CHAPTER II
PATHOGENIC GRAM POSITIVE COCCI

Objectives (summary)

Upon completion of this unit of instruction, the student will be able to:

- Discuss the basic characterisic of gram positive cocci
- Describe the virulent factor of Gram positive cocci
- Discuss pathogenicity, clinical manifestations, laboratory diagnosis, prevention & control of members of the Gram positive cocci

- There are two most important genera of **gram-positive cocci**
  - The Genus ***staphylococcus***
  - The Genus ***Streptococcus***

The Genus staphylococcus

- Staphylococci make a very large contribution to man's commonsal flora and also account for a high proportion of his acute and chronic suppurative lesions.

General properties /characteristics

- Gram positive in reaction
- Arranged in grape like irregular clusters
  - **Single cocci, pairs, tetrads / rarely chains**
- The genus:
  - Has greater than 30 Spp
  - Contain 3 main Spp of Clinical importance:
    - *S. aureus*
    - *S. epidermis*
    - *S. saprophytius*

- Grow on many types of media
- Some are members of normal flora of:
  - Skin & mucous member of humans
  - Others cause a variety of infections
- Pathogenic staphylococci:
  - Hemolyze blood
  - Coagulate plasma
  - Produce extra cellular enzymes/toxins
- Food poisoning: Heat stable stap.cocci enterotoxin
- Resistance to some anti-microbial agents

Identification

A. Culture

- Grow on most bacteriological media under:
  - Aerobic & micro aerophilic conditions
  - Grow rapidly at 35 - 370C
  - Colonies on solid media are round, smooth & raised
  - *S. aureus* forms gray to deep golden yellow colonies

- *S. epidermis* produces **gray to white colonies**

B. Growth characteristics

- Produce catalase
- Ferments carbohydrate (release of lactic a cid & not gas)
- Varies proteolytic activity
- Produce many extra cellular substances
- Resistant to:
    - Drying
    - Heat (50 0C for 30 min)
    - 9% NaCl
    - Variably sensitive to many antibiotics (ABCs)
    - Some are resistance to certain ABCs
        - **B - lactamase** production (Due to plasmid genes)
- Resistant to Ampicillin Tetracycline, Amino glycosides

N.B: **Plasmids** are small and specialized genetic elements that can

carry and confer resistant genes to certain bacteria

Antigenic structure

Staphylococci contain:

- Antigenic polysaccharides
- Proteins
- Other substances, like capsules & coagulase in *S. aureus*

N.B: * **Capsules** inhibit phagocytosis by phagocytic leukocytes

* **Coagulase** binds to fibrinogen & aggregate bacteria

- Antigenic polysaccharide on cell wall of Stap.cocci:
  - Peptidoglycan:
    - Pathogenesis of infection & endotoxin like activity
  - Teichoic acids Polymers of glycerol

Toxins & enzymes

1.Enzymes

A. Catalase

- Staphylococci produce catalase
- Converts H2 02 into H20 & 02
  - Catalase test differentiates:
    - Staphylococci --- Catalase positive
    - Streptococci ---- Catalase negative

B. Coagulase

- Produced by *S. aureus*
- Clots oxalated /citrated plasma
- Deposit fibrin on the surface of stap.cocci
  - Inhibits phagocytosis
  - Associated with pathogenicity

C. Other enzymes

- Hyaluronidase: Spreading factors
- Staphylokinase: Cause fibrinolysis
- Proteinases
- Beta- lactamase: Disrupts the Beta -lactame rings

2.Toxins

A. Exotoxins

- Several toxins that are lethal
- Cause necrosis in skin
- Contain soluble hemolysin (alpha - toxin)
- Lyse RBCs, damage platelets &vascular smooth muscle
- ß - toxin is toxic for human RBCs

B. Leukocidin

- This toxin of *S. aureus* kills WBCs

3. Exfoliative toxin

- Desquamation of staphylococci scalded skin

4. Toxic shock syndrome toxin

- Produced by most *S. aureus*
- Associated with: fever, shock and skin rash

5. Enterotoxins

- 6 (A-F) soluble toxins produced by *S.aureus* strains
- Bind to MHC class II molecules
  - Inhibit T- cell activation
- Heat stable (Resist boiling for 30min.)
- Resistant to gut enzymes
- Responsible for food poisoning
- Produced when *S.aureus* grows on carbohydrate & protienous food
- Plasmid may carry a protein that regulates toxin prod’n

