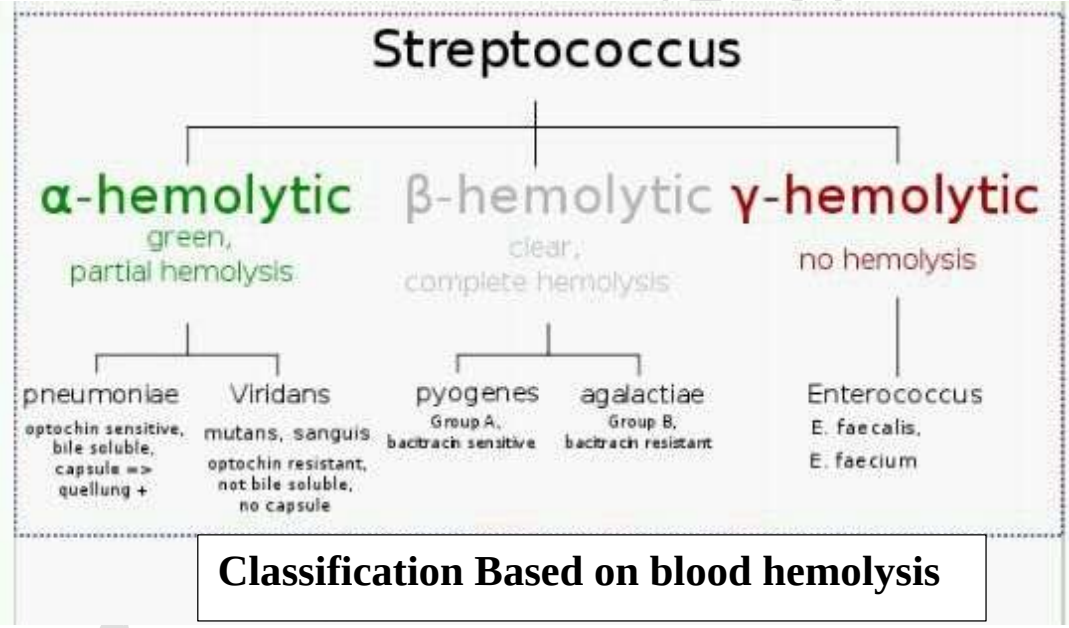
Lecture-3D: Streptococcus:

Morphology and General characteristics: 1- They are **Gram positive cocci** arranged in **chains**, **non-motile** and **non-sporing**. **2**- They require media **enriched with blood serum for growth**. **3**- They are **human pathogens** causing pyogenic infection. **4**- They are responsible for **non-suppurative lesions** like **acute rheumatic fever and glomerulonephritis**. **5- Group A streptococci have a hyaluronic acid capsule.**

Pathogenesis: Virulence factors of group A streptococci include (**1**) **M** protein and **lipoteichoic acid for attachment.** (**2**) a **hyaluronic acid capsule that inhibits phagocytosis**. (**3**) **extracellular** products, such as **pyrogenic toxin** (**erythrogenic**), which **causes the rash of scarlet fever**; and (**4**) **streptokinase**, **streptodornase** and **streptolysins**. It causes **immune-mediated sequelae** (**acute rheumatic fever and glomerulonephritis**)

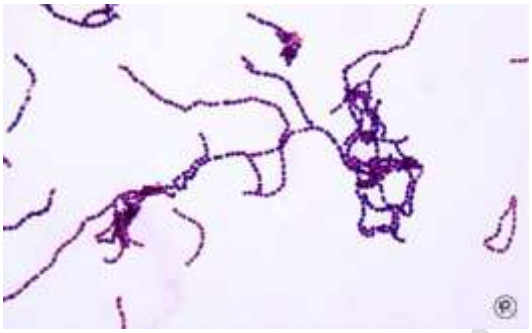
Classification of streptococcus: Several systems of classification have been employed as the following: **1**. Classification based on **morphology**. **2**. On **cultural characters**. **3**. On **biochemical reactions**. 4- On antigenic structure.



