GROUP A STREPTOCOCCAL INFECTION AMONG CHILDREN WITH PHARYNGITIS IN JIMMA TOWN, SOUTHWEST ETHIOPIA

BY: GETNET TESFAW (BSc.)

A RESEARCH THESIS SUBMITTED TO THE DEPARTMENT OF MEDICAL LABORATORY SCIENCES AND PATHOLOGY, COLLEGE OF PUBLIC HEALTH AND MEDICAL SCIENCES, JIMMA UNIVERSITY IN PARTIAL FULFILLMENT FOR THE REQUIERMENTS OF MASTERS OF SCIENCE IN MEDICAL MICROBIOLOGY

June, 2014

JIMMA, ETHIOPIA

GROUP A STREPTOCOCCAL INFECTION AMONG CHILDREN WITH PHARYNGITIS IN JIMMA TOWN, SOUTHWEST ETHIOPIA

BY: GETNET TESFAW (BSc.) [1]

ADVISORS:

ALEMSEGED ABDISSA (MSc, PhD Fellow) [2]

GEBRE KIBRU (MSc) [3]

DEMEKE MEKONNEN (MD) [4]

June , 2014

JIMMA, ETHIOPIA

---

[1]*MSc Candidate, Tel: 0913 65 85 77, Email: getnet.tesfaw@ju.edu.et*

[2]*Assistant Prof. Department of Medical Laboratory Sciences and Pathology, Jimma University Tel: 09 11 40 9074,E-mail: alemseged.abdissa@ju.edu.et*

[3]*Assistant Prof. Department of Medical Laboratory Sciences and Pathology, Jimma University Tel: 09 11 31 01 88, E-mail: gebre.tiga@ju.edu.et*

[4]*MD (Pediatrician), Department of Pediatrics, Jimma University, Tel: 09 11 56 90 74, E-mail: demekemm@yahoo.com*

ABSTRACT

Background: *Group A Streptococcus (GAS) is an important cause of morbidity and mortality with clinical presentation ranges from pharyngitis and pyodrema, to life threatening immunological complications such as acute rheumatic fever and glomerulonephritis. GAS is the most common cause of bacterial pharyngitis responsible for 20–30% in children and 5 – 15% in adults.*

Objective: *To determine prevalence, antimicrobial susceptibility pattern and clinical predictors of GAS among children with pharyngitis in Jimma Town Southwest, Ethiopia.*

Methods: *A cross sectional study was conducted on 355 children (5-15 years old) with pharyngitis attended in two selected Health Centers in Jimma town from May 8-December 31, 2013. Demographic and clinical data were collected by using questionnaire and checklist. Throat swabs were collected using sterile cotton swab, inoculated on blood agar plates and incubated for 24-48 hrs at 35-37^{0}C with 5% CO_2. β- hemolytic colonies that were susceptible for 0.04U bacitracin and pyrrolidonylarylamidase (PYR) positive were considered as GAS. Disc diffusion method was used for antimicrobial sucesbtibility testing for selected antibiotics. McIsaac score was used to determine the diagnostic perfomnance of modified centor score for the diagnosis of GAS. Descriptive statistics and multivariate logistic regression analysis was done by SPSS version 20. P-value less than 0.05 was considered as statistically significant at 95% confidence level.*

Results: *The sex profile of 355 children with pharyngitis showed that about 57.7% were females. Majority (66%)* of the children were 5-9 years old giving mean ± SD age of 8.5 ± 2.7

years. *The prevalence of GAS was 11.3%. All isolates of GAS were 100% susceptible to penicillin, amoxicillin, erythromycin, clindamycin, chloramphenicol and ceftriaxone but 52.5% were resistant to tetracycline. Absence of cough [AOR 3.77, 95% CI 1.73-8.22], tonsillar swelling or exudate [AOR 4.48, 95% CI 1.63-12.31], temperature >38^{0}C [AOR 3.47, 95% CI 1.61-7.49] (p<0.05) were found independent predictors for GAS infection. The sensitivity and specificity of a total* McIsaac score ≥ *four was 65% and 87.9% respectively compared to culture results.*

***Conclusions**: The prevalence of GAS was low. The seasonality of GAS infection may understimate the prevalence in this study, so that large-scale prospective study in the entire season and in various settings is required to understand the actual burden of GAS infection among children's with pharyngitis. In addition, future studies on children with pharyngitis should focus on estimation of rheumatic heart disease cases that follows from pharyngitis complications. The use of a McIsaac score had a good diagnostic performance to identify GAS infection, which can be considered for the diagnosis in resource-limited settings where culture facilities and rapid antigen tests are not affordable.*

***Key words**: Group A Streptococcus, Pharyngitis, Children, Jimma, Ethiopia*

ACKNOWLEDGMENTS

I am grateful to my advisors Mr. Alemseged Abdissa, Mr. Gebre Kibru and Dr. Demeke Mekonnen for their fruitful guidance and assistance throughout the development of this research thesis.

Moreover, I would like to express my heartfelt gratitude to Mr. Fasil Tesema for his support in the data analysis.

I would like to thank Jimma University, College of Public Health and Medical sciences, Department of Medical Laboratory Sciences and Pathology for giving me this opportunity to upgrade my professional career.

The last but not the least I am thankful for the study participants who had been involved in this study.

TABLE OF CONTENTS

Contents **page**

ABSTRACT I

ACKNOWLEDGMENTS III

TABLE OF CONTENTS IV

ABBREVIATIONS AND ACRONYMS VII

OPERATIONAL DEFINITIONS VIII

LIST OF TABLES IX

LIST OF FIGURES IX

1. INTRODUCTION 1

    1.1. Background 1

    1.2. Statement of the Problems 3

    1.3. Significance of the Study 6

2. LITERATURE REVIEW 7

    2.1. Epidemiology and Clinical predictors of Group A *Streptococcus* Pharyngitis 7

    2.2. Laboratory Diagnosis of Group A *Streptococcus* Pharyngitis 10

    2.3. Treatment and Drug Susceptibility Pattern of Group A *Streptococcus* 13

3. OBJECTIVES 16

    3.1. General Objective 16

3.2. Specific Objectives 16

4. MATERIALS AND METHODS 17

4.1. Study Area and Period 17

4.2. Study Design 17

4.3. Population 17

4.4.1. Source Population 17

4.4.2. Study Population 18

4.4. Variables 18

4.7.1. Dependent variables 18

4.7.2. Independent variables 18

4.5. Exclusion Criteria 18

4.6. Sample Size and Sampling Technique 18

4.7. Data Collection Methods 21

4.7.1. Collection of Demographic and Clinical Data 21

4.7.2. Collection, Transportation, and Handling of Throat Swab 21

4.7.3. Isolation and Identification of Group A Streptococcus (GAS) 22

4.7.4. Antimicrobial Susceptibility Testing 22

4.8. Data Entry and Analysis 24

4.9. Quality Control 25

4.10. Ethical Consideration....25

5. RESULTS....27

5.1. Socio-demographic Characteristics and Prevalence of GAS....27

5.2. Antimicrobial Susceptibility Pattern of GAS Isolates....30

5.3. Clinical Predictors for GAS Pharyngitis in Children....30

5.4. The McIsaac score Diagnostic performance....32

6. DISCUSSION....34

7. CONCLUSIONS AND RECOMMENDATIONS....40

7.1. Conclusions....40

7.2. Recommendations....40

8. REFERENCES....41

9. ANNEXES....48

Annex-I Information Sheet....48

Annex-II: Questionnaires and Checklist....58

Annex-III: Laboratory Procedures....63

ABBREVIATIONS AND ACRONYMS

ARF: Acute Rheumatic Fever

ASO: Anti-Streptolysin O

ATCC: American type culture collection

BHI: Bain Hearth Infusion Broth

CLSI:Clinical Labortory Standard Institute

CSA: Central Statistical Agency

EUCAST: European Comittee of Antimicrobial susceptibility Testing

GAS: Group A *Streptococcus*

GBS: Group B *Streptococcus*

PSGN: Post streptococcal glomerulonephritis

PYR: Pyrrolidonylarylamidase

RADTS: Rapid Antigen Detection Tests

RHD: Rheumatic Heart Disease

ROC: Receiver Operating Characteristics

STX: Trimethoprim-Sulfamethoxazole

TSS: Toxic Shock Syndrome

OPERATIONAL DEFINITIONS

Children: Children who are aged between 5-15 years old with a peak incidence of GAS pharyngitis and high risk of developing acute rheumatic fever (1, 2).

Pharyngitis: Also called pharyngotonsillitis is an acute infection (inflammation) of the oropharynx and/or nasopharynx that is caused by group A *Streptococcus* (3, 4).

LIST OF TABLES

Table 1: The prevalence of GAS with respect to socio-demographic characteristics among children with pharyngitis in Jimma town, southwest Ethiopia from May-December 2013.. 28

Table 2: The prevalence of GAS in relation to medical and treatment history of children with pharyngitis in Jimma town, southwest Ethiopia from May-December 2013. 29

Table 3: Bivariate and multivariate logistic regression analysis of clinical predictors of GAS among children with pharyngitis in Jimma town, southwest Ethiopia from May-December 2013. 31

Table 4: The proportion of culture positive for GAS with respect to McIsaac score value among children with pharyngitis in Jimma town, southwest, Ethiopia from May-December 2013. 32

LIST OF FIGURES

Figure 1: Conceptual framework of prevalence of GAS among children with pharyngitis in Jimma town southwest ,Ethiopia May-December 2013. 5

Figure 2: Laboratory flow chart for the identification of GAS from throat culture. 24

Figure 3: ROC curve of with cutoff value McIsaac score ≥four. 33

1. INTRODUCTION

1.1. Background

Historically, Billroth coined the name *Streptococcus* in 1874 in his observation of chain forming cocci in wounds. By the end of 19th century, the importance of gram-positive chain-forming cocci in infections of humans and animals had been established. The name *Streptococcus* had been used in the generic sense by Rosenbach in 1884 to describe such bacteria isolated from suppurative lesions in man which he called *Streptococcus pyogenes* (5).

Group A *Streptococcus* (GAS) or *Streptococcus pyogenes* classified under group A in Lancefield grouping is a gram positive bacterium that grows in pairs or chains and causes complete hemolysis when cultured on blood agar. GAS causes different spectrum of human infections, ranging from pharyngitis and pyodrema, to life threatening immunological complications such as acute rheumatic fever (ARF), rheumatic heart disease (RHD) and post-streptococcal glomerulonephritis (PSGN), as well as severe invasive illness, including toxic shock syndrome (TSS) and necrotizing fasciitis (6, 7).

Globaly, it is estimated that about 600 million cases of symptomatic GAS pharyngitis occur annually among people aged over four years and over 550 million of these occur in less developed countries. The greatest global burden of GAS disease is due to RHD which follows GAS pharigitis, where 15 million cases and 349,000 deaths occur worldwide annually. Ninety five percent of the disease burden from RHD is in low and middle income countries where it continues to have a significant impact on the health of children and young adults (8).

GAS is the most common bacterial cause of acute pharyngitis, responsible for 5–15% of sore throat in adults and 20–30% in children (9, 10). The prevalence of GAS pharyngitis in developing countries is higher than in industrialized nations (11). A meta-analysis on fourteen studies among children with sore throat on 2010 showed that, the pooled estimate prevalence of GAS pharyngitis was 37% from both industrialized and developing countries. From this review, prevalence rates ranged from 23% in the United States to 58% in a study from the Netherlands had been reported. In the same review, two studies from developing countries reported prevalence rates of 45% in Sri Lanka and 33% (Egypt/ Croatia/Brazil) (12).

Prevalence data on GAS pharyngitis from developing countries specially in Africa are largely lacking when compared to industrialized nations. Studies in Tunisia (13), South Africa (14), Egypt (15) reported a prevalence of GAS pharyngitis 17.7, 21.6 and 26% respectively. Similarly, in Ethiopia, there is only one study among asymptomatic children (16) and another study 20 years back among children with tonsillitis (17) reported a prevalence of GAS 9.7 and 40.6%, respectively.

This showed that, in Africa data on prevalence of GAS pharyngitis, antimicrobial susceptibility pattern and clinical predictors among children with pharyngitis remained scarce with substantial heterogeneity prevalence rates among studies. This study was therefore, aimed at determining the prevalence, antimicrobial susceptibility pattern and clinical predictors of GAS among children with pharyngitis in Jimma town, where no study ever conducted in this issue. In addition, the study evaluated diagnostic performance of McIsaac score for the diagnosis of GAS among children with pharyngitis.

1.2. Statement of the Problems

Streptococcus pyogenes causes different spectrum of clinical disease of which acute pharyngotonsillitis is a more frequently occurring childhood illness with a highest incidence during the winter and early spring among children between 5-15 years. The major cause of bacterial pharyngitis is attributed by this pathogen which accounts for over 15-30% all cases of pharyngitis (18).