- Pathogenesis of Stap.cocci is combined effect of:
  - Extra cellular factors
  - Toxins
  - Invasive properties of the strain

Clinical findings

- *S. aureus* infection results from direct contamination of a wound

E.g. * Postoperative staphylococcal wound infection

* Infection following trauma

E.g: * Chronic osteomyelitis subsequent to open fracture

* Meningitis following skull fracture

- Food poisoning caused by enterotoxins
  - Characterized by:
    - Violent nausea
    - Vomiting
    - Diarrhea with out fever

- Toxic shock syndrome toxin is manifested by:
  - High fever
  - Hypertension
  - Vomitiny
  - Cardiac & renal failure
  - Diarrhea
  - Myalgia

Lab. Dx

Potential specimens:

- Pus
- Tracheal aspirates
- Blood
- CSF

Lab. techniques

A. Smears

- Gram’s stain (Typical organisms)
- Not helps to distinguish among different Spp.

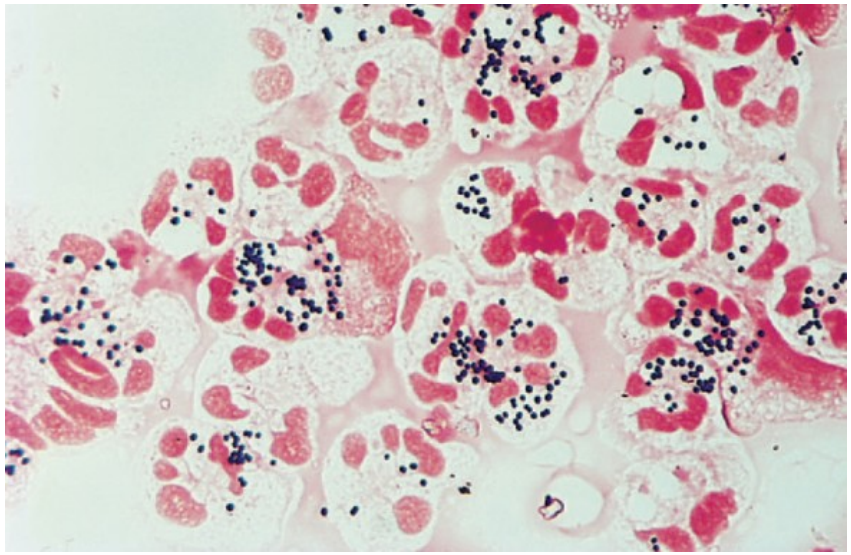


Figure: Gram positive cocci

B. Culture

- Blood agar plates give typical colonies in 18 hrs at 370C
- Media containing 7.5% NaCl inhibits the growth of

other bacteria, but not *S. aureus*

N.B. Hemolysis & pigment production can stay for several days

C. Catalase Test

- A drop of H202 (+) small amount of bacterial growth on a slide
- Formation of bubbles (release of 02) ----- Positive test

D. Coagulase Test

- Human / citrated rabbit / plasma diluted 1:5 (+) equal volume of growth from colonies from broth culture
- Incubated at 370C
  - **Positive test**: If clots form in 1- 4 hrs

N.B. All coagulase positive staphylococci are considered pathogenic to humans

E. Susceptibility Testing

- Disk disffusion susceptibility testing
- to penicillin detected by a pos. test for ß -lactamase

F. Serological Testing

- Anti-bodies to teichoic acid (Chronic infections)

