CHAPTER ONE

1.1Introduction

Group A beta hemolytic streptococcus(GAS) is an important species of gram positive extracellular bacteria that colonize throat or skin (Skin infection appear as cellulites erysipelas and impetigo) and responsible for a number of suppurative infection and non suppurative sequelae. Commonly known as group A streptococcus pyogen [1,2]

Throat infection is commonly called sore throat or pharyngitis. It's one of the most common presenting problem in emergency department [3] and primary care clinics . [4]

The most common infection in children between 5 – 15 years age attributable group A streptococcus is pharyngitis .[1]

An estimated 700 million group A beta hemolytic infections occur world wide each year , over 650,000 of these cases are sever and invasive , and have amortality rate of 25% early recognition and treatment are possible but diagnostic failure can result in sepsis and death. [5]

The pathogenesis of streptococcus group A Beta hemolytic is mediated by a variety of factors, one of them is streptolysin O toxin, which damage cell membrane and accounts for hemolysis which can be demonstrated on sheep blood agar.

The prevalence of sever GAS associated disease is estimated to be 18.1 million cases world wide with and estimated yearly. incidence of GAS pharyngitis of 616 million with 517.000 death due to GAS associated disease . [5]

The global estimate of pharyngitis is 600 million case annually, with over 550 million of these occurring in less developing countries.[2]

Recent research reported in Sudan that the prevalence rates of GAS disease and carriage varied by age ; children who were younger than 5 years had lower rates of throat cultures that were positive for GAS ,more than 8% to 40% of children and 5% to 9% of adolescents who have sore throat, fever ,and tonsilopharyngeal inflammation have GAS infection.[6]

1.2. Rational:

In River Nile state in Barber we noticed that there is high prevalence of streptococcus pyogen infection in children 5-15 years which can be complicated to Rheumatoid fever.

Accurate diagnosis of GAS pharyngitis followed by appropriate antimicrobial therapy is important for the prevention of acute RF and other complicated disease, because in developing countries, especially those of Africa, rheumatic heart disease is identified as major cause of death among school children .

To reduction in transmission of GAS to other people and prevent administration of wrong antimicrobial. [7]

1.3.Objective:

1.3.1.*General objective*

. To determine the prevalence of group A beta hemolytic streptococcus in children (5-15) years old in river Nile state [Berber] from April to July in 2017.

1.3.2.*Specific objective*

- To identify streptococcus pyogen that cause infection among children (5_15) years.
- To estimate the number of affected children by group A beta hemolytic streptococcus in river Nile state in Barber.
- to evaluate health state and incidence according to the result which will be found .

CHAPTER TWO

Literature review

2.1. Classification of streptococci

The genus **Streptococcus** consists of Gram-positive, aerobic , which appear as chains under microscope. The organisms in this genus is characterized by a coccus appearance, a thick cell wall, and aerobic utilization of glucose. Over many years, the classification of Streptococci into major categories has been based on a series of Observation. colony morphology and hemolytic reaction on blood Agar. serologic specificity of the cell walls group-specific substance (Lancefield Classification) and other cell wall or capsular antigens biochemical reaction and resistance to physical and chemical factors; and ecological feature. Additional biochemical tests and molecular genetics also have been used to study the relationships of streptococcal species to each other [8] . The classification of streptococci summarized in Table 1.

2.2. Streptococcus pyogen:

Group A Beta-hemolytic Streptococcus pyogen (GAS) is the most common bacterial cause of pharyngitis. It accounts for only 10 - 20 % of sore throats [9, 10, 11], and it is most common in children 5-15 years old [12], as well as the only cause of pharyngitis for which antimicrobial therapy is indicated [13] specially during its accidental suppurative or non-suppurative sequelae [14,15]

2.3. Morphology and Staining:

Gram- positive spherical cocci, occurring in chains, capsulate in very young cultures, non-motile and non-spore forming.

2.3.1. Gram Stain

The gram staining reaction is used to help identify pathogens in specimens and cultures by their gram reaction (gram positive or gram negative) and morphology. Pus cells can also be identified in gram smears.

