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# **STREPTOCOCCI**

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## **Learning objectives**

To know in detail about

- Morphology, cultural and biochemical characteristics of streptococci
- Classification of streptococci
- Toxins and virulence factors of streptococci
- Diseases caused by streptococci in domestic animals
- Distinguish between streptococci and staphylococci
- General approaches used to isolation and identification streptococci
- Dick test, scarlet fever and CAMP test
- Strangles in horses

## **• SYSTEMATICS**

<b>Domain</b>	<b>Bacteria</b>
Phylum	Firmicutes
Class	Bacilli
Order	Lactobacillales
Family	Streptococcaceae
Genus	Streptococcus
Species	<i>S. agalactiae</i> , <i>S.dysgalactiae</i> , <i>S. equi subsp zooepidemicus</i> , <i>S.uberis</i> , <i>S. equi subsp equi</i> , <i>S. canis</i> , <i>S.suis</i> , <i>S. pyogenes</i> (human)



## HISTORY

- Rivolta (1873) described chain forming organisms in pus from a case of strangles in horses.
- In 1878-79, Pasteur recognized this organism as a pus-forming agent.
- In 1903, Hugo Schottmuller introduced blood to differentiate various types of hemolysis.
- In 1928, Rebecca Lancefield reported a serological method of grouping streptococci.

## HABITAT

- Streptococci are world wide in distribution.
- Most of the streptococci of Veterinary interest live as commensals in the mucosa of the upper respiratory and lower urogenital tracts.
- They do not survive for long away from the animal hosts.

## MORPHOLOGY

- Streptococci are Gram positive, spherical or ovoid cells, arranged in chains or pairs.
- Chain formation is due to the cocci dividing in one plane only and the daughter cells failing to separate completely. Each coccus is about 1 µm in diameter.
- They are facultative anaerobes, catalase negative, oxidase-negative, and non-spore forming and non-motile with exception of some of the enterococci.
- Capsulation is not a regular feature of streptococci but some strains of *S.pyogenes* and some group C strains have capsules composed of hyaluronic acid while polysaccharide capsules are encountered in members of group B and D.
- Protoplasts (L-forms) may be induced by penicillin or phage associated lysine and may be propagated on hyper tonic media.

## CLASSIFICATION

- Several systems of classification have been employed. Based on growth characteristics, type of haemolysis and biochemical activities they can be divided into 6 principal categories.

Group	Examples
Pyogenic Streptococci	<i>S. pneumoniae</i> , <i>S.pyogenes</i> , <i>S.equi</i> , <i>S. dysgalactiae</i>
Oral Streptococci	<i>S.salivarius</i>
Enterococci	<i>S.faecalis</i> , <i>S.avium</i> , <i>S. gallinarum</i>
Lactic acid Streptococci	<i>S.lactis</i>
Anaerobic Streptococci	<i>S. monbillorum</i>
Other Streptococci	<i>S.bovis</i> , <i>S.uberis</i> , <i>S.equi</i> subsp <i>zooepidemicus</i>

- The aerobic and facultative anaerobic streptococci are classified, based on their haemolytic properties.
- Brown (1919) established this method by employing meat infusion peptone agar with 5% horse blood. He recognized three types of reactions.

- alpha - haemolytic streptococci: They produce a greenish discolouration with partial haemolysis around the colonies.
- The zone of lysis is small (1or2 mm wide), within which the unlysed erythrocytes are seen.
- These α - streptococci are generally commensals in the throat. Because of the distinctive green color, they produce; they are called as greening or viridans streptococci. Eg. *S.pneumoniae*
- Beta haemolytic streptococci produces a sharply defined, clear, colourless zone of haemolysis, 2 or 4mm wide, within which the red cells are completely lysed.
- Most of the pathogenic streptococci fall into the beta group and are called as the haemolytic streptococci.
- Gamma or non-haemolytic streptococci produces no change in the medium.
- The gamma streptococci includes the faecal streptococci ( *S. faecalis*) and related species. They are called the enterococcus or indifferent streptococci.
- Another **important** way in which the beta haemolytic streptococci were classified by Rebecca lancefield (1933) was based on the nature of a carbohydrate (C) antigen on the cell wall. They are known as Lancefield groups, 19 of which have been identified so far and named by the capital letters A-U (without I and J)

