

Streptococcus

- Characters of Streptococci

- Gram positive cocci
- 1 μ m in diameter
- Chains or pairs
- Usually capsulated
- Non motile
- Non spore forming
- Facultative anaerobes
- Fastidious
- Catalase negative (**Staphylococci are catalase positive**)



Morphology and Identification

Gram-positive cocci arranged in chains or pairs.

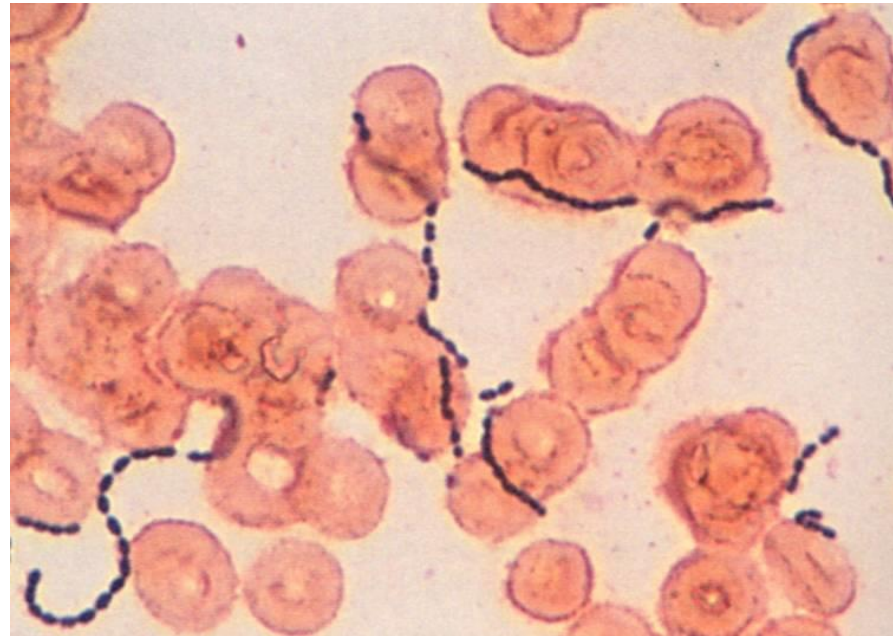
Most group A, B, and C strains produce capsules.

Most strains grow as discoid colonies, 1-2 mm in diameter.

Catalase-negative.

Grow better in media enriched with blood or tissue fluid.

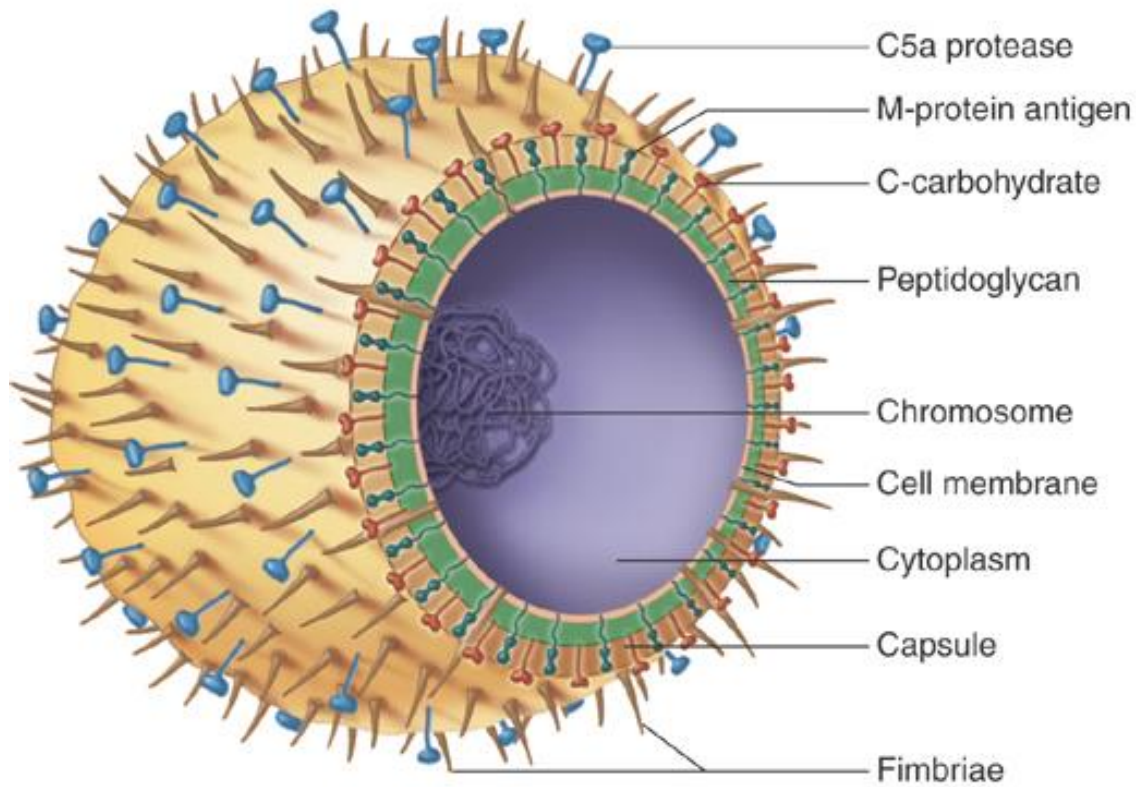
Most are facultative anaerobic and some are capnophilic. For most species growth and hemolysis are aided by incubation in 10% CO₂.



