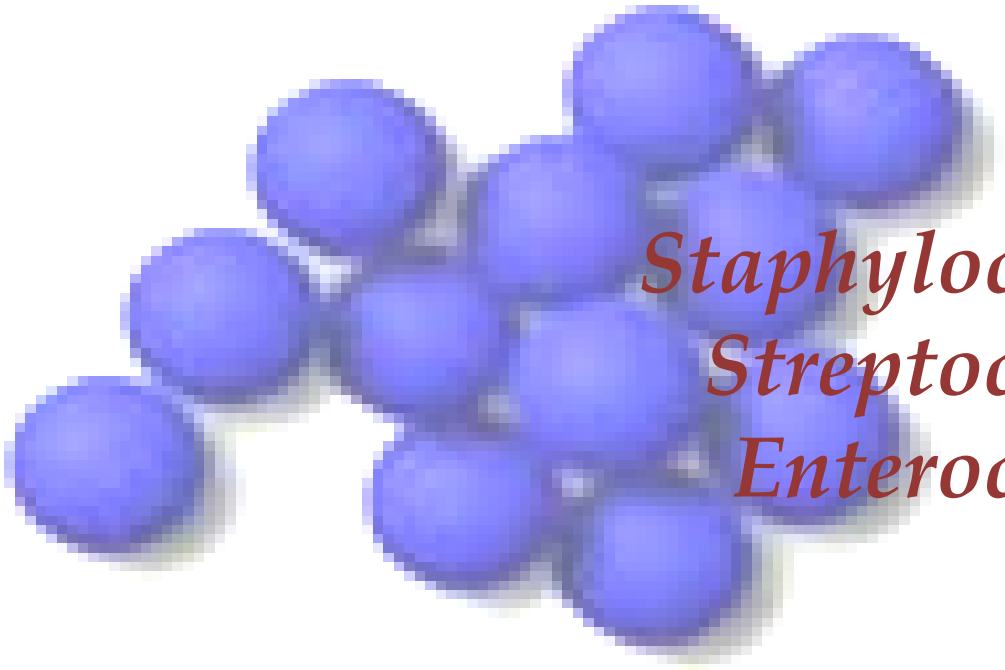
SPECIAL BACTERIOLOGY

Gram-positive and Gram-negative cocci

Hemophilic and other ‘fastidious’ Gram-negative bacilli

Gram-positive cocci

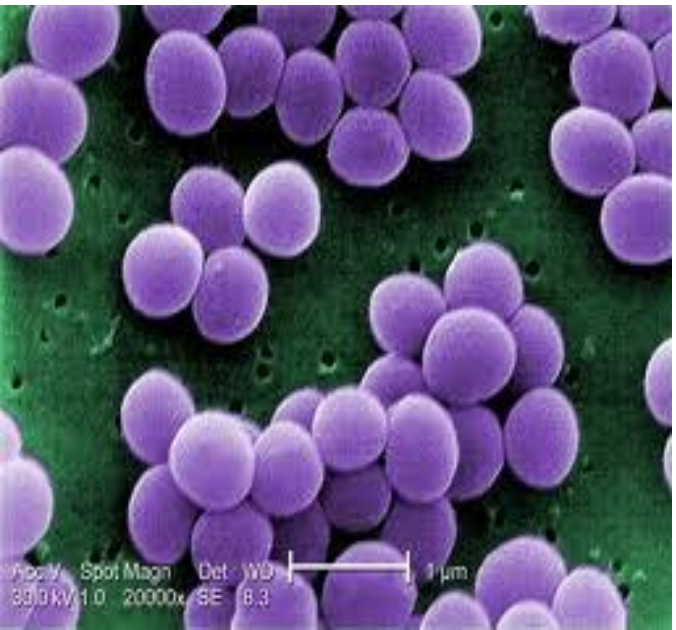


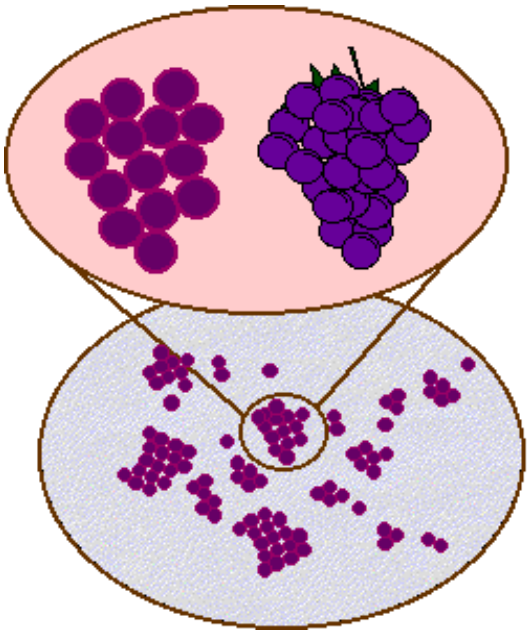
Staphylococcus spp.
Streptococcus spp.
Enterococcus spp.

Staphylococcus spp.
- general characteristics -

Staphylococci are Gram-positive ball-shaped bacteria (cocci), grouped in clusters

Staphylo- comes from the Greek word for grape clusters





Pathogenic and opportunistic staphylococci

Staphylococcus aureus is one of the major causes of pyogenic infections in humans. It colonizes the nasal mucosa of 30-40% of people and causes serious diseases.

Staphylococcus epidermidis - the most common ubiquitous bacterium that inhabits the skin of most people and rarely causes disease in healthy people. It causes numerous infections in the hospital environment, in patients with various implants.

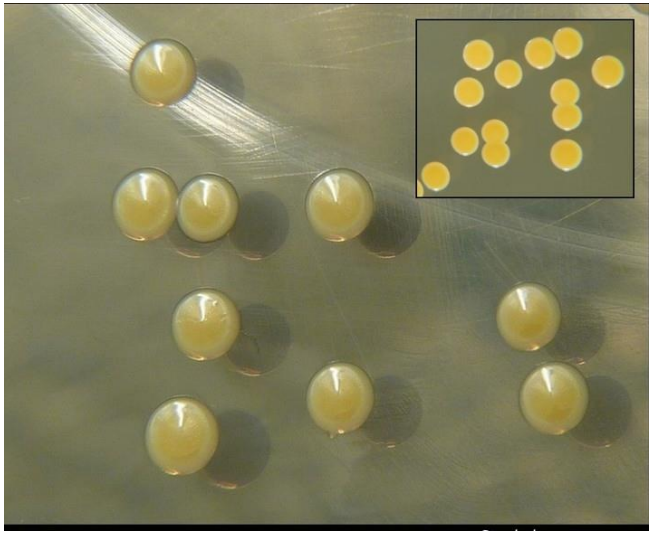
Staphylococcus saprophyticus exclusively causes urinary tract infections..

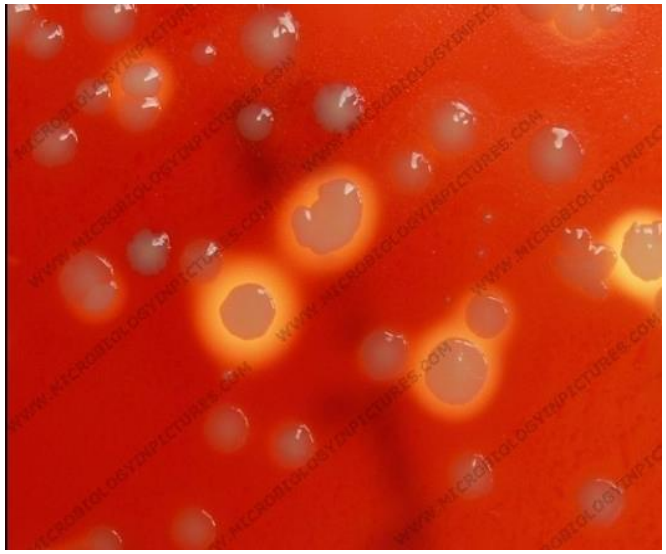
Staphylococcus spp.
-identification-

All staphylococci **form colonies on artificial nutrient media** and **can be Gram-stained**.

S. aureus is named after the characteristic pigment that gives its colonies a golden yellow color (colonies of other species of staphylococci are white).

All staphylococci produce catalase, an enzyme that breaks down hydrogen peroxide.





β-hemolytic *S. aureus*

Culture characteristic of *S.aures*

Colony morphology on many types of agars:

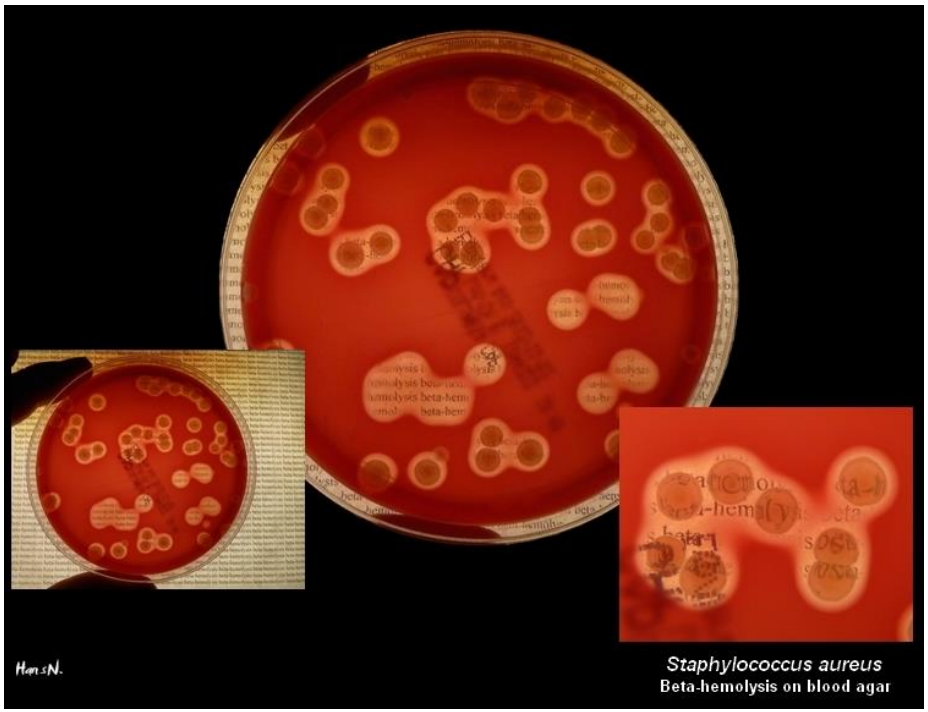
On nutrient agar

S. aureus colonies are:

- Large, circular, smooth and are pigmented (golden-yellow)

On blood agar:

β-hemolysis/ clear zone around the colonies



Staphylococcus aureus

The coagulase test is used to distinguish between S. aureus and other types of staphylococci.

Species	Coagulase	Color of colonies	Novobiocin resistance
Staphylococcus aureus	+	White to golden yellow	-
Staphylococcus epidermidis	-	White	-
Staphylococcus saprophyticus	-	White	+

Staphylococci are divided into:-

- **Coagulase positive staphylococci**:- *S.aureus (most* pathogenic species).
- **Coagulase negative staphylococci:** -*S.epidermidis* and *S .saprophyticus* are the most important coagulase-negative species that can cause infections in man.

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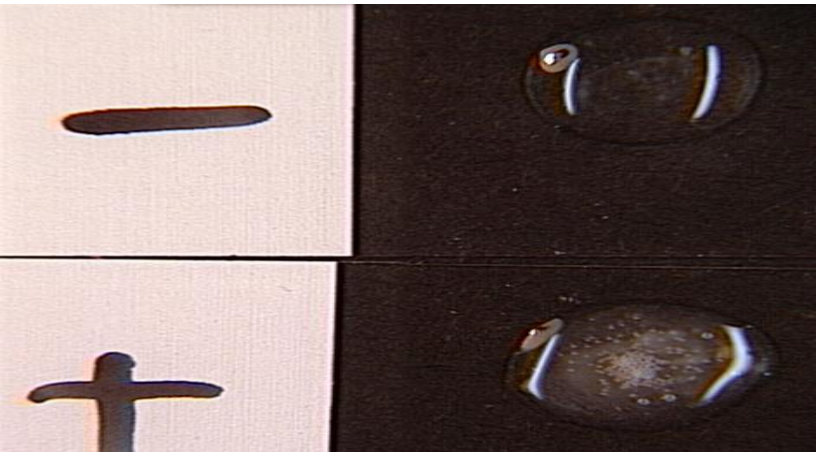
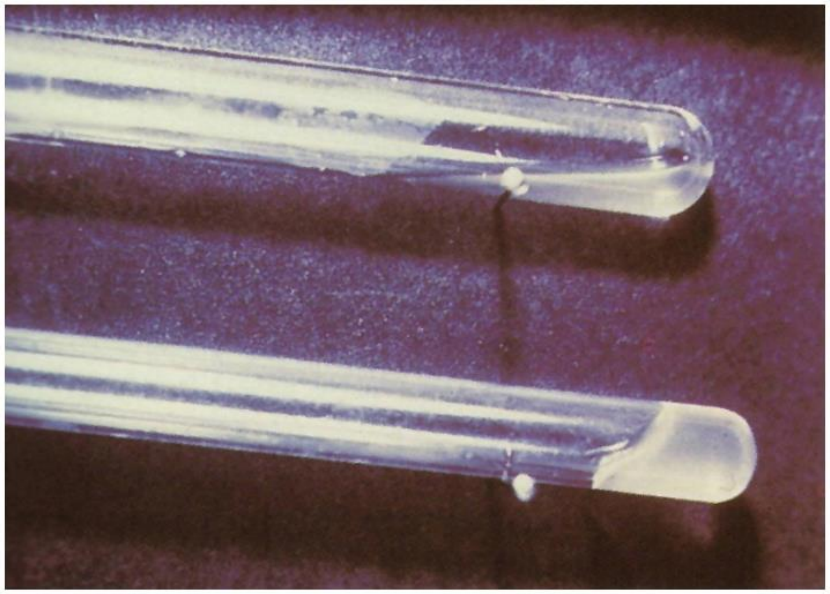


S. aureus releases free coagulase, an enzyme that coagulates plasma, therefore *S. aureus* is a coagulase-positive staphylococcus. Other types of staphylococci do not produce free coagulase and these are coagulase-negative staphylococci.



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S. aureus possesses clumping factor (bound coagulase) that induces the formation of coagulum around bacteria in plasma.

The coagulase test

(on a plate and in a test tube)

-Novobiocin Susceptibility Test

- This test is used to differentiate coagulase-negative staphylococci.

Staphylococcus saprophyticus

Staphylococcus epidermidis

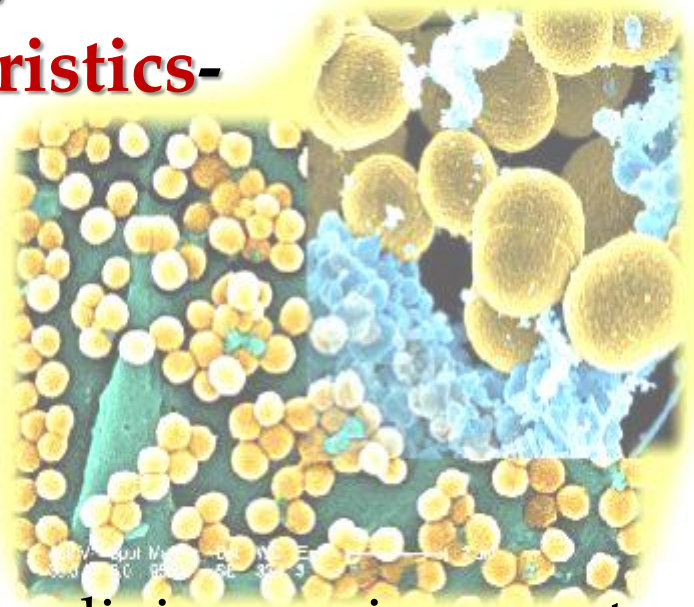
Classification

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

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

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

Staphylococcus spp.
-epidemiological characteristics-



- ✓ Staphylococci survive in living and non-living environments (bedding, doorknobs and clothing). They are resistant to drying and high temperature.
- ✓ **Humans** are the major reservoir of *S. aureus*. This bacterium colonizes the nasal mucosa. It transiently colonizes the skin, oropharynx, vagina and feces.

Staphylococcus aureus

- Natural habitat: Nostril and skin

- **Gram positive** cocci, 0.5-1.5 μm in diameter
- Form irregular **grapelike clusters**
- Non-motile, non-sporing and few strains are **capsulated**

...

Staphylococci successfully colonize the skin because they can grow at high salt and lipid concentrations.

Staphylococci produce enzymes, lipases and hydrolases, that break down the protective lipid layer of the skin.

S. aureus colonizes the skin and mucosa using bacterial surface molecules (**MSCRAMMs**, *Microbial Surface Components Recognizing Adhezive Matrix Molecules*):

S. aureus MSCRAMMs can promote binding to fibronectin, fibrinogen, and collagen. Most strains express two related fibronectin-binding proteins:

- fibronectin-binding proteins **(FnbpA** и **FnbpB)**

Carrier state...

Staphylococci are transmitted through direct contact or by aerosol.

Some people are **carriers** for a long period of time, while others are only occasional carriers.

People of a certain profession (doctors, nurses and other hospital staff) are prone to colonization by *S. aureus*.

Diabetics, patients on hemodialysis, as well as those who are given intravenous drugs for a longer period of time, are at higher risk of becoming carriers.

Staphylococcal diseases

Invasive:

intense suppuration

and local necrosis

- local: superficial and deep infections
- generalized

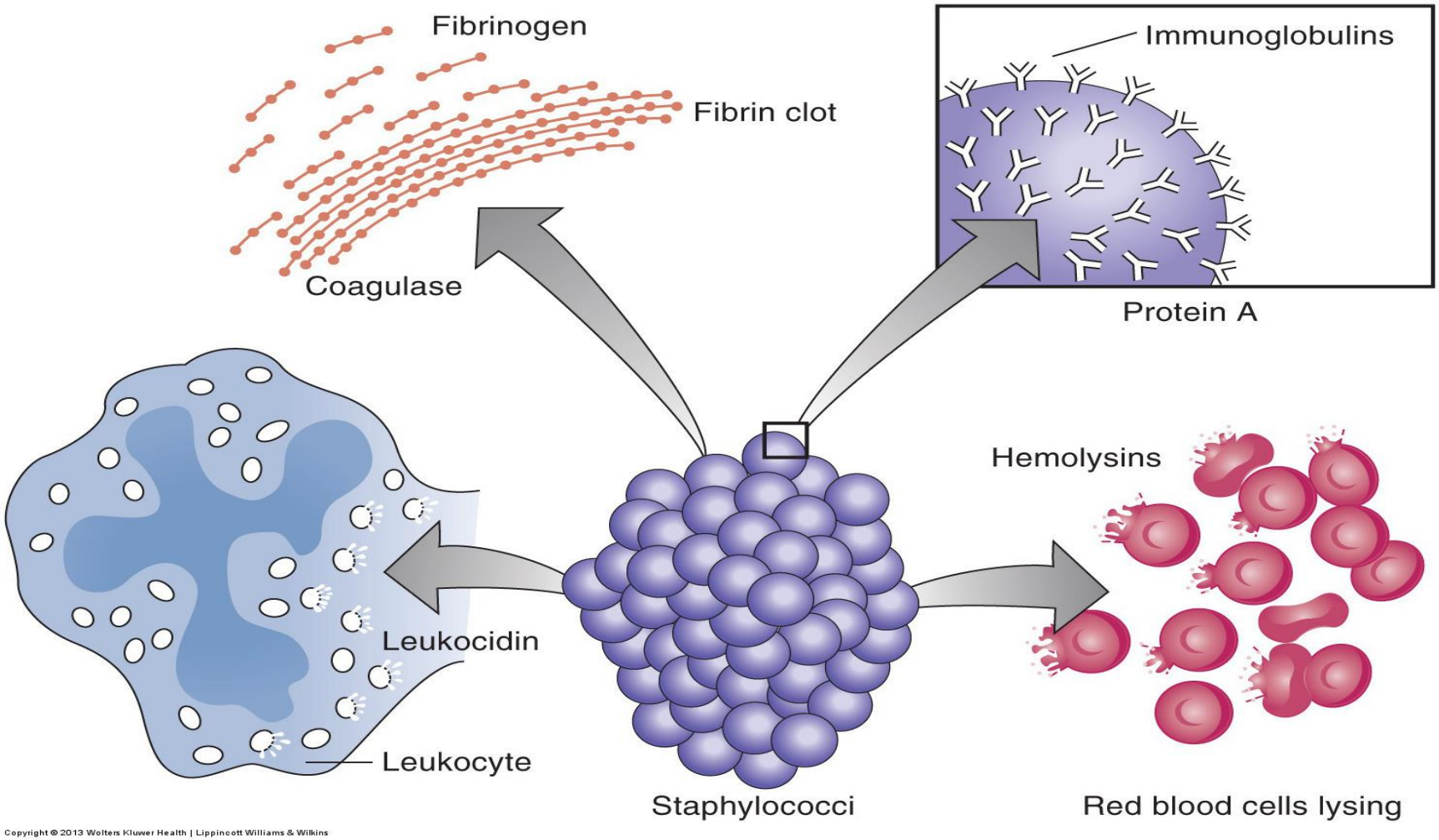
Toxic:

systemic damage (staphylococcal toxins)

- toxic effects away from the site of infection

Pathogenesis of purulent staphylococcal infections

First, an **acute inflammatory reaction** develops with a rapid and extensive influx of leukocytes (neutrophils)



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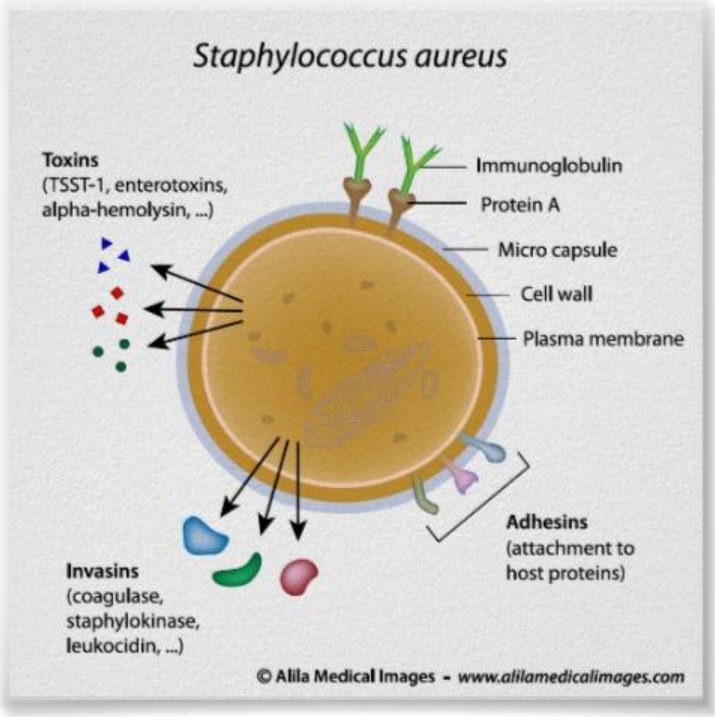
Pus and abscess formation. *S. aureus* infection induces a rapid and extensive leukocyte influx. Chemotactic factors of bacterial origin, as well as complement components are released in large quantities. *S. aureus* secretes coagulase which causes clot formation. The released protein A binds to antibodies and thus reduces opsonization. *S. aureus* secretes cytolysins (leukocidins and hemolysins) which lyse neutrophils and erythrocytes at the site of infection. Lysed neutrophils release large amounts of lysosomal enzymes responsible for damaging the surrounding tissue.

Other *S. aureus* virulence factors



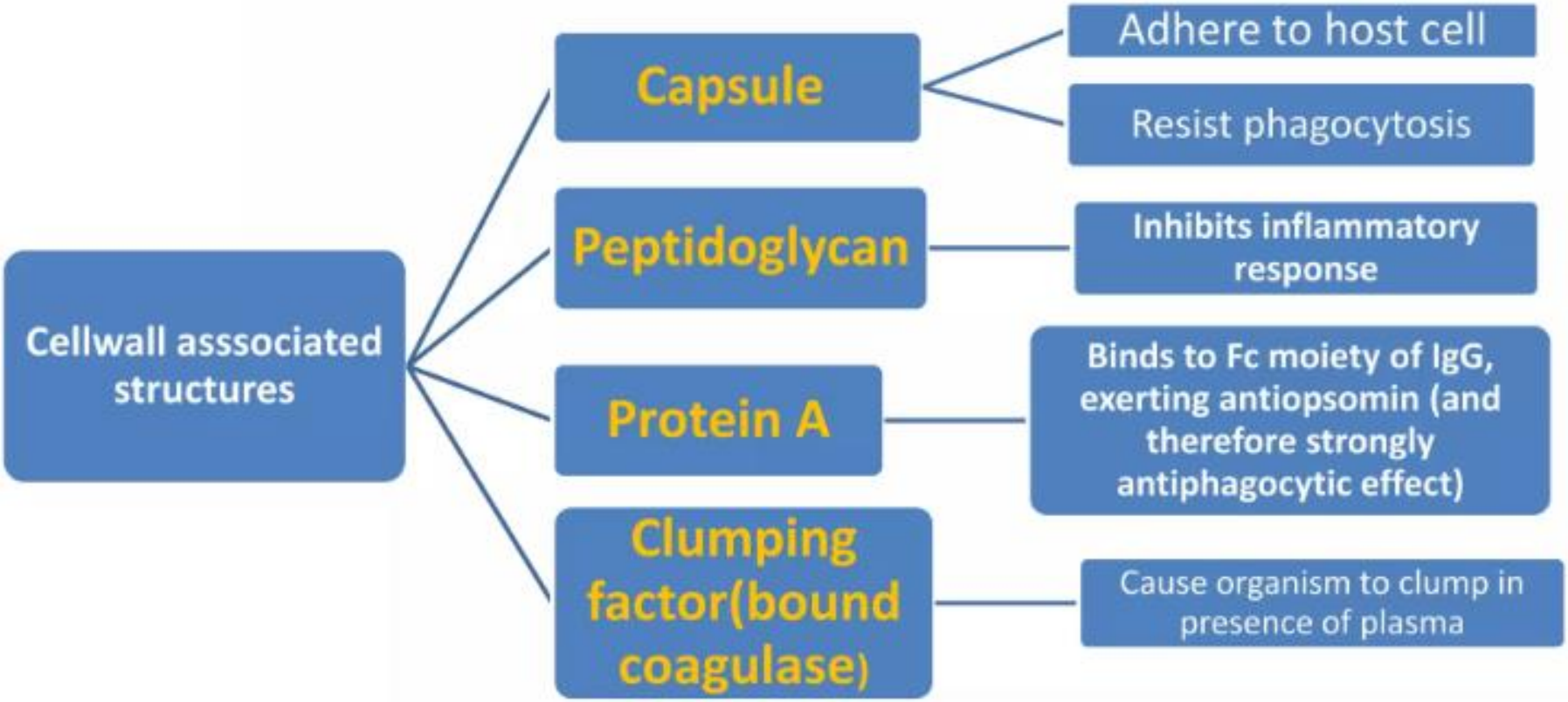
Structural components:

- More than 90% of disease-causing strains of *S. aureus* are surrounded by a **capsule** that inhibits phagocytosis;
- **Peptidoglycan** - activates complement by the alternative pathway and thus contributes to the inflammatory response;
- **Lipoteichoic acid** and **teichoic acid** - involved in complement activation as well as bacterial adhesion to mucosal surfaces;
- **Protein A** - reduces bacterial opsonization.

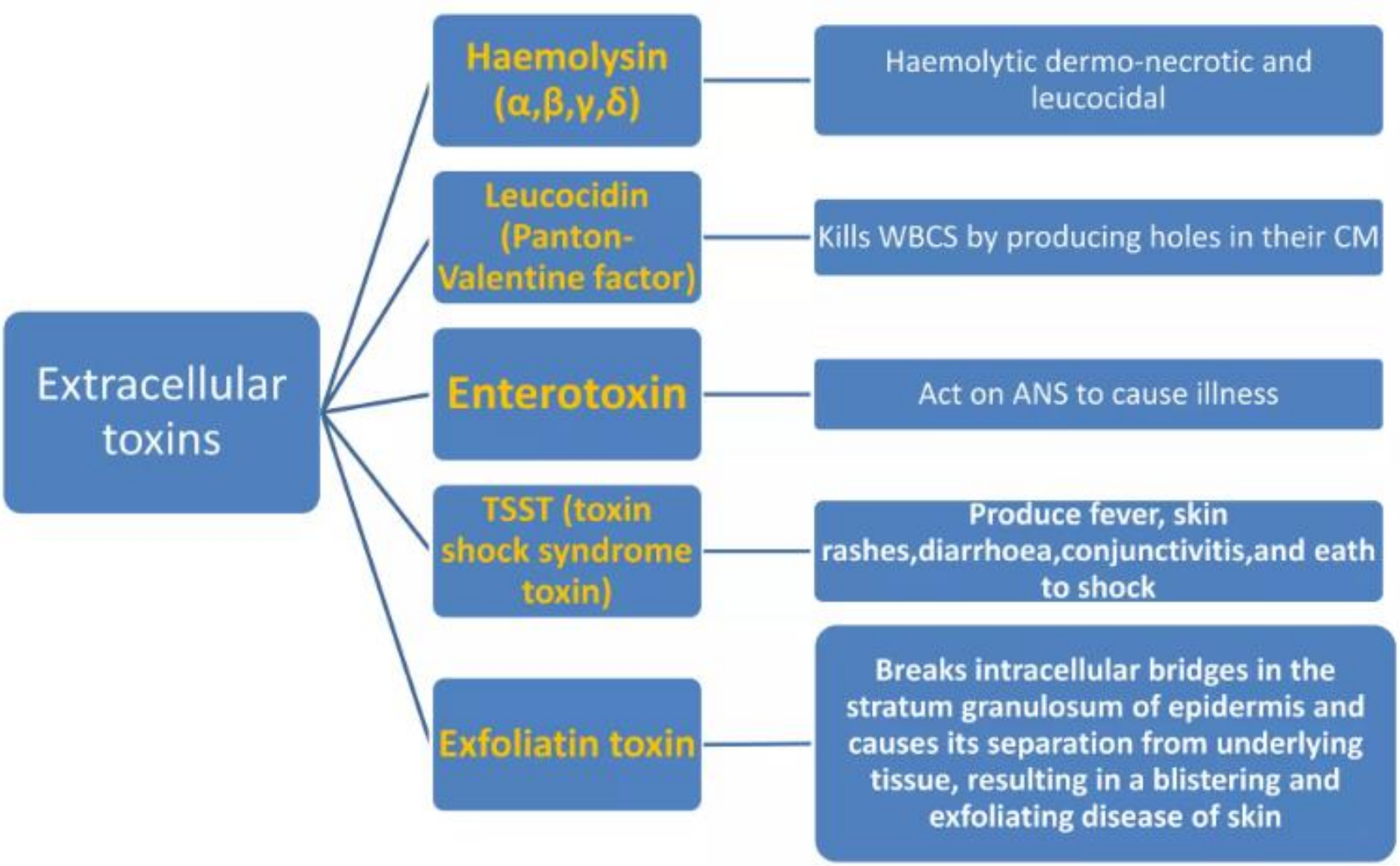
Enzymes and toxins:

- **Catalases** - interfere with the killing ability of neutrophils;
- **Panton-Valentine leukocidins** - effective in damaging neutrophils;
- **Pore-forming toxins** - α-, γ- and δ-toxins;
- **Hyaluronidase** hydrolyzes the connective tissue matrix. Most strains also release lipases, proteases, deoxyribonucleases (DNases).

