further classified into more than 100 serotypes based on M protein present in their cell wall.

Genotyping: Based on *emm* gene (gene encoding M protein); group A streptococci can be typed into >200 genotypes.

STREPTOCOCCUS PYOGENES

S. pyogenes is the only species under Lancefield's group A *Streptococcus* (GAS). It is associated with a variety of suppurative infections and can also trigger post-infectious nonsuppurative complications such as acute rheumatic fever and acute glomerulonephritis.

Virulence Factors and Pathogenicity

Virulence factors of *S. pyogenes* can be categorized into cell wall antigens, toxins and enzymes.

Cell Wall Antigens

Cell wall of *S. pyogenes* is composed of (Fig. 22.2):

- Inner **thick peptidoglycan layer**: It confers cell wall rigidity, induces inflammatory response and has thrombolytic activity
- Middle layer of group specific **C-carbohydrate antigen**
- Outer layer of **protein** and **lipoteichoic acid** (helps in adhesion).

Outer Protein Layer

Several protein antigens such as M, T and R proteins have been identified in the outer protein layer.

M protein: It is the principle virulence factor of group A *Streptococcus*.

- It inhibits phagocytosis (by inhibiting opsonization via alternate complement pathway)
- It binds to fibrinogen which together bind to β2 integrins of neutrophils leading to release of inflammatory mediators that induce vascular leakage; causing streptococcal toxic shock syndrome
- Antibody to M protein is protective in nature and promotes phagocytosis
- Based on M protein (especially, its variable amino terminal end), GAS can be typed into around 100 serotypes
- M protein is further divided into two classes—Class I and Class II. **Antibodies to class I M protein** are responsible for pathogenesis of rheumatic fever.

T and R proteins are other outer proteins of GAS; they are not associated with pathogenesis.

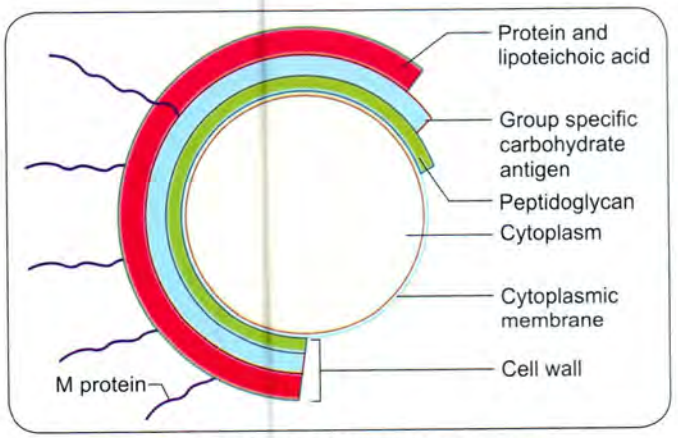


Fig. 22.2: Cell wall of *Streptococcus pyogenes*

Other Cell Wall Proteins

Other cell wall proteins include:

- M associated protein
- Hair-like fimbriae (consist of M protein along with teichoic acid)—project from the cell wall and help in adhesion
- F factor (fibronectin binding protein) helps in adhesion.

Capsule

Some strains of group A *Streptococcus* are capsulated, made up of hyaluronic acid. These strains produce mucoid colonies. Capsule is antiphagocytic, but not antigenic. It helps group A streptococci to colonize the pharynx by binding to CD44, a hyaluronic acid-binding protein expressed on human pharyngeal epithelial cells.

Toxins

Hemolysins

β hemolytic streptococci such as group A, C and G produce two hemolysins—streptolysin-O and streptolysin-S (Table 22.1). They cause RBC membrane lysis, that leads to complete β hemolysis surrounding the colonies.

Streptococcal Pyrogenic Exotoxin (SPE)

It is so named because it induces fever (pyrogenic). It is responsible for the pathogenesis of certain streptococcal infections such as scarlet fever, necrotizing fasciitis and toxic shock syndrome.

- It can be typed into three antigenic distinct subtypes—SPE-A, B and C
- SPE-A and C are bacteriophage coded; whereas SPE-B is chromosomally mediated
- SPE-A and C are superantigens; like staphylococcal toxin (TSST-1), they also act as T cell mitogens which induce a massive release of cytokines causing fever, shock and tissue damage
- **Dick test:** SPE was previously called as **erythrogenic** or **scarlet fever** toxin because its intradermal injection in susceptible children produced local erythema.

Table 22.1: Differences between streptolysin-O and streptolysin-S

Streptolysin (SL-O)	Streptolysin (SL-S)
• Oxygen labile (hence named as streptolysin-O) • Heat labile	• Oxygen stable • Serum soluble (hence named as streptolysin-S)
Hemolysis is seen only in deep colonies (pour plate) as it is inactivated in presence of oxygen	Causes hemolysis on the surface of blood agar plate
It is cytotoxic for neutrophils, platelets and cardiac tissue	It has leukocidal activity
Strongly antigenic	Not antigenic
Antistreptolysin-O antibodies (ASO) are raised in most of the streptococcal infections and are used as a standard marker for retrospective diagnosis of streptococcal infections (except in glomerulonephritis and pyoderma where ASO titer is low)	Not useful for serological diagnosis of streptococcal infections
Streptolysin-O is structurally and functionally similar to: • Tetanolysin of *Clostridium tetani* • Pneumolysin of *S. pneumoniae* • Theta toxin of *Clostridium perfringens* • Listeriolysin O of *Listeria* • Cereolysin of *Bacillus cereus*	

This test was previously used to identify the individuals susceptible to scarlet fever.

Enzymes

Streptokinase (Fibrinolysin)

It activates plasminogen to plasmin, thus breaks down the fibrin barrier around the infected site, there by facilitating the spread of infection.

- Antibodies to streptokinase can be used for retrospective diagnosis of streptococcal infection
- **Therapeutic use:** Being fibrinolytic, this toxin can be used in the treatment of myocardial infarction and other thromboembolic disorders.