Worldwide it is estimated that there are at least 517, 000 deaths each year due to severe non-suppurative GAS diseases like ARF, RHD, PSGN, and invasive infections. There are 600 million incident cases of GAS pharyngitis per year. The prevalence of severe GAS disease is 18.1 million cases, with 1.78 million new cases each year. RHD as a chronic complication; leading to congestive heart failure, strokes, endocarditis, and finally death. Globally, prevalence of RHD in patients with endocarditis is estimated to be 76.6%. There are over 15 million cases of RHD worldwide, with 282,000 new cases and 349,000 deaths annually (8, 19).

GAS is an important cause of morbidity and mortality with variation in disease burden between populations in which the greater burden of the disease occurs in developing countries, particularly those located in the tropics (20). Rheumatic fever in children aged 5–15 is the leading cause of childhood heart disease in which more than 80 cases per 10,000 children is found in developing countries (7).

The incidence and prevalence of ARF and RHD have been decreasing in developed nations since the early 1900s; but continued to be major causes of morbidity and mortality among

young people in developing nations (19). In low income countries of the world , ARF and RHD are estimated to affect nearly 20 million people and are the leading causes of cardiovascular death during the first five decades of life and accounts more than 95% of the estimated 349,000 deaths due to RHD (21, 22). There are 2.4 million affected children between five and fourteen years of age in developing countries, one million of whom live in Sub-Saharan Africa, making the continent the major ARF/RHD hotspot (8).

Ethiopia as one of the African countries shares the burden of ARF where 50-64 % of all cases of heart disease among children are of rheumatic origin (17). In a study conducted among cardiac admission in Ethio-Swedish Children's Hospital RHD accounted for 54.5% (23). Studies in Addis Ababa and Jimma reported prevalence rate of RHD 39.6% and 32.8%, respectively among cardiac patients (24, 25).

Generally, the burden of GAS pharyngitis and its post infectious complications are immense in developing countries like Ethiopia. To the best of our knowledge, there is no adequate information on the prevalence, antimicrobial susceptibility pattern and clinical predictors of GAS among children with pharyngitis in Jimma where the diagnosis of GAS pharyngitis is merely based on the clinical judgment.

Therefore, conducting this study in this area brings the problem in to light and helps to design effective diagnostic and treatment options to prevent the GAS pharyngitis and its complications. In addition to that, it can be used as baseline data for further research on pharyngitis among children.

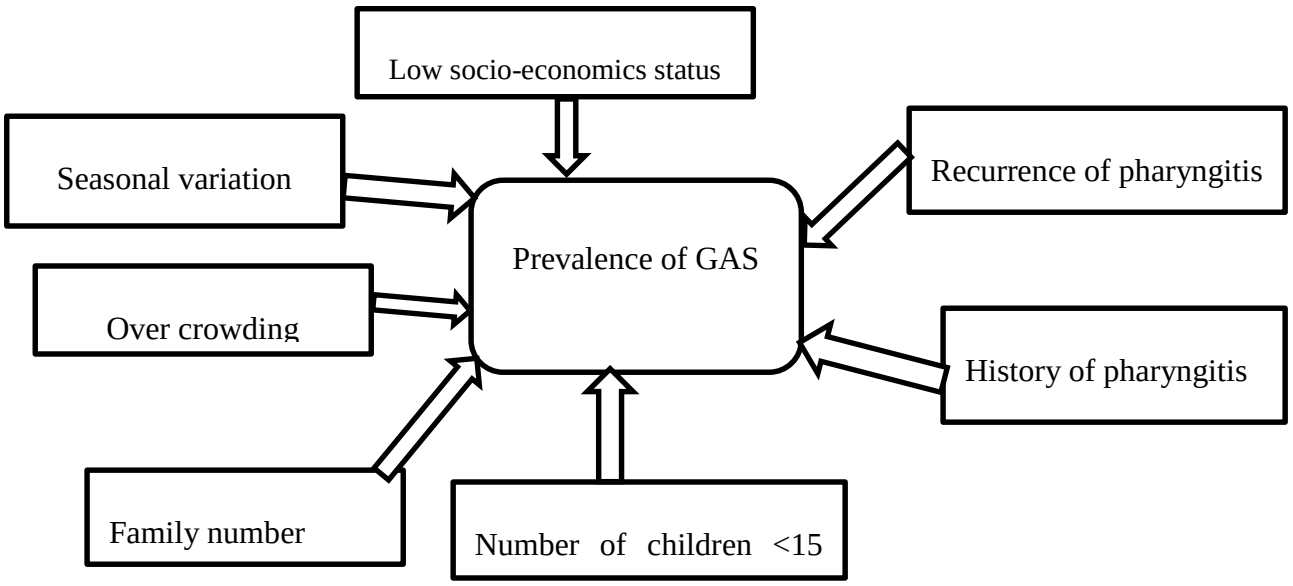


Figure 1: Conceptual framework of prevalence of GAS among children with pharyngitis in Jimma town southwest, Ethiopia May-December 2013.

1.3. Significance of the Study

Annually up to one million case of RHD have been documented in Africa (8). High rate of rheumatic heart disease is reported in the countries of the region such as Nigeria and Zimbabwe, 9.8 and 25.1% respectively (26). Ethiopia as one of the African countries shares the burden of rheumatic fever where 50-64 % of all cases of heart disease in children are of rheumatic origin (17). Study findings in Addis Ababa (24) and Jimma (25) reported prevalence rates of RHD was 39.6% and 32.8% among cardiac patients respectively. Though there is high burden of streptococcal pharyngitis and its severe immunological sequelae, much is not done to investigate the epidemiological, antimicrobial susceptibility pattern and clinical information regarding to the disease.

Therefore, this study was aimed at providing the actual burden of the disease, antimicrobial susceptibility pattern and its clinical predictors of streptococcal disease in children with clinical presentation of pharyngitis. The study outcome in turn helps to design effective diagnostic and treatment options, control and preventive strategies of the disease in that locality. In addition, we have evaluated the McIsaac score for the diagnosis of GAS pharyngitis in children, which is thought to be helpful to improve the efficiency of clinical diagnosis and empirical treatment.

2. LITERATURE REVIEW

2.1. Epidemiology and Clinical predictors of Group A *Streptococcus* Pharyngitis

Globaly, over 600 million cases of symptomatic GAS pharyngitis occur annually among people aged over four years and over 550 million of these occur in less developed countries (8). The overwhelming burden of GAS disease is found in low-income countries, where more than 95% cases of invasive GAS disease and more than 95% of the estimated deaths due to RHD occur in this regions (22).

Group A streptococcal pharyngitis occurs most commonly among children between 5-15 years of age with prevalence ranged from 20-30% with the highest incidence in winter and early spring in temperate climates (27). Although viruses cause most acute pharyngitis episodes, in meta-analysis GAS causes 37% of cases of acute pharyngitis in children older than five years. Highly heterogeneous prevalence of GAS pharyngitis reported from developed and developing countries. In this meta- analysis a prevalence of GAS was 23% in the United States and 58% in a study from the Netherlands. In the same review, a prevalence of 45% in Sri Lanka and 33% (Egypt/ Croatia/Brazil) was reported (12).

In a hosptial based study in Scotland from January to March 2003 on 213 childern with pharyngitis, the prevalence of GAS was 15.9%. In this study, history of sore throat, rash, and pyrexia $>38^0$C were significantly associated with GAS pharyngtis (28). Eleswhere, in Turkey studies reported a prevalence of 11% (29) and 14.7% (30) of GAS pharyngitis among

childern. In another prospective hospital based study in Brusseles among children with pharyngitis showed 20% prevalence of GAS (31).

Studies in USA reported different prevalence of GAS among childern with pharyngitis. In 2003, a study in Colorado on a total 887 children with pharyngitis, 23.7% tested positive for GAS. Clinical findings scarlatina rash, tonsillar exudate and anterior cervical adenitis were significantly associated with GAS pharygitis (32). A study in the same country on childern with pharyngitis reported a prevalence of 27% and identified clinical predictors of GAS as tonsillar exudates, swollen anterior cervical lymph nodes, tonsillar swelling, history of fever in the previous 24 hours, absence of cough, absence of rhinorrhea, swollen posterior cervical lymph nodes, exposure to GAS and temperature above 38^0C (33). Similarly, a prospective cohort study showed that from 587 children, 218 (37%) positive for GAS. This study also showed that clincal variables like scarletinifrom rash, tonsillar swolling, lymphadenopathy and absence of coryza were independent predictor for GAS pharyngitis (34). A prevalece of 58% of GAS also reported in Columbus among childern with sore throat (35). In the same region in Canada 34.1% (36) and 34.8% (37) prevalence of GAS were reported among childern with pharyngitis.

Cross sectional survey in India on total of 579 school children aged 5-15 years with pharyngitis showed 2.8% prevalence of GAS (38) and a prevalence of 7.9% was also reported in Indonesia (39) . A study in Taiwan 2012 among 342 children with pharyngitis a prevalence of 4.1% was reported. The study aslo identified that, skin rash as a positive predictor and cough and runny nose as negative predictors for GAS pharyngtis (40). Elsewhere in Hong Kong, the prevalence of GAS was 38.6% among childern with pharyngitis and identifiedd

absence of cough was significantly associated with GAS pharyngitis. But there was no statistically significant association with tempreture ≥38ºC and rhinorrhoea and GAS pharyngitis (41).

In another study in Yemen on childern with acute pharyngitis the prevalence of GAS was 41.5%. Clinical variables including history of fever, anterior tender cervical lymphadenopathy, petechiae on soft palate and the presence of a red uvula were found independent predictrors for GAS pharyngitis (42).

Study in Brazil, among 2194 Children 3-15 years of age 12% prevalece of GAS reported. In this study the mean of the people per houshold were 4.3 with mean of 1.8 childern less than 15 years old (43). In the same country, prosepective study on a total of 220 children (0–15 years) showed 26% prevalence of GAS (44).

In a multicountry prospective study on children with sore where a total of 2598 children (Brazil n = 294, Croatia n = 404, Egypt n = 1642, Latvia n = 258) sampled, 2472 cases whre eligible for analysis. The proportion of children with a positive GAS throat culture was 24.5%, 26.4%, 29.5% and 39.4% in Brazil, Egypt , Lativia and Croatia, respectively. In Brazil and Egypt, absence of cough, pharyngeal or tonsillar exudates, Cervical lymphadenopathy; in Croatia, absence of cough and Cervical lymphadenopathy; and Latvia only Cervical lymphadenopathy were significantly associated with GAS culture positivity (15).

In African countries data on prevalence and clinical predictorss of GAS are limited. Studies conducted in Egypt (45) Tunisia (13) and South Africa (14) showed the prevalence of GAS was 17, 17.7% and 21.6% respctively. In the study that was conducted in Eygypt clinical

variabeles like dysphagia, vomiting, fever, watery eyses, pharyngeal exudate and scarlitiniform rash were independent predictors of GAS infection in childern with pharyngitis. In this study a high incidence of GAS was reported on early spring and eary winter (45).

There is only single study condcuted 20 year back in Ethiopia among 143 childen with tonsillitis, where 58 (40.6%) were positive for GAS. In this study isolation rate was highest from Feberuary to May (17).

2.2. Laboratory Diagnosis of Group A *Streptococcus* Pharyngitis

Accurate diagnosis of streptococcal pharyngitis followed by appropriate antimicrobial therapy is important for the prevention of acute rheumatic fever and suppurative complications. Diagnosis of GAS remains a subject of controversy due to the best standard for diagnosis has not definitively established. The diagnostic methods that are currently used for the diagnosis of GAS includes; throat culture, Gen-Probe Group A S*treptococcus* direct Test, rapid antigen test, clinical diagnosis by clinician, McIsaac score method based on five clinical criteria (tonsillar exudates, tender anterior cervical lymphadenopathy or lymphadenitis, absence of cough, age 3-15 years and fever >38.5^{0}C), and anti-streptolysin O antibodies (9, 10).

Culture of a throat swab on a sheep blood agar remains the gold standard for the confirmation of streptococcal pharyngitis. A single throat swab on a blood agar plate has a sensitivity of 95% and a specificity of 99% for the detection of GAS pharyngitis. Several variables affect the accuracy of throat culture results, including the manner in which the swab obtained, receiving anti-antibiotics shortly before the throat swab, the use of selective media, the incubation condition and period. The time delay to start antibiotics therapy is the major

disadvantage for throat culture (10, 46). The isolation of GAS in throat culture can be enhanced up to 5% by the use of selective blood agar containing colistin, nalidixic acid, oxolinic acid and trimethoprim-sulfamethoxazole (47, 48).

The development of rapid tests for detection of GAS antigen has useful advantage over culture by reducing the delay of results and can be done with in a minutes (1). The rapid antigen detection tests (RADTs) which are based on carbohydrate antigen from GAS has sensitivity between 70%-90% and 95% specificity compared to culture results. The negative results could not rule out the absence of infection, which means all negative results must be confirmed with culture. But in case of a positive RADT, the diagnosis is considered accurate and follow-up culture is not required (49). In addition to the low sensitivity, the RADTs are expensive, challenging to perform, require multiple manual steps and difficult for interpretation (50).