Table [1] Classification of streptococcus pyogen

Name	Group specific substance	hemolysis	habitat	Common disease
Streptococcus pyogen	A	Beta	Throat , Skin	Pharyngitis , RF
Streptococcus agalactiae	B	Beta	Female genital tract	Neonatal sepsis
Streptococcus viridians	D	Alpha	Mouth , Throat	Endocarditis, abscess
Streptococcus faecalis	D	None	Colon	Abdominal abscess
Streptococcus pneumonia	None	Alpha	Throat	Pneumonia
Peptostreptococci	None	None	Mouth , Colon	Abscess

2.3.2. Gram reaction;

Gram reaction differences in gram reaction between bacteria is thought to be due to differences in the permeability of the cell wall of gram positive and gram negative organisms during the staining process. Following staining with a triphenyl methane basic dye such as crystal violet and treatment with iodine, the dye–iodine complex is easily removed from the more permeable cell wall of gram negative bacteria but not from the less permeable cell wall of gram positive bacteria. Retention of crystal violet by gram positive organisms may also be due to the more acidic protoplasm of these organisms binding to the basic dye (helped by the iodine).

2.4. Cultural characters:

Culture media a nutrient material prepared for the growth of microorganism in laboratories .

Facultative anaerobe, optimum temperature of growth 37°c it grows best on nutrient agar with blood or serum. Colonies are small (after 24 h incubation), semi-transparent, low convex, clear, often wide zones of hemolysis surround colonies on horse or sheep blood agar. Hemolysis is variable with human or rabbit blood.

2.4.1. Blood agar:

Blood agar is both a differential medium and enriched one.
It distinguished between hemolytic and no hemolytic bacteria .Hemolytic bacteria (e.g. streptococci) produce clear zone around their colonies because of red blood cell destruction.

2.4.2. Amie's transport media:

This medium is transport media for swab that preserve it from dryness and inhibit normal flora.

2.5. Enzymes and Toxins:

1- Erythrogenic (Dick) toxin: causes rash of scarlet fever.
2- Hemolysins: affect other kinds of cells. Two types of streptolysins O and S are available.
3- Deoxyribonucleases: are produced by streptococci groups A, C and G.
4- Other enzymes include: streptokinase, hyaluronidase, nicotinamide Adenine dinucleotidase (NADase), and pyrrolidonyle peptidase(PYRase).

2.6. Streptococcal Antigens:

2.6.1. Cellular antigens: are group specific and of carbohydrate origin. Group A antigen is characteristic of S-pyogen.

2.6.2. M protein: it impedes phagocytosis, promotes attachment to epithelial cells, and is essential for virulence

2.6.3. T protein: is used as a typing marker in epidemiological studies, but it is not related to pathogenicity.

2.6.4. R- Protein: present in few strains of group A serotypes, their role is unknown.

2.7. Clinical features:

Mild redness of tonsils and pharynx may be the only sign, but the classical picture is infection and edema involving the faces and soft palate with exudates giving acute follicular tonsillitis that is most common in 5-8 years old children [6].Streptococcal pharyngitis cannot be distinguished clinically from viral pharyngitis [16].

This pathogen causes a variety of inflammatory and supportive conditions such as sore throat, a scarlet fever, cellulitis , erysipelas, impetigo, puerperal fever, otitis media, septicemia, wound infections .

2.8. Complications of streptococcal infection:

2.8.1. Local complication:

These include otitis media, streptococcal rhinitis, sinusitis and peritonsillar abscess [17].

- **Otitis media**: is an upper respiratory infection involving the middle ear by extension of infection up the Eustachian tube. It is predominantly a disease of children, and the main symptom is ear ache.
- **Sinusitis**: It is a mild discomfort over the frontal or maxillary sinuses. Symptoms include: severe pain, tenderness, and a purulent nasal discharge that requires treatment.
- **Streptococcal rhinitis**: It is an infection of the nasal passages.
- **Peritonsillar abscess:** It is a complication of tonsillitis, and most common in children older than 5 years and young adults, it is important to treat this infection because it can spread to adjacent tissues, and may erode the carotid artery to cause an acute hemorrhage. Streptococcus pyogen and viridian streptococci may be involved. [18]

2.9. Post streptococcal sequelae:

2.9.1. Rheumatic fever:

This is a disease of connective tissue which is of immunological origin. It may be the result of antibodies produced against protein and polysaccharide cell wall antigens of S. pyogen, cross-reacting with connective tissue in the heart and elsewhere. Clinical features include: acute onset of fever, pain, swelling of the joints, and pericarditis. [8]

