



## 15

## STREPTOCOCCUS

## 15.1 INTRODUCTION

## Streptococci

Streptococci are Gram-positive cocci arranged in chains or pairs. They are part of the normal flora of humans and animals. Some of them are human pathogens. The most important of them is *Streptococcus pyogenes* causing pyogenic infections, with a characteristic tendency to spread, as opposed to staphylococcal lesions, which are typically localized. It is also responsible for the nonsuppurative lesions, acute rheumatic fever and glomerulonephritis which occur as sequelae to infection.



Fig. 15.1

Cocci in chains were first seen in erysipelas and wound infections by Billroth (1874), who called them *Streptococci* (streptos, meaning twisted or coiled). Ogston (1881) isolated them from acute abscesses, distinguished them from staphylococci and established their pathogenicity by animal inoculation. Rosenbach (1884) isolated the cocci from human suppurative lesions and gave them the name *Streptococcus pyogenes*.



## OBJECTIVES

After reading this lesson, you will be able to:

- describe the morphological and physiological characteristics of bacteria in the genus *Streptococcus*
- list the feature by which *Streptococcus pyogenes*, *Streptococcus agalactiae*, *Streptococcus mutans* and *Enterococcus* (formerly *Streptococcus*) *faecalis* can be identified.

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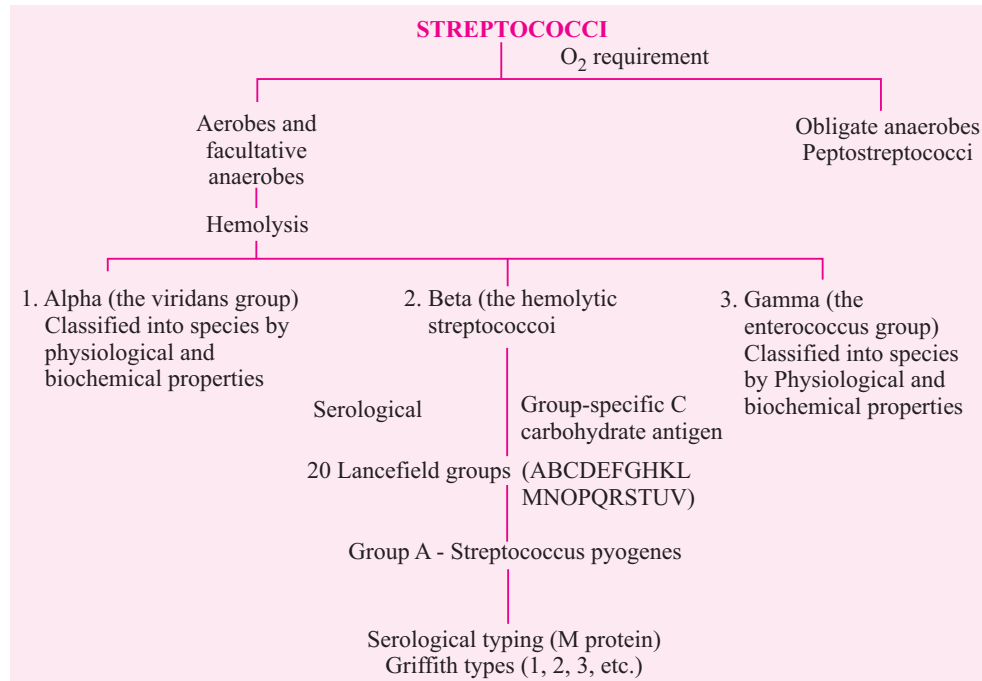


Notes

## Streptococcus

- describe the spectrum of diseases caused by the various *streptococci*.
- describe & compare suppurative & nonsuppurative Infections of *streptococci*.

### 15.2 CLASSIFICATION



Several systems of classification have been employed but in medical bacteriology the following method is useful.

Streptococci are first divided into obligate anaerobes and facultative anaerobes. The former are designated peptostreptococci and are discussed in a later chapter. The aerobic and facultative anaerobic streptococci are classified on the basis of their hemolytic properties. Brown (1919) categorized them into three varieties based on the growth in 5% horse blood agar pour plate cultures.

