13

Microbiology

Doctor 2017 | Medicine | JU

Sheet Slides

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DOCTOR

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Streptococci

They are a diverse collection of **gram-positive cocci** typically arranged in **pairs** or **chains** *(in contrast to the **clusters** formed by **Staphylococcus**).* Also, they are **catalase negative** *(while **Staphylococcus** is **catalase positive**).*

Classification:

The classification of more than 100 species within the genus Streptococcus is complicated because **three** different overlapping schemes are used:

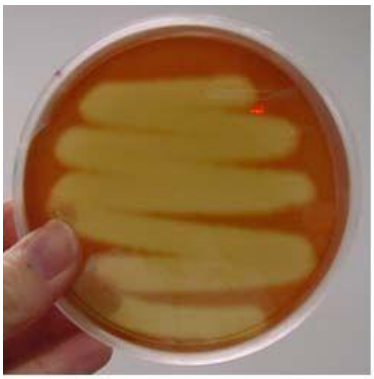
1) **Hemolytic patterns on a blood agar plate**.

   Streptococci are able to hemolyze red blood cells in **varying** degrees:

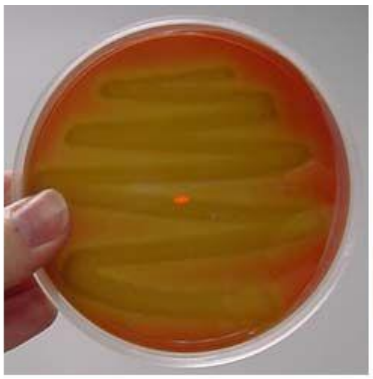
   a- **Complete hemolysis** of erythrocytes with clearing of the blood around the bacterial growth → **β-hemolysis.**

   b- **Incomplete hemolysis** of erythrocytes with reduction of hemoglobin and the formation of **green** pigment → **α-hemolysis.**

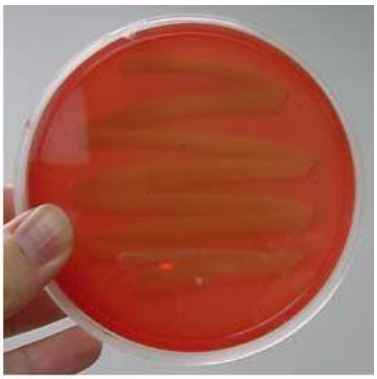
   c- **No hemolysis occurred** → **γ-hemolysis**



β-hemolysis



α-hemolysis



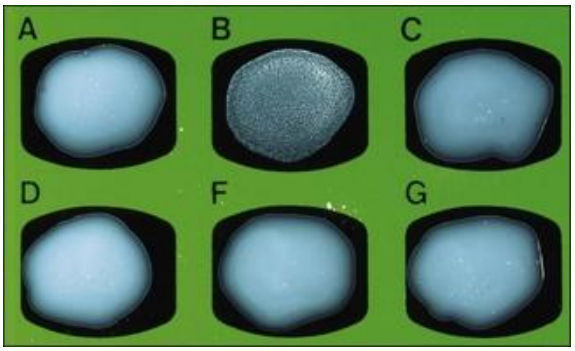
γ-hemolysis

2) **Serologic Specificity**

   In **Lancefield Grouping**, Streptococci are classified depending on **carbohydrates** attached to their **cell wall** into groups from A-W.

   Other bacteria that do **not** apply to Lancefield Grouping such as **S. pneumoniae,** are classified according to their **capsular antigens**. **Antibodies** are used to detect and identify these **antigens**.

In the picture to the right, antibodies that are specific to certain group antigens were added to a sample of streptococcus in different slots. Since antibody-B indicated a reaction where **agglutination** (clumping) showed, this means that the bacteria had **group B antigen → Group B Streptococcus.**

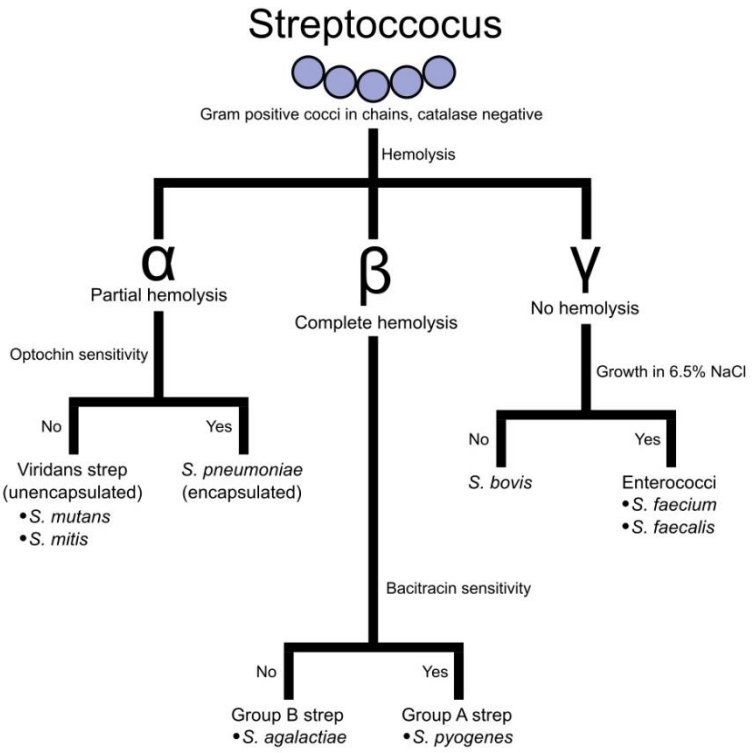


3) Biochemical (physiologic) properties

Biochemical tests include tests for the **presence of enzymes**, tests for the **resistance to certain chemical agents / antibiotics** and tests of **growth** in certain mediums *(e.g. in a 6.5% NaCl).*