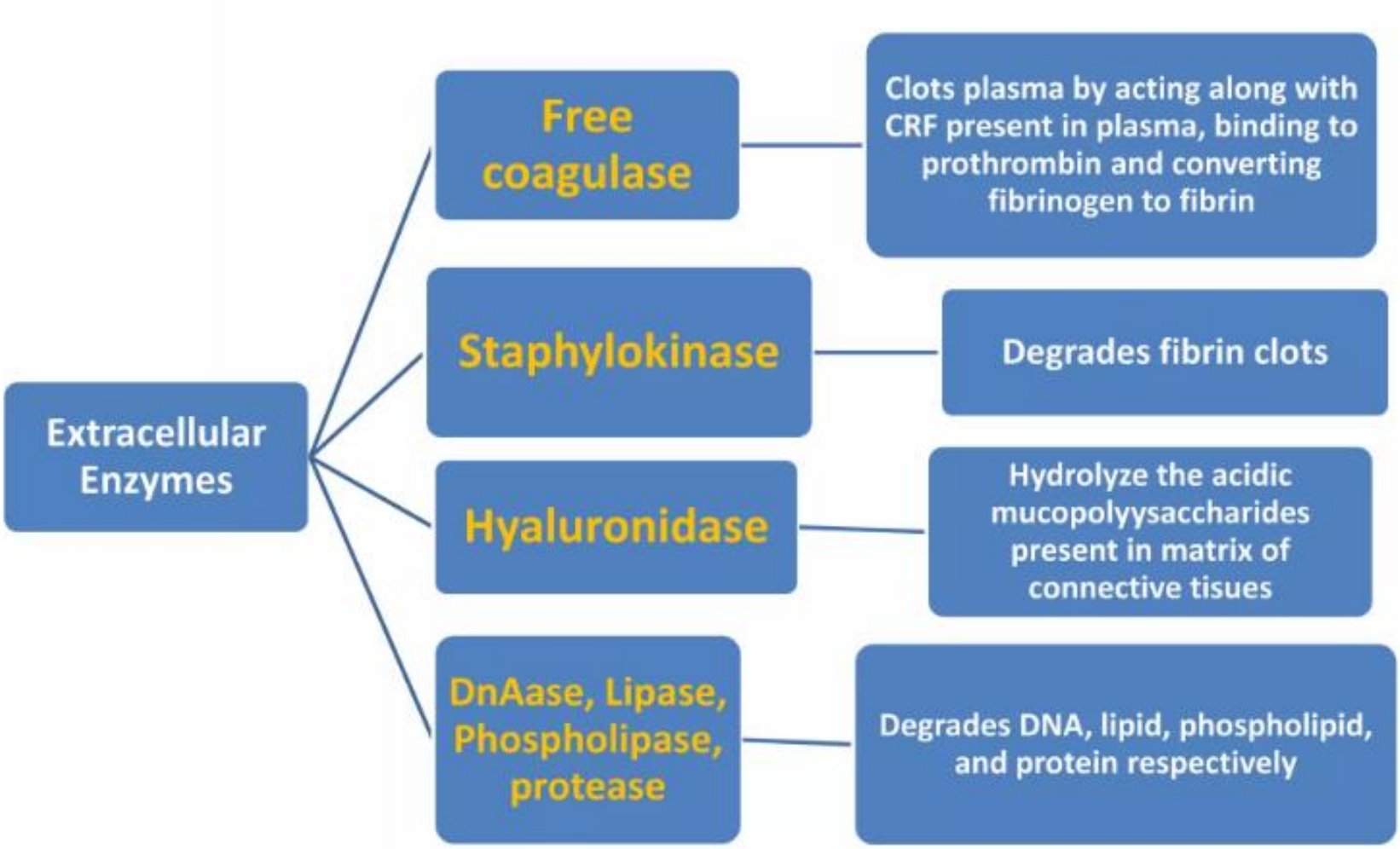
Virulence Factors



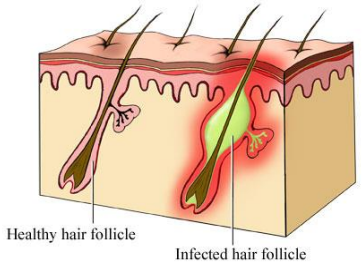
Virulence Factors



Virulence Factors

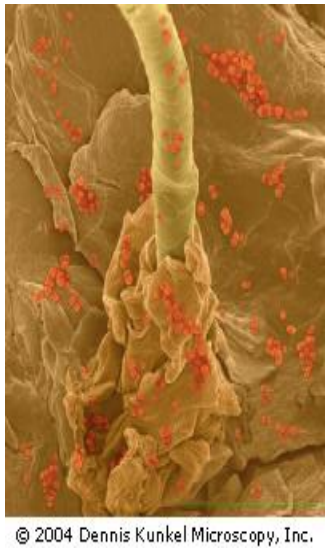


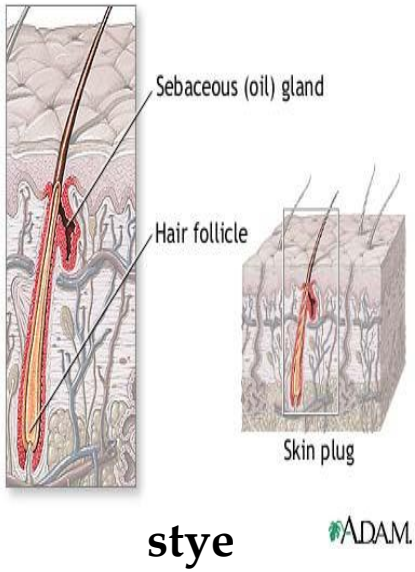
1. Invasive diseases caused by *S. aureus*



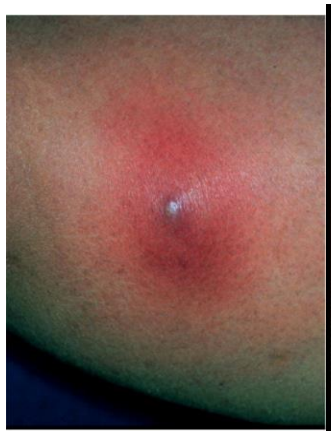
Skin and soft tissue infections:

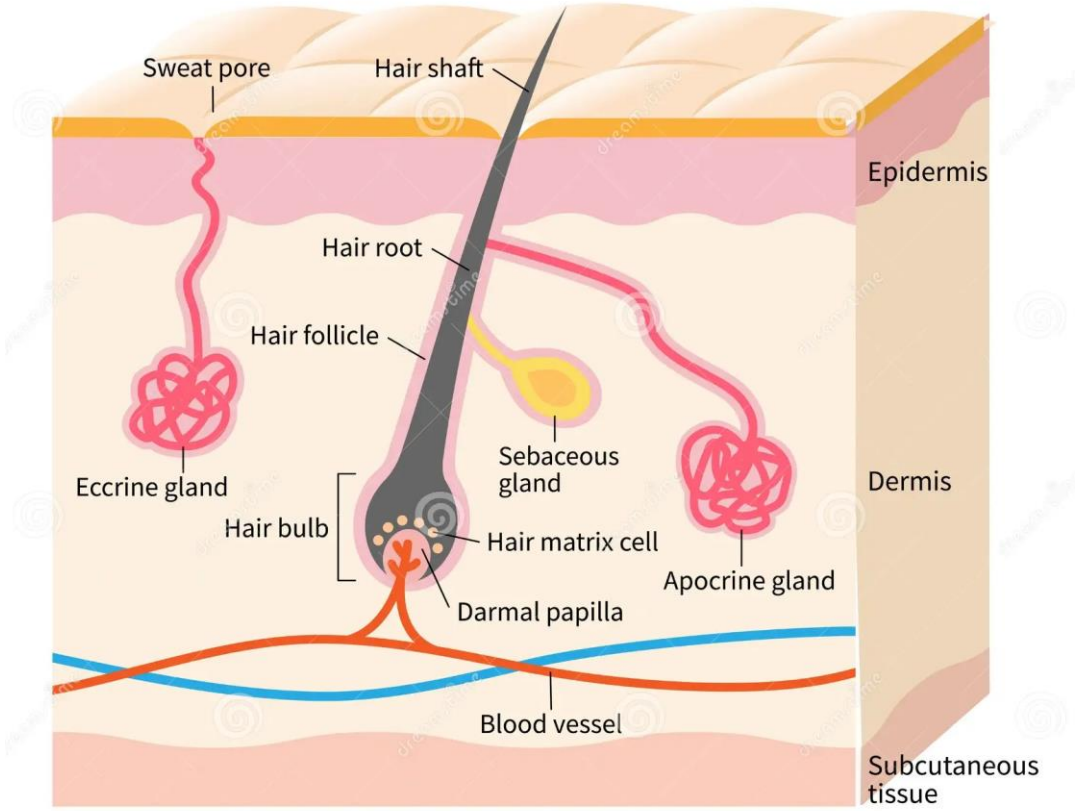
- Most local staphylococcal infections induce the formation of a pus collection called an **abscess**. An abscess in the skin is called a **boil** or **furuncle**. A boil is a superficial infection of the skin that develops in the hair follicle or in the area of sweat and sebaceous glands (closed excretory ducts predispose development of this infection).
- Multiple associated boils form a **carbuncle**. Staphylococci from the skin can spread to the subcutaneous tissue, which results in diffuse inflammation called **cellulitis**.





stye





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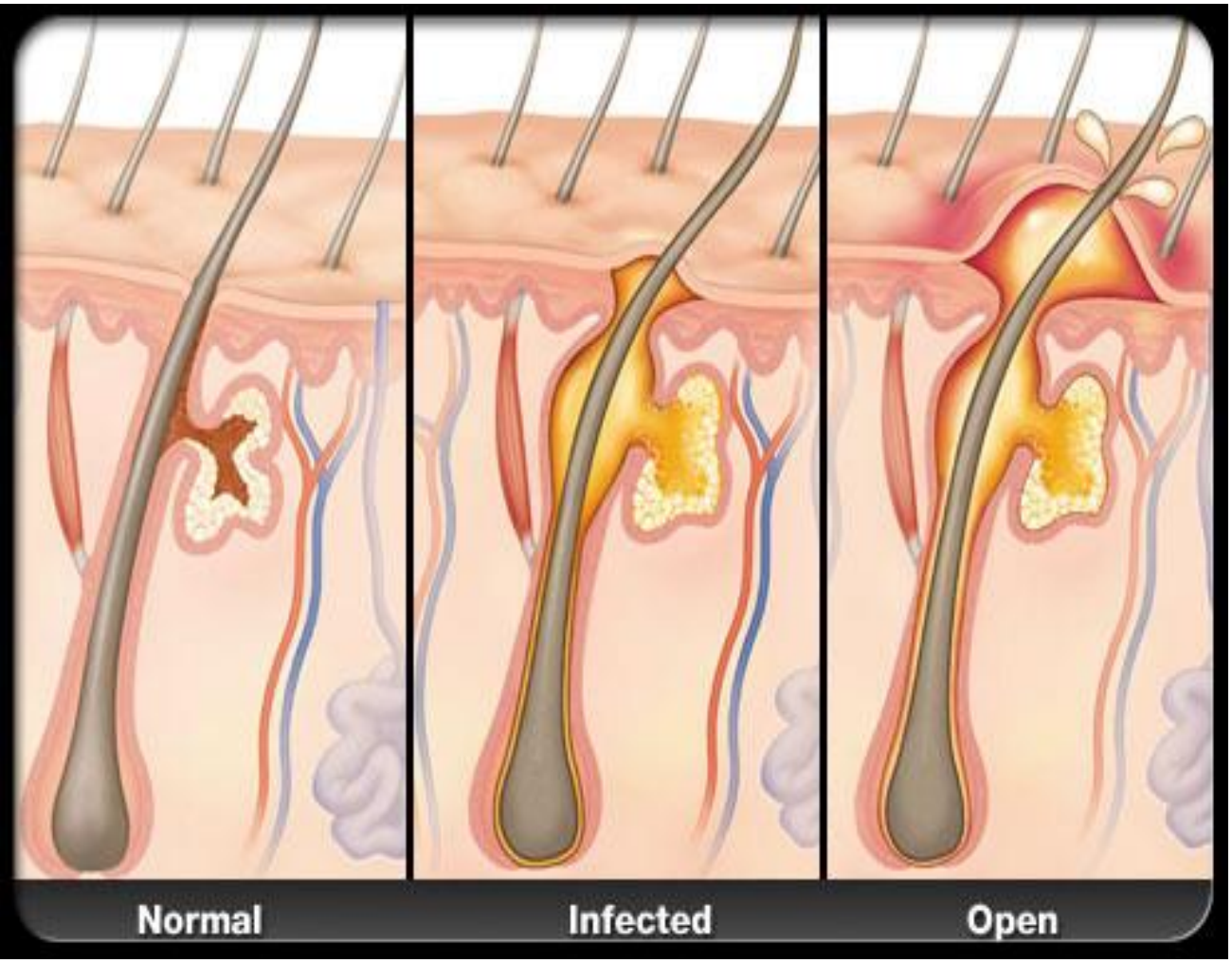
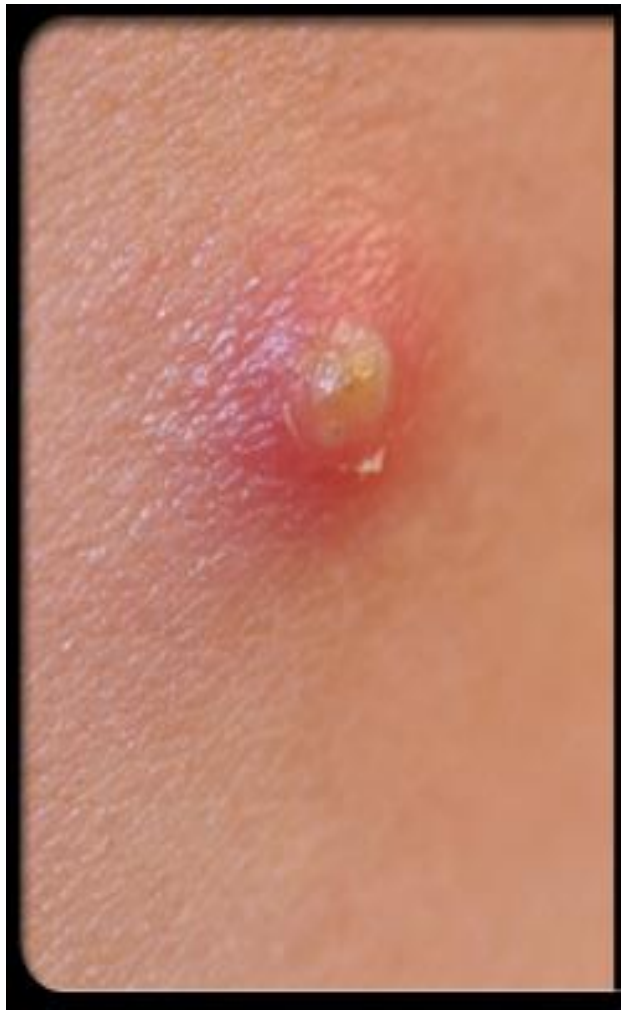
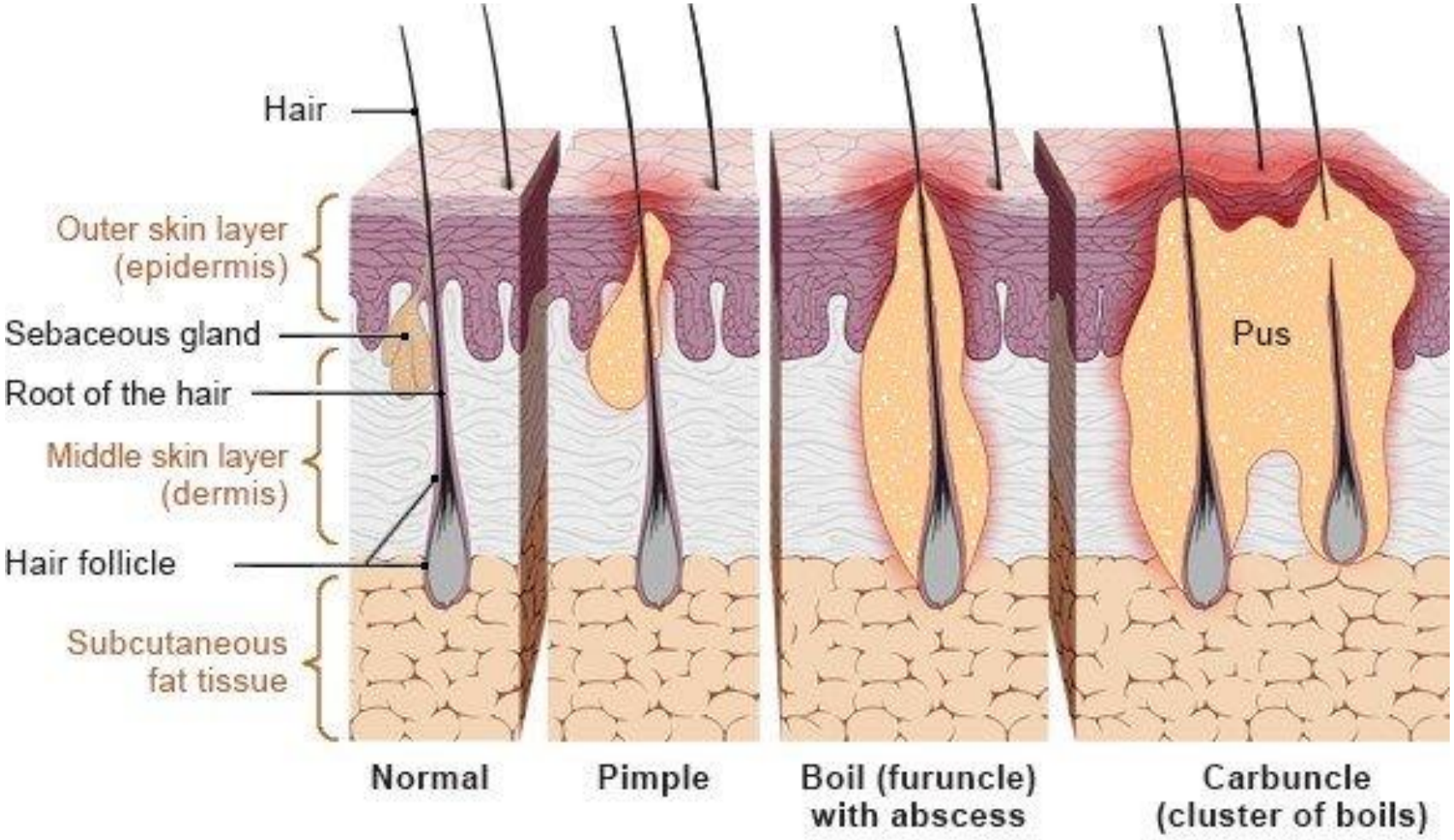
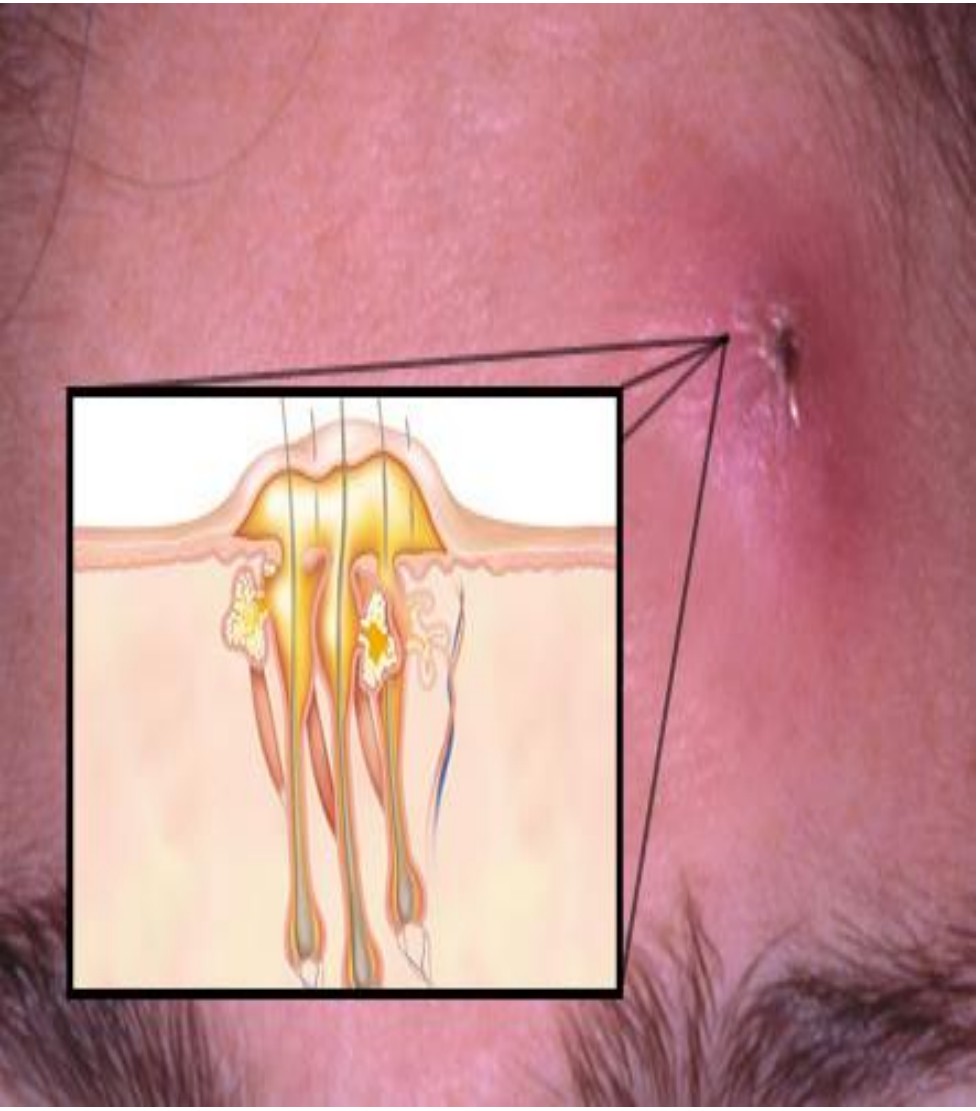
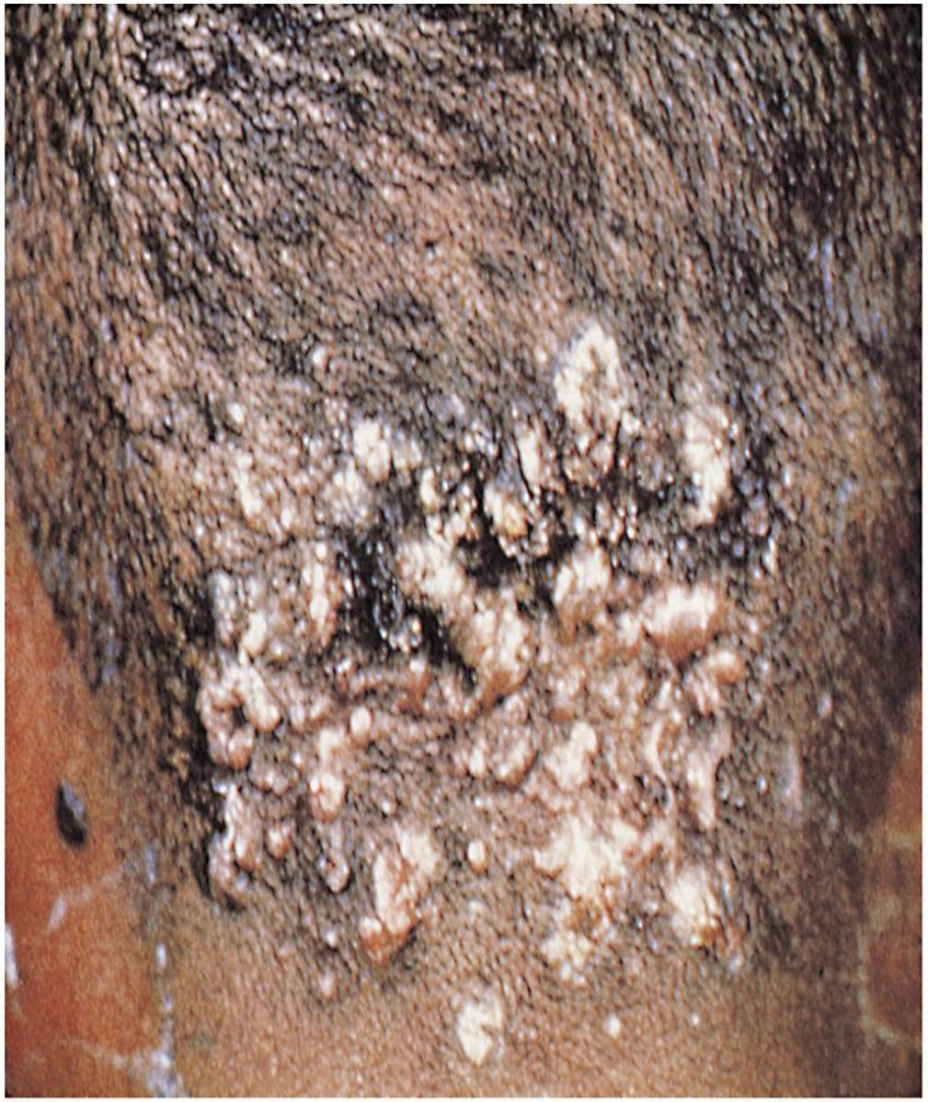




Furuncle


Hair
Outer skin layer (epidermis)
Sebaceous gland
Root of the hair
Middle skin layer (dermis)
Hair follicle
Subcutaneous fat tissue
Pus
Normal
Pimple
Boil (furuncle) with abscess
Carbuncle (cluster of boils)





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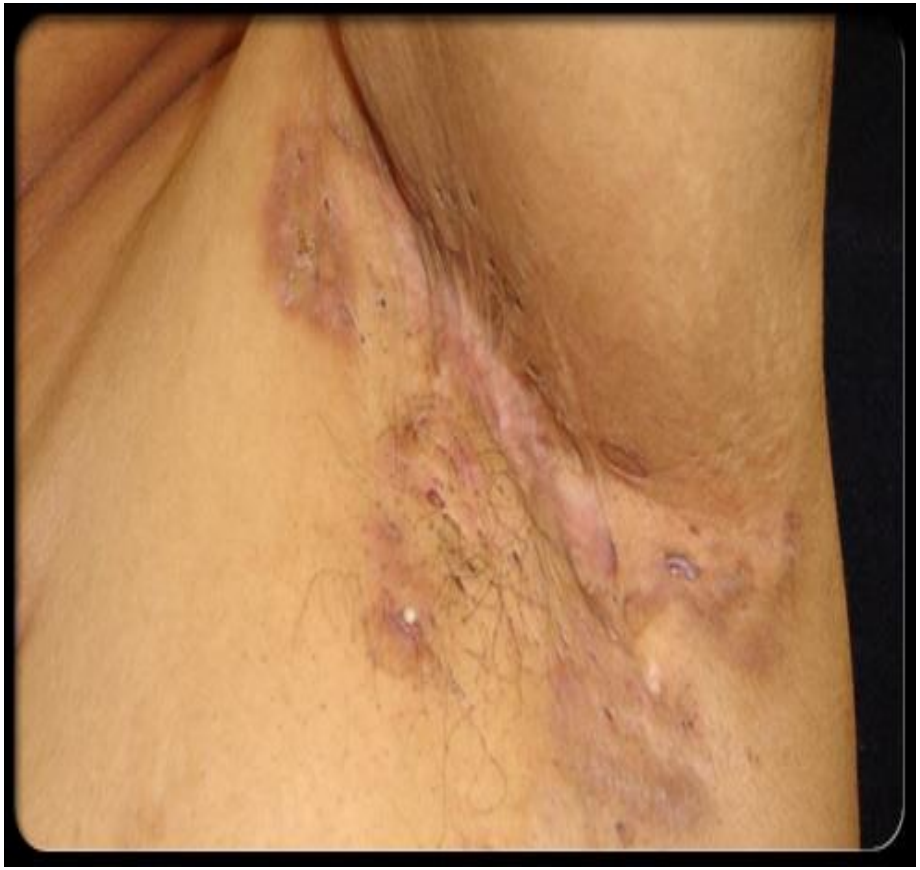
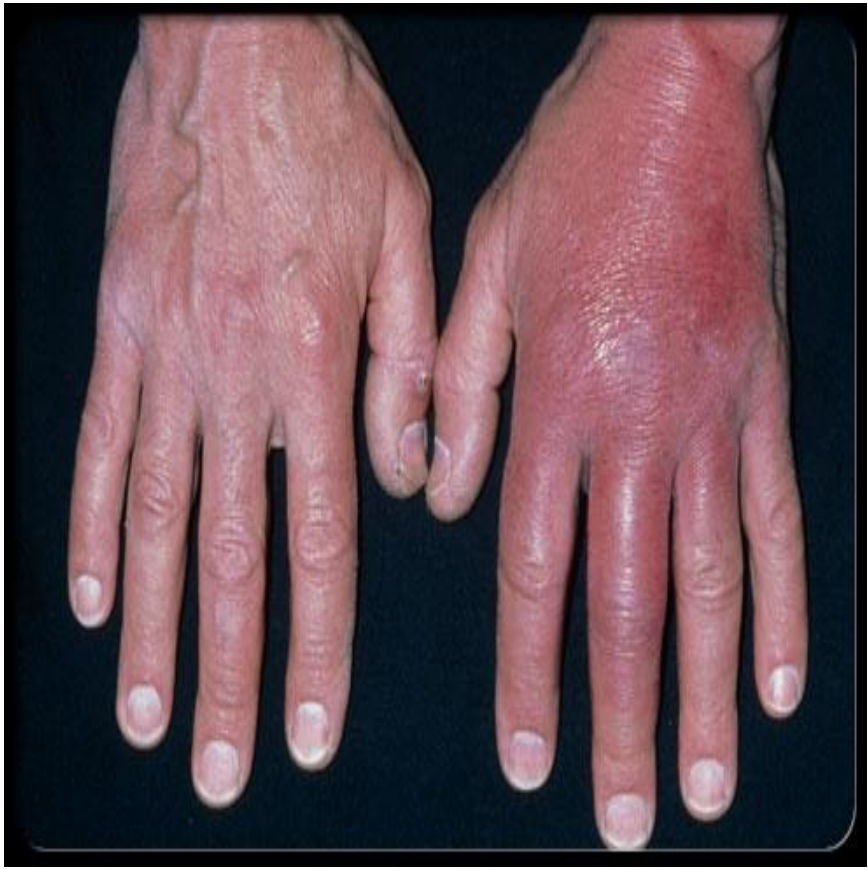
Carbuncle

Hidradenitis Suppurativa



Staphylococcal sweat gland infection

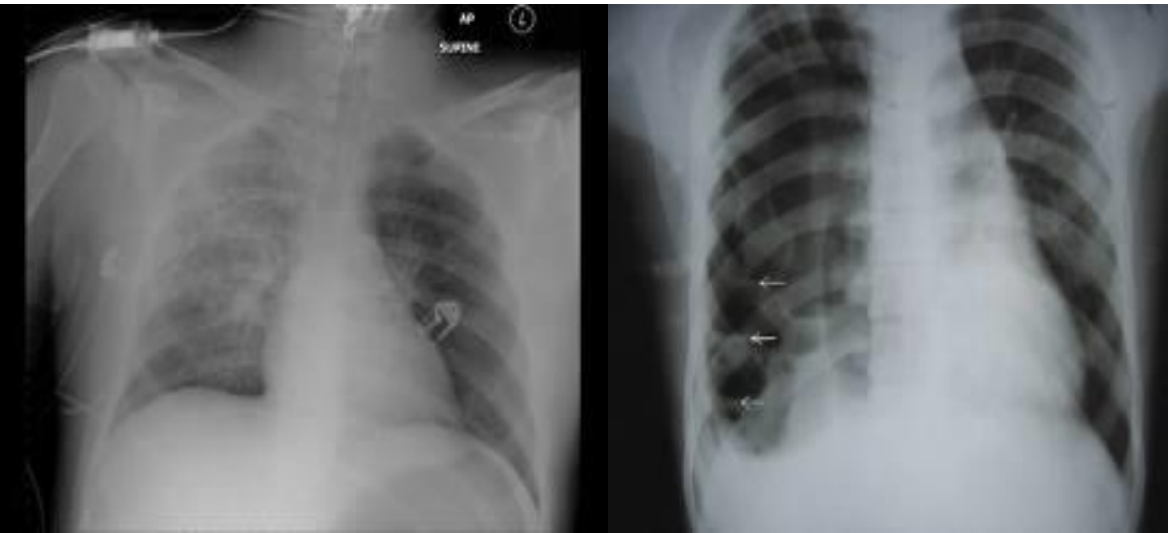
Cellulitis



Staphylococcal infections of deeper tissues

When it reaches deeper tissues, *S. aureus* manages to survive and trigger an inflammatory reaction that occurs in a similar way to that during the development of skin abscesses.

The main targets: **bones**, **lungs** and **heart**.

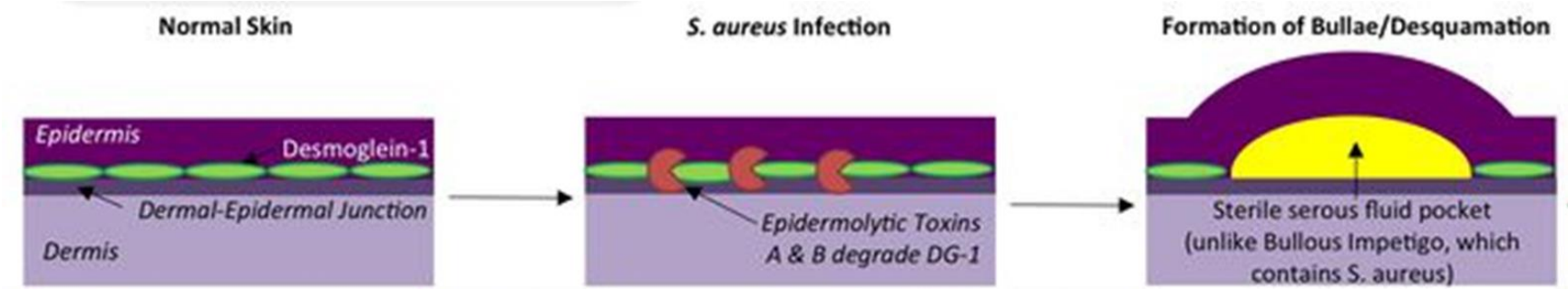


2. Toxic infections caused by *S. aureus*

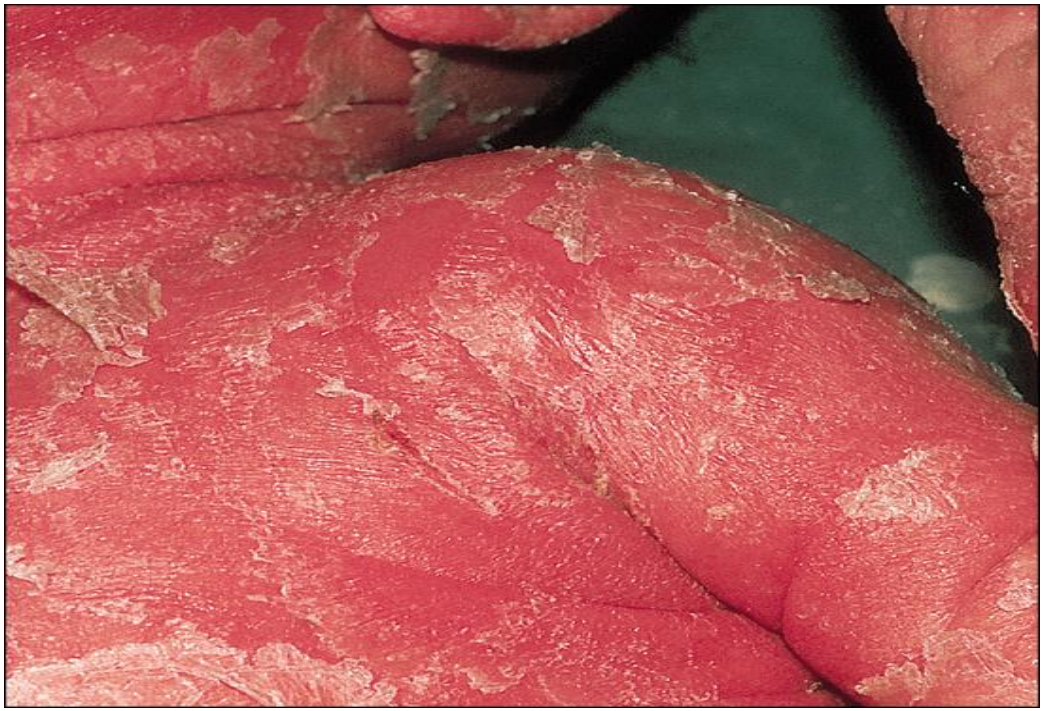
- Staphylococcal scalded skin syndrome -

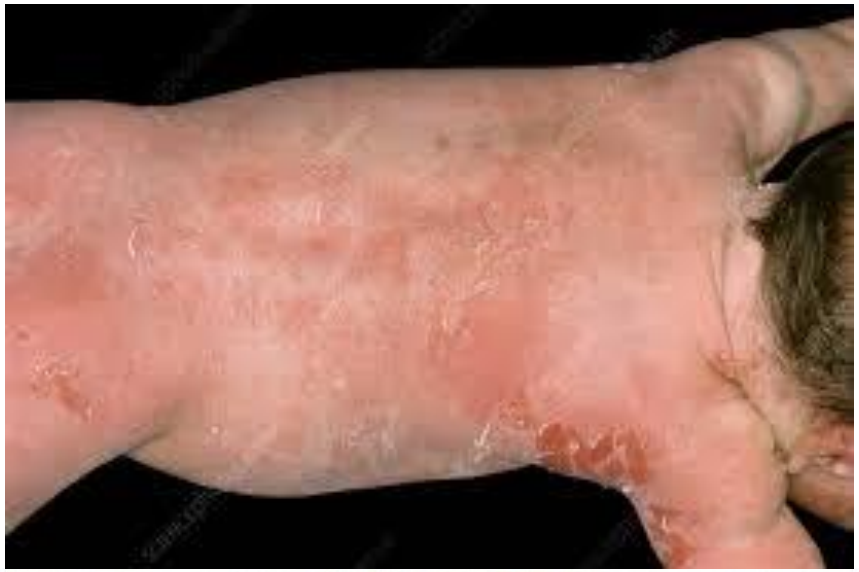
The disease is characterized by extensive peeling of the skin and mainly occurs in newborns. It is mediated by the exotoxins **exfoliative toxins A** and **-B**. These toxins are highly tissue-specific serine proteases that act at the desmosomes resulting in the separation of the epidermal layers.

