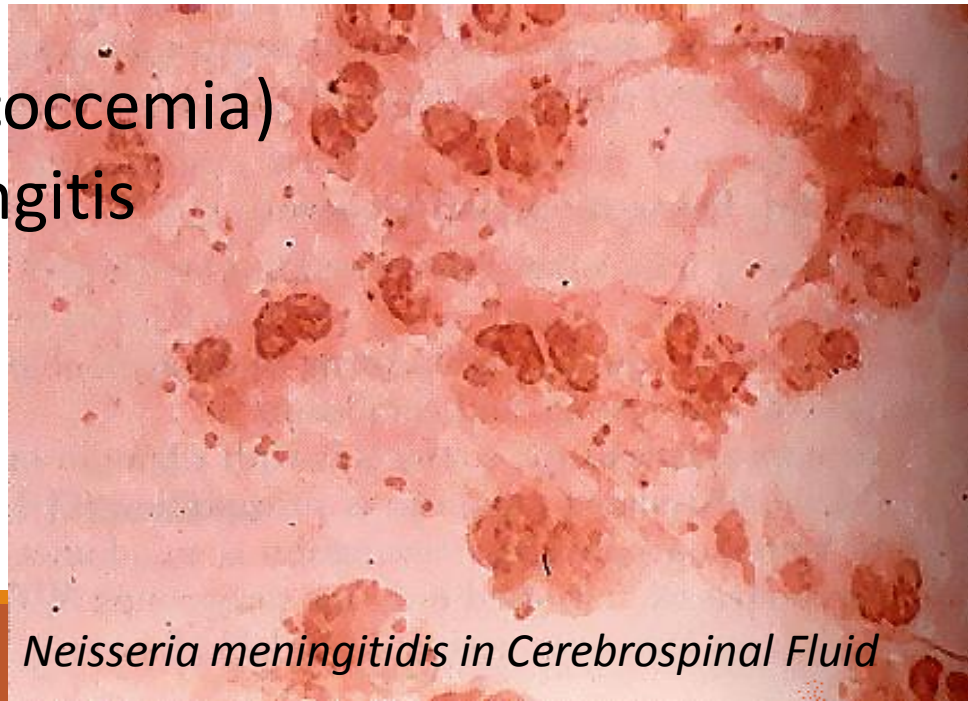


General Overview of Neisseria meningitidis

- Encapsulated small, gram-negative diplococci
- Second most common cause (behind *S. pneumoniae)* of community-acquired meningitis in previously healthy adults; swift progression from good health to life-threatening disease
- Pathogenicity:
  - Pili-mediated, receptor-specific colonization of nonciliated cells of nasopharynx
  - Antiphagocytic polysaccharide capsule allows systemic spread in absence of specific immunity
  - Toxic effects mediated by hyperproduction of lipooligosaccharide
- Serogroups A, B, C, Y, W135 account for about 90% of all infections

Diseases Associated with Neisseria meningitidis

- Following dissemination of virulent organisms from the nasopharynx:
  - ✓ Meningitis
  - ✓ Septicemia (meningococcemia) with or without meningitis
  - ✓ Meningoencephalitis
  - ✓ Pneumonia
  - ✓ Arthritis
  - ✓ Urethritis



Neisseria meningitidis in Cerebrospinal Fluid

Epidemiology of Meningococcal Disease

- Humans only natural hosts
- Person-to-person transmission by aerosolization of respiratory tract secretions in crowded conditions
- Close contact with infectious person (e.g., family members, day care centers, military barracks, prisons, and other institutional settings)
- Highest incidence in children younger than 5 years and particularly those younger than 1 year of age as passive maternal antibody declines and as infants immune system matures
- Commonly colonize nasopharynx of healthy individuals; highest oral and nasopharyngeal carriage rates in school-age children, young adults and lower socioeconomic groups

- **Reservoir and Habitat**
  - Upper respiratory tract of humans

- **Transmission**
  - Direct contact and air borne droplets
  - Close contact with infectious person (e.g., family members, day care centers, military barracks, prisons, and other institutional settings)
- **Incubation period:** 1-7 days
- **Carriage**
  - 5-30% of normal persons may harbor meningococci in nasopharynx

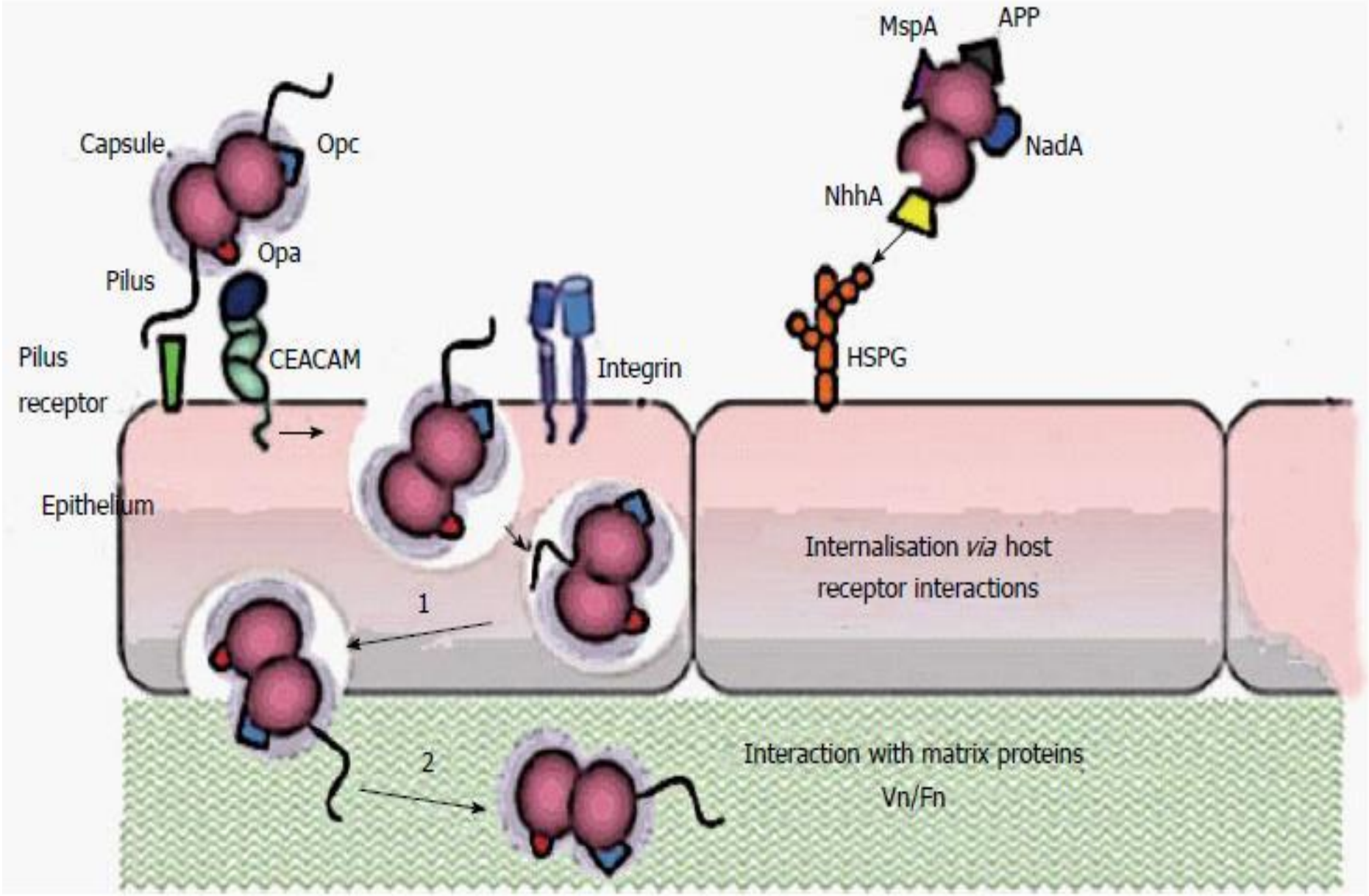
Pathogenesis of Meningococcal Disease

- Specific receptors (GD1 ganglioside) for bacterial fimbriae on nonciliated columnar epithelial cells in nasopharynx of host
- Organisms are internalized into phagocytic vacuoles, avoid intracellular killing in absence of humoral immunity and complement system (patients with late complement deficiencies are particularly at risk)
- Replicate intracellularly and migrate to subepithelial space where excess membrane fragments are released
- Hyperproduction of endotoxin (lipid A of LOS) and blebbing into surrounding environment (e.g., subepithelial spaces, bloodstream) mediates most clinical manifestations including diffuse vascular damage (e.g., endothelial damage, vasculitis (inflammation of vessel walls), thrombosis (clotting), disseminated intravascular coagulation (DIC)