Biochemical tests are most often used to classify streptococci **after** the colony growth and hemolytic characteristics have been observed. Biochemical tests also are used for species that typically **cannot** be classified according to **Lancefield Grouping** *(e.g. Streptococcus pneumonia).*

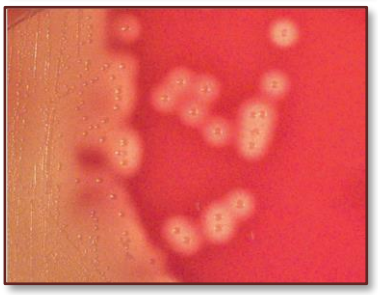
Observe the following plot of **Streptococcus classification**:



Note: *Each Streptococci specie mentioned in the plot will be **discussed** in this sheet, so yes **memorize** it.*

We will now discuss the streptococci species that are of medical interest until the end of this sheet:

1) Streptococcus Pyogenes



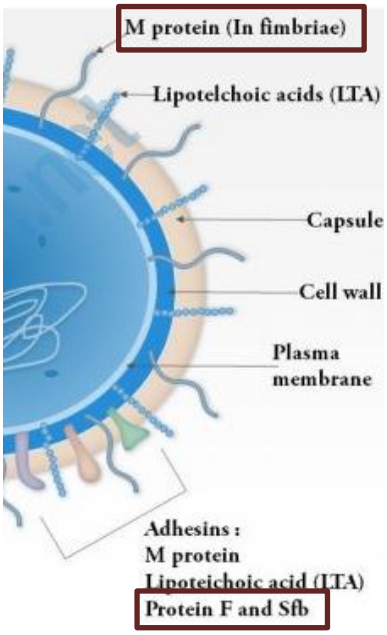
- They are **spherical** cocci, **1-2 µm in diameter**, arranged in **chains**.
- They contain the Lancefield group **A antigen**, and are considered **Group A Streptococcus** (GAS).
- They are **sensitive** to **Bacitracin** (antibiotic).
- After 24hrs of incubation, they cause large zones of **β-hemolysis** around colonies of 1-2mm.

<u>They have multiple mechanisms and features to avoid opsonization and phagocytosis:</u>

a- Hyaluronic Acid Capsule

It is a **poor immunogen** *(immunogen is a substance that is able to elicit an immune response)* thus **minimizing** the **recognition** of phagocytic cells **disrupting phagocytosis**.

b- M Protein



It is a filamentous structure anchored to the cell membrane that penetrates and projects from the streptococcal cell wall. It **blocks the binding of C3b** (opsonin) hence **disrupting phagocytotic**. It also **facilitates adherence** and **mediate invasion** to host cells with the help of **F Protein** *(which is also attached to the cell membrane).*

> ***<u>Note:</u> Opsonization** is a process of **enhancing phagocytosis** by using **Opsonins** (C3b) which coats substances making them **easily** recognized by **phagocytic** cells. Recall pathology.*

c- C5a peptidase

S. pyogenes have **C5a peptidase** on their **surface** which **inactivates C5a**.

> ***<u>Note:</u>** C5a is a **chemoattractant** which attracts **phagocytic cells**.*

Many Toxins & Enzymes are produced by S. pyogenes, including the following:

a- Pyrogenic Exotoxins (Spe, also called Erythrogenic Toxin)

It is secreted by **lysogenic** S. pyogenes *(which were lysogenized by having their chromosome **integrated** with a section of a **phage's chromosome**).*

These toxins act as **superantigens** which are antigens that cause **exaggerated immune response**.

b- Streptolysins S

It is **stable** in the presence of oxygen (**oxygen-stable** haemolysin), does not trigger immune responses *(Non-immunogenic)* and a cell-bound exotoxin that can **lyse erythrocytes**, **leukocytes**, and **platelets**.

It is the agent **responsible** for the **β-hemolysis zones** around streptococcal colonies growing on the surface of blood agar plates.

c- Streptolysin O

It is **not stable** *(altered)* in the presence of oxygen (**oxygen-labile** haemolysin), capable of **lysing erythrocytes**, **leukocytes**, **platelets**, and **cultured cells** *(cells grown under controlled conditions).*

Anti-Streptolysin O, is an antibody that appears in the human body after infection with any streptococci that **produce** streptolysin O. This antibody **prevents** haemolysis by streptolysin O.

When a serum sample has an **abnormal ASO concentration**, this indicates that the patient has had a recent **Group A streptococcal infection** *(e.g. S. Pyogenes infection).*

> ***Note:*** *Streptolysin O is **irreversibly inhibited** by **cholesterol** in **skin lipids**, therefore patients with **cutaneous infections** do not develop **ASO antibodies**.*

d- Streptokinase (Fibrinolysin)