Treatment

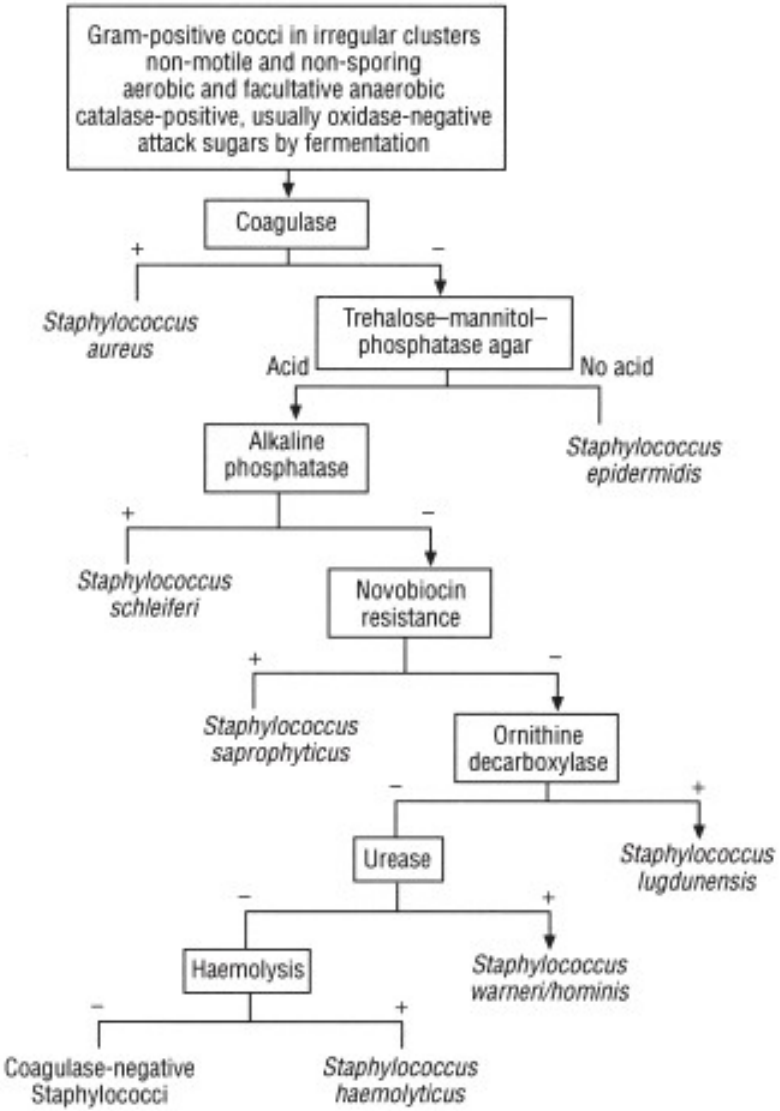
- Tetracycline
- Amino glycosides
- Cephalosporins
- Erythromycins
- Vancomycine

- Identification of an isolate as a staphylococcus relies largely on microscopic and colony morphology, and catalase positivity.
  - Bacteria stain strongly gram-positive, and are frequently seen in grapelike clusters.
  - S. aureus is distinguished from the coagulase-negative staphylococci primarily by coagulase positivity.
  - In addition, S. aureus colonies tend to be yellow and β-hemolytic, rather than gray and non hemolytic like the coagulase-negative staphylococci.
  - S. aureus is also distinguished from most coagulase-negative staphylococci by being mannitol-positive.

Table: Biochemical characteristic of staphylococci

Species	Frequency of disease	Coagulase	Color of colonies	Mannitol fermentation	Novobiocin resistance
S. aureus	Common	+	Golden yellow	+	–
S. epidermidis	Common	–	White	–	–
S. saprophyticus	Occasional	–	Variable	–	+

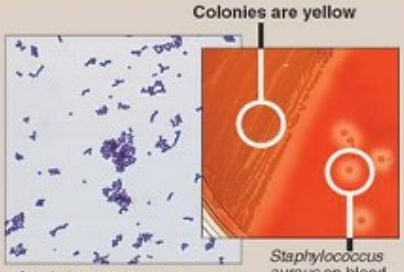
Fig. Flow diagram for the preliminary identification of human *Staphylococcus* species



Prevention & control

- Early detection & treatment
- Personal hygiene
- Proper storage of foods

Staphylococcus species



Staphylococcus aureus cultured from a wound infection

Staphylococcus aureus on blood agar surrounded by zone of beta hemolysis.