#### 1. Alpha ( $\alpha$ ) hemolytic streptococci

Alpha ( $\alpha$ ) hemolytic streptococci produce a greenish discoloration with partial hemolysis around the colonies. The zone of lysis is small (1 or 2 mm wide) with indefinite margins, and unlysed erythrocytes can be made out microscopically within this zone. These are known as 'viridans streptococci' or *Streptococcus viridians* (from 'viridis' meaning green). The alpha streptococci are normal commensals in the throat, but may cause opportunist infections rarely. Pneumococcus (*Streptococcus pneumonia*) is also an alpha hemolytic streptococcus.

## 2. Beta ( $\beta$ ) hemolytic streptococci

Beta ( $\beta$ ) hemolytic streptococci produce a sharply defined, clear, colourless zone of hemolysis, 2-4mm wide, within which red cells are completely lysed. The term 'hemolytic streptococci' strictly applied only to beta lytic strains. Most pathogenic streptococci belong to this group.

## 3. Gamma ( $\gamma$ ) or nonhemolytic streptococci

Gamma ( $\gamma$ ) or nonhemolytic streptococci produce no change in the medium and so are sometimes referred to as 'indifferent streptococci'. They include the fecal streptococci (enterococci, *Str faecalis*) and related species. They are called the 'enterococcus group'.

Hemolytic streptococci were classified by Lancefield (1933) serologically into groups based on the nature of a carbohydrate (C) antigen on the cell wall. These are known as Lancefield groups, twenty of which have been identified so far and named A-V (without I and J). The great majority of hemolytic streptococci that produce human infections belong to group A. Hemolytic streptococci of group A are known as *Streptococcus pyogenes*. These may be further subdivided into types based on the protein (M, T and R) antigens present on the cell surface (Griffith typing). About eighty types of *Str pyogenes* have been recognized as far (types 1,2,3 and so on).

Table 1 shows the medically important streptococci and their characteristics.

## 15.3 STREPTOCOCCUS PYOGENES

**Morphology :** The individual cocci varies in size from 0.5 – 1.00 mm in diameter whereas they are spherical and oval in shape. They are arranged in chains because chain formation is due to cocci dividing in one plane & daughter cell failing to separate completely. Streptococcus has been classified as *Str. longus* (long chain) & *Str. brevis* (short chain.). Streptococcus are gram positive, non-motile, non-spore forming and capsulated.

**Culture Characteristics :** It is an aerobes & facultative anaerobes growing best at 37°C (range 22 – 42). It is exacting in nutritive requirements, growth occurring in media containing fermentable carbohydrate & enriched with blood & serum. When cultured on blood agar it gives colonies which are circular, semitransparent, low convex disc with area of clear hemolysis around it. Virulent strain on fresh isolation from lesion, produce a 'matt' (finely granular) colony, while avirulent strains form 'glossy' colonies. Strains with well marked capsules produce 'mucoid' colonies, corresponding in virulence to the matt type. When cultured in liquid medium like glucose or serum broth it shows granular turbidity with powdery deposit. No pellicle is formed.



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**Table 15.1:** Medically important streptococci and their characteristics

Species or Common name	Lancefield group	Hemolysis	Habitat in human hosts	Laboratory tests	Common diseases caused
Str. pyogenes	A	Beta	Throat, Skin	Bacitracin sensitive; PYR test positive; Ribose not fermented	URTI, Pyoderma, RF, Glomerulonephritis
Str. agalactiae	B	Beta	Female genital tract, rectum		Neonatal meningitis, septicemia
Str. equisimilis	C	Beta	Throat		Pharyngitis, endocarditis
Str. anginosus	A,C,F,G Untypable	Beta (alpha, gamma)	Throat, colon, female genital tract		Pyogenic infections
Enterococcus sp (Str faecalis and other enterococci)	D	Gamma (alpha, beta)	Colon		UTI, endocarditis, suppurative infections
Nonenterococcal Group D species (str bovis)	D	Gamma Alpha (gamma)	Colon		Endocarditis
Viridans streptococci (many species)	Not types		Mouth, colon, female genital tract	Optochin resistant, species classification, biochemical properties	Endocarditis (Str. sanguis); dental caries (str. mutans)



### INTEXT QUESTIONS 15.1

1. Cocci in chains were first seen in which infections ?
2. What is the basis for classification of Hemolytic streptococci ?
3. Which type of colonies are produced by Beta Hemolytic Streptococci ?