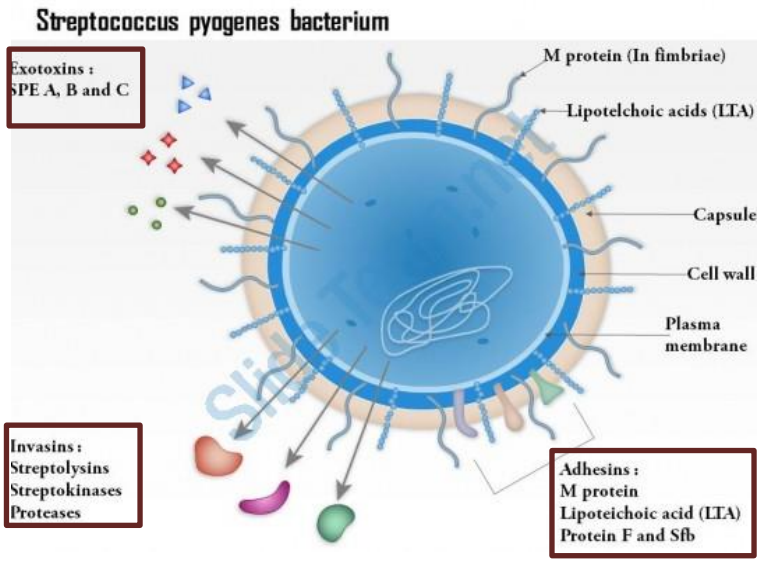
This enzyme **lyses clots** and **fibrin deposits**. Thus, **facilitating** the **rapid spread** of S. pyogenes in the infected tissue.

> ***Note:*** ***Streptokinase** has been used as a treatment to **remove blood clots** that may cause problems.*

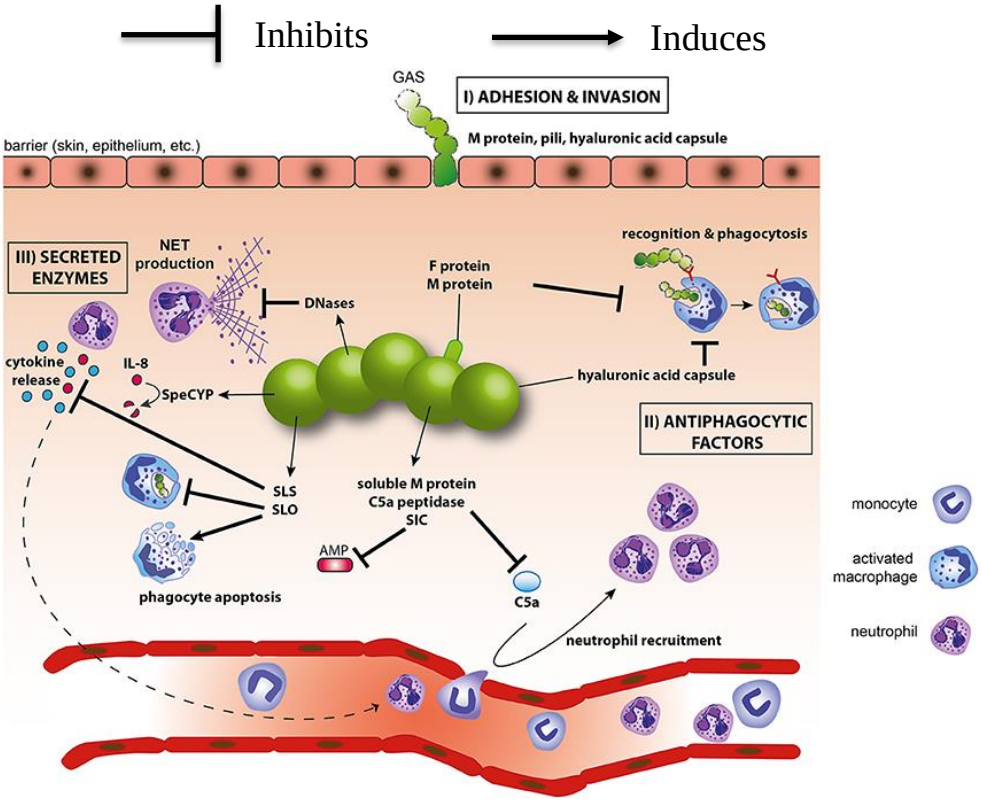
e- Deoxyribonucleases (DNases)

Streptococcal DNases *(A, B, C, and D)* **degrade** *(depolymerise)* the DNA released by **neutrophils**, and similar to streptokinase they **facilitate** the **spread** of streptococci in tissue.

> ***Extra Explanation:*** *Recall pathology, we took that **PMNs** undergo **NETosis** where they dispose of their DNA forming **NETs** which traps bacteria, also **histones** with the DNA can be **antibacterial**. Here, DNases **degrade** these traps.*



The following graph summarizes all the substances Group A Streptococci (GAS) produces, others, and their roles:



Streptococcus Pyogenes Epidemiology

Epidemiology is the study of the determinants, occurrence, and distribution.

- The Centers for Disease Control and Prevention (CDC) has estimated that at least **10 million cases** of non-invasive disease *(diseases that usually **do not spread** to or damage other organs and tissues)* occur annually, with **pharyngitis** *(swelling in the back of the throat (pharynx))* and **pyoderma** *(skin infection with formation of pus)* as the **most common** infections caused by **GAS**.
- Group A streptococci mostly colonize in the **oropharynx** of **children and young adults without** causing a clinical disease because the body produces specific antibodies against them.
- However, S. pyogenes disease is caused by recently learnt **types** that can establish an **infection of the pharynx** or **skin before** specific antibodies are produced or competitive organisms **are able to proliferate**. Also, S. pyogenes **can** cause **infections** depending on the **virulence factors** acquired by the **bacteria** and **the host's immune system state**.
  *(Patients with **impaired** immune system are **more** vulnerable to infections even from **normal** resident bacteria)*
- The pathogen is **spread** from person to person **through respiratory droplets** *(sneezing or coughing)*. **Crowding**, such as in **classrooms** and **day-care facilities**, increases the opportunity for the organism to spread too, particularly during the **winter** months.