Classification Based on blood hemolysis

Streptococcus pyogenes

Morphology: It is arranged in **chain**. Chain formation is due to cocci **dividing in one plane only and failure of daughter cell to separate completely**. The length of a chain depends upon medium in which organism is grown. It is usually **encapsulated**, **non-spore forming and non-motile**. When capsule is present it is composed of **hyaluronic acid.**

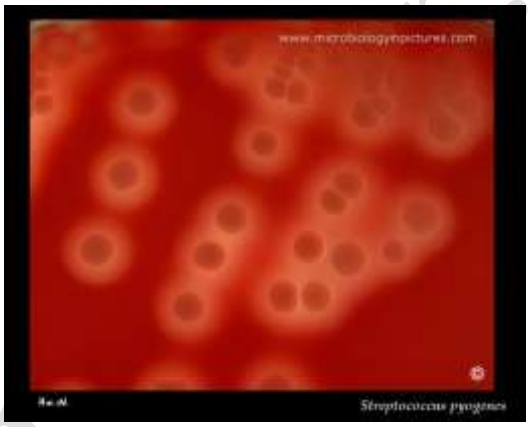


Cultural characters: *Streptococcus pyogenes* is aerobic and facultative anaerobes with optimum temperature of growth being 37°C. **Enriched media with whole blood, serum, or glucose favors rapid growth.**

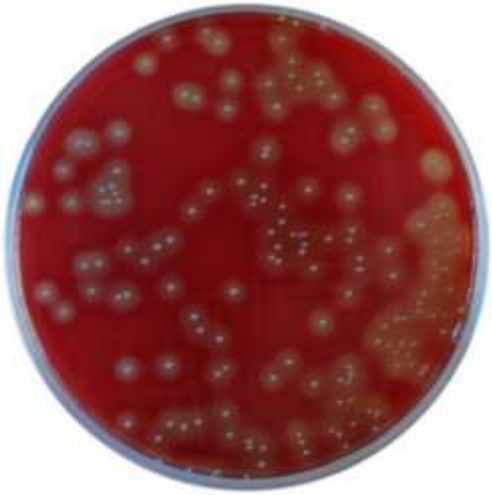
a. ***Fluid media:*** Serum broth, 24 hours after culture shows **granular** growth with **powdery deposits**.

b. ***Blood agar:*** After 24 hours' incubation colony is small, 0.5 to 1 mm (**pin point colonies**), **circular**, **transparent**, low **convex** with area of **hemolysis**.

Strains with capsules produce **mucoid colonies**.