In developing countries, especially those of Africa, South America and Central America, rheumatic heart disease is identified as a major cause of death among school children. [19]

Complication of rheumatic fever includes:

Damage to heart values.
Endocarditis.
Heart failure.
Arrhythmias.
Pericarditis.
Sydenham's chorea. [20]

2.9.2. Acute glomerulonephritis:

This is a more common cause of acute nephritis in comparison with rheumatic fever, which can be caused by a wide range of serotypes of Streptococcus pyogen. Acute glomerulonephritis is produced by a much narrower range of serotypes. [21]

Clinically, acute glomerulonephritis presents 1-3 weeks after a streptococcal throat infection, giving rise to hematuria, albuminuria, and edema.

The disease is a result of an immunological process. It occurs because components of the glomerular basement membrane are immunologically similar to the cell membranes or nephrogenic β-hemolytic streptococci. Group C streptococci may also be involved as a causative agent [21].

2.10. Laboratory diagnosis of Streptococcal Infection:

2.10.1. Collection and transport of specimens:

Either cotton, Dacron or calcium alginate-tipped swabs are suitable for collecting specimens for isolating most upper respiratory tract microorganisms [18]. Within two hours of collection, the swab is delivered with a request form to the laboratory. If this not possible, transport specimen in a transport media. [18, 22]

2.10.2. Culture:

The microbe that grow and multiply in or on a culture medium are referred as culture.

Because the cause of bacterial pharyngitis is Streptococcus pyogen, most laboratories routinely screen throat cultures for this organism. Throat swabs have been plated onto 5% sheep blood agar. [16] The plate should be incubated at 37°C for 18-24 hours aerobically . [18]

If sufficient number of pure colonies are not available for identification, a subculture for it and additional incubation is necessary. By placing a 0.04unit differential Bacitracin filter paper disk on the area of inoculation, presumptive identification of S. pyogen may be made.

New selective agar media such as streptococcal selective agar, have been developed. [16]

2.10.3. Direct visual examination:

A gram stain does very little to help diagnosis. Characteristic pattern of Fusiform and spirochetes may be visualized, and can be used to identify the agents of Vincent's angina.

Direct fluorescent antibody stains have been used to identify Group A beta hemolytic streptococci in throat specimens.[16]

2.10.4.Rapid antigen detection tests of streptococcal infection:

In 1978, El kholy et.al published the results of a study describing the identification of GAS directly from tonsil scrapings within 30 minutes, by using a modified nitrous extraction procedure coupled with an immune precipitin reaction.[23]

A rapid antigen kit for GAS, utilizing a latex agglutination method was later developed. Overtime more kits were employed e.g. the co-agglutination, enzyme immunoassay techniques. The specificity of all rapid antigen tests for GAS is generally › 97% [24]. The American Heart Association has recommended that all negative rapid antigen tests should be confirmed by culture [24].

2.10.5. PYRase test (pyrrolidonyl peptidase):

PYRase can be carried out on the isolated beta hemolytic colonies. It is a rapid test that detects production of pyrrolidonyl peptidase by Group A streptococci and enterococci. It gives a result within 15 minutes. [16]

2.10.6.Nucleic acid testing:

There are two very distinct ways to perform nucleic acid testing for GAS using commercially available kits. The Group A streptococci direct test utilizes a direct non- amplified nucleic acid probe methodology. The assay utilizes a real-time PCR technology for detection of the amplified GAS nucleic acid.

The group A Streptococcus direct test is intended for the detection of GAS directly from pharyngeal specimens. The test uses a non-isotopic, chemiluminescent, single -stranded DNA probe that is complementary to RNA target of the GAS. Sensitivity has ranged from 88.6 to 94.8% compared to culture, and all had specificity of ›97%.