Streptodornase (DNase)

It breaks down the DNA, thus helps in liquefying the thick pus (containing large amount of DNA derived from nuclei of necrotic cells) and may be responsible for the serous nature of streptococcal exudates.

- **Therapeutic use:** Preparation containing streptodornase and streptokinase can be used to liquefy the thick exudates in empyema cases
- **Subtypes:** Streptodornase has four distinct subtypes DNase-A, B, C, and D; of which type-B is most antigenic
- **Diagnostic use:** Anti-DNase B antibodies can be used for retrospective diagnosis of the infection, particularly the skin infections (pyoderma) and acute glomerulonephritis where ASO titer is low.

Other Enzymes

- **Hyaluronidase (spreading factor):** It breaks down the hyaluronic acid present in tissues, thus helps in the spread of infection along the intercellular space. It is usually secreted by noncapsulated strains (such as M type 2 and 22)
- **NADase:** It acts on the coenzyme NAD (nicotinamide adenine dinucleotide). It is produced by group A, C and G. It is antigenic and leukotoxic
- **Serum opacity factor:** It is a lipoproteinase produced by a few M serotypes of *S. pyogenes*, causes opacity when applied on agar gel containing serum
- **SpyCEP:** It is a serine protease that inactivates interleukin 8, which is a neutrophil chemoattractant
- **C5a peptidase:** It is a serine protease that cleaves C5a; which is also a neutrophil chemoattractant
- Others include neuraminidase, N-acetyl glucosaminidase, esterase and phosphatase. Their pathogenic role is uncertain.

Clinical Manifestations

Group A *Streptococcus* (GAS) produces both suppurative and non-suppurative manifestations (Table 22.2).

Suppurative Complications

Respiratory Infections

Throat is the primary site of invasion by GAS. Infection occurs through respiratory droplets.

Pharyngitis (Sore Throat)

Sore throat is the most common streptococcal disease; may be localized (tonsillitis) or diffuse (pharyngitis).

- GAS is the most common cause of pharyngitis in children (20–40% of all cases)
- It is characterized by erythema and swelling of pharyngeal mucosa with purulent exudate formation
- Younger children (<3 years) manifest with a syndrome of fever, malaise, and lymphadenopathy without exudative pharyngitis
- Complications occur due to spread of infection from the pharynx to deeper tissues by direct extension, hematogenous or lymphatic routes which may lead to quinsy (peritonsillar abscess), sinusitis, otitis media, meningitis, bacteremia and postviral pneumonia.

Scarlet Fever

Scarlet fever is mediated due to streptococcal toxins such as SPE-A, B, and C. It is characterized by pharyngitis, with:

Table 22.2: Suppurative and non-suppurative manifestations of *Streptococcus pyogenes*

Suppurative	Non-suppurative
Respiratory infections: • Pharyngitis/sore throat • Pneumonia • Empyema	Acute rheumatic fever
Scarlet fever	Acute glomerulonephritis
Skin and soft tissue infections: • Impetigo (pyoderma) • Cellulitis and erysipelas	Guttate psoriasis Reactive arthritis
Deep soft tissue infections: • Necrotizing fasciitis • Streptococcal myositis • Toxic shock syndrome	PANDAS (Pediatric Autoimmune Neuropsychiatric Disorders Associated with Streptococcal infections)
Bacteremia leading to toxic shock syndrome, osteomyelitis, meningitis, etc.	
Complications: Puerperal sepsis, otitis media, quinsy, Ludwig's angina, pneumonia (postviral), etc.	

- **Characteristic rash with sandpaper feel:** Rashes may be due to direct action of the circulating toxin or as a result of hypersensitivity reaction
- Strawberry tongue (enlarged papillae on a coated tongue)
- Rash in skin folds (called Pastia's lines).

Scarlet fever has become less common now, although strains producing SPE continue to be prevalent in the community. Reasons are not clear.

Skin and Soft Tissue Infections

Impetigo

It is a superficial infection of the skin, caused primarily by group A *Streptococcus* and occasionally by other streptococci or *S. aureus*.

- Risk factors include young children, warmer months, tropical climates, poor hygiene, colonization of group A *Streptococcus* and minor trauma
- Most common sites involved are face (nose and mouth) and legs
- Individual lesions begin as red papules, which evolve quickly into vesicles and then pustular lesions that break down and coalesce to form characteristic thin papery **honeycomb-like crusts** (Fig. 22.3A). Lesions are painless and not associated with fever.

Cellulitis

It is an infection involving the skin and subcutaneous tissues.

Erysipelas: It is a form of cellulitis, characterized by a tender, bright red, swollen and indurated peau d'orange texture of involved skin (due to involvement of the superficial lymphatics) along with fever and chills. Superficial blebs or bullae may form later.

Figs 22.3A and B: Streptococcal skin infections. **A.** Impetigo; **B.** Erysipelas on malar area of face (peau d'orange skin)

Source: A. wikipedia/Åsa Thörn, B. Public Health Image Library, Atlanta, ID# 2874/ Dr. Thomas F. Sellers, Emory University/Centers for Disease Control and Prevention (CDC) (*with permission*).

- Most common sites are malar area of the face and the lower extremities (Fig. 22.3B)
- Recurrences are common, occur after many years, involving the same site.

Deep Soft Tissue Infections

Necrotizing Fasciitis

It is also known as **hemolytic streptococcal gangrene**. It involves the superficial and/or deep fascia invading the muscles (Fig. 22.4).

- **Source of the infection may be of two types:**
  1. Traumatized skin: Most commonly caused by group A *Streptococcus* alone or in mixture with *S. aureus*
  2. Gastrointestinal tract breach: It occurs due to abdominal surgery releasing the bowel flora. It is polymicrobial, involving anaerobic flora and gram-negative bacilli like *E. coli*.
- GAS is the most common cause, accounting for nearly 60% of total cases of necrotizing fasciitis. Common serotypes include M types 1 and 3 which produce streptococcal pyrogenic exotoxins
- The onset is acute and rapid, and is marked by severe pain with minimal erythema at the site of involvement. Patients present with malaise, fever, chills, and a toxic appearance in contrast to cellulitis, where the skin appears more abnormal, but tenderness is mild
- Later on (over several hours), disease tends to be more severe. Skin becomes dusky or develops mottled erythema and anesthetized (due to infarction of the cutaneous nerves induced by spreading inflammatory process) with extensive necrosis of subcutaneous tissue, fascia and muscle (Hence, GAS is also called as **flesh eating bacteria**).

