ADDIS ABABA UNIVERSITY

COLLEGE OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

Prevalence And Drug Susceptibility Pattern Of Group A Streptococcus Among Patients With Tonsillopharyngitis Attending Yekatit 12 Hospital, Addis Ababa, Ethiopia

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A Research Thesis Submitted To the Department Of Medical Laboratory Sciences College Of Health Science Addis Ababa University In Partial Fulfillment Of Master Of Science Degree In Clinical Laboratory Sciences (Diagnostic And Public Health Microbiology)

March, 2021

Addis Ababa, Ethiopia

Addis Ababa University

School Of Graduate

This is to certify that Terefe Selbane thesis, entitle:

Prevalence And Drug Susceptibility Pattern Of Group A Streptococcus Among Patients With Tonsillopharyngitis Attending Yekatit 12 Hospital, Addis Ababa, Ethiopia submitted To the Department Of Medical Laboratory Sciences College Of Health Science Addis Ababa University In Partial Fulfillment Of Master Of Science Degree In Clinical Laboratory Sciences (Diagnostic And Public Health Microbiology) which compiles the regulation of the university and meets the standards with respect to originality and quality.

Signed by the examining committee;

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ACKNOWLEDGMENT

First of all, I want to pass my gratitude to Addis Ababa University, Department of Medical Laboratory Sciences for providing me good inspiration in scientific teaching and for providing me a chance to conduct research which is a chance to learn and to contribute.

I would like to express my sincere gratitude to my principal advisor, Dr Abraham Tesfaye (he who fed me skill and knowledge about SPSS) and co-advisor Mrs. Regasa Diriba for their continuous support, motivation, patience and accessibility from the early development of the proposal to the write-up of my thesis.

I would like to thank the Yekatit 12Hospital Microbiology Department for all contributions and permitting me in order to conduct my study and providing me the necessary resources for the study to be carried out successfully.

I would also like to thank my study participants for their willingness to take part in study voluntarily.

Last but not least to my wife Misrak Nire for her continuous support, encouragement, and patience

LIST OF FIGURES

LIST OF TABLES

ABBREVIATIONS

AAU	Addis Ababa University
ARF	Acute Heart Disease
ASO	Anti-Streptolysins O
AST	Antibiotic Susceptibility Testing
ATCC	American Type Culture Collection
CI	Confidence Interval
CLSI	Clinical and laboratory Standards Institute
COPD	Chronic Obstructive Pulmonary Disease
DRERC	Departmental Research and Ethics Review Committee
ENT	Ear, Nose and Throat
GABS	Group Aβ-Streptococcus
GAS	Group A Streptococcus
MHA	Muller Hinton Agar
OR	Odds Ratio
PI	Principal Investigator
PSGN	Post Streptococcal Glomerulonephritis
RDDMH	Ras Desta Damtew Memorial Hospital
RHD	Rheumatic Heart Disease
SOP	Standard Operating Procedure
WHO	World Health Organization
Y12H	Yekatit 12 Hospital

ABSTRACT

Background: The group A streptococcus causes different spectrum of human infections, ranging from pharyngitis and pyoderma, to life threatening immunological complications such as acute rheumatic fever, rheumatic heart disease, post streptococcal glomerulonephritis, toxic shock syndrome and necrotizing fasciitis. The greatest global burden of group A streptococcus disease is due to Rheumatoid Heart Disease which follows group A streptococcus pharyngitis, where 15 million cases and 349,000 deaths occur worldwide.

Objective: To determine the prevalence, drug susceptibility pattern of group A streptococcus and possible associated risk factors among patients with Tonsillopharyngitis attending Yekatit 12 Hospital, Addis Ababa, Ethiopia.

Methods: A cross-sectional study was conducted on 192 participants with Tonsillopharyngitis who attended Yekatit 12 hospital Ear Nose Throat clinic in the city of Addis Ababa, Ethiopia from July 2020 to October 2021 and throat swab sample was collected. A standard microbiological method was used to isolate and identify group A streptococcus from throat swabs obtained from study subjects. The disk diffusion method was used for antimicrobial susceptibility testing. Descriptive statistics and multivariate logistic regression analysis were done by Statistical Package Social Science version 26.

Results: In this study, the overall prevalence of group A streptococcus was 3.65% (7/192).All isolates of GAS were susceptible to all penicillin (10unit), Clindamycin (2µg), Chloramphenicol (30 µg), Vancomycin (30µg), erythromycin (15 µg), Tetracycline (30 µg) while resistance to Trimethoprim-sulfametoxazole (1.25/23.75µg). None of the risk factors were found to be significantly associated with strep-throat Tonsillopharyngitis [P>0.05].

Conclusion: In this study the prevalence of group A streptococcus was relatively low. All group A streptococcus isolates remained susceptible to penicillin, Clindamycin, erythromycin, Chloramphenicol, tetracycline and Vancomycin indicating that those are still the drug of choice for the treatment of GAS infection.

Key Words*: Group A Streptococcus, Bet–hemolytic, Drug Resistance, Prevalence, Tonsillopharyngitis, Ethiopia*

TABLE OF CONTENTSPAGES

1-INTRODUCTION

1.1 Background

Group A Streptococcus (GAS), streptococcus pyogenes, is a Gram-positive spherical bacterium that causes different spectrum of human infections, ranging from pharyngitis and pyoderma, to life threatening immunological complications such as acute rheumatic fever (ARF), rheumatic heart disease (RHD), post streptococcal glomerulonephritis (PSGN), toxic shock syndrome (TSS) and necrotizing fasciitis. Group A streptococci are extracellular bacterial pathogens that produce a variety of pyogenic infections, hence called ***pyogenes***, involving the mucous membranes, tonsils, skin, and deeper tissues, including pharyngitis, impetigo/pyoderma, erysipelas, cellulites, necrotizing fasciitis, toxic streptococcal syndrome, scarlet fever, septicemia, pneumonia, and meningitis. Infections may be mild to extremely severe. Complications such as sepsis, pneumonia, and meningitis can occur, which may lead to a fatal outcome(1–3).