Skin Lesions of Meningococcemia

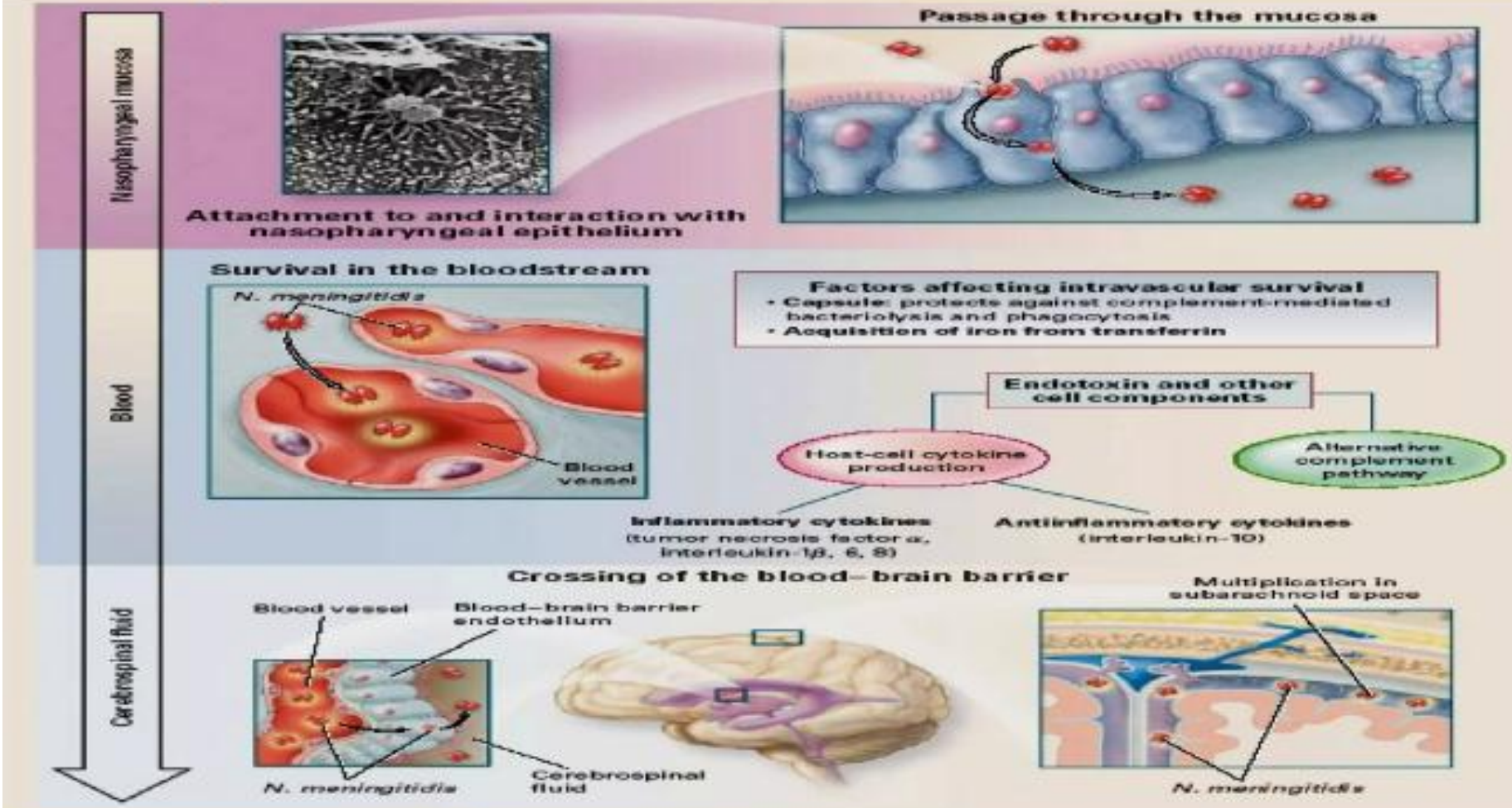
- Common cause of meningitis between the ages of **2-18 years**
- Highest incidence in **children 1 - 5 years** of age
- **Group A** meningococci
  - 90% of outbreaks
- **Group C**
  - Africa, Asia and South America
- **Group W135**
  - Occasionally

NOTE: Petechiae have coalesced into hemorrhagic bullae.

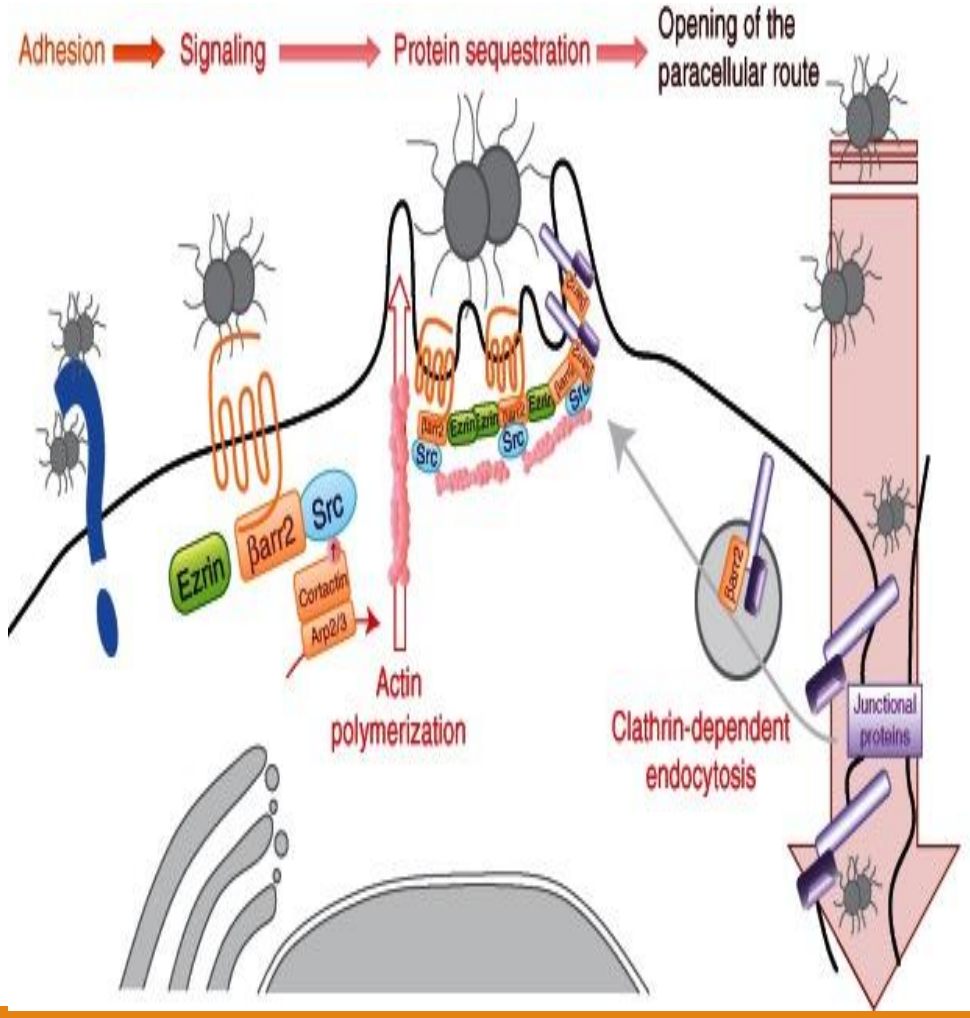

MspA
APP
NadA
NhhA
Capsule
Opc
Opa
Pilus
Pilus
receptor
CEACAM
Integrin
HSPG
Epithelium
Internalisation *via* host
receptor interactions
1
2
Interaction with matrix proteins
Vn/Fn