Another diagnostic method based on molecular techniques, called Gen-Probe Group A *Streptococcus* Direct Test (GP-ST) permits detection of GAS directly in pharyngeal specimens with a nucleic acid probe and provide a result within a minute with no need of conformation with culture in case of negative result. The GP-ST uses a chemiluminescent, single-stranded DNA probe that is complementary to the rRNA of the target GAS. The selection reagent used in the assay differentiates the nonhybridized probe from the hybridized probe in a liquid-phase reaction. The molecular based diagnostic method has sensitivity 86-93% and specificity >95% (35, 51).

The serological test, ASO test is a neutralization assay based on the fact that specific anti-streptolysin O antibodies in the serum neutralize the hemolytic activity of the streptolysin -O

toxin when tested in the presence of erythrocytes (52). GAS pharyngitis also diagnosed by determination of anti-streptolysin O antibodies of four-fold rise in titer on paired serum samples taken at an interval of 7 to 14 days apart indicate an acute infection (53).

In addition to the above aforementioned diagnostic methods of GAS pharyngitis, GAS is frequently diagnosed by clinical judgment which results in 80 to 95% overestimate of the disease, and consequently, an overprescribing of antibiotics (54). Clinician based on clinical sign and symptom to reach diagnosis of GAS pharyngitis including , sudden onset of sore throat, age 5–15 years, fever, tonsillopharyngeal inflammation, tonsillopharyngeal exudates, anterior cervical adenitis, winter and early spring presentation, history of exposure to strep pharyngitis and Scarlatiniform rash (10). It is evidenced that contribution of experienced physician to predict positive throat culture has sensitivity ranging from 55 to 74% and specificity ranging from 58-76% (9).

Clinical prediction rules have been proposed as a way to increase the accuracy of clinical diagnosis (55). The most widely used clinical prediction rule for GAS pharyngitis diagnosis in children is the McIsaac score, which validate from the original Centor score by considering age as an additional criteria. To determine the score, assigns one point for each of the following: temperature greater than 38°C, absence of cough, tender anterior cervical adenopathy, tonsillar swelling or exudate, and age less than 15 years. Zero is assigned for age 15 years. If the total score is one or less, antibiotic therapy and culture of throat swab are not recommended. If the total score is two or three, culture of a throat swab is recommended, and a decision about antibiotics should be based on the culture. Patients with a score of four or

more have the highest likelihood of disease, and either initiating treatment with an antibiotic or taking a throat swab for culture is appropriate (37, 56).

A number of researchers validated the diagnostic performance of the McIsaac score in different countries for the diagnosis of GAS pharyngitis among children. Studies done in Canada showed that, the sensitivity of a McIsaac score ≥four was ranged from 92.6% to 100% and specificity of ranged from 67.2% to 90.3% (36, 37, 56). Elsewhere, in USA reported McIsaac score ≥four had 17% sensitivity and 98% specificity (34).

Studies in developing countries showed that the McIsaac score had sensitivity and specificity of [66.7%, 87.6%] in Indonesia (39), [71% , 87.6%] in Taiwan (40) and [93% , 90 %] in Yemen (42).

2.3. Treatment and Drug Susceptibility Pattern of Group A *Streptococcus*

Patients with acute GAS pharyngitis should be treated with an appropriate antibiotic at an appropriate dose for duration likely to eradicate the organism from the pharynx and to prevent the immunological complications. Based on their narrow spectrum of activity, lack of adverse reactions, and modest cost, penicillin or amoxicillin is the recommended drug of choice for those non-allergic to these agents. However, many people are allergic to penicillin, macrolides (erythromycin, clarithromycin or azithromycin), first generation cephalosporin, clindamycin used as alternatives (10).

Despite decades of primary treatment of GAS with penicillin and the long term use of penicillin for GAS prophylaxis in high risk populations strong resistance this pathogen has not reproted .Specific GAS strains exhibit resistance to almost all categories of antibiotics

aside from penicillin, though most strains exhibit only one or a few of the possible specific resistance phenotypes. Antibiotic resistance elements in GAS are of diverse origin and are highly mobile, many being transferred readily from strain to strain by phage-mediated recombination (57).

A study in Turkey assesed the antimicrobila suscebtibility pattern of 263 strains isolated from childern with tonsillopharyngitis showed, all strains were found to be susceptible to penicillin G, ampicillin, cefazolin, cefuroxime, ceftriaxone,ofloxacin and vancomycin. On the otherhand, 3.8, 4.2, 4.2 and 3% were resistant to erythromycin, clarithromycin, azithromycin and clindamycin respectively (29).

A study done in Brazill showed that all GAS isolates were 100% susceptible for penicillin, erythromycin, clarithromycin, cefalexin, cefaclor, clindamycin, chloramphenicol. But 50% resistant rate was detected for tetracycline (58).

On a study on a total of 1313 GAS isolated from patients with clinical symptoms of pharyngitis in southern India during 1986–2002 reported 2.7% (36) over all resistance of erythromycin but all isolates were susceptible for penicillin (59).

A totalal of 466 GAS clincal isolates from China showed, all of the isolates were sensitive to penicillin, cefradine and ofloxacin. The highest rate of resistance was against clarithromycin 98.1% followed by erythromycin 97.6%, azithromycin and clindamycin (both 97.2%) and tetracycline (94.0 %) (60).

In Africa study done in Tunisia 103 isolates were 100% susceptible to penicillin G, amoxicillin, pristinamycin, vancomycin, and teicoplanin. About 0.9% and 2.9% of isolates

were resistant to rifampin and bacitracin, respectively. Only 5% of strian were resistant to erythromycin and clindamycin. High level of tetracycline resistance (70%) was reported (61). Similarly, a study done in Egypt reported no resistance to penicillin or erythromycin and only one percent resistance to cephadroxil (45).

In a study done in Ethiopa showed that all isolates of GAS were 100% susceptible to penicillin, ampicillin, chloramphinicol, clyndamycin, erythromycin; but, 68% resistant to tetracycline (17). Similarly, GAS isolates from healthy school children showed that 100% sensitive to oxacillin, penicillin, erythromycin, clindamycin and trimethoprim-sulphamethoxazole. In this investigation lower frequency of resistance observed against tetracycline and vancomycin (16).

Generally, there is only limitted data on the prevalence, antimicrobal susceptibilty pattern and clinical predictors of GAS among chilern with pharyngitis. Likewise, there is inadequate data on the diagnostic performance of McIsaac score for the diagnosis of GAS pharyngitis in children. Therefore, this study was aimed at determining the prevalence, antimicrobial susceptibility pattern, clinical predictors of GAS among children with pharyngitis. Moreover, the study evaluated the diagnostic performance of McIsaac score for the diagnosis of GAS among children with pharyngitis.

3. OBJECTIVES

3.1. General Objective

- To determine prevalence, antimicrobial susceptibility pattern and clinical predictors of Group A *Streptococcus* among children with pharyngitis in Jimma town, southwest Ethiopia from May-December 2013.

3.2. Specific Objectives

- To determine the prevalence of Group A *Streptococcus* among children with pharyngitis.
- To asses antimicrobial susceptibility pattern of Group A *Streptococcus* isolates from children with pharyngitis.
- To determine clinical predictors for Group A *Streptococcus* among children with pharyngitis.
- To determine diagnostic performance of McIsaac score for the diagnosis of Group A *Streptococcus* among children with pharyngitis.

4. MATERIALS AND METHODS

4.1. Study Area and Period

The study was conducted in two health centers namely Jimma town Health center and Higher-2 Health center between May 8-December 31, 2013 which are found in Jimma town. Jimma town is located 355km southwest of Addis Ababa and found at latitude and longitude of 7°40′N 36°50′E. The town is located in an area of average altitude of about 1789m above sea level. It lies in the climatic zone locally known as Woyna Daga with a mean annual maximum temperature of 30°C and a mean annual minimum temperature of 14°C. The annual rainfall ranges from 1138 mm to 1690 mm with an area of 100.2 square kilometers. The total projected population of the town from 2007 central statistical agency (CSA) census report is 120,960, of whom 60,824 are men and 60,136 women. There are two government Hospitals (Jimma University Specialized and Shenen Gibe Hospitals) and three public health centers (Jimma town, Higher-2, and Menedera Kochi Health centers) that provide health service facility for the residents of Jimma town (*Jimma town health office).*

4.2. Study Design

A facility based cross-sectional study was conducted.

4.3. Population

4.4.1. Source Population

All children who visited the selected government health centers of Jimma town.

4.4.2. Study Population

The study participants were 5-15 years old children with pharyngitis who consecutively presented at Jimma towen Health Center and Higher-2 Health Center from May 8- December 31, 2013.

4.4. Variables

4.7.1. Dependent variables

- GAS infection
- Antimicrobial susceptibility pattern of GAS

4.7.2. Independent variables

- Age
- Sex
- Family size in the household
- Family income
- History of sore throat
- Clinical variables

4.5. Exclusion Criteria

Children who have taken antibiotics within the last 7 days before their visit to the selected health facilities were excluded from the study (49).

4.6. Sample Size and Sampling Technique

The sample size was calculated based on the prevalence of GAS among Ethiopian children i.e.40.6% (17) at 95% confidence level.

$$n = \frac{(Z\alpha_{/2})^2 P(1-P)}{d^2}$$

n = 370.58 ≈ 371

$Z\alpha_{/2}$= Indicates standard normal value at 0.05 level of significance

P = Prevalence of GAS among Ethiopian Children

d = margin of error 0.05

4.7. Data Collection Methods

4.7.1. Collection of Demographic and Clinical Data

Patients demographic and clinical information was collected using pre-tested questionnaire and checklist. All study participants were examined by trained health officers.

4.7.2. Collection, Transportation, and Handling of Throat Swab

The posterior pharynx and tonsils swabbed trained health officers with a sterile cotton swab. However, swabbing the cheeks, tongues, lips or other areas was avoided. The swabs were placed immediately in Amies transport medium (Oxoid, England) and transported to Jimma University microbiology laboratory, and plated on 5% sheep blood agar within 2 hours of collection (4, 62).

4.7.3. Isolation and Identification of Group A Streptococcus (GAS)

All laboratory procedure done by laboratory technologist. Throat swabs obtained from children with pharyngitis were inoculated onto 5% sheep's blood agar plates and incubated for 24 hrs at 37^0C in candle jar to provide an atmosphere of 5% CO_2. Identification of GAS isolates was based on hemolytic activity on sheep's blood agar, colony characteristics, Gram reaction, catalase production , 0.04-U bacitracin disc susceptibility and PYR (Hardy Diagnosistics, USA) test. After 24 hours of incubation, each plate was checked for GAS characteristics colonies. Culture plates negative for β-hemolytic colonies were incubated for additional 24 hours to allow for the recovery and detection of slow growers. Isolated β-hemolytic colonies were subcultured onto blood agar plates to obtain a pure growth. After overnight incubation, the pure colonies were tested for their Gram reaction and catalase. Moreover, all β-hemolytic Gram positive and catalase negative colonies were inoculated into BHI broth (Oxoid, England) and incubated overnight at 35- 37^0C in candle jar. Then, bacterial suspension was taken from BHI broth and evenly spread using sterile cotton swab onto Muller Hinton agar (Oxoid, England) supplemented with 5% sheep blood. At last, 0.04 U bacitracin disc was placed on the inoculated surface and incubated for 18-24 hours at 37^0C in 5% CO_2. Those bacitracin sensitive and PYR positive colonies were considered as GAS isolate. All such isolates were stored in triplicate in Tryptone soy broth (Oxoid, England) with 15% glycerol at -80^0C (3, 4, 53, 63).

4.7.4. Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was done using disc diffusion method according to criteria set by CLSI and EUCAST. The pure colony of GAS bacterial suspension which

turbidity matched with 0.5 McFarland standard was evenly spread onto Muller-Hinton agar supplemented with 5% sheep blood using sterile cotton swab. Soon after, antibiotic discs selected based on prescription pattern and recommendations from CLSI (64) and EUCAST (65) were placed on the inoculated plate. The following antimicrobial disks with respective concentration were used: penicillin (P,1unit), ceftriaxone (CRO, 30µg) and chroramphinicol (C, 30µg) all from [Becton Deckinson BD, USA Company], amoxicillin (Aml, 25µg), erythromycin (E, 15µg), clindamycin (DA, 2µg), tetracycline (TE, 30µg) all from [Oxoid, England] (29, 64, 66). Finaly the plates were incubated at 35- 37^0C in candle jar overnight. The interpretation of the results of the antimicrobial susceptibility was made based on the criteria as sensitive, intermediate and resistant by measuring the zone diameter of inhibition.

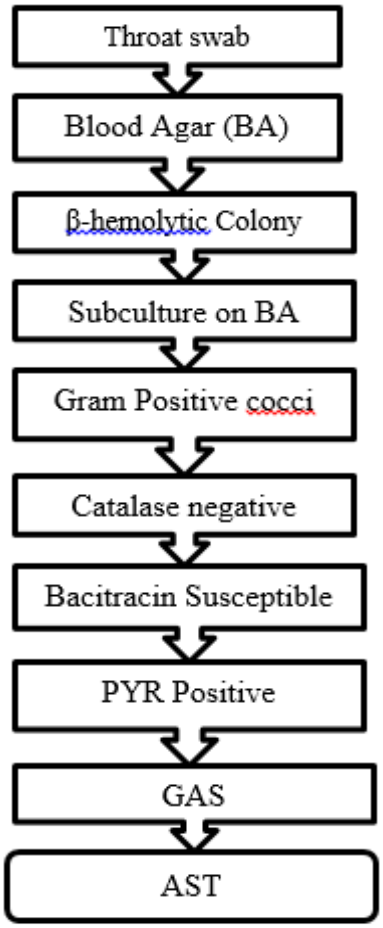


Figure 2: Laboratory flow chart for the identification of GAS from throat culture.