Streptococcus pyogenes or Lancefield group A streptococcus (GAS) is the most common pathogenic species in the genus. Group A streptococci are spherical or ovoid cells 0.6-1.0 micrometer in diameter and occur as pairs or as short to moderate sized chains in clinical specimens. GAS are facultative anaerobic, nutritionally fastidious and are cultivated in complex media, often supplemented with blood or serum. When cultured on blood agar plates, GAS appears as white to grey colonies 1-2 mm in diameter surrounded by zones of complete β-hemolytic(4).

The detection and appropriate treatment for GAS Tonsillopharyngitis reduce the risk of spread of infection, shorten symptom duration, reduce school absenteeism and prevent the non-suppurative sequelae of acute rheumatic fever (ARF), rheumatic heart disease (RHD) and acute glomerulonephritis(2).

The (GAS) is a common infective agent in children that causes the widest range of clinical diseases in humans of any age. The spectrum of GAS diseases can be divided into superficial, invasive, toxin-mediated and post-infectious diseases(5).

Molecules produced by bacteria, which contribute to the degree of their disease causing capacity, are known as virulence factors. The outcome of an infection depends on the interaction between the virulence factors of the bacterium and the host immune system. GAS has evolved an impressive range of strategies to evade the non-specific and specific immune defense mechanisms of its human host. Functionally, the M-protein inhibits phagocytosis, which is a primary virulence mechanism for survival in tissue(4).

GAS has a complex and sophisticated regulatory mechanism that has several virulence factors interacting with the host at many levels. These virulence factors enable it to escape host defenses, allowing it to successfully colonize and thrive. Factors such as M-Proteins and hyaluronic acid capsule, mimic human tissue, this enables GAS to escape being engulfed by the human defense system(6).

Also, the M-Protein alongside virulence factors such as F-Proteins, fimbrial proteins and lipoteichoic acid, enables GAS to adhere to epithelial cells. Lastly the M-Protein with capsules, the DNases, streptokinase, hyaluronidase and SpeB enable GAS to invade tissue and internalize the host. As a result, all these factors work together to mediate adherence to host cells, by avoiding phagocytosis, fostering invasion and internalization(7).

Once the GAS bacterium reaches the host's organism level, it releases invasion and exotoxins virulence factors such as streptokinase, streptodornase, hyaluronidase and streptolysins, to facilitate dissemination throughout the host and induce systemic toxicity. Notably most of these virulence factors are known to work at several stages of infection. Acute Tonsillopharyngitis is one of the most common infectious diseases particularly in children. Among responsible bacteria, Streptococcus pyogenes(group A streptococcus) is one of the most common pathogens(7,8).

Of the two hemolysis produced by group A streptococci, streptolysins S and streptolysins O, only the latter is antigenic. Streptolysins O is a cytolytic toxin that is produced by group A streptococci. Its biologic properties include hemolysis of erythrocytes and other eukaryotic cells; it may also damage leukocytes. The antibody produced by the human host against this toxin,

ASO, is the most widely used and the most standardized of the group A streptococcal antibody tests available. This antibody does not have a protective role in the host.

As data on prevalence and antimicrobial susceptibility of *S.pyogenes* to antibiotics needed to update in Ethiopia, I assessed the prevalence and susceptibility of this pathogen isolated from patients with acute and chronic Tonsillopharyngitis to a broad spectrum of antibiotics using the standard susceptibility-testing method(9,10).

1.2 Statement of The problem

Acute respiratory infections are still a major health problem in most developing countries. Group-A streptococci are the leading cause of acute bacterial pharyngitis and primary skin infections. Impetigo is the most frequently occurring disease, especially in tropical climates. GAS is by far the most common bacterial cause of acute Tonsillopharyngitis, accounting for approximately 15-30 percent of cases in children and 5-10 percent of cases in adults(2,11).

Globally, it is estimated that about 600 million cases of symptomatic GAS pharyngitis occur annually among people aged over 4 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 pharyngitis, where 15 million cases and 349,000 deaths occur worldwide annually 95% 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. There are 2.4 million affected children between 5 and 14 years of age in developing countries, 1 million of whom live in Sub-Saharan Africa, making the continent the major ARF/RHD Hotspot(1).

Data from three studies that documented age-specific RHD prevalence rates confirm that the prevalence increases beyond the adolescent years, peaking in the third and fourth decades of life. This finding accords with the natural history of ARF and RHD. Although the peak incidence of first episodes of ARF occurs at approximately age 12 years, RHD is increasingly likely to develop after subsequent ARF recurrences. Moreover, RHD is a chronic disease. Survivors may retain heart lesions for many years, while new cases accumulate as a result of recurrent ARF episodes in older adolescents and young adults(12).

Following Tonsillopharyngitis, RHD and PSGN constitute the biggest burden of GAS disease following Tonsillopharyngitis resulting in more than 15 million cases and 349,000 deaths worldwide annually; 95% of RHD cases occur in low socio-economic group children. There are 2.4 million affected children between 5 and 14 years of age in developing countries; 50% - 64% of heart disease among children was reported to be of rheumatic origin(8).

Although penicillin remains active on *S. pyogenes*, in recent years, the increase in the incidence of strains that are resistant to current antibacterial agents highlights the need to assess the evolution of resistance of this pathogen to antibiotics. Inappropriate antibiotic treatment for Tonsillopharyngitis is becoming a major issue; hence only true group-A beta-hemolytic streptococcal infections proven by culture should be treated with antibiotics. The clinical diagnosis of group A beta-hemolytic streptococcal pharyngitis cannot be made with certainty even by the most experienced physicians, and bacteriologic confirmation is required(2,13,14).

Despite the high burden of GAS Tonsillopharyngitis and its severe immunological sequelae, much was not done on prevalence, antimicrobial susceptibility pattern and clinical information regarding the disease. Therefore to update the data which were already done in Ethiopia, this study was aimed to determine the prevalence and drug sensitivity testing of group A streptococcus causing Tonsillopharyngitis among patients attending Y12H.

1.3 Significance Of The Study

The finding of this study will redound to the benefit of patients with Tonsillopharyngitis considering that culturing of group A streptococcus and doing antibiotic susceptibility testing (AST) plays an important role in treating patients with the right antibiotics after bacteriological confirmation; this helps prevent the development of sequelae. Hence to help clinicians treat patients with Tonsinsillopharyngitis appropriately and timely, protocol/ policy should be formulated the one which clinicians depend on to prescribe medications for the right patients.