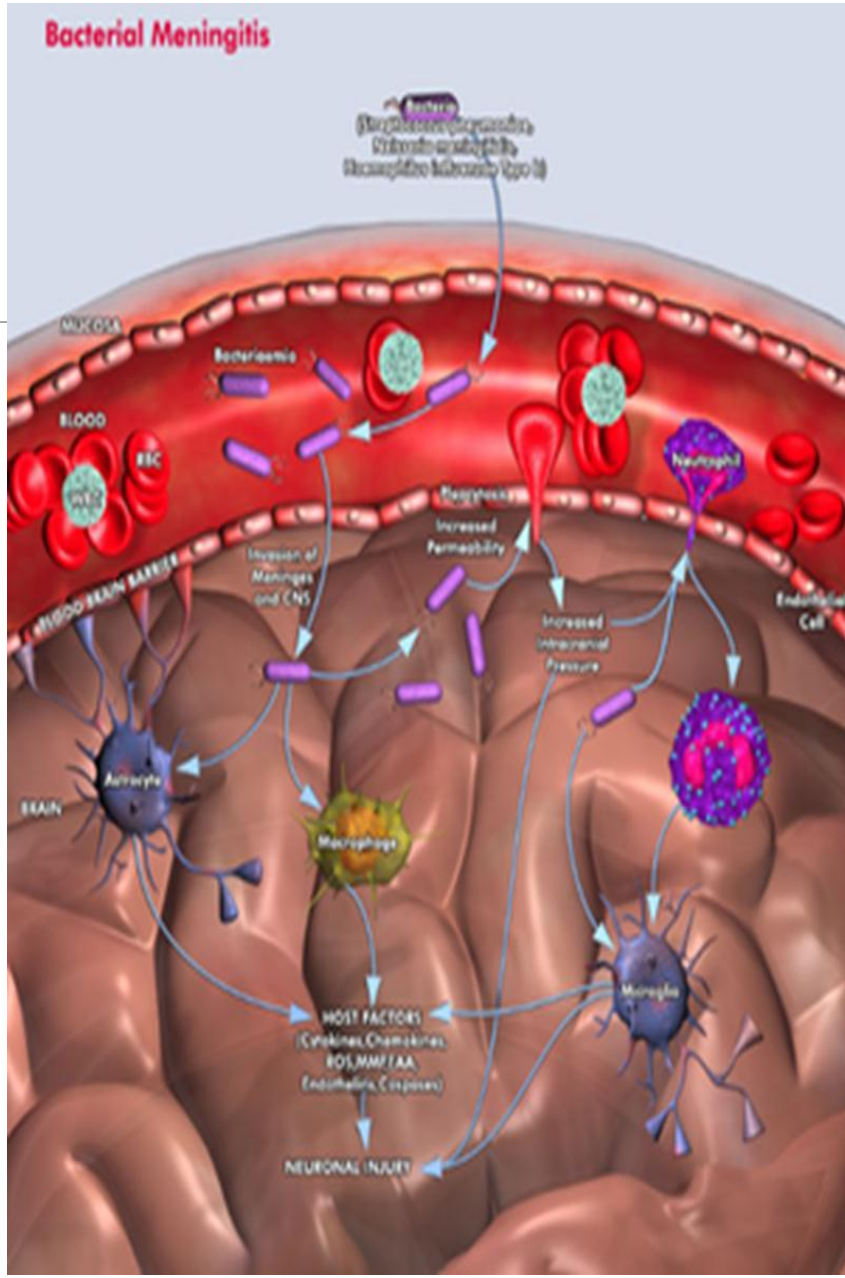
Schematic representation of the interaction mechanisms of Neisseria meningitidis with cellular receptors. The first adherence phase would be a reversible process in which Van der Waals and electrostatic forces are responsible for a wide range of interactions, including chemical bonding. Finally we added a summary at the endding, dipolar interaction and hydrophobicity. Pili extending beyond the capsule are considered to mediate the primary interaction with epithelial cells. Opa proteins may bind to carcinoembryonic antigen-related cell-adhesion molecule (CEACAMs) and heparan sulphate proteoglycan (HSPGs), and Opc proteins can interact with HSPGs and, via vitronectin and fibronectin, to their integrin receptors. Engagement of CEACAMs, integrins and HSPGs can result in meningococcal internalization by epithelial cells. MSP: Meningococcal serine protease A; App: Adhesion and penetration protein; NadA: Neisserial adhesin; NhhA: Neisseria hia/hsf homologue A.

Pathogenesis



Pathogenesis





Pathogenic Event	Host Defense	Bacterial Evasion Mechanism
Colonization and mucosal invasion	1. Secretory IgA 2. Cellular cilia activity 3. Mucosal epithelium	IgA protease secretion Ciliostasis Adhesive pili
Survival in the blood stream	Activation of Complement Pathways	Blockage of Alternative Complement Pathway
Crossing the blood-brain barrier	Cerebral endothelium	Passage through tight junctions between cells
Survival within the CSF	Poor opsonic activity	Rapid bacterial replication

C5-C9 deficiency: *pathophysiology and clinical findings*

Authors: Heather Yong
Reviewers: Jessica Tjong, Crystal Liu, Yan Yu*, Nicola Wright*
** MD at time of publication*

Normal complement response

The complement pathway is a non-specific response to bacterial pathogens

Bacterial infection

Classical, alternative, or lectin pathway activation

Complement cascade

MAC formation on bacterial surface
C5b, C6, C7, C8, C9

Complement proteins create trans-membrane channels within bacterial cell walls/cell membranes

Critical bacterial proteins leak out of the cell, breakdown of entire cell

Bacterial lysis
Especially gram (-)ve bacteria like *Neisseria*

Varied bactericidal action via other complement proteins

Abbreviations:
- **MAC:** Membrane Attack Complex
- **CH50:** Classic Hemolytic Complement Test
- **AH50:** Alternative Hemolytic Complement Test
- **CNS:** Central Nervous System
- **CSF:** Cerebrospinal Fluid

Primary (hereditary) causes
All are autosomal recessive

Secondary (acquired) causes
Biologic therapy ex. *eculizumab*

Absence or suboptimal functioning of ≥1 terminal complement proteins

C5-C9 deficiency

CH50 ± AH50 assay
No lysis

Total complement activity assay
<10% activity C5, C6, C7, C9
<50% activity C8

Inability to form MAC

↑ susceptibility to systemic *Neisseria* infection
Commonly *N. meningitidis*
Rarely *N. monorrhoeae*

Nasopharyngeal colonization of *N. meningitidis*, ↑ susceptibility to bacteremia

Note:
- Risk of invasive meningococcal disease is 1000-10000X higher in C5-C9 deficiency than in the general population
- Reason is unknown
- C5-C9 deficient patients are **not** at greater risk for contracting other gram (-) infections
- Clinical meningitis in C5-C9 deficiency is less severe and fatality is rare

Bacteria cross the blood-brain barrier, causing swelling and damaging brain tissue

Fatigue, fever, headache, altered mental status, *etc.*

Inflammation of CSF and meninges

Activation of dura and pia mater fibres

Headache, neck stiffness

Bacteria release toxins

Damage to surface blood vessels

Maculopapular rash

Exact mechanism unknown

Recurrent meningitis

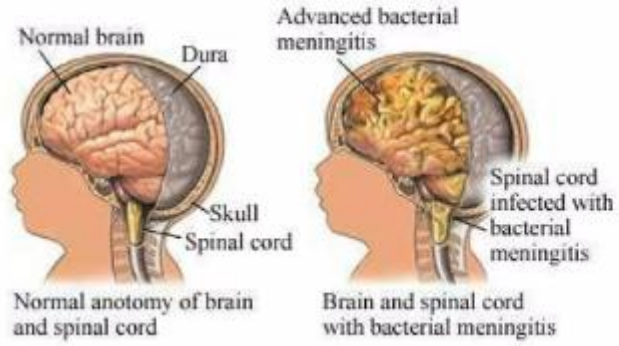
CNS damage due to recurrent meningitis

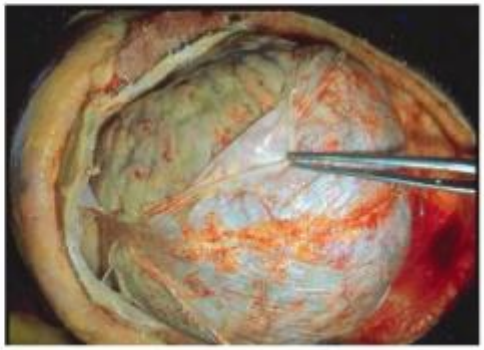
Sepsis

Published **February 16, 2019** on www.thecalgaryguide.com

DISEASES

- Meningitis
- Meningococcemia
  - Septicemia with or without meningitis
- Meningoencephalitis
- Pneumonia
- Bacteremia
- Arthritis
- Urethritis