- **Gram-positive**, staining darkly
- Round cocci tending to occur in bunches like grapes
- True facultative anaerobic organisms
- Cultured on enriched media containing broth and/or blood

Staphylococcus aureus

- Skin infections
- Osteomyelitis
- Endocarditis
- Food poisoning
- Septicemia
- Necrotizing pneumonia
- Toxic shock syndrome

PENICILLINS[1]: 1 Oxacillin; 1 Nafcillin

CEPHALOSPORINS
TETRACYCLINES
AMINOGLYCOSIDES
MACROLIDES
FLUOROQUINOLONES

OTHER: 2 Vancomycin[2]

OTHER: 3 Quinupristin-dalfopristin[3]; 3 Linezolid[3]; 3 Daptomycin[3]

[1] Most isolates resistant to penicillin G
[2] Used in methicillin-resistant isolates
[3] Used in vancomycin-resistant isolates

Staphylococcus epidermidis

- Infections of catheters and heart valves

PENICILLINS[1]: 1 Oxacillin; 1 Nafcillin

CEPHALOSPORINS
TETRACYCLINES
AMINOGLYCOSIDES
MACROLIDES
FLUOROQUINOLONES

OTHER: 2 Vancomycin[2]

[1] Most isolates resistant to penicillin G
[2] Used in methicillin-resistant isolates

Staphylococcus saprophyticus

- Cystitis in women

PENICILLINS: 1 Penicillin G

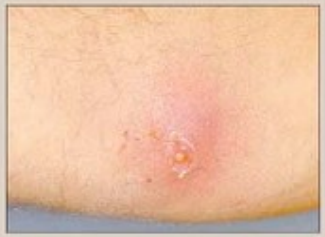
CEPHALOSPORINS
TETRACYCLINES
AMINOGLYCOSIDES
MACROLIDES
FLUOROQUINOLONES
OTHER



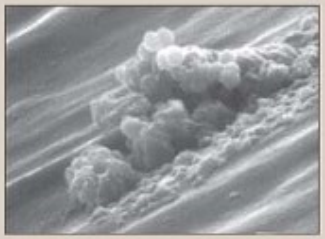
Folliculitis caused by *Staphylococcus aureus*



Carbuncle caused by *Staphylococcus aureus*



Furuncle caused by *Staphylococcus aureus*



Scanning electron micrograph of cardiac pacemaker lead colonized by *S. aureus*



Staphylococcal scalded skin syndrome



Superficial impetigo

Genus Streptococci

Properties

- Gram positive in reaction
- Forms pairs / chains during growth
- Ubiquitous
- Contain normal flora & pathogenic Spp.
- Produce extra cellular substances & enzymes

- Heterogeneous group of bacteria
  - No system suffice to classify
  - Spp characterized by:
    - Colony characteristics
    - Hemolysis (alpha/ beta) patterns on blood agar
    - Antigenic composition of group specific cell wall subs.
    - Biochemical reactions

Identification

- Group A, B & C strains possess capsular polysaccharide
- Streptococcal cell wall contains:
  - M, T and R proteins
  - Group specific Carbohydrates
  - Peptidoglycans
    - Pili of Grp A strep cocci consists of partly M - protein
    - Possess lipotechoic acid
      - Bacterial attachment to epithelial cells

- **Culture & growth characteristics**
  - Most grow an solid media
  - Media should be supplemented with blood / tissue fluids
  - Strains with capsular materials produce mucoid colonies
  - Growth & hemolysis are aided by incubation in 10% CO2
  - Most hemolytic strep. cocci grow best at 370C
  - Most strep cocci are facultative anaerobes

Antigenic structure

1. **Groups specific** c**arbohydrate**
   - Contained in the cell wall of many strep.cocci
   - Forms basis of serologic grouping (Lance field groups A-U)
   - Serologic specificity of the group specific carbohydrate is determined by amino sugar

Eg. * **For grp A** strep cocci: rhamnose N - acetyl glucosamine

* **For grp B**: rhamnose - glucosamine polysaccharide

2. M - protein

- Major virulence factor for grp A *S. pyogenes*
- Grp A strep. cocci that lack M- protein are not virulent
- **> 80** types of M-proteins