4. What type of colonies formed by Virulent & Avirulent strain of streptococcus?
5. Which is the Common Causative URTI Organism for ?

**Biochemical reaction :** Streptococci ferments all the sugars like Sorbitol, Maltose, Lactose & Mannitol producing acid but no gas. It is Catalase negative.

**Resistance :** Str. Pyogenes is a delicate organism, easily destroyed by heat (54°C for 30 minutes). It dies in a few days in cultures, unless stored at a low temperature (4°C), preferably in Robertson's cooked meat medium. It can, however, survive in dust for several weeks if protection from sunlight. It is rapidly inactivated by antiseptics. It is more resistant to crystal violet than many bacteria, including Staph aureus.

**Toxins, Enzymes & other virulence factors :** Streptococcus *pyogenes* produces several types of exotoxins & enzymes those act as virulence factors. Also young protein act as a virulence factor by inhibiting phagocytosis. The C polysaccharide has been shown to have a toxic effect on connective tissue in experimental animals.

**Hemolysins :** Streptococci produce two hemolysins, streptolysin 'O and 'S'. Streptolysin O is so called because it is oxygen labile. It is inactive in the oxidized form but may be reactivated by treatment with mild reducing agents. On blood agar, streptolysin O activity is seen only in pour plates and not in surface cultures. It may be obtained in the active state by growing streptococci in broth containing reducing agents such as sodium hydrosulphite. It is also heat labile. It appears to be important in contributing to virulence. It is lethal on intravenous injection into animals and has specific cardiotoxic activity. It has leucotoxic activity also. In its biological action, streptolysin O resembles the oxygen labile hemolysins of *Cl. perfringens*, *Cl. tetani* and the *pneumococcus*.

Streptolysin O is antigenic and antistreptolysin O appears in serum after streptococcal infection, which is very important in diagnosis. Streptolysin S is so called because it is soluble in serum. It shows stability with oxygen, dry heat. It is responsible for hemolysis seen on the surface of the blood agar plates. It also has leucocidal activity.

Pyrogenic exotoxin (erythrogenic or dick or scarletinal toxin) is a toxin named erythrogenic because its intradermal inoculation in susceptible individual causes erythematous reaction. This test is known as "dick test". This test is used to identify the children susceptible to scarlet fever so, named as "Scarletinal toxin". This toxin induces fever so named as streptococcal pyrogenic exotoxin. It has three types A, B, C. It is a super antigen so act as T-cell mitogens and causes rapid release of inflammatory cell which cause wide spread manifestation.

**Notes**



## Notes

Streptokinase is an enzyme which acts as a toxin which promotes the lysis of human fibrin clot by activating plasminogen to plasmin. It acts as a diagnostic marker as it is antigenic in nature so antibodies are produced in convalescent areas. This is helpful in retrospective study. It shows biological significance during infection, by breaking down the fibrin barrier around tissue & thus helps in spread of infection. It shows therapeutic significance in myocardial infarction & other thromboembolic disorders.

**Deoxyribonuclease (streptodornase, DNAase) :** It is an enzyme which causes depolymerisation of DNA also showing diagnostic significance as Streptokinase. It shows biological significance because pyogenic exudates contain large amount of DNA. DNAase causes liquefaction of pus & its serous character. It also liquefies thick pus in empyema which is a therapeutically important.

**NADase (Diphosphopyridine nucleotidase) :** It is an enzyme which acts on NAD & liberates nicotinamide from it. It is a diagnostic as it is antigenic in nature. Biological significance is not known.