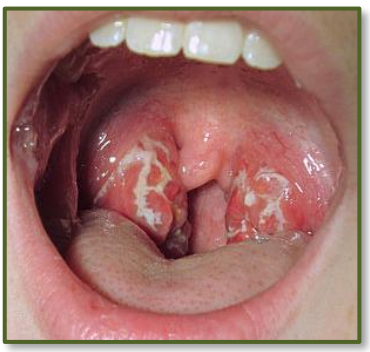
Streptococcus Pyogenes Clinical Correlation

A variety of distinct disease processes are associated with S pyogenes infections, which can be:

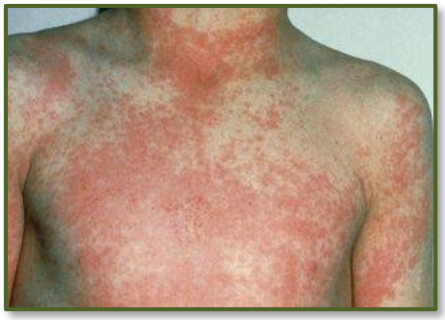
1) Suppurative infections *(involves pus formation)*, such as:

a- Pharyngitis

Redness and **swelling** of **tonsils** and **pharynx**, with **exudate** *(pus)* generally present. Also, **cervical lymphadenopathy** can be prominent *(enlargement of cervical lymph nodes)*.



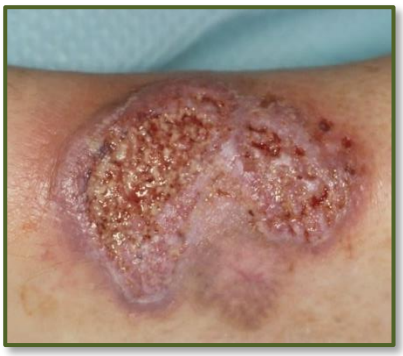
b- Scarlet Fever



It is a complication of streptococcal pharyngitis that occurs when the infecting strain is **lysogenized** by a bacteriophage that mediates production of a **pyrogenic exotoxin** *(SPE, as mentioned before).*

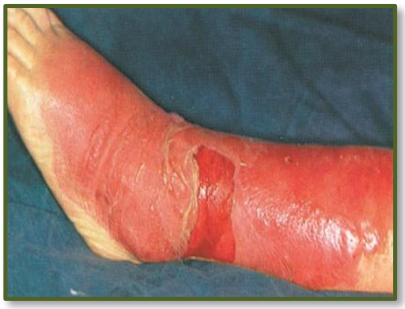
An **erythematous** *(red)* rash initially appears on the upper chest and then **spreads** to the extremities *(limbs)* **The face**, is usually flushed, most prominently in the cheeks, with a ring of paleness **around the mouth**.

c- Pyoderma (Impetigo)



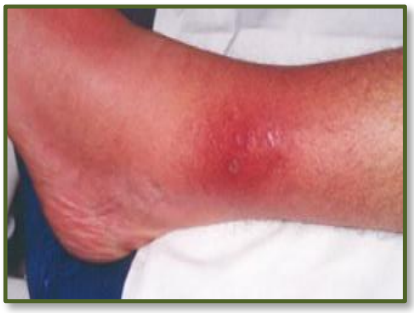
It is **not** a systemic disease *(affecting the entire body)*, it is a **localized** skin infection where **vesicles develop**, becoming **pustules** *(pus-filled vesicles).*

d- Erysipelas



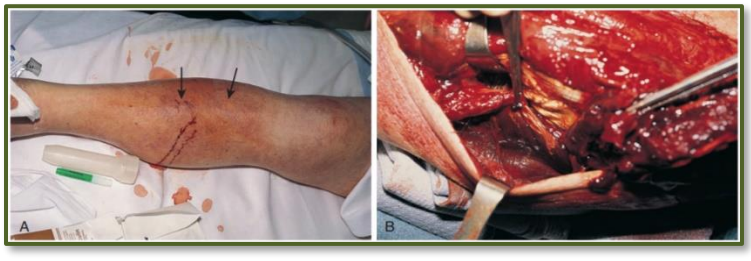
Localized skin infection with **pain**, **inflammation** *(erythema, warmth)*, **lymph node enlargement**, and **systemic signs** *(chills, fever).* It occurs when the skin's inegrity is **compromised** *(following a cut, wound, etc.)*

e- Cellulitis



Unlike erysipelas, cellulitis typically is **more serious** and involves both the **skin and deeper subcutaneous tissues** infections.

f- Necrotizing fasciitis *(also called streptococcal gangrene)*



It is an infection that occurs **deep in the subcutaneous** tissue that involves **extensive destruction** of muscle and fat.

g- Streptococcal Toxic Shock Syndrome

Multiorgan systemic infection resembling Staphylococcal Toxic Shock Syndrome. However, in contrast with staphylococcal disease, most patients with **streptococcal** disease are **bacteremic** *(bacteria is abnormally present the blood)*, and have **necrotizing fasciitis**.

> ***Note:*** ***Streptococcal pyrogenic exotoxins*** *(Spe) is believed to be* ***responsible*** *for many of the severe streptococcal infections, including* ***necrotizing fasciitis*** *and* ***streptococcal toxic shock syndrome****, as well as the rash observed in patients with* ***scarlet fever****.*

2) Nonsuppurative infections *(doesn't involves pus formation).*

a- Rheumatic Fever

Characterized by inflammatory changes of the **heart** *(pancarditis)*, **joints** *(arthralgias to arthritis)*, **blood vessels** and **subcutaneous tissues**.
It is very serious since if it is not treated, it can result in **damage** to the heart **muscle** and **valves**.