4.8. Data Entry and Analysis

Data was edited, cleaned and checked for its completeness and entered SPSS version 20 for analysis. Patients' demographic and clinical characteristics were described by using descriptive statistics. Bivariate and multivariate logistic regression analysis was done to identify the clinical predictors for GAS pharyngitis and P-value less than 0.05 was taken as statistically significant at 95% confidence level.

In addition , McIsaac score modification method was used to calculate modified centor score value by assigning a value of one for each variables (i.e. body temperature >38°C, no cough, tender anterior cervical adenopathy, tonsillar swelling or exudate, age 5-14 years) and zero for age 15 year. The sensitivity, specificity and predictive value of McIsaac score ≥four using culture as gold standard was determined (37, 56).

4.9. Quality Control

To assure the quality of the data generated during the study, standard operating procedures were followed during media preparation and other laboratory procedures. Sterility check was performed to avoid the possibility of contamination. All reagents were checked for their expiry date and prepared according to the manufacturer's instruction. The data was kept for rechecking and 5% of the tests were repeated randomly to check the accuracy and reliability of the working procedure and performance of the data collectors to minimize technical and observer bias. Half-day training was given for data collector by pediatrician to minimize inter personal variation in identification of clinical signs and symptoms. In addition, GAS (ATCC 19615) and GBS (ATCC 13813) strains that are susceptible and resistance for bacitracin respectively was used as a quality control.

4.10. Ethical Consideration

Ethical clearance was obtained from Jimma University Ethical Review Board. Letter of permission to conduct the study was secured from the health facilities managements. Written informed consent and assent was obtained from the parents /caregivers and children respectively before recruitment of study participants in to the study. The purpose of the study

was clearly described to the study participants including the benefits and risks (Annex –I). Any information concerning the patients was kept confidential and the specimens collected from the patients were analyzed for the intended purpose only. Patients with positive result were communicated about their result.

5. RESULTS

5.1. Socio-demographic Characteristics and Prevalence of GAS

A total of, 355 children between ages of 5-15 years with pharyngitis were enrolled from May 8- December 31, 2013 with response rate of 95.7%. Majority of study participants 193 (54.4%) were from Jimma Health Center. Relatively high proportion of the study participants were females 205 (57.7%) and urban residents 330 (93.0%). Sixty-six percent of the children were 5-9 years old with mean ± SD age of 8.5 ± 2.7. Moreover, the average family size of the target population was 4.6 with mean of 1.8 children less than fifteen years per household (Table -1).

In this study, the overall prevalence of group A *Streptococcus* (GAS) was 11.3% (40/355). The prevalence of GAS was 11.7% (24/205) among females (P=0.759), 14.2% (17/120) in children 10-15 years (P=0.219), 16% (4/25) among rural residents (P= 0.441) and 14.8 % (19/128) children with family income ≤500 Birr/month (P=0.713) (Table -1).

Two hundred twenty four (63.1%) children had an episode of sore throat in the previous year of which 27 (12.1%) were culture positive for GAS. The highest proportion of the study participants 75 (33.5%) had experienced single episode of sore throat in the previous year. Relatively high prevalence of GAS (24%) was observed among children who had sore throat episode five times in the previous year. Out of those study participants who experienced episode of sore throat, 215 (96.0%) received treatment of which 203 (94.3%) had been treated in health institutions. One hundred ninety-five (54.9%) study participants had been sick for 1-2 days before their visit to the health institutions of which 24 (12.3%) of them were positive for GAS (Table-2).

Table 1: The prevalence of GAS with respect to socio-demographic characteristics among children with pharyngitis in Jimma town, southwest Ethiopia from May-December 2013.

Characteristics		Culture result for GAS		Total № (%)	P-Value
		Negative № (%)	Positive № (%)		
Sex	Male	134 (89.3)	16 (10.7)	150 (42.3)	0.759
	Female	181 (88.3)	24 (11.7)	205 (57.7)	
Age in years	5-9	212 (90.2)	23 (9.8)	235 (66.2)	0.219
	10-15	103 (85.8)	17 (14.2)	120 (33.8)	
Residence	Urban	294 (89.1)	36 (10.9)	330 (93.0)	0.441
	Rural	21 (84.0)	4 (16.0)	25 (7.0)	
Schooling* status	Yes	290 (87.9)	40 (12.1)	330 (93.0)	0.998
	No	25 (100.0)	0 (0.0)	25 (7.0)	
Educational status of the family*	Illiterate	45 (84.9)	8 (15.1)	53 (14.9)	0.967
	Read & Write	16 (100.0)	0(0.0)	16 (4.5)	0.998
	1-4 grade	42 (95.5)	2 (4.5)	44 (12.4)	0.102
	5-8 grade	79 (87.8)	11(12.2)	90 (25.4)	0.595
	9-12 grade	89 (89.0)	11(11.0)	100 (28.2)	0.440
	>12 grade	44 (84.6)	8 (15.4)	52 (14.6)	
Family* income per month (birr)	≤500	109 (85.2)	19 (14.8)	128 (36.1)	0.713
	501-999	120 (93.8)	8 (6.2)	128 (36.1)	0.082
	≥1000	86 (86.9)	13 (13.1)	99 (27.9)	
Total		315 (88.7)	40 (11.3)	355 (100.0)	

Family*=Mother, Father or Guardian, Schooling*= current schooling status of the children

Table 2: The prevalence of GAS in relation to medical and treatment history of children with pharyngitis in Jimma town, southwest Ethiopia from May-December 2013.

Medical and treatment history		Culture Result for GAS		Total	P-value
		Negative № (%)	Positive № (%)	№ (%)	
Sore throat episode in previous year	Yes	197 (87.9)	27 (12.1)	224 (63.1)	0.541
	No	118 (90.1)	13 (9.9)	131 (36.9)	
Total		315 (88.7)	40 (11.3)	355 (100.0)	
Recurrent sore throat episode in the previous year	1- Times	64 (85.3)	11 (14.7)	75 (33.5)	
	2 –Times	58 (93.5)	4 (6.5)	62 (27.7)	0.278
	3- Times	34 (87.2)	5 (12.8)	39 (17.4)	0.029
	4 –Times	22 (95.7)	1 (4.3)	23 (10.3)	0.254
	5-Times	19 (76.0)	6 (24.0)	25 (11.2)	0.085
Total		197 (87.9)	27 (12.1)	224 (100.0)	
Treatment measure for the episode taken	Yes	188 (87.4)	27 (12.6)	215 (96.0)	
	No	9 (100.0)	0 (0.0)	9 (4.0)	0.999
Total		197 (87.9)	27 (12.1)	224 (100)	
Place treatment measure taken	Clinic	178 (87.7)	25 (12.3)	203 (94.4)	
	traditional Healer	10 (83.3)	2 (16.7)	12 (5.6)	0.660
Total		188 (87.4)	27 (12.6)	215 (100.0)	
Uvulectomy	Yes	10 (90.9)	1 (9.1)	11 (3.1)	
	No	305 (88.7)	39 (11.3)	344 (96.9)	0.817
Total		315 (88.7)	40 (11.3)	355 (100.0)	
Duration of symptom for the current sore throat in days	1-2 days	171 (87.7)	24 (12.3)	195 (54.9)	0.829
	3-4 days	114 (90.5)	12 (9.5)	126 (35.5)	0.700
	≥5 days	30 (88.2)	4 (11.8)	34 (9.6)	
Total		315 (88.7)	40 (11.3)	355 (100.0)	

5.2. Antimicrobial Susceptibility Pattern of GAS Isolates

In this study, 320 (90.1%) and 35 (9.9%) of study participants were received amoxicillin and benzantine penicillin before the throat culture result was issued respectively. The susceptibility test results showed that all GAS isolates were 100% susceptible to penicillin G, amoxicillin, erythromycin, clindamycin, chloramphenicol and ceftriaxone. Whereas from all GAS isolates 21 (52.5%) were resistant, 9 (22.5%) intermediate and 10 (25%) susceptible to tetracycline.

5.3. Clinical Predictors for GAS Pharyngitis in Children

Bivariate logistic regression analysis was done to identify clinical signs and symptoms predictors for throat culture. From all of the clinical variables that entered to bivariate analysis only cough, tonsillar swelling or exudate, temperature >38^0c, drooling and rhinorrhea had a p-values less than 0.25 and enlarged anterior cervical lymph node due to its clinical importance were included in the multivariate analysis. Clinical predictors not significant at P-value of 0.05 were removed from the final model. Finally, three clinical predictors were found to be independent predictors for GAS in children with pharyngitis. These were absence of cough [AOR 3.77, 95% CI 1.73-8.22, P=0.001], tonsillar swelling or exudate [AOR 4.48, 95% CI 1.63-12.31, P=0.004] and temperature >38^0C [AOR 3.47, 95% CI 1.61-7.49, P=0.001] (Table- 3).

Table 3: Bivariate and multivariate logistic regression analysis of clinical predictors of GAS among children with pharyngitis in Jimma town, southwest Ethiopia from May-December 2013.

Clinical Signs & Symptoms		Crude OR (95% CI*)	P-value	Adjusted OR* (95% CI)	P-value
Cough	Yes	1.00		1.00	
	No	3.608 (1.740-7.480)	0.001	3.778 (1.735-8.225)	0.001
Tonsillar swelling exudate	Yes	4.605 (1.757-12.074)	0.002	4.482 (1.631-12.314)	0.004
	No	1.00		1.00	
Body Temperature	$>38^0$	3.275 (1.645-6.520)	0.001	3.479 (1.616-7.490)	0.001
	$\leq 38^0$	1.00		1.00	
Enlarged anterior cervical lymph	Yes	0.955 (0.494- 1.845)	0.891	2.060 (0.983-4.316)	0.056
	No	1.00		1.00	
Drooling	Yes	2.015 (0.821-4.947)	0.126	2.364 (0.864-6.471)	0.094
	No	1.00		1.00	
Rhinorrhea	Yes	1.00		1.00	
	No	3.416 (0.797-14.631)	0.098	3.870 (0.826-18.139)	0.086
Dysphagia	Yes	0.635 (0.284-1.415)	0.266		
	No	1.00			
Hoarseness	Yes	0.715 (0.327-1.561)	0.399		
	No	1.00			
Abdominal pain	Yes	1.00	0.446		
	No	0.762 (0.378-1.533)			
Nausea	Yes	1.169 (0.604-2.264)	0.643		
	No	1.00			
Vomiting	Yes	0.949 (0.477-1.891)	0.883		
	No	1.00			
Otitis media	Yes	1.107 (0.408-3.007)	0.842		
	No	1.00			
Petechia on the palate	Yes	1.073 (0.396-2.911)	0.889		
	No	1.00			
Conjunctivitis	Yes	1.00	0.318		
	No	0.619 (0.241-1.589)			

NB: OR = odds ratio, CI*= confidence interval*

5.4. The McIsaac score Diagnostic performance

McIsaac score was used to calculate modified centor score value by assigning a value of one point for each variables (i.e. body temperature >38°C, no cough, tender anterior cervical adenopathy, tonsillar swelling or exudate, age 5-14 years) and zero for age 15 year.

Majority of children 159 (44.8%) had McIsaac score value of three followed by McIsaac score value of two, 132 (37.2%) and McIsaac score value of ≥ four, 64 (18%). According to the culture results, the prevalence of GAS was 4.5 % (6/132) among children with a McIsaac score of two, 5.0 % (8/159) for those with a McIsaac score three and 40.6 % (26/64) for those with a total of McIsaac score of ≥ four. (Table- 4).

Table 4: The proportion of culture positive for GAS with respect to McIsaac score value among children with pharyngitis in Jimma town, southwest, Ethiopia from May-December 2013.

McIsaac score value	**Culture Result for GAS**		**Total**
	Negative	**Positive**	
	№ (%)	**№ (%)**	**№** (%)
2	126 (95.5)	6 (4.5)	132 (37.2)
3	151 (95.0)	8 (5.0)	159 (44.8)
≥4	38 (59.4)	26 (40.6)	64 (18.0)
Total	315 (88.7)	40 (11.3)	355 (100.0)

The Receiver operating characteristic (ROC) curve with cutoff value of a McIsaac score ≥four had an area under the curve [0.765, 95% CI 0.678-0.856]. A total McIsaac score ≥four had a sensitivity of 65%, a specificity of 87.9%, positive predictive value of 40.6% and negative predictive value of 95.2% compared to culture results (Fig.3.).

