

# **CHAPTER ONE**

## **1. INTRODUCTION and LITERATURE REVIEW**

### **1.1. Streptococci**

#### **1.1.1. Description**

Streptococci are aerobic and facultatively anaerobic Gram-positive cocci (from the greek streptos: chain and coccus: a grain or berry). They require complex nutrients for their growth (Greenwood *et al.*, 2002). Streptococci are part of the normal flora in humans and animals. They are non-motile, non-spore forming, spherical or ovoid cocci, and have a hyaluronic acid capsule. They are catalase negative by which they are distinguished from Staphylococci. Streptococci are relatively fastidious bacteria requiring enriched medium such as blood agar for growth (Sherrie *et al.*, 2004).

#### **1.1.2. History**

Streptococci were discovered in 1874 by Billroth from cases of erysipelas and wound infections. Pasteur was the first to report isolation of these bacteria from the blood in a woman with puerperal sepsis in 1884. The organism was designated *Streptococcus pyogenes* by Rosenbach in the late 19th century. In the early 1900s, Dochez, George, and Dick identified that scarlet fever was caused by hemolytic streptococci (Subhash Chandra Parija., 2009).

### **1.1.3. Habitat**

Streptococci are normal flora of the oral cavity, nasopharynx, skin, fingernails, perianal region, intestine and upper respiratory tract of humans (Subhash Chandra Parija., 2009).

### **1.1.4. Pathogenicity**

Infected human cases are the reservoir of organisms. Respiratory and salivary secretions in the form of droplets and contaminated fomites are the source of *Streptococcus pyogenes* infection. Children with untreated acute infection spread organisms by their salivary droplets and nasal discharge. Streptococcal carrier rate as high as 20-40% has been reported. However, these carriers with chronic asymptomatic pharyngeal and nasopharyngeal colonization are not usually at risk of spreading disease, as they mostly inhibit virulent organisms. Person to person transmission is the main route of transmission. The infection is transmitted from person to person through respiratory droplets (Subhash Chandra Parija., 2009). The infection is due to antigenic epitopes of M protein. The acquired immunity against a particular M type of streptococci lasts longer in untreated persons than in treated persons. Although such antibodies (Abs) protect from infection against a homologous M protein type, they confer no immunity against other M serotypes (Subhash Chandra Parija., 2009).

### **1.1.5. Classification**

The streptococci based on their oxygen requirements are classified into aerobes, obligate anaerobes, and facultative anaerobes.

#### **1.1.5.1. Based on hemolysis in blood agar**

##### **a. Alpha-hemolytic Streptococci**

These cocci produce colonies surrounded by a narrow zone (greenish zone) of hemolysis with persistence of some partially lysed red blood cells. The greenish discoloration is due to the formation of a reduced product of hemoglobin. Alpha hemolytic streptococci are known as Viridians streptococci. These are found as commensals in the upper respiratory tract of humans and may cause opportunistic infections. *Streptococcus pneumoniae* also belongs to alpha-hemolytic group (Jawetz *et al.*, 2010).

##### **b. Beta-hemolytic Streptococci**

These cocci produce a well-defined, clear, colorless zone of hemolysis (2-4 mm wide) around the colonies. RBCs in the zone of hemolysis are completely used. This lysis is due to the liberation of enzymes streptolysin O and streptolysin S. The term hemolytic streptococci is applicable only to beta hemolytic streptococci. Most of pathogenic streptococci belong to this group, and among them *Streptococcus pyogenes* is the most important one (Jawetz *et al.*, 2010).

### **c. Gamma-hemolytic Streptococci**

These Streptococci do not produce any hemolysis or discoloration on blood agar. These group are generally found as comensals. *Streptococcus faecalis*(*Enterococcus faecalis*) belong to this group (Jawetz *et al.*, 2010).

#### **1.1.5.2. Based on antigenic structures**

Lancifield classification is a serological classification of the beta hemolytic streptococci. It is based on the presence of group specific carbohydrates antigen (C-antigen) on the cell wall of streptococci (Jawetz *et al.*, 2010).These carbohydrates can be extracted by any of the following methods (Jawetz *et al.*, 2010).

- I. Acid extraction with HCL (Lancifield's method).
- II. Formamide extraction 150°C (Fuller's method).
- III. Autoclaving (Rant and Randall's method).
- IV. Enzyme extraction (Maxted's method).

The beta hemolytic streptococci are classified into 21 serological groups known as Lancifield from A to V (with exception of I and J).The majority of hemolytic streptococci that cause human infections belong to group A (*Streptococcus pyogenes*) (Subhash Chandra Parija., 2009).

Based on the M, T and R antigens present on cell wall surface, *Streptococcus*

*pyogenes* have been further classified into 80 serotypes, this classification is known as Griffith typing. M protein is the most important type specific antigen. This serotyping is important for epidemiological studies (Subhash Chandra Parija.,2009).