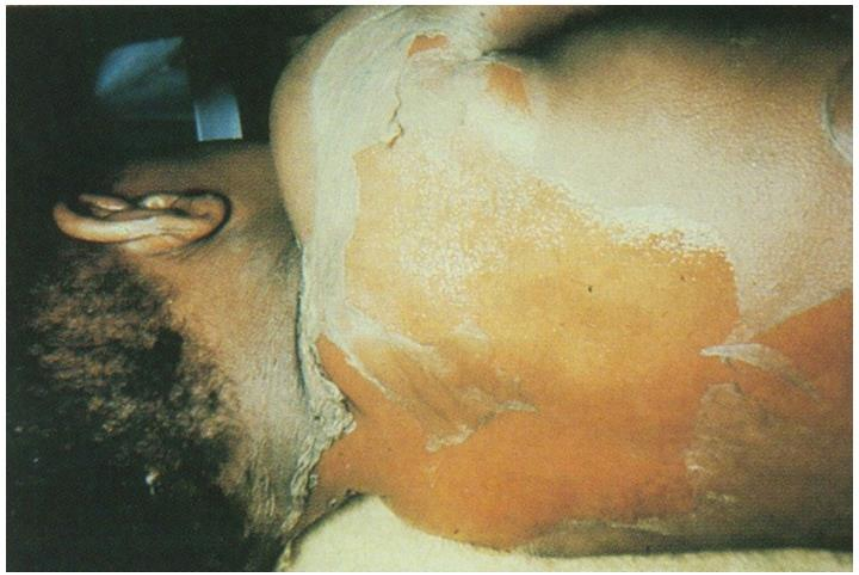
A layer of **serous** fluid forms beneath the epidermis and separates it.
The epidermis **peels off** at the slightest touch (resembling extensive burns).



Staphylococcal scalded skin syndrome







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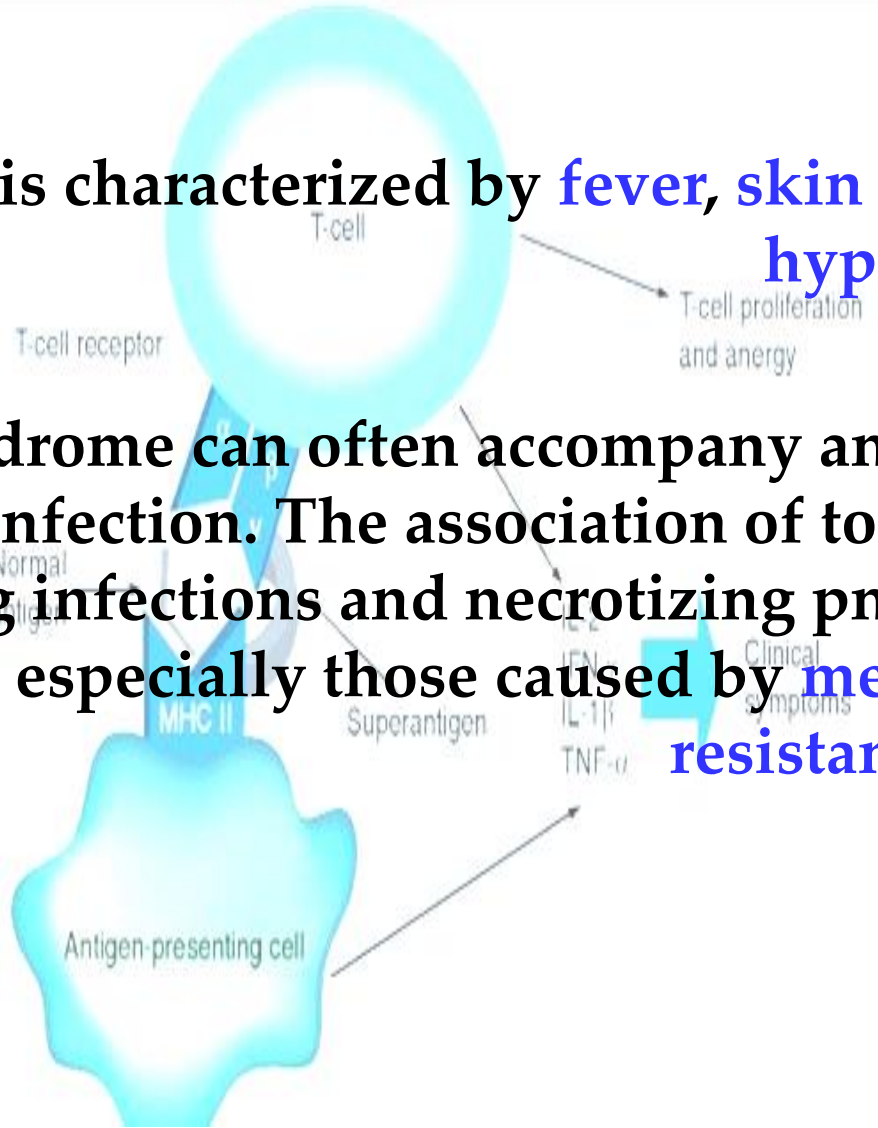
- Toxic shock syndrome-

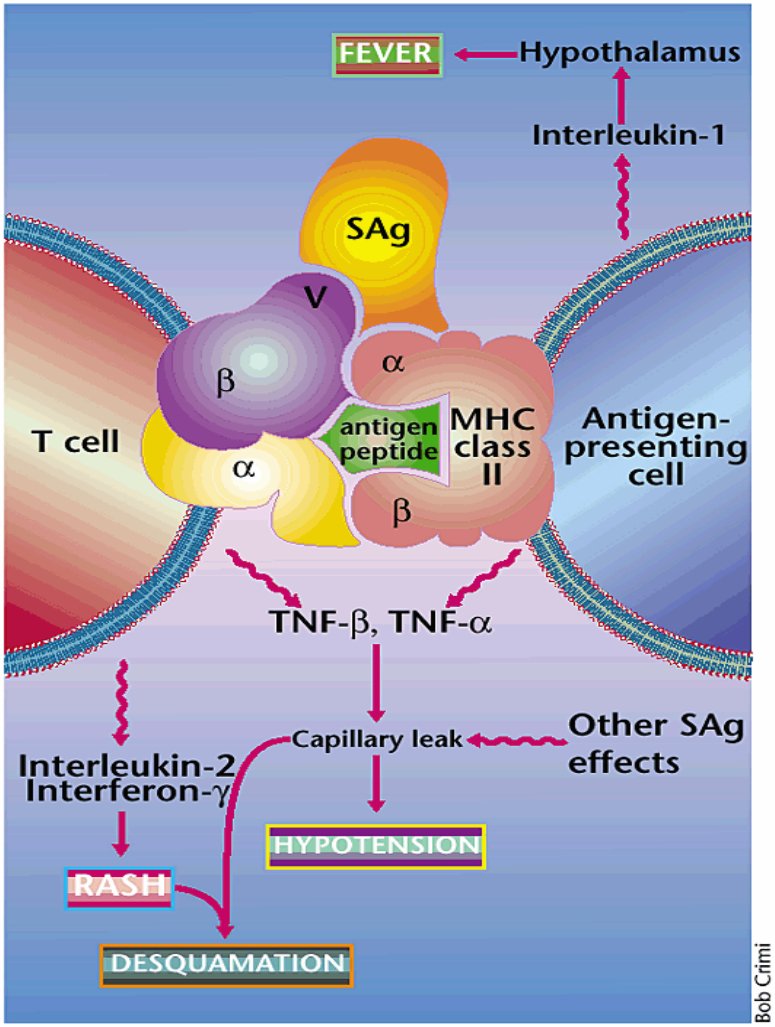
Toxic shock syndrome is characterized by fever, skin rash, and hypotension.

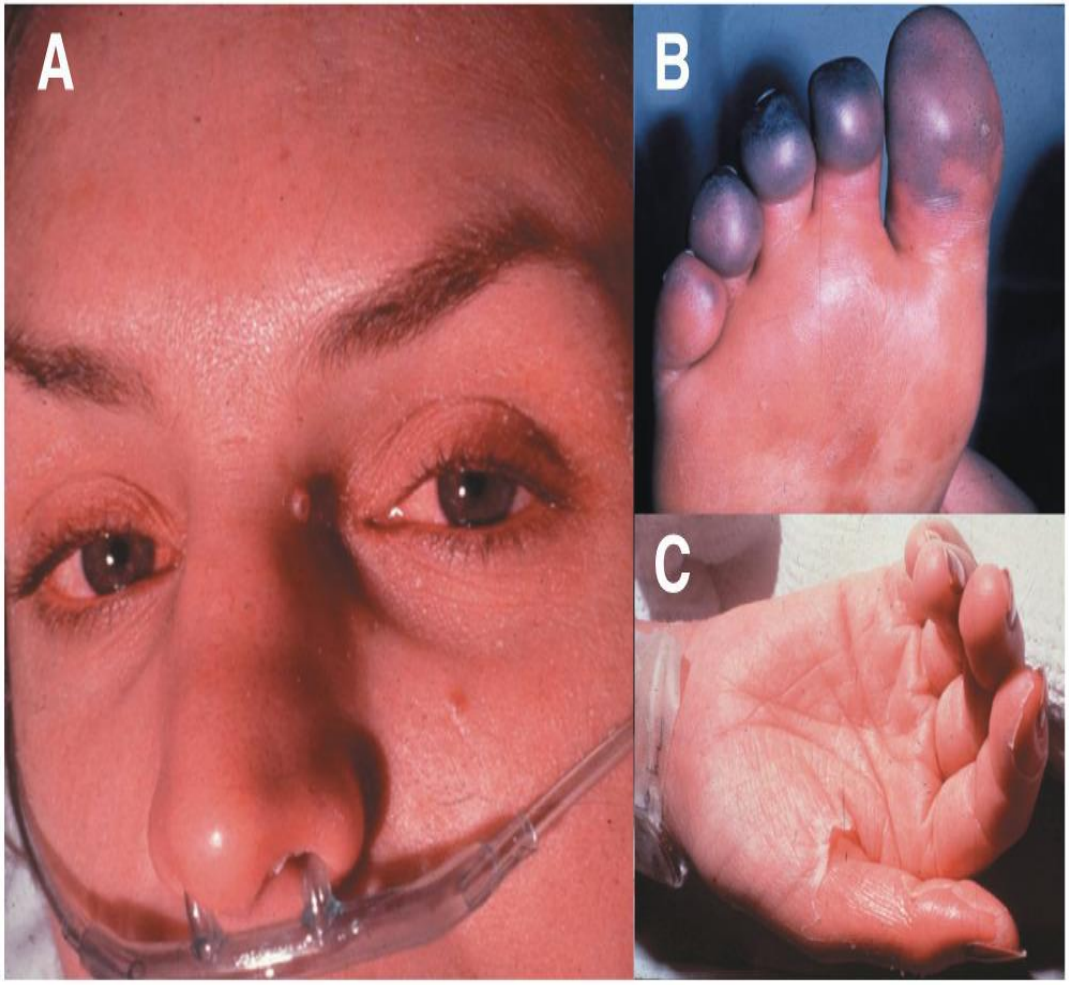


Toxic shock syndrome can often accompany any type of staphylococcal infection. The association of toxic shock syndrome with lung infections and necrotizing pneumonia has increased, especially those caused by methicillin-resistant strains.

Toxic shock syndrome pathogenesis

Exotoxins, key in the development of toxic shock syndrome, are **TSST-1**, and staphylococcal enterotoxins, especially **enterotoxin serotypes -B** and -**C**. Bacteria release them in high concentrations, which induces the development of **T**oxic **S**hock **S**yndrome. These toxins function as **superantigens**.





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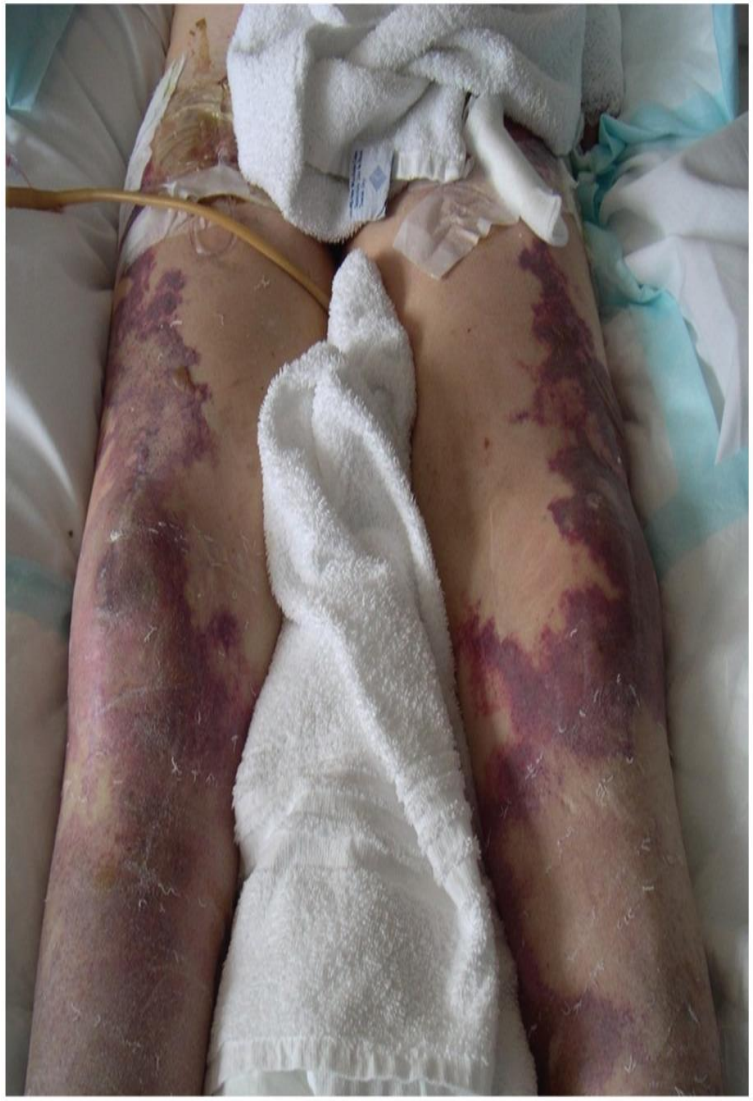

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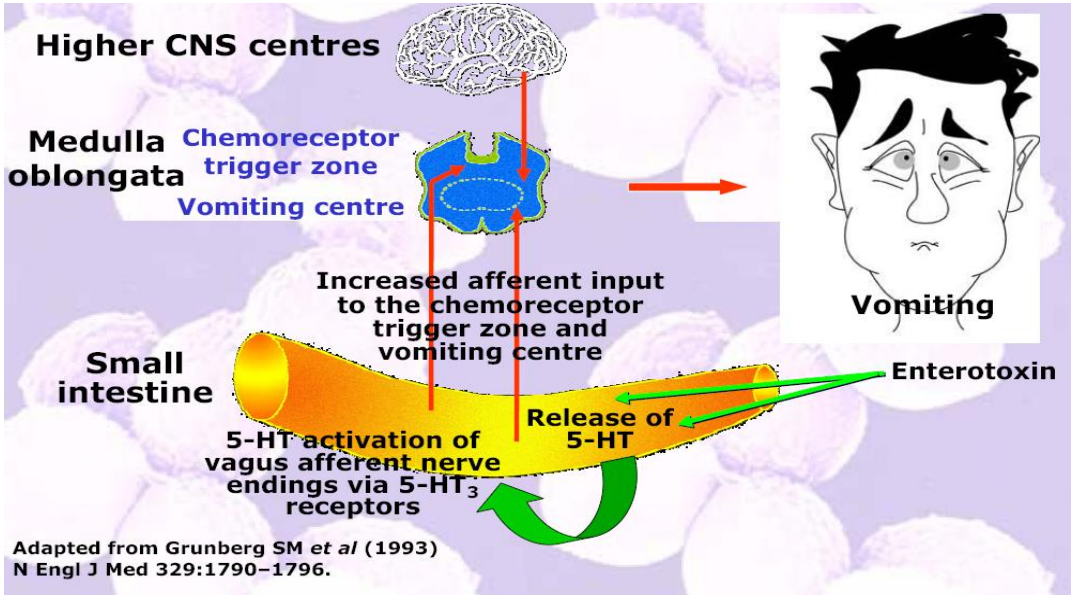
Toxic shock syndrome

-Food poisoning-

Staphylococcal enterotoxin serotypes -A, -E and -I are the leading cause of food poisoning. This **alimentary intoxication** is caused by ingestion of contaminated food.

Toxins are heat stable and resistant to proteases. Staphylococci that produce these toxins are killed by cooking.

Toxins increase intestinal peristalsis and act directly on the vomiting center in the brain.



Prevention

Wash your hands

Keep wounds covered

Reduce tampon risks

Avoid sharing personal care items

Cooking and storing food properly

Laboratory Diagnosis

A. Haematological Investigation:

1. TLC (Total leukocyte count):

Normal: 4000-10000 cells/mm^3

In case of infection: > 10000 cells/mm^3

2. DLC (Differential leukocyte count):

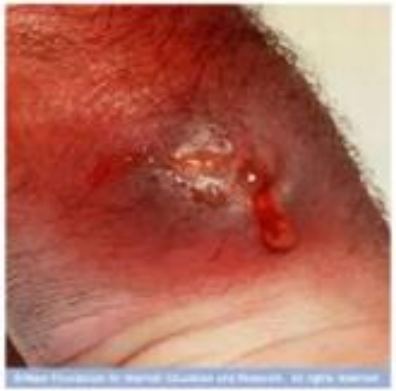
Normal neutrophil : 80%

In case of infection: > 80%

Laboratory Diagnosis

B. Bacteriological Investigation:

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



Gram +ve (purple/blue) cocci

Catalase test

Catalase +ve (clusters)
Staphylococcus

Catalase –ve (chain) → HAEMOLYSIS
streptococcus

Coagulase –ve

S. epidermidis* OR *S. saprophyticus

Coagulase

Novobiocin or Colistin (Polymyxin)

Coagulase +ve
S. aureus

S. saprophyticus
sensitive

S. epidermidis
resistant

Staphylococcal disease treatment

Choice of antibiotic therapy based on antibiogram

Antibiotics that act on various processes that are essential for bacterial survival, such as bacterial cell-wall synthesis (e.g. **β-lactams**), folic acid metabolism (**sulfonamides**), and bacterial protein synthesis (e.g. **macrolides**, **lincosamides** and **aminoglycosides**) are used for the treatment of infections caused by *S. aureus*.

Antibiotic resistance

- *S. aureus* produces - **β-lactamases** and **penicillin-binding protein 2A** (**PBP2A**). β-lactamases are powerful enzymes that degrade beta-lactams. PBP2A makes bacteria resistant to all penicillins and cephalosporins.
- Bacteria that possess PBP2A are called **methicillin-resistant *S. aureus* (MRSA)**.
- More than 70% of *S. aureus* strains causing nosocomial infections and 30% of strains causing community-acquired infections are methicillin-resistant, due to the presence of PBP2A.
- **Vancomycin-resistant *S. aureus* (VRSA)** has also been identified.

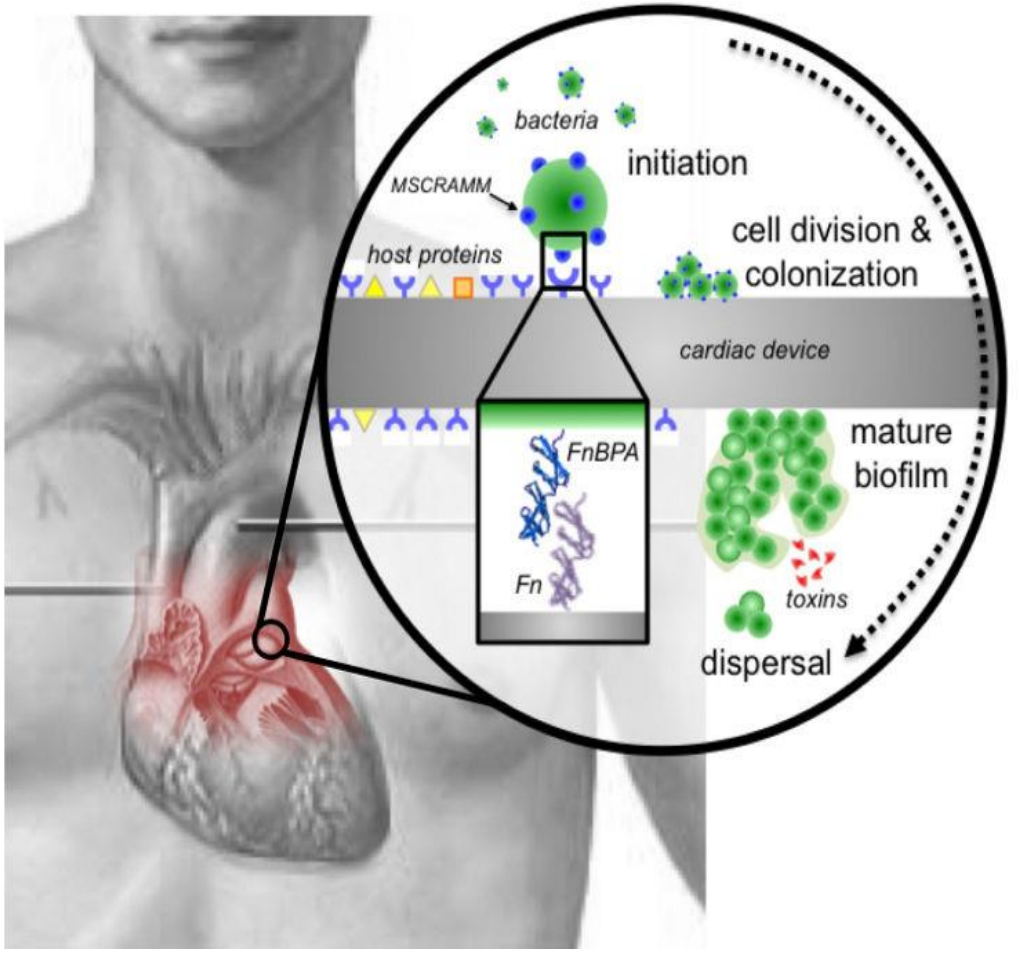
Staphylococcus epidermidis

S. epidermidis inhabits the skin and rarely causes diseases. The incidence of infections caused by *S. epidermidis* is increasing in patients with implants (it can form **biofilm** on artificial heart valves or catheters).

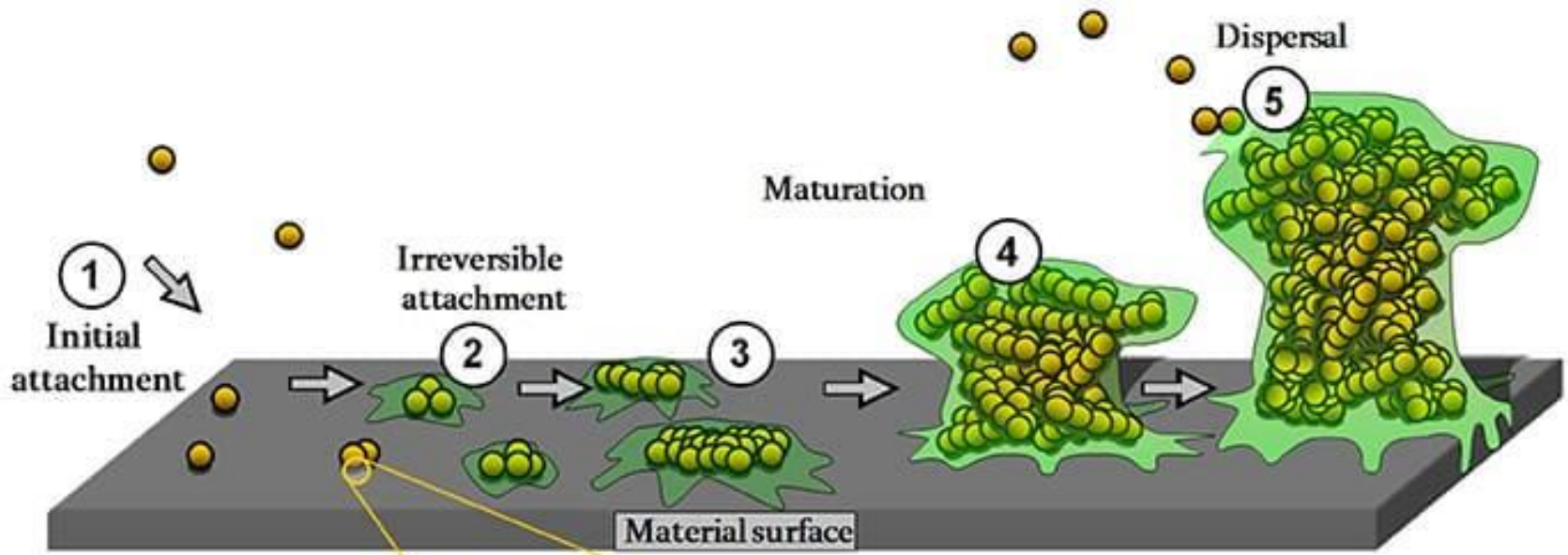
S. epidermidis can cause severe infections: **septicemia** and **endocarditis**.

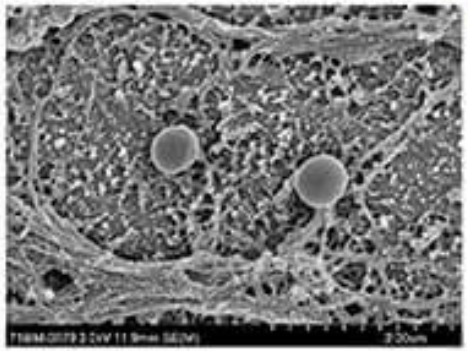
A potential virulence factor is **peptidoglycan** and the **exopolysaccharide slime layer** that are present in more than 80% of disease-causing *S. epidermidis* isolates.

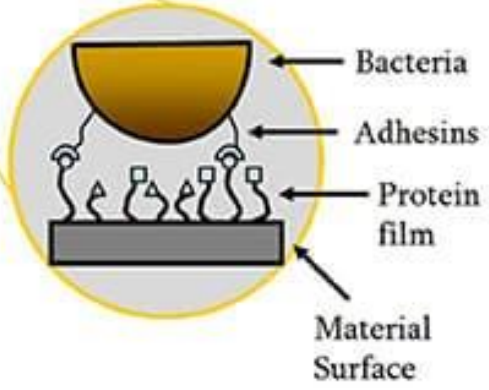
This slime layer allows these bacteria to bind to the smooth surface of the implant.

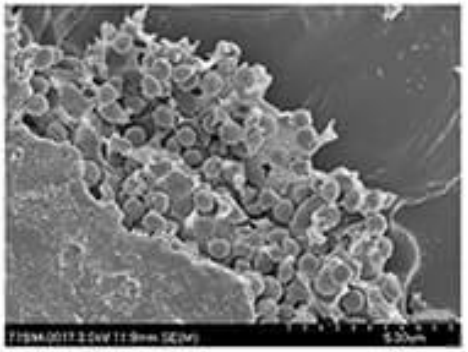


Biofilm formation is the most important virulence factor of S. epidermidis which causes various infections. Biofilm helps colonization, immune evasion as well as antibiotic resistance of the organism.









Staphylococcus saprophyticus

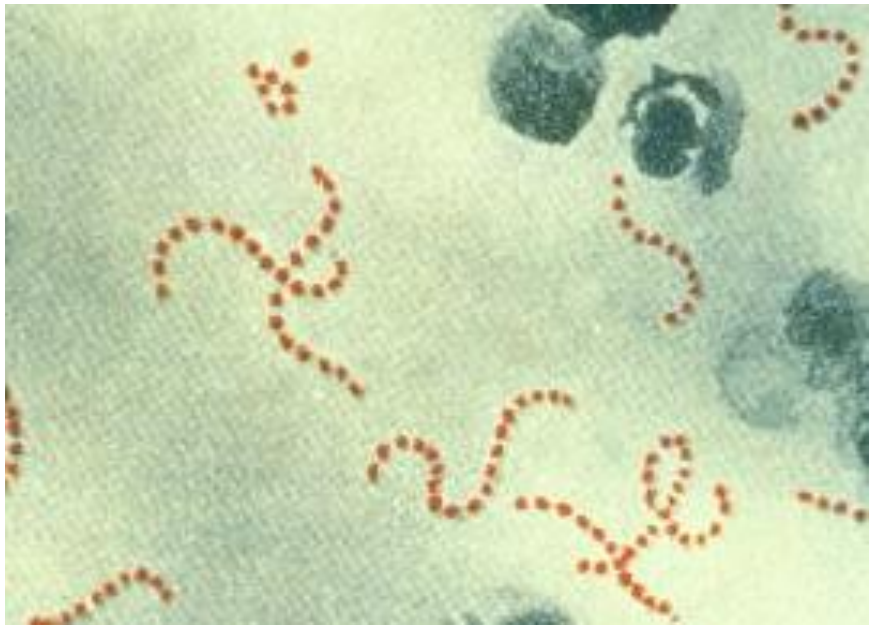
The causative agent of **opportunistic infections of the urogenital system.**

S. saprophyticus is a highly specialized bacterium that causes urinary tract infections, particularly cystitis in young women.

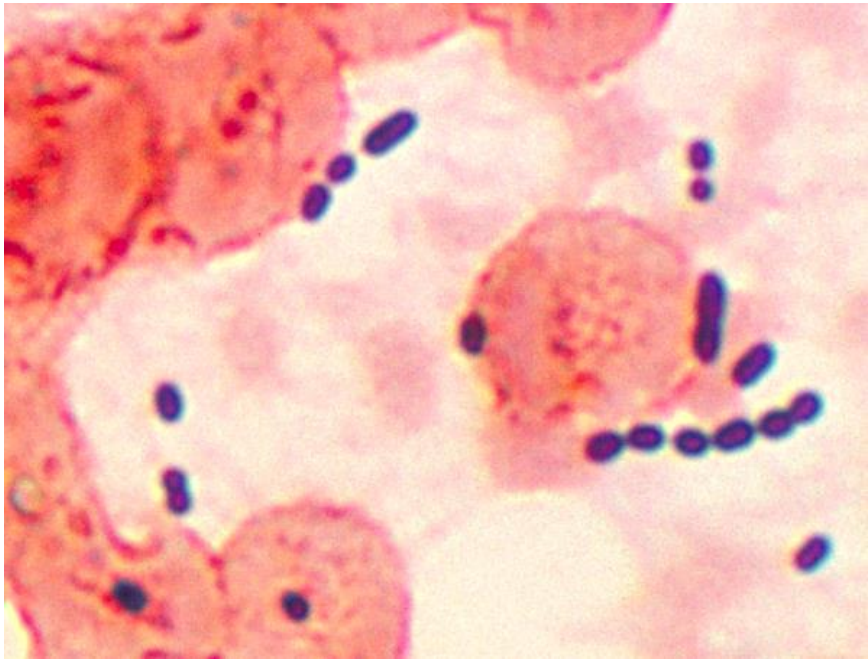
Staphylococcus epidermidis	**sensitive**
Staphylococcus saprophyticus	**resistant**


Streptococcus spp.
Enterococcus spp.

Streptococci are ***Gram*+ cocci**, grouped in **chains**.



Enterococci are ***Gram*+ cocci** that grow in **pairs** (**diplococci**) or **short chains**



Classification of streptococci and enterococci

- **Based on hemolysis:** streptococci and enterococci form colonies on blood agar that can be surrounded by a zone of hemolysis.

- **Based on group-specific antigens:** different streptococci / enterococci are classified into groups from A to U.

- **Based on the species:** various types of streptococci can be identified using **biochemical tests** that analyze the metabolism and presence of enzymes in bacteria. Newer molecular techniques can distinguish types of streptococci based on **DNA sequences**.

Hemolysis on Blood Agar

- **α-hemolysis**
- Partial hemolysis
- Green discoloration around colonies
- e.g. S. pneumoniae & S. viridans
- **β-hemolysis**
- Complete hemolysis
- Clear zone of hemolysis around colonies
- e.g. S. pyogenes & S. agalactiae
- **γ-hemolysis**
- No hemolysis
- e.g. Enterococcus sp.