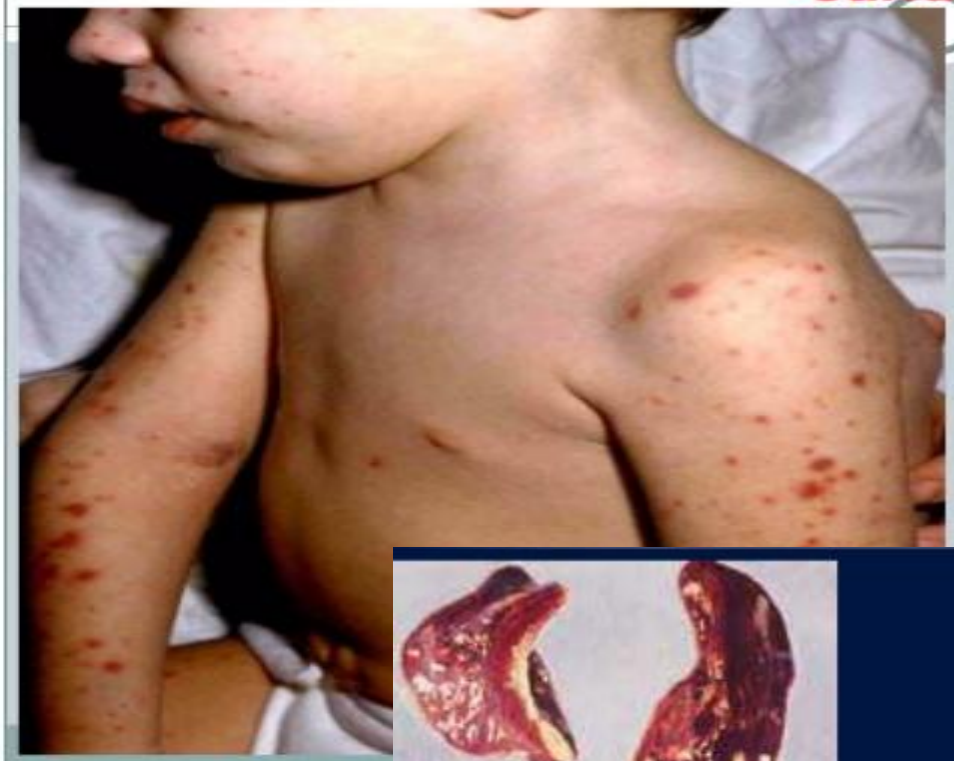


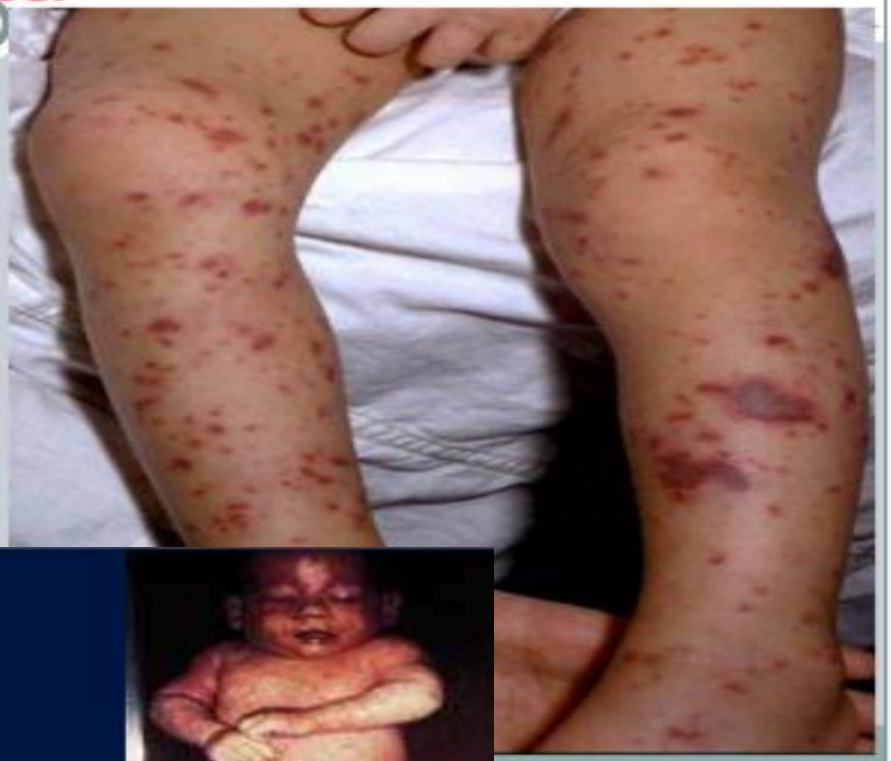


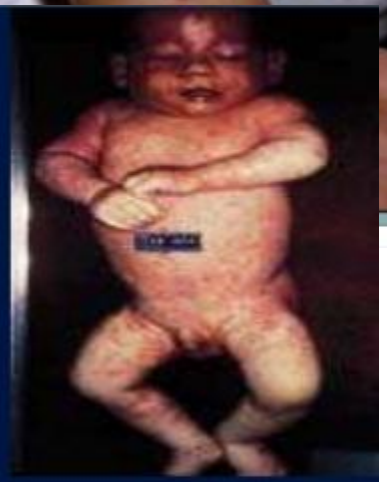
SIGNS

- Neck and back stiffness
- Positive Kernig's and Brudzinski's signs
- In children, there is usually presence of neck rigidity with bulging fontanelle

Meningococcemia showing striking involvement of the extremities with relative sparing of the skin of the child's body surface.



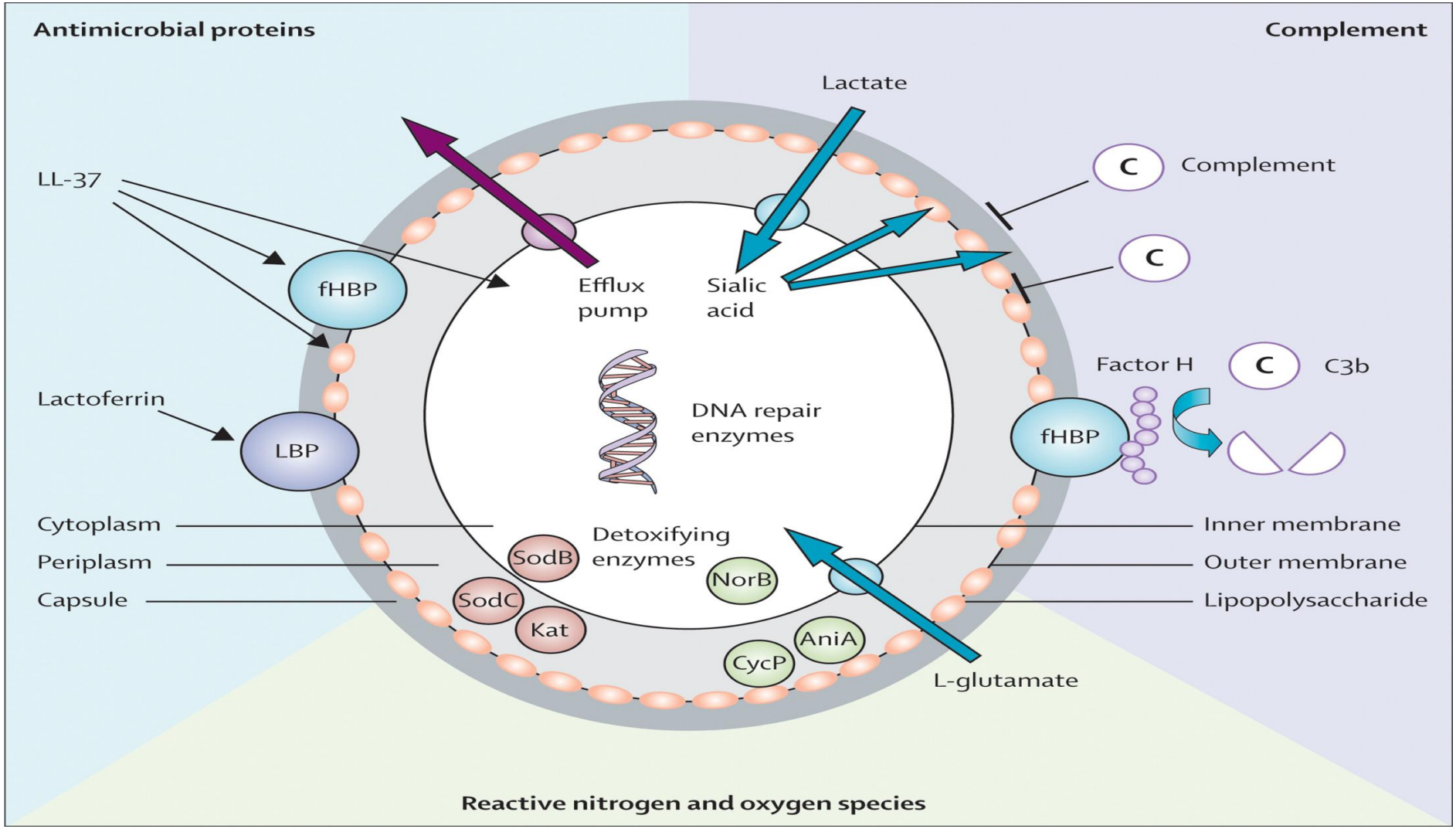




Hemorrhage in the adrenal glands in Waterhouse-Fridericksen syndrome

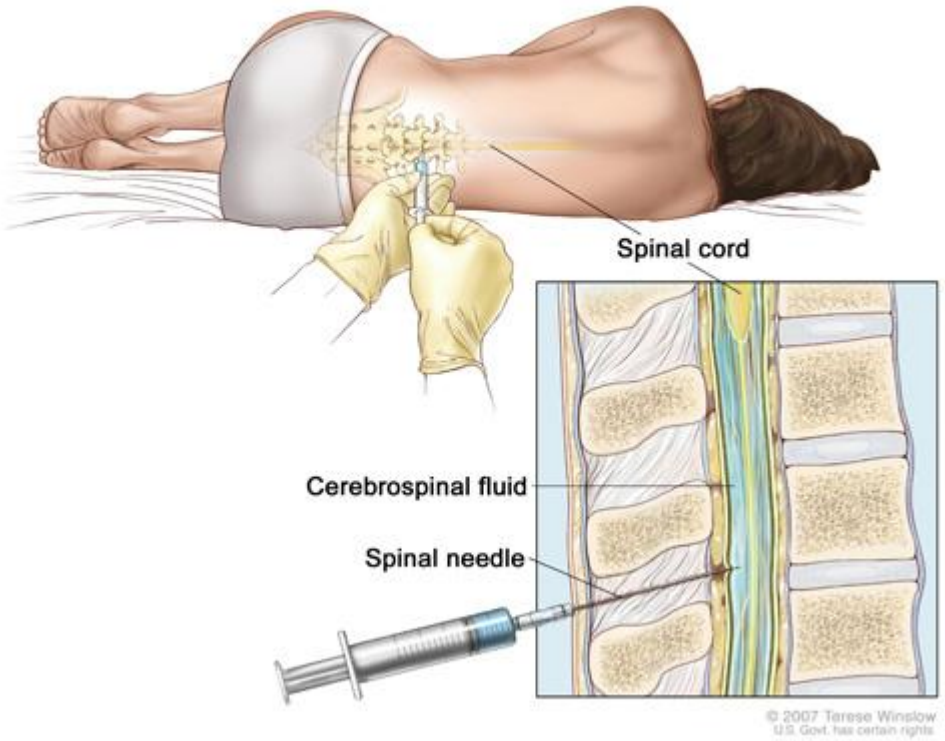
Meningococcal disease is favoured by defieciency of the terminal complement components (C_5-C_9)