Fig. 22.4: Necrotizing fasciitis of leg

Source: Department of Microbiology, JIPMER, Puducherry (*with permission*).

TREATMENT **Necrotizing fasciitis**

- It involves early drainage of inflammatory fluid and debridement of involved necrotic area along with antibiotics
- The drug of choice is penicillin G plus clindamycin.

Bacteremia

Streptococcal bacteremia occurs secondary to necrotizing fasciitis, rarely with pharyngitis or cellulitis or pneumonia. It leads to variety of focal infections including endocarditis, meningitis, septic arthritis, osteomyelitis, peritonitis, visceral abscesses and toxic shock syndrome.

Toxic Shock Syndrome (TSS)

Group A *Streptococcus* producing pyrogenic exotoxins may cause TSS secondary to soft tissue infection such as necrotizing fasciitis.

- In contrast to patients with staphylococcal TSS, the majority with streptococcal TSS are bacteremic
- The case definition of TSS includes: (i) isolation of *S. pyogenes* plus (ii) hypotension plus (iii) multiorgan (≥2) involvement.

Puerperal Sepsis

Being colonizer of female vagina, streptococci are often associated with infectious complications of childbirth, usually endometritis and associated bacteremia. Group B streptococci and anaerobic streptococci are more common to cause puerperal sepsis than GAS.

Non-suppurative Complications

Streptococcal antigens show molecular mimicry with human antigens (Table 22.3). Due to antigenic cross reactivity, antibodies produced against previous streptococcal infections cross react with human tissue to produce lesions. This accounts for a number of non-suppurative complications such as:

- Acute rheumatic fever
- Post-streptococcal glomerulonephritis (PSGN)
- Guttate psoriasis
- Reactive arthritis
- Pediatric Autoimmune Neuropsychiatric Disorders Associated with *Streptococcus pyogenes* (PANDAS).

Table 22.3: Antigenic cross reactivity between streptococcal antigens and the corresponding human antigens

Streptococcal antigen	Human antigen
Cell wall **M** protein (of serotypes M1, M5, M6, and M19)	**M**yocardium (tropomyosin and myosin)
Cell wall **C** carbohydrate	**C**ardiac valves
Cytoplasmic membrane	Glomerular vascular intima
Peptidoglycan	Skin antigens
Hyaluronic acid	Synovial fluid

Acute Rheumatic Fever

Acute rheumatic fever (ARF) occurs in people previously infected with streptococcal sore throat.

- **Pathogenesis:** It is unclear. It may be due to:
  - **Autoimmune theory:** Streptococcal antibodies cross react with the human tissue antigens (e.g. heart and joint)
  - **Cytotoxicity theory:** Streptococcal toxins (e.g. SPE) and enzymes (e.g. SL-O) are directly cytotoxic for human cardiac cells.
- **Clinical manifestations and laboratory diagnosis:**
  - It affects heart, joints, and skin. The cardiac lesions include degeneration of heart valves and formation of inflammatory myocardial lesions called as **Aschoff nodules**
  - Acute rheumatic fever is diagnosed by modified Jones criteria (Table 22.4)
  - Prognosis is variable, repeated attacks are common, hence long-term penicillin prophylaxis is indicated
  - The ASO titer is much higher in patients with ARF than that seen in patients with group A *Streptococcus* infections without ARF.

Post-streptococcal Glomerulonephritis (PSGN)

PSGN typically occurs 2–3 weeks following either pyoderma (usually by M serotypes–49, 53-55 and 59-61) or rarely following pharyngitis (caused by M serotypes 1 and 12) (Table 22.5).

- PSGN results from lodging of antigen antibody complexes on the glomerular basement membrane, followed by complement activation. As a result urine retention and renal insufficiency occurs that leads to edema, hypertension, hematuria and proteinuria

Table 22.4: Revised Jones criteria for acute rheumatic fever

Criteria	Manifestations
Major manifestations	**S**ubcutaneous nodules
	Pancarditis
	Arthritis (migrating polyarthritis)
	Chorea (CNS manifestation)
	Erythema marginatum (skin lesion)
Minor manifestations	• Clinical: Fever, arthralgia • Laboratory: Elevated ESR and C-reactive protein • ECG: Prolonged P-R interval
Supporting evidence (of previous streptococcal infection)	Elevated ASO, or A positive throat culture, or Rapid antigen test for GAS, or Recent scarlet fever
Rheumatic fever is diagnosed if:	Two major manifestations or one major and two minor manifestations plus Any one evidence of previous streptococcal infection

Abbreviations: CNS, central nervous system; ESR, erythrocyte sedimentation rate; ECG, electrocardiography; ASO, antistreptolysin O; GAS, group A *Streptococcus*.

- Patients usually have elevated streptococcal anti-DNase B antibodies
- PSGN usually occurs in children (5–12 years) and has a good prognosis.

Epidemiology

Humans are the natural reservoir for group A *Streptococcus*. It is highly communicable, affecting all age groups. Disease in neonates is uncommon, due to protective maternal antibody. Pharyngitis is more common in children of 3–15 years of age. Outbreaks occur commonly in areas with close contacts, such as schools and military barracks, etc.

LABORATORY DIAGNOSIS — *Streptococcus pyogenes*

- **Specimen collection and transport:** Depends on the site of the infection
- **Transport medium:** Pike's medium
- **Direct smear microscopy:** Pus cells with gram-positive cocci in short chains
- **Culture:**
  - Blood agar: Pinpoint colony with a wide zone of β-hemolysis
  - Selective media: Crystal violet blood agar and PNF media
  - Liquid media: Granular turbidity with powdery deposit.
- **Culture smear microscopy:** Gram-positive cocci in short chains
- **Biochemical identification**
  - Catalase negative
  - Bacitracin sensitive
  - PYR test positive.