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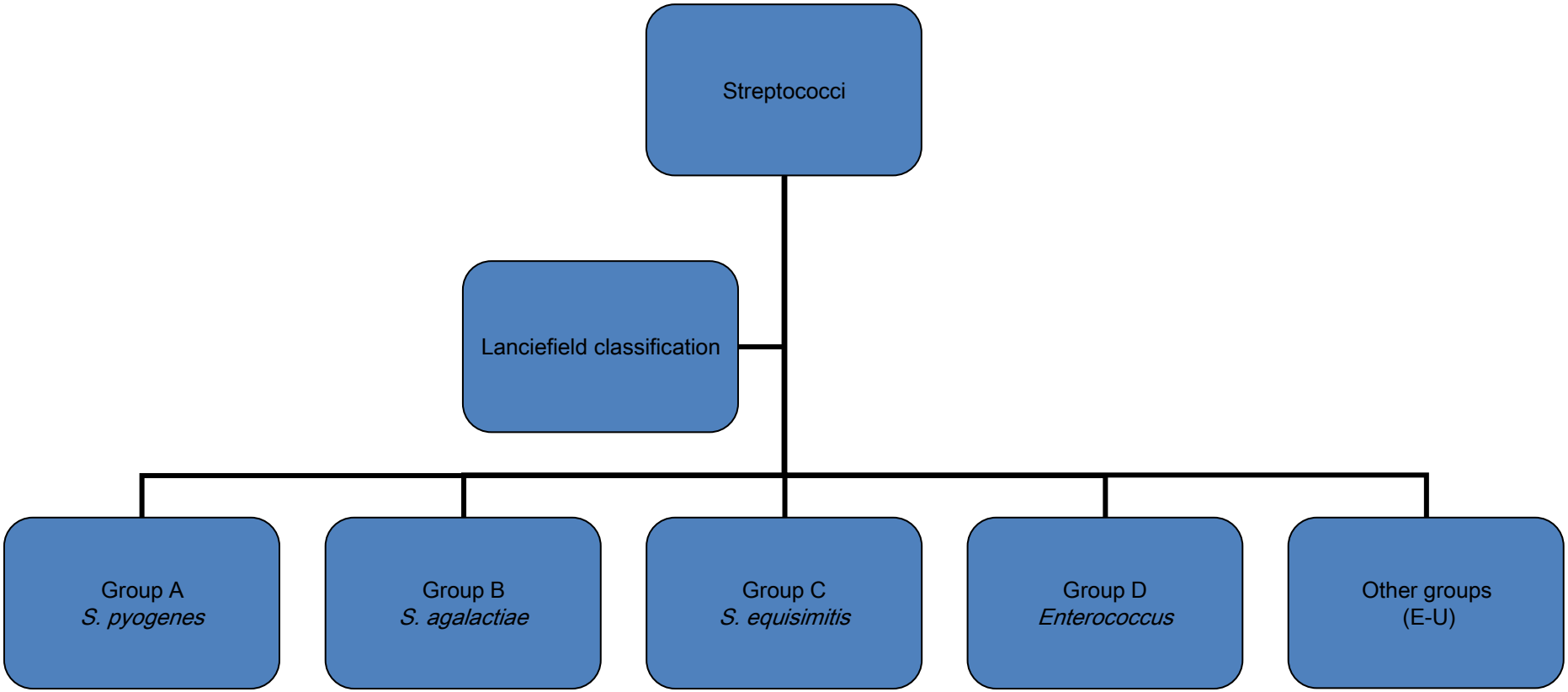
Hemolytic classification of streptococci. **α-hemolysis** (*viridans* streptoocci). **γ-hemolysis** (*E. faecalis*). **β-hemolysis** (*S. piogenes*)

Lancefield Classification- β hemolytic



- **Classification based on C antigen of cell wall**

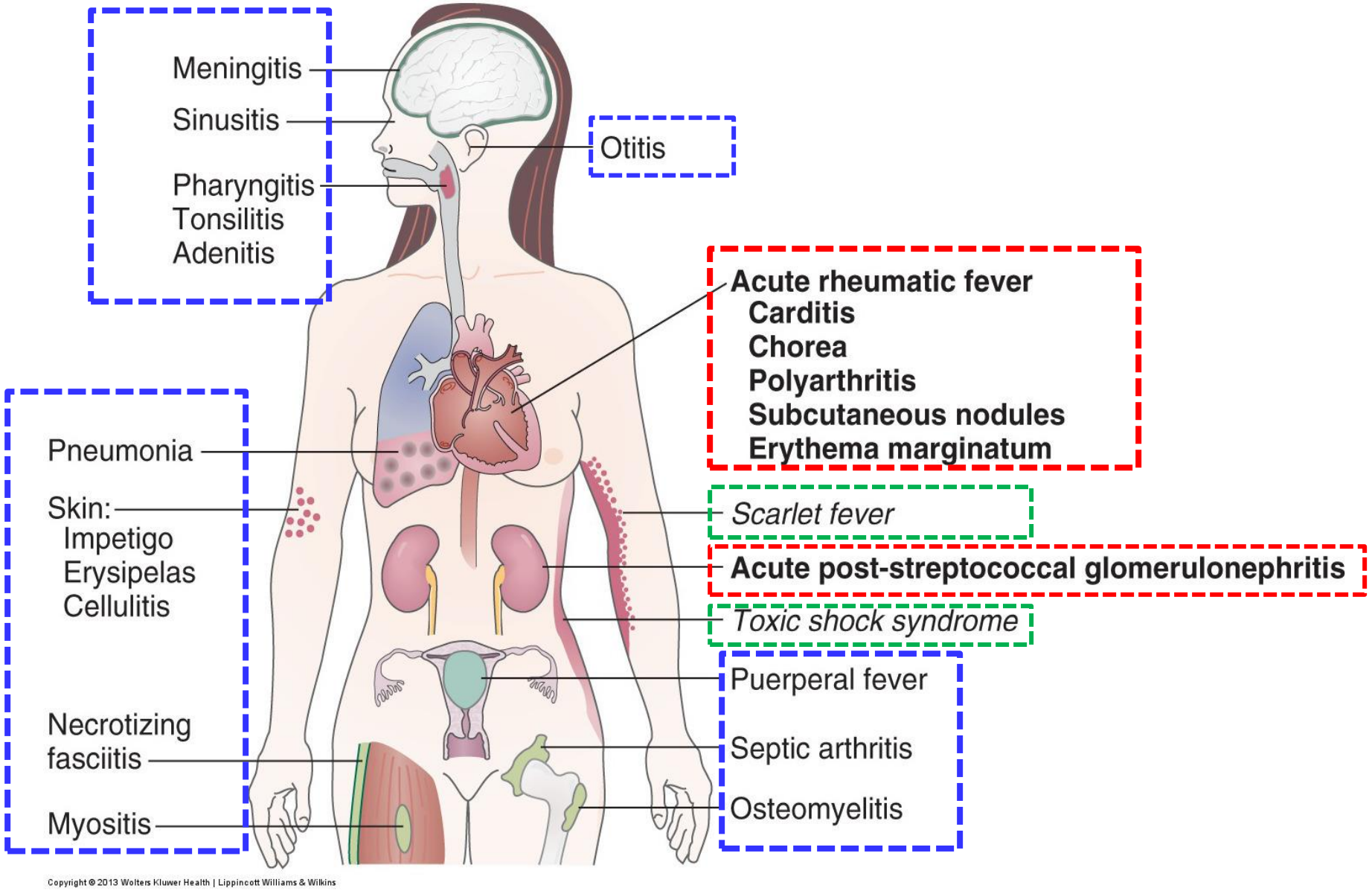
Group A streptococci (*Streptococcus pyogenes*) - epidemiological characteristics-

S. piogenes is ubiquitous in the human population with worldwide distribution.

Group A streptococci are usually present in the **nasopharynx** and on the **skin**. During the winter months, as many as 20% of school-aged children are **carriers** (these streptococci are present in the pharynx), many of whom have no symptoms of infection.

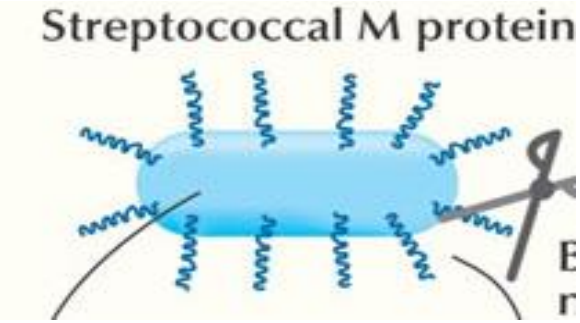
The infection is **transmitted by** respiratory droplets, or by direct contact in case of skin infections.

Recurrent ping-pong infections are common in families.



Diseases caused by *Streptococcus pyogenes*

Streptococcus pyogenes
- colonization of the portal of entry-



Bacteria bind to various structures using adhesins:

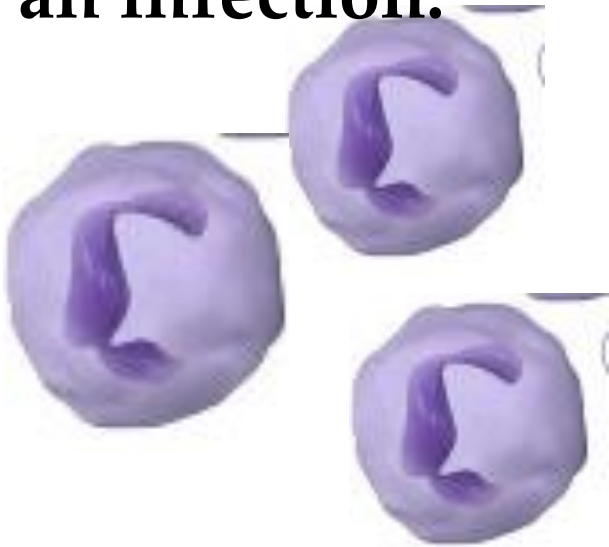
- In the case of **streptococcal pyoderma**, the bacteria reach deeper layers of the skin through skin lesions.
  - **M protein** and **hyaluronic acid** on the streptococcal capsule bind to keratinocytes.

- In case of **streptococcal throat infection**:
  - **The hyaluronic acid** of the capsule binds to the CD44 receptor on the epithelial cells of the pharynx and skin keratinocytes.

- Streptococci also bind to extracellular matrix molecules using adhesins (**lipoteichoic acid** and **streptococcal fibronectin-binding proteins**).

Once group A streptococci enter the host, they must evade phagocytosis and the immune response in order to multiply and establish an infection.

They evade phagocytosis using...



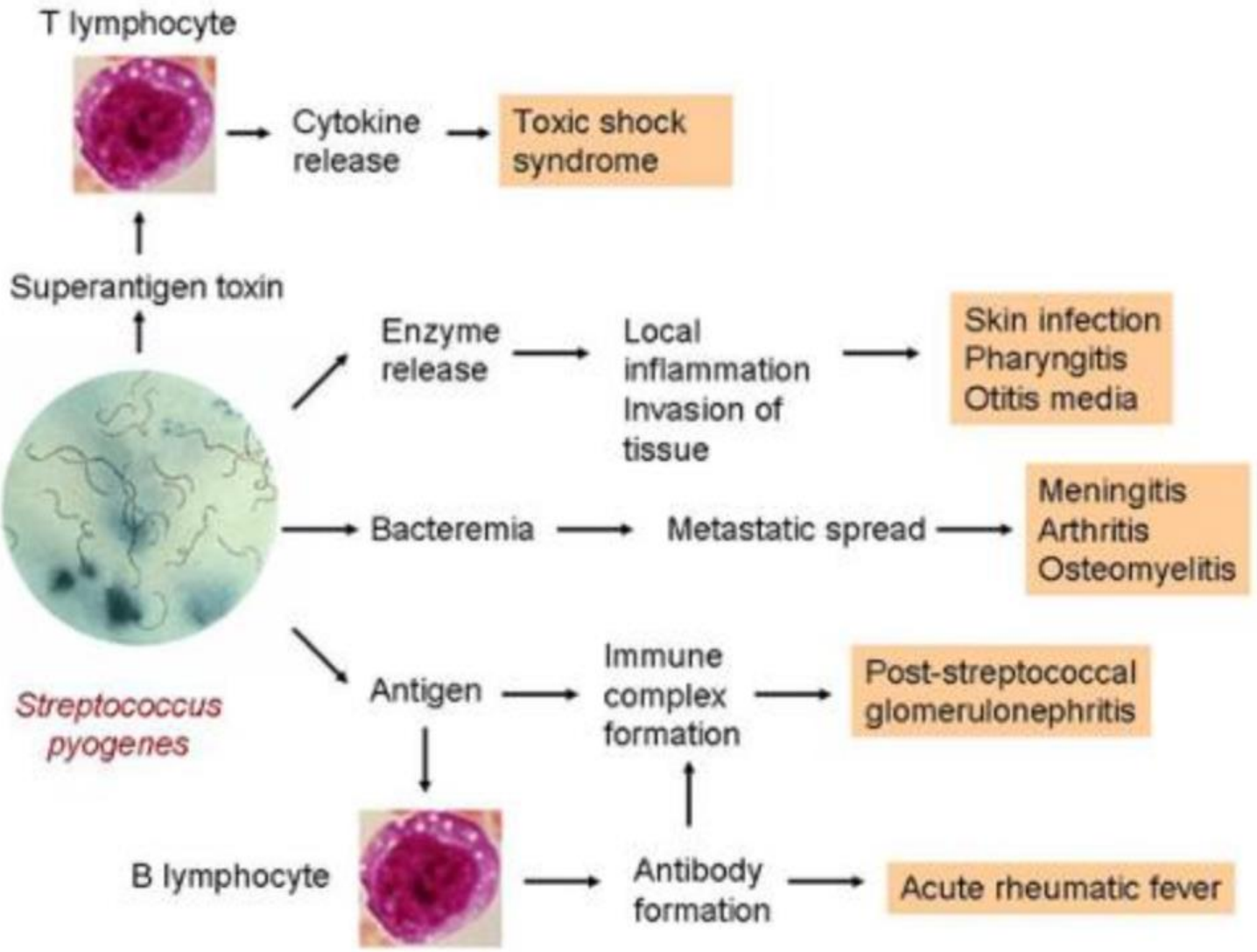
...M protein and **hyaluronic acid of the capsule**

...**chemotaxin-degrading proteins**

...**proteins** that **inactivate** or **degrade antibodies**

... **proteins** that **block** the **antimicrobial peptide function**.

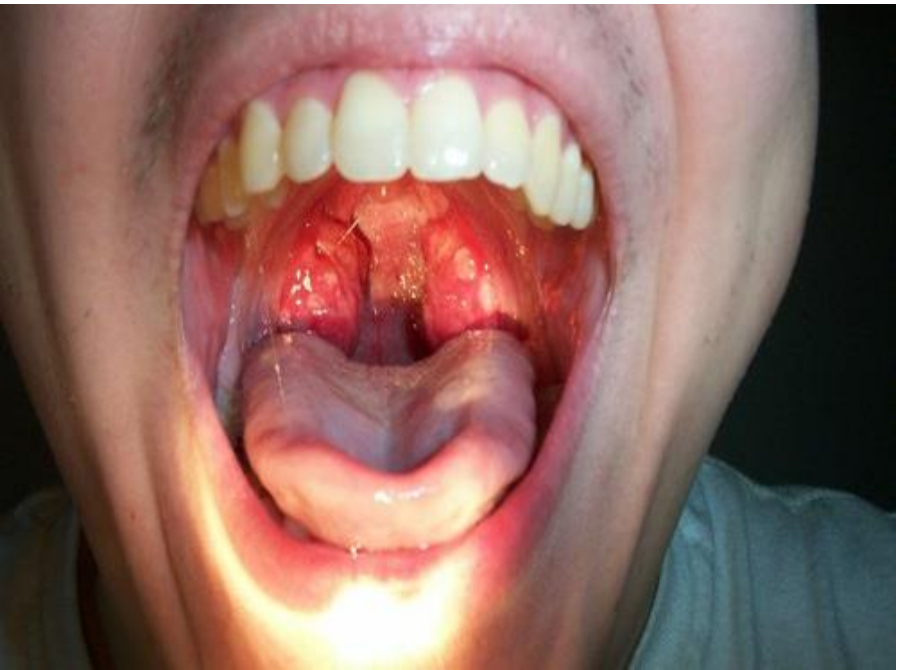
Strep pyogenes infections

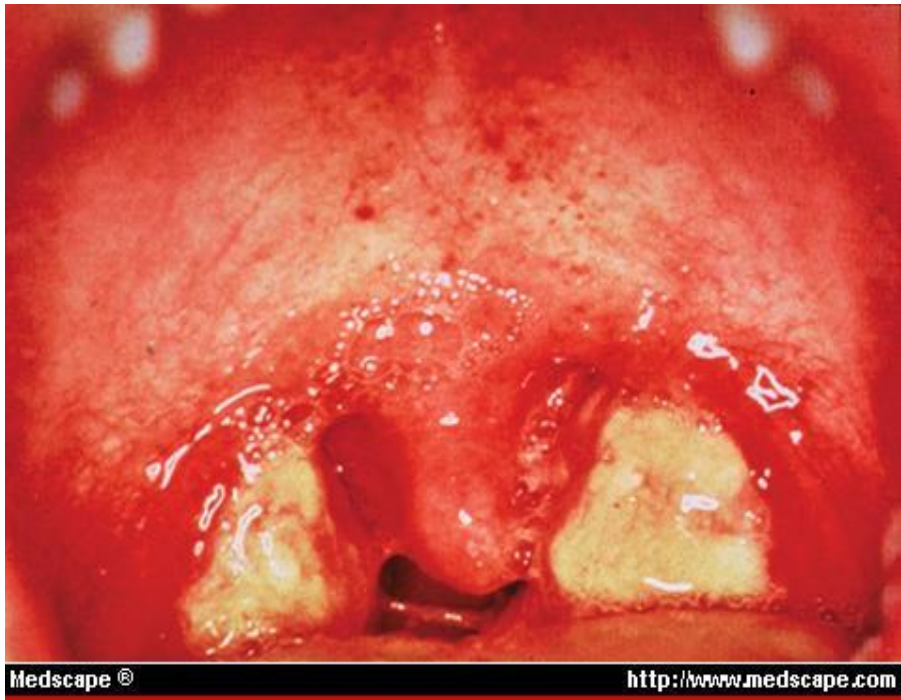


1. Invasive diseases caused by *S. pyogenes*

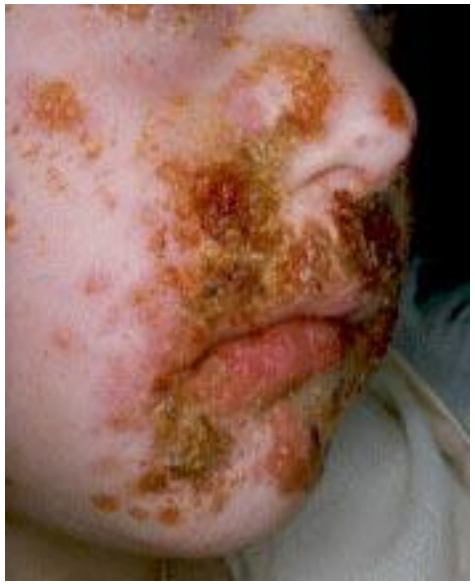
-Tonsillopharyngitis (''strep throat'')-

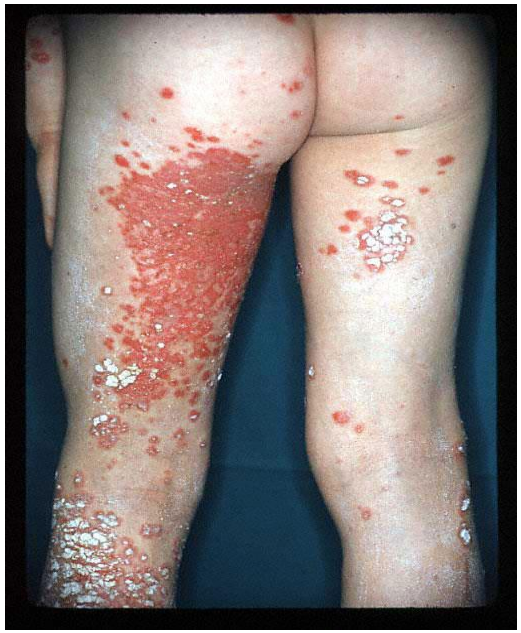
Group A streptococci induce an intense inflammatory reaction in tissues and usually remain localized to the site of initial infection.



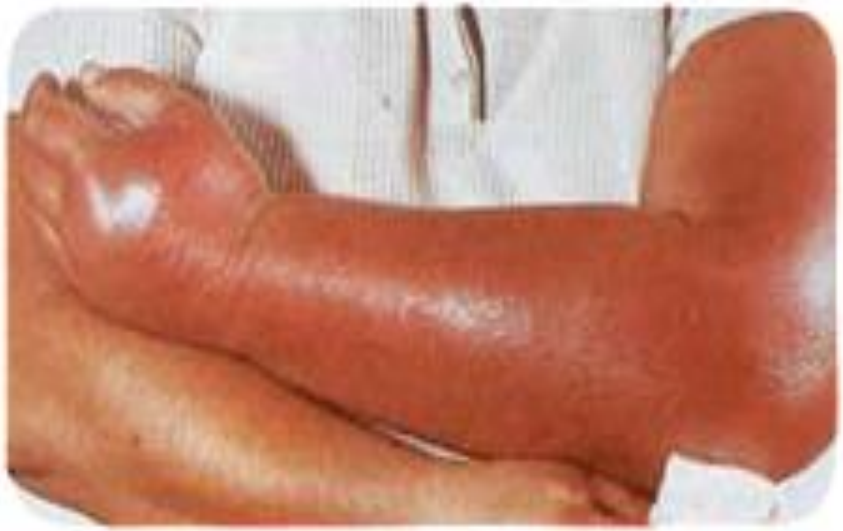


-Bullous impetigo (pyoderma)-



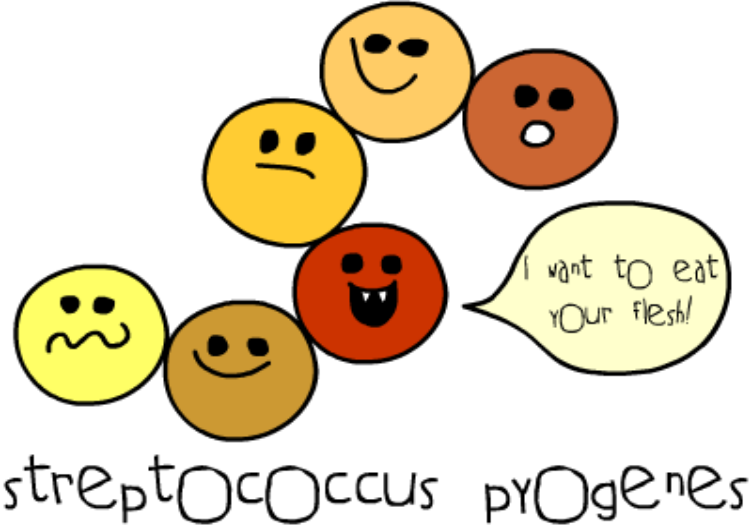


Impetigo is a common and highly contagious skin infection that causes sores and blisters. It's not usually serious and often improves within a week of treatment or within a few weeks without treatment.



-Cellulitis-

Cellulitis is an infection of the deeper layers of skin and the underlying tissue. It can be serious if not treated promptly.

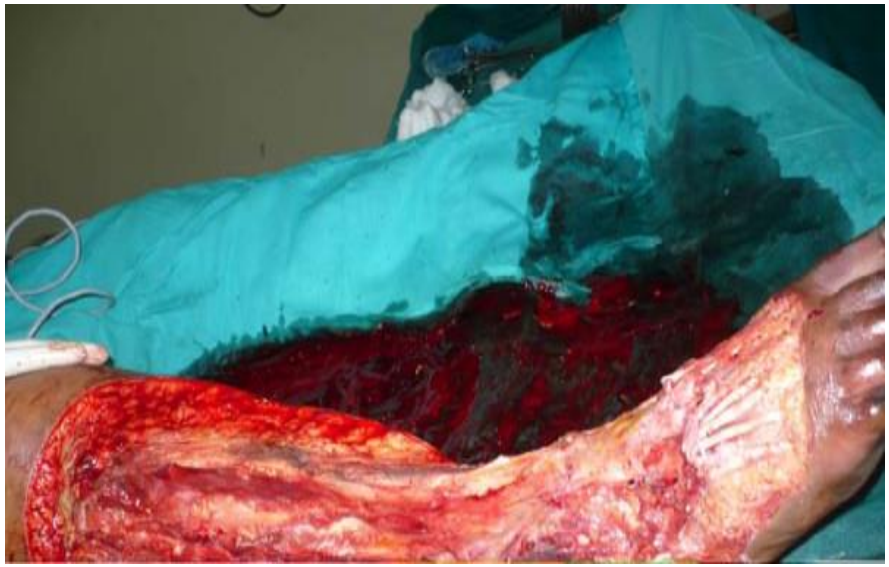


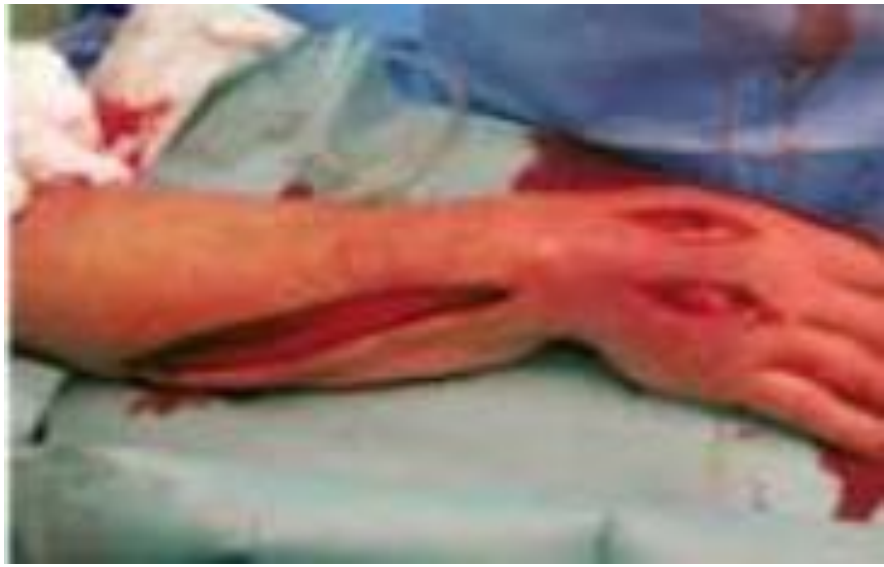
"*flesh eating*" bacterium

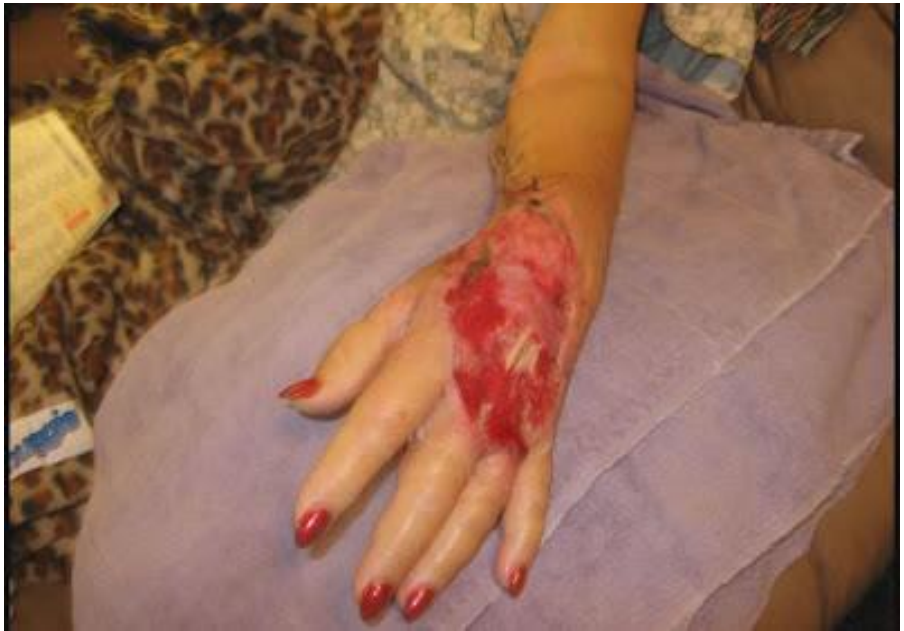
S. pyogenes secretes enzymes that promote its spread along the fascia, namely **proteases**, **hyaluronidases**, **deoxyribonucleases** (DNases) and **streptokinase**. Streptokinase can bind to plasminogen to form a catalytic complex that converts plasminogen to plasmin which then binds to the surface of group A streptococci. Such plasmin-coated streptococci can degrade fibrin and spread through it.

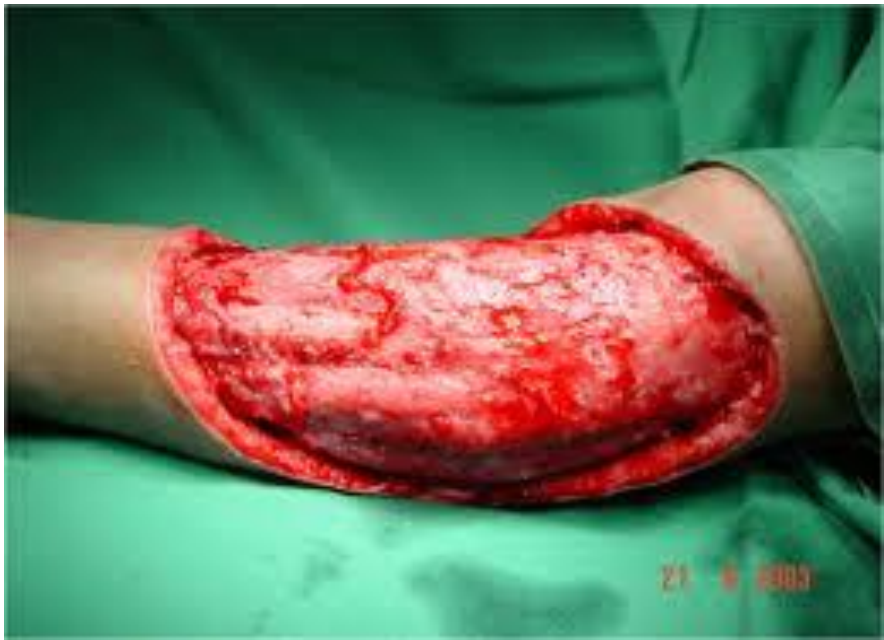
S. pyogenes also secretes **streptolysin- S** and **-O**, hemolysins that lyse the membrane of various host cells.

-Necrotizing fasciitis and myositis-



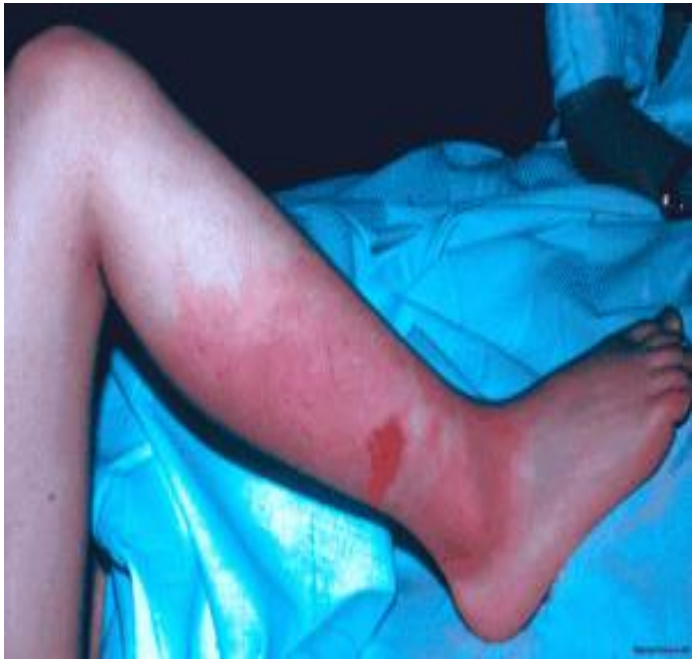


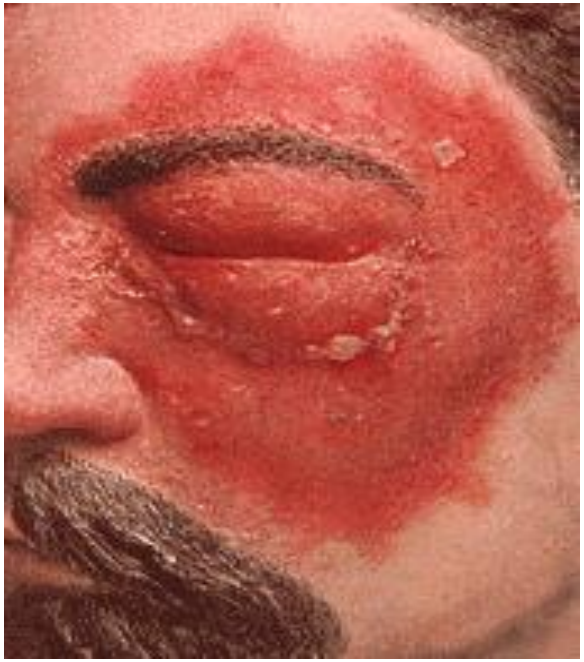


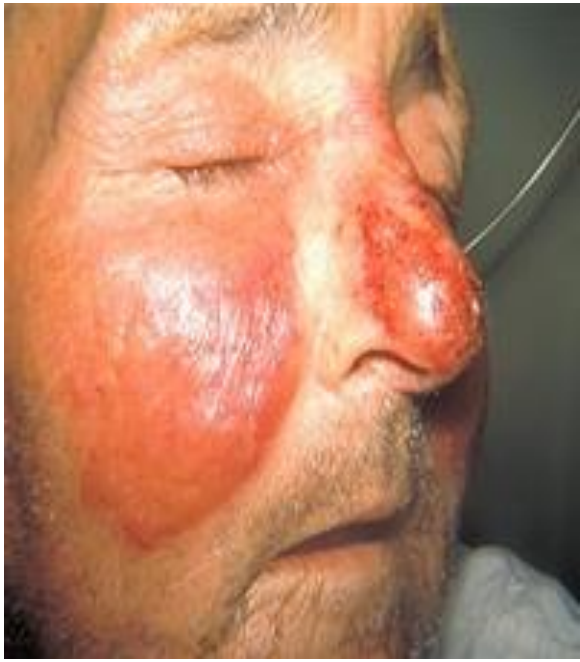


2. Toxoinfections caused by *S. pyogenes*

-Erysipelas (''Holy fire'')-

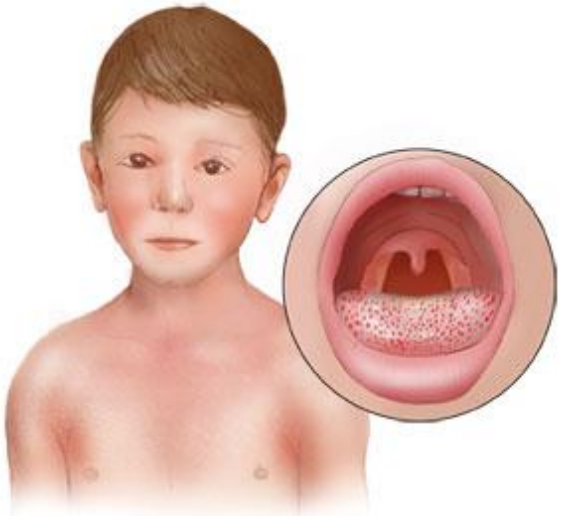


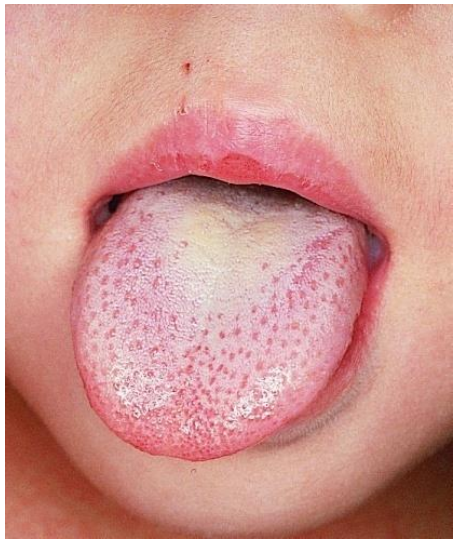


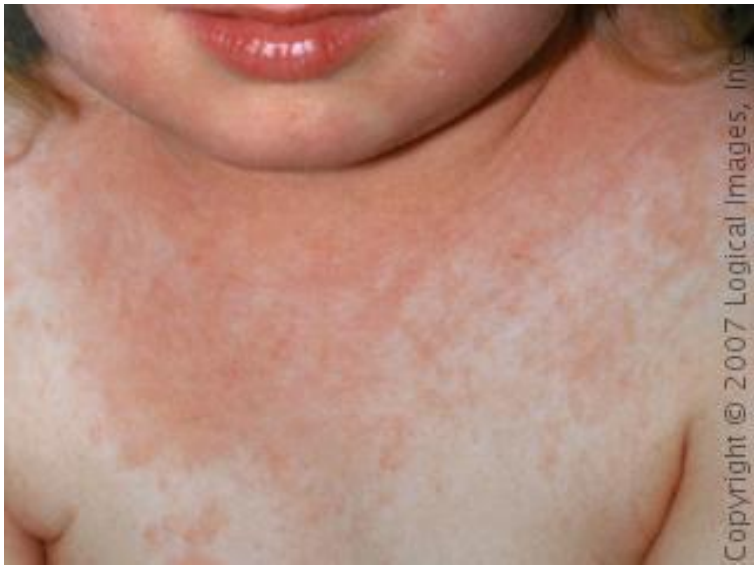


- Scarlet fever -

- Scarlet fever (*scarlatina*) is **local infection (purulent angina)** accompanied by **general intoxication**.
- The three toxins responsible for the pathogenesis of scarlet fever are the **streptococcal pyrogenic exotoxins -A, -B**, and **-C**. In addition to causing the rash that is characteristic of scarlet fever, SPE-A and SPE-C are also bacterial superantigens.