3. T - substance

- No relation ship to virulence
- Un like M-protein; acid and heat labile

4. Nucleoproteins

- Make up most of strep. coccal cell body

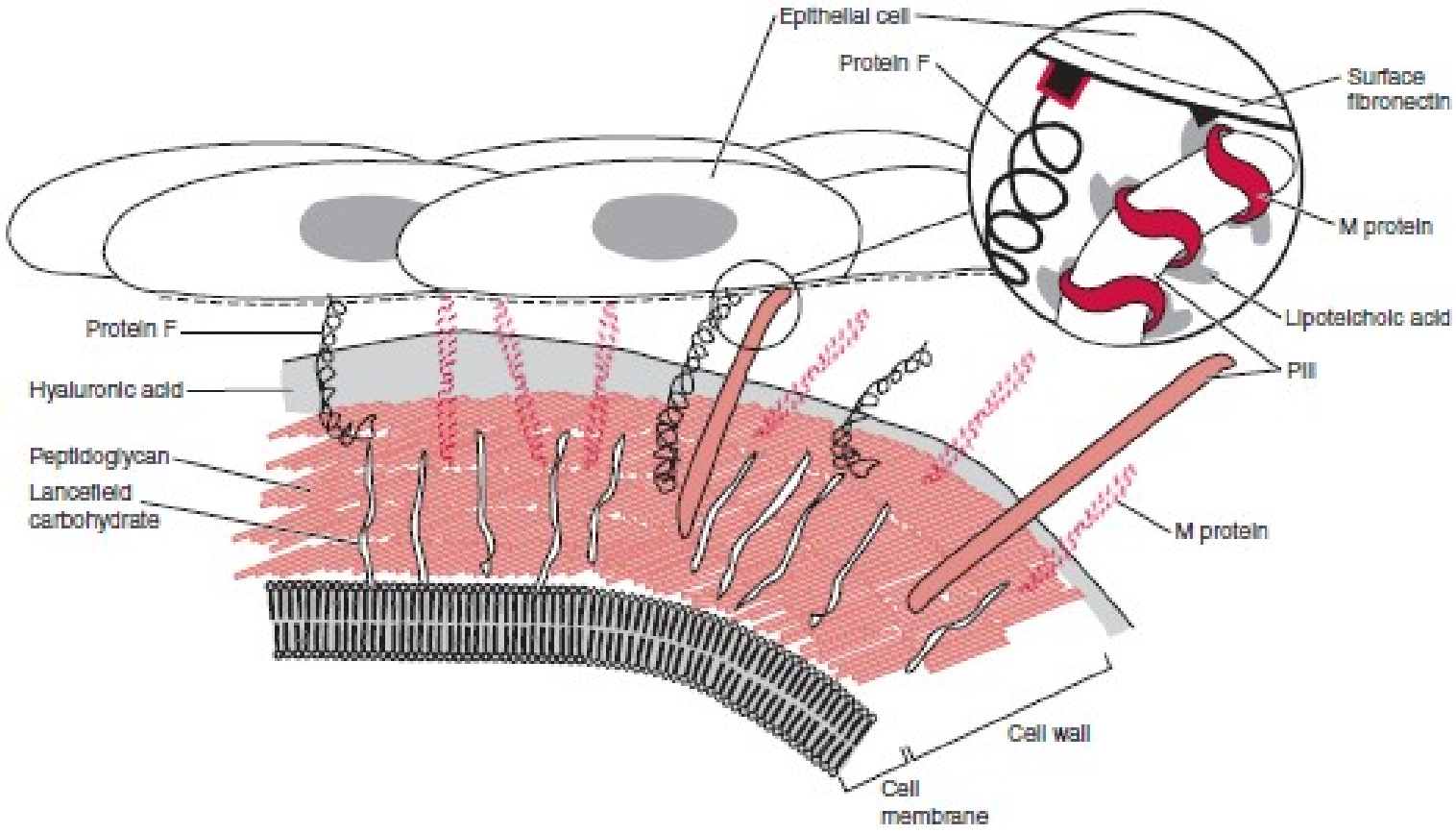


Figure: Antigenic structure of *S. pyogenes* and adhesion to an epithelial cell. The location of peptidogl
and Lancefield carbohydrate antigen in the cell wall is shown in the diagram. M protein and lipoteicho
acid are associated with the cell surface and the pili. Lipoteichoic acid and protein F mediate
binding to fibronectin on the host surface.