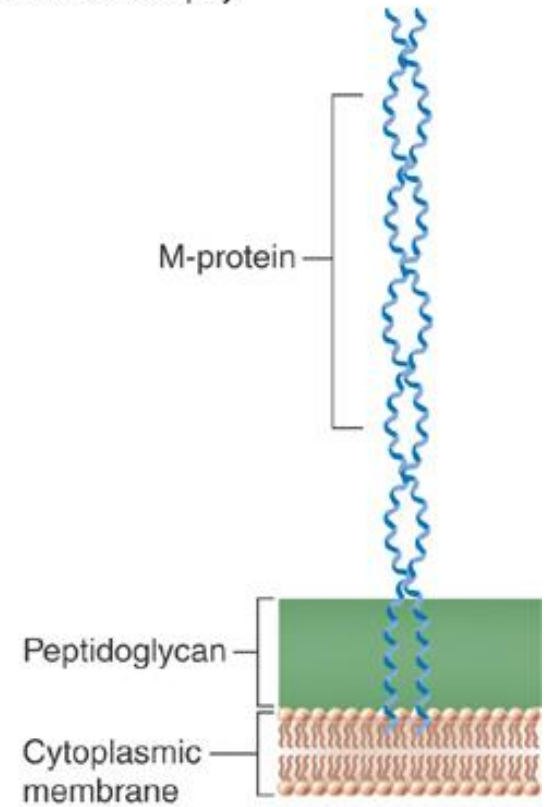
General Characteristics of Streptococci

- Gram-positive spherical/ovoid cocci arranged in long chains; commonly in pairs
- Non-spore-forming, nonmotile
- Can form capsules and slime layers
- Facultative anaerobes
- Do not form catalase, but have a peroxidase system
- Most parasitic forms are fastidious and require enriched media.
- Small, nonpigmented colonies
- Sensitive to drying, heat and disinfectants
- 25 species

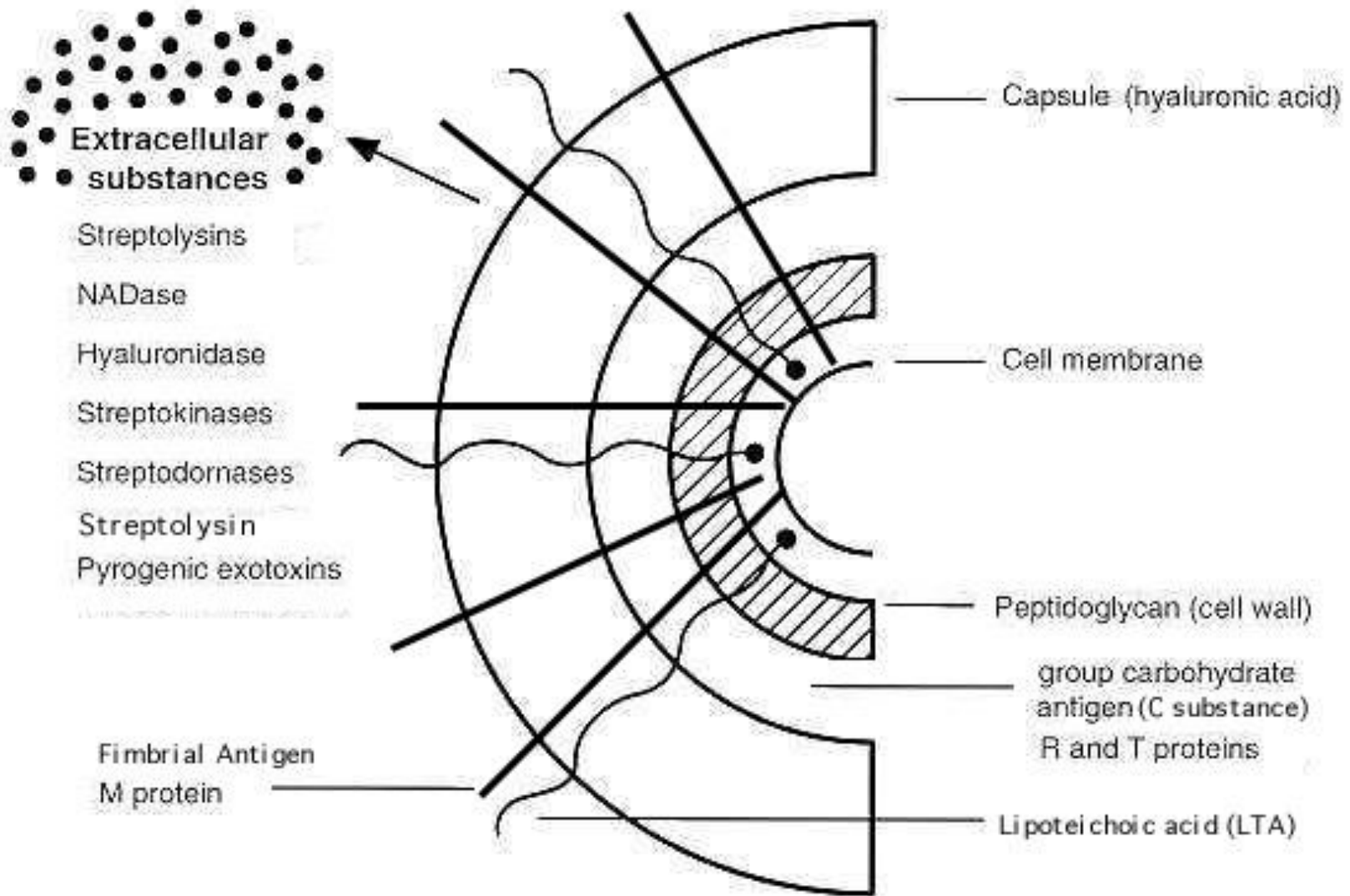
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(a)