The light cycler Strep. Assay utilizes a real time PCR method for the detection of GAS from throat swabs. Utilizing the light cycler technology, a single light cycler can test 32 samples (Test and controls) per run in 1.5 hours using culture as a gold standard, some workers, determined the light cycler Strepto. Assay to have a sensitivity of 93% and a specificity of 98%. In addition, a much shorter time is needed for completion than traditional cultures. [35]

2.10.7.Serological tests:

These tests with paired sequential sera from the patient are used to detect a rise in the titer of antibody to one of the extracellular products of S. pyogen. They may be used to confirm primary infections, but are more commonly used for diagnosis of the non-suppurative sequelae of S. pyogen infections, such as rheumatic fever or glomerulonephritis.

Anti-streptolysin O (ASO) test:

It is more frequently used. The upper limit of normal anti-body is 200 units/ml.

Anti-DNase B estimation:

May also be useful. The rise in DNase B antibody usually occurs later than the ASO antibody.

Other tests:

They employ anti-streptokinase, anti-hyaluronidase, and anti-DNase enzymes. [22]

2.11. Epidemiology:

2.11.1. Sources of infection:

1- Throat carriers of S. pyogen and nasal carriers.
2- Acute clinical cases of streptococcal infections.
3- Patients with streptococcal otitis media, involving rhinitis and infected skin lesions Incubation period: 1-3 days [26]

2.11.2. Predisposing factors:

.Poverty, over-crowding and bad social and environmental Conditions.

.Absence of health education.

.Closed and semi -closed communities, e. g: troops, schools children, nurseries, etc. [26]

2.12. Control of Streptococcal Infection:

1- Early treatment of cases.
2- Early diagnosis of organism, by taking throat swabs.
3- Early search for the source of infection, e. g: nasal, throat, and saliva carriers, or patients with otitis media or skin lesions. All such cases should be treated at once.
4- In closed and semi-closed communities, all bedding, clothing and floors should be washed regularly and disinfected.
5- Dust control measures in hospitals and barracks. [26]

2.13. Treatment:

This is needed to prevent both the suppurative and non suppurative sequelae, as well as to decrease mortality [18]
Penicillin was the drug of choice for the treatment of GAS pharyngitis for more than four decades [27, 28] Patients hypersensitive to penicillin are given erythromycin. However, in recent years, some investigators have reported an increasing incidence of treatment failures with penicillin therapy [29, 30, 31].
There are several common reasons for apparent penicillin treatment failure. These include:

(I) Presence of beta- lactamase, producing organisms in the Oropharynx that inactivate penicillin.
(ii) Cases of repeated infection, which may be of viral etiology.

(iii) Poor patient compliance in taking oral medication.
(iv) Possible patient re-exposure to a GAS- positive family member or associate.
(v) Patient carrier of GAS. [32]

Reports from various countries estimate that antibiotics are prescribed in 30% to 75% of visits [33,34] This suggests that antibiotics are often prescribed more than necessary.

CHAPTER THREE

Material and method

3.1. Study design:

Cross sectional Study.

3.2. Study area:

This study conducted in river Nile state Barber city .

3.3. Study population:

Children between 5-15 years' old.

3.4. Inclusion and Exclusion criteria:

This study includes any children between 5-15 years suffer from upper respiratory infection or skin rash. Exclude any patient do not suffer from upper respiratory tract infection or skin rash.

3.5. Data collection:

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

3.6. Sample size:

Sampling equation

$$N = \mathbf{4pq \div L2}$$

P: estimated prevalence {percentage}(20%)
q: 1-p [100-20]
L: allowable errors 8%
N=4*20*80/64= 100 sample

100 sample

3.7. Sample selection:

The participant selected randomly.

3.8. Collection of sample:

In under light and using tongue depressor, the throat was examined for inflammation, and for presence of membrane exudates or pus, with sterile cotton wool swabs 100 throat swab sample were collected from children between 5-15 years from Barber State, inserted directly in Amie's media.

3.9. Data Collection Method:

Data were collected as per a structural interview questionnaire specially designed to collect and maintain all information of each patient examined**.**

3.10. Data analysis:

The gathered data was analyzed with SPSS (statistical package of social science), the test will be used for calculating, degree of variation, personal and clinical data will be collected by direct interviewing questionnaire from each subject.

3.11. Variable:

Gender, Age, Symptoms, Signs.

3.12. Ethical consideration:

Permission to carry out the study will be obtain from the collage of health science Al-sheikh Abdullah Al-Badry university. All parent come to hospital with their children will be informed for purpose of the study before collection of the samples and verbal consent will be taken from them.