Contd...

Table 22.5: Differences between acute rheumatic fever and post-streptococcal glomerulonephritis

Features	Acute rheumatic fever (ARF)	Post-streptococcal glomerulonephritis (PSGN)
Prior history of infection with	Pharyngitis strains	Mainly pyoderma, or rarely pharyngitis strains
Serotype	Most of the strains of group A *Streptococcus*	Pyodermal strains-49, 53–55, 59–61 and pharyngitis strains-1, 12
Immune response	Marked	Moderate
Complement level	Unaltered	Low (due to deposition in glomeruli)
Genetic susceptibility	Present	Absent
Repeated attack	Common	Uncommon
Penicillin prophylaxis	Indicated	Not indicated
Course	Progressive	Spontaneous resolution
Prognosis	Variable	Good
Hypersensitivity reaction	Type II	Type III

Contd...

LABORATORY DIAGNOSIS — *Streptococcus pyogenes*

- **Typing:**
  - *Lancefield grouping:* Shows group A *Streptococcus*
  - *Typing of group A Streptococcus:* Griffith and *emm* typing.
- **Serology:**
  - ASO antibodies
  - Anti-DNase B antibodies.
- **Antimicrobial susceptibility testing.**

Laboratory Diagnosis

Specimen Collection and Transport

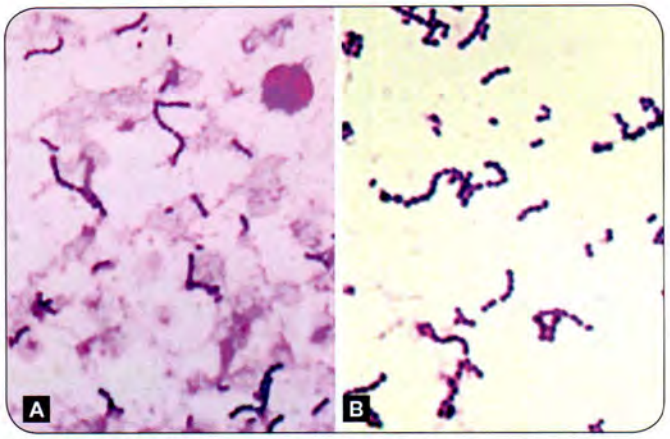
It depends on the site of the lesion. Common specimens are throat swab, pus swab, exudates and blood. Specimens are transported immediately after collection or in **Pike's transport media** (broth containing crystal violet and sodium azide).

Direct Smear Microscopy

Gram staining of pus or wound swab reveals pus cells with gram-positive cocci (0.5–1 μm) in chains (Fig. 22.5A). However, direct microscopy is not useful when *S. pyogenes* is a part of normal flora in the sample (e.g. throat swab).

Culture

The specimens are inoculated onto various media and incubated overnight at 37°C aerobically in presence of 5–10% CO_2. *S. pyogenes* is fastidious, does not grow on MacConkey agar and basal media like nutrient agar or



Figs 22.5 A and B: Streptococci **A:** In Gram stained smear of pus **B:** In culture smear showing gram-positive cocci in chains

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (*with permission*).

peptone water broth. It grows only in media enriched with blood, serum or carbohydrate.

- **Blood agar:** Colonies are small 0.5–1 mm, pinpoint, circular, semitransparent, low convex with a wide zone of β hemolysis (Fig. 22.6A). Stabbing of blood agar plate while inoculating may enhance streptolysin-O induced hemolysis. Colonies of capsulated strains are mucoid
- **Liquid media** can be used such as glucose or serum broth or brain heart infusion broth**.** Growth appears as granular turbidity with powdery deposit
- **Selective media** used are as follows:
  - **Crystal violet blood agar:** Crystal violet (0.1%) inhibits the growth of staphylococci and other bacteria
  - **PNF media:** This medium is composed of horse blood agar with polymyxin B, neomycin and fusidic acid.

Table 22.6: Differences between *Streptococcus pyogenes* and *S. agalactiae*

Characters	*S. pyogenes*	*S. agalactiae*
Lancefield grouping	Group A	Group B
Bacitracin sensitivity test	Sensitive	Resistant
PYR test	Positive	Negative
Hippurate hydrolysis test	Negative	Positive
CAMP test*	Negative	Positive
β hemolytic colonies	0.5–1 mm, pin point	Mucoid, slightly larger (2 mm)

*CAMP: Christie, Atkins, and Munch-Peterson test.

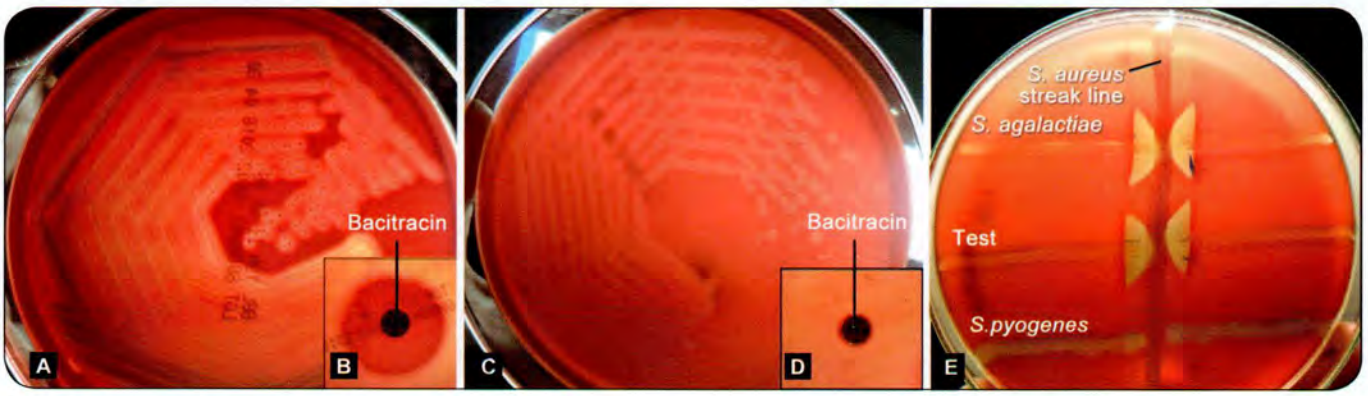
Culture Smear Microscopy

Gram stained smear from the colonies show gram-positive spherical cocci (0.5–1 μm), arranged in chains (Fig. 22.5B). Hanging drop reveals non-motile cocci.