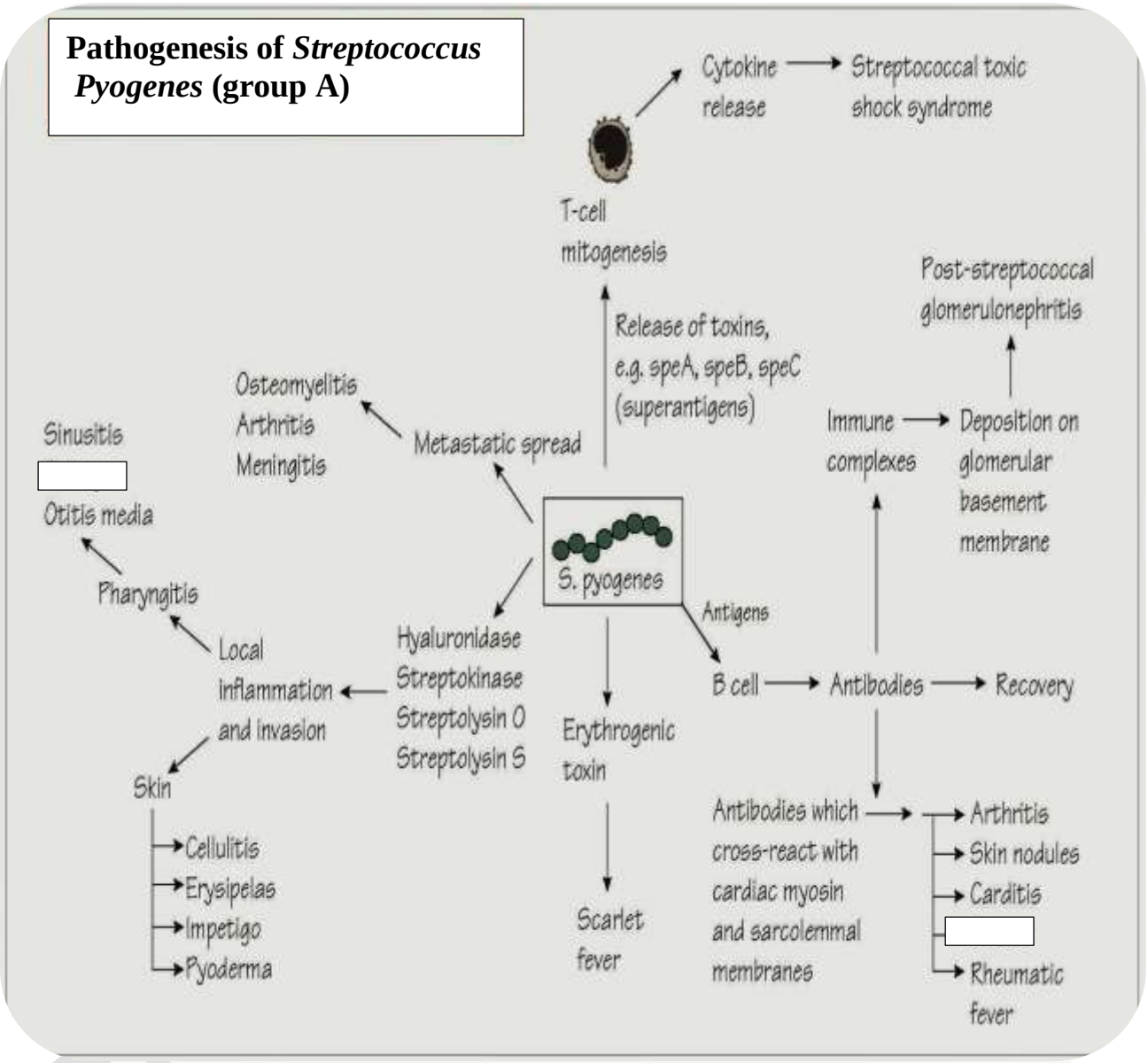


Streptococcus pyogenes **growth of blood agar medium, Beta-hemolysis**



Columbia Agar Base with 5% Defibrinated Horse Blood & Streptococcal Selective Supplement.
It is **selective medium for the isolation of *Streptococcus* spp.** from clinical samples.
It is made selective by the addition of **Colistin** and **Oxolinic Acid**.

Biochemical reactions: 1- **It ferments lactose, glucose, salicin, sorbitol, maltose, dextrin, etc. producing acid but no gas. 2- <u>It is catalase negative</u>. 3- <u>It does not soluble in bile salt</u>. 4- <u>It hydrolyzes pyrrolidonyl naphthyl amide (PYR test), producing red colors.**



……See Lancefield groups……

Table 25.1: Characteristics and clinical significance of important streptococci and enterococci