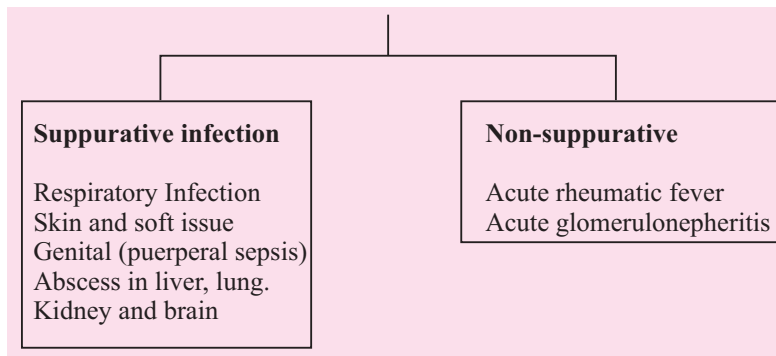
**Hyaluronidase :** It is an enzyme which breaks down the hyaluronic acid of tissue. It is a biological significance it helps in spread of infection.

**Serum opacity factor :** Some M types of Str. Pyrogen produce lipoproteinase that produces opacity when applied to agar gel containing the horse serum. That is known as Serum opacity factor.

**Antigenic Structure :** The capsule when present inhibits phagocytosis. It is not antigenic in human beings. The cell wall is composed of an outer layer of protein and lipoteichoic acid, a middle layer of group-specific carbohydrate and an inner layer of peptidoglycan. The peptidoglycan (mucoprotein) is responsible for cell wall rigidity. It also has some biological properties such as pyrogenic and thrombolytic activity. Serological grouping of streptococci depends on the C carbohydrate. Str. pyogenes belongs to group A. As this antigen is an integral part of the cell wall, it has to be extracted for grouping by a precipitation test with group antisera. Several protein antigens have been identified in the outer part of the cell wall. Str. pyogenes can be typed based on the surface proteins M, T and R. The M Protein is the most important of these. It acts as a virulence factor by inhibiting phagocytosis. It is antigenic. The antibody to the M protein promotes phagocytosis of the coccus and is therefore protective. The M protein is heat and acid stable but susceptible to tryptic digestion. It can be extracted by the Lancefield acid extraction method and typing is done with type-specific sera. The T protein is an acid labile, trypsin resistant antigen present in many serotypes of Str. pyogenes. It may be specific but many different M types possess the same T antigen. It is usually demonstrated by the slide agglutination test using trypsin-treated whole streptococci. Hair-like pili (fimbria) project through the capsule of group A streptococci. The pili consist partly of M protein and are covered with lipoteichoic acid which is important in the attachment of streptococci to epithelial cells.

**Pathogenicity**

Str. Pyrogen produces pyrogenic infection that spread locally along with lymphatic & blood serum. They produce mainly two types of lesions.



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**Respiratory Infection:** The primary site of invasion of the human body of Str pyogenes is the throat. Sore throat is the most common of the streptococcal diseases. It may be localized as tonsillitis or may involve the pharynx more diffusely (pharyngitis). Virulent group A streptococci adhere to the pharyngeal epithelium by means of lipoteichoic acid covering the surface pili. The glycoprotein fibronectin on the epithelial cells probably serves as the lipoteichoic acid ligands. Tonsillitis is more common in older children and adults than in younger children, who commonly develop diffuse pharyngitis. Localisation is believed to be favoured by hypersensitivity due to prior contact.

**Chronic Tonsillitis**

From the throat, streptococci may spread to the surrounding tissues, leading to suppurative complications such as otitis media, mastoiditis, quinsy, Ludwig's angina and suppurative adenitis.

**Skin and soft tissue infections**

Str pyogenes causes a variety of suppurative infections of the skin, including infection of wounds or burns, with a predilection to produce lymphangitis and cellulitis. Infection of minor abrasions may at times lead to fatal septicemia.

The two typical streptococcal infections of the skin are Erysipelas and Impetigo.