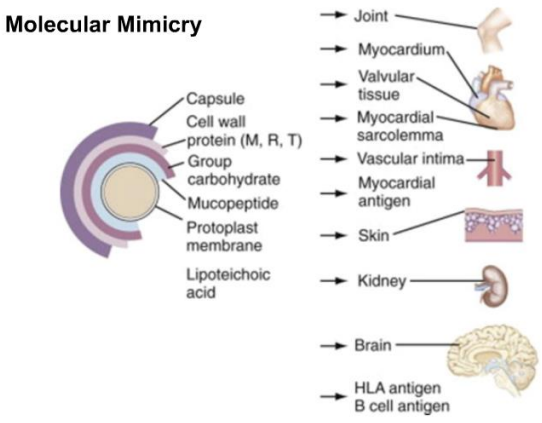
b- Acute Glomerulonephritis

Characterized by **acute inflammation** of the **renal glomeruli** with **edema**, **hypertension**, **hematuria** *(presence of blood in the urine)*, and **proteinuria** *(presence of protein in the urine).*

Molecular Mimicry

An infection is **not** necessarily caused by **bacteria** and its **exotoxins**, it can also result due to **molecular mimicry**, which happens when organs in the body *(joints, skin, kidney, etc.)* have **similar antigens** to the ones present on streptococci.

For example, Certain strains of group A streptococci contain cell membrane antigens that **mimic** the human **heart tissue antigens**, which is what causes **Rheumatic Fever.**



----------*Up to now we only discussed S. pyogenes*----------

2) Streptococcus Agalactiae

- S. agalactiae is the **only** species that has the **group B antigen**, usually found in **chains**.
- They are **resistant** (not sensitive) to **Bacitracin** unlike S. pyogenes.
- They typically are **β-hemolytic** and produce zones of hemolysis that are only **slightly larger** than the colonies.
- In general, Group B Streptococcus (GBS) is a **harmless bacterium** being part of the human **microbiota** colonizing the **gastrointestinal** and **genitourinary tract** of up to 30% of healthy human adults. Also, GBS transient (lasting only for a short time) vaginal carriage has been **observed** in 10%-30% of **pregnant women** .

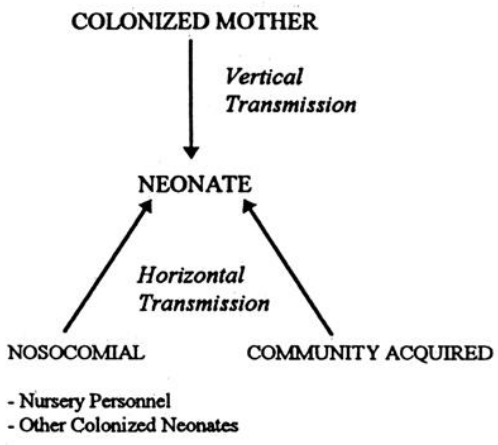
  Nevertheless, GBS can actually cause **severe invasive infections**:

  a- In **nonpregnant old adults** with **debilitating underlying conditions** *(conditions making someone very weak)* can develop invasive GBS infection, complications may include **pneumonia**, **skin and soft-tissue infection**, **bone and joint infection**, and **bacteremia**.

  b- In **pregnant women**, GBS severe infections can cause serious illness for the mother and the newborn.

GBS infections can be acquired by the neonates through 2 ways:

1) **Vertical Transmission:** GBS is transmitted from the mother to the neonate.
2) **Horizontal Transmission:** GBS is transmitted either by **Nosocomial spread** *(originating from the hospital)* in the **nursery** or **other colonized neonates**, or from **community sources** *(acquired in the community, in contrast to a nosocomial hospital-acquired).*



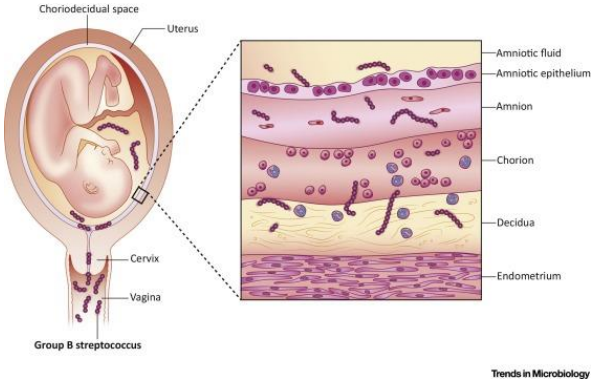
GBS infections in newborns are separated into 2 clinical disorders, where both most likely cause bacteremia or meningitis:

a- Early-onset disease (EOD)

EOD is acquired **in utero** or **at birth** by **Vertical Transmission**, through exposure of the fetus or the baby to GBS from the vagina of a colonized woman.

It occurs at **less** than **7 days of age.**

> ***Note:*** *Infants can be **infected** during passage through the **birth canal**. However, new-borns that acquire **GBS** through **this route** can also become **only colonized** (not harmful), and these colonized infants do **not develop EOD** if it was **managed**.*

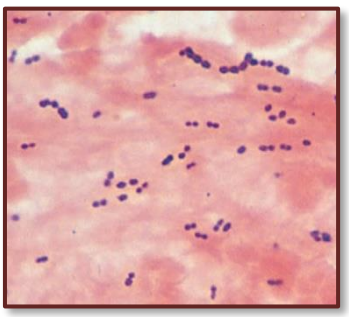


b- Late-onset disease (LOD)

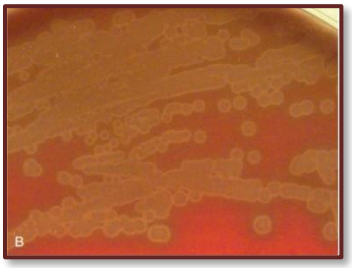
It is acquired from an **exogenous** source (e.g. mother or other infants) through **Horizontal Transmission** and develops between **1 week and 3 months of age**.