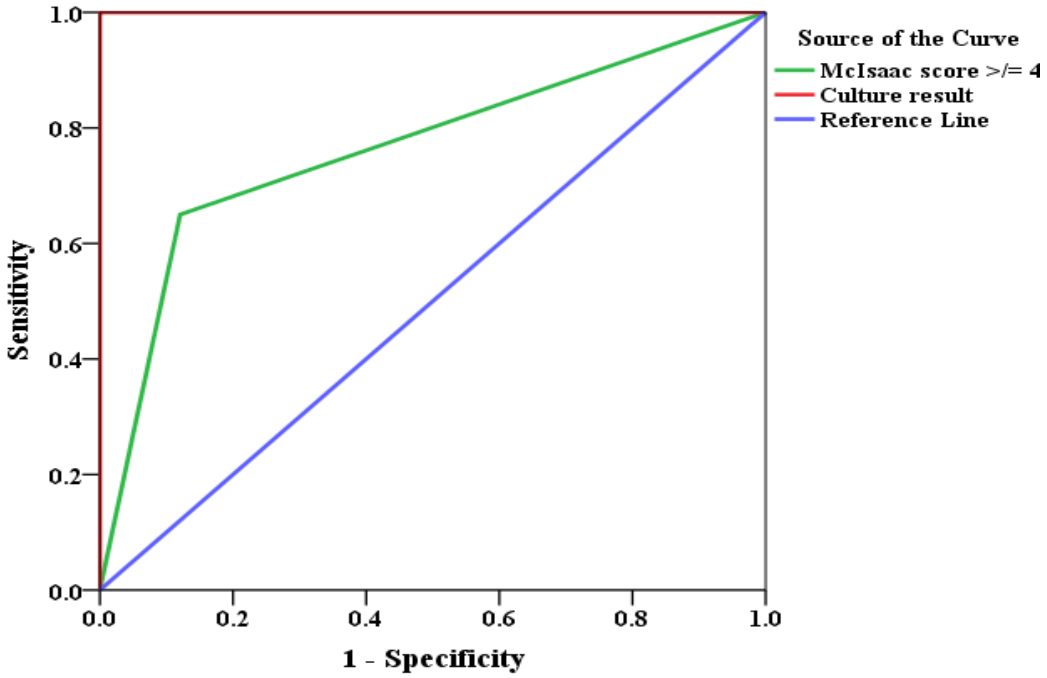


Figure 3: ROC curve of with cutoff value McIsaac score ≥four.

6. DISCUSSION

GAS is an important cause of morbidity and mortality with variation in disease burden between populations in which the greater burden of the disease occurs in developing countries, particularly those located in the tropics (20). In the current study, we presented the results on the prevalence, antimicrobial susceptibility pattern and clinical predictors for GAS isolates among 355 children between age ranges 5-15 years old with a mean ± SD age of 8.5 ±2.7.

In the present study, about 57.7 % of study participants were females children which is higher than 49.6% reported in Ethiopia (17), 42.2% in Egypt (45) and 43.9% in Indonesia (39). However, this higher proportion of females was not significantly associated with culture positivity for GAS (P=0.759).

The overall prevalence of 11.3% GAS in our study goes in line with 11% and 12% report made in Turkey (29) and Brazil (43) respectively. But, it was higher than prevalences of 2.8%, 4.1, and 7.9%, India (38),Taiwan (40) and Indonesia (39) respectively. On the other hand, the 11.3% prevalence of GAS in our study was much lower than 40.6% reported by Tewodros *et. al* in Ethiopia (17). This low prevalence of GAS in the current study may be due to difference in seasons where the previous study in Ethiopia (17) was conducted from Feberuary to May when the carriage rate and infection of GAS reaches to the maximum. Moreover, civil war during 1992 in Ethiopia may contributed for an influx of people to the big cities, causing crowding and lower standards of living which might be the reason for observed high prevalence of GAS in the former study (17).

In addition to this, the low prevalence rate in our study probably due to the trends of over prescription of antibiotics in health facilities as it was demonestrated in our data where 100% of children with pharyngitis received either amoxacillin or penicillin. Such indiscriminate use of antibiotics might contribute for the low carriage rate of GAS in the community. Similarly, 11.3% prevalence of GAS in our study was lower than the findings in Egypt (45), Tunisia (13), Yemen (42), Brazil (15) and USA (35) where average prevalence of 17 to 58% was reported. This difference may be due to methodological differences where Colistin-Oxalinic acid, nalidixic acid and colistin, and Todd Hewitte broth (LIM broth) enhanced culture in STX blood agar was used as a selection media in Egypt, Tunisia and USA, respectively. In addtion to this, seasonal variation of GAS is documnetd in different litratures (17, 45). So that, the low prevalence may be due to the data collection period of our study which was not under taken in the entire season. On the otherhand, to studies done in Egypt (45), Tunisia (13) Yemen (42) and Brazil (15) were conducted in the entire season which includes the peak periods of GAS infcetion. There is aslo high possiblity that the prevalence of GAS in the current study may represent the actual prevalence in this locality for fact that most of acute pharyngitis has viral etiology (1).

In this study, the socio-demographic and childern's medical and treament histroy including, residence, schooling, educational and economic status of the family, previous exposure for GAS, number of episode in the previous year, treatemnt histroy, place of treatemnt taken, tonsilloctomy status, duration of symptom for the current sore throat had no significant associtions with GAS prevalence in childern ($P>0.05$). This showed that these were not the only determinat factors for the epidemiology of GAS rather seasonal variation as suggested in previous studies may play the major role in the distribution of GAS (17, 45) . Unlike present

study, rural residence, recent exposure to GAS and duration of symptom were significantly associated from studies in Egypt (45), Canada (56) and USA (33) repectively .

Regarding the anti-microbial susceptibility patteren, our result showed that all GAS isolates were 100% susceptible to penicillin, amoxicillin, erythromycin,clindamycin, chloramphenicol and ceftriaxone, which goes in agreement with previous studies in Ethiopia (16, 17) where no β-lactams and macrolides drug resistant noted. These findings indicated that β-lactams and macrolides remains as the drug of choice for the treatment of GAS infection. It is known that erythromycin and clindamycin usually used as alternative treatment for patients allergic to penicillin. Fortunately in this study, no single isolate of GAS become resistant to erythromycin and clindamycin which was in agreement with findings elsewhere in Ethiopia (16, 17), Egypt (45) and Brazil (58). However, various level of resistant to erythromycin and clindamycin was reported elsewhere. For instance GAS resistance rate of [3.8%, 3%] from Turkey (29), [5%, 5%] Tunisia (61) and [97.6%, 97.2%] China (60) was reported respectively. In the present study, more than half the isolates (52.5%) were resistant to tetracycline which was comparable with 68% reported in Ethiopia (17) and 50% in Brazil (58), but much lower than 70% and 94% resistance rate documented in Tunisia (61) and China (60), respectively. It is a well documnted fact that tetracycline is not recommended for the therapy of GAS infection because of high prevalence of resistant strians of GAS emerged starting from its released into the market (10) which is similar to our study where 52.5% strian were resistant for tetracycline. Another possible reason for high level of tetracycline resistance could be the fact that GAS strains share common resistance genes with various species of the oral flora that the gene transfers could occur in the oral cavity (58) .

Multivariate logistic regression was done to identify clinical sign and symptoms predictors for GAS among children with pharyngitis. Accordingly, absence of cough, presence of tonsillar swelling or exudate and body temperature $>38^0$ ($p<0.05$) were found to be independent predictors for GAS infection among children. Studies in different setting reported almost similar findings with our study including, studies conducted in Canada (56), USA (33, 34, 67) and Yemen (42). However, these studies also reported enlarged anterior cervical lymph node as independent predictor for GAS. This absence of enlarged anterior cervical lymph node as an independent predictor of GAS in our study may be due to viral infection rather than GAS infection, which causes the enlargement of anterior cervical lymph node. In addition to this, geographical and strain variation may contribute for presentation of different clinical sign and symptoms. However, absence of independent predictor of enlarged anterior cervical lymph node for GAS also found in a study in Egypt (45). Moreover, scarlatina rash reported as idependent predictor for GAS in different studies such as in USA (32, 34, 67) and Egypt (45) which is incontrary to our finding in which none of the study participants had scarlatina rash. In a study done in Egypt, dysphagia and vomiting found to be predictor for GAS, whereas watery eye as protective for GAS infection (45). On the other hand, a study showed that the four clinical variables that frequently reported as indepdendt predictros for GAS did not have a value in the diagnosis of GAS among chileren with sore throat in Brussel (68). This shows that, the clinical varibales that are predictor for GAS infection may be variable among different GAS strian, geographic area and immunity profile of the study population (45).

In this study, majority (44.8%) of the children had a total McIsaac score value of three. This value was similar to the study findings in Indonesia (39) and Canada (36) which reported 50

% and 60.8% of children had a total McIsaac score value of three. In contrary to our findings, the study done in Toronto, Canada reported high proportion (43.3%) of children with had a total McIsaac score value of four (56).

The area under the ROC curve (AUC) is commonly used as indicator of diagnostic performance. The area under curve (AUC) values between 50% (no discriminative value) and 100% (best discriminative value). The area under the rock curve (AUC) for a total McIsaac score ≥four in our study was 76% indicating good diagnostic performance in our settings which was away from the line of 50% and nearly 100% (69).

In our study, a McIsaac score ≥four had low sensitivity compared to the specificity. The sensitivity of a McIsaac score ≥four was 65% which is lower than reports from three studies in Canada which reported sensitivity ranged from 92.6% to 100% (36, 37, 56) but higher than 17% sensitivity reported from USA (34). In this particular study, the lower sensitivity compared to the specificity helps to reduce unnecessary antibiotic prescription which may contribute reduce unnecessary adverse reactions, decrease antibiotic resistance in both *Streptococcus* and other upper airway organisms (15). However, such low sensitivity in our setting where the prevalence of GAS in the community is unknown and rheumatic fever is still common in children may misleads in under estimation the burden of rheumatic fever by reducing prescription of antibiotics. The specificity of a McIsaac score value of ≥four was 87.9%, which is similar to study done in Indonesia with specificity of 87.6% (39). However, higher than study done in Canada with a specificity of 67.2% and 72.3% (37, 56) and lower than 90.3% and 98% specificities reported from Canada and USA respectively (34, 36). The

higher specificity of this study indicates for a total of McIsaac score ≥four, there is higher probability that pharyngitis is highly likely caused by GAS.

7. CONCLUSIONS AND RECOMMENDATIONS

7.1. Conclusions

The prevalence of GAS was low. GAS isolates remains susceptible to penicillins and macrolides. Clinical variables: absence of cough, presence of tonsillar swelling or exudate, and body temperature >38^0C was independent predictors for GAS infection among children with pharyngitis. A total McIsaac score value ≥four had a good diagnostic performance to identify GAS among children with pharyngitis.

7.2. Recommendations

The seasonality of GAS infection may understimate the prevalence in this study, so that large-scale prospective study in the entire season and in various settings is required to understand the actual burden of GAS infection among children's with pharyngitis. In addition, futures studies on children with pharyngitis should focus on estimation of RHD cases that follows from pharyngitis complications. Even if it is not necessary to test susceptibility of GAS isolates to penicillin on a routine basis, it is crucial to look for emergence of resistance to penicillin and more importantly to erythromycin where reports of drug resistance is on an increase in different settings. In resource-limited settings like Ethiopia where culture facilities and rapid antigen tests are not affordable, the McIsaac score can be considered for the diagnosis of GAS infections.

8. REFERENCES

1. Bisno AL. Acute pharyngitis: Etiology and Diagnosis.*Pediatrics*. 1996;97(6):949-54.
2. Steer AC, Batzloff MR, Mulholland K, Carapetis JR. Group A Streptococcal Vaccines: Facts Versus Fantasy. *Current Opinionin Infectious Diseases.* 2009;22:544–52.
3. Bisno AL, Gerber MA, Gwaltney JM, Kaplan EL, Schwartz RH. Practice Guidelines for the Diagnosis and Management of Group A Streptococcal Pharyngitis. Clinical Infectious Diseases. 2002;35:113–25.
4. Carroll K, Reimer L. Microbiology and Laboratory Diagnosiso of Upper Respiratory Tract Infections. *Clinical Infectious Diseases*. 1996;23:442-8.
5. Hardie JM, Whiley RA. Classification and Overview of the Genera *Streptococcus* and *Enterococcus*. *Journal of Applied Microbiology Symposium Supplement*. 1997;83.
6. Langlois DM, Andreae M. Group A Streptococcal Infections. *Pediatrics in Review.* 2011;32(10):423-9.
7. Lynskey NN, Lawrenson RA, Sriskandan S. New Understandings in S*treptococcus Pyogenes*. *Current Opinion in Infectious Diseases*. 2011;24:196– 202.
8. Carapetis JR, Steer AC, Mulholland EK, Weber M. The Global Burden of Group A Streptococcal Diseases. *The Lancet Infectious Diseases*. 2005;5(11):685-94.
9. Cooper RJ, Hoffman JR, Bartlett JG, Besser RE, Gonzales R, Hickner JM, *et al*. Principles of Appropriate Antibiotic Use for Acute Pharyngitis in Adults: Background. *Annals of Internal Medicine*. 2001;134(6):509-17.
10. Shulman ST, Bisno AL, Clegg HW, Gerber MA, Kaplan DL, Lee G, *et al.* Clinical Practice Guideline for the Diagnosis and Management of Group A Streptococcal Pharyngitis: 2012 Update by the Infectious Diseases Society of America. *Clinical Infectious Diseases*. 2012.
11. Steer AC, Danchin MH, Carapetis JR. Group A Streptococcal Infections in Children. *Journal of Paediatrics and Child Health*. 2007;43(4):203-13.
12. Shaikh N, Leonard E, Martin JM. Prevalence of Streptococcal Pharyngitis and Streptococcal Carriage in Children: A Meta-analysis. *Pediatrics*. 2010;126(3).