## **1.2. Objectives**

1.1.2.1. To collect throat swabs from patients with sore throat.

1.1.2.2. To cultivate and incubate the collected swabs correctly.

1.1.2.3. To isolate the causative agent in pure culture.

1.1.2.4. To identify *Streptococcus pyogenes*.

### 1.3. Literature review

One hundred and eighty-six throat swabs were collected from patients with sore throats and 164 throat swabs were collected from healthy controls. All swabs were investigated bacteriologically, and sensitivity tests were performed on all pathogenic isolates. Group A *Streptococcus pyogenes* was the predominant pathogenic organism (24.7%) and *Proteus vulgaris* the least predominant organism (0.5%) isolated from patients with sore throats.

*Streptococcus pyogenes* infection was found to be most common among school children between the ages of 9 and 12 (61.5%). The pathogenic organisms were found to be sensitive to erythromycin (92.6%) (Omer *et al.*, 2010).

As beta-haemolytic streptococci can be cultured in people with and in those without a sore throat, a case-control study was set up in 43 family practices in the Netherlands. The association was tested between the number of colony counts, specific T/M types and exotoxin genes and an acute sore throat. Duplicate throat swabs were taken from 663 sore-throat patients, selected by clinical criteria, and from 694 healthy controls. They were cultured for beta-haemolytic streptococci by combining several updated laboratory methods. Approximately 40% of the controls and 80% of the patients had beta-haemolytic streptococci-positive cultures. When focusing

on cultures with high colony counts, not only group A (46%), but also non-group A streptococci (20%), predominated significantly in adult patients compared with controls. No T/M or exotoxin gene type was significantly more prevalent in patients than in controls. Thus, semiquantitative analysis, but not T/M and exotoxin gene typing, showed an association between beta-haemolytic streptococci and active disease. Groups A, C and G streptococci were found to be potentially pathogenic in adult sore-throat patients, and should be included in the discussion on the use of rapid antigen detection tests and penicillin treatment in primary care (Zwart *et al.*, 2001).

Of 1449 patients recruited during the 6-month study period, only 44 (3.0%) had positive throat cultures for group A beta-haemolytic streptococcus. The majority of group A beta-haemolytic streptococci were isolated from patients between the age of 3 and 60 years. Clinical findings other than an absence of cough were found to be unhelpful in predicting group A beta-haemolytic streptococcal throat infection. The sensitivity of the rapid group A streptococcal antigen detection test was 52.6% and the specificity was 98.2% (Wong *et al.*, 2003).

The bacterial growth in patients presenting with a sore throat was assayed and four clinical features were tested in order to reliably differentiate between beta-haemolytic streptococci group A and other micro-organisms.



For 2 years, 53 general practitioners in The Netherlands took throat swabs from all patients, aged 4-60, presenting with a sore throat lasting 14 days or less. Four clinical features: fever (history), (tonsillary) exudate, anterior cervical lymphadenopathy and absence of cough were registered. In 70% of the 598 patients one or more micro-organisms were cultured from throat specimens. In 48% of the patients beta-haemolytic streptococci were found (32% group A, 7% group C, 4% group G, 5% others). Enterobacteriaceae were cultured in 5%, *Candida albicans* in 5%, *Staphylococcus aureus* in 4%, various other in 8% of the patients. In 30% of the patients cultures remained negative. Of the 270 patients with three or four clinical features, 46% (95% CI, 40-52%) harboured GABHS in their throats, while in 328 patients with less than three features 21% (95% CI, 16-25%) were GABHS positive. However, this relationship between presence or absence of clinical features and culture result was not found in the youngest age category (4-14 years old). Culture results were not related to sex, smoking habits or the insurance mode of the patient. The clinical relevance of several micro-organisms, other than beta-haemolytic streptococci, remains to be determined. The four mentioned signs and symptoms were helpful in predicting the probability of GABHS in patients aged 15 years and older (Dagnelie *et al.*, 2007)

## **CHAPTER TWO**

### **2. MATERIALS and METHODS**

#### **2.1. Type of study**

This is descriptive cross sectional study.

#### **2.2. Study area**

This study was done at Ear, Nose and Throat (ENT) hospital in Khartoum.

#### **2.3. Inclusion criteria**

People who have sore throat manifestations.

#### **2.4. Exclusion criteria**

Adult patients and children patients who do not having tonsillitis or who undergoes tonsillectomy or received antibiotic therapy recently.

#### **2.5. Data collection**

The information related to the study such as age, gender, symptoms and signs were collected using structured questionnaire.

#### **2.6. Ethical consideration**

The present study was proved by the board of medical laboratory science, Sudan University of Science and Technology. The participants were informed about the purpose of the research before sample collection and verbal or sign agreement were obtained from them.