2-LITERATURE REVIEW

2.1 Globally

A meta-analysis conducted in Europe Australia on 285 eligible studies show the prevalence of GAS+ve pharyngitis was 24.1% (95% CI: 22.6±25.6%) in clinical settings (which used `passive recruitment' methods), but less in sore throat management programmes (which used `active recruitment', 10.0%, 8.1±12.4%). GAS+ve pharyngitis was more prevalent in high-income countries (24.3%, 22.6±26.1%) compared with low/middle-income countries (17.6%, 14.9±20.7(15).

A systematic review and meta-analysis of 9 randomized controlled trials involving 2113 patients in America New York comparing cephalosporin with penicillin for treatment of group A beta-hemolytic streptococcal (GABHS) Tonsillopharyngitis in adults show the summary odds ratio (OR) for bacteriologic cure rate significantly favored cephalosporin, compared with penicillin (OR,1.83; 95% confidence interval [CI], 1.37–2.44); the bacteriologic failure rate was nearly 2 times higher for penicillin therapy than it was for cephalosporin therapy (*P*=.00004)(16).

A meta-analysis carried out in Seattle, Washington on 266 articles retrieved, 29 met all inclusion criteria. Among children of all ages who present with sore throat, the pooled prevalence of GAS was 37% (95% confidence interval [CI]: 32%–43%). Children who were younger than 5 years had a lower prevalence of GAS (24% [95% CI: 21%–26%]). The prevalence of GAS carriage among well children with no signs or symptoms of pharyngitis was 12% (95% CI: 9%–14%)(17).

A study conducted in Netherlands shows that throat swab culture was positive in 13 of 41 patients (32%), especially 12 patients without previous antibiotic treatment and in patients younger than 40 years of age (culture positive<40 years: 12 patients; culture negative <40 years: 14 patients; culture positive >40years: one patient; culture negative >40 years: 14 patients; odds ratio 7.3). In patients without previous antibiotic pre-treatment, throat culture became positive in 11 of 21 cases (52%); whereas in patients with antibiotic pretreatment, throat culture became positive in only two of 20 patients (10%): one each GAS and GCS; this difference was significant (p<0.001)(18).

According to a study conducted in Yemen a total of 730 children with acute Tonsillopharyngitis were enrolled between August 2006 and July 2007. Their mean (SD) age was 11.8 (3-4) years, with 406 (55.6%) aged between 11 and 15 years. Participants recruited from schools comprised 553 (75.8%) of the total, and the remainder were from policlinics. Eighty-three (11.4%) of the participants had a history of prophylactic penicillin because of a previously diagnosed ARF or RHD(13).

The study conducted in Turkish on 3127 children showed that they were diagnosed with Tonsillopharyngitis. *Streptococcus pyogenes* was isolated from 345 throat swabs (11.0%). Because of inadequate data from the questionnaire, contamination or missing of isolated strains, 263 patients were found eligible for the analysis of risk factors. One hundred and forty-nine children (56.7%) were male and 114 (43.3%) children were female. The mean age of the children in the study was 85.6+/-8.6 months(range 10 months–15 years (20).

A study conducted in Germany showed that a total of 4782 patients, ages 1–18 years, with acute Tonsillopharyngitis and a positive throat culture for GABHS, were enrolled in the study to receive a 5-day oral treatment with cephalosporin (cefuroxime axetil, ceftibuten, loracarbef), macrolides (erythromycin, clarithromycin), amoxicillin/clavulanate, or the standard therapy with oral penicillin V given for 10 days. Three hundred patients (6.3%) were eliminated from the data analysis; 230 (4.8%) failed to return for post-treatment assessments, and an additional 70 (1.5%) were excluded because they did not complete the full treatment course as determined by diary cards and medication returned. Because compliance of the 230 patients who failed to return is not known, the true compliance rate is between 93.7% and 98.5%(21).

The eradication rate of pharyngeal GABHS at the first visit after completion of treatment was 83.7% and was equivalent in the 2 treatment groups (*P*0.022): 83.3% in the 5-day group versus 84.4% in the 10-day group. The persistence of GABHS in throat cultures, with clinical symptoms, was similar in the 5-day and 10-day treatment groups. At the late follow-up visit, 6–7 weeks after end of therapy, the eradication of primary isolates was 85.6%, and the difference between the 2 treatment groups was not statistically significant (85.0% in the 5-day group and

86.8% in the 10-day group. No isolate of group A streptococcus was documented to be resistant to penicillin, oral cephalosporin, or amoxicillin/clavulanate used in this study. Macrolide resistance has been documented for 6.0% of all strains tested (p=4.698), and 6.8% have shown intermediate susceptibility. There have been geographic areas where the rate of susceptible strains decreased to 81.6% or 77.1%(21).

A study conducted in Turkey among the study participants in the study group, the majority were female (n=170, 60.3%). Furthermore, 40.4% (n=114) of the study group were children between 7 and 15 years of age. When the visiting dates of the patients were reviewed, it was revealed that most of them were made in spring, especially in April (n=59, 20.9%), which is in accordance with the literature. GABHS (Group A-beta Hemolytic Streptococcus) infections, in which antibiotic treatment is certainly indicated, are encountered in children at a proportion of 15–30% and in adults 5–15%. However, studies show that acute Tonsillopharyngitis is the cause of over 70% of unnecessary antibiotic prescriptions by primary-care physicians(22).

2.2 In The African Context

According to the systematic review conducted in Africa from May 2008-September 2011, a total of 742 subjects were enrolled in the study. GAS was recovered from 160 participants yielding a culture positivity rate of 21.6% (23).

According to the study conducted in Egypt on children in 2015 throat swab culture of cases found that 67 (47.2%) yielded no growth of pathogenic bacteria, 15 (10.6%) yielded growth of β hemolytic streptococci identified as non-group A streptococci (they were bacitracin and sutrim resistant) mostly group C (Streptococcus dysagalatiae), and 60 (42.2%) showed growth of β-hemolytic streptococci confirmed to be GAS on the basis of a battery of tests. They exhibited beta-hemolysis on blood agar. Gram stain showed gram positive cocci arranged in chains. They were catalase and latex agglutination test positive and bile esculin negative. They were sensitive to bacitracin and resistant to sutrim(24).