13. Mzoughi R, Bouallegue O, Selmi H, Ben Said H, Essoussi A, Jeddi M. Group A Streptococci in Children with Acute Pharyngitis in Sousse, Tunisia. *Eastern Mediterranean Health Journal*. 2004;10:488-93.
14. Engel ME, Muhamed B, Whitelaw AC, Musvosvi M, Mayosi BM, Dale JB. Group A Streptococcal *emm* Type Prevalence Among Symptomatic Children in Cape Town and Potential Vaccine Coverage. *The Pediatric Infectious Disease Journal.* 2014;33(2):208-10.
15. Rimoin AW, Walker CLF, Hamza HS, Elminawi N, Ghafar HA, Vince A, et al. The Utility of Rapid Antigen Detection Testing for the Diagnosis of Streptococcal Pharyngitis in Low-resource Settings. *International Journal of Infectious Diseases*. 2010;14:e1048–e53.
16. Abdissa A, Asrat D, Kronvall G, Shitu B, Achiko D, Zeidan M, et al. Throat Carriage Rate and Antimicrobial Susceptibility Pattern of Group A Streptococci (GAS) in Healthy Ethiopian School Children. *Ethiopian Medical Journal*. 2011;49(2):125-30.
17. Tewodrosw, Muhe L, Daniel E, Schalen C, Kronvall G. A One-year Study of Streptococcal Infections and Their Complications among Ethiopian Children. *Epidemiol Infect*. 1992;109:211-25.
18. Stjernquist-Desatnik A, Orrling A. Pharyngotonsillitis. *Periodontology 2000*. 2009;49:140-50.
19. Seckeler MD, Hoke TR. The Worldwide Epidemiology of Acute Rheumatic Fever and Rheumatic Heart Disease. *Clinical Epidemiology*. 2011;3:67-84.
20. Steer AC, Magor G, Jenney AW, Kado J, Good MF, McMillan D, *et al. emm* and C-repeat Region Molecular Typing of Beta-hemolytic Streptococci in a Tropical Country: Implications for Vaccine Development. *Journal of Clinical Microbiology*. 2009;47(8):2502-9.
21. Gerber MA, Baltimore CRS, Eaton CB, Gewitz M, Rowley AH, Shulman ST, et al. Prevention of Rheumatic Fever and Diagnosis and Treatment of Acute Streptococcal Pharyngitis. *Circulation*. 2009;119:1541-51.

22. Steer AC, Law I, Matatolu L, Beall BW, Carapetis JR. Global *emm* Type Distribution of Group A Streptococci: Systematic Review and Implications for Vaccine Development. *The Lancet Infectious Diseases*. 2009;9(10):611-6.
23. Ephrem D, Abegaz B, Muhe L. Profile of Cardiac Diseases in Ethiopian Children. *East African Medical Journal*. 1990;67(2):113-7.
24. Hodes R. Pattern of Heart Disease in Ethiopia as Seen in a Cardiology Referral Clinic. *Cardiology*. 1988;75(6):458-64.
25. Habte B, Alemseged F, Tesfaye D. The Pattern of Cardiac Diseases at the Cardiac Clinic of Jimma University Specialised Hospital, Southwest Ethiopia. *Ethiopian Journal of Health Sciences*. 2010;20(2).
26. Sani MU, Karaye KM, Borodo MM. Prevalence and Pattern of Rheumatic Heart Disease in the Nigerian Savannah: An Echocardiographic Study. *Cardiovascular Journal of Africa.* 2007;18(5):295-9.
27. Wessels MR. Streptococcal Pharyngitis. *The New England Journal of Medicine*. 2011;364:648-55.
28. Van Limbergen J, Kalima P, Taheri S, Beattie TF. Streptococcus A in Paediatric Accident and Emergency: Are Rapid Streptococcal Tests and Clinical Examination of any Help? *Emergency Medicine Journal*. 2006;23(1):32-4.
29. Ciftci E, Dogru U, Guriz H, Asev AD, Ince E. Antibiotic Susceptibility of *Streptococcus Pyogenes* Strains Isolated from Throat Cultures of Children With Tonsillopharyngitis. *Journal of Ankara Medical School*.2003;25(1):15-20.
30. Gur E, Akkus S, A. A, Guzeloz S, Can G, Diren S, et al. Prevalence of Positive Throat Cultures for Group A Beta-Hemolytic Streptococci Among School Children in Istanbul. *Indian pediatrics*. 2002;39:569-73.
31. Smeesters PR, Vergison A, Campos Dc, Aguiar Ed, Deyi VYM, Van L, et al. Differences Between Belgian and Brazilian Group A *Streptococcus* Epidemiologic Landscape. *PloS One*. 2006;1(1).
32. Gieseker KE, Roe MH, MacKenzie T, Todd JK. Evaluating the American Academy of Pediatrics Diagnostic Standard for *Streptococcus pyogenes* Pharyngitis: Backup Culture Versus Repeat Rapid Antigen Testing. *Pediatrics*. 2003;111(6):666.

33. Fine AM, Nizet V, Mandl KD. Large-scale Validation of the Centor and McIsaac Scores to Predict Group A Streptococcal Pharyngitis. *Archives of Internal Medicine*. 2012;172(11):847-52.
34. Attia MW, Zaoutis T, Klein JD, Meier FA. Performance of a Predictive Model for Streptococcal Pharyngitis in Children. *Archives of Pediatrics & Adolescent Medicine.* 2001;155(6):687-91.
35. Fox JW, Marcon MJ, Bonsu BK. Diagnosis of Streptococcal Pharyngitis by Detection of *Streptococcus Pyogenes* in Posterior Pharyngeal Versus Oral Cavity Specimens. *Journal of Clinical Microbiology*. 2006;44(7):2593-4.
36. McIsaac WJ, Kellner JD, Aufricht P, Vanjaka A, Low DE. Empirical Validation of Guidelines for the Management of Pharyngitis in Children and Adults. *The Journal of the American Medical Association*. 2004;291(13):1587-95.
37. McIsaac WJ, Goel V, To T, Low DE. The Validity of a Sore Throat Score in Family Practice. *Canadian Medical Association Journal*. 2000;163(7):811-5.
38. Kumar R, Vohra H, Chakraborty A, Sharma YP, Bandhopadhya S, Dhanda V, *et al.* Epidemiology of Group A Streptococcal Pharyngitis & Impetigo: A Cross-sectional & Follow up Study in a Rural Community of Northern India. *The Indian Journal of Medical Research.* 2009;130:765-71.
39. Malino IY, Utama DL, Soenarto Y. McIsaac Criteria for Diagnosis of Acute Group-A β-hemolytic Streptococcal Pharyngitis. *Paediatrica Indonesiana*. 2013;53(5):259.
40. Shiha C-T, Lin C-C, Lud C-C. Evaluation of a Streptococcal Pharyngitis Score in Southern Taiwan. *Pediatrics and Neonatology*. 2012;53:49-54.
41. Wong M, Chung C. Group A Streptococcal Infection in Patients Presenting with a Sore Throat at an Accident and Emergency Department: Prospective Observational study. *HKMJ*. 2002;8(2):92-8.
42. Ba-Saddik I, Munibari A, Alhilali A, Ismail S, Murshed F, Coulter J, et al. Prevalence of Group A Beta-haemolytic *Streptococcus* Isolated from Children with Acute Pharyngotonsillitis in Aden, Yemen. *Tropical Medicine & International Health*. 2014;19(4) 431–439.

43. Tartof SY, Reis JN, Andrade AN, Ramos RT, Reis MG, Riley LW. Factors Associated with Group A *Streptococcus emm* Type Diversification in a Large Urban Setting in Brazil: A Cross-sectional Study. *BMC Infectious Diseases.* 2010;10(1):327.
44. Smeesters PR, Campos D, Jr., Van Melderen L, de Aguiar E, Vanderpas J, Vergison A. Pharyngitis in Low-resources Settings: A Pragmatic Clinical Approach to Reduce Unnecessary Antibiotic Use. *Pediatrics*. 2006;118(6):e1607-11.
45. Basilli A, Barakat S, E.L.Sawaf G, Zaher s, Zeki a, Ez.E LDS. Identification of Clinical Characteria for Group A -β Hemolytic Streptococcal Pharigitis in Childern Living in Rheumatic Fever Endemic Area. *Journal of Tropical Pedaitrics* 2002;48:285-93.
46. Kellogg JA. Suitability of Throat Culture Procedures for Detection of Group A Streptococci and as Reference Standards for Evaluation of Streptococcal Antigen Detection kits. *Journal of Clinical Microbiology*. 1990;28(2):165.
47. Petts DN. Colistin-oxolinic Acid-blood Agar: A New Selective Medium for Streptococci. *Journal of Clinical Microbiology*. 1984;19(1):4-7.
48. Gunn BA, Ohashi DK, Gaydos CA, Holt ES. Selective and Enhanced Recovery of Group A and B Streptococci from Throat Cultures with Sheep Blood Agar Containing Sulfamethoxazole and Trimethoprim. *Journal of Clinical Microbiology.* 1977;5(6):650-5.
49. Maltezou HC, Tsagris V, Antoniadou A, Galani L, Douros C, Katsarolis I, *et al.* Evaluation of a Rapid Antigen Detection Test in the Diagnosis of Streptococcal Pharyngitis in Children and Its Impact on Antibiotic Prescription. *Journal of Antimicrobial Chemotherapy*. 2008;62(6):1407-12.
50. Uhl JR, Adamson SC, Vetter EA, Schleck CD, Harmsen WS, Iverson LK, et al. Comparison of LightCycler PCR, Rapid Antigen Immunoassay, and Culture for Detection of Group A Streptococci from Throat Swabs. *Journal of Clinical Microbiology*. 2003;41(1):242–9.
51. Steed LL, Korgenski EK, Daly JA. Rapid Detection of *Streptococcus Pyogenes* in Pediatric Patient Specimens by DNA Probe. *Journal of Clinical Microbiology*. 1993;31:2996-3000.

52. Shet A, Kaplan EL. Clinical Use and Interpretation of Group A Streptococcal Antibody Tests: A Practical Approach for the Pediatrician or Primary Care Physician. *The Pediatric Infectious Disease Journal*. 2002;21(5):420-6.
53. Brahmadathan K, Gladstone P. Microbiological Diagnosis of Streptococcal Pharyngitis: Lacunae and Their Implications 92. *Indian Journal of Medical Microbiology*.2006; 24(2):92-6.
54. Borchardt RA. Diagnosis and Management of Group A Beta-hemolytic Streptococcal Pharyngitis. *Journal of the American Academy of Physician Assistants*. 2013;26(9):53-4.
55. Centor RM, Witherspoon JM, Dalton HP, Brody CE, Link K. The Diagnosis of Strep Throat in Adults in the Emergency Room. Medical Decision Making: *An International Journal of the Society for Medical Decision Making*. 1980;1(3):239-46.
56. McIsaac WJ, White D, Tannenbaum D, Low DE. A Clinical Score to Reduce Unnecessary Antibiotic Use in Patients with Sore Throat. *Canadian Medical Association Journal*. 1998;158(1):75-83.
57. Metzgar D, Zampolli A. The M protein of group A *Streptococcus* is a Key Virulence Factor and a Clinically Relevant Strain Identification Marker. *Virulence.* 2011;2(5):402-12.
58. De Melo MCN, Figueiredo AMS, Ferreira-Carvalho BT. Antimicrobial Susceptibility Patterns and Genomic Diversity in Strains of *Streptococcus Pyogenes* Isolated in 1978–1997 in Different Brazilian Cities. *Journal of Medical Microbiology*. 2003;52(3):251-8.
59. Brahmadathan KN, Anitha P, Gladstone P. Increasing Erythromycin Resistance among Group A Streptococci Causing Tonsillitis in a Tertiary Care Hospital in Southern India. Clinical Microbiology and Infection. *Clinical Microbiology and Infectious Diseases.* 2005;11(4):335-7.
60. Liang Y, Liu X, Chang H, Ji L, Huang G, Fu Z, et al. Epidemiological and Molecular Characteristics of Clinical Isolates of *Streptococcus Pyogenes* Collected Between 2005 and 2008 from Chinese Children. *Journal of Medical Microbiology*. 2012;61:975–83.
61. Hraoui M, Boubaker IB-B, Doloy A, Redjeb SB, Bouvet A. Molecular Mechanisms of Tetracycline and Macrolide Resistance and *emm* Characterization of *Streptococcus Pyogenes* Isolates in Tunisia. *Microbial Drug Resistance*. 2011;17(3):377-82.