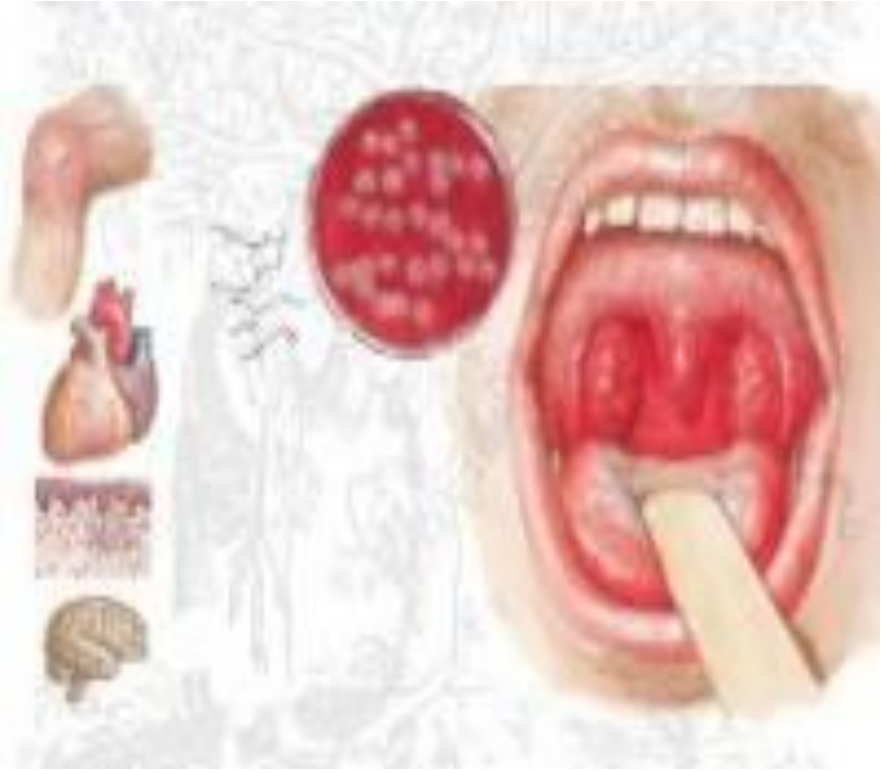






3. Poststreptococcal nonsuppurative (sterile) sequelae

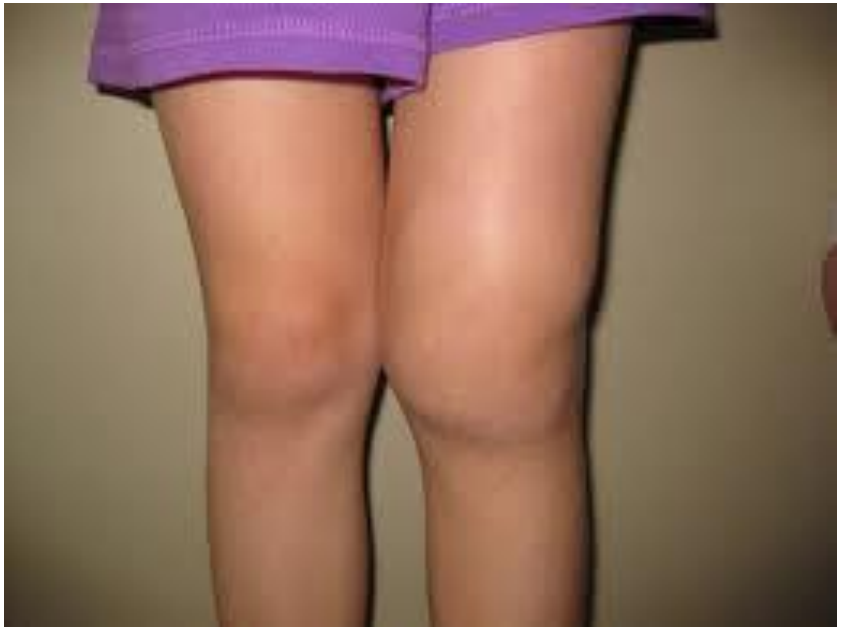
- Acute rheumatic fever -

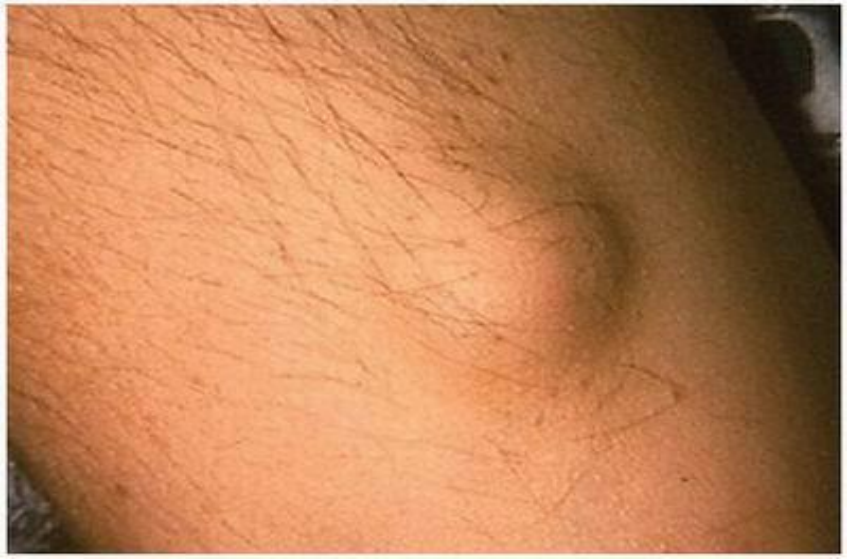


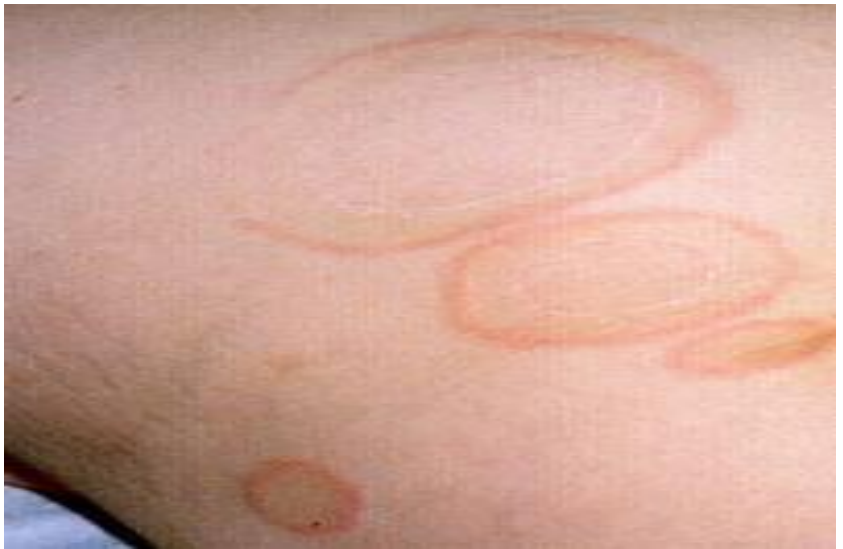
Tonsillopharyngitis is the only type of streptococcal infection that can lead to acute rheumatic fever.

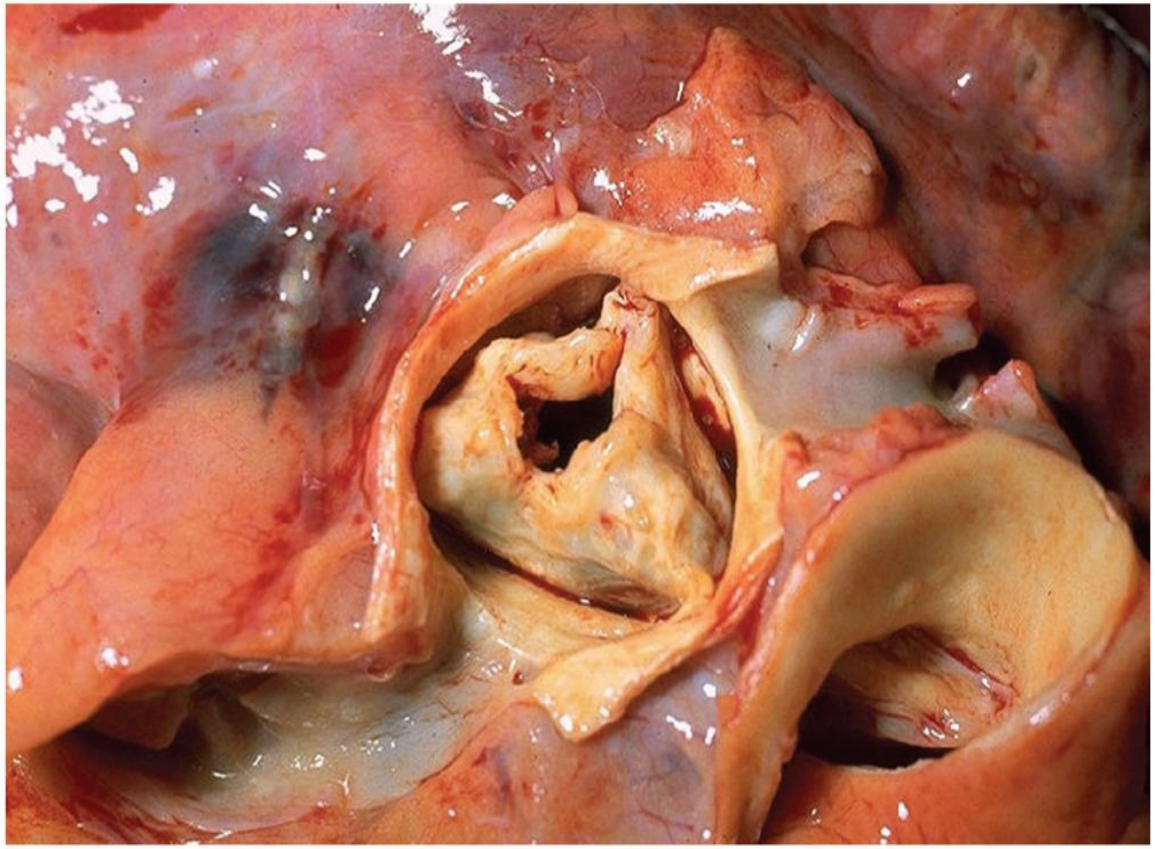
Clinical manifestations appear 1 to 4 weeks after pharyngitis. These are **carditis**, **polyarthritis**, **chorea**, **subcutaneous nodules** and **erythema marginatum**.

Rheumatic fever





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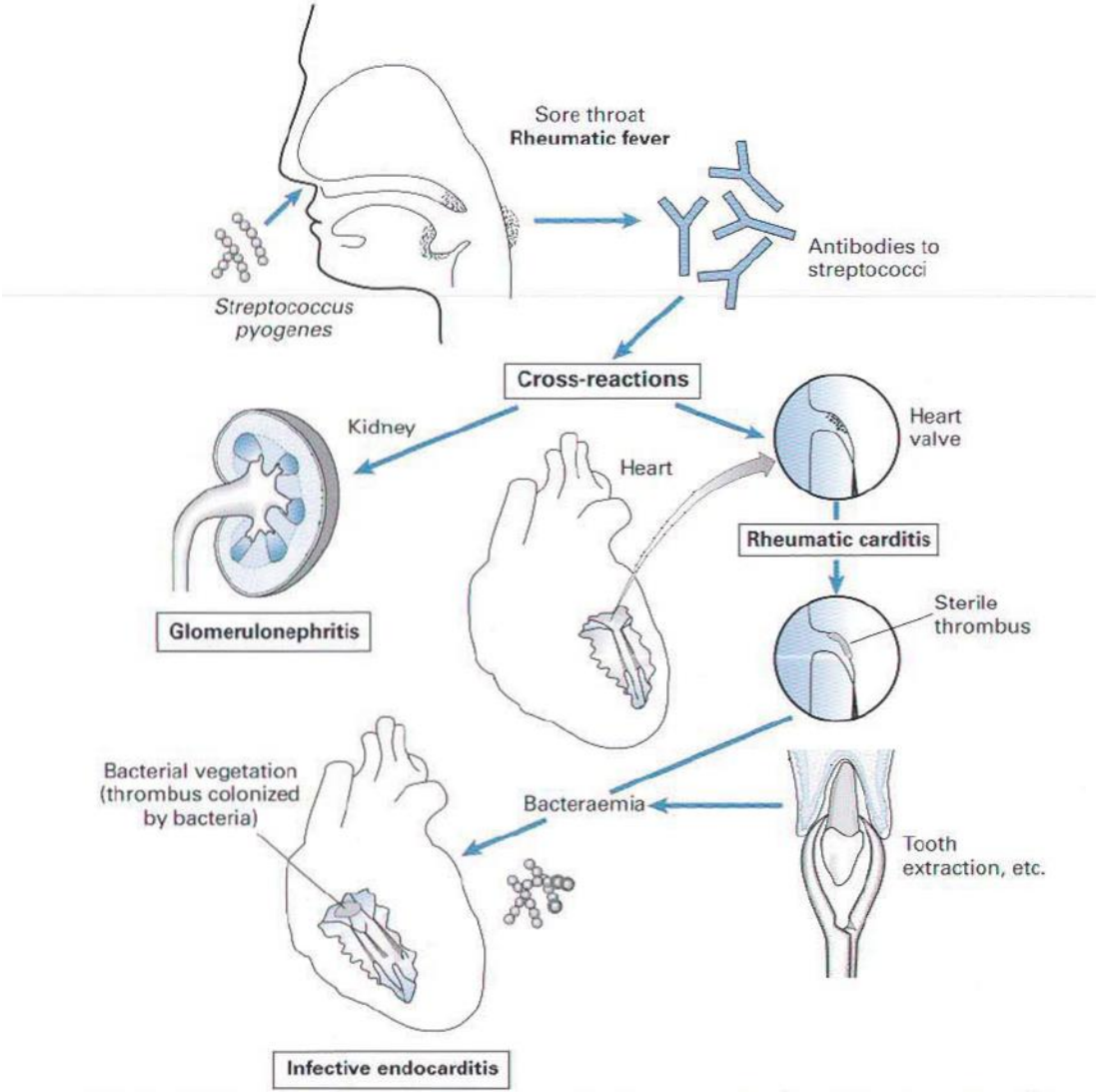
Extensive **scarring on the mitral and/or aortic valve** and **stenosis**. This manifestation, known as **"rheumatic heart disease",** causes a functional heart disorder.

- Acute poststreptococcal glomerulonephritis -

- This sequela can occur either after **pharyngitis** or **pyoderma**.
- Acute poststreptococcal glomerulonephritis usually occurs 1 to 4 weeks after infection. Patients develop renal failure.

Poststreptococcal sterile sequelae
-Immune mechanisms-



- Cross-reaction

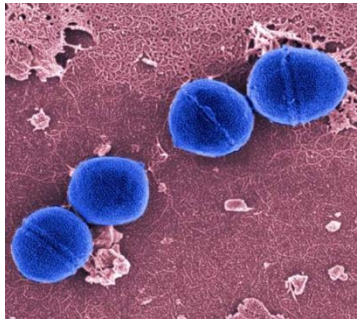
- Immune complexes

Treatment of streptococcal diseases

Penicillin is the medication of choice. Due to the possibility of developing sterile sequelae, the treatment must not be shorter than 10 days.

Erythromycin or other **macrolide antibiotics**.

Group B streptococci (*S. agalactiae*)



- They inhabit the lower parts of the gastrointestinal tract, as well as the genital tract in women.

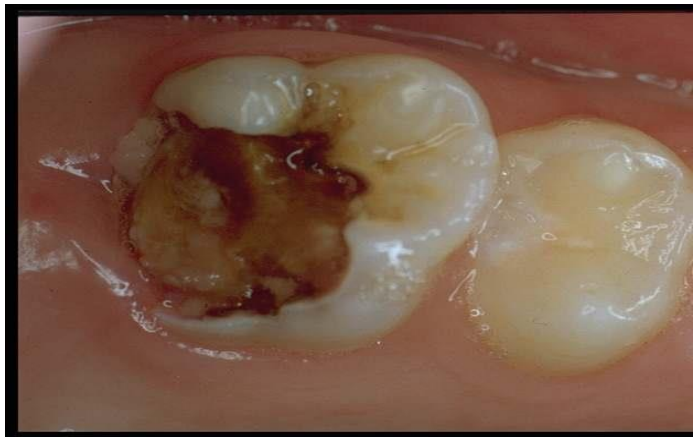
- Group B streptococci are still the leading cause of **neonatal sepsis** and **meningitis**.
- The colonization rate in pregnant women ranges from 15 to 40%, so screening for these infections is performed from the **35th to the 37th week of pregnancy.**

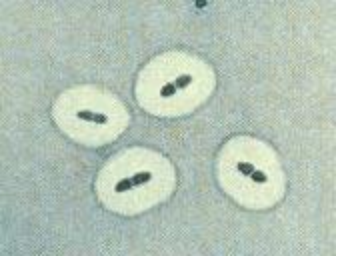
- **In pregnant women,** group B streptococci can cause **urinary tract infection, endometriosis and amnionitis**.
- Group B streptococci are sensitive to penicillin.

Other pathogenic streptococci

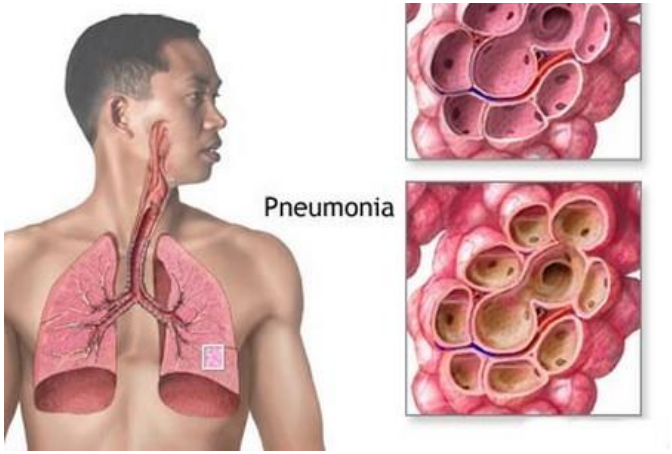
- **Group C and group D streptococci** (*S. disgalactiae*) - cause respiratory infections, deep tissue infections and nonsuppurative sterile sequela - acute glomerulonephitis.

- **Viridans streptococci -** bacteria of the oral cavity. They produce greenish discoloration on blood agar around the colonies, ("*viridis*" means green in Latin).
  - *S. mitis, S. sanguinis, S. gordonii, S. mutans* и *S. sobrinus* (endocarditis).
  - *S. milleri* (abscesses of the brain, liver, and abdominal cavity.



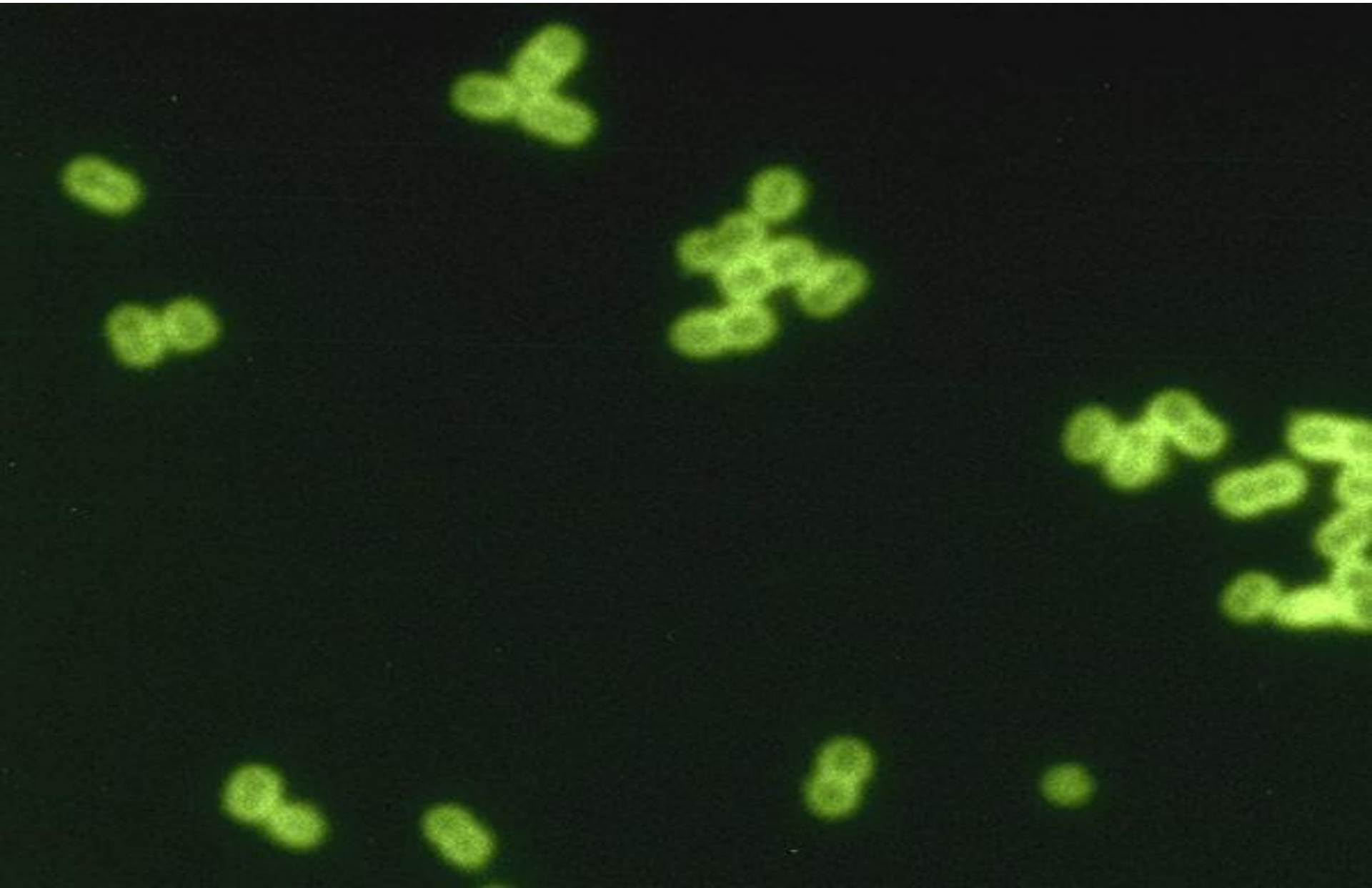


Streptococcus pneumoniae (pneumococcus)



… is the leading cause of severe infections, especially bacterial pneumonia, worldwide

Streptococcus pneumoniae (diplococcus). Fluorescent stain



General characteristics of pneumococcus

Pneumococcus....

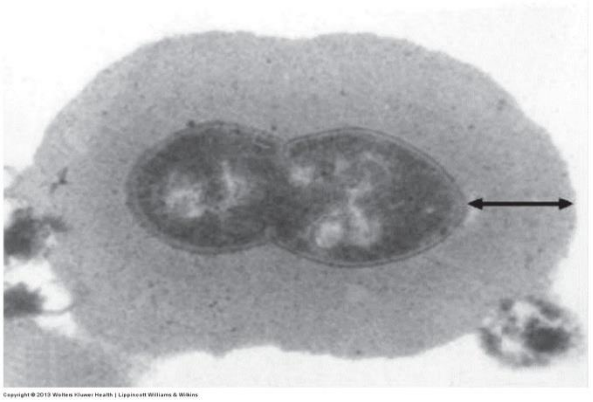
... is **an aerobic** streptococcus.

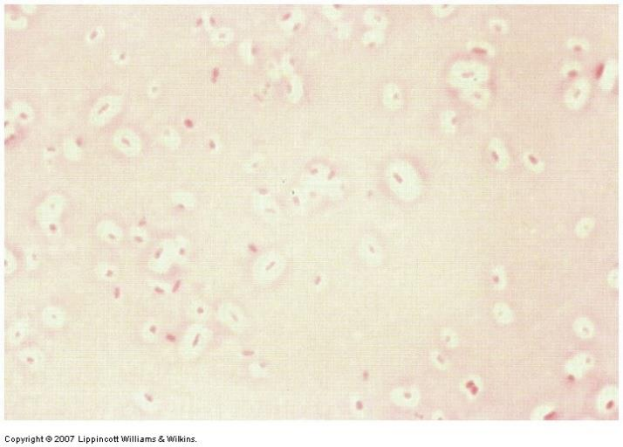
... it **does not produce** the enzyme **catalase**, so a large amount of **hydrogen peroxide** is formed during the metabolism of this bacterium.

... synthesizes **pneumolysin** which together with hydrogen peroxide inhibits the growth of competitive members of the microflora and also causes tissue damage and inhibits effective host defense.

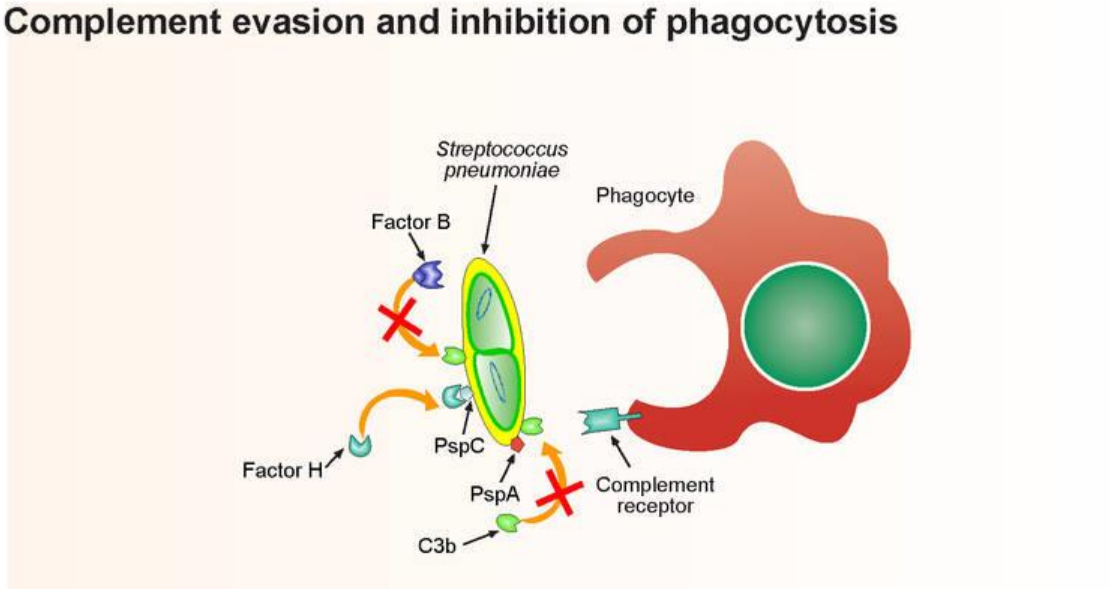
... in the stationary phase of growth, expresses the enzyme **autolysin**, which degrades its own cell wall.

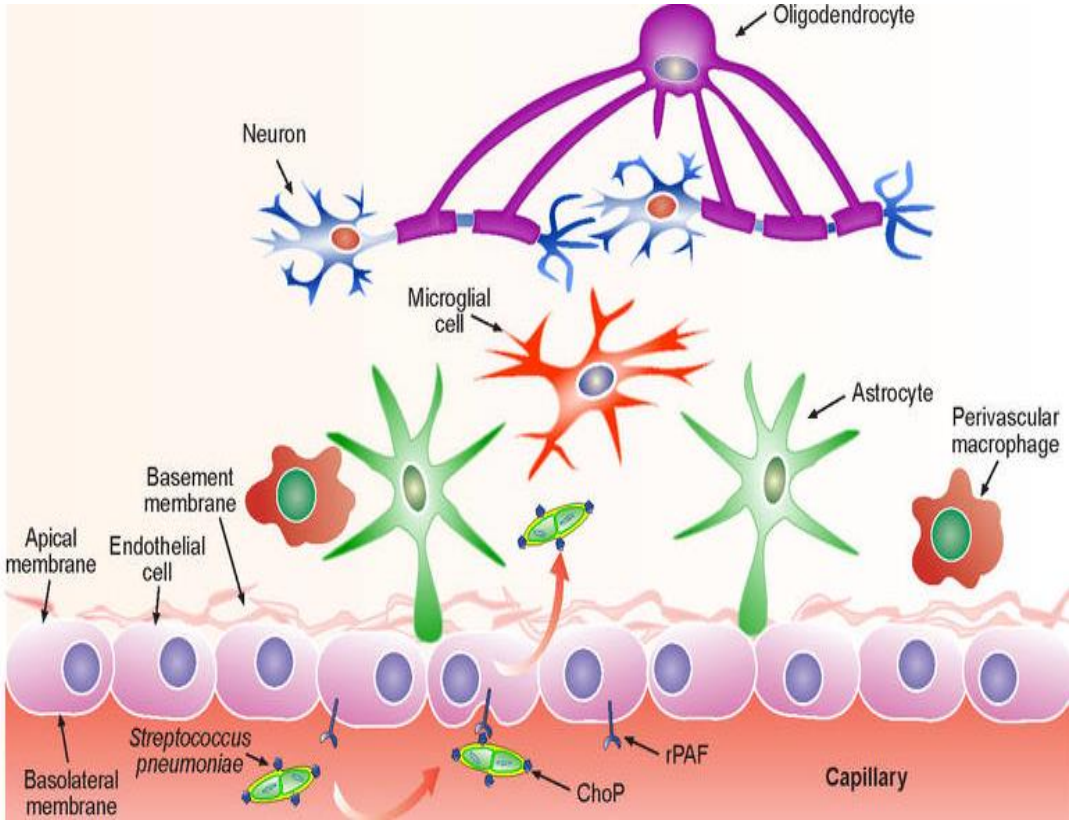
.... is surrounded by a **thick cell wall** whose surface molecules are recognized by the components of innate immunity, stimulating a strong inflammatory response.





Pneumococcus has a '**mucoid**' or '**smooth**' capsule composed of **polysaccharides**. This thick polysaccharide capsule plays an important protective role because it reduces complement activation ...

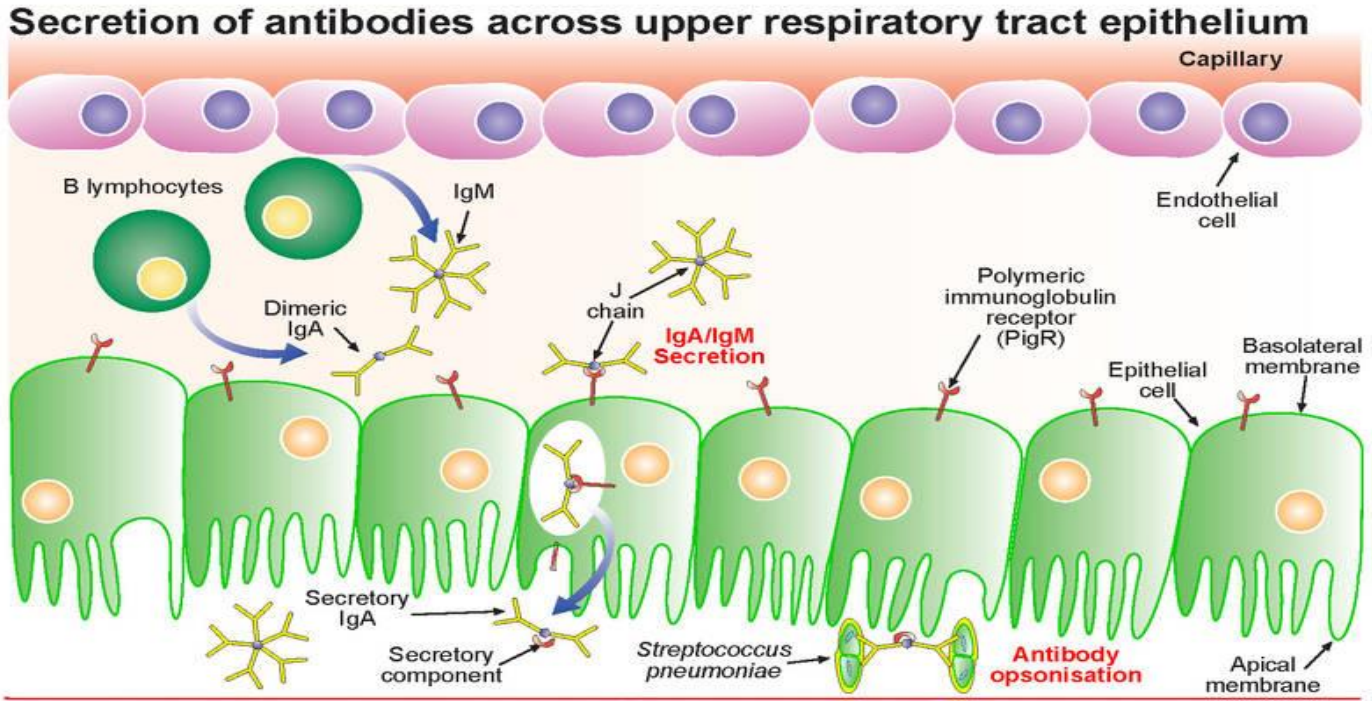




.... The capsule is especially important for pneumococci because it allows them to **survive in the bloodstream**, where complement activity is most effective. Mostly **encapsulated pneumococci** invade the blood and induce **bacteremia** during which they **pass through the blood-brain barrier** and reach the central nervous system causing **meningitis**.

Capsular polysaccharides are highly **immunogenic**. Because they are targets of the immune system, pneumococcal capsular polysaccharides are particularly susceptible to **antigenic variation** (resulting in more than 90 serotypes).