A study conducted in Mali from May 2006- September 2009 showed that 1,418 students at the participating schools contributed 1,759 episodes of Tonsillopharyngitis. GAS was isolated in 449 episodes (25.5%) experienced buy 421(29.7%) students. GAS was isolated 3 times from 5 students and twice from 18 students. GAS was more commonly isolated from girls with pharyngitis than boys. There was no evidence of an association between age and GAS isolation (p=0.88).The percentage of cases positive for GAS was similar from season to season (26.8% in the cold season,23.6% in the hot season and 25.3% in the rainy season) (25).

A study conducted in Nigeria from April to November 2014,a total of 3386 children aged between 3 and 15 years were seen in the general outpatient clinics of three centers.
Among these 30 children met the inclusion criteria and recruited into the study, giving the prevalence of 0.8% for acute sore throat in this age group. Among the age groups,71%,66% and 60%,respectivelyof age 3-6,7-10, and >10 years yielded GABHS- positive throat cultures(26).

According to the study conducted in South African showed the most up-to date data from Africa was collected more than 30 years ago with rates ranging from 23.2% to 45.5% of GAS pharyngitis in school children of all ages within Africa(27).

Study carried out in Uganda by cross-sectional study revealed an overall prevalence of GAS carriage of 15.9% (79/496, 95% confidence interval 12.8–19.5%). Among 532 children enrolled in the prospective cohort study, 358 (67%) reported 528 sore throats, with 221 (41.1%) experiencing at least one GAS-positive sore throat(28)

2.3 In the Ethiopian Context

A study carried out in Ethiopia show that the throat culture positivity rate for BHS was 24 % (56/233). Among the BHS bacterial strains isolated, four were characterized as S. pyogenes 1.7% and another four as S. dysgalactiae subsp. equisimilis (Lancefield group A, C and G). All BHS were susceptible to penicillin except one isolate of S. agalactiae. Among 233 children enrolled, 46(19.7 %) showed increased ASO titer(29).

A study conducted at Hawassa Southern Ethiopia among 287 school children from May – October 2018 showed the prevalence of GAS was 12.2% with 95% CI in their throat swabs(30).
A study conducted at Jimma from May 8 to December 31,2013 among 355 children with pharyngitis attended Health Centers of Jimma show that the overall prevalence of group A Streptococcus (GAS) was 11.3% (40/355). The prevalence of GAS in females was 11.7%, in children aged 10–15 years 14.2%, among rural residents 16% and in children with family income<25 USD/month 14.8%(1).

3-OBJECTIVES

3.1 General Objective;

Prevalence, Drug Susceptibility Pattern Of Group A Streptococcus and possible associated risk factors Among Patients With Tonsillopharyngitis Attending Yekatit 12 Hospital, Addis Ababa, Ethiopia.

3.2 Specific Objectives;

- ✓ To determine the prevalence of group A Streptococcus isolates.
- ✓ To determine the antibiotic susceptibility pattern of group A Streptococcus isolates
- ✓ To determine possible associated risk factors

4-MATERIALS AND METHODS

4.1 Study area.

Yekatit 12 Hospital, located in Arada Sub-city of City Government of Addis Ababa, was established in 1915 E.C with a total of 25 beds and 37 health professions. It is a teaching Hospital and managed under Addis Ababa City Administrative Health Bureau. According to the information obtained from the Hospital, currently, the Hospital has 725 health professionals and 375 administrative staff with around 272 beds and provides different medical services for around 4 million people. This clinic provides service for around 50 patients per day.

4.2. Study design and period

A cross-sectional study was carried out on 192 participants from July 2020 to October 2020at Yekatit 12 Hospital (Y12H).

4.3. Population

4.3.1. Source population

All patients attended the ENT clinic during my study period at Yekatit 12 Hospital, Addis Ababa Ethiopia.

4.3.2. Study Population

All patients presented with signs and symptoms of Tonsillopharyngitis and ruled out as Tonsillopharyngitis by senior physicians during the study period.

4.4 Selection and enrollment of participants

4.4.1 Inclusion criteria;

- ✓ participants of all age
- ✓ the participant presented with Tonsillopharyngitis
- ✓ written informed consent and assent for study participant obtained

4.4.2 Exclusion criteria

- ✓ A Participant who was on antibiotics and who has been treated with antibiotics in the week preceding the study period.

4.5. Study variables

4.5.1. Dependent variables

- ✓ the prevalence of group A Streptococcus isolates of the study participants
- ✓ the antibiotic susceptibility pattern of group A Streptococcus isolates
- ✓ possible associated risk factors

4.5.2. Independent variables:

- Age
- Sex
- Smoking
- Recurrent throat infection
- Discontinuation of medication
- Sharing utensils
- Recent close contact
- Hand washing
- Any chronic illness

4.6. Sample size calculation and Sampling method

4.6.1. Sample size calculation

The sample size was calculated using the formula for a single proportion sample size calculation by taking a 95% confidence level and a 5% margin of error and proportion or prevalence from a previous study conducted at Jimma which was 0.113[Jimma]and calculated in the following way;

Using the formula, $N = \frac{(\boldsymbol{Z\alpha/2})\mathbf{2}\boldsymbol{P}(\mathbf{1}-\boldsymbol{P})}{\boldsymbol{D^2}}$

Where **Z**=standard normal value corresponding to **95%** confidence interval for a two-sided test=**1.96**

P=estimated prevalence of GAS in patients of all ages(11.3%) from a study carried out in Jimma on the prevalence of GAS Tonsillopharyngitis [*Demeke D et al]* this was estimated to be **11.3%.D**= margin of error =**5%**, substituting into the formula, **n=154** but I have added 3/4th of my sample size to increase the chance of detecting GAS from patients who came to my study area which was 192.