62. McDonald M, Towers R, Fagan P, McKinnon M, Benger N, Andrews R, *et al.* Recovering Streptococci from the Throat, a Practical Alternative to Direct Plating in Remote Tropical Communities. *Journal of Clinical Microbiology*. 2006;44(2):547-52.
63. M081-1 Throat: Group A *streptococcus* /*Streptococcus pyogenes*: Clinical Microbiology Proficiency Testing: *Clinical Bacteriology*. 2008.
64. Cockerill FR, Wikler MA, Bush K, Dudley MN, Eliopoulos GM, Hardy DJ, *et al.* Performance Standards for Antimicrobial Susceptibility Testing Twenty-First Informational Supplement 2011.
65. European Committee on Antimicrobial Susceptibility Testing. Breakpoint tables for Interpretation of MICs and Zone Diameters. 2014;Version 4.0, valid from 2014-01-01.
66. Rijal K, Dhakal N, Shah R, Timilsina S, Mahato P, Thapa S, *et al*. Antibiotic Susceptibility of Group A *Streptococcus* Isolated from Throat Swab Culture of School Children in Pokhara, Nepal. *Nepal Medical College Journal*. 2009;11(4):238-20.
67. Magdyattia, Theokliszaoutis, Stepheneppes, Joelklein, Frederickmeier. Multivariate Predictive Models for Group A Beta-hemolytic Streptococcal Pharyngitis in Children. *Academic Emergency Medicine*. 1999;6(1):9-13.
68. Roggen I, van Berlaer G, Gordts F, Pierard D, Hubloue I. Centor Criteria in Children in a Paediatric Emergency Department: For What it is Worth. *BMJ open*. 2013;3(4).
69. Erkela ARv, Pattynama PMT. Receiver Operating Characteristic (ROC) Analysis: Basic Principles and Applications in Radiology. *European Journal of Radiology*.1998;27:88 – 94.
70. Cheesbrough M. District Laboratory Practice in Tropical Countries: C*ambridge University press*; 2006.

9. ANNEXES

Annex-I Information Sheet

English Version

Principal investigator: Getnet Tesfaw (BSc, MSc Candidate)

Advisors: ALEMSEGED ABDISSA (MSc., PhD Fellow)

GEBRE KIBRU (MSc.)

DEMEKE MEKONNEN (MD)

Organization: Jimma University

Sponsor: Jimma University.

Project Title: Group A Streptococcal Infection among Children with Pharyngitis in Jimma Town, Southwest Ethiopia

PART-1. **Information sheet**

Introduction:

Dear participant /Parent /Guardian

I am _____ studying my MSc degree in medical microbiology working and with researchers from Jimma University. We are doing a study on the burden of Group A *Streptococcus* antimonial susceptibility and clinical predictors among children with pharyngitis.

Purpose and length of the study

Group A *Streptococcus* among children with pharyngitis is the major public health problems in Ethiopia. Even if the high burden of the disease and its complication the actual

epidemiology of infection is not well documented in Jimma. So this study will determine the prevalence and its clinical predictors and drug susceptibility pattern among children with sore throat.

Procedures

We invite you to take part in this study. If you are willing, you will be required to provide throat swab of specimen and fill questionnaires related to the disease.

Risks and Discomfort

There is no serious risk associated with the procedure but there may be nausea during throat swab which relief immediately.

Benefits

If your throat swab becomes positive, you result will be immediately communicated to your physician. Any finding from your sample will be taken care of by our study health officer and you will get appropriate treatment.

Incentives

We will not pay you for taking part in this study. However, we will thank you for your participation.

Confidentiality

Your specimen will be analyzed only for the intended purpose. Any data regarding you will be kept confidential and accessible only to the researchers.

Right to refuse or withdraw

You do not have to take part in this research if you do not wish to do so, and refusing to participate will not have any effect on your medical care. You do have full right to withdraw

from this study at any point if you wish. Your withdrawal will have no influence what so ever on your further treatment.

Whom to contact

If you have any questions, you may ask the nurse/doctor now or later. If you wish to ask questions later, you may contact:

Mr.Getnet Tesfaw (+251 913658577)

Part 2. Consent form

Code number of the participant _____

Nam of the child_____

Name of parent/guardian_____

I the parent/guardian of _____have been requested for the participation of my child in the study and to give his/her throat swab for diagnosis of "Group A *Streptococcus*." The information in "part-I" have been read to me. I have had the opportunity to ask questions about it and any questions I have asked have been answered to my satisfaction. I consent voluntarily to give:-

My child's throat swab ☐

Print name of participant, date and signature participant

_____ ____/____/____ (dd/mm/yy)

Print name of principal investigator, date and signature of researcher

_____, ____/____/____ (dd/mm/yy)

Amharic Version

ክፍል አንድ፡ ለጥናቱ መረጃና ተሳታፊነት መግለጫ ቅጽ

ዋና ተመራማሪ ፡ ጌትነት ተስፋው

አማካሪዎች ፡ አቶ ዓለምሰገድ አብዲሣ

አቶ ገብሬ ክብሩ

ዶ/ር ደመቀ መኮንን

የድርጅቱ ስም: ጅማ ዩኒቨርሲቲ

የገንዘብ ድጋፉን የሰጠው ድርጅት፡ ጅማ ዩኒቨርሲቲ

የጥናቱ ርዕስ ፡ ‘‘ግሩፕ ኤ ስትርፕቶኮከሳይ በጅማ ከተማ የጉረሮ ህመም ባለባቸው ህጻናት ላይ ያለው ስርጭት፣በሽታውን ለማዎቅ የሚረዱ ምልክቶች እና መድሃኒቶችን የመቋቋም ባህርይ’’

ጤይና ይስጥልኝ፡ እኔ _____ እባላለሁ፡፡ ይህንን ጥናት የምንሰራው በጅማ ዩኒቨርሲቲ የድህረ መረቃ ተምህርታቸውን በማጠናቀቅ ላይ ያሉት ዋና ተመራማሪውን ጨምሮ ሀኪሞች እና ተመራማሪውች ነን።

የጥናቱ ዓላማ

የጉረሮ በሽታ ፤የልብ ህመም እንዲሁም ለተለያዩ ህመሞች መንስኤ የሆነውን ‘‘ግሩፕ ኤ ስትርፕቶኮከሳይ’’ በመባል የሚታዎቀውን ጀርም በጅማ ከተማ የጉረሮ ህመም ባለባቸው ህጻናት ላይ የለውን ስርጭት፣በሽታውን ለማዎቅ የሚረዱ ምልክቶች እና መድሃኒቶችን የመቋቋም ባህርይ ለማዎቅ የሚረዳ ጥናት ነው፡፡

የአሰራሩ ሂደት

በዚህ ጥናት ዉስጥ ይሳተፉ ዘንድ እንጠይቆታለን፡፡ለመሳተፍ በጥጥ አማካኝነት ከልጅዎ ጉረሮ ናሙና እንዲዎሰድ መፍቀድ እና ከበሽታው ጋር ተያያዥ የሆኑ መጠይቆችን መመለስ ይጠበቅበዋል።

በጥናቱ ሊከሰቱ የሚችሉ ተያያዥ ችግሮች

በጥናቱ መሳተፍ ምናልባት በጥቂት ልጆች ላይ ወዲያውኑ የሚጠፋ የማቅለሽ ስሜት ሊያስከትል ይችላል።

በጥናቱ በመሳተፍ የሚገኝ ጥቅም

በልጅዎ የጉረሮ ናሙና ዉሰጥ በሽታው ከተገኘ ልጅዎ ነጻ የህክምና እርዳታ ያገኛል፡፡

ክፍያን በተመለከተ

ልጅዎ በጥናቱ በመሳተፉ ምንም አይነት ክፍያ አይከፈልዎት።በአንጻሩ ከልብ ልናመሰግንዎት እንወዳለን፡፡

የጥናቱ መረጃዎች ሚስጥራዊነት

በጥናቱ ውስጥ የተሰበሰቡ ማናቸውም የልጅዎ መረጃዎች ሚስጥራዊነታቸው የተጠበቀ ይሆናል፡፡

ከጥናቱ ስለመውጣትና ስለማቋረጥ

ይህ ጥናት በፈቃደኝነት ላይ የተመሰረተ እንደመሆኑ መጠን በማንኛውም ወቅት በፈቃድዎ ልጅዎን ከጥናቱ ማስዎጣት ይችላሉ፡፡ ከጥናቱ ልጅዎ ቢወጣም እንኳን የተለመደውን የህክምና እርዳታ በጤና ተቋሙ ውስጥ በማንኛውም ጊዜ ልጅዎ የማግኘት መብት አለው፡፡

ከጥናቱ ጋር በተያያዘ ማናቸውም ጥያቄ ቢኖርዎት በሚከተው አድራሻ ጥያቄዎን ማቅረብ ይችላሉ።

አቶ ጌትነት ተስፋው (ስልክ:0913658577)

ክፍል ሁለት-የስምምነት ቅጽ

የተሳታፊው ህጻን መለያ ቁጥር_____

የተሳታፊው ህጻን ሙሉ ስም_____

የተሳታፊው ህጻን ወላጅ/አሳደጊ ሙሉ ስም_____

እኔ የ_____ ወላጅ/አሳዳጊ ልጅ በዚህ ጥናት እነዲሳተፍ እና በጥጥ አማካኝነት ከልጅ ጉረሮ ናሙና እንድሰጥ ተጠይቄዐለሁ፡፡በክፍል አንድ ዉስጥ ያለዉ መረጃ ተነቦልኛል፡፡መጠየቅ የምፈልገዉን ጥያቄ በአግብቡ ጠይቄ አስፈላጊዉን ምላሽ በአጥጋቢ ሁኔታ ተመልሶልኛል፡፡ ስለሆነም በፈቃደኛነት ልጅ በጥናቱ ተሳታፊ እንዲሆን እና የልጅን የጉረሮ ናሙና ለ‘‘ግሩፕ ኤ ስትርፕቶኮከሳይ’’ ምርመራ እንዲዎሰድ መፍቀዴን በፊረማዬ አረጋግጣለሁ።

የወላጅ/ያሳዳጊ ፊርማ _____

የዋና ተመራማሪ ፊረማ_____

Afan Oromo Version

kutaa tokkoffa: uunkaa Informeshiinii Hirmaatota qorannootif dubifamu

Qorataa hayuduree : Obboo Geetinnat Tasfawu

Gorsitootin : 1. Obboo Alamsaggad Abdiisaa

2. Obboo Gabruu Kibruu

3. Dr.Damaqaa Makooniin

Maqaan dhabbataa: Yunivarsitii Jimmaa

Dhabbatin qoranno kana isponsara godhe : Yuunivarsitii Jimmaa

Mata duree qorannaa: Giruup A Istiriptookoksay payojin magalaa jimmatti ijoolle dhukkuba qonqoo qaban jidduti ; Faca'insi ,mallatoolee dhukkuba kana qorachuuf nama gargaranii fi hala qorichaan walbaruu maal akka fakkatu qorachuudha.

Sensa : Harka funee akkam jirtu ani Ani maqaan kiyya **Getinnat Tasfawu** jedhama**.** Yunivarsitii Jimmaa, Dipaartimantii saaynsii laaboraatorii fayyaa fi paatologiitti barataa digirii lammafati .

kayyoo: kayyoon qo'anna kanaa baakteriyaa istirptokookas payoojin A jedhamuun beekamu kan dhukkuba qonqoo fi dhukkuba onnee fidu; facainsin isaa, mallatollee dhibee

inni fiduun wal qabatanii fi akkasumas halaa qorichaan wal baruu isaa daa'iman dhibee qoonqon qabamanii jiddutti maal akka fakkatu qo'achuudha.

Adeemsa qoranno

Qo'anna kanatti akka hirmatan kabajaan isin gafanna . Ragaleen hawasumma fi medicalaa dhukkuba kanaaf sababa tahu danda'an ni funaanamu. . Qo'annicha kana keessati himaatuuf jibriitti fayyadamuun qoonqoo ilma keessanii irra akkeen fudhamuun qorannon laaboraatorii adda addaa taasifinuuf akka nuuf eeyyamtan ni gafanna .

Rakkoolee qo'annaa kanaan walqabatanii dhufuu danda'an

Ijollen qo'anna kan irratti hiraatan irratti yeroo gabaadatti kan badu mallatton hoqisiisuu mul'achu ni danda'a .

Faydaa qo'annoo kana irraa argamu

Yoo dhukkubain kun qoonqoo daa'ima keesanii kessatti argame yaaliin tolaa ni taasifammaf.

Kaffaltii ilaalchisee

Daa'imni keessan qo'anna kanatti himatuu isaatin /isitin faydalee arman olitti eeramanin alatti kaffaltiin adda hin kaffalamuuf .