Immunity


Antimicrobial proteins
Complement
Lactate
LL-37
C
Complement
fHBP
Efflux pump
Sialic acid
C
Factor H
C
C3b
Lactoferrin
DNA repair enzymes
fHBP
LBP
Cytoplasm
Detoxifying enzymes
Inner membrane
Periplasm
SodB
NorB
Outer membrane
Capsule
SodC
Lipopolysaccharide
Kat
AniA
CycP
L-glutamate
Reactive nitrogen and oxygen species

ACUTE BACTERIAL MENINGITIS

- **CSF** (Lumbar puncture)
  - Cloudy or purulent
  - Elevated pressure
  - Increased protein
  - Decreased glucose
  - Cell count
    - Usually >1000 cells/μL with Neutrophils predominating
  - Gram stain
    - Gram negative intracellular diplococci



Laboratory diagnosis

- **Specimens**
  - Blood and CSF for smear and culture
  - Nasophyrangeal swab for carrier state
- **Culture media**
  - Blood agar
  - Chocolate agar
  - Selective medium

    (Modified Thayer-Martin medium)
    - To avoid contamination vancomycin, amphotericin B and colistin are added

- Oxygen Requirement
  - Aerobic or facultative anaerobic
- Temperature
  - 37°C
- Growth promoted by
  - 5-10% CO_2
- Colony morphology
  - 1-2 mm dia, convex, grey, translucent, non-pigmented and non-hemolytic
  - After 48 hours, colonies are larger with an opaque raised centre and transparent margins

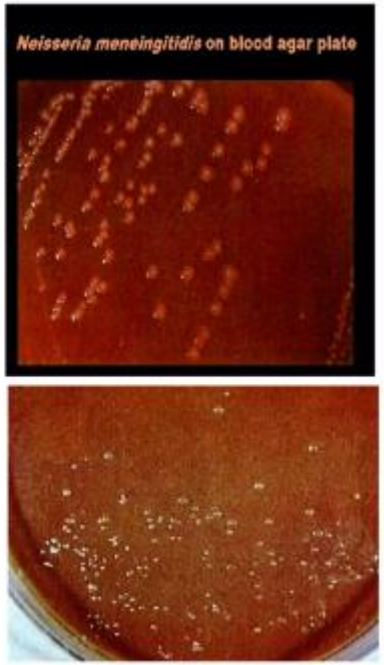
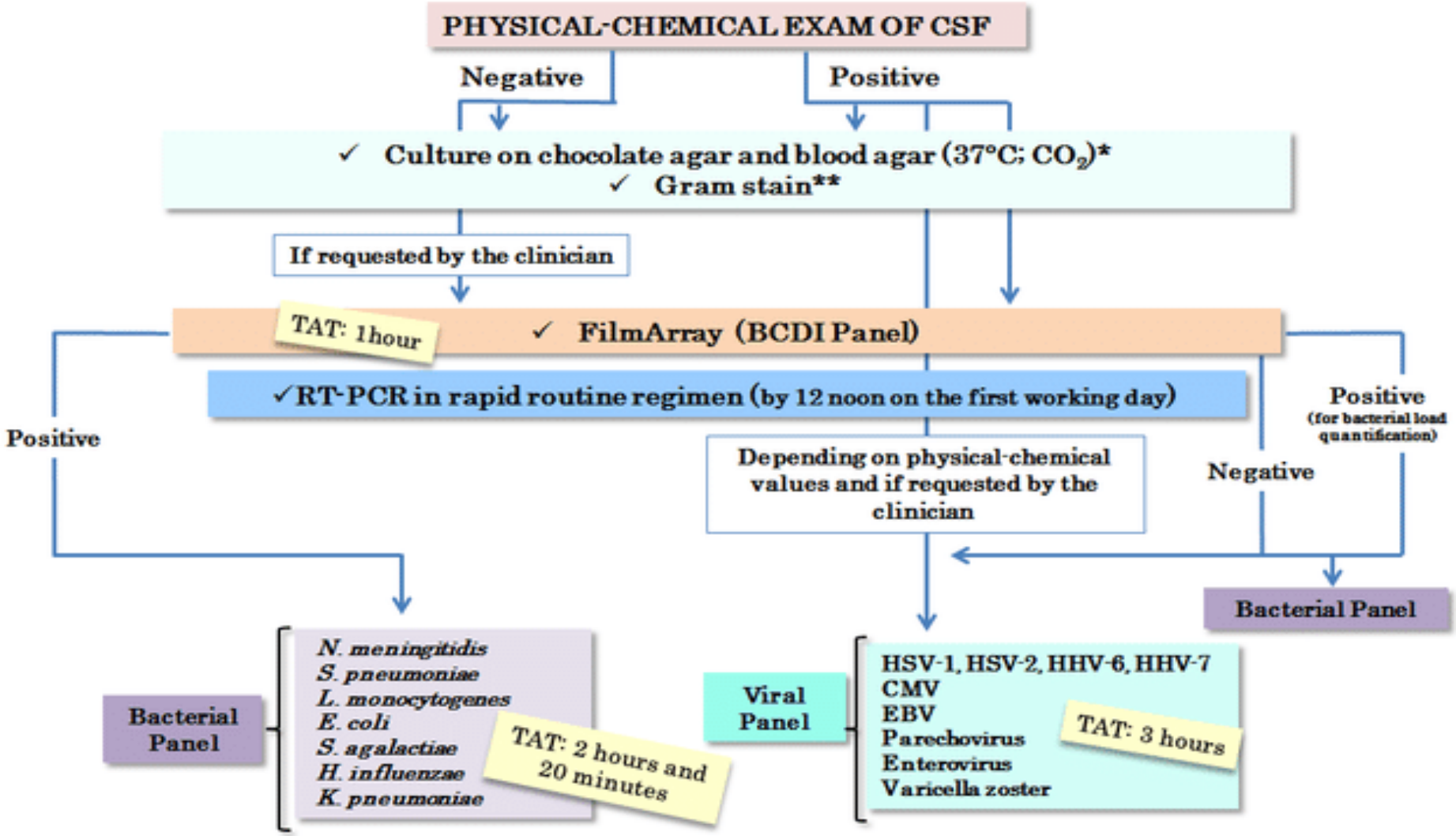


Table 7.4: Colony morphology of *Neisseria Meningitides* on media

Name of the media	Colony Morphology
Chocolate agar	Colonies are large, colorless to grey opaque colonies.
Mueller Hinton agar	Colonies are small, round, convex grey, translucent with entire edges. The colonies are butyrous in consistency and easily emulsified.

DIAGNOSTIC PATHWAY IN SUSPECTED BACTERIAL/VIRAL MENINGITIS



* in blood culture bottles and automatically processed when the Microbiology Laboratory is closed
** stained and reported by 12 noon on the firts working day by the microbiologist

Immunogenicity of Neisseria meningitidis

- Following colonization of the nasopharynx, protective humoral immunity develops against the same or closely related organisms of the same serogroup, but not against other serogroups
- Bactericidal activity of the complement system is required for clearance of the organisms
- Cross-reactive protective immunity acquired with colonization by closely related antigenic strains and with normal flora of other genera (e.g., *E. coli* K1); progressive disease can occur in absence of serogroup-specific immunity

Prevention and Treatment of Meningococcal Disease

- Penicillin is drug of choice for treatment in adjunct with supportive therapy for meningeal symptoms
  - Increasing MIC mediated by genetic alteration of target penicillin binding proteins is being monitored)
  - Chloramphenicol or cephalosporins as alternatives
- Chemoprophylaxis of close contacts with rifampin or sulfadiazine (if susceptible)
- Polyvalent vaccine containing serogroups A, C, Y, and W135 is effective in people older than 2 years of age for immunoprophylaxis as an adjunct to chemoprophylaxis
  - Serogroup B is only weakly immunogenic and protection must be acquired naturally from exposure to cross-reacting antigens

Treatment

The "go-to" drug used to treat meningococcal meningitis is **penicillin**. Though penicillin is unable to cross the blood-brain barrier, it can penetrate the barrier when the meninges are acutely inflamed. For those who are allergic to penicillin, **chloramphenicol** or a **third-generation cephalosporin** can be taken.