**Erysipelas :** It is a diffuse infection involving the superficial lymphatics. The affected skin, which is red, swollen and indurated, is sharply demarcated from the surrounding healthy area. While erysipelas is rare and seen only in older patients, impetigo is found mainly in young children. The skin area shows erythema with edema. One attack does not give protection & recurrent infection in same area occurs in some person.

**Impetigo :** It is caused by Str pyogenes it is a superficial crushed spot, especially in children usually less than 1 Inch in diameter. Impetigo and streptococcal infection of scabies lesions are the main causes of acute glomerulonephritis in

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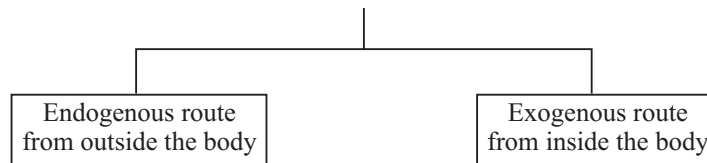


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children in the tropics. It last for 1-2 week. It heals spontaneously without leaving scar.

**Genital Infections :** Both aerobic & anerobic streptococci are normal inhabitants of female genital tract. They are important causative organism of puerperal sepsis.



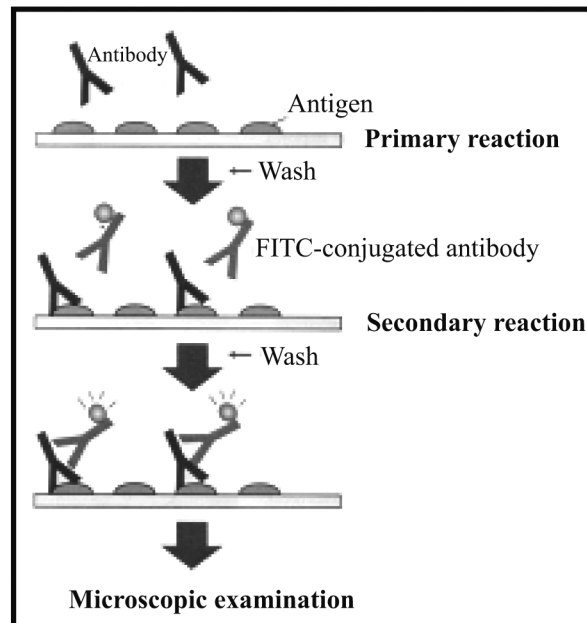
**Other suppurative infections :** Str pyogenes may cause abscesses in internal organs such as the brain, lungs, liver and kidneys, and also septicemia and pyemia.

**Nonsuppurative complications :** Str pyogenes infections lead to two important nonsuppurative sequelae – acute rheumatic fever and acute glomerulonephritis.

	Acute rheumatic fever	Acute Glomerulonephritis
1 Site of infection	Throat	Skin or throat
2 Latent period	Longer (> 2-5 week)	Shorter (1-3 weeks)
3 Prior sensitization	Essential	Not necessary
4 Serotypes	Any	Pyodermal types (49, 52, 53, 54, 57, 61) Throat infection types (12, 21, 25)
5 Repeated attack	Common	Absent
6 Manifestation	Swelling of joints & pancarditis.	Hematuria, albuminuria & Edema.
7 Pathology	Connective tissue disease Typical lesion : Aschoff's nodules	Increases cellularity of Glomerulus with larger Deposit on outer membrane & smaller deposit on inner Membrane of GBM.
8 Pathogenesis	Antibody produced against protein & polysaccharide, which cross-react with myocardial & heart valve tissue.	Mechanism is not clear.
9 ASO titer	Markedly raised	Moderately raised or low
10 Complement level	Unaffected	Spontaneous resolution
11 Course	Progressive/static	Spontaneous resolution
12 Prognosis	Variable	Good
13 Penicillin Prophylaxis	Essential	Not indicated



**Laboratory diagnosis of streptococci :** In acute infections, diagnosis is established by culture, while in the nonsuppurative complications, diagnosis is mainly based on the demonstration of antibodies.