4.6.2. Sampling Method

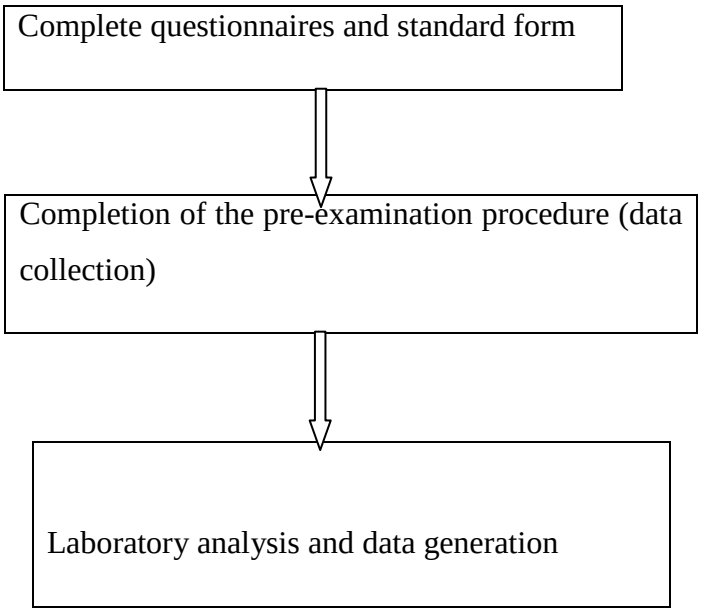
A convenient sampling technique was used and a total of 192 samples were collected. The sample collected was a throat swab for culture. During sample collection, participants presenting with Tonsillopharyngitis and visited ENTOPD clinics of my study area were sampled from July 2020 to October2020.

4.7. Measurement and data collection procedures

4.7.1. Data collection

After I obtained written consent from participants, data were collected using a well-structured questionnaire designed to obtain socio-demographic data and other relevant information.

Figure 1: Data collection frame-work



4.7.2 Specimen collection

Sample collection was performed according to the World Health Organization (WHO) standard for diagnosis of GAS. A sterile cotton swab was used to take the throat swab from the tonsil and pharynx. Necessary care was taken not to swab the cheeks, tongues, lips or other areas of the mouth. Socio-demographic data was recorded on the prepared questionnaires during the recruitment and all data forms and laboratory request forms were clearly labeled with each patient's identification number and unique code. The collected samples were sent immediately to Y12 Hospital Microbiology Department for culture.

4.7.3 Culture and Identification

The throat swabs were inoculated on 5%sheep blood agar and inoculated at 35-37°C for 18-24hrs in candle jar plates which can provide an atmosphere of 5% CO2. Culture plates negative for beta-hemolytic colonies were incubated for additional 24hrs to allow the growth of slow growers. All catalase-negative, Gram-positive cocci were further tested for susceptibility to bacitracin. All bacitracin susceptible, Gram-positive, catalase-negative cocci were classified as GAS. Identification of GAS isolates was made based on the standard microbiological techniques which include a β-hemolytic activity on 5% sheep blood agar, small colony characteristics, Gram-positive cocci, catalase production negative, and 0.2-U bacitracin disc susceptible(1).

4.7.4 Antibiotic susceptibility testing (AST)

Antimicrobial susceptibility testing of the isolates was performed by Kirby-Bauer disk diffusion test on Muller-Hinton agar. The bacterial suspension turbidity was matched with 0.5 McFarland standards. Then a sterile cotton swab was dipped in to the suspension and squeezed free from excess fluid against the side of test tube. The test organisms uniformly seeded on the surface of Muller-Hinton agar. Using sterile forceps, the antibiotic discs were placed on agar plate by considering 24 mm distance between each disk and 15 mm from border according to clinical and laboratory standard institute (CLSI).

The medium was incubated at 35oC for 18-24 hours. Interpretation of AST: Grades of susceptibility pattern was interpreted by comparison of the zone of inhibition as indicated in the manufacturer's guide. The diameters of the zone of inhibition were measured using a metric scale reported as susceptible (S), intermediate (I) ,resistance(R).

4.8. Data Quality Assurance

4.8.1. Pre-analytical

Socio-demographic characteristics of the participants were collected appropriately after getting consent and assent. This phase included the selection of appropriate site and samples, collection, identification, and labeling (every sample container had a label that included information about: subject code, ID number, Date and time), handling, and transportation of biological samples. All these steps were performed according to the standard operating procedure (SOP). Aseptic techniques were implemented in all the steps of specimen collection and inoculation on the culture media to minimize contamination.

4.8.2. Analytical

All materials, equipment and procedures were adequately controlled. All culture media were prepared according to the direction of the manufacturer. Culture media were tested for sterility and performance. The sterility of media was checked by incubating overnight at 37°C. Moreover, culture growth, biochemical test and antimicrobial susceptibility test results were confirmed by specially trained experienced microbiologists working in the microbiology unit of my study area.

4.8.3. Post-analytical

All of the extracted information (filled questionnaire, laboratory findings) were checked for legibility, completeness, consistency and recorded before entry to the statistical tool. The data were also put in secure location. All laboratory isolates were stored as per the SOP of the study site.

4.9. Data analysis and interpretation

The finding was analyzed using descriptive statistics SPSS (the Statistical Package for Social Sciences), chi-square test and $p \leq 0.05$ was considered to see the statistically significant risk factors. The variables from the demographic data analysis were performed and computed to determine the prevalence of GAS.

4.10. Operational Definitions

Beta- hemolytic: When a bacterium completely destructs red blood cells to release hemoglobin, it is classified as β-Hemolytic. In blood media this will appear transparent and light yellow

Lysis: Destruction of red blood cells with the release of hemoglobin

Necrotizing fasciitis: Commonly known as flesh-eating disease

Rheumatic fever: inflammation and pain in and around the joints, and by inflammatory involvement of the pericardium and heart valves, occurs chiefly in children and adolescents following inadequately treated Group A streptococcal infection of the upper respiratory tract (such as in strep throat) and is characterized by fever, by inflammation and pain in and around the joints, by inflammatory involvement of the pericardium and heart valves.

Rheumatic heart disease: is a condition in which permanently damages heart valves; is caused by rheumatic fever. The heart valve is damaged by a disease process that generally begins with a strep throat caused by bacteria called Streptococcus, and may eventually cause rheumatic fever.

Post-streptococcal glomerulonephritis: acute inflammation of the kidney caused by an immune response and can develop after group A strep infections.

Sequelae: a condition that is the consequence of a previous disease or injury.

Tonsillopharyngitis: also known as strep throat is an infection of the back of the throat including the tonsils caused by group A streptococcus (GAS).