3.13. Sterilization of glassware:

Glassware such as petri dishes, test tubes, as well as flasks were sterilized in the hot air oven at 100°C for one hour.

3.14. Preparation of culture media:

Amie'stransport media and Blood agar media prepared according to the information in the bottle of each one.

3.15. Sterilization of media:

Blood agar and amies transport media were sterilized by autoclave at 121°C for 15minute .

3.16. Preservation of media:

All media were checked for contamination and then kept in a refrigerator at 2-8°C for several weeks

3.17. Culture media:

Sample that collected in Amie's cultured in blood agar; to see the action of streptolysin if beta hemolytic is present in sample. If beta hemolytic isolated from the culture, the biochemical test will be done.

3.18. Gram stain:

Gram stain done after culture to confirmed gram positive cocci in chain.

3.19. Biochemical test:

3.19.1. Catalase test:

This test is used to differentiate those bacteria that produce the enzyme catalase, such as staphylococci, from non-catalase producing bacteria such as streptococci.

Principle:

Catalase acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. An organism is tested for catalase production by bringing it into contact with hydrogen peroxide. Bubbles of oxygen are released if the organism is a catalase producer. The culture should not be more than 24 hours old.

Required:

Hydrogen peroxide, 3% H_2O_2 (10 volume solution)

Method:

1- Pour 2–3 ml of the hydrogen peroxide solution into a test tube.
2- Using a sterile wooden stick or a glass rod [not a nichrome wire loop] remove several colonies of the test organism and immerse in the hydrogen peroxide solution.

Important: Care must be taken when testing an organism cultured on a medium containing blood because catalase is present in red cells. If any of the blood agar is removed with the organism, a false positive reaction may occur.

3- Look for immediate bubbling.

Results:

Active bubbling . . . . . Positive catalase test
No bubbles . . . . . Negative catalase test

3.19.2. Bacitracin test:

Principle:

-Bacitracin test is used for presumptive identification of group A streptococcus

-To distinguish between S. pyogen (susceptible to B) & non group A such as S. agalactiae (Resistant to B)

-Bacitracin will inhibit the growth of group A Strep. pyogen giving zone of inhibition around the disk

Procedure:

-Inoculate BAP (Blood Agar Plate) with heavy suspension of tested organism.

-Bacitracin disk (0.04 U) is applied to inoculated BAP.

-After incubation, any zone of inhibition around the disk is considered as susceptible

CHAPTER FOUR

Result

This is a descriptive cross sectional study about the prevalence of streptococcus pyogen conducted in River Nile State –Barber- during the period from April to July 2017. The result of our study shows in the tables bellow.

Result

	Frequency	Percentage
Valid positive	73	73 %
Negative	27	27 %
Total	100	100 %

Gender

	Frequency	Percentage
Male	30	41.1 %
Female	43	58.9 %
Total	73	100 %

Age

	Frequency	Percentage
Valid 5-10	41	56.2 %
10-15	32	43.8 %
Total	73	100 %

Resident Barber

	Frequency	Percentage
Valid Algambarat	47	64.4 %
Algadawab	14	19.2 %
Al-Hajana	6	8.2 % %
Al-Makaylab	0	0 %
Darmali	0	0 %
Kanoor	6	8.2 %
Total	73	100 %

Recurrent Infection

	Frequency	Percentage
Valid Yes	57	68.1 %
No	16	21.9 %
Total	73	100 %

Treated with antibiotic for their condition

	Frequency	Percentage
Valid Yes	23	31.5%
No	50	68.5%
Total	73	100%

Experience fever with their condition

	Frequency	Percentage
Valid Yes	68	93.2%
No	5	6.8%
Total	73	100%

Experience bad breath with their condition (halitosis)

	Frequency	Percentage
Valid Yes	45	61.6%
No	28	38.4%
Total	73	100%

Experience swallowing problem

	Frequency	Percentage
Valid Yes	56	76.7%
No	17	23.3%
Total	73	100%

Skin rash

	Frequency	Percentage
Valid Yes	0	0%
No	73	73%
Total	73	73%

CHAPTER FIVE

5.1. Discussion

This is cross sectional community based study regarding prevalence of streptococcus pyogen among children 5-15 years old conducted in river Nile state Barber from April to July 2017.