Biochemical Tests for Identification

- **Catalase test:** Streptococci are catalase negative. This test differentiates them from staphylococci which are catalase positive
- **Bacitracin sensitivity testing:** Group A *Streptococcus* is sensitive to bacitracin 0.04 U disk (any zone of inhibition around the disk is considered as positive test), while most of other β hemolytic streptococci are resistant. Hence, it can be used as a rapid diagnostic test for GAS (Fig. 22.6B).

Various tests to differentiate GAS from group B β-hemolytic streptococci are tabulated in Table 22.6.



Figs 22.6A and B: *Streptococcus pyogenes:* **A.** Growth on blood agar with wide zone of beta hemolysis around the pin point colonies; **B.** Bacitracin sensitive

Source: Department of Microbiology, JIPMER, Puducherry (*with permission*).

Figs 22.6C to E: *Streptococcus agalactiae:* **C.** Growth on blood agar with wide zone of beta hemolysis around the colonies; **D.** Bacitracin resistant; **E.** CAMP test positive

Source: Department of Microbiology, JIPMER, Puducherry (*with permission*).

Lancefield Grouping

The biochemical identification of Group A *Streptococcus* can be further confirmed by Lancefield grouping. Lancefield grouping is extremely useful in epidemiological studies. Here, the β hemolytic streptococci are grouped serologically based on C-carbohydrate antigen. Test involves extraction of C-carbohydrate antigen followed by testing with group specific antisera.

- C-carbohydrate antigen extraction is done—either by hydrochloric acid (Lancefield's acid extraction), or by formamide (Fuller's method) or by autoclaving (Rantz and Randall method) or by an enzyme produced by *Streptomyces albus* (Maxted's method)
- Extracted antigen is tested with commercially available group specific antisera by latex agglutination test (commonly used) and ring precipitation (used previously).

Typing of Group A Streptococci

Group A *Streptococcus* can further be typed based on two methods; phenotypic method, i.e. serological (Griffith typing) and genotypic method (*emm* typing).

- **Griffith typing:** Based on M protein (especially, its variable amino terminal end), GAS can be typed into around 100 serotypes. M protein can be extracted by Lancefield's acid extraction method and typing is done with type specific antisera
- ***emm* typing:** A few strains of GAS are untypable serologically. Hence, gene coding for M protein (*emm* gene) is widely used for typing. This method is almost replacing the conventional serological typing. More than 200 emm types and >750 subtypes of GAS have been identified so far.

Serology

In rheumatic fever and poststreptococcal glomerulonephritis (PSGN), a retrospective diagnosis of streptococcal infection may be established by detecting antibodies in patient's serum.

- **ASO (Anti-streptolysin O) antibodies:** ASO titer is elevated (>200 IU/mL) in most of the streptococcal infections except pyoderma and PSGN. Previously neutralization test was followed for ASO detection, however, currently it is detected by latex agglutination test
- **Anti-DNase-B antibodies**: Titer more than 300–350 units/mL is diagnostic of PSGN and pyoderma
- Other antibodies elevated are antihyaluronidase antibodies and anti-streptokinase antibodies.

Antimicrobial Susceptibility Test

Antimicrobial susceptibility test is carried out on Mueller Hinton blood agar by disk diffusion test.

Table 22.7: Treatment of streptococcal infection

Conditions	Treatment recommended
Pharyngitis	Benzathine penicillin G, IM single dose; or Oral penicillin V for 10 days
Erysipelas/cellulitis	Mild: Procaine penicillin Severe: Penicillin G
Necrotizing fasciitis	Surgical debridement (most crucial) + Penicillin G + Clindamycin
Pneumonia and empyema	Penicillin G + drainage of empyema
Streptococcal toxic shock syndrome	Penicillin G + Clindamycin + immunoglobulin (to streptococcal pyrogenic exotoxin)
Rheumatic fever	Benzathine penicillin G, IM single dose; or Oral Penicillin V for 10 days Long-term maintenance therapy—with penicillin, once a month for duration: • 5 years or until 21 years of age (without carditis) • 10 years (with carditis) • up to 40 years of age/lifelong (with residual heart disease)
Poststreptococcal glomerulonephritis	Benzathine penicillin G, IM single dose; or oral penicillin V for 10 days
Treatment of asymptomatic carriers	
Pharyngeal carriers	Oral clindamycin for 10 days Penicillin V + rifampicin
Rectal carriers	Vancomycin + rifampicin

Abbreviation: IM, intramuscular.

TREATMENT ***Streptococcus pyogenes***

Penicillin is the drug of choice for pharyngeal infections as well as for suppurative complications. Resistance to penicillin is not reported yet.

- However, failure to penicillin treatment may occur due to: (1) noncompliance, if discontinued before 10 days of full course of oral penicillin V, (2) β lactamases produced by normal throat flora such as *Moraxella*.
- Macrolide, such as erythromycin is given to patients allergic to penicillin. However, resistance to macrolides is common.

Treatment of streptococcal infections is outlined in Table 22.7.

Prophylaxis

Long-term maintenance therapy with penicillin (alternative-sulfadiazine or erythromycin in penicillin allergy) is required for children who develop early signs of rheumatic fever. This prevents streptococcal reinfection and further damage to heart.