4.11 Ethical considerations

Ethical clearance was obtained from the Departmental Research and Ethics Review Committee (DRERC) of Medical Laboratory Sciences, from Addis Ababa Health Bureau Ethical Review Committee before to data collection and permission letter was obtained from study hospital administration. The purpose and procedure of the study were explained for each study participant at the study site. Those who agreed to participate were asked to sign consent form. Written and informed consent was obtained. The confidentiality of the information collected was maintained by using code numbers for participants. Any participant who was not voluntary was not forced to be included as a study subject.

4.12. Dissemination of the result

The finding of this study was presented to the department of Medical Laboratory Sciences for public defense. The result was also communicated to Addis Ababa Health Bureau and to the study site Y12Hospital. It is expected that this work would contribute to the knowledge base of the prevalence of Tonsillopharyngitis GAS, thus potentially influencing policy and practice in this area. An effort will be made to publish the finding in peer reviewed journals.

5-RESULTS

5.1 Socio-Demographic Of Participants

A total of 192 participants including all ages of patients with both acute and chronic Tonsillopharyngitis were enrolled from July 2020 to October 2021 at Y12 Hospital; with mean ± SD age of 13.67 ± 10.24. Females accounted for 49% while males accounted for 51% which shows that in this study males are a little bit more prone to throat infection than females.

5.2 Prevalence of the isolated Group A Streptococcus

Among age groups mentioned in this thesis, (5-15) age group contained the dominant participants complaining of Tonsillopharyngitis. In this study, the overall prevalence of group A streptococcus (GAS) was 3.65% (7/192).The prevalence of GAS in females was 3.2% and in males it was 4.1% in participants aged through (0-4), (5-15), (16-25) and (26-36) years with the values of 3.7%, 3.8%, 3.2%, 5.6% respectively (Table 1).

Table 1: The prevalence of GAS to age and sex among patients with Tonsillopharyngitis in Y12 Hospital Addis Ababa Ethiopia from July 2020 to October 2021.

Characteristics	Culture result of GAS			Total
		Positive	Negative	
Sex	Male	4(4.1%)	94(95.9%)	98(51%)
	Female	3(3.2%)	94(49%)	94(49%)
	Total	7(3.65%)	185(96.35%)	192(100%)
Age in years	[0-4]	1(3.7%)	26(96.3%)	27(14.1%)
	[5-15]	4(3.8%)	102(3.8%)	106(55.2%)
	[16-25]	1(3.2%)	30(3.2%)	31(16.1%)
	[26-35]	1(5.6%)	17(94.6%)	18(9.4%)
	[36-45]	0 (0%)	10 (100%)	10(100%)

Total	7(3.65%)	185(96.35%)	192(100%)

5.3 Associated Risk factors

None of the risk factors mentioned in this thesis found to be associated with Tonsillopharyngitis caused by GAS. Even though in binary logistic regression analysis sharing utensils and not hand washing found to be significantly associated risk factors of strep throat with OR value of 0.191 CI 95% (0.045-0.819);but in the multivariate analysis [P >0.05 (P=0.24)] and with OR value of 0.952 CI 95% (0.919-0.987);but in multivariate analysis [P >0.05 (P=0.997)] respectively, it was disproved that both of them are not a significant risk factor of strep throat-Tonsillopharyngitis (see Table 2).

Table 2: Multivariate logistic regression analysis of possible associated risk factors of GAS among patients with Tonsillopharyngitis in Y12Hospital Addis Ababa Ethiopia from July 2020 to October 2021.

Possible associated risk factors	S.E	P-value	Crude OR	Adjusted OR	95% CI for adjusted OR	
					Lower	Upper
Sex	.846	.805	1.279	.812	.155	4.265
Smoking	11736.746	.999	.962	.000	.000	.
Sharing Utensils	1.142	.240	.191	3.824	.408	35.865
RT in Family	1.135	.465	.301	2.289	.248	21.157
Recent Close Contact	1.280	.974	.737	1.042	.085	12.801
Medication Discontinuation	.965	.678	.538	1.492	.225	9.892
Hand Washing	5650.952	.997	.952	.000	.000	.
Chronic Illness	9974.475	.999	.961	.000	.000	.
Age Groups	.495	.233	.926	1.804	.684	4.758

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5.4 AST of the isolated GAS

Antimicrobial susceptibility testing of the isolates was done by using the disc diffusion method according to Clinical Laboratory and Standard Institute (CLSI). The pure colony of GAS bacterial suspension from sheep blood agar was evenly spread onto Muller Hinton Agar supplemented with 5% sheep blood using a sterile cotton swab.

Soon after antibiotic discs were placed on the inoculated plate, the plates were incubated at 37º C in a candle jar overnight. The following antimicrobial discs with respective concentration were used: penicillin (10unit), Clindamycin (2µg), Chloramphenicol (30 µg), Vancomycin (30µg), erythromycin (15 µg), Trimethoprim-sulfametoxazole (1.25/23.75µg), Tetracycline (30 µg).The antibiotic discs were selected based on prescription pattern and recommendations from CLSI. Zone of inhibition diameters was interpreted as sensitive, intermediate and resistant according to the principles established by CLSI.

Table 3: Antimicrobial Susceptibility Test of GAS by Drugs Disk diffusion

Drugs	Susceptible (S)	Intermediate (I)	Resistant (R)
Clindamycin (2µg)	7(100%)	0	0
Chloramphenicol(30 µg)	7(100%)	0	0
Co-Trimoxazole (1.25/23.75µg)	6 (85.7%)	0	1(14.3%)
Co-Trimoxazole (15 µg)	7(100%)	0	0
Penicillin (10U)	7(100%)	0	0
Tetracycline (30 µg)	7(100%)	0	0
Vancomycin (30 µg)	7(100%)	0	0

6-DISCUSSION

GAS is by far the most common bacterial cause of acute Tonsillopharyngitis, accounting for approximately 15-30 percent of cases in children and 5-10 percent of cases in adults (2, 7).

The overall prevalence of GAS 3.65% detected in my study was nearly comparable with GAS prevalence in Ethiopia in which all isolates were susceptible to penicillin (1.7%)(29).

The overall prevalence of GAS 3.65% detected in my study was comparable with GAS prevalence detected in Chile, Pakistan, Mali (2,3,19)**.**

A systematic review conducted in Africa shows that the prevalence of GAS Tonsillopharyngitis was 21.7% (23) which is much higher than GAS prevalence seen in my study (3.65%).