## **2.7. Collection of specimens**

Throat swabs were taken from tonsils with special care to avoid contamination, the collection was done by using dry sterile cotton wool swab, and then specimens were labeled and delivered to the laboratory immediately. The suitable vein puncture site was cleaned with 70% alcohol and using sterile syringe, 5 ml of blood was collected, the blood was allowed to clot at room temperature then centrifuged at 5000 rpm for 5 min the obtained sera were stored at -20 °C until used.

## **2.8. Preparation of culture media**

Blood agar, crystal violet blood agar, nutrient agar plates and slope were prepared according to the instruction of manufacturers (Hi media).

## **2.9. Inoculation of culture media**

The isolation and identification of *Streptococcus pyogenes* was carried out according to Cheesbrough (2009) scheme. Each throat swab sample was inoculated into blood agar with bacitracin disc under aerobic and anaerobic conditions and incubated at 35-37°C for 24 hours, then the suspected organism was subcultured on crystal violet blood agar using streaking technique under aseptic conditions, at the same time nutrient agar slopes supplemented with serum were stabbed in order to preserve the isolates and every 72 hrs the preservation process was renewed.

## **2.10. Morphology**

The suspected *Streptococcus pyogenes* produced smooth, small, colorless, shine and Beta-hemolytic colonies on blood agar. On crystal violet blood agar produced smooth, small, colorless and shine while on nutrient agar showed smooth, small whitish colonies. Suspected organism was inoculated into nutrient agar plate and incubated at 35-37°C for 24 in order to perform biochemical reaction.

## **2.11. Catalase test**

This test was used to differentiate between those bacteria which produce the enzyme catalase such as *Staphylococcus* species from non catalase producers such as *Streptococcus* species. Catalase acts as catalyst in the breakdown of hydrogen peroxide to oxygen and water. The test was performed according to Cheesbrough (2009).

## **2.12 Voges-proskauer (v-p) test**

This test was used to differentiate between *Streptococcus pyogenes* and *Streptococcus agalactiae*. The test organism was cultured in glucose phosphate peptone water for 48 hours. Sodium hydroxide 40% and small amount of creatine were then added. Under alkaline condition and exposure to the air, the acetoin produced from the fermentation of glucose is oxidized to diacetyl which forms a pink compound creatine.

### **2.13. Sugar fermentation**

*Streptococcus pyogenes* have ability to ferment lactose, Sucrose, Starch and gives variable results with mannitol (strain of M-type 6 and M-type 66 ferment mannitol)

### **2.14. Data analysis**

Data were analyzed by the statistical package for social sciences (SPSS) software program namely excel and presented in form of tables and graphs.

## **CHAPTER THREE**

### **3. RESULTS**

A total of fifty (50) throat swabs were collected from patients suffering from sore throat. The patients containing twenty four (24) males and twenty six (26) females were analyzed for *Streptococcus pyogenes* infection (Table 1). Three (3) *Streptococcus pyogenes* were isolated, giving percentage of *Streptococcus pyogenes* infection to be 6%. Two (2) isolates from females and one (1) from male. Table 2 and figure 1 show the frequency and percentage of culture results. Table 3 shows frequency and percentage of all samples types grown as *Streptococcus pyogenes*. The other bacteria isolated from the specimens were from the oral cavity which represent the oral normal flora .