When preventing the spread of the infection, chemoprophylactic agents are used, with **rifampin** being the most popular option to date

Cocci		
Bacteria	**Disease**	**Treatment**
Neisseria meningitidis	• Meningococcemia • Waterhouse-Friderichsen syndrome • Meningitis	• Ceftriaxone • Ampicillin • Rifampin
Neisseria gonorrhoeae	• Gonorrhea (urethritis and PID) • Neonatal conjunctivitis • Septic arthritis • Fitz-Hugh-Curtis syndrome	• Ceftriaxone

Prevention

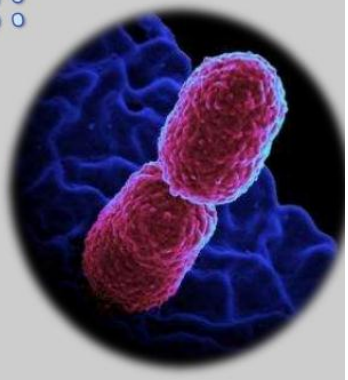
Vaccine Brand	Course commenced at age 6 weeks to ≤ 5 months of age	Course commenced at 6 to ≤ 11 months	Course commenced at ≥12 to 23 months	Course commenced at ≥ 2 years of age
Nimenrix®†	2 doses (minimum 8 weeks apart) + 1 booster dose¥^#	1 dose + 1 booster dose¥^#	1 dose¥#	1 dose#
Menveo®†	2 doses (minimum 8 weeks apart) + 1 booster dose¥^	1 dose + 1 booster dose¥^	2 doses (minimum 8 weeks apart) ¥	1 dose
Additional booster doses				
Further booster doses are not routinely recommended for healthy individuals. In circumstances where someone has previously received a primary course of meningococcal ACWY and is offered a further dose in year 10 in line with the NIP, it is acceptable to receive this dose.				

Taxonomy :

- Domain = Bacteria
- Phylum = Proteobacteria
- Class = Gammaproteobacteria
- Order = Enterobacteriales
- Family = Enterobacteriaceae
- Genus = *Klebsiella*
- Species =k.pneumonia , k.ozaenae
  k.rhinoscleromatis.

Klebsiella pneumoniae

Characteristics:



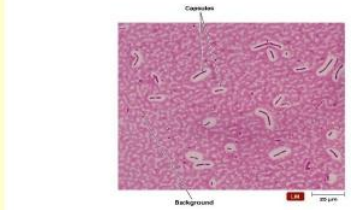
1. gram-negative
2. Non motile
3. Lactose fermenting
4. Oxidase negative
5. Rod shaped organism
6. Facultative anaerobe
7. Surrounded by thick capsule
8. Act as oppurtunistic human pathogen

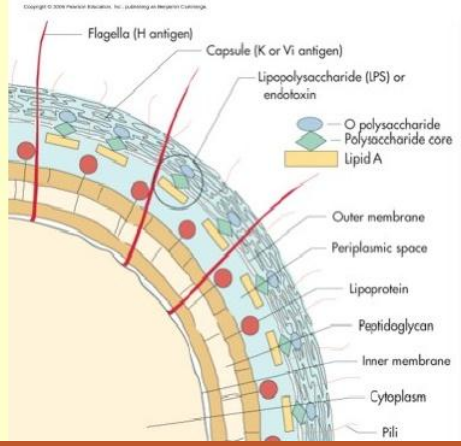
Antigenic structure

- 80 Capsular (K) antigens
  - Gram stain
  - Capsular ‘swelling’ reactiion
  - CCIE
  - ELISA
- 5 Somatic (O) antigens

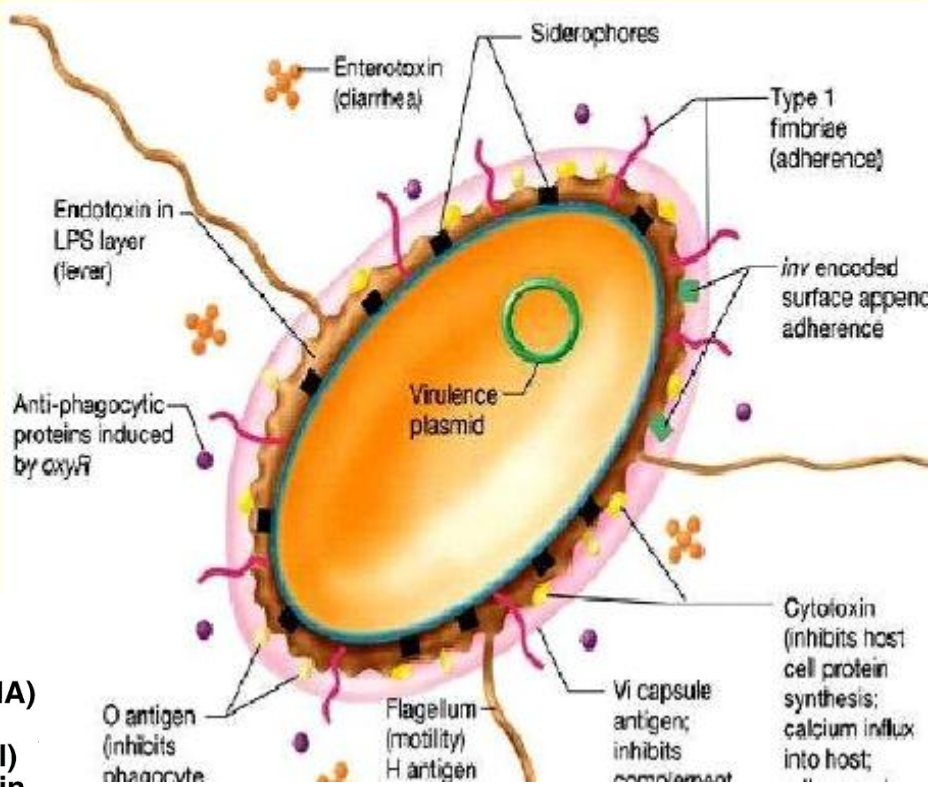
Virulence factors and Pathogenesis

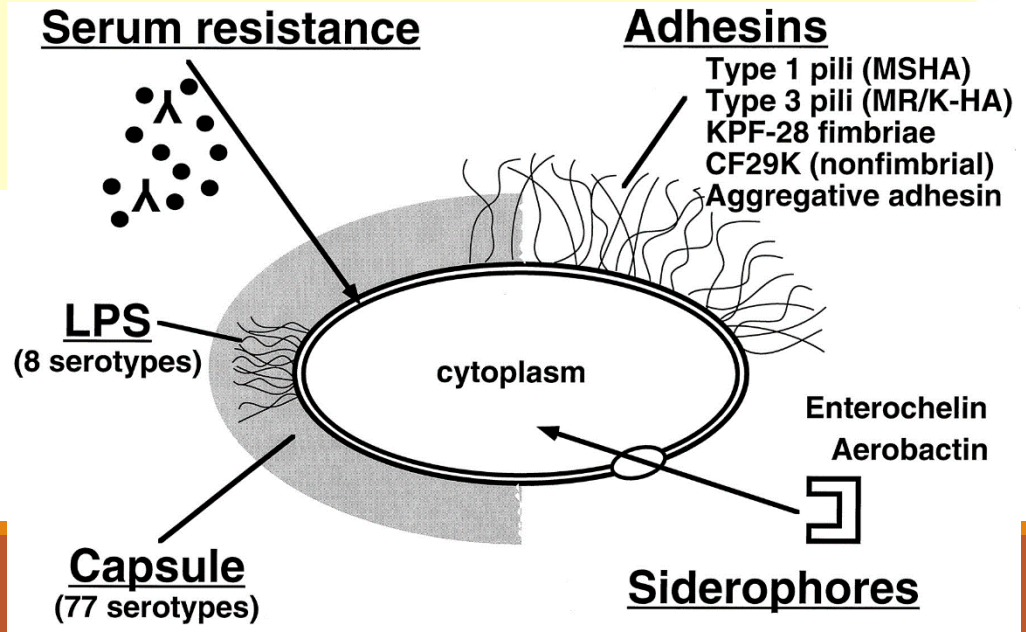
- Capsule
  - Anti phagocytic
  - Prevents from complement mediated bacteriolysis
- LPS
  - Prevent from complement mediated bacteriolysis
- Adhesins (Fimbrial and non-fimbrial)
  - Type-I and Type-III
  - Adhesion to host tissues





- Toxins
  - Heat labile and heat stable toxins
  - Role not well defined
- Enzymes
  - β-lactamase and ESBL





KLEBSIELLA

India ink capsule stain

-The background will be dark.
-The bacterial cells will be stained purple.
-The capsule (if present) will appear clear against the dark background.

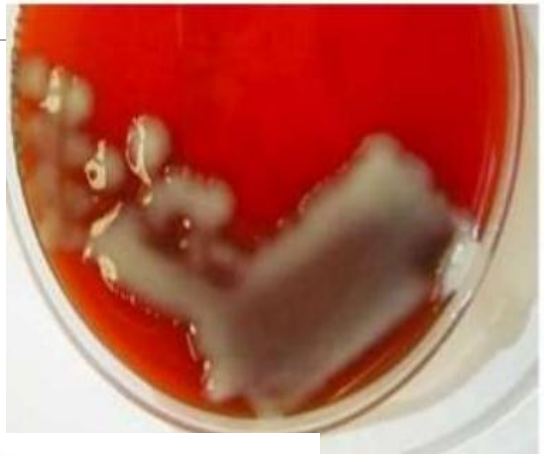
India Ink Capsule Stain of *Klebsiella pneumoniae* showing white capsules (Glycocalyx) surrounding purple cells

On EMB

Klebsiella species produces large, mucoid, pink to purple colonies with no metallic green sheen on EMB agar.