Iciitii ragaalee qo'annoo

Ragaallen qo'annof daa'ma keessan irra funannaman hundi iciitin isaan ni eegama

Mirga didu ykn addan kutuu

Qo'anna kanatti hirmaatuun ijoolee keessan gutuun gututti fedhii keessan irratti kan hundahedha. Diduufis tahee addan kutuuf mirga gutuu qabdu . Hirmannan ijoolee kessanii fayidaa yala isaan argatuu qabdaniin walitti hidhata hin qabu . Hirmatanus dhiftanuus mirga yalamuu isaanii ni argatu .

Odeefannoo dabalataa

qoo'anoo kana ilaalichisee gaffii yoo qabattan gaffachuu dandessu. Gaffiilee dabalattaatif namotaa armaan gaditti eeraman kana haasofsisuu ni dandessu.

Obbo **Getinnat Tasfawu** (lakk. bilbila :0913658577)

kuutaa lammaffa: uunkaa waliigaltee

lakkosa daa'ina hirmaatuu _____

maqaa guutuu daa'ina hirmaatuu _____

maqaa guutuu matii(guddiftuu) daa'iimaa_____

Ani _____maatiin /guuddisan(fitun) daa'imin kiyya qo'anna kanaati akka hirmaatuu fi jibritti fayyadammun qoonqoo daa'ima kiyya irra sampliin akka fudhamu gafatamee jira .armaan olitti raggan jiru naaf ibsamee jira.Gaffiiwwan gaffachuu barbadu hunda seeraan gafadhee deebii quubsa argadhee jira . Haaluma kanaan daa'imni kiyya qo'anna kanatti akka

hirmaatu fi qoranoo guruup A istirptookookasitif qoonqoo isaa /isii irraa sampilii akka fudhamu kiyyan fedhiidhaan murteesse kiyyaa mallaattoo kiyyan ni mirkaneessa.

Mallatto maatii (guuddiftuu) daa'imaa _____

mallaroo hayu-duree garee qo'anna _____

Milka'ina qo'anna kanatiif gaffiilee armaan gadiitiif deebii sirrii tahe akka nuf kennitan kabajaan isin gaffana .

Galatomaa!

Annex-II: Questionnaires and Checklist

Jimma University

College of public health and medical sciences

These questionnaire and checklist are prepared to assess the burden, clinical predictors and antimicrobial susceptibly pattern of GAS among children with pharyngitis at Jimma town Health Centre and Higher -2 Health Center from May 8-December 31, 2013.

Name of the children _____ card number_____

Code Number _____Address_____phone number_____

- Include all children from 5-15 years old and who did not take antibiotics in the last 7 days.

Part I. Sociodemographic Data		
Sr.no	**Questions to Be Asked**	**Response**
01	Sex	1. Male 2. Female
02	Age(in year)	_____
	Place of residence	1. Urban 2. Rural
03	Height _____weight_____	
04	Religion	1. Orthodox 2. Muslin 3. Protestant 4. Other

O5	Ethnicity	1. Oromo 2. Amahara 3. Dawuro 4. Yem 5. Others
06	Is the child is at school/day care center currently?	1. Yes 2. No
07	Educational status of (father) __ (Mother) ____	1. Illiterate 2. Can read and write 3. 1-4 grade 4. 5-8 grade 5. 9-12grae 6. >12 grade
08	Family income in birr/month?	_____
09	Family size of the household?	_____
10	Number of children in the family less than 15years?	_____
	Part II. History of Sore Throat	
11	Have you encountered sore throat in the last one year?	1. Yes 2. No
12	If yes for Q. NO.13. How many times?	_____
13	Had treatment measure taken?	1. Yes 2. No

14	If yes for Q. NO.15 from where it had been taken?	1. Visit a clinic 2. Traditional Hear
15	Have you been subjected to uvulectomy?	1. Yes 2. No 3. Don't know
Part III. Clinical Data (sign and symptoms)		
16	Body temperature in ^{0}C	_____
17	For how long the current sore throat last.	_____
18	Do you have dysphagia?	1. Yes 2. No
19	Do you have cough?	1. Yes 2. No
20	Tender or Enlarged anterior cervical lymph node	1. Yes 2. No
21	Tonsillar swelling or Exudate	1. Yes 2. No
22	Scarlatina rash	1. Yes 2. No
23	Otitis media	1. Yes 2. No
24	Hoarseness	1. Yes 2. No
25	Drooling	1. Yes 2. No
26	Petechia on the palate	1. Yes 2. No
27	Abdominal pain	1. Yes 2. No

28	Nausea	1. Yes 2. No
29	Vomiting	1. Yes 2. No
30	Conjunctivitis	1. Yes 2. No
31	Rhinorrhea	1. Yes 2. No
32	Diarrhea	1. Yes 2. No
33	type of treatment given	_____

	Part IV. To be Completed by the Principal Investigator			
34	McIsaac score criteria		Point	
			yes	No
	1. Temperature > 38°C		1	0
	2. Tender or enlarged anterior cervical adenopathy		1	0
	3. cough		0	1
	4. Tonsillar swelling or exudate		1	0
	5. Age in year	5-14	1	
		15	0	
	Total score		_____/5_____	

	Part V. Laboratory Result of the Throat Culture	
35	β-Hemolytic Colony	1. Yes 2. No
36	Gram positive Cocci	1. Yes

		2. No
37	Catalase	1. Positive 2. Negative
38	Bacitracin susceptibility	1. Susceptible 2. Resistant
38	PYR	1. Positive 2. Negative
39	GAS identified	1. Yes 2. No

Annex-III: Laboratory Procedures

1. Throat Swab Collection Procedure (4)

1. Preparing the patient facing a light source.
2. Depress the tongue of the patient with tongue depressor.
3. Apply a sterile cotton swab and rube vigorously over the tonsil and back wall of the pharynx.
4. Place the swab in to Amies transport medium.

2. Preparation of Amies transport medium (*Manufacturer Instruction*)

1. 23gm Amies transport medium of suspended in 1liter of distilled water and boiled.
2. Dispense aseptically 5 ml of medium in to 15 ml of test tube.
3. Sterilize the suspension by autoclaving at121^0C for 15 minutes.

3. Preparation of 5% Sheep Blood Agar (*Manufacturer Instruction*)

1. 40gm blood agar base suspended in 1liter of distilled water and boiled.
2. Sterilize the suspension by autoclaving at121^0C for 15 minutes and transfer to a 50^0C water bath.
3. Add 50ml of sheep blood to the suspension and mix homogenously.
4. Dispense aseptically 15 ml amounts in sterile petri dishes.

4. Preparation of 5% Mueller Hinton Agar (*Manufacturer Instruction*)

1. 38gm Mueller Hinton agar of suspended in 1liter of distilled water and boiled.
2. Sterilize the suspension by autoclaving at121^0C for 15 minutes and transfer to a 50^0C water bath.
3. Add 50ml of sheep blood to the suspension and mix homogenously.

4. Dispense aseptically 60–70 ml of medium per plate into 15 x 150-mm plates.

5. Inoculation of Plates and Incubation (16,54,64)

1. Roll firmly the throat swabs over one-sixth of the plate to deposit the specimen.
2. Streak using sterile loop the inoculum over the surface of the plate and the plate incubated at 37^0cin a candle jar.

6. Catalase Test (70)

1. Pour 2 – 3 ml of the 3% hydrogen peroxide on microscopic slide.
2. Using a sterile wooden stick or a glass rod, remove several colonies of the test organism & immerse in the hydrogen peroxide solution.
3. Look for immediate active bubbling. Active bubbling indicates positive test.

7. Bacitracin Susceptibility Test (16, 70)

1. Inoculate catalase negative isolate into Brain Heart Infusion (BHI) broth and incubate at 37^oC in 5% CO2 overnight in order to obtain a bacterial suspension.
2. Evenly spread the bacterial suspension onto blood agar plate using sterile swab.
3. Place bacitracin disc (0.04 U) aseptically on the inoculated surface and incubated for 18-24 hour at 35^0 C in 5% CO2.
4. Examine the plate for the presence of a zone of inhibition around the disc.
5. Any zone of inhibition surrounding the disc indicates of a presumptive GAS.

8. PYR Hydrolysis Procedure (*Manufacturer Instruction*)

1. Bring disks to room temperature
2. Place PYR disc on a disposable glass microscopic slide.
3. Moisten the disc by adding a small volume of sterile normal saline (5-10 uL) directly to the disc.

4. With a sterile loop or stick, pick up several well-isolated colonies from an 18-24 hour sheep blood plate and gently rub a heavy visible inoculum onto small area of the disc.
5. Incubate the inoculated disc at room temperature for 2 minutes
6. Dispense one drop of color developer onto the disc.
7. A positive result is indicated by the development of a pink or cherry-red color within 1 minute after addition of the color developer
8. A negative result is indicated by no color change within one minute after addition of the color developer.

9. Preparation of Turbidity Standard (70)

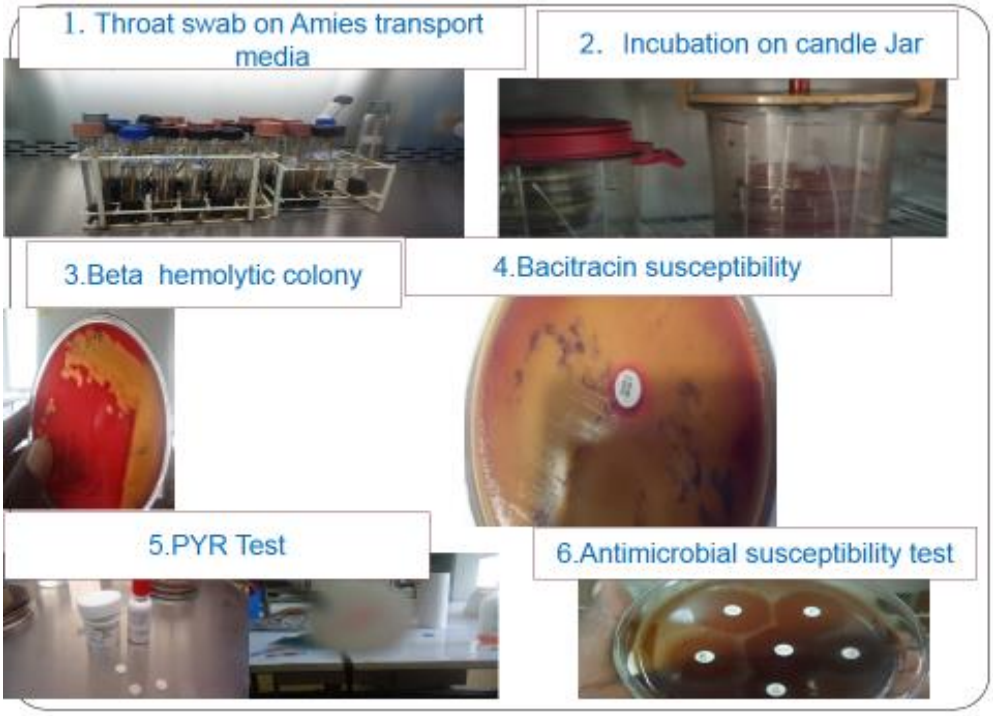
1. Prepare a 1% v/v solution of sulphuric acid by adding1 ml of concentrated sulphuric acid to 99 ml of water and mix well.
2. Prepare a 1% w/v solution of barium chloride by dissolving 0.5 g of dehydrate barium chloride (.2H2O) in 50 ml of distilled water.
3. Add 0.6 ml of the BaCl2 solution to 99.4 ml of the sulphuric acid solution & mix.
4. Transfer a small volume of the turbid solution to a capped tube or screw cap bottle of the same type as used for preparing the test and control inocula.

10. Antimicrobial Susceptibility Testing by Disc Diffusion Method (16,70)

1. Prepare a suspension of the test organism by emulsifying several colony of the organism in a small volume of Brain Heart Infusion (BHI) broth much the turbidity of suspension with 0.5 McFarland turbidity standard
2. With a sterile swab, take sample from the suspension (squeeze the swab against the side of the test tube to remove the excess fluid).

3. Spread the inoculum evenly over the Muller-Hinton agar plate supplemented with 5% sheep blood with the swab using a sterile forceps or needle; place the antimicrobial disc on the inoculated plate.
4. Incubate the plate at 35-37^{0}C for 18-24 hours in candle jar.
5. Read the test after checking that the bacterial growth is neither heavy nor light. Measure the radius of the inhibition zone.
6. Interpret the reaction of the test organism to each antibiotics used as sensitive, intermediate, or resistance as per the standard.

11. Photographs



Declaration

I, the undersigned, declare that this thesis is my own work and it has not been presented in other universities, colleges or other institutions for similar degree or other purpose. In case of others work has been used, it has been carefully acknowledged and referenced in accordance of with requirements.

Name of the principal investigator **Signature** **Date**

Getnet Tesfaw (BSc, MSc candidate) _____ _____

Approval of Advisors

1. Alemseged abdissa (MSc,Assistant professor) _____ _____
2. Gebre kibru (MSc,Assistant professor) _____ _____
3. Demeke Mekonnen (MD, Pediatrician) _____ _____

Approval of External Examiner

1. Abraham Haileamlak (Professor of Pediatrics and Child Health)_____ _____

Approval of Internal Examiner

1. Tesfaye Kassa (PhD, Microbiology) _____ _____