Vaccine

There are no licensed vaccines available for *S. pyogenes*. The advanced vaccine trials are the M protein-based 30

Table 22.8: Early and late onset group B *Streptococcus* disease in neonates

Characteristics	Early-onset disease	Late-onset disease
Age of onset	0–6 days of birth	7–90 days of birth
Increased risk following obstetric complications	Prematurity and prolonged labor	Not associated
Mode of transmission to the baby	During or before birth from the colonized maternal genital tract	Contact with a colonized mother and nursing personnel
Common clinical manifestations	Pneumonia and/or respiratory distress syndrome followed by meningitis	Bacteremia and meningitis (most common)
Common serotypes	Ia, III, V, II, Ib	Type III (most common)
Case fatality rate	4.7%	2.8%

valent vaccine (StreptAnova) and conserved M protein vaccines (the J8 vaccine and the StreptInCor vaccine).

OTHER β HEMOLYTIC STREPTOCOCCI

Group B Streptococci (S. agalactiae)

Pathogenesis and Clinical Manifestations

Approximately 30% of women are vaginal or rectal carriers of group B *Streptococcus* (GBS). Hence, the GBS infection is common in neonates and in pregnancy.

- Group B *Streptococcus* has been recognized as a major cause of neonatal sepsis and meningitis. Neonatal sepsis can be of two types—early onset and late onset type (Table 22.8)
- Infections in pregnancy can lead to peripartum fever in women
- Infections in adults generally involve elderly or people with underlying chronic illness, such as diabetes mellitus or malignancy. Common infections are cellulitis and soft tissue infections (including infected diabetic skin ulcers), urinary tract infection, pneumonia and endocarditis
- Group B *Streptococcus* has a capsular polysaccharide which can be typed into nine serotypes.

Laboratory Diagnosis

It is catalase negative like all streptococci, but exhibits the following biochemical properties that differentiate it from group A *Streptococcus* (Table 22.6).

- **CAMP positive:** CAMP factor (named after the discoverers—Christie, Atkins-Munch-Petersen) is a phospholipase produced by GBS that causes synergistic hemolysis with β hemolysin produced by *S. aureus*. When GBS is streaked on blood agar plate perpendicular to *S. aureus*, an enhanced arrowhead-shaped hemolysis is produced at their junction, pointing towards *S. aureus* streak line (see Fig. 22.6E)
- Hippurate hydrolysis test positive
- Bacitracin resistant (see Fig. 22.6D)
- PYR (Pyrrolidonyl-beta-naphthylamide) is negative
- Orange pigment production—enhanced in Islam's medium
- β hemolytic colonies are mucoid and slightly larger (2 mm) then group A streptococci (see Fig. 22.6C).

TREATMENT **Group B Streptococci**

Penicillin is the drug of choice for all GBS infections. GBS is less sensitive to penicillin than GAS, hence a higher dose of penicillin is recommended.

Prevention

Screening for anogenital colonization of GBS is recommended at 35–37 weeks of pregnancy and prophylactic ampicillin or penicillin is given to carrier mothers during delivery to reduce the risk of infection to the newborn.

Group C Streptococci

Group C streptococci commonly cause infection in animals and comprise of four species: *S. equi, S. equisimilis, S. dysgalactiae, S. zooepidemicus*. Human infection is rare.

- *S. equisimilis* can cause pharyngitis especially epidemic food-borne pharyngitis after ingestion of contaminated animal products (milk).
  Other deep infections include skin and soft tissue infections, osteomyelitis, pneumonitis, infective endocarditis, bacteremia, meningitis, epiglottitis, pericarditis, urinary tract infections and puerperal sepsis
- *S. equisimilis* is a common source of streptokinase, which is used for thrombolytic therapy.

Group F Streptococci

They are also called **minute streptococci**. They grow poorly on blood agar, occasionally cause suppurative infection.

Streptococcus MG is an α hemolytic strain belonging to this group. Demonstration of antibodies to *Streptococcus* MG in the patient's sera has been used for diagnosis of primary atypical pneumonia (caused by *Mycoplasma pneumoniae*).

Group G Streptococci

They are throat commensals, occasionally cause puerperal sepsis, neonatal infection, skin and soft tissue infections, tonsillitis, and endocarditis.

Group D Streptococci

Group D streptococci comprise of enterococci (fecal streptococci, described below) and non-enterococci

(*S. gallolyticus* and *S. equinus*). They possess the common group D lipoteichoic acid antigen.

S. gallolyticus (formerly *S. bovis*) is commensal of intestine of animals. It has been occasionally associated with various human infections such as endocarditis, colorectal cancer and spontaneous bacterial peritonitis.

ENTEROCOCCUS

Enterococci were initially grouped under group D *Streptococcus*, but later, it has been reclassified as a separate genus *Enterococcus*. Based on the molecular structure; it is now placed under a new family; Enterococcaceae.

Both enterococci and non-enterococcal group D streptococci give a positive **bile aesculin hydrolysis test** (they grow in the presence of 40% bile and hydrolyse aesculin to aesculetin that combines with ferric chloride to produce black colored complex). However, they differ in many other properties (Table 22.9).

Virulence Factors

Enterococci are part of normal flora of human intestine, biliary tract and to lesser extent vagina and male urethra. At the same time, enterococci are also becoming increasingly important agents of human disease especially in hospitals mainly because of their resistance to antibiotics. *E. faecalis* is the most common species found in clinical specimens, whereas *E. faecium* is more drug resistant than *E. faecalis*. They exhibit a number of virulence factors such as:

- **Cytolysin/hemolysin:** They lyse the sheep and human RBCs
- **Aggregation substances or pheromones:** They help in clumping of adjacent cells to facilitate plasmid exchange (transfers drug resistance)
- **Extracellular surface protein (ESP):** It helps in adhesion to bladder mucosa
- **Common group D lipoteichoic acid antigen:** It induces cytokine release such as tumor necrosis factor α (TNFα)
- **Coccolysin:** It inactivates endothelin, a vasoactive peptide.