Hundred throat swab samples were collected from children suffer from throat infection in different village in Berber, analyzed in department of microbiology in Elshaikh Abdullah Elbadry .

Most of children their age between 5-15 years old, had Primary School Education and live in variously socioeconomic condition, most of them have no information about the disease and its complication.

73 of the children are positive and 27 are negative, 30 of them are male (41.1%), 43 female (58.9%). 41 positive sample from age 5-10 years old has percentage 56.2% , and 32 positive sample from age 10-15 years old by percentage 43.8%.

Depend on residence in Algamberat, Algadawab,Alhajana ,Kanoor the percentage is (64.4%), (19.2%), (8.2%), (8.2%) respectively .

This study similar to the study of Screening for streptococcal pharyngitis discussed By Mohamed Rodwan Ali Hassan B.Sc. Faculty of Natural Resources and Environmental Study University of Kordofan (collected 86 sample, 57 of them were positive (66.3%), 29 were negative (33.7%).

5.2. Conclusion

This study represented high prevalence of streptococcus pyogen in Berber (73%), most of positive result isolated from Gambarat village and most of them were female between 5-10 years old.

5.3. Recommendation

GAS was concerned in this study and by other studies than other pathogens due to their serious complications, so to prevent their serious complication the under lining recommendation should be regarded.

-Early diagnosis of streptococcus pyogen in early stage to prevent their spread to other children in same area.

-Provident the advanced method for diagnosis.

-Increase educational health about infectious disease specially streptococcus pyogen which can lead to heart failure.

-Develop health center in Berber and increase their number to be easy to reach it.

-Treatment of sore throats that must be based on the isolation and identification of the causative organism and determination of its sensitivity pattern.

-The establishment of national RF and RHD remain significant health problem.

CHAPTER SIX

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APPENDIX {1}

QUESTIONNAIRE

PREVALENCE OF GROUP A BETA HEMOLYTIC STREPTOCOCCUS AMONG CHILDREN (5-15) YEARS IN RIVER NILE STATE FROM APRIL TO JULY IN 2017 .

QUESTIONNAIRE

- SERIAL NUMBER .....
- DATE.....
- RESIDENCE.....

- **Gender male** ☐ ☐ **female**

- **Is there recurrent infection Yes** ☐ ☐ **No**

- **Were you treated with antibiotic for your condition when the diagnosis was mad?**

  ☐ **Yes** ☐ **No**

- **Age** ☐

- **Any appearance of skin rash** YES NO ☐ ☐

- **Did you experience fever with your condition?**

  ☐ **YES** ☐ **NO**

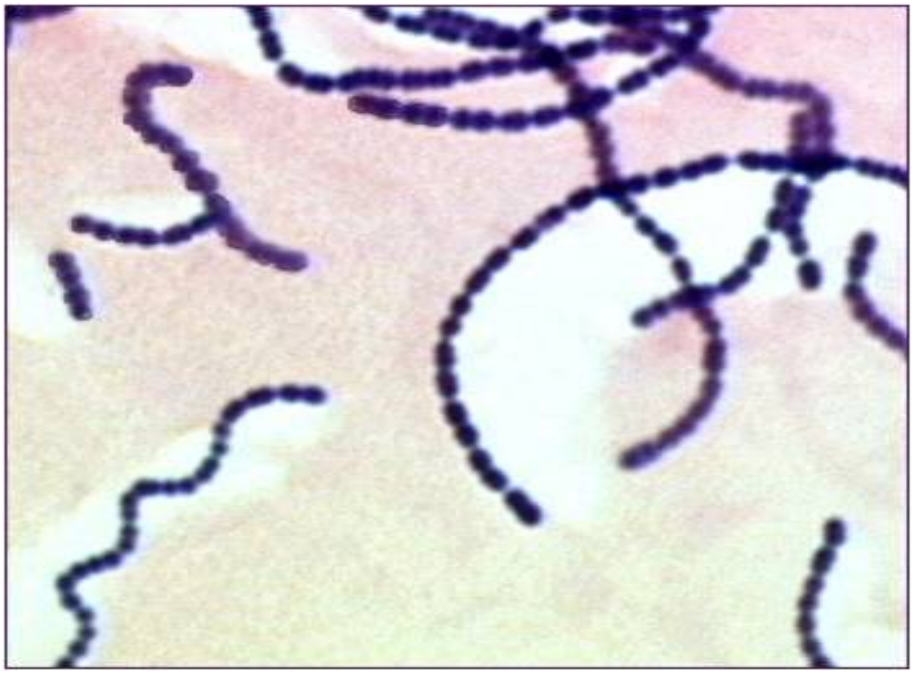
- **Did you experience bad breath(Halitosis)?**