Toxins & enzymes

1. Streptokinase (Fibrinolysin)

- Produced by strains of grp.A ß - hemolytic strep cocci
- Active proteolytic enzyme that digests fibrin & other proteins

2. Strepto dornase (strp.coccal deoxyribonvclease)

- Depolymerises DNA
  - Confirmed by decreased viscosity of known DNA solution

- **3.Hyaluronidase**
  - Splits hyaluronic acid
  - Important component of connective tissue
  - Helps as spreading factor
- **4. Pyrogenic exotoxins A – C (Erythrogenic toxin)**
  - Soluble & destroyed by boiling for 1hrs
  - Causes rash that occur in scarlet fever
  - Only strains containing this toxin cause scarlet fever
- **5. Diphosphopyridine Nucleotidase**
  - Seen in some strep. cocci
  - Related to organism’s ability to kill leukocytes

6. Hemolysin

- Hemolyze RBCs *in vitro*
- Beta - hemolysis
  - Complete disruption of RBCs with release of Hgb
    - Alpha - hemolysis
  - Incomplete lysis of RBCs with the formation of **green pigment**
- ß – hemolytic grp. A *S. pyogenes* produce two hemolysins/ streptolysins

A. Streptolysin O

- Rapidly inactivated in the presence of O2
- Hemolyze RBCs,
- Antigenic in nature
- Antistreptolysin - O (Blocks hemolysis by streptolysin - O)

2. Streptolysin - S

- Responsible for hemolytic zones (on blood agar plate)
- Not antigenic

Classification of Strep. cocci

Based on:

1. Colony morphology & hemolytic rxns on blood agar
   - Alpha, beta - hemolysis or non - hemolysis
2. Serologic specificity of the cell wall grp specific carbohydrate
   - Lance field classification
3. Capsular antigens: *S. pneumonia* into 84 types
4. Battery of Biochemical Tests:
   - Sugar fermentation rxns
   - Tests for the presence of enzymes
   - Tests for susceptibility / resistance to certain ABCs

N.B: Biochemical tests are used for Spp that do not react with the commonly used antibody preparations for the Grp. specific subs.

5. Resistance to physical & chemical factors
6. Ecologic features
7. Molecular techniques (Gene amplification & Sequencing)

Table: Classification of Streptococci and Enterococci

Group/Species	Common Term	Hemolysis	Major Antigens/Structures: Lancefield Cell Wall	Major Antigens/Structures: Surface Protein	Major Antigens/Structures: Capsule	Virulence Factors	Disease
Streptococci							
Pyogenic							
Streptococcus pyogenes	Group A strep, GAS	β	A	M protein (80+)	Hyaluronic acid	M protein, leipoteichoic acid, streptococcal pyrogenic exotoxins, streptolysin O, streptokinase	Strep throat, impetigo, pyogenic infections, toxic shock, rheumatic fever, glomerulonephritis
S. agalactiae	Group B strep, GBS	β, –	B	–	Sialic acid (9)	Capsule	Neonatal sepsis, meningitis, pyogenic infections
S. equi		β	C	–	–	–	Pyogenic infections
S. bovis		–, α	D	–	–	–	Pyogenic infections
Other species		β, α, –	E-W	–	–	–	Pyogenic infections
Pneumococcus							
S. pneumoniae	Pneumococcus	α	–	Choline-binding protein	Polysaccharide (90+)	Capsule, pneumolysin, neuraminidase	Pneumonia, meningitis, otitis media, pyogenic infections
Viridans and Nonhemolytic							
S. sanguis		α	–	–	–	–	Low virulence, endocarditis
S. salivarius		α	–	–	–	–	Low virulence, endocarditis
S. mutans		α	–	–			Dental caries
Other species		α, –	–	–	–	–	Low virulence, endocarditis
Enterococci							
Enterococcus faecalis	Enterococcus	–, α	D	–	–	–	Urinary tract, pyogenic infections
E. faecium	Enterococcus	–, α	D	–	–	–	Urinary tract, pyogenic infections
Other species		–, α	D, –	–	–	–	Urinary tract, pyogenic infections

- **Group A beta -Hemolytic Streptococci (*Streptococcus pyogens)***
  - S. pyogenes, the most clinically important member of this group of gram-positive cocci,
  - is one of the most frequently encountered bacterial pathogens of humans worldwide.
  - It can invade apparently intact skin or mucous membranes, causing some of the most rapidly progressive infections known.
  - A low inoculum suffices for infection. Some strains of S. pyogenes cause postinfectious sequelae, including rheumatic fever and acute glomerulonephritis.