----------*Up to now we discussed S. agalactiae*----------

3) Streptococcus Pneumoniae



- S. Pneumoniae is an **encapsulated gram-positive coccus**.
- The cells are **0.5 to 1.2 µm in diameter**, **oval**, and arranged in **pairs** (diplococci) or **short chains**.
- They are **sensitive** to **Optochin** (chemical substance). They do **not** apply to Lancefield Grouping.



- They produce **pneumolysin**, which is an enzyme that **degrades hemoglobin**, producing a **green product** *(which may be responsible for the green colored **phlegm** associated with **diseases** from this bacteria)*, resulting in the **α-hemolytic** pattern.

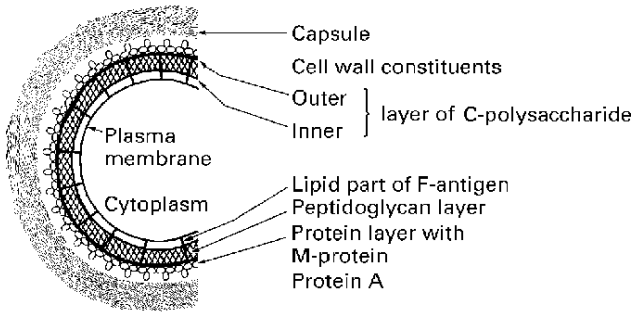
Streptococcus Pneumoniae Structure

1) Capsule

It is present in **virulent strains** of S. pneumoniae, made up of a **complex polysaccharide**, which is used for the **serologic classification** of them.

Polyvalent vaccines *(immunize against two or more strains)* are **derived** from the **capsular** polysaccharide of S. Pneumoniae.

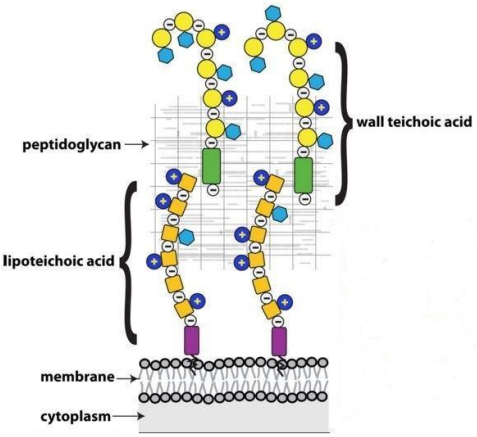
2) Cell wall



a- **C Polysaccharide** is a **major** cell wall component. It has a **high binding affinity** to a serum globulin protein ***(C-reactive protein)*** precipitating it in the serum, which causes the **immune response**.

C Polysaccharide, is a **teichoic acid**, which is a major **antigenic** determinant.

b- **Two** forms of **teichoic acid** exist in the pneumococcal cell wall, one **exposed** on the **cell surface** *(wall teichoic acid / C Polysaccharide)* and a similar structure covalently **bound** to the plasma membrane **lipids** *(lipoteichoic acid)* which is called **F antigen.**



Streptococcus Pneumoniae Pathogenesis

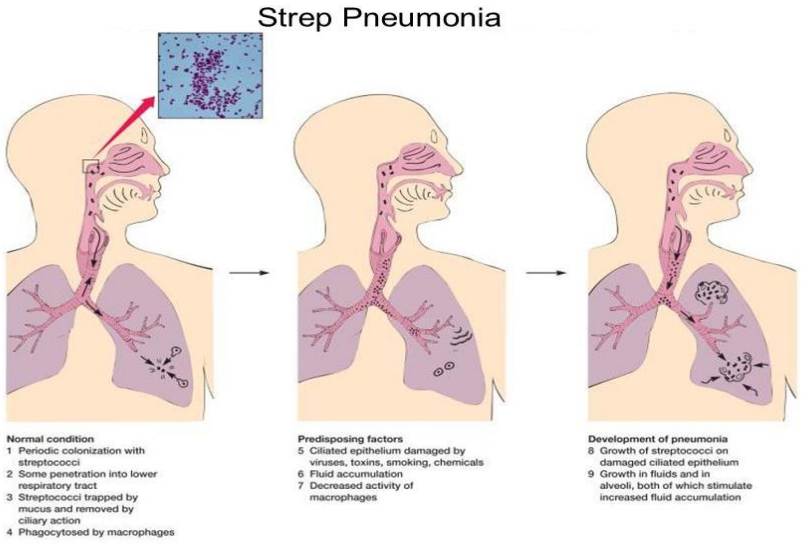
The disease manifestations are caused **primarily** by the **host response** to the infection rather than the **production** of organism-specific **toxic** factors.

S. pneumoniae is a human pathogen that initially **colonizes** the **upper respiratory tract** *(oropharynx)* and then **migrates** to the **lower respiratory** tract where **secretory IgA** *(an immunoglobulin found in the mucus)* Fc regions' recognizes the **bacteria** and **bind** it with **mucin**. The bacteria are **enveloped** in **mucus** and removed from the airways by the **action** of **ciliated epithelial cells.**

Note: *If the ciliated epithelial cells were* ***defected*** *due to* ***smoking*** *or such, the body is at a* ***high risk*** *of developing* ***S. pneumoniae infections.***

However, S. pneumoniae can prevent the previous interaction by producing IgA proteases. IgA proteases **degrade** secretory IgA. Therefore, the bacteria **won't** get trapped inside the mucus and thus **migrating** to the **lungs** and other organs causing **infections**.