<b>Lan cefi eld gro up</b>	<b>Species</b>	<b>Host</b>	<b>Disease</b>
<b>A</b>	<i>Streptococcus pyogenes</i>	Human s	<b>Scarlet fever</b> , Septic sore throat, erysipelas, abscesses and rheumatic fever
<b>B</b>	<i>S. agalactiae</i>	Cattle, sheep and goats	Chronic mastitis
		Human and dogs	Neonatal septicaemia
		Cats	Kidney and uterine infections
<b>C</b>	<i>S. dysgalactiae</i>	Cattle	Acute mastitis
		Lambs	Polyarthrits
	<i>S. dysgalactiae subsp. equismilis</i>	Horse	Abscesses, endometritis and mastitis
	<i>S. equi subsp. equi</i>	Horse	<b>Strangles</b> , genital and suppurative conditions, Mastitis and purpura haemorrhagica
	<i>S. equi subsp. zooepidem</i>	Horse	Mastitis, abortion, secondary Pneumonia and navel

	<i>icus</i>		infections
		Cattle	Metritis & Mastitis
		Pigs	Septicaemia & arthritis in 1-3 wk old piglets

		Poultry	Septicaemia & Vegetative Endocarditis
		Lambs	Pericarditis and Pneumonia
<b>D</b>	<i>Enterococcus faecalis</i>	Many species	Opportunistic infections
	<i>S. equines</i> and <i>S. bovis</i>	Many species	Opportunistic infections
<b>E (P,U, V)</b>	<i>S. Porcinus</i>	Pigs	Jowl abscesses and lymphadenitis
<b>G</b>	<i>S. canis</i>	Carnivores	Neonatal septicaemia, genital, skin and wound infections
		Cattle	Occasional mastitis
<b>N</b>	<i>Lactococcus lactis</i>	Cattle	unknown
<b>Q</b>	<i>Enterococcus avium</i>	Many species	unknown
<b>R</b>	<i>S.suis type 2</i>	Pigs (4 to 6 months)	Meningitis and arthritis
<b>S</b>	<i>S.suis type 1</i>	Pigs (2 to 4wks old)	Meningitis and arthritis
<b>Un group able</b>	<i>S.uberis</i>	Cattle	Mastitis
	<i>S.pneumoniae</i>	Guinea pigs, rats and primates	Pneumonia

- Haemolytic streptococci of group A are known as *S.pyogenes* . These may be further subdivided into types based on the protein (M,T and R) antigens present on the cell wall.
- The M protein is acid and heat labile and the T protein is acid labile and trypsinresistant.
- Some of the Lancefield groups may be further subdivided by means of the agglutination test and designated by Arabic numbers-Griffith typing

### CULTURAL CHARACTERISTICS

- It is an aerobe and facultative anaerobe, growing best at a temperature of 37°C. They grow best in media enriched with blood, serum and fermentable carbohydrates.
- On blood agar after incubation for 24hrs small, circular, semitransparent colonies with an area of clear haemolysis are produced.

- Virulent strains from fresh isolates produce matt (finely granular) colony and avirulent strains form glossy colonies. Strains producing capsules form mucoid colonies.
- In glucose or serum broth, growth occurs as a granular turbidity with a powdery deposit. No pellicle is formed.
- In Edwards's medium (selective media) it produces dewdrop like black colonies.

- Edwards medium containing blood agar, crystal violet and aesculin (differentiate among different species of streptococci which do or do not hydrolyse aesculin).
- *S. pneumoniae* is alpha haemolytic, produces mucoid or flat colonies with smooth borders and a central concavity after 48-72hrs on blood agar (draughts man colonies).
- Ability to grow in 0.1 % tellurite broth is characteristic of *S. faecalis*. Majority of streptococcal species do not grow on Mac Conkey agar except *Enterococcus faecalis*.

## BIOCHEMICAL PROPERTIES

- Streptococci are catalase and oxidase negative . ([Click here for visual - catalase test](#))
- Ferment several sugars producing acid but no gas.
- They ferment sorbitol, trehalose, lactose, maltose, dextrin, and mannitol.
- Gelatin not liquified.
- Nitrates not reduced.
- Indole is negative.
- They are not soluble in 10% bile unlike pneumococci

## RESISTANCE

- It is a delicate organism easily destroyed by heat (54° C for 30 minutes).
- It can survive in dust for several weeks, if protected from sunlight.
- It is rapidly inactivated by antiseptics.
- It is more resistant to crystal violet and susceptible to sulphonamides and other antibiotics.
- Sensitivity to bacitracin is employed as a convenient method for differentiating *S. pyogenes* from other hemolytic streptococci.