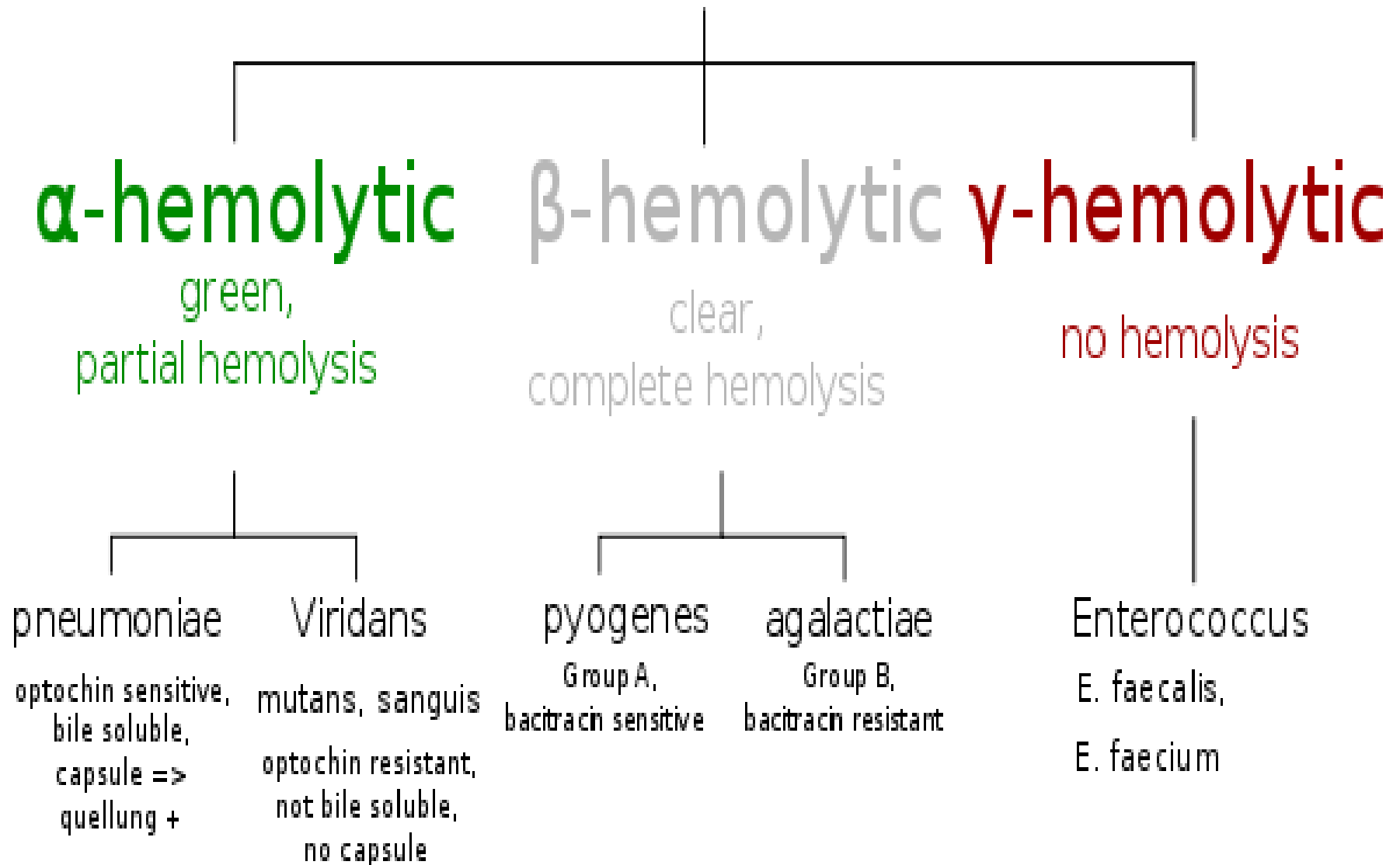
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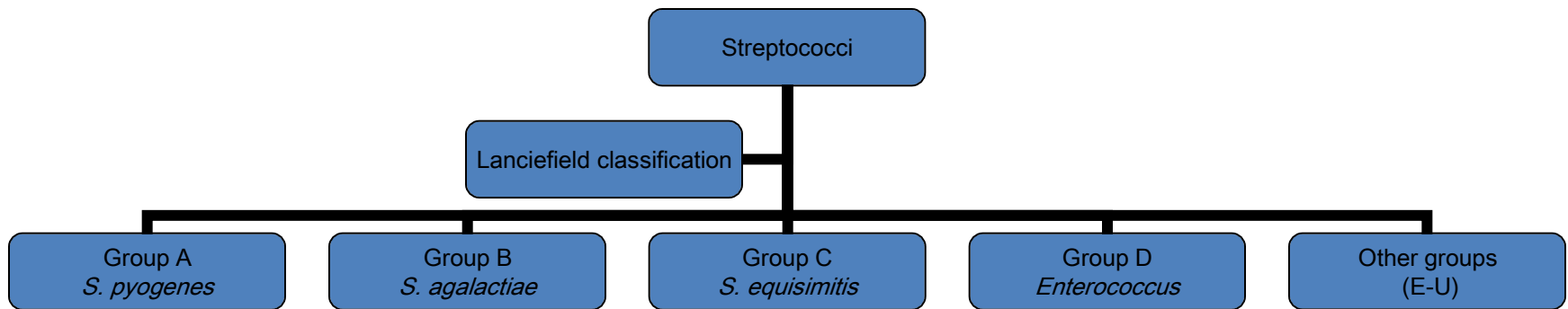
Classification of Streptococci