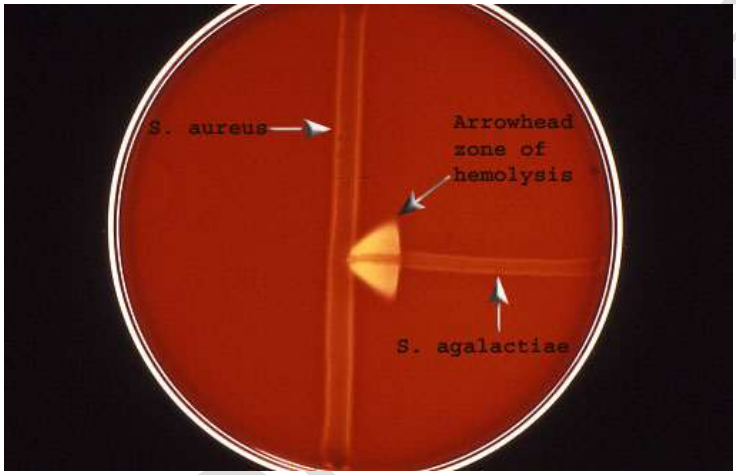
Species	*Lancefield group*	*Hemolysis*	*Natural habitat*	*Associated diseases*	*Laboratory tests*
Str. pyogenes	A	beta	Throat, skin	Pharyngitis, scarlet fever, pyoderma, erysipelas, cellulitis, necrotizing fasciitis, streptococcal toxic shock syndrome, bacteremia, rheumatic fever, glomerulonephritis	Bacitracin sensitive; PYR test positive; Ribose not fermented
Str. agalactiae	B	beta	Female genital tract, rectum	Neonatal sepsis, meningitis, puerperal fever, pyogenic infections	Hippurate hydrolysis, CAMP test
S. equisimilis	C	beta	Throat	Pharyngitis, endocarditis	Ribose and trehalose fermentation
Enterococcus sp. (*Enterococcus faecalis* and other enterococci)	Group D	variable hemolysis	Gastrointestinal tract, oral cavity, gallbladder, urethra, and vagina	Urinary tract infections, endocarditis, bacteremia, abdominal infections	Growth in 6.5% NaCl; PYR positive
Nonenterococcal group D species (*Streptococcus bovis*)	Group D	alpha-hemolytic or nonhemolytic	Gastrointestinal tract	Neonatal meningitis	No growth in 6.5% NaCl
Str. anginosus group	A, C, F, G, untypable	Beta (alpha, gamma)	Throat, colon, female genital tract	Pyogenic infections	Group A strains, bacitracin resistant, PYR negative colony variants of other groups
Viridans streptococci (*Str. mitis*, *Str. mutans*, *Str. salivarius*, and many other species)	Not typed	Alpha (gamma)	Mouth, throat, colon, female genital tract	Dental caries; endocarditis	Optochin resistant, species classification on biochemical properties

Streptococcus agalactiae

***Streptococcus agalactiae* belongs to Lancefield group B**. Human pathogenic group B strains possess a polysaccharide capsule which appears to confer virulence.

Identification of agalactiae:

Identification method is based on their ability to; 1- **rapid hydrolyse hippurate. 2- CAMP** reaction (Christie, Atkins and Munch-Peterson), which can be demonstrated as a **zone of hemolysis (arrowhead-shaped area of enhanced hemolysis) when *Str. agalactiae* is inoculated perpendicular to a streak of *Staph. aureus* grown on blood agar (see the fig., below, please)**. ***S. agalactiae* produces a CAMP factor that enhances the lysis of sheep red cells by staphylococcal β-lysin.**



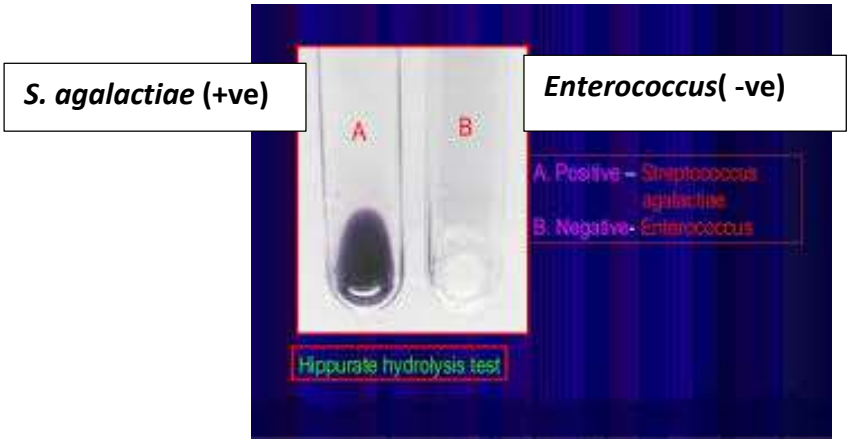
CAMP reaction

Enterococcus

The enterococci ("**enteric cocci**") **were previously classified as group D streptococci.** This group consists of **gram-positive cocci**, **non-motile and non-capsulated,** that are natural **inhabitants of the intestinal tracts of humans and animals.**

Characteristics of Enterococci (**Distinctive Features)**

1- **The enterococci grow in the presence of 6.5 percent NaCl, 40% bile, at pH 9.6, at 45°C. It survives heating at 60°C for 30 min, a feature distinguishing it from streptococci. On MacConkey medium they produce deep pink colonies. Enterococci are PYR test positive. They do not hydrolyze hippurate.**



Viridans streptococci: The **viridans** streptococci are **commensals of mouth and upper respiratory tract infection.** The viridans group of streptococci are a **heterogeneous collection of α-hemolytic and non-hemolytic** streptococci.