On blood agar

-slimy appearance of the colonies



On MacConkey agar

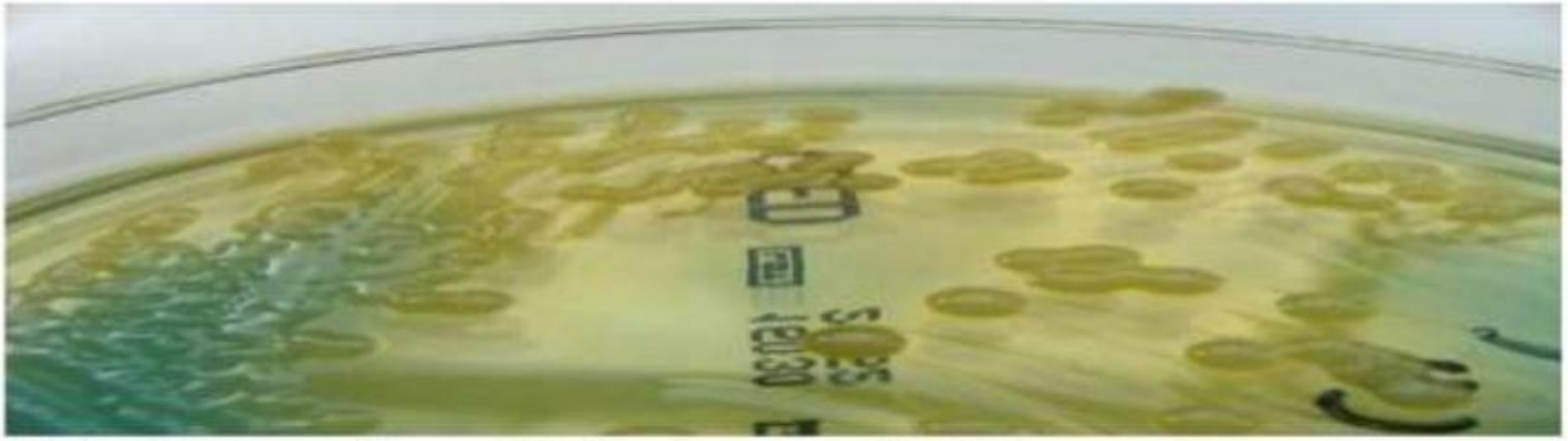
- red/pink colonies

Klebsiella pneumoniae and *Salmonella enterica* on MacConkey agar:

Mucous, lactose positive colonies of *Klebsiella pneumoniae* on

On CLED AGAR

- This medium supports the growth of urinary pathogens and provides distinct colony morphology.



Klebsiella pneumoniae on CLED Agar. Large, mucoid colonies.
Bromothymol blue indicator in the agar changes to yellow due to acidification of the medium due to lactose fermentation by bacterial growth.
Lactose fermenters appear yellow. Non Lactose fermenters remain a translucent blue.

Diseases Caused by Klebsiella :

1) urinary tract infections
2)pneumonia
3) Specticaemia
4) nosocomial infections
5) soft tissue infections.

Where it is found?

1)Found in the normal flora of the nose, mouth, skin, GI tract and intestines.
2)It is also found in soil and water.

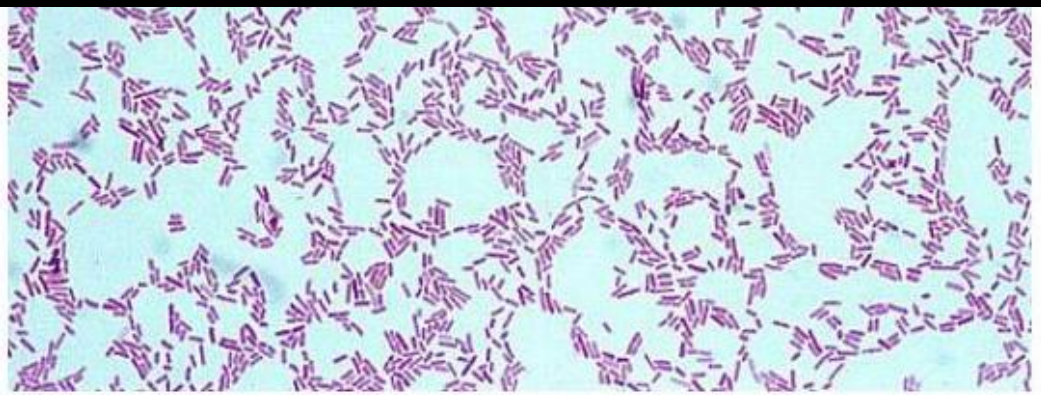
Generally, Klebsiella infections are seen mostly in people with a weakened immune system.

Some Klebsiella bacteria have become highly resistant to antibiotics.

Klebsiella pneumoniae produce an enzyme known as a carbapenemase (referred to as KPC-producing organisms).

PSEUDOMONAS

- A large group of aerobic, non sporing gram negative bacteria motile by polar flagella
- Found I nature water, soil, other moist environments
- Some of them are pathogenic to plants
- Creation of new genera such as Burkholderia. Stenotrophomnonas



Pseudomonas aeruginosa is a Gram negative, non-sporing motile rod

GENERAL CHARACTERISTICS

- Widely distributed in soil and water
- Gram negative rods
- Aerobic
- Motile
- Produce water-soluble pigments
- Opportunistic pathogens

Pseudomonas aeruginosa causing

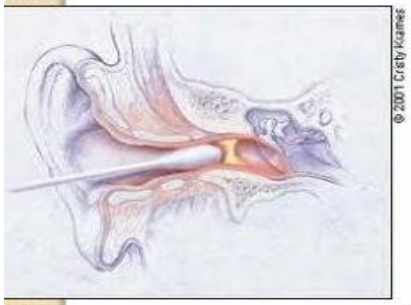
- Skin infections (wound swab)*

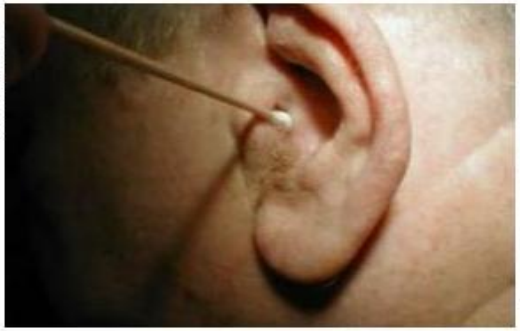
* Specimens depending on the site of infect