- Streptococci can be classified according to:
 - Oxygen requirements
 - Anaerobic (*Peptostreptococcus*)
 - Aerobic or facultative anaerobic (*Streptococcus*)
 - Serology (Lanciefield Classification)
 - Hemolysis on Blood Agar (BA)

Streptococcus



Serology: Lancefield Classification



- Streptococci classified into many groups from A-K & H-V
- One or more species per group
- Classification based on C- carbohydrate antigen of cell wall
 - Groupable streptococci
 - A, B and D (more frequent)
 - C, G and F (Less frequent)
 - Non-groupable streptococci
 - *S. pneumoniae* (pneumonia)
 - viridans streptococci
 - e.g. *S. mutans*
 - Causing dental carries

Classification

Group A - *Streptococcus pyogenes*

Group B - *Streptococcus agalactiae*

Group C - *Streptococcus equisimilis*, *Streptococcus equi*, *Streptococcus zooepidemicus*, *Streptococcus dysgalactiae*

Group D - *Enterococci*, *Streptococcus bovis*

Group E - *Streptococcus milleri* and *mutans*

Group F - *Streptococcus anginosus*

Group G - *Streptococcus canis* and *Streptococcus dysgalactiae*

Group H - *Streptococcus sanguis*

Group L - *Streptococcus dysgalactiae*

Group N - *Lactococcus lactis*

Group R&S - *Streptococcus suis*

other *Streptococcus* species are classified as non-Lancefield Streptococci

TABLE 13-1 Medically Important Streptococci

Type species	Lancefield serogroup	Normal habitat	Significant human disease
<i>S pyogenes</i>	A	Humans,	Acute pharyngitis and others
<i>S agalactiae</i>	B	Cattle, humans	Neonatal meningitis and sepsis and infections in adults
<i>S equisimilis</i>	C	Wide human and animal distribution	Endocarditis, bacteremia, pneumonia, meningitis, mild upper respiratory infection
<i>E faecalis</i> <i>S bovis (nonenterococcus)</i>	D	Human and animal intestinal tracts, dairy products bacteremia	Biliary or urinary tract infection, endocarditis,
<i>S anginosus</i>	F, G ^a	Humans, animals	Subcutaneous or organ abscesses, endocarditis, mild upper respiratory infection
<i>S sanguis</i> ^b	H	Humans	Endocarditis, caries
<i>S salivarius</i>	K	Humans	Endocarditis, caries
None	O	Humans	Endocarditis
<i>S suis</i>	R	Swine	Meningitis
"viridans" <i>S mitis</i> , <i>S mutans</i> ^c	None identified	Humans	Caries, endocarditis
Anaerobic or micro-aerophilic	None identified	Wide human and animal distribution	Brain and pulmonary abscesses, gynecologic infections
<i>S pneumoniae</i>	None identified	Humans	Lobar pneumonia and others

^a Strains of the "S milleri" group (*S constellatus*, *S intermedius*, *S anginosus*, *minute strains*) may possess antigens of groups A, C, F, or G, or no identifiable Lancefield group antigens; a heterogeneous group, genetically related but with a wide variety of phenotypic and biochemical characteristics

^b Disparate grouping undergoing further definition.

^c Other viridans streptococci (*S sanguis*, *S salivarius* "S milleri," *S bovis*) have identified group antigens(s); nutritionally variant streptococci may be included in this diverse category.

Streptococci

S. pyogenes
(group A)

Pharyngitis, scarlet fever, pyoderma, erysipelas, cellulitis, necrotizing fasciitis, streptococcal toxic shock syndrome, bacteremia, rheumatic fever, glomerulonephritis

S. agalactiae
(group B)

Neonatal infections (meningitis, pneumonia, bacteremia), urinary tract infections, amnionitis, endometritis, wound infections, pneumonia, bacteremia

Other β -hemolytic
streptococci

Pharyngitis, abscess formation, bacteremia

Viridans group
streptococci

Bacteremia, endocarditis, abscess formation, dental caries

S. pneumoniae

Pneumonia, meningitis, sinusitis, otitis media, bacteremia

Classification of Streptococci Based on Hemolysis on Blood Agar

■ Hemolysis on BA

– α -hemolysis

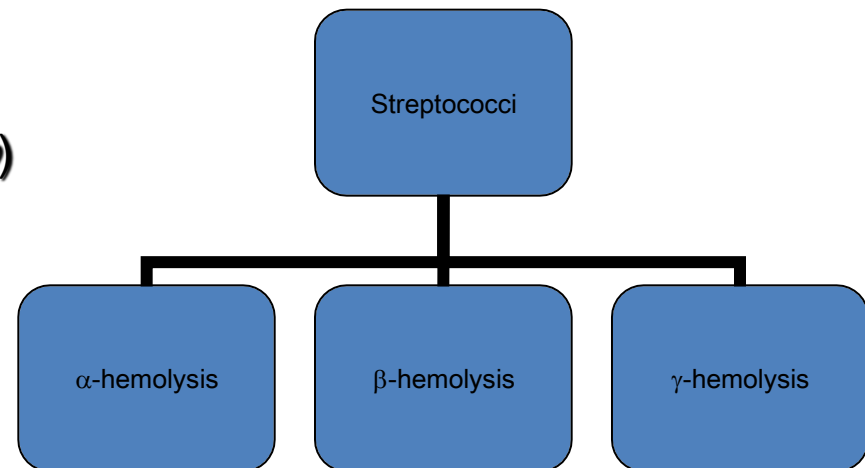
- Partial hemolysis
- Green discoloration around the colonies
- e.g. non-groupable streptococci (*S. pneumoniae* & *S. viridans*)