Pneumonia and Pneumococci (*Streptococcus pneumoniae*)

Morphology: Pneumococci are: 1-**gram-positive cocci in pairs** (**diplococci**). The cocci are **2- slightly elongated cocci**, **with one end rounded**. They may occur **singly**, in **pairs**, or in **short chains but most often are seen as pairs (diplococci)**. **3**- They are **non-motile and non-sporing. 4**- All freshly isolated strains are **capsulated**. **5- The capsule encloses each pair**.

The capsule may be demonstrated as a clear halo in Indian ink or by use of type-specific antibody in the Quellung reaction.

Cultural Characteristics

1-They are **aerobes and facultative anaerobes**. **2- It grows best in air or hydrogen with 5-10 percent CO_2. 3-** Optimum temperature being **37°C** and **pH 7.8. 4- Has complex nutritional requirements and grow only in enriched media** (supplemented with serum, blood or heated blood). **5- On blood agar, the colonies are small** (0.5-1 mm), **dome shaped and glistening, with an area of green discoloration (alpha hemolysis) around them similar to the greenish discoloration observed with the viridans streptococci. 6- On further incubation, the colonies become flat with raised edges and depressed center,** due to **autolysis by amidase enzyme of bacteria within the flat pneumococcal colonies. Autolysis is enhanced by bile salts. 7- Encapsulated strains** form very large capsules, tend to form larger, **mucoid colonies.**

Biochemical Reactions

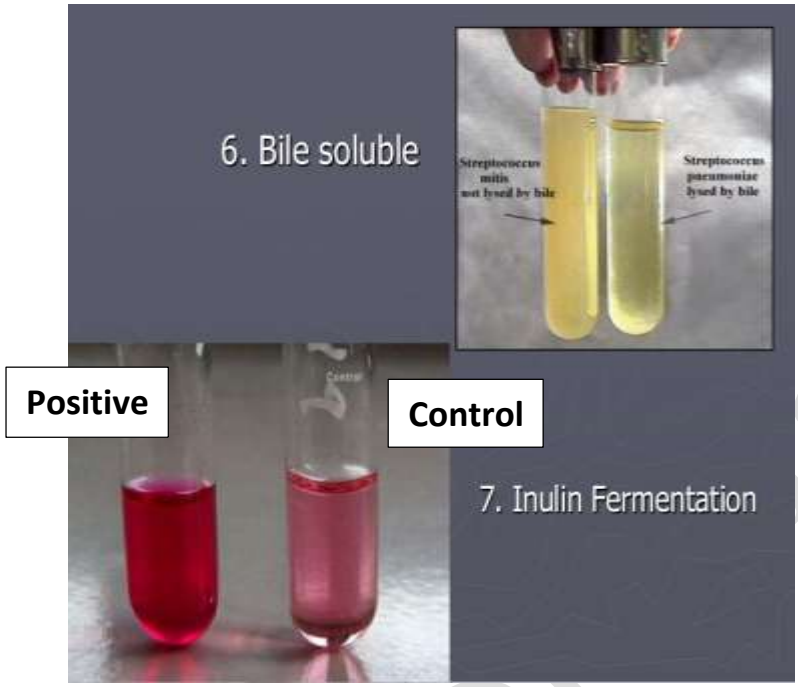
1. Inulin Fermentation: Pneumococci ferment inulin with the production of **acid without gas.** Fermentation is tested in Hiss's serum water or serum agar slopes. **Fermentation of inulin by pneumococci is a useful test for differentiating them from streptococci as the latter do not ferment it.**

2. Bile Solubility Test:

1- Grow the isolate to be tested for 18 hours at 37°C in 5 ml serum, digest broth or infusion broth.

2- While still warm, add 0.5 ml of 10 percent, bile salt (sodium deoxycholate solution) and re-incubate at 37°C. <u>Pneumococci are lysed within 15 minutes and the initially turbid culture becomes clear and transparent.</u>

<u>Pneumococci are soluble in bile; viridans and other streptococci are not.</u>



3. Pneumococci are Catalase and Oxidase negative

4- Optochin Sensitivity

Pneumococci are highly sensitive to killing by **optochin,** in a concentration of 1/500,00 and is **<u>useful in distinguishing them from viridans streptococci</u>**. **For testing, place a paper disk containing 5 µg of optochin on an area of a blood agar plate inoculated with pneumococci colonies from the primary diagnostic plate.** A growth of pneumococci will be inhibited in a zone extending radially for at least 5 mm from the margin of the disk on incubation. Viridans streptococci will grow right up to the disk.