But it was much higher than the prevalence of 0.8% in Nigeria (14). The reason may be due to unnecessary over-prescription of antibiotics in Nigeria.

On the other hand, A meta-analysis conducted in Seattle Washington DC show a very high prevalence of 37% and also the prevalence of 3.65% GAS in this study was lower than the prevalence of 11.4% in Yemen,11.0% in Turkish,14.60% in Ponchindary, 10.6% in Egypt, 21.6% in Cape town,12.2% in Hawassa and 11.3% in Jimma(1,9,17,20,24,31).

The overall prevalence of GAS 3.65% detected in my study was much lower than the prevalence detected 52% in Netherlands, 47.2% in Egypt, 25.5% in Mali (2,5).

This low prevalence of Group A Streptococcus (GAS) in the current study may be due to the trends of over-prescription of antibiotics in health facilities as it was demonstrated in my data where most of my participants with Tonsillopharyngitis received some common antibiotics. Such indiscriminate use of antibiotics might contribute to the low prevalence rate of GAS in the community. Probably the other reason may be due to the shortness of my study period and low sample. To determine the approximate prevalence of GAS; conducting study throughout the year is advisable. An improvement in the habit of hand washing and the improvement in the habit of not sharing utensils may also be the reason.

Antimicrobial resistance is an important problem in the management of patients with infectious diseases. Interestingly, S.pyogenes remains susceptible to penicillin in many countries of the world. The reason for this unique lack of development of resistance to penicillin is unknown. In this study none of the isolates were resistant to penicillin which is concordant with studies around the world, which once again confirms that penicillin is the reference molecule for the treatment of GAS infections.

On the other hand, numerous reports have demonstrated a significant prevalence of erythromycin-resistant S.pyogenes around the world(21,32,33).

In this study, all GAS isolates were susceptible to Clindamycin, Chloramphenicol, erythromycin, penicillin, tetracycline, Vancomycin, which agrees with the previous studies in Ethiopia and also concordant with the many studies conducted all over the world(16). It is known that erythromycin and Clindamycin are usually used as an alternative treatment for patients allergic to penicillin(31,34).

GAS showed resistance to Trimethoprim-sulfametoxazole which agrees with the study conducted in Ethiopia (31).

❖ 7-STRENGTHEN AND LIMITATION

❖ Strengths

- ❖ Conduction of the study during this difficult time (the era of COVID)
- ❖ Conduction of the study in the resource limited condition

Limitations

- ❖ Low sample size
- ❖ Short study period

8-CONCLUSION

In this study the prevalence of GAS was relatively low. However, the over-prescription of antibiotics for Tonsillopharyngitis might underestimate the prevalence, so that large-scale prospective study in the entire season and various settings is required to understand the actual burden of infection. In my study, all GAS isolates remained susceptible to penicillin, Clindamycin, erythromycin, Chloramphenicol, tetracycline and Vancomycin indicating that those are still the drug of choice for the treatment of GAS infection.

9-RECOMMENDATION

- This finding indicated that even though children are more prone to be infected than adults all ages can be infected by group A streptococcus bacterium.
- Since untreated Tonsillopharyngitis caused by GAS, mistreatment and over-prescription can cause resistance to medications; clinicians should consider seriously this issue to treat their patients timely and they should be alert in order to combat the sequelae.
- Health education should be given to the community to create awareness on the way of transmission among family, school children and other places expected to be a source of infection.

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ANNEX I. GENERAL INFORMATION FOR THE STUDY PARTICIPANTS (ENGLISH VERSION)

Introduction

My name is Terefe Selbane and I am MSC student of Addis Ababa University, School of Medical Laboratory Sciences. I am doing research entitled Prevalence and Drug Susceptibility Pattern of Group a Streptococcus among Patients with Tonsillopharyngitis Attending Y12 H, Addis Ababa, Ethiopia. Tonsillopharyngitis is highly transmittable throughout the whole community infects all age groups especially in children. So this study will indicate Prevalence and Drug Susceptibility Pattern of Group a Streptococcus among Patients with Tonsillopharyngitis Attending Y12 H.

What is the reason of this study? **The objective of this research is to study the prevalence and Drug Susceptibility Pattern of Group a Streptococcus among Patients with Tonsillopharyngitis Attending Y12 Hospital Addis Ababa, Ethiopia. All consented participants who agree to participate in the study will give a throat swab for culture and venous blood for ASO as per the standard and they will be also interviewed about some clinical information.**

Will the information be confidential? All the data obtained will be kept confidentially; only the study personnel will have accesses to the files. Anonymous testing will be undertaken, that means samples will be coded and positive results will not be identified by names.

What are the costs? All the investigations performed for the participants of this study will be free of charge.

What about compensation? You will not be compensated for your participation in this study but the patient and you may benefit from the study because more intensive investigations will be done which may allow detecting and treating an infection earlier than otherwise.

What about my rights to decline participation or withdraw from the study?

Your participation in this study is purely voluntary, and you may stop the participation or you may refuse to answer some of the questions if you feel uncomfortable. You are free to participate

in this study or you can withdraw your consent anytime, which will not involve any penalty or loss of benefits to which you are entitled.

What about the harm which may happen in the study?

This research involves taking throat swab from participants selected in the study. There are no major risks to participants and will not cause any harm to the hospital.

Assurance of the principal investigator

I put my signature below to confirm you that I take over the responsibility for the scientific ethical and technical conduct of the research project and for provision of reports for all stakeholders of the research project.

Terefe Selbane (PI): signature_____Date_____

Note: if you have any questions about this study, feel free to ask now or anytime throughout the study by contacting:

PI Address: Terefe Selbane: Department of Medical Laboratory Sciences, College of Health Sciences, Addis Ababa University, Addis Ababa, Ethiopia.