– β -hemolysis

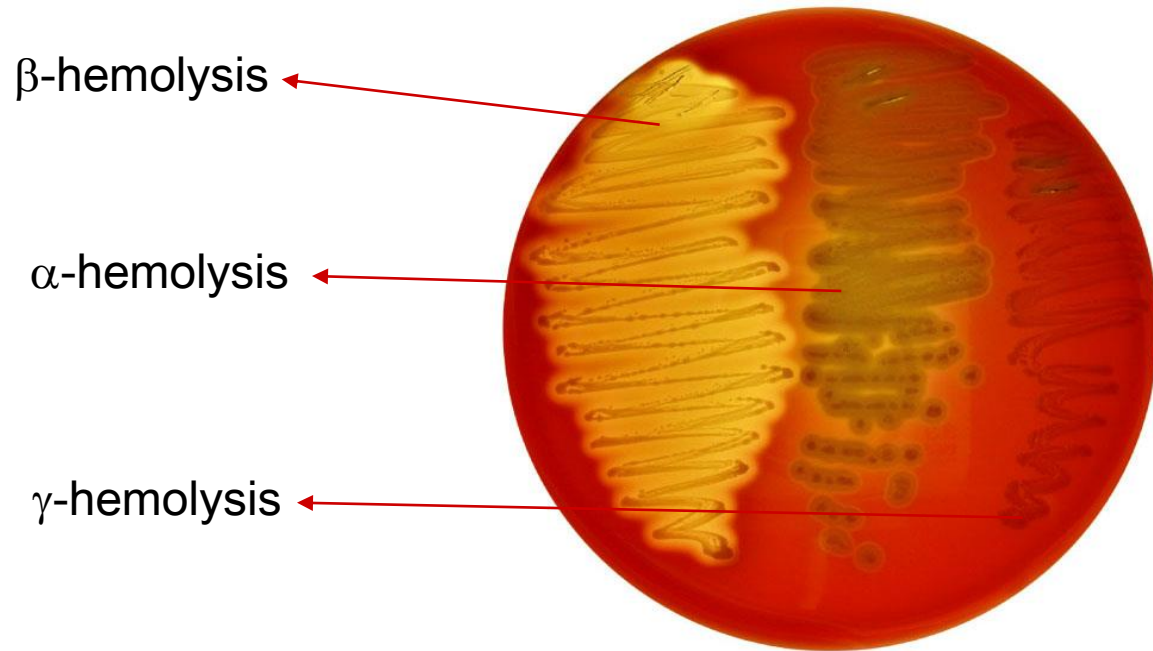
- Complete hemolysis
- Clear zone of hemolysis around the colonies
- e.g. Group A & B (*S. pyogenes* & *S. agalactiae*)

– γ -hemolysis

- No lysis
- e.g. Group D (*Enterococcus spp*)



Hemolysis on Blood agar



Biochemical Classification	Serologic Classification	Hemolysis Patterns
<i>S. pyogenes</i>	A	Beta
<i>S. anginosus</i> group	A, C, F, G, nongroupable	Beta; occasionally alpha or nonhemolytic
<i>S. agalactiae</i>	B	Beta; occasionally nonhemolytic
<i>S. dysgalactiae</i>	C, G	Beta
<i>S. bovis</i>	D	Alpha; nonhemolytic; occasionally beta
Viridans group streptococci	Nongroupable	Alpha or nonhemolytic
<i>S. pneumoniae</i>	Nongroupable	Alpha

Group A streptococci

- Include only *S. pyogenes*
- Group A streptococcal infections affect all ages peak incidence at 5-15 years of age
- 90% of cases of pharyngitis

Pathogenesis and Virulence Factors

- Structural components
 - M protein M, which interferes with opsonization and lysis of the bacteria
 - Lipoteichoic acid & F protein → adhesion
 - Hyaluronic acid capsule, which acts to camouflage the bacteria
- Enzymes
 - Streptokinases
 - Deoxynucleases
 - C5a peptidase

→ facilitate the spread of streptococci through tissues
- Pyrogenic toxins that stimulate macrophages and helper T cells to release cytokines
- Streptolysins
 - Streptolysin O lyse red blood cells, white blood cells, and platelets
 - Streptolysin S

Streptococcus pyogenes

Capsule: antiphagocytosis. The capsule of group A streptococci is composed of hyaluronic acid.

Group-specific cell wall antigen (Lancefield group A)

Carbohydrate

A dimer of N-acetylglucosamine and rhamnose.

M protein

T protein: type-specific; function unknown.

M-like proteins: binds IgM, IgG and α 2-macroglobulin; interfere with phagocytosis.

Lipoteichoic acid: binds to epithelial cells.

Protein F: a major adhesin of *S. pyogenes*, binding with fibronectin.