  ☐ **YES** ☐ **NO**

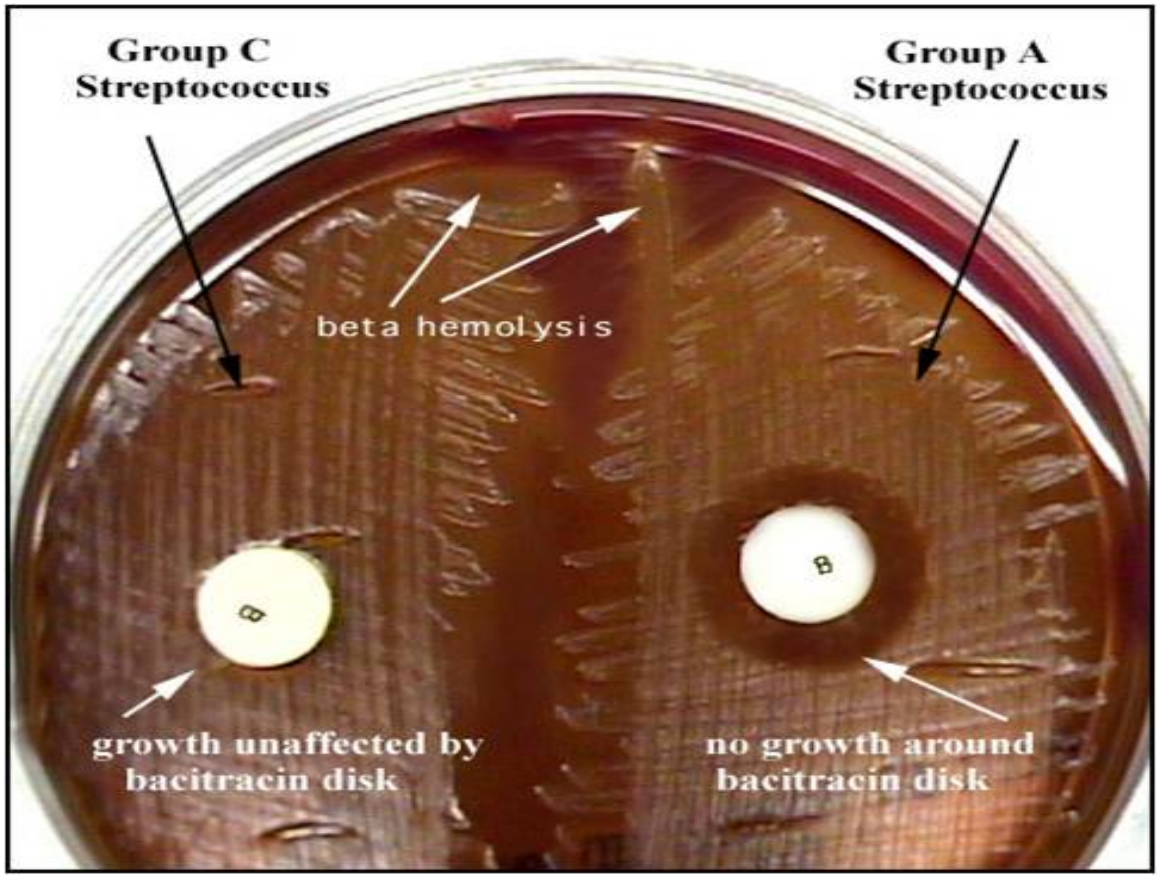
- **Did you experience swallowing problem?**

APPENDIX {2}

PICTURE 1 :Gram Stain of streptococcus in chain



PICTURE 2: Sensitivity of streptococcus to bacitracin disk .



PICTURE 3: Beta Hemolysis streptococcus on blood agar.

PICTURE 4: Catalase Test