- Nasopharyngeal carriage is common especially in colder months and particularly among children.
- its habitat is infected patients, and also normal human carriers in whom the organism resides on skin and mucous membranes.

Morphology

- S. pyogenes occur as long chains when recovered from liquid culture, but may appear as individual cocci, pairs, or clusters of cells in Gram stains of samples from infected tissue.
- **Toxin production**: these bacteria produce the following toxins
  - Streptolysin O
  - Streptolysin S
  - Streptokinase
  - Hyaluronidase etc

Clinical significance

- S. pyogenes is a major cause of cellulitis. Acute pharyngitis or pharyngotonsilitis:Impetigo, Erysipelas, Puerperal sepsis (a newborn),
- Acute rheumatic fever: (This autoimmune disease occurs two to three weeks after the initiation of pharyngitis. It is caused by cross-reactions between antigens of the heart and joint tissues, and the streptococcal antibody).
- Acute glomerulonephritis: (Antigen-antibody complexes on the basement membrane of the glomerulus initiate the disease). and
- Streptococcal toxic shock syndrome.

Laboratory identification

- Rapid latex antigen kits for direct detection of group A streptococci in patient samples are widely used.
- Specimens from patients with clinical signs of pharyngitis and a negative antigen detection test should undergo routine culturing for streptococcal identification.
- Depending on the form of the disease, specimens for laboratory analysis can be obtained from throat swabs, pus and lesion samples, sputum, blood, or spinal fluid.

- S. pyogenes forms characteristic small, opalescent colonies surrounded by a large zone of beta hemolysis on sheep blood agar. [Note: Hemolysis of the blood cells is caused by streptolysin S, which damages mammalian cells resulting in cell lysis.]
- This organism is highly sensitive to bacitracin,
- Serologic tests detect a patient's antibody titer to streptolysin-O (ASO test) after group A streptococcal infection.

- **Group B beta-Hemolytic Streptococci (*Streptococcus agalactiae)***
  - S. agalactiae is found in the vaginocervical tract of female carriers, and the urethral mucous membranes of male carriers, as well as in the GI tract.
  - Transmission occurs from an infected mother to her infant at birth, and venereally (propagated by sexual contact) among adults.
  - Group B streptococci are a leading cause of meningitis and septicemia in neonates, with a high mortality rate.

- Samples of blood, cervical swabs, sputum, or spinal fluid can be obtained for culture on blood agar.
- Group B streptococci are β-hemolytic, with larger colonies and less hemolysis than group A.
- Most isolates remain sensitive to penicillin G and ampicillin, which are still the antibiotics of choice.

- **Streptococcus Pneumoniae (Pneumococcus)**
  - S. pneumoniae are gram-positive, nonmotile, encapsulated cocci.
  - They are lancet-shaped, and their tendency to occur in pairs accounts for their earlier designation as **Diplococcus pneumoniae.**
  - In tissue, pus or sputum pneumococci are typically arranged in pairs (diplococci) each coccus some what elongated, and pointed at one end but rounded at the other (lanceolate) and the two members of a pair point away from each other. They are surrounded by a polysaccharide capsule.
  - S. pneumoniae is the most common cause of pneumonia and otitis media and an important cause of meningitis and bacteremia

Laboratory identification

- Specimens for laboratory evaluation can be obtained from a nasopharyngeal swab, blood, pus, sputum, or spinal fluid.
- *Str.pneumonia* resembles the streptococci already described on its nutritional and environmental requirements except its growth is facilitated in 5-10%CO2 atmosphere.
- α-Hemolytic colonies appear when S. pneumoniae is grown on blood agar overnight under aerobic conditions at 37°C.