## ANTIGENECITY

- The hyaluronic acid capsule of *S. pyogenes* inhibits phagocytosis.
- The cell wall is composed of an outer layer of fimbria containing proteins and lipoteichoic acid, a middle layer of group specific carbohydrate and an inner layer of peptidoglycan.
- The Cell wall polysaccharide has been shown to have a toxic effect on connective tissue in experimental animals.
- The peptidoglycan is responsible for cell wall rigidity. Several protein antigens have been identified in the cell wall (M, T and R).
- They are responsible for type specificity in *S. pyogenes*. Among these the M protein acts as a virulence factor by inhibiting phagocytosis.
- Hair-like pili 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.

## TOXINS AND VIRULENCE FACTORS

- Streptococci form several exotoxins and enzymes, which contribute to its virulence.

### Extra cellular toxins Hemolysins

- Streptolysins O and S are produced by groups A, C and G. **Streptolysin O**
- It is so called because it is oxygen labile. It is an antigenic protein and is active in the reduced form.
- On blood agar, its activity is seen only in pour plates and not in surface cultures. It is also heat labile.
- It is lethal on I/V inj. into animals and has a specific cardiotoxic activity. It is a general cytotoxin.
- Red cells of all animal species except mouse are lysed. Streptolysin O is antigenic and antistreptolysin regularly appears in sera following Streptococcal infection.
- Estimation of this antibody (ASO) titre is a standard serological procedure for the retrospective diagnosis of infection with *S.pyogenes*.

### Streptolysin S

- It is an oxygen stable haemolysin and so is responsible for the beta haemolysis seen around streptococcal colonies on the surface of blood agar plates.
- It is called Streptolysin S since it is soluble in serum. Addition of serum to broth increased the yield of haemolysin.
- It is protein but not antigenic. It has been shown experimentally to be nephrotoxic. Erythrogenic toxin (Streptococcal pyogenic exotoxins/ Dick toxin)
- Four erythrogenic toxins are known and most strains of *S. pyogenes* produce one or more. They are pyogenic and enhance susceptibility to lethal shock by endotoxin.
- The toxin is thermostable and antigenic. The intradermal injection in rats leads to development of erythema.
- This reaction is called as -Dick test or Schultz-charlton reaction and it is useful for diagnosis of scarlet fever.

### Enzymes

- Streptokinase (Fibrinolysin): Filtrates of streptococci gp, A, E & G produces fibrinolysin. This toxin promotes the lysis of fibrin clots by activating a plasminogen.
- Fibrinolysin plays a biological role in streptococcal infections by breaking down the fibrin barrier around the lesions and facilitating the spread of infection.
- Deoxyribonucleases (Streptodornase): These cause depolymerisation of DNA, pyogenic exudates contains large amount of DNA, derived from the nuclei of necrotic cells.
- Streptodornase helps to liquefy the thick pus and may be responsible for the thin serous character of streptococcal exudates.
- Four antigenically distinct streptodornase, A, B, C & D have been recognized.
- *Hyaluronidase*: This enzyme breaks down the hyaluronic acid of the tissues. This might favour the spread of infection along intercellular spaces.
- Streptococci possess a hyaluronic acid capsule and also elaborate a hyaluronidase- a seemingly self-destructive process. This is produced by group A, C, G and B Streptococci.
- *Proteinase*: This is another instance of an apparently self-destructive enzyme, since it is capable of breaking down the M protein, streptokinase and hyaluroindase.
- The enzyme is, however, produced only under special conditions such as an acidic pH (5.5 –6.5). Such conditions may be produced by tissue destruction, as in abscesses.
- Most strains of *S. pyogenes* form proteinase.
- *Neuraminidase*: This activity is detected in streptococci groups A, B, C, G and L. This enzyme is a virulence factor for pathogens surviving on mucosal surface.



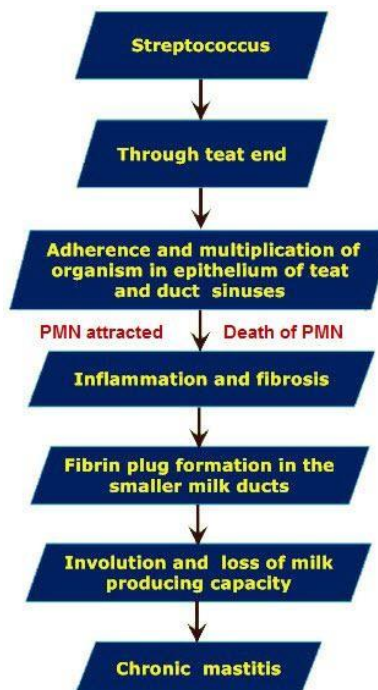
In addition to this M types of *S.pyogenes* produce NADase and other many strains also produce esterase, amylase and N-acetyl glucosaminidase.