Streptococcus pyogenes

Pathogenesis (via invasiveness and production of toxins)

Adherence to the epithelial cells;

>10 adhesion molecules

invasion into the epithelial cells;

mediated by M protein and protein F

important for persistent infections and invasion
into deep tissues

avoiding opsonization and phagocytosis;

M protein, M-like proteins, and C5a peptidase

producing enzymes and toxins

Streptococcus pyogenes

Enzymes and toxins

Streptokinase (fibrinolysin)

Can lyse blood clots and may be responsible for the rapid spread of the organism.

Used (IV injection) for treatment of pulmonary emboli, coronary artery thrombosis and venous thrombosis.

Streptodornase (DNases A to D)

Decreases viscosity of DNA suspension. A mixture of this and streptokinase is used in enzymatic debridement-liquifies exudates and facilitates removal of pus and necrotic tissue.

Hyaluronidase (spreading factor):

Destroys connective tissue and aids in spreading infecting bacteria.

C5a peptidase

Prevents streptococci from C5a-mediated recruitment and activation of phagocytes, and is important for survival of *S. pyogenes* in tissue and blood.

Streptococcal pyrogenic exotoxins (Spe)

Produced by both the scarlet fever strains and new invasive *S. pyogenes* strains.

More than four serologically distinct toxins (SpeA, B, C and F).

They are **superantigens** (except for SpeB, which is a cysteine protease) and may exhibit the following biological activities:

Enhances release of proinflammatory cytokines
(pyrogenicity)

causes skin rash

Immunosuppression

Spe is associated with streptococcal toxic shock syndrome or other invasive *S. pyogenes* diseases.

Hemolysins

Streptolysin O: O₂-labile; causes hemolysis deep in blood agar plates. ASO (antistreptolysin O) titer >160-200 units suggests recent infection or exaggerated immune response to an earlier respiratory infection. However, skin infection does not induce ASO.

Streptolysin S: O₂-stable. Causes β-hemolysis on the surface of blood agar plates. Cell-bound, not antigenic. Produced in the presence of serum. Kills phagocytes by releasing the lysosomal contents after engulfment.

Disease caused by *S. pyogenes*

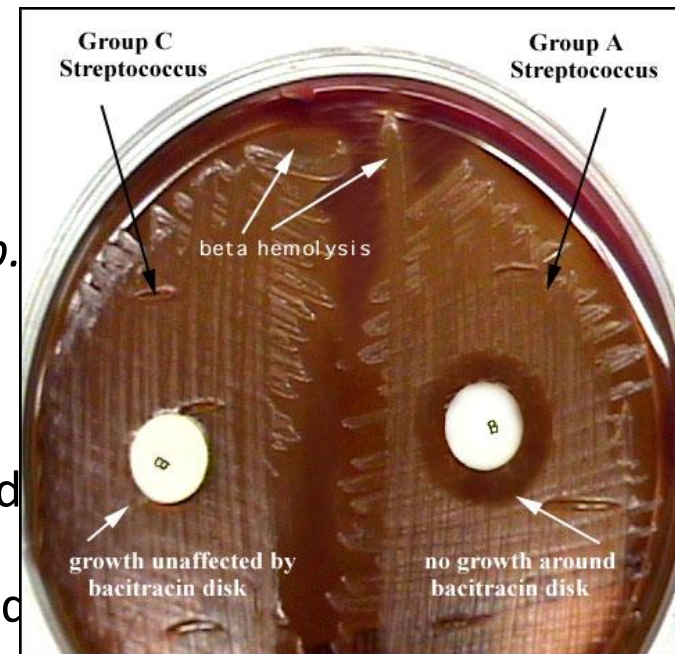
- Suppurative
 - Non-Invasive
 - Pharyngitis (“strep throat”)-inflammation of the pharynx
 - Skin infection, Impetigo
 - Invasive
 - Scarlet fever-rash that begins on the chest and spreads across the body
 - Pyoderma-confined, pus-producing lesion that usually occurs on the face, arms, or legs
 - Necrotizing fasciitis-toxin production destroys tissues and eventually muscle and fat tissue
- Non Suppurative
 - Rheumatic fever: Life threatening inflammatory disease that leads to damage of heart valves muscle
 - Glomerulonephritis
 - Immune complex disease of kidney
 - inflammation of the glomeruli and nephrons which obstruct blood flow through the kidneys

Differentiation between β -hemolytic streptococci

- The following tests can be used to differentiate between β -hemolytic streptococci
 - Lancefield Classification
 - Bacitracin susceptibility Test
 - Specific for *S. pyogenes* (Group A)
 - CAMP test
 - Specific for *S. agalactiae* (Group B)

Bacitracin sensitivity

- **Principle:**
 - Bacitracin test is used for presumptive identification of group A
 - To distinguish between *S. pyogenes* (susceptible to B) & non group A such as *S. agalactiae* (Resistant to B)
 - Bacitracin will inhibit the growth of gp A *Strep. pyogenes* giving zone of inhibition around the disk
- **Procedure:**
 - Inoculate BAP with heavy suspension of tested organism
 - Bacitracin disk (0.04 U) is applied to inoculated BAP
 - After incubation, any zone of inhibition around the disk is considered as susceptible