**Fig. 15.2:** Fluorescent antibody technique

The sample collection require for acute conditions are throat swab, pus or blood for isolation of *Str. Pyogen* & Vaginal Swab, blood, CSF, ear swab for *Str. Agalactiae*, Urine & blood for enterococci.

There are different methods of demonstrating organisms in direct method of organisms.

In Microscopy Gram Staining is done for Gram Positive Cocci which formed chain and non motile which is indicative of Streptococci. Microscopy don't have any value in throat & genital infections because these streptococci are part of resident flora.

**Culture :** In this method different media is used. In Spikes (pike's) medium it is blood agar containing in 10,00,000 crystal violet & 1 in 16,000 sodium azide. It is a transport medium. While Blood agar is a most common culture medium used for isolation. A specimen is collected under Aseptic Precaution. It is transported a lab in pike's medium. It is plated on blood agar & incubated at 37°C anaerobically with CO<sub>2</sub>. Colonies & hemolysis appear. Seriological testing for definite Lancefield group & Griffith typing is done. Sheep blood agar is preferable as it is inhibitory to hemophilus hemolyticus, which may be confused with colonies of streptococci. For rapid identification of *Str. pyrogen* Maxtod's method is used. In Maxtod method *Str. progen* is more sensitive to bacitracin than others in which filter paper disc soaked in solution of bacitracin is applied on the inoculated blood agar, which shows wide zone of inhibition.



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Antigen detection, Serologic test & Typing are used as indirect method for demonstrating organisms. In antigen detection rapid diagnostic test kits are used in which streptococci A antigen is available but Serologic test is not helpful in acute infection for detection of antibody. Typing is required for epidemiological purposes.

For Diagnosis of Nonsuppurative conditions like Acute rheumatic fever & Acute Glomerulonephritis. Aim is Demonstration of high levels of antibody against streptococcal toxins. Streptococci produce two types of hemolysin streptolysin O & streptolysin S. Streptolysin O is antigenic & antibody against it (antistreptolysin O) appears in serum. Estimation of ASO titer in serum is the standard procedure for diagnosis. ASO titer is raised in few nonsuppurative conditions like Penumococcal pneumonica, Tuberculosis, Gonorrhoea, Hepatitis & Rheumatoid arthritis.

Estimation of Anti-DNAase B titer is considered significant when is it a more than 300-350.

Anti-Hyaluronidas test is important in pyoderma where ASO is not important.

Streptozyme test is passive slide hemagglutination in which erythrocytes sensitize with extracellular antigen of streptococci added to patient's serum. It produces agglutination, which is taken as positive test. This test is positive in all types of streptococcal infections.

### 15.4 OTHER HEMOLYTIC STREPTOCOCCI

#### Group B (*Str. agalactiae*)

It is single most cause of Neonatal meningitis is west.

Their ability to hydrolyze hippurate acts as presumptive identification method.

#### Group C

Streptococci of this group are mainly animal pathogen & divided into four species.

Group C pathogens from human sources are mainly *str. quisimilis* species.

It causes upper Respiratory tract infection as well as deep infection.

It differs from *Str. pyrogen* that it ferments ribose

It is commercial source of thrombolytic therapy.

#### Group F

These group poorly on blood agar unless incubated under CO<sub>2</sub> they have been called "minute streptococci". One member of this group is *Streptococcus MG* which is an alpha-hemolytic strain isolated from cases of primary atypical pneumonia.

**Group G**

These are commensals in the throats of human beings, monkeys or dogs. They may occasionally cause tonsillitis, endocarditis and urinary infections in human beings.

**Group D**

They mainly of two types

Enterococci (E. faecalis) & Non-enterococci ( Str. bovis, Str. equines)

Enterococci shows Distinctive features is ability to grow in presence of 40% bile, 6.5% sodium chloride, At pH 9.6 & temperature 45OC and in 0.1% methylene blue milk.

E faecalis is most common species isolated from human.

It can be identified by its ability to ferment mannitol, sucrose, sorbitol and aesculin & to grow on tellurite blood agar producing black colony. It mainly causes UTI, Wound infection & endocarditis.