Table 22.9: Comparing enterococci and Group D streptococci

Features	Enterococci	Non-enterococcal Group D streptococci
Group specific D Ag	Present	Present
Bile aesculin hydrolysis	Positive	Positive
In presence of • 6.5% NaCl • pH 9.6 • at 45°C • at 10°C	Grows	Does not grow
PYR test	Positive	Negative
Drug resistance	Marked	Uncommon
Existence as normal intestinal flora	More common	Less common
Pathogenicity	Marked	Less

Abbreviation: PYR, Pyrrolidonyl-beta-naphthylamide.

Clinical Manifestations

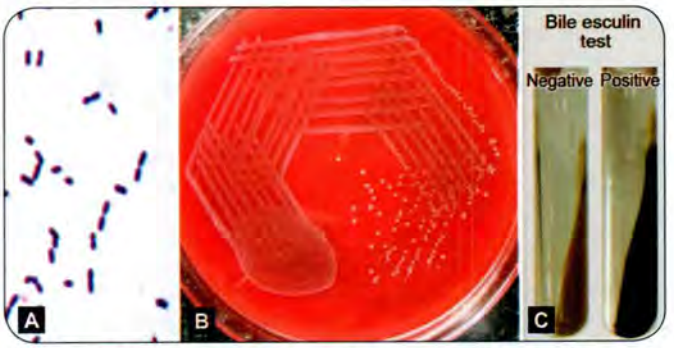
Enterococci are one of the major hospital acquired pathogen, produce various infections such as:

- Urinary tract infections (cystitis, urethritis, pyelonephritis and prostatitis)
- Bacteremia and mitral valve endocarditis (in intravenous drug abusers)
- Intra-abdominal, pelvic and soft tissue infections
- Late-onset neonatal sepsis and meningitis
- Infection on burn surface.

Laboratory Diagnosis

Enterococci show the following characteristics that help in their identification:

- They are gram-positive oval cocci (Fig. 22.7A) arranged in pairs; cocci in a pair are arranged at an angle to each other (**spectacle-shaped** appearance)
- Non-motile cocci (except *E. gallinarum* and *E. casseliflavus*)
- **Blood agar:** It produces non-hemolytic (Fig. 22.7B), translucent colonies (rarely produces α or β hemolysis)
- **MacConkey agar:** It produces minute magenta pink colonies
- **Nutrient agar:** It grows poorly
- Bile aesculin hydrolysis test is positive (Fig. 22.7C)
- PYR (Pyrrolidonyl-beta-naphthylamide) test is positive
- They can grow in presence of extremes of conditions such as—6.5% NaCl, 40% bile, pH 9.6, 45°C and 10°C



Figs 22.7A to C: *Enterococcus.* **A.** Gram-positive oval cocci in pairs; **B.** translucent non-hemolytic colonies on blood agar; **C.** Bile aesculin hydrolysis test (left—negative, right—positive result, black color due to aesculin hydrolysis)

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (*with permission*).

Table 22.10: Comparison between *Enterococcus faecalis* and *E. faecium*

Features	*E. faecalis*	*E. faecium*
Mannitol	Fermented	Fermented
Arabinose	Not fermented	Fermented
Sorbitol	Fermented	Not fermented
Pyruvate	Fermented	Not fermented

- **Heat tolerance test:** They are relatively heat resistant, can survive 60°C for 30 minutes
- **Groups:** According to Facklam and Collins classification, enterococci can be divided into five groups—group I to V based on mannitol fermentation and arginine dihydrolase test. *E. faecalis* and *E. faecium* belong to group II, which can be further differentiated by several biochemical properties (Table 22.10).

TREATMENT ***Enterococcus***

Enterococci are resistant to penicillins, aminoglycosides, sulfonamides, cephalosporins and cotrimoxazole.

- Resistance to penicillin and aminoglycoside is overcome by combination therapy (e.g. ampicillin plus gentamicin) due to synergistic effect and this remains the standard therapy for life-threatening enterococcal infections
- This combination therapy fails if the isolate is found resistant to either ampicillin or high level aminoglycoside in vitro
- Vancomycin is usually indicated in such cases, but resistance to vancomycin has also been reported
- If resistant to vancomycin: then treatment options available are linezolid, streptogramins (active against *E. faecium*, but not to *E. faecalis*) and daptomycin.

Vancomycin Resistant Enterococci (VRE)

Vancomycin resistance in enterococci has been increasingly reported now a days.

- The prevalence of VRE varies with time and place. A report in 2016 revealed that among hospitalized patients the VRE frequency is high in America (35%) and low in Europe (4%) and moderate (10–15%) in Asian countries. In India, the VRE rate varies from 5–10%
- VRE is mediated by *van* gene, which alters the target site for vancomycin present in the cell wall; i.e. D-alanyl-D-alanine side chain of peptidoglycan layer (which is the usual target site for vancomycin), is altered to D-alanyl-D-serine or D-alanyl-D-lactate. This altered side chains have less affinity for binding to vancomycin
- ***Van* gene has 11 genotypes:** (*van* A, B, C1-C3, D, E, G, L, M and N). The *van* A and *van* B genotypes predominate worldwide; expressed by *E. faecalis* and more commonly by *E. faecium*
- All *van* genes are located on transposons and are inducible; except type C and N (chromosomal and constitutive).
  - Strains with *van* A gene show high level resistance to both glycopeptides vancomycin and teicoplanin

Contd...

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  - Strains with *van* B gene show low level resistance to vancomycin, but sensitive to teicoplanin
  - *E. gallinarum* and *E. casseliflavus* possess *van* C genes and they show intrinsic resistance to both the glycopeptides.

VRE Carriers

VRE often colonizes the intestine and poses a risk of transmitting to other patients.

- Screening for VRE: It is recommended for high risk patients from ICUs and transplantation units
- Detection: Rectal swab is collected and subjected to (i) Sodium azide agar added with 6 µg/mL of vancomycin or (ii) chromogenic media or (iii) PCR for detection of *van* gene
- Management: Ensure infection control measures such as hand hygiene and isolation precautions (refer Chapter 53). Treatment (i.e. decolonization) is not recommended for VRE carriers.