Table 1. Distribution and frequency of specimens according to the gender

Sample	Frequency
Males	24
Females	26
Total	50

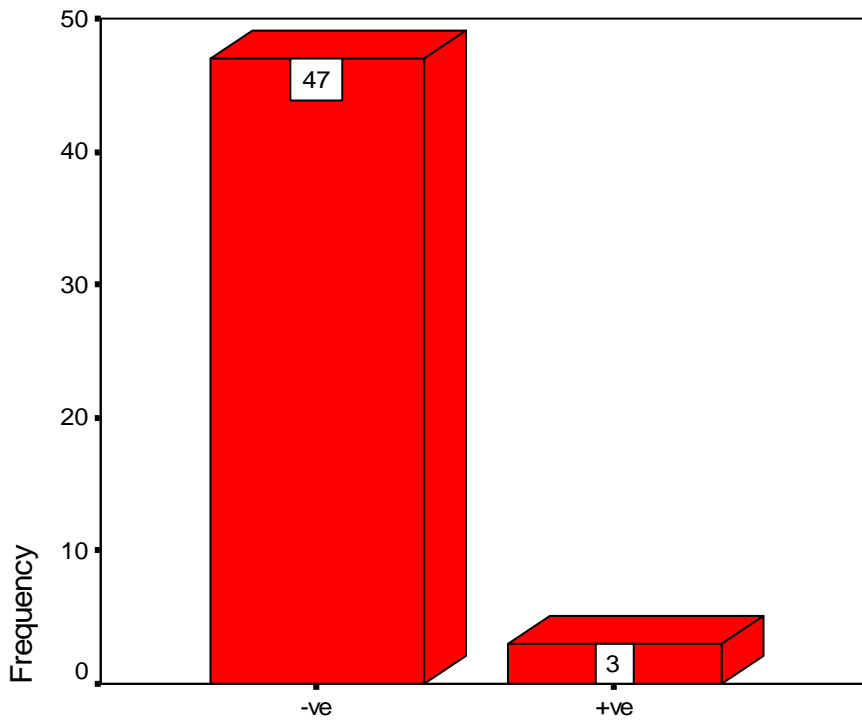


Fig (1): Frequency of culture result

Table 2. Frequency and percentage of *Streptococcus pyogenes*.

Type of growth	Frequency	Percentage
<i>Streptococcus pyogenes</i>	3	6%
Others	47	94%

Table3. Distribution and frequency of *Streptococcus pyogenes* isolated during this study according to gender.

Gender	Frequency	Percentage
Males	1	2%
Females	2	4%
Total	3	6%



## CHAPTER FOUR

### 4. DISCUSSION

Some sore throat infections may be due to viral agents and some may be due to fungal invasion. Only 5-10% of such complaints in adults and 15-20% in children are associated with bacterial infections (Omer *et al.*, 2010). Upper respiratory tract infections have been the focus of much attention for the last two decades since they are cause of morbidity and mortality in the age groups between 5 to 15 years despite all the advances made by medical science. Many reports have shown the emergence of *Streptococcus pyogenes* as an important pathogen of upper respiratory tract in the last two decades (Jawetz *et al.*, 2010). The present study aimed to determine the frequency of *Streptococcus pyogenes* among patients with upper respiratory tract infection (tonsillitis). Out of 50 significant growth, the *Streptococcus pyogenes* was responsible for 3 (6%) of tonsillitis infection, 1 (2%) strains was isolated from male and 2 (4%) from females. All isolates (6%) were sensitive to bacitracin. Our result showed that females 26 (52%) were more suspected to tonsillitis than males 24 (48%), these results were in agreement with (Wong MC *et al.*, 2003) report in Hong Kong.

## **Conclusion**

*Streptococcus pyogenes* was found to constitute about 6% respiratory tract infected patients, they were sensitive to bacitracin.

## **Recommendation**

Further research is required to study the actual percentage of *Streptococcus pyogenes* from patients with sore throat.

## References

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## Appendixes



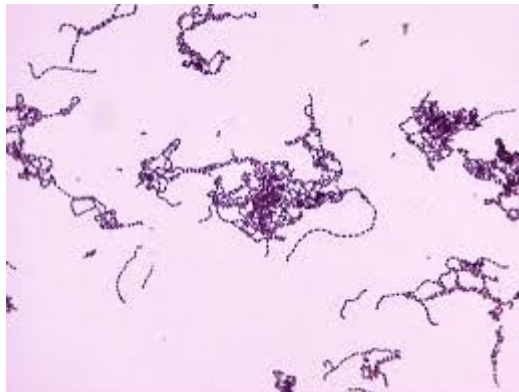
Cotton swab used in specimen collection



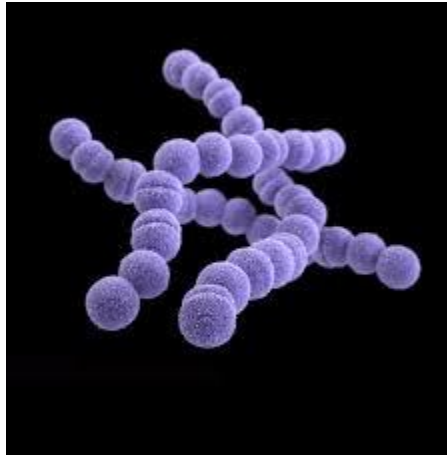
Wire loop used for bacterial culture



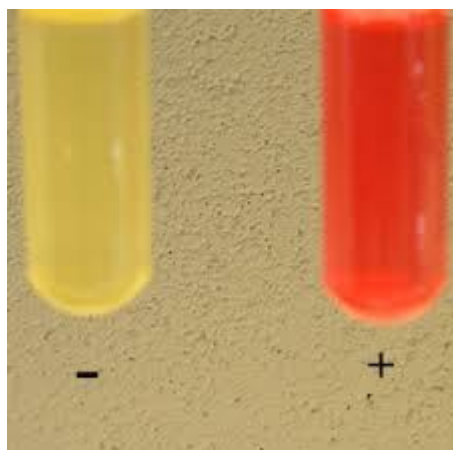
Growth pattern of *S.pyogenes* in blood agar media



Gram reaction of *S.pyogenes*



*S.pyogenes* by electron microscopy



Voges-Proskauer test negative for *S.pyogenes*