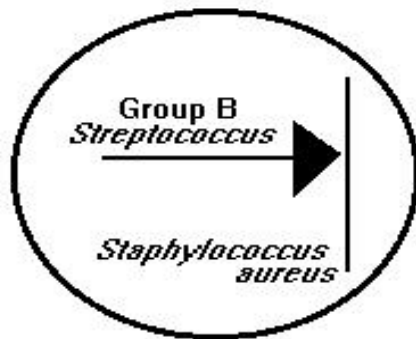


CAMP test

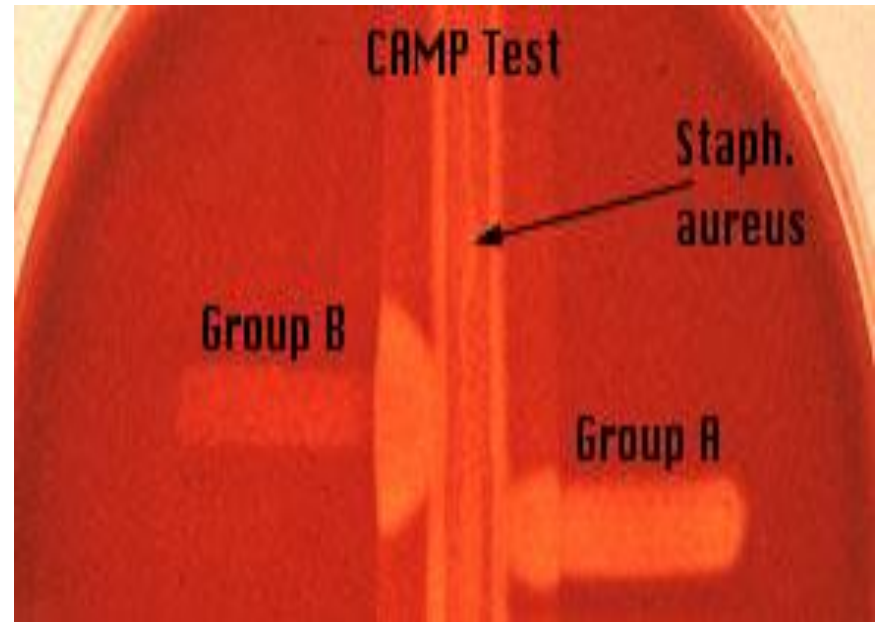
- **Principle:**
 - Group B streptococci produce extracellular ceramide binding protein (CAMP factor)
 - CAMP act synergistically with staph. β -lysin to cause lysis of RBCs
- **Procedure:**
 - Single streak of *Streptococcus* to be tested and a *Staph. aureus* are made perpendicular to each other
 - 3-5 mm distance was left between two streaks
 - After incubation, a positive result appear as an arrowhead shaped zone of complete hemolysis
 - *S. agalactiae* is CAMP test positive while non gp B streptococci are negative

CAMP test

Positive Control:
S. agalactiae



Negative Control:
Group A or Group D Strep.



Differentiation between α -hemolytic streptococci

- The following definitive tests used to differentiate between *S. pneumoniae* & viridans streptococci
 - Optochin Test
 - Bile Solubility Test
 - Inulin Fermentation

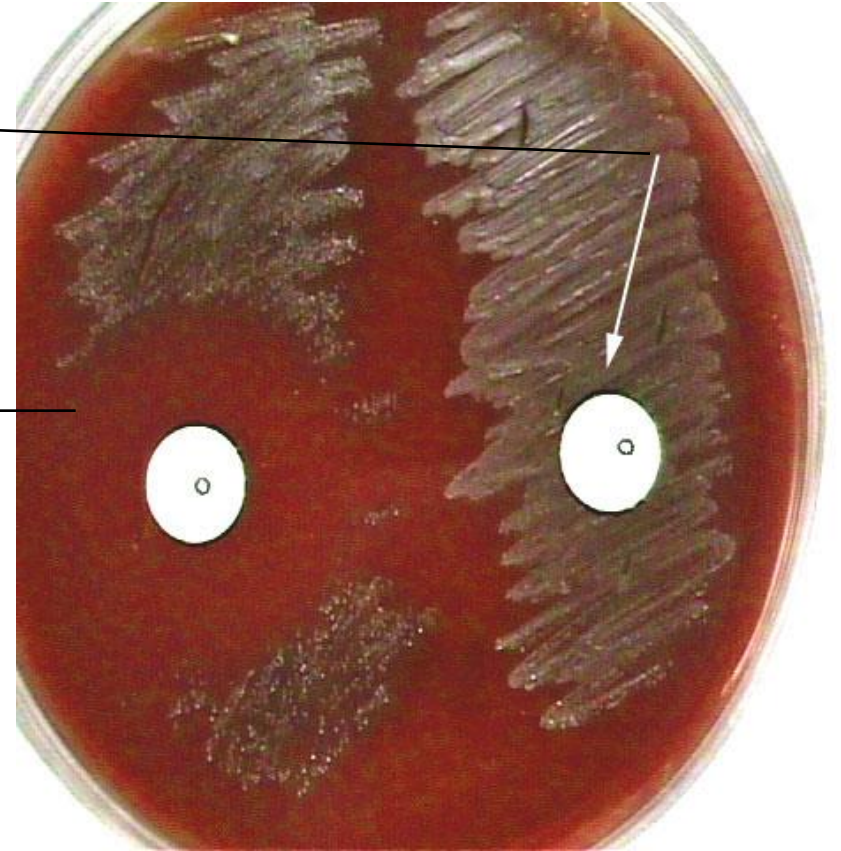
Optochin Susceptibility Test

- **Principle:**
 - Optochin (OP) test is presumptive test that is used to identify *S. pneumoniae*
 - *S. pneumoniae* is inhibited by Optochin reagent (<5 µg/ml) giving a inhibition zone ≥14 mm in diameter.
- **Procedure:**
 - BAP inoculated with organism to be tested
 - OP disk is placed on the center of inoculated BAP
 - After incubation at 37°C for 18 hrs, accurately measure the diameter of the inhibition zone by the ruler
 - ≥14 mm zone of inhibition around the disk is considered as positive and ≤13 mm is considered negative
- *S. pneumoniae* is positive (S) while *S. viridans* is negative (R)