### Serum opacity factor

- When group A. streptococci is grown in horse serum it produces an opalescence of the serum. This opacity factor is a protein.
- This is a lipoproteinase and opalescence is a result of an agglomeration of the bacterial antigen.

## PATHOGENESIS

- The natural habitat of the species of streptococci are skin, nose, throat, digestive and urogenital tracts of man and animals.
- *S.pyogenes* are present in human nose and throat without causing any disease, while *S.agalactiae* and *S.uberis* can exist in bovine udder without causing mastitis.



## PATHOGENECITY

### Cattle: Bovine mastitis

- It is caused by *S. agalactiae* (group B), *S. dysgalactiae* (grp C), *S. equi* subsp. *zooepidemicus* (grp. C), *S. uberis* (grp C, D, E, P, V).
- Mastitis arises from the multiplication of streptococci in the teat sinus and extends into the ducts.
- It causes parenchymatous mastitis, which is characterized by progressively chronic condition resulting in fibrosis.
- In acute stages milk is composed of purulent exudate, dead tissue cells, coagulated milk protein and bacteria.
- *Peptostreptococcus indolicus* is an anaerobic streptococcus, which is responsible

for summer mastitis in cattle in association with *Arcanobacterium pyogenes*.

### **Horse**

- *S. equi* and *S. equisimilis* are the main causes of strangles in young horses.
- It is characterized by a catarrhal discharge, with inflammation of the nasal mucous membranes, followed by swelling of pharyngeal LN's in which abscesses develop.
- The infection spreads through lymph channels. It also causes metritis and cervicitis in horses.
- Purpura haemorrhagica, considered to be an immune mediated disease, occur in horses 1 to 3 weeks after illness.
- Bastard strangles – in which abscesses developed in many organs. It is a very serious complication.

### **Chicken**

- *S. gallinarum* causes typical acute septicemia with peritonitis in chicken.

### **Dogs**

- *S. canis* is considered to be the cause of acid milk in puppies and canine tonsillitis. It is also associated with neonatal septicaemia and toxic shock syndrome.

### **Pigs**

- *S. suis* causes porcine cervical lymphadenitis and also isolated from pneumonia, septicaemia, arthritis, endocarditis, meningitis and reproductive tract infections.
- It also causes erosive arthritis in young pigs.

## **DIAGNOSIS**

- It involves clinical, microscopical and bacteriological examination.

### **Clinical examination**

- Palpation of the udder and supramammary lymphnodes will be helpful in distinguishing the chronic and insidious form of mastitis produced by *S. agalactiae* and *S. uberis*.
- In contrast *S. dysgalactiae* and *S. zooepidemicus* causes sudden onset of acute inflammation of one quarter only with an acute systemic disturbance followed by joint infections and lameness.

### **Microscopical examination**

- When long chains of organisms are detected in milk samples from chronic mastitis, it is caused by *S. agalactiae*.

### **Based on type of haemolysis on blood agar**

### **Bacteriological examination**

- When 0.1 ml of secretions inoculated on Edward's medium (blood agar, crystal violet and aesculin) *S.agalactiae* produces bluish-grey colonies and *S.uberis* produces dark color colonies.
- **CAMP test (Christie, Atkins, Munch and Peterson, 1944)**
  - This test is based on the observation that ruminant red blood cells lysed by the beta toxin of staphylococci at 37°C are completely lysed in the presence of *S.agalactiae* (group B).
  - Differentiation between the pneumococcus and *S.viridans* organisms can be achieved by bile solubility and the optochin test.
- **Bile solubility test**
  - Autolysis of pneumococcal cultures takes place within 15 minutes at 37°C in the presence of 10 per cent sodium deoxycholate.
  - These substances have no effect on *S. viridans* organisms.
- **The optochin test**
  - The majority of pneumococcal strains are sensitive to optochin (Ethyl hydrocuprein hydrochloride). Whereas *S.viridans* organisms are not.
  - This test consists of placing a small circular piece of filter paper, impregnated with 1:4000 aqueous solution of optochin, in the center of a blood agar plate after inoculating the test cultures in streaks across the full width of the medium.
  - The growth of pneumococcal strains will be inhibited to a distance of some 5mm from the circumference of the filter paper.

## **CONTROL AND PREVENTION**

- In streptococcal infections in animals, including mastitis, the most satisfactory method of control is antibiotic therapy using penicillin preparations to other substances because streptococci develop resistance to penicillin comparatively infrequently.
- Vaccines are of very limited value for the immunization of animals against streptococcal infections, with the possible exception of equine strangles.

*S.pyogenes* (group. A) is the most usual cause of septic sore throat and scarlet fever in humans.