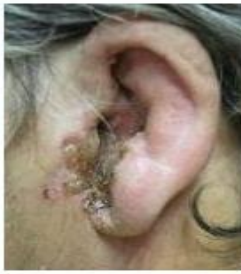
- Urinary Tract infection (urine)*

- Respiratory infections (sputum & effusions)*

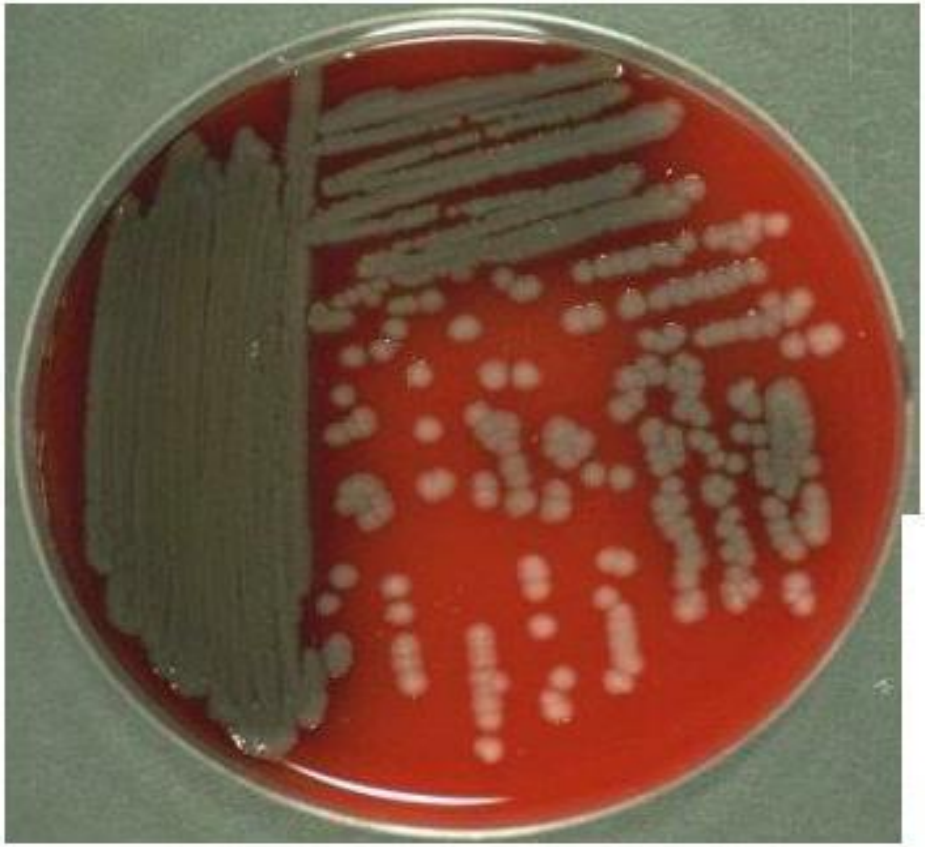
- Otitis Externa (ear swab)*







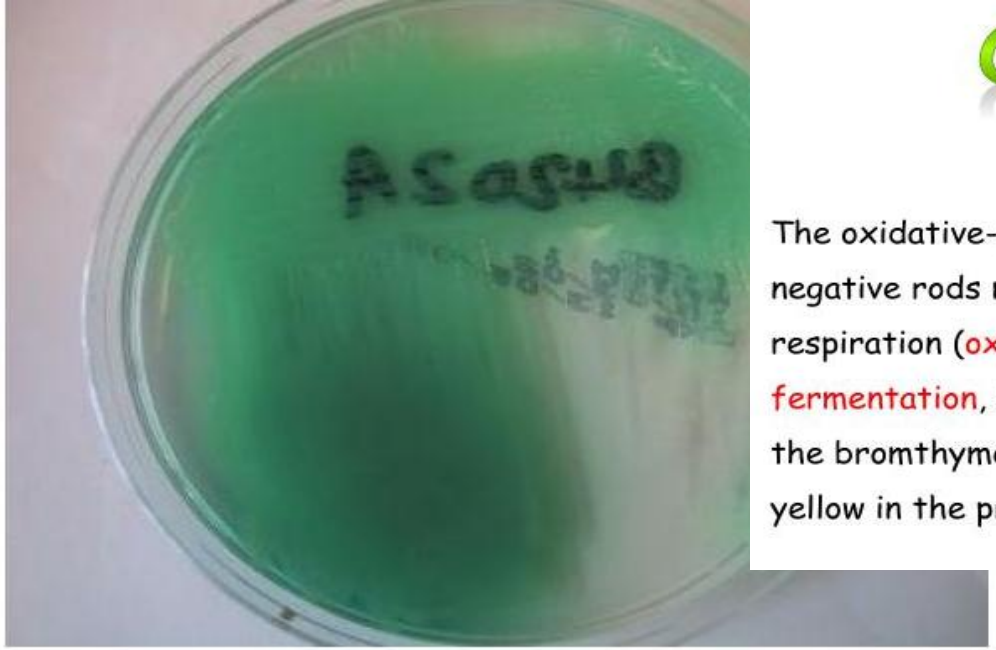
* Specimens depending on the site of infection.



P. aeruginosa produces large, flat, spreading colonies which are often haemolytic and usually pigment-producing.

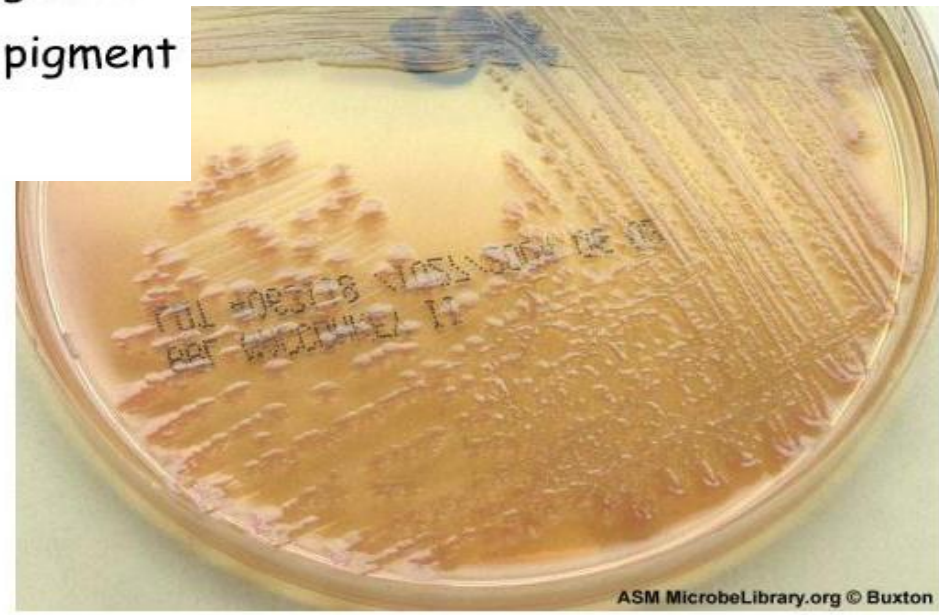
The pigments diffuse into the medium giving it a dark greenish-blue colour

O-F TEST



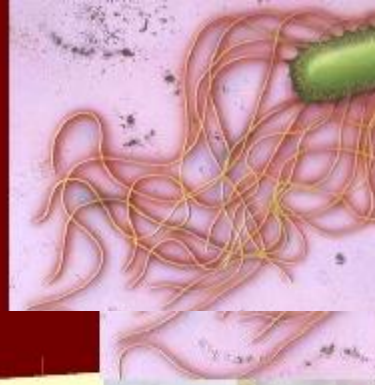
The oxidative-fermentative test determines if certain gram-negative rods metabolize glucose by fermentation or aerobic respiration (oxidation). During the anaerobic process of glucose fermentation, the high concentration of acid produced will turn the bromthymol blue indicator in OF media from green to yellow in the presence or absence of oxygen

On Nutrient agar *P. aeruginosa* can be recognized by the pigments, it produces a blue-green pigment (pyocyanin).



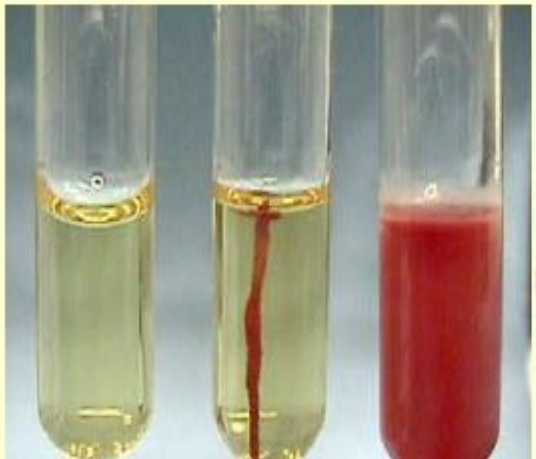
P. aeruginosa produces pale coloured colonies on MacConkey

Species of Proteus



- ***Proteus mirabilis***
- ***Proteus vulgaris***
- *Proteus myxofaciens*
- *Proteus penneri*

Motility test



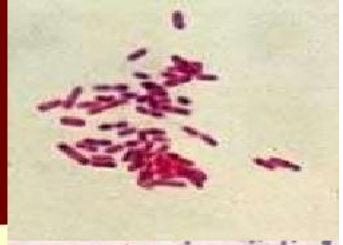
Virulence Factors

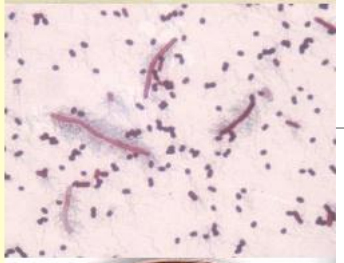
- Urease activity
- Protease
- Fimbriae
- Haemolysins
- Motility
- Swarming

Pathogenicity

- *P. mirabilis* -70-90 %
- UTI – Commonest site
  - Young / elderly patients
  - High concentration of Urea in urine
- Superficial septic lesions
- Meningitis
- Osteomyelitis
- Septicemia
- Otitis media

Lab Isolation and Identification





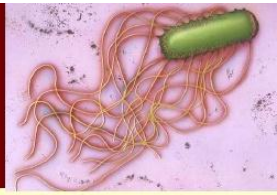
Morphology

- GNR, 1 – 3 um
- Motile-peritrichate flagella

Cultural Characteristics

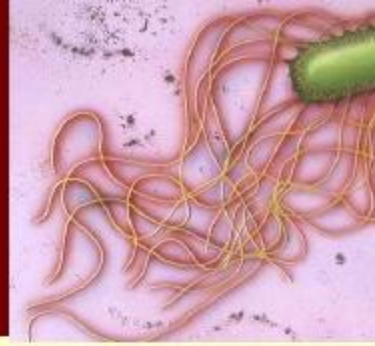
- Grow well on ordinary media
- Swarming
  - Continuous
  - Discontinuous
- Faint ammonia / fishy odor

Swarming



- Characteristic but not unique
  - *Serratia marcescens*
  - *Vibrio parahaemolyticus*
  - Bacillus
- Continuous swarming
- Discontinuous swarming
- Ascending infection

Anti-swarming Agents



- Increasing Agar concentration 3-4 %
- Incorporation into media of a polyvalent-H anti-sera
- Incorporating growth inhibitors
  - Sulphonamides
  - Chloral Hydrate
  - p-Nitrophenyl Glycerol
  - Neomycin
  - Barbiturates
- Incorporation of
  - Detergents
  - Bile Salts-MacConkey Agar
- Electrolyte Deficiency- CLED