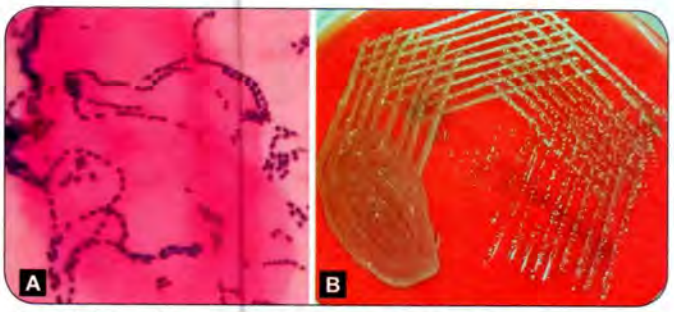
VIRIDANS STREPTOCOCCI

Viridans streptococci are commensals of mouth and upper respiratory tract. Usually, they are nonpathogenic, however occasionally cause disease such as:

- **Dental caries**: It is mainly caused by *S. mutans* which breaks down dietary sucrose to acid and dextrans. Acid damages the dentine, while adhesive dextran binds together with food debris, mucus, epithelial cells and bacteria to produce **dental plaques**
- **Subacute bacterial endocarditis (SABE):** Viridans streptococci are the most common cause of SABE. The commensal viridans streptococci (*S. sanguis*) in the oral cavity can enter blood to cause transient bacteremia while chewing, tooth brushing and dental procedures that can account for the predilection of these organisms to cause endocarditis
- ***S. milleri* group** (includes *S. intermedius*, *S. anginosus*, and *S. constellatus*): Produce suppurative infections particularly abscesses of brain and abdominal viscera.

Laboratory Diagnosis

- On Gram stain, they appear as long chains of gram-positive cocci (Fig. 22.8A)
- They produce minute α hemolytic green color (rarely non-hemolytic) colonies on blood agar (*"viridis"* means green Fig. 22.8B)
- They can be differentiated from *S. pneumoniae* (which is also α hemolytic) by a number of tests (Table 22.11).



Figs 22.8A and B: Viridans streptococci. **A.** Gram-positive cocci in long chains; **B.** α hemolytic colonies on blood agar

Source: Department of Microbiology, Pondicherry Institute of Medical Sciences, Puducherry (*with permission*).

Table 22.11: Differences between *Streptococcus pneumoniae* and Viridans streptococci

Features	*S. pneumoniae*	Viridans streptococci
Arrangement	Gram-positive cocci in pairs	Gram-positive cocci in long chains
Morphology	Lanceolate or flame shaped	Round/oval
Capsule	Present	Absent
On blood agar	Draughtsman or carom coin colony	Convex-shaped colony
Liquid medium	Uniform turbidity	Granular turbidity
Bile solubility	Soluble in bile	Insoluble in bile
Inulin fermentation	Fermenter	Non fermenter
Optochin	Sensitive	Resistant
Mice pathogenicity	Pathogenic	Non-pathogenic

TREATMENT **Viridans streptococci**

They are usually sensitive to penicillin except in neutropenic patients with bacteremia, where vancomycin is given.

PNEUMOCOCCUS

Streptococcus pneumoniae (commonly referred to as pneumococcus) is the leading cause of lobar pneumonia, otitis media in children and meningitis in all ages. They are α hemolytic and may present as commensals in human upper respiratory tract. They differ from α hemolytic viridans streptococci in many ways such as their shape (lanceolate-shaped diplococci), bile solubility, optochin sensitivity and presence of a polysaccharide capsule (Table 22.11).

Virulence Factors and Pathogenesis

S. pneumoniae possesses a number of virulence factors such as:

- **Capsular polysaccharide:** It is the principal virulence factor, protects the cocci from phagocytosis. It is type specific (about 95 capsular serotypes are recognized). Being soluble, it diffuses into culture media, tissue and exudates, hence also called **soluble specific substance**
- **C carbohydrate antigen** (C-polysaccharide or C-substance): It is species specific, made up of ribitol, teichoic acid linked to fragments of peptidoglycan. In sera of patients with acute inflammation, a beta globulin appears (synthesized by liver) that precipitates with pneumococcal C-antigen, hence it is named as **C-reactive protein (CRP)**. However, it is not an antibody to C-antigen. CRP is a non-specific acute phase reactant protein, can be raised in many inflammatory conditions (infective as well as noninfective conditions, such as malignancies) and disappears once the inflammation subsides
- **Pneumolysin**: It is a membrane damaging toxin, which inhibits neutrophil chemotaxis and phagocytosis, similar to streptolysin-O
- **Autolysin:** It is an amidase enzyme that cleaves its own peptidoglycan leading to autolysis of cells. The activity is enhanced in presence of bile salts and other surface active agents. This property is responsible for characteristic bile solubility and draughtsman appearance of pneumococcal colonies. Release of cell wall fragments lead to a self-perpetuating inflammatory response that contributes to the pathogenesis
- **Other virulence factors:**
  - **Pneumococcal surface protein A (PspA):** It prevents complement activation. It shows some similarities to the M protein of *S. pyogenes*
  - **IgA protease:** It cleaves IgA, present in the respiratory mucosa, thus facilitates entry
  - **Pneumococcal surface protein C (PspC):** It is also known as *choline-binding protein A* (CbpA). It binds to factor H and accelerates the breakdown of C3 complements
  - **Adhesins:** These include sialidase (neuraminidase) and pneumococcal surface adhesin A (PsaA).
  - Choline-binding protein- helps in adhesion.

Clinical Manifestations

Pneumococci colonize the human nasopharynx at an early age. From the nasopharynx, the bacteria spread either via the bloodstream to distant sites (e.g. brain, joint, bones and peritoneal cavity) or spread locally to cause otitis media or pneumonia.

Various manifestations include:

- **Lobar pneumonia:** *S. pneumoniae* is the most common cause of lobar (alveolar) pneumonia. Though starts as noninvasive illness due to contiguous spread from the nasopharynx, it soon becomes bacteremic and invasive. Patients present with productive purulent cough, fever and chest pain. Important signs are dullness