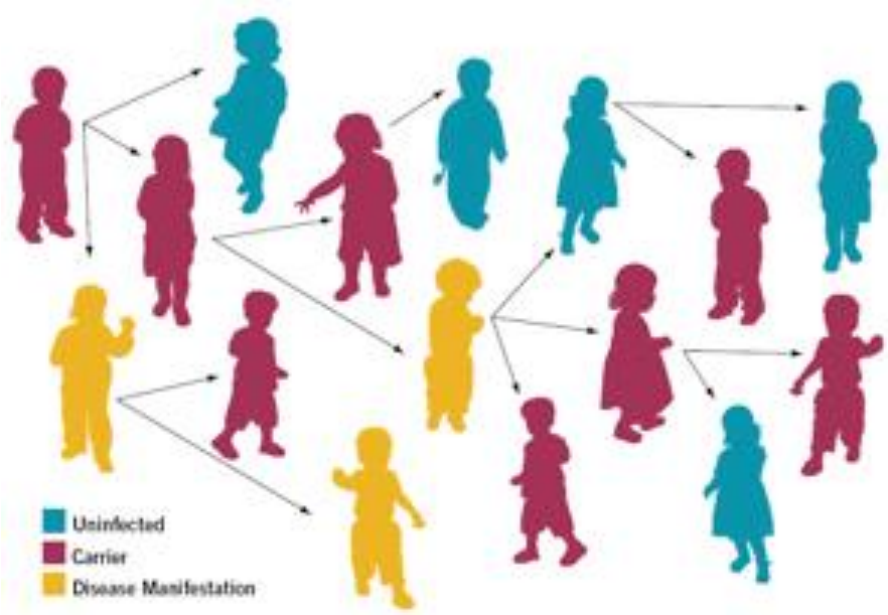
Antibodies specific for these capsular polysaccharides neutralize bacteria or serve as opsonins. These antibodies play an important role in protection against infections caused by pneumococci of the same serotype but have minimal impact in protection against other serotypes.



Epidemiological characteristics of pneumococcus

The reservoir of *S. pneumoniae* are humans.

It colonizes the **nasopharyngeal mucosa**. The outcome of this colonization depends on the virulence of the pneumococcal strain and the efficiency of host defense mechanisms.



Pneumococci are present in two thirds of **preschool children**. The incidence of colonization decreases with age.

Transmission of pneumococcus from a sick person, or more often from a healthy carrier, occurs through **droplets** of respiratory secretion. It is also transmitted by **contaminated hands**. Transmission occurs directly from person to person (in families and in collectives).

Diseases caused by pneumococcus

Pneumococcal pneumonia is the most common form of bacterial pneumonia and one of the leading causes of death in humans.

Pneumococcus is the leading cause of other infections such as **otitis media**, **acute sinusitis** and **chronic bronchitis** as well.

During bacteremia, pneumococci can reach other normally sterile regions and cause pneumococcal infection of the peritoneal cavity (**peritonitis**), joints (**septic arthritis**) or heart valves (**endocarditis**).

Encapsulated strains of pneumococcus cause **meningitis**.

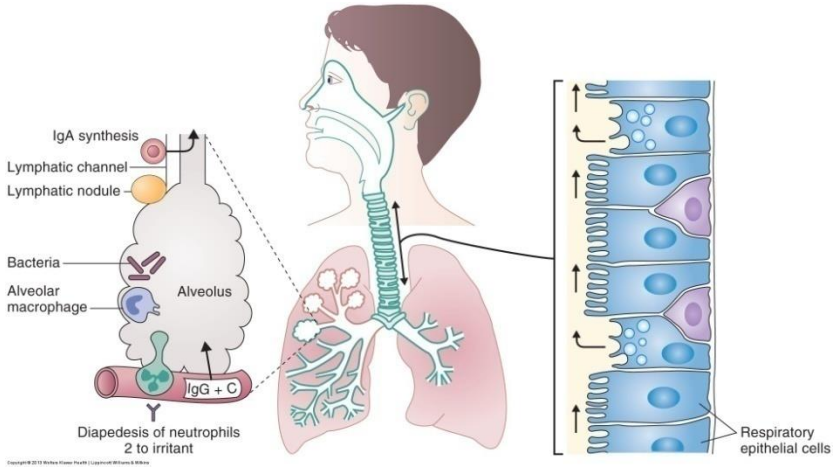
After *N. meningitidis* and *H. influenzae*, pneumococcus is the most common cause of bacterial meningitis.

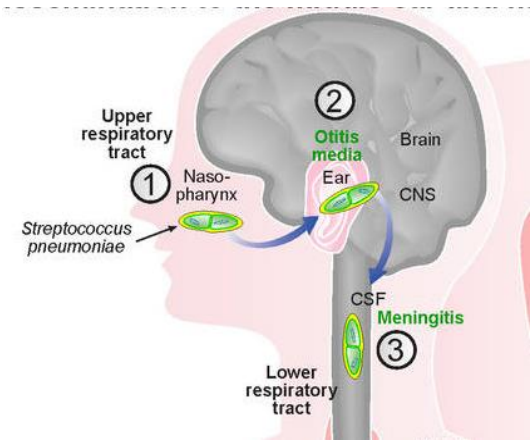
Pneumococcus portal of entry

The defense mechanisms of the host are :

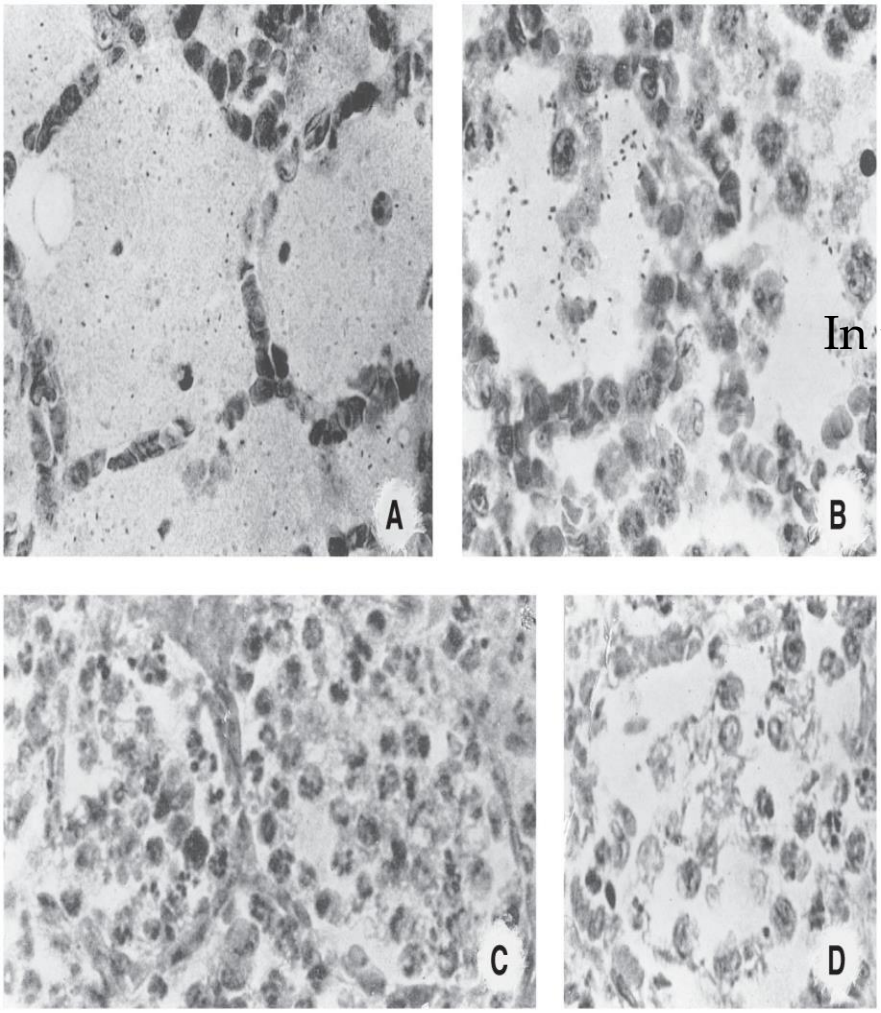
- ✓ **epiglottis;**
- ✓ **cough reflex;**
- ✓ a layer of **sticky mucus;**
- ✓ **cilia of the respiratory epithelium;**
- ✓ **alveolar macrophages.**

The disease occurs when the bacterium spreads from the nasopharynx to the normally sterile parts of the airway, and this spread is usually influenced by multiple host factors.





Bacterial spread, multiplication and tissue damage



In the first stage of pneumococcal pneumonia, the **alveoli become filled with serous fluid** containing a large number of bacteria, but few inflammatory cells. This leakage of fluid can compromise the basic function of the lungs - gas exchange.

In the second stage, called **early consolidation**, the alveoli are infiltrated by neutrophils and erythrocytes. Strong **chemotactic signals**, induced by pneumococcus and the alternative pathway of **complement** , lead to the recruitment of large numbers of neutrophils and **the acute inflammatory process**. The capsule allows pneumococci to resist phagocytosis. **The extent of successful phagocytosis determines the outcome of the infection.**

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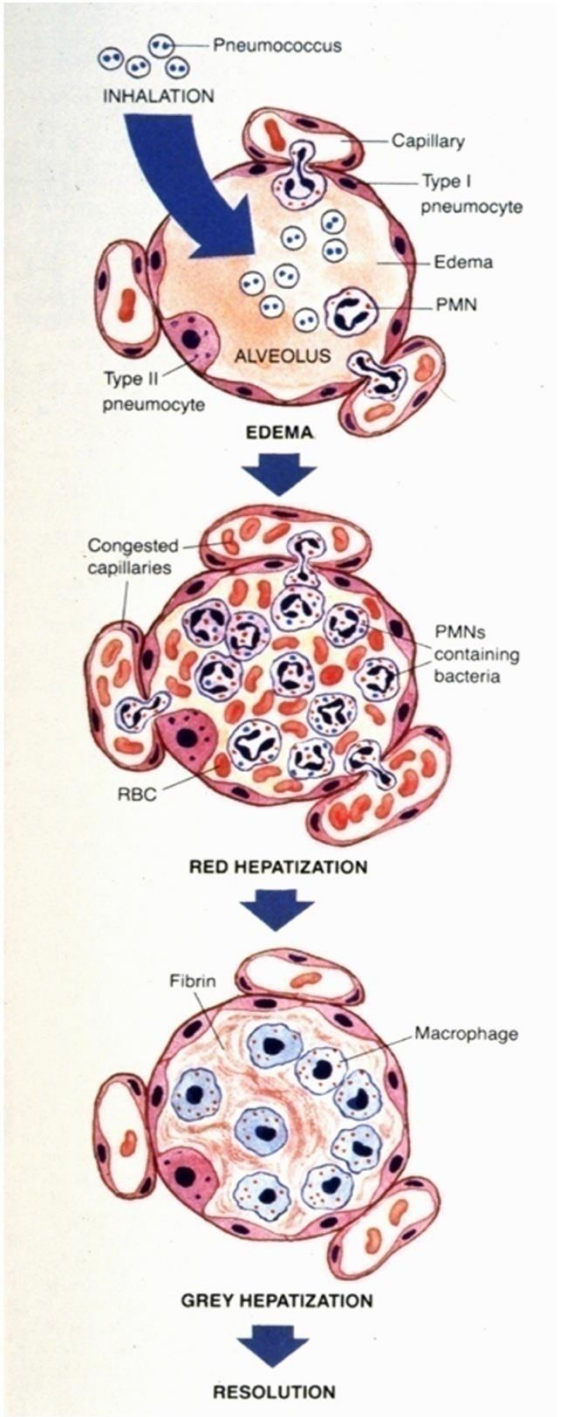

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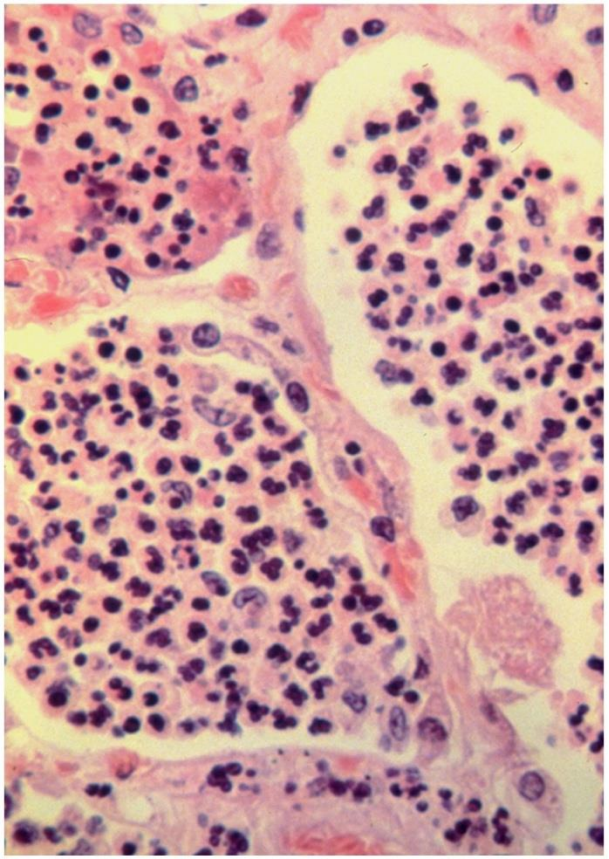
The third stage of pneumococcal pneumonia is called **late consolidation**. The alveoli are filled with neutrophils and a few remaining pneumococci. Macroscopically, the damaged regions of the lungs resemble the liver (this phenomenon is called **hepatization**).

In the fourth final phase, or **resolution phase**, neutrophils are replaced by macrophage "scavengers", which remove debris created during inflammatory processes. In most cases, the lung architecture eventually returns to normal.



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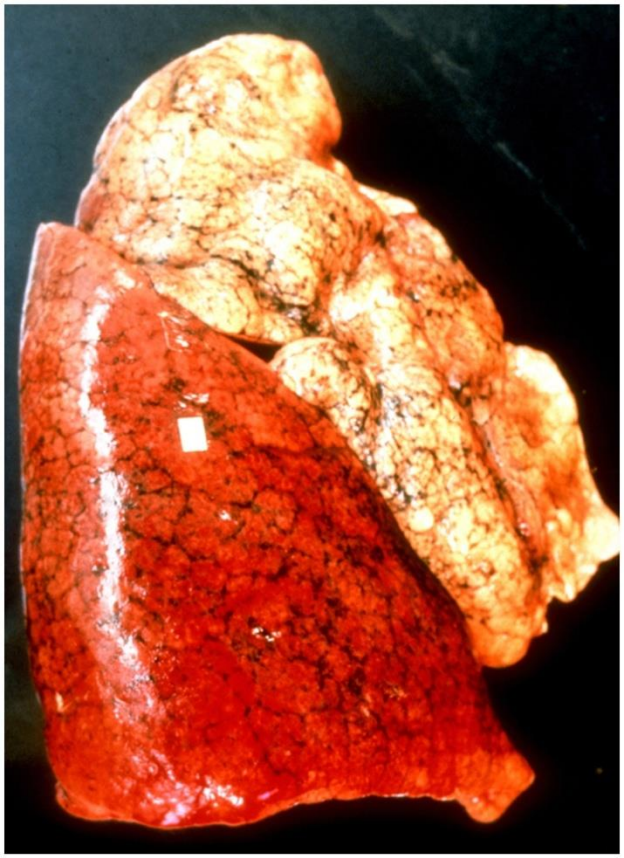
Lungs stained with H&E



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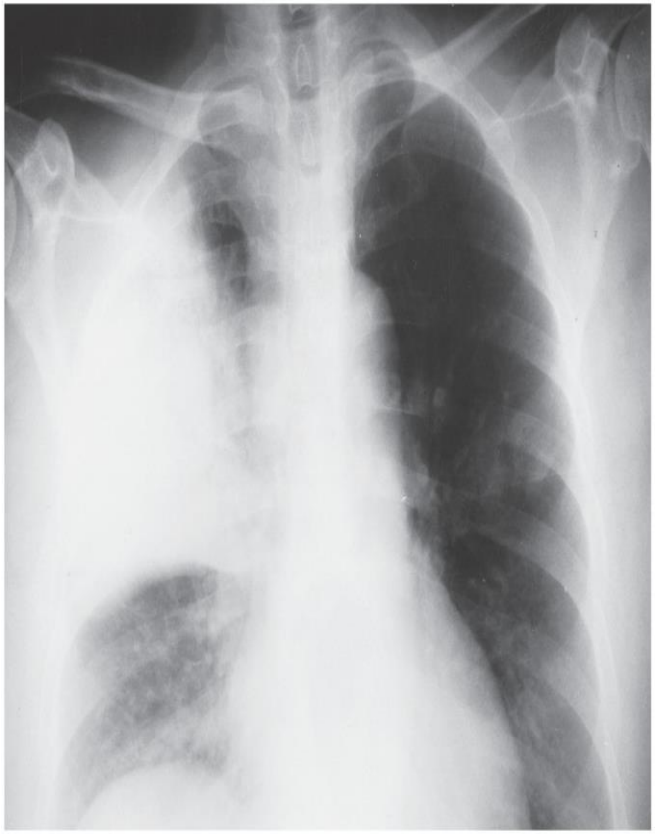
Lung lobe consolidation with red hepatization



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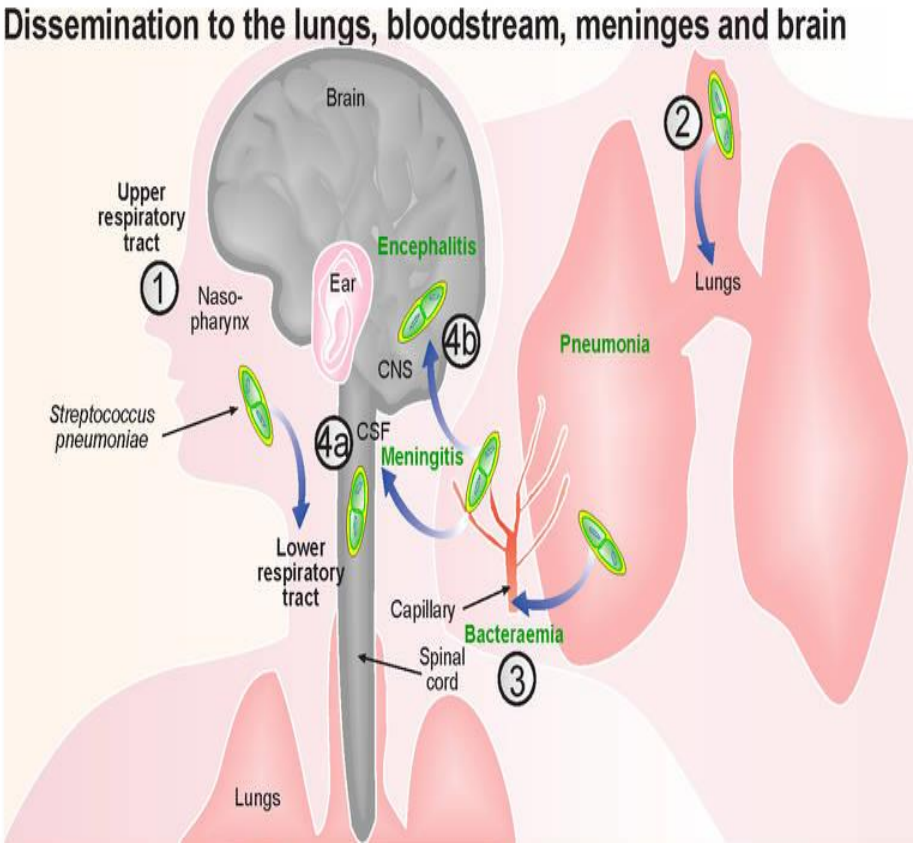
Pneumococcal pneumonia may lead to local complications:

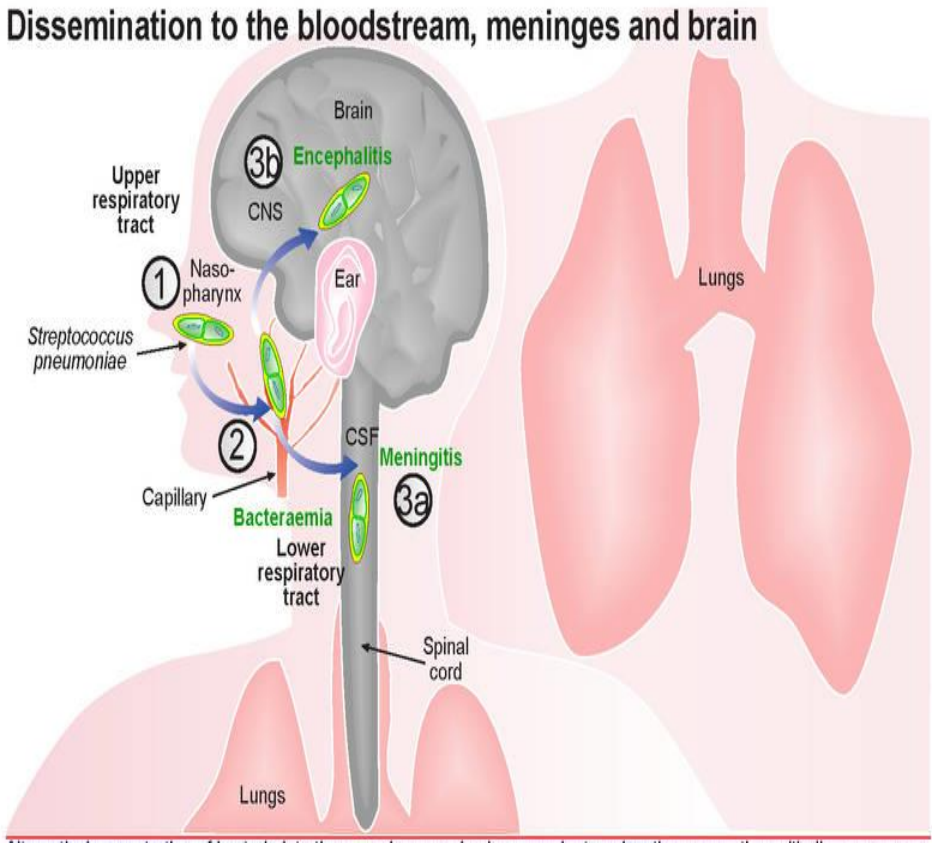
pleural effusion

empyema

or distant complications:

secondary metastatic foci (infection of the meninges).





Fulminant progressive pneumococcal bacteremia can occur in individuals after **splenectomy**.

Treatment and prevention of pneumococcal infection

Penicillin is the medication of choice for treatment of pneumococcal infection.

Macrolides or **selected fluoroquinolones** (in case of penicillin allergy).

Mechanism of **penicillin-resistance** of pneumococci - resistant pneumococci have **altered penicillin-binding proteins**.

Pneumococci take over and integrate bacterial DNA from their environment through a process known as **transformation**.

Vaccination is especially important for people with the highest risk of developing pneumococcal disease, namely younger children, the elderly, as well as people with predisposing conditions.

The vaccine contains **capsular polysaccharides** (23 most common serotypes) - it stimulates the synthesis of protective antibodies.

Pneumococcal conjugate vaccine - contains up to 13 serotypes, most commonly found in children, linked to a protein carrier resulting in more efficient antibody production. This conjugate vaccine reduces carriage of pneumococcus and has been shown to be highly effective against invasive pneumococcal diseases and, to some extent, in preventing pneumonia and otitis media.

Enterococci

Enterococci are part of the normal flora of the gastrointestinal and urogenital tracts.

They are non-pathogenic in immunocompetent patients.

They are one of the most important causes of **nosocomial (hospital-acquired) infections** and have developed vancomycin resistance.

E. faecalis and ***E. faecium*** - cause urinary tract infections, wound infections, endocarditis, intra-abdominal abscesses and bacteremia.

Characteristics of enterococci ...

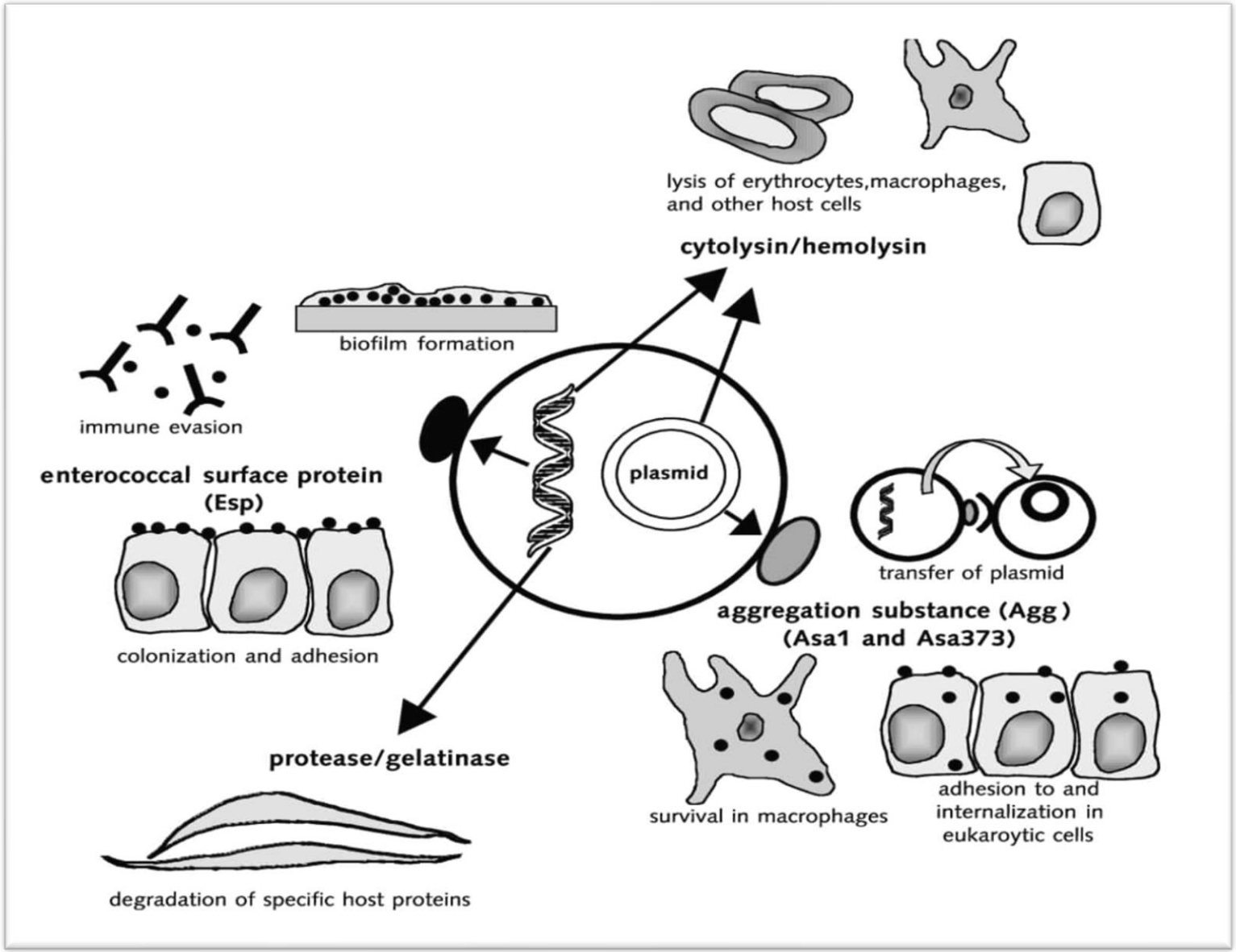
... the ability to form **biofilm** and produce **cytolysin.**

…*E. faecalis* can develop biofilm over catheters and other implanted medical devices and on the host's tissue providing protection to the bacteria against immune response and antimicrobials and supporting persistent infection.

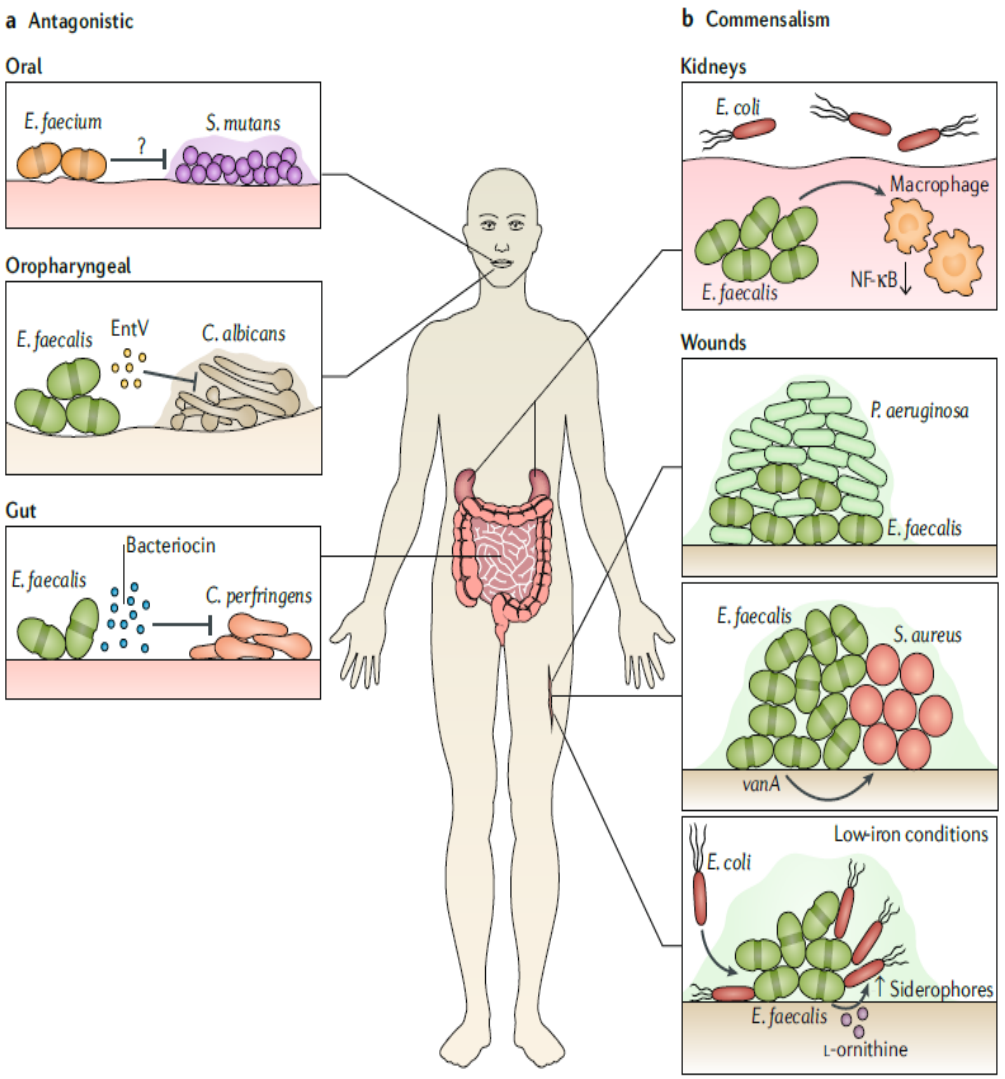
.... **intrinsic (innate) resistance** to several classes of antibiotics (penicillin, cephalosporin, aminoglycosides).

.... vancomycin resistance genes taken from exogenously acquired plasmids and transposons (**vancomycin-resistant enterococci**).

E. Faecalis virulence factors

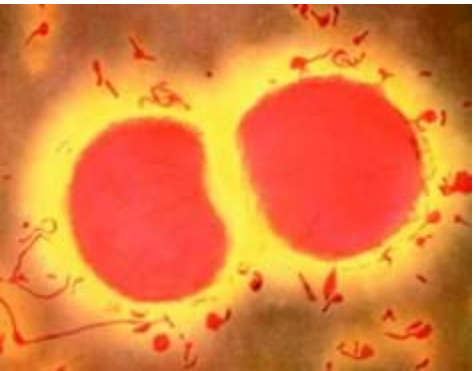


Interactions of enterococci with other species in polymicrobial biofilms



- **Antagonistic polymicrobial interactions**
  - *Enterococcus faecium* inhibits *Streptococcus mutans* in the oral cavity, *Candida albicans* in the oropharynx, *Clostridium perfringens* in the gut
- **Commensal polymicrobial interactions:**
  - *E. faecalis* can increase the virulence *E. coli* in the urinary tract and in wounds.
  - E. faecalis increases secretion of biofilm matrix components by *P. aeruginosa.*
  - In wounds, vancomycin-resistant *E. faecalis* can transfer resistance to *Staphylococcus aureus*

Gram-negative cocci



Neisseria gonorrhoeae (gonococcus)

Neisseria meningitidis (meningococcus)

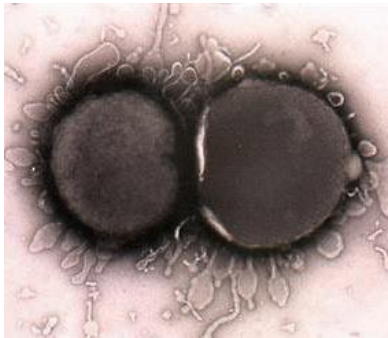
General characteristics of *Neisseria*

Neisseria spp have the cell wall, typical for Gram-negative bacteria, and contain **outer membrane proteins** and endotoxin. This **endotoxin** or **lipopolysaccharide** (**LPS**) **does not have the repeating O-antigenic subunits** and is therefore classified as a **lipooligosaccharide** (**LOS**).

The genus *Neisseria* includes numerous nonpathogenic bacteria that often inhabit the mucous membranes of healthy people, especially the nasopharynx.

Neisseria are **sensitive** and cannot survive in the external environment for long.

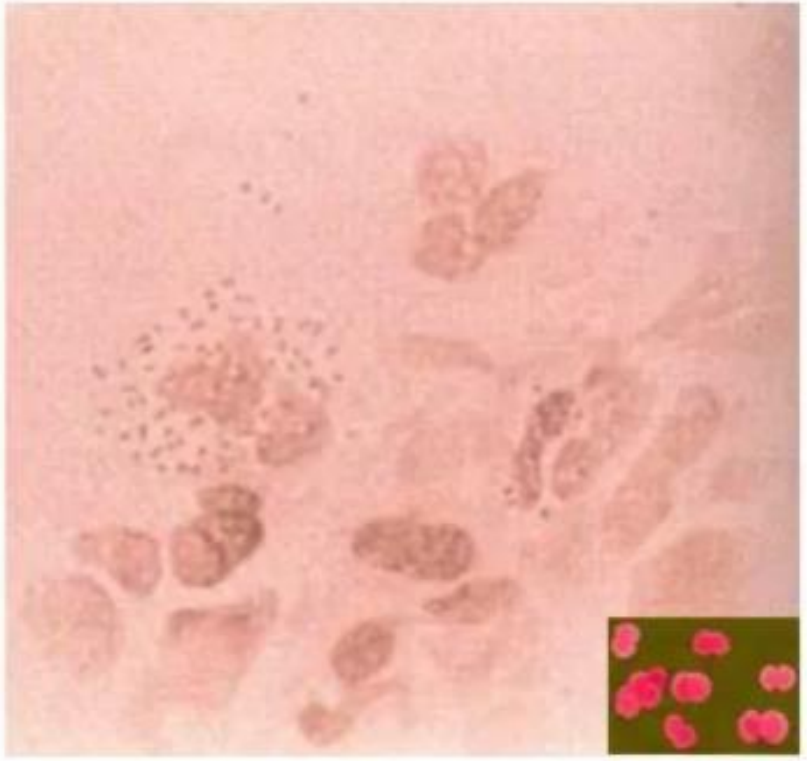
The only **source of infection** is an infected person.