Laboratory Diagnosis

1. Specimens: **Sputum**, **lung aspirate**, **pleural fluid**, **cerebrospinal fluid** (**CSF)** or **blood** are collected **according to the site of lesion**. **Sputum specimens must be mucus expectorated from the lungs rather than samples of saliva.**

2. Collection and Transport

All the specimens should be collected in sterile containers under all aseptic conditions. They should be processed immediately. CSF specimen should never be refrigerated in case of delay and should be kept at 37°C **(*H. influenzae*, another causative agent of pyogenic meningitis may die at cold temperature).**

3. Microscopy and Antigen Detection

Gram stain of sputum specimens is a rapid way to diagnose pneumococcal disease. **If the smears are positive for gram-positive lancet-shaped diplococci, a presumptive diagnosis of pneumococcal pneumonia may be made**. A centrifuged deposit of the CSF should be examined immediately in a Gram film in case of meningitis and presumptive diagnosis may be made by finding gram-positive diplococci both inside the polymorphs and extra-cellularly.

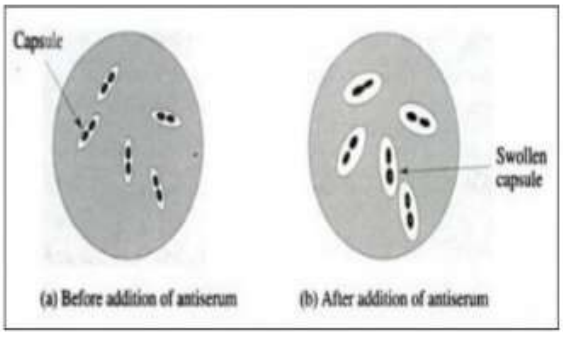
Pneumococcal antigen is often detectable by **co-agglutination (COA)**, **latex agglutination (LA)** and ELISA. COA test for antigen gives positive result in larger proportion of specimens than either Gram film or culture.

4. Capsule Swelling Tests (Quellung reaction)

If typing sera are available, the most, simple, rapid, and accurate method for the identification of pneumococci by direct examination is the **Quellung reaction.** In this test, **polyvalent anti-capsular antibodies are mixed with the bacteria**, and then the mixture is examined under microscope. A greater refractiveness around the bacteria is a positive reaction for *S. pneumoniae.*

DIAGNOSIS:

- Positive Quellung test: swelling when tested against antiserum containing anti-capsular antibodies
  - Quellung reaction: technique used to detect encapsulated bacteria (such as S. pneumonia and H. influenza)



Quellung Antibody reaction

5. Culture: Specimen is inoculated on plates of **blood agar** and **heated blood agar (chocolate agar)** and incubated in air with 5-10% CO_2 for 18-24 hours. The colonies are small (0.5-1 mm), **dome** shaped and **glistening**, with an area of **green discoloration** (**α-hemolysis**) around them. On further incubation (old culture), the colonies have **draughtsman** appearance.

6. **Identification**: Procedures commonly used to distinguish *S. pneumoniae* from the viridans streptococci are **1) optochin susceptibility, 2) bile solubility, and 3) the Quellung reaction. *S pneumoniae* is susceptible to optochin,** whereas other α-hemolytic species are resistant. Additional biochemical, serologic, or molecular diagnostic tests can be performed for a definitive identification, as in the table.

Differences between Viridans Gp & Pneumococci

Point	Pneumococci	Viridans Gp
Morphology	Capsulated, lanceolate, diplococci	Oval or rounded in chains
Quellung test	+	-
Colonies	Dome shaped→ Draughtsman	Dome shaped
Growth in liquid	Uniform turbidity	Granular turbidity with powdery deposits
Bile solubility	+	-
Inulin fermentation	+	-
Optochin sensitivity	+	-
Intraperitoneal inoculation in mice	Fatal Infection	Non-pathogenic

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