- **Groups C & G**
  - Occur in nasopharynx
  - Cause sinusitis, bacteremia, or endocarditis
  - ß – hemolytic on blood ajar
  - Identified by rxns with specific antisera for Grps C & G
- **Entero coccus fecalis**
  - Part of normal enteric flora
  - Occasionally alpha - hemolytic

- Lancet-shaped, gram-positive diplococci are observed on a Gram stain of the sample.
- Growth of these bacteria is inhibited by low concentrations of the optochin, and the cells are lysed by bile acids (bile solubility positive).
- Capsular swelling is observed when the pneumococci are treated with type-specific antisera (the **Quellung reaction**).

Table: Usual Hemolytic, Biochemical, and Cultural Reactions of Common Streptococci and Enterococci*a*

	Susceptibility to		Bile	Bile/Esculin	
	Bacitracin	Optochin	Solubility	Reaction[b]	PYR[c]
Streptococci					
β-Hemolytic					
Lancefield group A	+	−	−	−	+
Lancefield groups B, C, F, G	−	−	−	−	−
α-Hemolytic					
S. pneumoniae	−	+	+	−	−
Viridans group	−	−	−	−	−
Nonhemolytic	−	−	−	−	−
Enterococci	−	−	−	+	+

[a] All are tests commonly substituted for serologic identification in clinical laboratories.

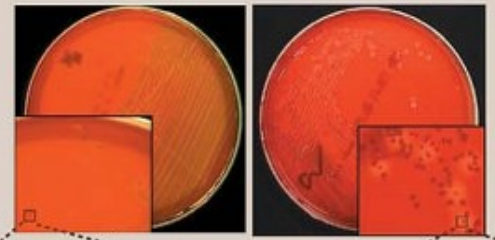
[b] Tests for the ability to grow in bile and reduce esculin.

[c] PYR = pyrrolidonyl arylamidase test.

Gram (+) cocci

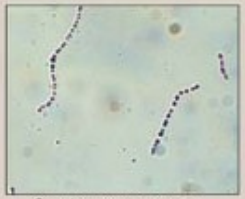


Streptococcus species





α-Hemolytic streptococci on blood agar



Streptococcus pyogenes (Gram stain)



β-Hemolytic streptococci on blood agar

- **Gram-positive**
- Ovoid to spherical in shape, occurring as pairs or chains
- Nonmotile, catalase-negative
- Most are facultative anaerobes, but grow fermentatively even in the presence of oxygen
- Culture on blood agar

Streptococcus pyogenes
(Group A, β-hemolytic)

- Acute pharyngitis or pharyngotonsillitis
- Acute rheumatic fever
- Erysipelas
- Puerperal sepsis
- Invasive group A streptococcal (GAS) disease

PENICILLINS (CEPHALOSPORINS, TETRACYCLINES, AMINOGLYCOSIDES) — **1** Penicillin G[1]

MACROLIDES (FLUOROQUINOLONES, OTHER) — **2** Clarithromycin or azithromycin[2]

[1] *S. pyogenes* has not acquired resistance to penicillin G.
[2] For penicillin-allergic patient

Streptococcus agalactiae
(Group B, β-hemolytic)

- Meningitis and septicemia in neonates
- Endometritis
- Septicemia or pneumonia in individuals with impaired immune systems
- Diabetic foot infections

PENICILLINS (CEPHALOSPORINS, TETRACYCLINES) — **1** Penicillin G[3]

AMINOGLYCOSIDES (MACROLIDES, FLUOROQUINOLONES, OTHER) — **2** An aminoglycoside[4]

[3] All isolates remain sensitive to penicillin G and ampicillin.
[4] In life-threatening infections, an aminoglycoside can be added to the regimen.

Streptococcus pneumoniae
(α-hemolytic)

- Acute bacterial pneumonia
- Otitis media
- Meningitis

PENICILLINS — **1** Penicillin G[5]

Cephalosporins (CEPHALOSPORINS, TETRACYCLINES, AMINOGLYCOSIDES, MACROLIDES, FLUOROQUINOLONES) — **1** Cefotaxime; **1** Ceftriaxone

OTHER — **2** Vancomycin[6]

[5] Penicillin G has been the drug of choice, but resistant strains are regularly seen.
[6] Most resistant strains remain sensitive to vancomycin.



Facial erysipelas



Impetigo



Streptococcal pharyngitis