Morfology

Gram-negative cocci (kidney-shaped), usually in pairs.

Human pathogens (i.e. *N. gonorrhoeae* and *N. meningitidis*) are typically found associated with or inside PMN cells.



Gonococcus and **meningococcus** belong to the genus *Neisseria* and **cause diseases** in humans.

Gonococci and meningococci are taxonomically close, these two bacteria show about 90% similarity in their DNA sequences, both have similar virulence factors (pili, outer membrane proteins and lipooligosaccharide). Both gonococci and meningococci cause purulent infections.

While gonococcal infections are usually local and rarely lethal (even in the case of spreading to the bloodstream), meningococcal infection of the bloodstream is a systemic and life-threatening disease.

Important differences between N. gonorrhoeae and N. meningitidis

I have got a **polysaccharide capsule**

N.meningitidis

- Meningococcus
- Respiratory Tract
- Polysaccharide Capsule
- Maltose fermentation +
- No β-lactamase
- Has Vaccines

I have got an **antibiotic resistant plasmid**

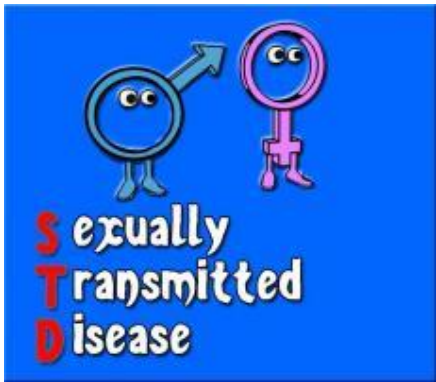
N. gonrrhoeae

- Gonococcus
- Genital Tract
- No Capsule
- Maltose fermentation –
- β-lactamase (Penicillinase)
- No Vaccine

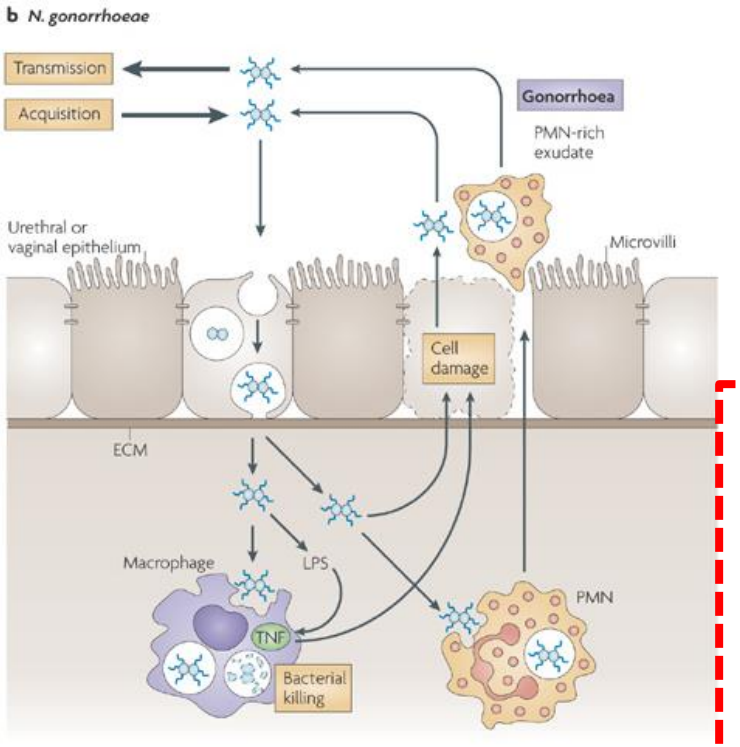
Epidemiological characteristics of gonococcus

- Humans are the only known **reservoir** for gonococci.
- Gonococcus is transmitted by **sexual contact**.
- Both men and women can be gonococcal **carriers** without visible symptoms, although the prevalence of asymptomatic carriage is higher in **women**.

It is important to test and treat all persons who have been in sexual contact with a person with gonorrhea.



Portal of entry....



Gonococci **bind** to the **epithelial cells of the distal part of the urethra** or **cervix** and **multiply**. They possess pili, surface proteins and lipooligosaccharides that all together help gonococci to attach to host cells.

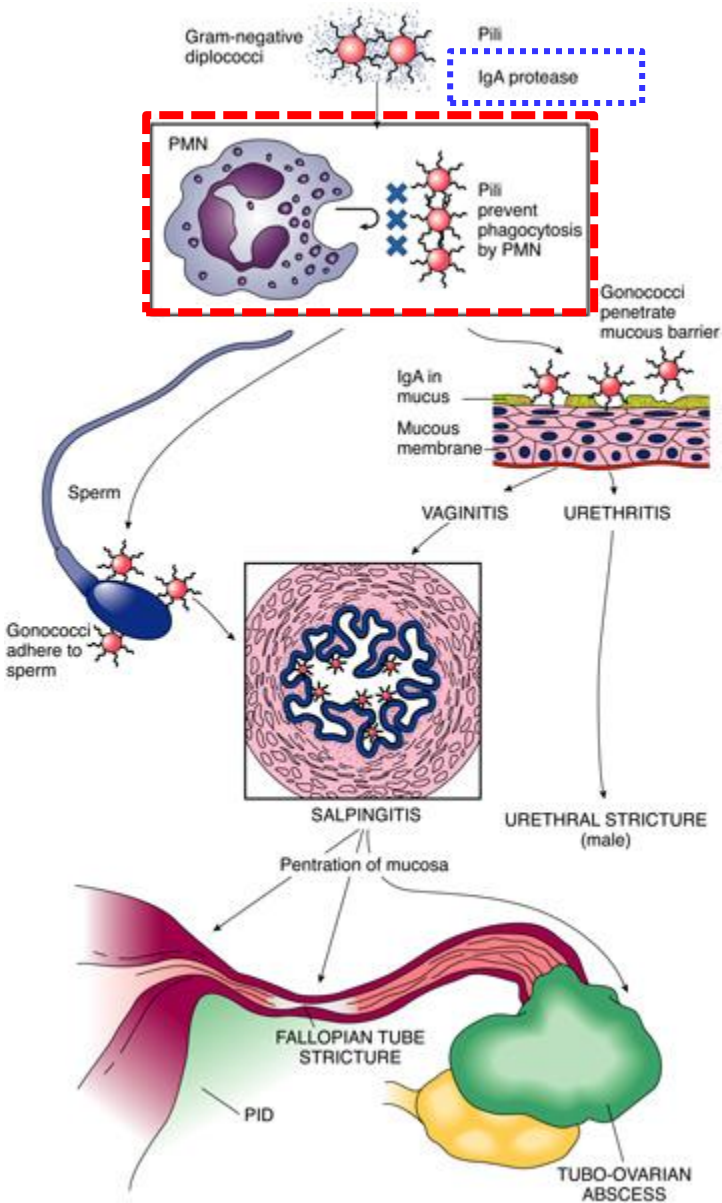
Pili and **surface proteins** are **immunodominant**. But these structures are highly **variable**, which makes them bad targets for the immune system.

The genetic mechanisms of bacteria enable them to express or not express many of the adherence factors (**phase variation**) or to change their surface structures (**antigenic variation**).

Neutrophils cannot bind those gonococci that do not have **Opa proteins** (colony opacity-associated proteins). Gonococci lacking Opa proteins are most often associated with **pelvic inflammatory disease**, **disseminated gonococcal infection** and **arthritis**.

Spread and multiplication....

- After colonization of the mucosa, gonococci multiply rapidly and are **shed in** large numbers into the **genital secretions** of infected men and women.
- **Genital secretions contain IgG, and IgA1 and IgA2 as well.**
- Gonococci produce extracellular **proteases** that specifically **degrade IgA1**. These proteases help gonococci avoid phagocytosis by removing the Fc region of IgA molecules, **reducing gonococcal phagocytosis** that way.



Gonococcal infections: localization and types

Infections of the lower parts of the genital system

- cervicitis
- urethritis (men and women)
- abscess formation in glands near the vagina (eg Bartolini's glands)

Infections of the upper parts of the genital system

- endometritis
- epididymitis
- pelvic inflammatory disease (infection of the fallopian tubes, ovaries or adnexal tissues)

Other localized infections

- rectal gonorrhea (proctitis)
- pharyngitis
- *opthalmia neonatorum* (bilateral conjunctivitis)

Disseminated gonococcal infection

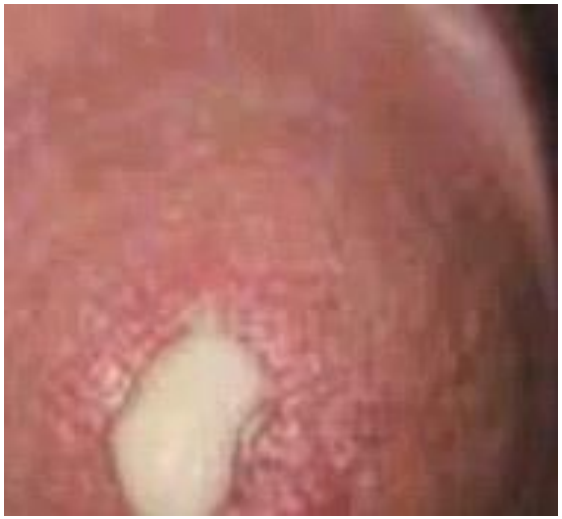
- dermatitis-arthritis-tenosynovitis syndrome: fever, polyarthritis and tenosynodermatitis
- monoarticular septic arthritis (one infected joint)
- rarely, endocarditis (infection of the heart valve) or meningitis (infection of the central nervous system)

Tissue damage ...

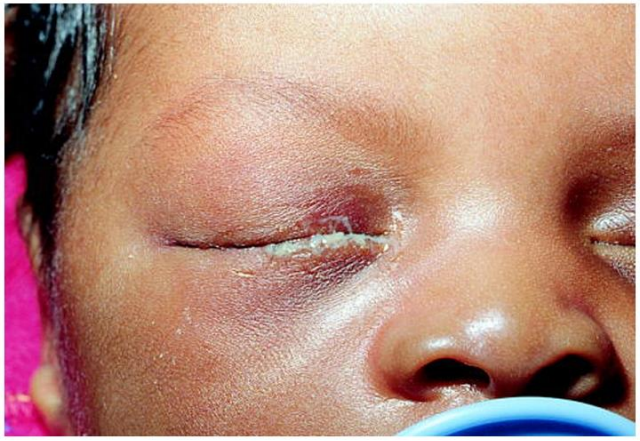
…is most likely caused by **LOS** and other bacterial cell wall components (**peptidoglycan**). Both LOS and peptidoglycan induce **the production of TNF-α** in different host cells. Ciliated and nonciliated cells, infected with gonococci are lysed, releasing tissue factors that mediate further inflammatory processes.

In men, the inflammatory response in the urethra is responsible for local symptoms of gonorrhea, such as pain on urination (**dysuria**) and **urethral discharge of pus**.

In women, **gonococcal cervicitis** is more likely to be **asymptomatic**.



genital gonorrhea ("the clap")



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Ophthalmia neonatorum

The outcome of gonococcal infection

Prompt treatment reduces the risk of disseminated infections and complications.

Local urogenital infections are asymptomatic in about 30% of women and often result in complications. **Chronic inflammation of the fallopian tubes** induces the formation of **scars and strictures**, resulting in long-term sequelae, such as chronic pelvic pain; ectopic pregnancy; recurrent pelvic inflammatory disease caused by chlamydiae, anaerobes and other bacteria; and infertility.

Treatment of gonococcal infection

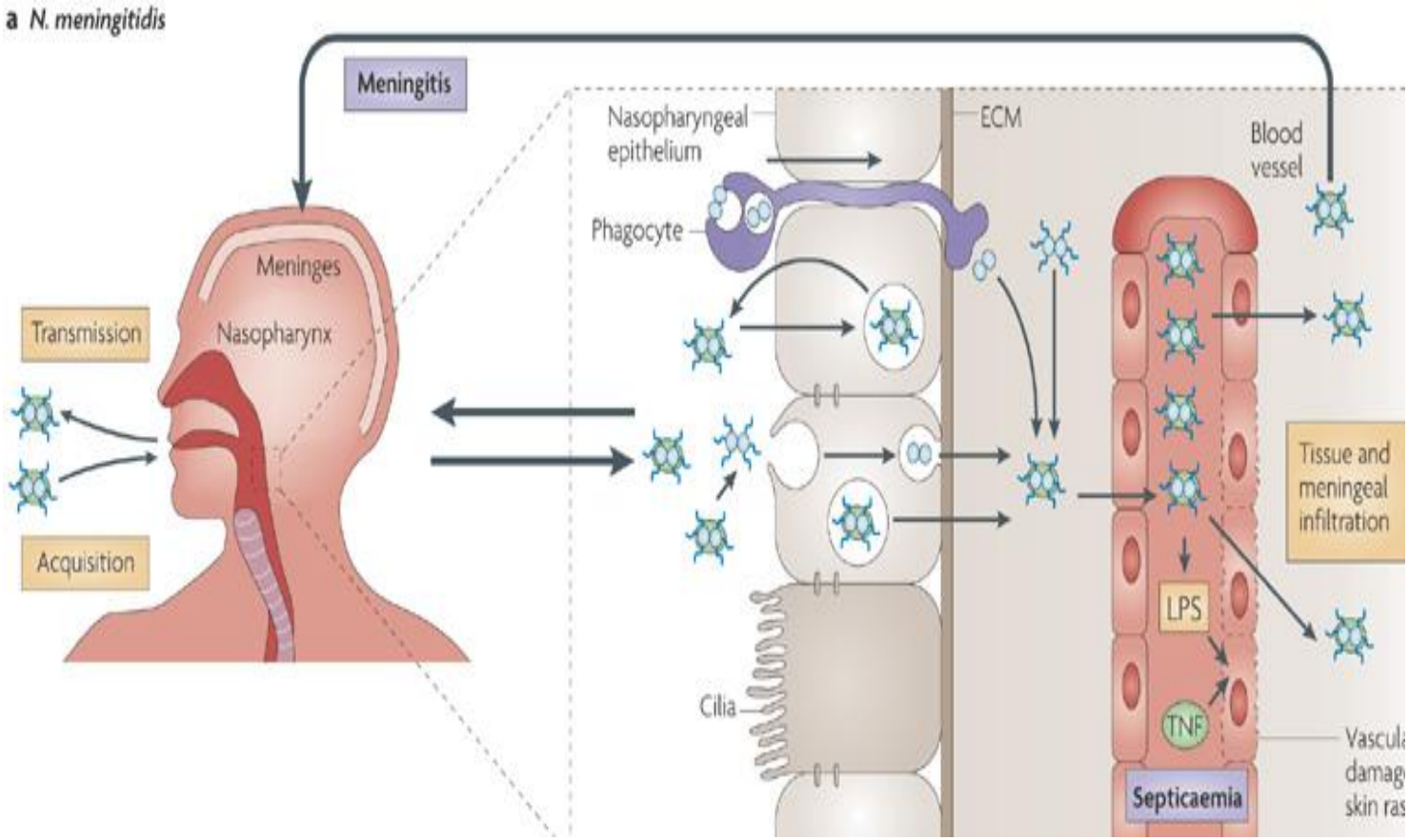
A relatively high percentage of gonococci bear a plasmid encoding **β-lactamase**, an enzyme that degrades penicillin.

β-lactamase resistant antibiotics are used (cephalosporin-cefixime or ceftriaxone).

Due to the isolation of strains resistant to extended-spectrum cephalosporins, there is a concern that gonococcus has achieved the status of a **"superbacterium".**

Individuals infected with *N. gonorrhoeae* are often coinfected with *Chlamydia trachomatis*. Therefore, it is recommended that people who are being treated for gonococcal infection also take azithromycin or doxycycline (drugs to which chlamydia is sensitive).

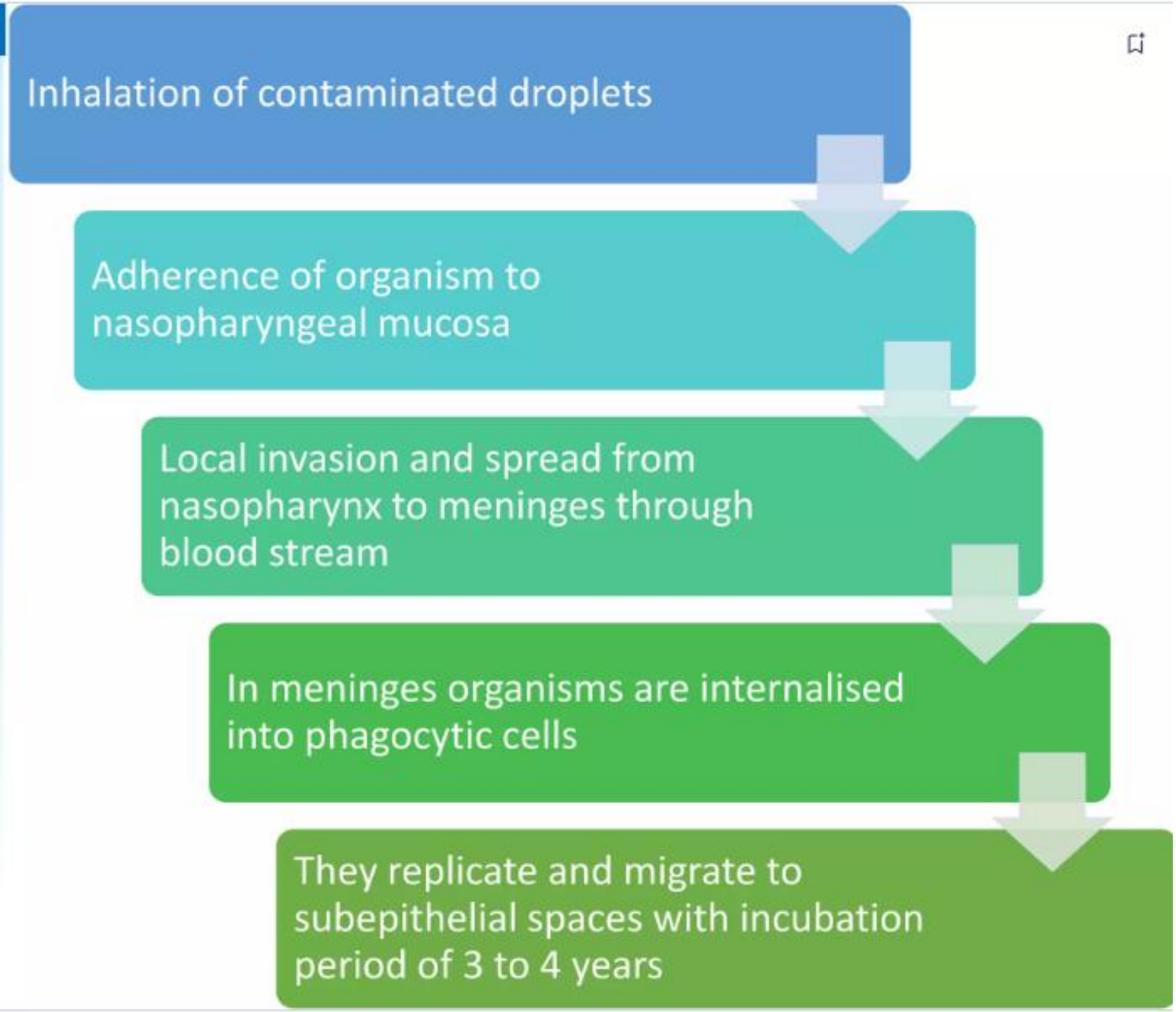
Meningococcus...



The main virulence factor of the meningococcus is the **capsule**. Meningococci colonize the **nasopharynx**, usually without local symptoms or systemic consequences.

If meningococci manage to avoid defense mechanisms in the bloodstream, they multiply rapidly and reach extremely high titers in the blood. The path of meningococcal spread to the meninges – they can spread directly through the lamina cribrosa along the olfactory nerve to the subarachnoid space. More often, meningococci leave the nasopharynx and enter bloodstream and then cross the blood-brain barrier and enter the cerebrospinal fluid.

Pathogenesis



...

Symptoms and signs of circulatory collapse, multiorgan dysfunction, and coagulopathy occur during **meningococcal sepsis**. Increased blood vessel permeability and vasodilation result in capillary permeability syndrome with peripheral edema.

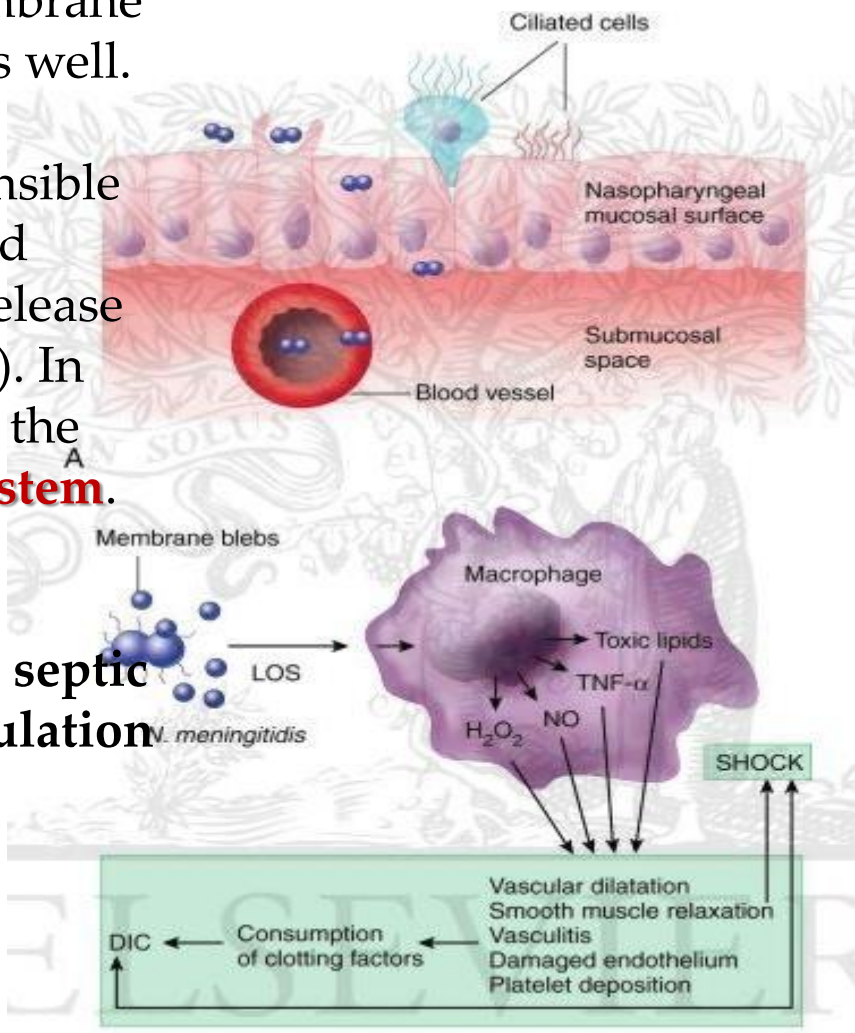
The release of fluid and plasma proteins from blood vessels results in hypovolemia and reduced venous blood flow to the heart, hypotension, and reduced perfusion of vital organs.

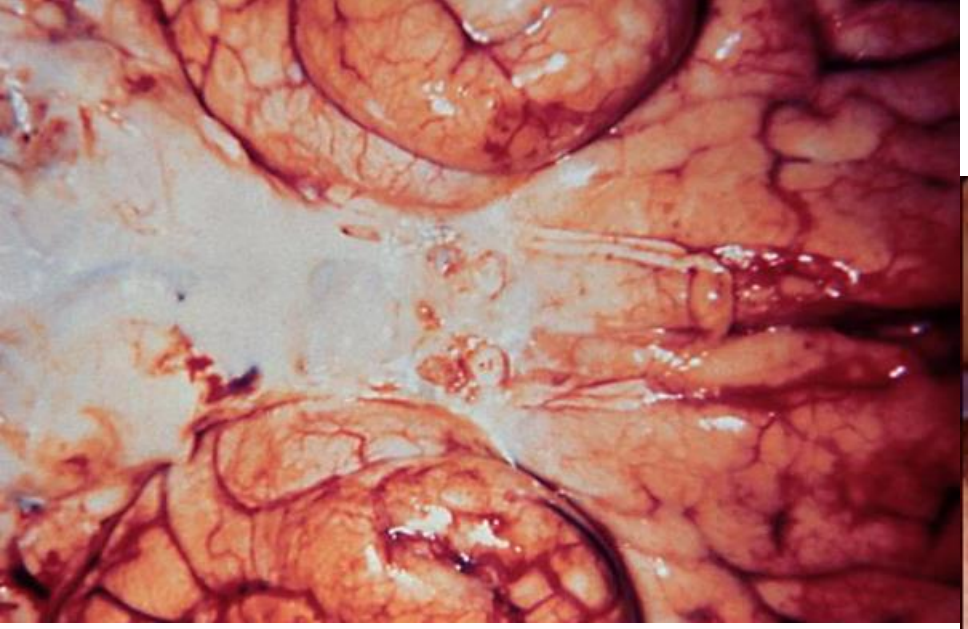
...

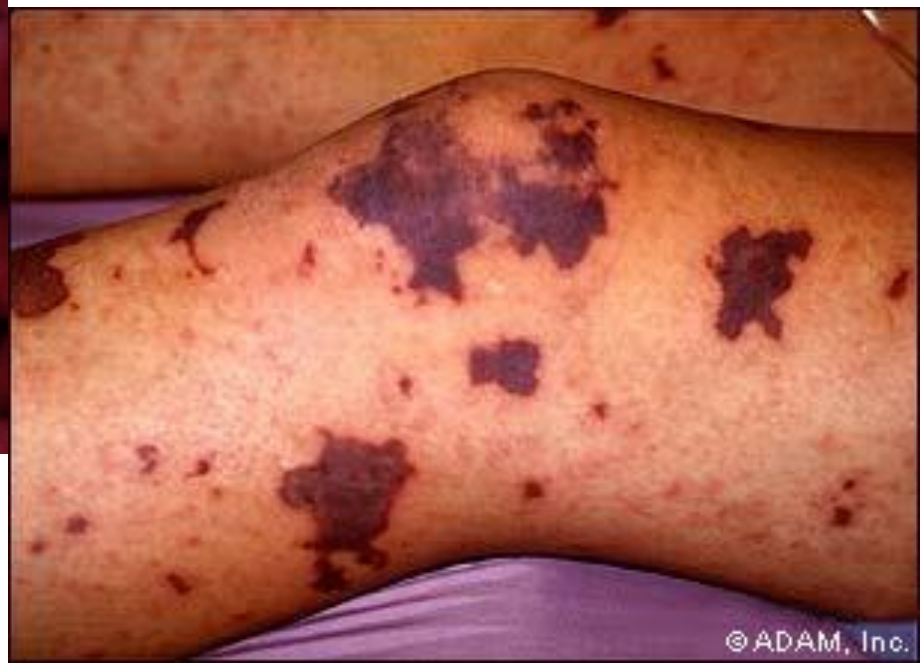
Live meningococci can **actively release** large amounts of **endotoxin** through external membrane vesicles that contain other surface proteins as well.

Meningococcal endotoxin is primarily responsible for septic shock, extensive tissue damage, and multiorgan dysfunction by stimulating the release of inflammatory mediators (**TNF-α** and **IL-1**). In addition, meningococcal endotoxin activates the **coagulation system and the complement system**.

That way, meningococci cause a devastating systemic disease characterized by **fulminant septic shock** and **disseminated intravascular coagulation** (**DIC**) with generalized skin manifestations (petechiae and ecchymoses).







... Meningococci cause a wide range of clinical manifestations: meningococcal sepsis with or without meningitis, bacteremia with or without sepsis, meningococcemia (fulminant purpura and Waterhouse-Friderichsen syndrome), pneumonia, septic arthritis...

Meningitis and sepsis are the most common clinical manifestations of meningococcal infection

Treatment of meningococcal infection

Treatment of meningococcal infection with **intravenous administration of antibiotics** (**ceftriaxone** or **penicillin**).

Meningococcal disease can be effectively prevented by **vaccines** containing capsular polysaccharide. The exception is disease caused by meningococci of serogroup B. The capsule of serogroup B strains is poorly immunogenic, and usually does not induce the production of protective antibodies.

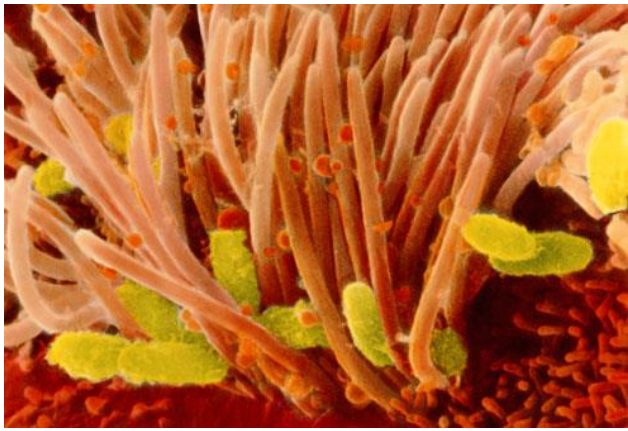
Gram-negative coccobacilli

Bordetella pertussis

Bordetella parapertussis

The causative agents of **whooping cough**

General characteristics of *Bordetellae*



B. pertussis and *B. parapertussis* infect **humans exclusively** and **cannot survive outside the body for long.**

Bordetellae have specific proteins (**adhesins**) on their surface that allow them to **bind to the ciliated epithelial cells** of the respiratory tract. These bacteria also secrete **toxins** that penetrate host cells and eventually cause the signs and symptoms of the disease.

Bordetellae contain **endotoxin** (lipopolysaccharide) which induces the **inflammatory response** and consequently symptoms of the disease, including fever.

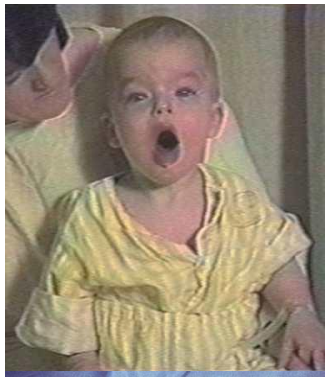
Epidemiological characteristics of *B. pertussis* and *B. parapertussis*

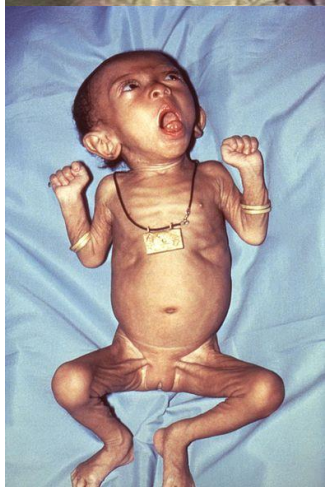
B. pertussis and *B. parapertussis* cause disease exclusively in humans.

Infants and **young children** usually develop **"classic" whooping cough (pertussis).**

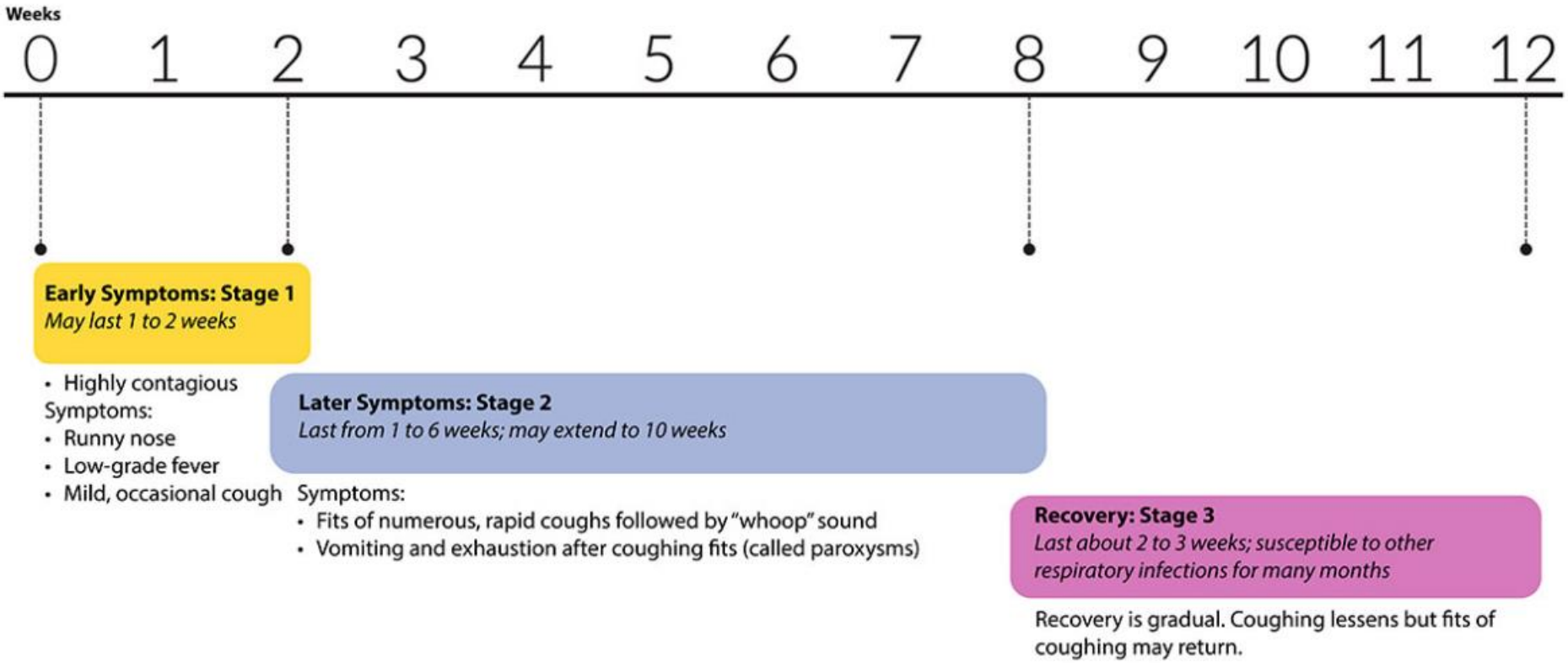
The catarrhal stage resembles the common cold. **The paroxysmal stage** is characterized by paroxysmal **coughing spasms**. Paroxysms are characterized by 10 or more forceful coughs during a single expiration, followed by a massive inspiratory effort against a closed epiglottis, which results in a classic “whoop".