Also, **Pneumolysin** *(a toxin similar to the streptolysin O in S. pyogenes)* **destroys** the **ciliated epithelial** cells and **phagocytic cells**, therefore, the bacteria cannot be removed from the airways as normally.



- A **characteristic** of pneumococcal infections is the **mobilization** of inflammatory cells to the **site of infection.**

  Amidase, which is a bacterial enzyme that **enhances** the release of the **cell wall components** *(teichoic acid and peptidoglycan fragments).* **Teichoic acid** and the **peptidoglycan fragments induce** the production of **C5a** *(Chemoattractant)*, which **mediates** the inflammatory process.

- **Phosphorylcholine** present in the bacterial cell wall **can bind** to receptors for **platelet-activating factor.** By binding these receptors, the bacteria **can enter** endothelial host cells *(invasion),* where they are **protected** from opsonization and phagocytosis.

- In a **viral co-infection** case, the virus may **increases** further migration of the bacteria causing **infection** due to increased **bacterial load** and **mucus production**.

- The virulence of S. pneumoniae is a direct **result** of its **capsule** which **inhibits phagocytosis**. Encapsulated (smooth) strains can **cause** disease in humans, whereas non-encapsulated (rough) strains are **avirulent**.

Streptococcus Pneumoniae Epidemiology

S. pneumoniae is a **common inhabitant** of the **throat** and **nasopharynx** in healthy people, with colonization **more** common in **children** than in adults and more common in adults living in a household with children.

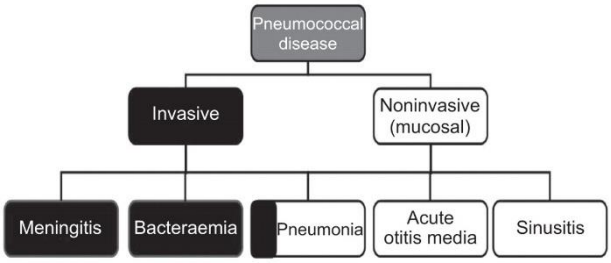
Pneumococcal disease occurs when organisms colonizing the nasopharynx and oropharynx **spread to the lungs** *(pneumonia)*, **paranasal sinuses** *(sinusitis)*, **ears** *(otitis media)*, or **meninges** *(meningitis).*

The introduction of vaccines for pediatric and adult populations has **reduced** the incidence of disease **caused** by S. pneumoniae.

Pneumococcal diseases can be:

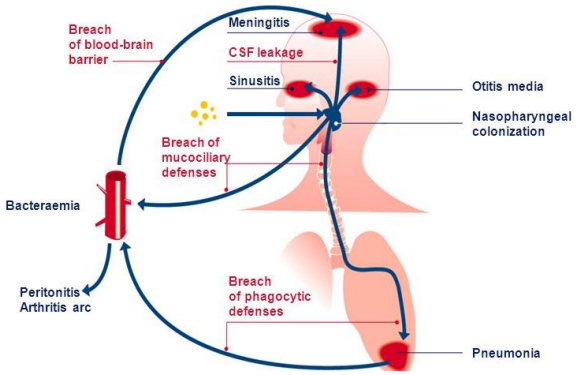
a- Invasive

It is the **invasion** of S. pneumoniae in **normally sterile sites** affecting many organs, such as the **bloodstream** *(Bacteraemia)* and the **meninges** *(Meningitis).*



b- Non-invasive

These may be **less serious** than invasive pneumococcal disease and occur in places **other** than the major organs or the blood, such as the **paranasal sinuses** (sinusitis), **ears** (otitis media).



----------*Up to now we discussed S. pneumoniae*----------

4) Viridans Streptococci

- The Viridans Streptococci is a heterogeneous collection (5 groups) of **α-haemolytic** and **nonhemolytic** streptococci. They produce a **green coloration** on blood agar plates, hence the name "Viridans", where “vĭrĭdis” in Latin means green.

- The Viridans streptococci **colonize** the **oropharynx**, **gastrointestinal tract**, and **genitourinary tract.**

- Each group has different species, each responsible for **specific diseases.**

- **S. Mitis** can cause **subacute endocarditis**, which is an infection of the inner surface of the **heart**, usually the **valves.**

- **S. Mutans & S. sobrinus** cohabit the **mouth**, both contribute to **oral disease** such as **dental caries** which breakdown the teeth.

Group	Representative Species	Diseases
Anginosus	*S. anginosus, S. constellatus, S. intermedius*	Abscesses in brain, oropharynx, or peritoneal cavity
Mitis	*S. mitis, S. pneumoniae, S. oralis*	Subacute endocarditis; sepsis in neutropenic patients; pneumonia; meningitis
Mutans	*S. mutans, S. sobrinus*	Dental caries; bacteremia
Salivarius	*S. salivarius*	Bacteremia; endocarditis
Bovis	*S. gallolyticus* subsp. *gallolyticus,* subsp. *pasteurianus*	Bacteremia associated with gastrointestinal cancer (subsp. *gallolyticus*); meningitis (subsp. *pasteurianus*)
Ungrouped	*S. suis*	Meningitis; bacteremia; streptococcal toxic shock syndrome

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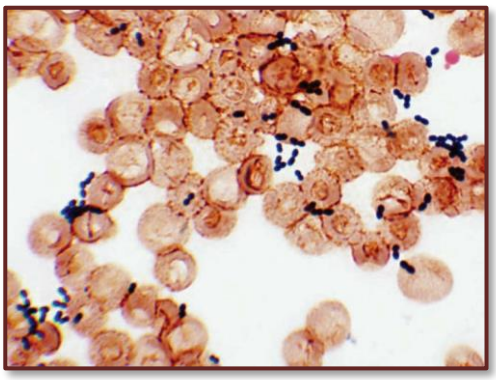
5) Enterococcus



- They are **gram-positive cocci**, typically arranged in **pairs** and **short chains.**

- They were **previously** classified as group D streptococci, but then they were considered as a **different genus**. In contrast to non-enterococcal group D streptococci, they **grow** well in 6.5% NaCl.