Optochin Susceptibility Test

Optochin resistant
S. viridans

Optochin susceptible
S. pneumoniae



Bile Solubility test

- **Principle:**

- *S. pneumoniae* produce a self-lysing enzyme to inhibit the growth
- The presence of bile salt accelerate this process

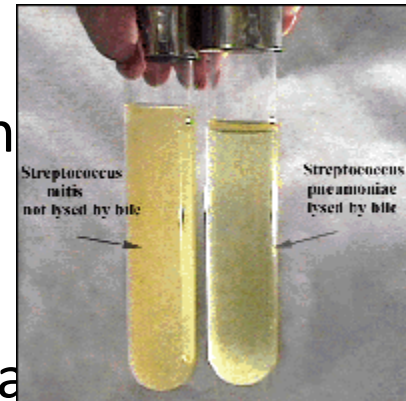
- **Procedure:**

- Add ten parts (10 ml) of the broth culture of the organism to be tested to one part (1 ml) of 2% Na deoxycholate (bile) into the test tube
- Negative control is made by adding saline instead of bile to the culture
- Incubate at 37°C for 15 min
- Record the result after 15 min

Bile Solubility test

- **Results:**

- Positive test appears as clearing in the presence of bile while negative test appears as turbid
- *S. pneumoniae* soluble in bile whereas *S. viridans* insoluble



Differentiation between β -hemolytic streptococci

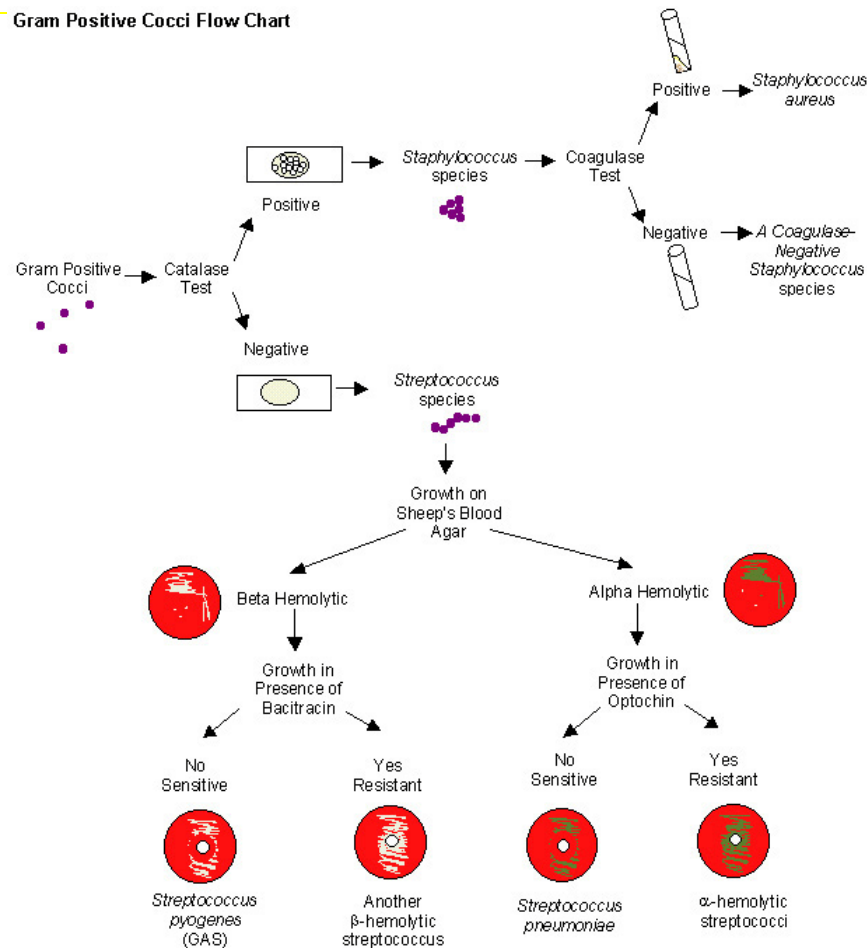
	Hemolysis	Bacitracin sensitivity	CAMP test
<i>S. pyogenes</i>	β	Susceptible	Negative
<i>S. agalactiae</i>	β	Resistant	Positive

Differentiation between α -hemolytic streptococci

	Hemolysis	Optochin sensitivity	Bile solubility	Inulin Fermentation
<i>S. pneumoniae</i>	α	Sensitive (≥ 14 mm)	Soluble	Not ferment
<i>Viridans strep</i>	α	Resistant (≤ 13 mm)	Insoluble	Ferment

Outline of differentiation between Gram-Positive cocci

Gram Positive Cocci Flow Chart



e.g. *S. epidermidis*

Bacterial Infection: Streptococci

- Environmental
 - *S. uberis*
 - *S. dysgalactiae*
 - *S. equinus*
- More subclinical mastitis
- Environment
- Predominant early and late lactation
- Contagious
 - *S. agalactiae*
- Clinical mastitis
- Resides in the milk and on the surface of the milk channel
- Cannot invade the tissue
- Accumulate Neutrophils
- Ducts and acinar epithelium damage
- Inter-alveolar tissue fibrosis
→ loss of secretory function
- Treated easily with penicillin