During **the convalescent stage** the frequency and intensity of paroxysms decrease.





Whooping Cough Disease Progression



cdc.gov/whoopingcough

U.S. Department of Health and Human Services
Centers for Disease Control and Prevention

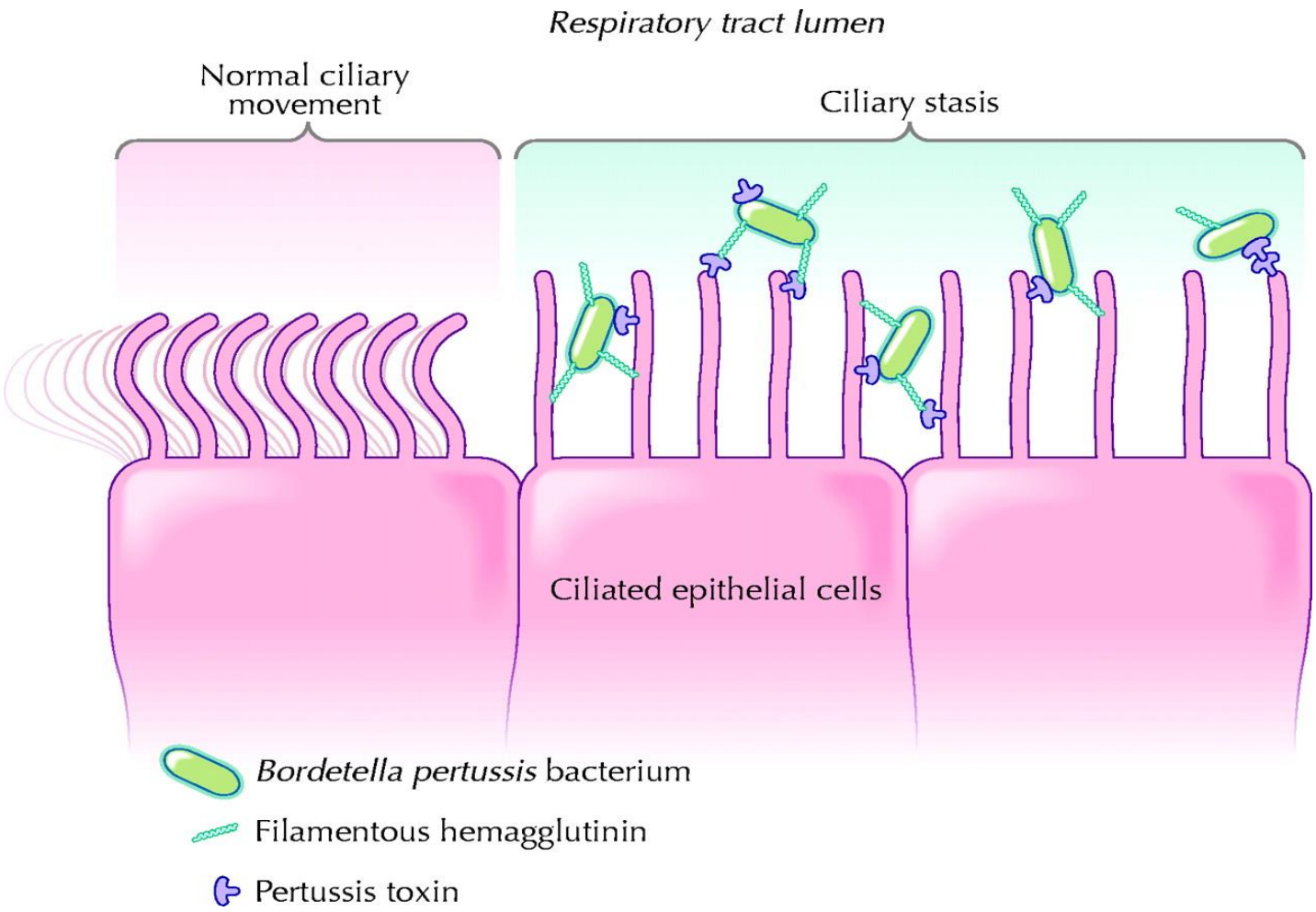
... Older adolescents, adults, younger children who are incompletely immunized, newborns and infants who are partially protected by maternal antibodies usually develop a **mild illness** or **asymptomatic infection** ...

... Neither immunization nor previous infection provides lifelong immunity. It is important to warn all family members of sick children that they may get sick or already have whooping cough.

B. pertussis and *B. parapertussis* portal of entry

B. pertussis and *B. parapertussis* usually enter the **trachea** and **bronchi** by **inhalation**. Bacteria bind specifically to the **cilia of epithelial cells in the airways**.

Whooping cough is a **superficial infection** in which bacteria remain on the mucosal surface and do not invade tissues, but **secrete toxins** that have dramatic effects on host cells and are responsible for most manifestations of the disease.



The surface proteins of *B. pertussis* and *B. parapertussis* function as **adhesins**: **fimbriae** (Fim), **filamentous hemagglutinin** (FHA), and **pertactin** (Prn).

Proteins of the integrin family on host cells serve as receptors to which these bacterial proteins bind.

Spread and multiplication of *B. pertussis* и *B. parapertussis*

During the first few weeks of infection, Bordetella multiplies dramatically and can **spread quickly** from the nasopharynx to the trachea, bronchi and bronchioles.

Masses of bacteria are trapped in the cilia and thick mucus.

Bacteria contribute to the formation of biofilm by secretion of **exopolysaccharide matrix**.

The submucosa beneath the bacteria **becomes more and more inflamed,** and the **peribronchial lymph nodes enlarge.**

Local manifestations of whooping cough are tracheitis and bronchitis, with accumulation of mucus, inflammatory cells, bacteria and dead epithelial cells in the airway lumen.

B. pertussis and *B. parapertussis* produce a wide range of virulence factors responsible for tissue damage

Major toxins and virulence factors of *B. pertussis* and *B. Parapertussis*

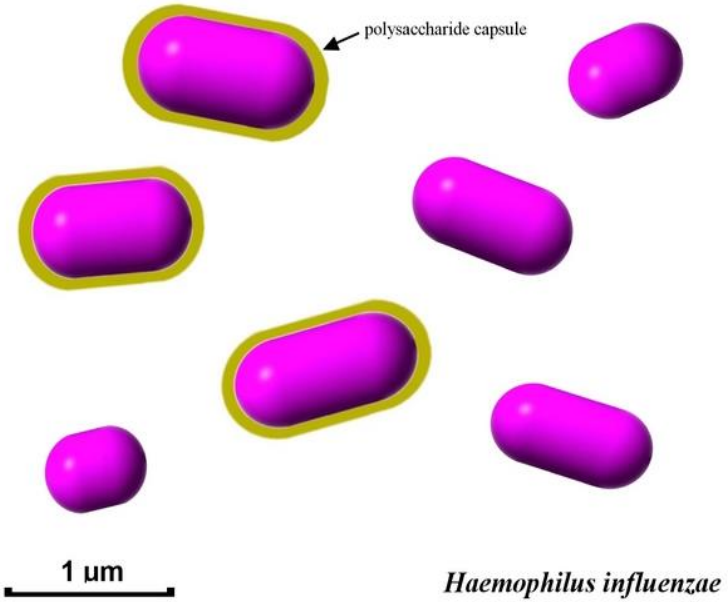
Name	Chemical nature	Site of action	Biochemical activity	Physiological effects
Pertussis toxin	Protein	Local and systemic	ADP-ribolization of G protein	Impairs neutrophil chemotaxis, phagocytosis, and bactericidal activity; lymphocytosis
Adenylate cyclase toxin	Protein	Local	Converts ATP to cAMP Membrane insertion	Increases capillary permeability leading to edema; Hemolytic activity
Tracheal cytotoxin	Murein	Local	Stimulates nitric oxide production	Cytopathological damage to tracheal epithelium; Kills ciliated epithelial cells; Adjuvant

Treatment and prevention of whooping cough

Macrolides and **tetracyclines** are effective against B. pertussis (most effective if given as early as possible in the course of disease).

The Di-Te-Per vaccine contains a suspension of whole killed B. pertussis cells along with diphtheria and tetanus toxoids.

The acellular vaccine contains inactivated toxins and adhesins.



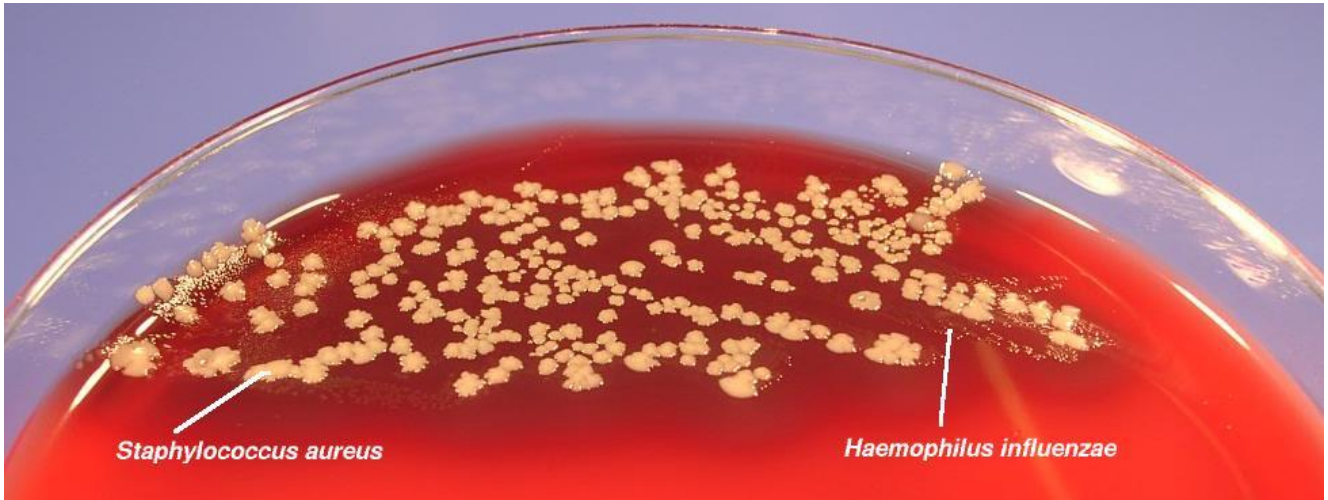
Haemophilic *Gram*-negative bacillus

Haemophilus influenzae

Haemophilus- comes from the Greek words "***haema***" – **blood** and "***philos***" – **loving**

- The cultivation of bacteria of the genus Haemophilus requires a nutrient medium that contains blood or blood products.
- Factors required for their growth, **factor X (hematin)** and **factor V (NAD),** are present in erythrocytes.

satellitism phenomenon



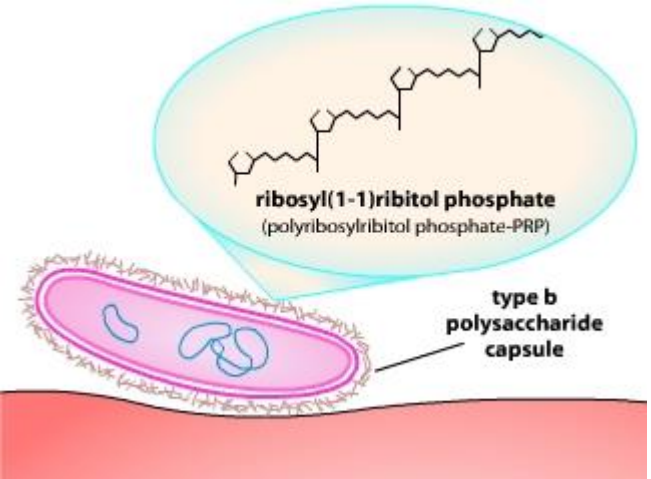
General characteristics of *H. influenzae*

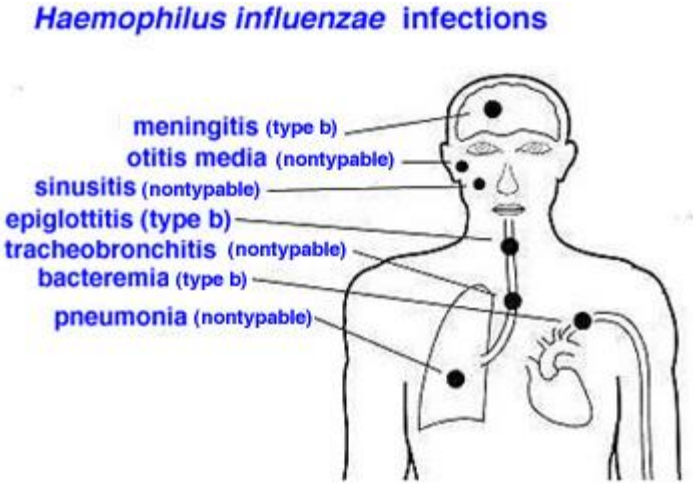
H. influenzae may or may not be **encapsulated**.

The *H. influenzae* type b capsule contains a polymer of ribose, ribitol and phosphate called **PRP** (PolyRibitol Phosphate).

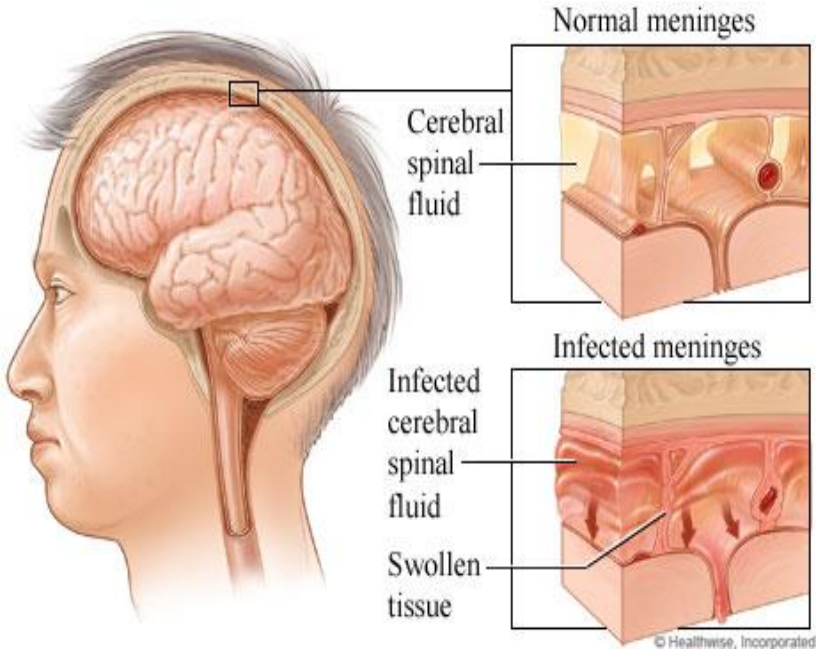
H. influenzae type b causes acute infections of the **central nervous system, epiglottitis**, as well as soft tissue infections, especially in **children**.

Nonencapsulated strains usually cause infections of the **bronchi**, **sinuses** and **middle ear**.





Epidemiological characteristics of *H. influenzae*

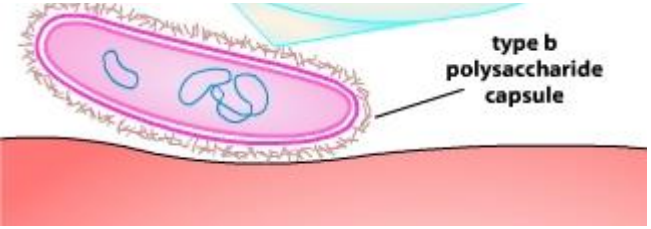


H. influenzae is present as the part of normal flora of the **nasopharynx** in 20-80% of healthy individuals.

Infection caused by this bacterium is transmitted by **respiratory droplets**.

Meningitis is the most common form of invasive disease (in the first 2 years of life). **Epiglottitis** and **pneumonia** show the highest peak of incidence from 2 to 5 years of age. More than 90% of these cases are caused by ***H. influenzae* type b.**

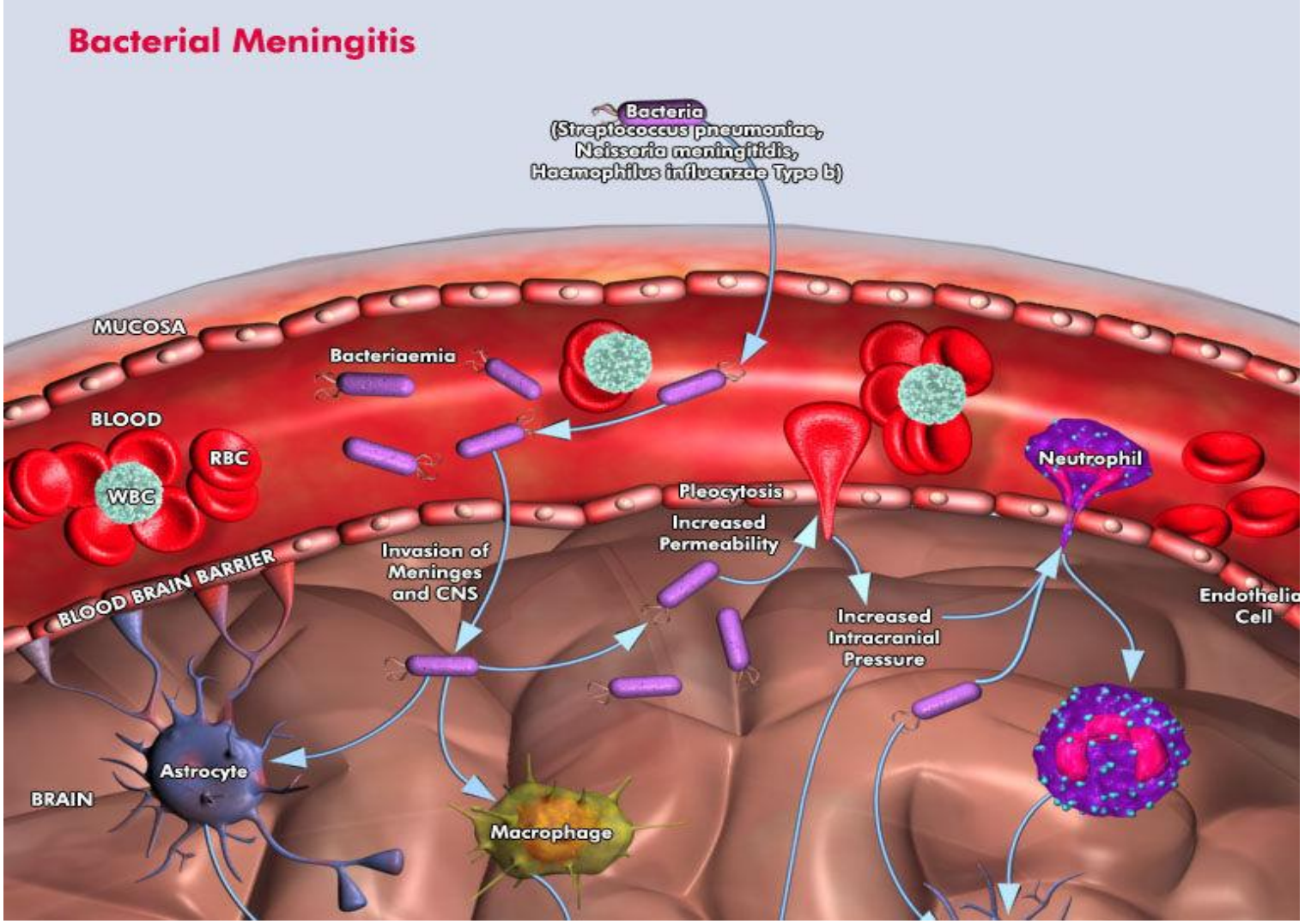
Invasive disease pathogenesis



H. influenzae strains that are part of the normal nasopharyngeal flora occasionally **invade deeper tissues**.

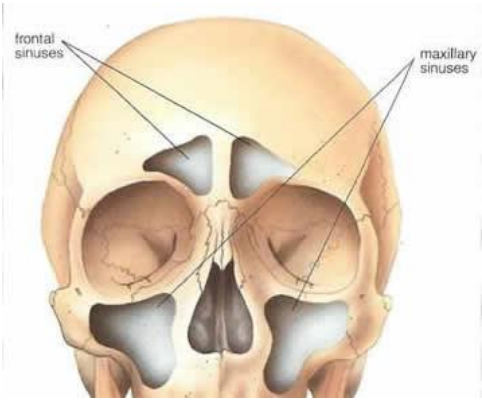
Binding to respiratory epithelial cells is mediated by **pili**.

Bacteremia allows the spread of *H. influenzae* to the **central nervous system** and the development of **metastatic infections** (bones and joints). Systemic spread is typical for **encapsulated *H. influenzae* strains**, more than 90% of invasive strains are **type b**.



Once bacteria pass through the mucosal barrier, the *H. influenzae* **capsule** protects them from opsonization and phagocytosis in the same way as other encapsulated bacteria (*S. pneumoniae* and *N. meningitidis*). Immunity to *H. influenzae* type b infection is mediated by **anticapsular (PRP) antibodies**. **The peak incidence of** *H. influenzae* type b **infection** is **between 6 and 18 months of age** when serum levels of these antibodies are the lowest.

Localized disease pathogenesis



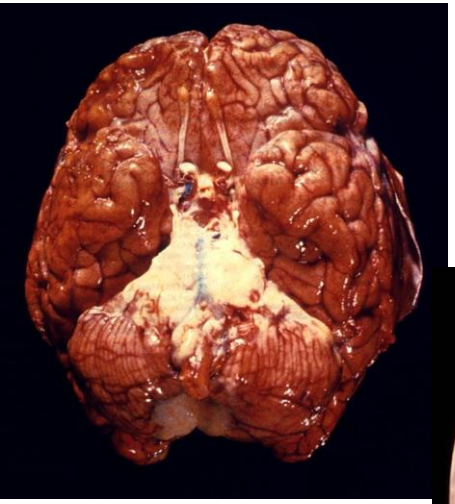
Nonencapsulated *H. influenzae* strains cause disease under certain conditions when, for example, they reach the middle ear, sinuses or bronchi from the upper respiratory tract. This occurs when the elimination of *H. influenzae* is impaired due to a viral infection or structural damage.

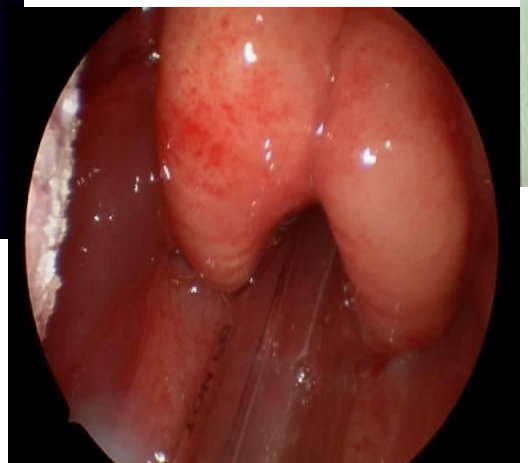
They usually cause **localized diseases**.

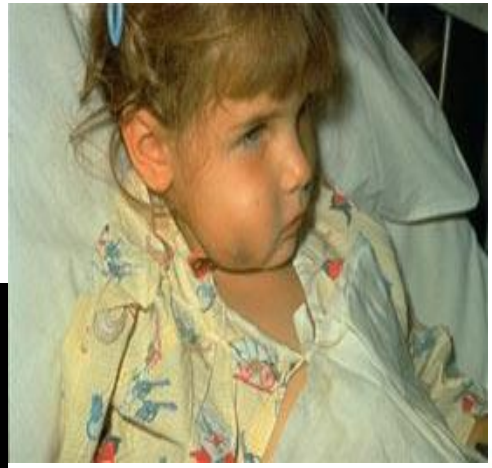
Nonencapsulated *H. influenzae* strains express high-molecular-weight **surface proteins** (**HMW1 and HMW2**) that mediate bacterial binding to epithelial cells.

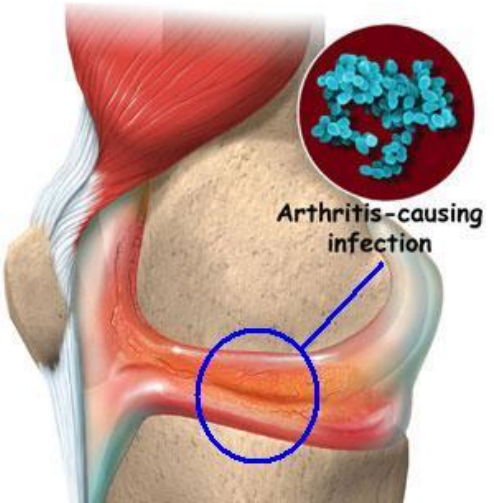
Diseases caused by *H. influenzae*

- **Meningitis** caused by *H. influenzae* type b is an acute purulent disease.
- **Acute epiglottitis** is a severe infection during which inflammation of the epiglottis and surrounding tissue develops resulting in airway obstruction (*H. influenzae* type b)
- **Cellulitis** caused by *H. influenzae* type b.
- **Arthritis** usually affecting one large joint.









H. influenzae is an important causative agent of **conjunctivitis**, **otitis media**, **sinusitis**. This bacterium also causes **exacerbation of chronic bronchitis** ....

... Most of these diseases are caused by **nonencapsulated strains** and usually remain **localized** **without bacteremia**.

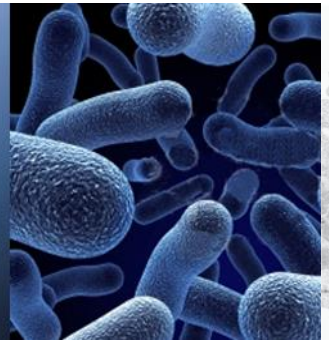
Pneumonia is caused by both **encapsulated** and **nonencapsulated strains** of *H. influenzae*.

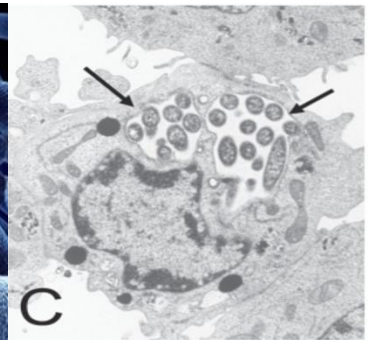
Treatment and prevention of diseases caused by *H. influenzae*

- *H. influenzae* is sensitive to ampicillin, cephalosporins, tetracyclines, aminoglycosides and sulfonamides.
- It is sensitive to penicillin and erythromycin to a lesser extent.
- A vaccine that contains purified PRP
- The conjugated vaccine contains PRP conjugated to proteins derived from *Corynebacterium diphteriae* or *N. meningitidis.*

Legionella spp.

Parasite of Amoebae and macrophages





Epidemiological characteristics of *L. pneumophila*

- ***The Legionellae*** are a diverse genus of **aerobic *Gram*-negative bacilli**.
- They parasitize **amoebae** (in water and soil), and **occasionally colonize** air conditioners and water supply systems.

Legionella is particularly adapted to hot water supply systems in buildings. The bacterium grows at a **temperatures up to 46 ° C**, and tolerates much higher temperatures. *Legionella* is **relatively resistant to chlorine**. It also forms **bacterial biofilm** that is resistant to biocides.

People become infected by **inhalation**. *L. pneumophila* is an opportunistic pathogen of **human macrophages** ...

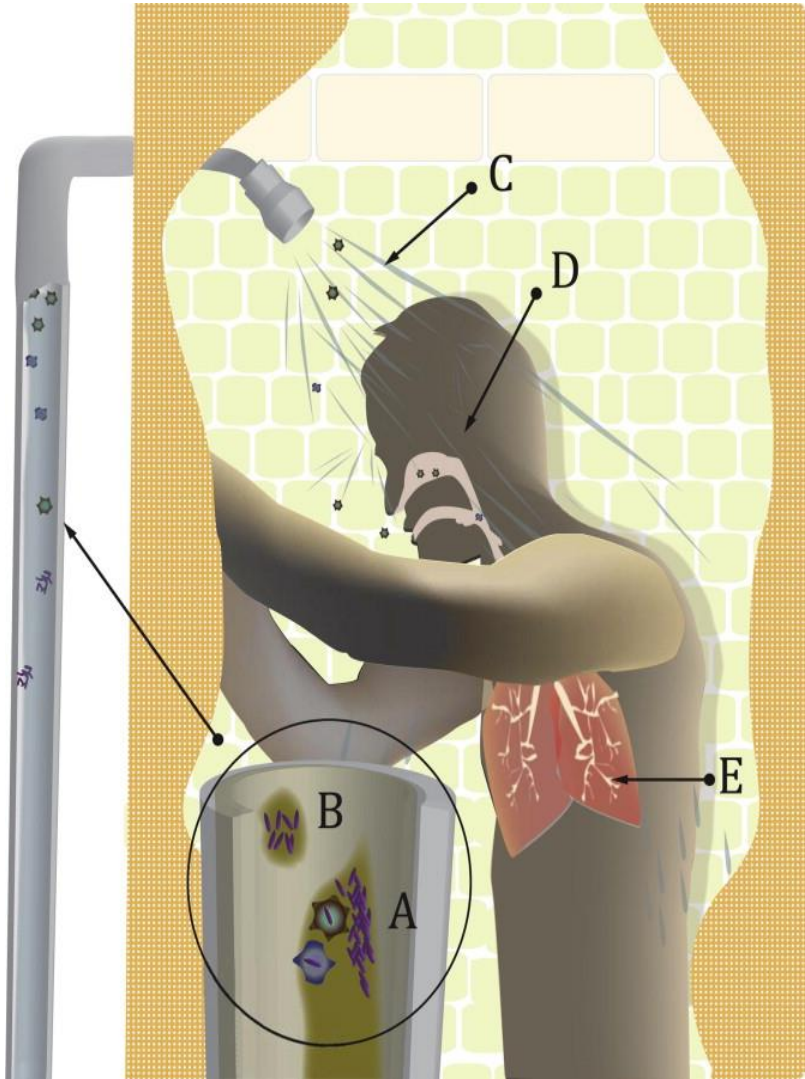
... People with immunodeficiency can develop a more **severe form of pneumonia** called **Legionnaires disease** ...

... Numerous outbreaks of Legionnaires disease in hospitals or hotels, and other large buildings are associated with **contamination of water supply systems** by *L. pneumophila*.

Legionnaires disease is a **primary pulmonary infection**

The pathogen is **never transmitted from person to person**, but people become infected from an **environmental source**, usually in contact with water supply systems that are colonized with this microorganism.

Showers, fountains, inhalation equipment, central air conditioning systems release **infectious aerosols**.

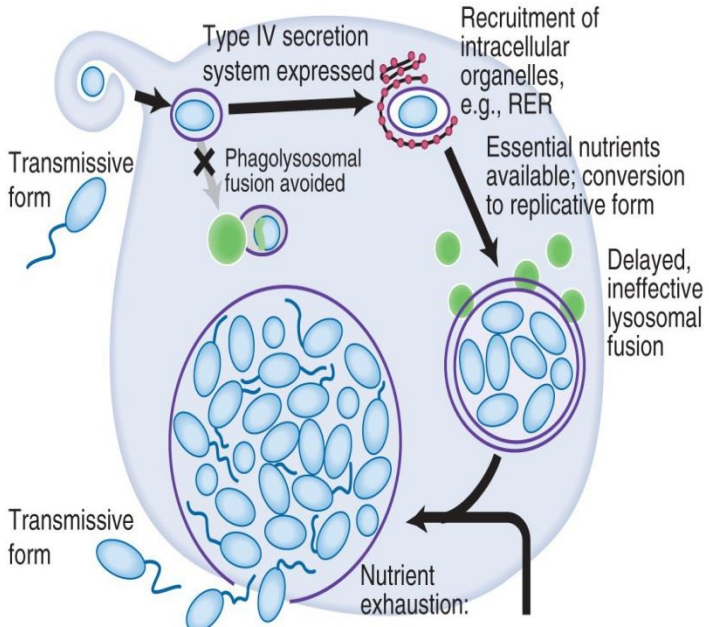


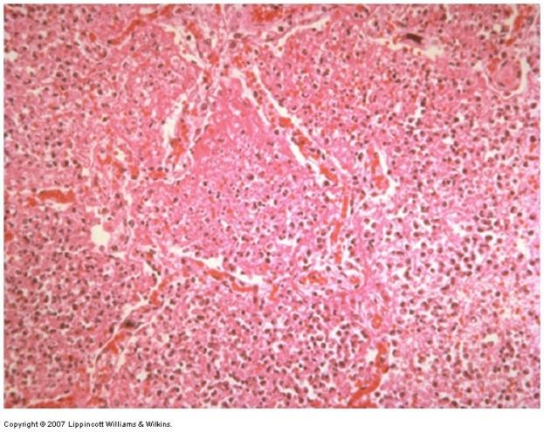
Intramacrophage survival

In the air spaces of the lungs, *Legionellae* are ingested by **resident alveolar macrophages**. **Phagocytes fail to kill** *L. pneumophila* in the lungs.

Phagosome containing *L. pneumophila* fuses with the **endoplasmic reticulum**. If nutrients are available within this compartment, *L. pneumophila* differentiates into a **replicative form**. When the amount of nutrients is reduced, the bacteria stop replicating and turn into a **transmissible (infectious) form**. *L. pneumophila* expresses factors that lyse the eukaryotic membrane and release the bacterial progeny from the infected host cell.

The infectious form is **resistant to osmotic shock**, which enables bacteria to survive extracellularly. Factors that **block the fusion of phagosomes with lysosomes** after ingestion by the next host cell are crucial for transmission.

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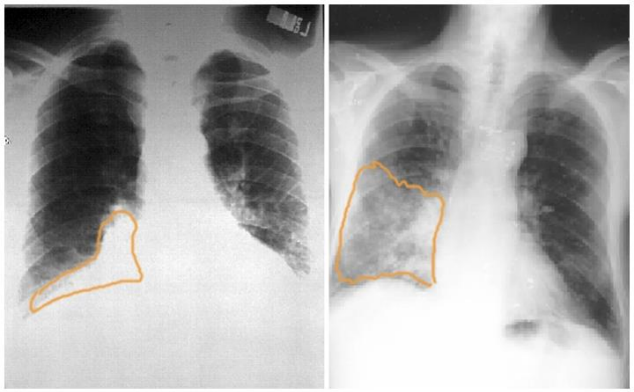
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Tissue damage

Macrophages infected with *L. pneumophila* **release cytokines** that increase the influx of monocytes and neutrophils from the blood into the air spaces of the lungs.

Nodular areas of infection enlarge and usually evolve into microapscesses that then coalesce to form cavites.

Most local damage is due to a strong inflammatory response in the presence of infection.

Treatment and prevention

- Drugs that can penetrate into infected cells, in which bacteria grow, such as **macrolides / ketolides, fluoroquinolones and tetracyclines.**

- Penicillin or cephalosporins penetrate poorly into the eukaryotic cell.

- Prevention of Legionnaires disease includes **monitoring** water systems and clinical surveillance for legionellosis cases.