- As their name implies, enterococci are **enteric** (occurring in the intestines) bacteria that are commonly found in **feces** collected from humans. They have many species, two of them are **E. faecalis** and **E. faecium.**

- E. faecalis is found in the large intestine in **high concentrations** (e.g. 10^5 to 10^7 organisms per gram of feces) and in the **genitourinary tract.**

- The distribution of E. faecium is **similar** to that of E. faecalis but is found in **lower** concentrations.

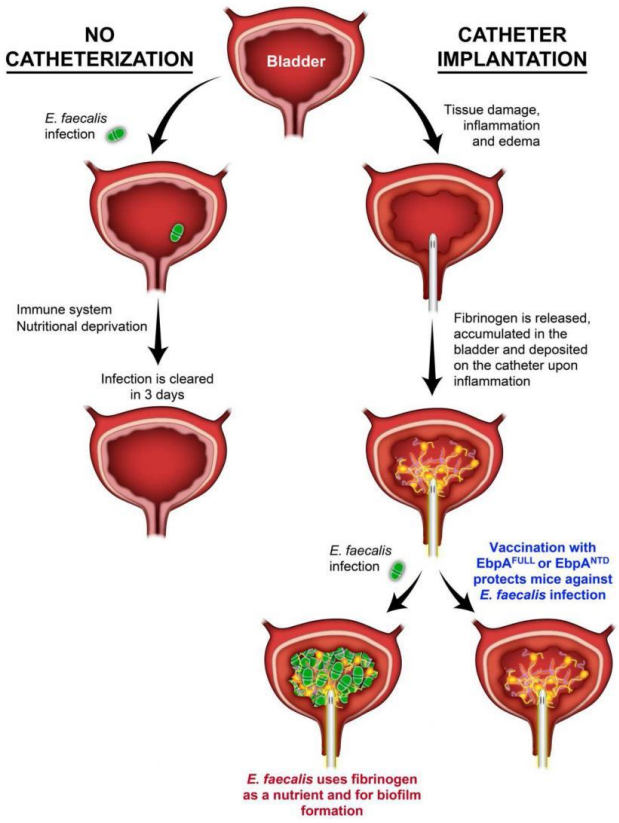
- They can grow both **aerobically** and **anaerobically** in a **broad** temperature range *(10° C to 45° C),* in a **wide** pH range *(4.6 to 9.9),* and in the **presence** of high concentrations of **sodium chloride** *(NaCl)* and **bile salts**. Thus there are very few clinical conditions that inhibit the growth of enterococci.

- They have **variable haemolysis patterns** *(nonhemolytic, α-hemolytic, or, rarely, β-haemolytic).*

- Virulence is mediated by **two general properties**:

  1) **Ability to adhere to tissues and form biofilms**
  2) **Antibiotic resistance.**

- Enterococci do **not cause** infections **normally**, however they are one of the most common causes of infections **acquired** in the **hospital** (nosocomial infection). The **urinary tract** is the most common site of enterococcal infections, and infections are frequently associated with **urinary catheterization** or **instrumentation**.



Summary

Bacteria	Morphology	Colonization	Disease
Streptococcus pyogenes (Group A)	Spherical, 1-2 µm, arranged in chains.	Skin surface and oropharynx of children and young adults.	**Suppurative;** pharyngitis, scarlet fever, pyoderma, erysipelas, cellulitis, necrotizing fasciitis and S. toxic shock syndrome. **Nonsuppurative;** rheumatic fever, glomerulonephritis.
Streptococcus agalactiae (Group B)	-	The female genital tract and oropharynx.	Neonatal infections. Pneumonia, bone and joint infections and skin and soft tissue infections.
Streptococcus pneumoniae	Oval, 0.5 -1.2 µm, arranged in pairs.	Oropharynx and nasopharynx; more common in children.	Pneumonia, sinusitis, otitis media and meningitis.
Viridans streptococci	Green coloration on blood agar plate.	Oropharynx, gastrointestinal and genitourinary tract.	**S. Mitis;** subacute endocarditis, sepsis **S. Mutans;** dental caries
Enterococci	Arranged in pair or short chains.	Gastrointestinal tracts	Urinary tract infection

Classification:

1) **Streptococcus pyogenes (Group A)**

   Gram (+) → Catalase (-) → β-hemolysis → Bacitracin sensitive

2) **Streptococcus agalactiae (Group B)**

   Gram (+) → Catalase (-) → β-hemolysis → Bacitracin resistant

3) **Streptococcus pneumoniae (encapsulated)**

   Gram (+) → Catalase (-) → α-hemolysis → Optochin sensitive

4) **Viridans streptococci**

   Gram (+) → Catalase (-) → α-hemolysis → Optochin resistant

5) **Enterococci**

   Gram (+) → Catalase (-) → γ-hemolysis → Grows in 6.5% NaCl

Best Wishes ♥