Non-enterococci are inhibited by 6.5% sodium chloride & bile they cause UTI & endocarditis.

**15.5 THE VIRIDANS GROUP**

This group, formerly called Streptococcus viridians, is a miscellany of streptococci normally resident in the mouth and upper respiratory tract, and typically producing greening (alpha lysis) on blood sugar – hence the name viridians.

Some of them may be nonlytic. They cannot be categorized under the Lancefield antigenic groups.

They are ordinarily nonpathogenic but can on occasion cause disease. In persons with preexisting cardiac lesions, they may cause bacterial endocarditis, Str sanguis being most often responsible. Following tooth extraction or other dental procedures, they cause transient bacteremia and get implanted on damaged or prosthetic valves or in a congenitally diseased heart, and grow to form vegetation. Prophylactic antibiotic cover is advisable in such persons before tooth extraction or similar procedures. While viridians streptococci are generally penicillin sensitive, some strains may be resistant. It is therefore essential that in endocarditis.

Str mutans (so called because it assumes a bacillary form in acid environments) is important in the causation of dental caries. It breaks down dietary sucrose, producing acid and a tough adhesive dextran. The acid damages dentine and the dextrans bind together food debris, epithelial cells, mucus and bacteria to form dental plaques, which lead to caries.

**Notes**

**Notes****INTEXT QUESTIONS 15.2**

1. Which type of Hemolysin produced by Streptococcus ?
2. What is the most common causative organism for Pharyngitis ?
3. Acute & Chronic infection diagnosed by which method ?
4. Which hemolytic streptococci is known as Minute Streptococci ?
5. Which streptococci is causative of dental caries ?

**WHAT YOU HAVE LEARNT**

- Streptococci are Gram-positive cocci arranged in chains or pairs. They are non-motile and non-sporing organisms
- Streptococci are divided into obligate anaerobes and facultative anaerobes based on their oxygen requirement.
- Based on hemolytic properties Streptococci are classified into alpha hemolytic Streptococci, beta hemolytic Streptococci, gamma or non-hemolytic Streptococci
- Most pathogenic Streptococci belong to beta hemolytic Streptococci group.
- Hemolytic Streptococci of group are known as str. pyogenes
- Streptococcus are classified as str. Longus and str Breyis and they are aerobes and facultative anaerobes
- Biochemically Streptococci ferment cell organs
- Str pyogenes is a delicate organisms easily destroyed by heat and rapidly inactivated by antiseptics
- Streptococci produce two hemolysis streptolysin O and S. Streptolysin O is oxygen labile and streptolysin S is stable in serum.
- Str pyogenes produces suppurative infections of respiratory tract, skin and soft tissues, genital tract, abscess of liver, lung, kidney and brain.
- Non-suppurative infections are acute rheumatic fever and glomerulonephritis.

**TERMINAL QUESTIONS**

1. Describe Morphology & physiological characteristics of streptococcus?
2. Classify the streptococcus on the basis of Hemolysis?
3. Describe medically important Streptococci & their characteristics?

## Streptococcus

4. Describe antigenic structure of Streptococci?
5. Compare Rheumatic Fever & Acute Glomerulonephritis?
6. Describe Lab Diagnosis of Streptococci?
7. Describe Etiopathogenesis of Soft Tissue Infection?
8. Describe other Hemolytic Streptococci & write their clinical significance?



## ANSWERS TO INTEXT QUESTIONS

### 15.1

1. Erysipelas and Wound infections
2. Nature of Carbohydrates C
3. Sharply, Clear, Colourless Zone of hemolysis. 2-4mm wide, within which red cells are completely lysed.
4. Virulent – Matt (Finely Granular), Avirulent – (Glossy Colonies)
5. Str. Pyogenes

### 15.2

1. Streptolysin 'O' & 'S'
2. Streptococci
3. Acute Infection by culture & Chronic Infection by Demonstration of antibodies.
4. Group 'F'.
5. Str. Mutans

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