E-mail: sinolterem@yahoo.com: Tele: +251917367622

ANNEX II. INFORMATION SHEET (AMHARIC VERSION)

መግቢያ

ስሜተረፈሰልባኔበአዲስአበባዩኒቨርሲቲበጤናሳይንስኮሌጅበሜዲካልላቦራቶሪትምህርትክፍልማስትሬትዲግሪተማሪስሆንበየካ
ቲት 12 ሆስፒታልከሚመጡታካሚዎችየጉሮሮፈሳሽናሙናበመውሰድበጉሮሮዉስጥበሚገኘውባክቴሪያላይጥናትእሰራለው፡፡
ስለዚህይህጥናትበራስእናበየካቲት 12 ሆስፒታል ታካሚዎችላይያለውንየባክቴሪያመጠንለማወቅይረዳኛል ፡፡

የጥናቱ ምክንያት

*ይህበሳይንቲፊክመጠሪያውS.pyogenes ባክቴሪያየሚባልሲሆንብዙጊዜበሆስፒታልየሚታከሙህሙማንንለሌላበ
ሽታናሞትከመዳረጉምበላይለብዙወጪህሙማንንእንደሚዳርግበብዙጥናቶችተረጋግጧል፡፡*
ስለዚህይህጥናትበየካቲት 12
*ሆስፒታልታካሚዎችላይያለውንየባክቴሪያመጠንለማየትይረዳኛል፡፡በዚህጥናትላይለመሳተፍፈቃደኛሆነውከተሰማ
ሙአዎወይምአይበማለትይግለጹት፡፡*

የጥናቱ ሚስጥራዊነት

*የተገኘዉመረጃሁሉበሚስጥራዊነትበተቆለፈክፍልወይምሳጥንዉስጥበማስቀመጥየጥናቱባለቤትብቻእንዲገኝይደረ
ጋል፡፡በምርመራወቅትየተለየኮድበመጠቀምየተመርማሪዉውጤትእንዳይታወቅእንከላከላለን፡፡*

የክፍያ ጉዳይ

በጥናቱ ላይበመሳተፎበሚደረጉሁሉምአይነትየላቦራቶሪምርመራዎችወጪከክፍያነፃናት፡፡

የማካካሻ ጉዳይ

*በጥናቱላይስለተሳተፉምንምዓይነትማካካሻአያገኙምነገርግንህሙማኑከሚደረገዉጥልቅምርመራበሽታዉበጊዜተገኝ
ቶሎትበፍጥነትእንዲታከሙይረዳታል፡፡*

ከጥናቱ ላይ ያለመሳተፍና የመገለልመብትጉዳይ

እርሶበጥናቱላይየተሳተፉትሙሉበሙሉበፈቃደኝነትላይየተመሰረተነዉ፡፡
ስለዚህእርሶበማንኛዉምሰዓትበጥናቱላይመሳተፍማቆምይችላሉ፡፡ያለመሳተፍምመብትዎነዉ፤
አንዳንድጥያቄዎችካልተመቾትያለመመለስምመብትአለዎት፡፡
ይህንበማድረግዎምንምአይነትቅጣትአያስከትልቦዎትም፤ማግኘትየሚገባዎትንሁሉንምነገርአያሳጣም፡፡

ከጥናቱ ጋር የተያያዙ ተጓዳኝ አደጋ ጉዳይ

ናሙናበሚወሰድበትጊዜከሚሰማዉያለመመቸትበስተቀርምንምአይነትየከፋጉዳትበጥናቱምክንያትአይከሰትም፡፡

የተመራማሪዉማረጋገጫ

እኔከስርፊርማየንያስቀመጥኩትሳይንሳዊጥናቱንበግብረገብበተሟላመልኩእንደማካሀድናየጥናቱንሪፖርትለሚመለከታቸዉባለድርሻአካላትእንደማቀርብበፊርማዬአረጋግጣለሁ፡፡

ተረፈሰልባኔ (ዋናተመራማሪ)፡ፊርማ----------ቀን----------

ማስታወሻ፡

ስለጥናቱማንኛዉምጥያቄካለዎትበሚቀጥለዉአድራሻነፃሆነዉይጠይቁን፡

የዋናተመራማሪአድራሻ፡*ተረፈሰልባኔ*

በአዲስአበባዩኒቨርሲቲ፣የጤናሳይንስኮሌጅ፣የህክምናላቦራቶሪትምህርትክፍል፤አዲስአበባኢትዮጲያ፡፡

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ስልክ: +251917367622

ANNEX III. CONSENT FORM FOR PARTICIPANTS (ENGLISH VERSION)

I have read the information about the study which plans to determine prevalence and drug susceptibility pattern of group A Streptococcus among patients with Tonsillopharyngitis attending Yekatit 12 Hospital, Addis Ababa, Ethiopia. I have been given the opportunity to ask questions and my questions have been answered to my satisfaction. The objective and the application of the study were briefly explained to me. I voluntarily consent that I would participate in this study.

I have been well informed of my right to refuse information, decline to cooperate and dropout of the study if I want.

Name of participant, date and signature of participant

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

Name of researcher, date and signature of researcher

Phone number

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

ANNEX IV. CONSENT FORM FOR PARTICIPANTS (AMHARIC VERSION)

በየካቲት 12

ሆስፒታልከሚመጡታካሚዎችየጉሮሮፈሳሽናሙናበመውሰድበብዛትታካሚዎችላይየሚገኝናበሆስፒታልየሚታከሙህሙማንን የሚያጠቃውባክቴሪያላይጥናትእሰራለሁስለሚለዉምርምርበቂመረጃአግንቻለሁ፡፡

የጥናቱአላማናአተገባበርበመጠኑተገልፆልኛል፡፡

በጥናቱላይያለመተባበርያለመሳተፍናከተሳተፍኩምበኋላየማቋረጥመብትእንዳለኝተረድቻለሁ፡፡

እኔባለመተባበርምክንያትምንምአይነትበደልበጤናተቋሙእንደማደርስብኝተገልፆልኛል፡፡

ስለዚህያለዉንሁኔታሙሉበሙሉተረድቼበሙሉፈቃደኝነትበጥናቱላይለመሳተፍናሙናእንዲወሰድምረምራእንዲከናወንስልፈቃ ደኛመሆነንአረጋግጣለሁ፡፡

የተወሰደውናሙናበሽታአምጭተዋሲያንንለመመርመርናተዋሲያኑለመድሃንትያላቸዉንምላሽለማወቅእንደሆነአዉቄተስማምቻ ለሁ፡፡ጥያቄዎችንየማቅረብእዲልአግኝቻለሁጥያቄየምእኔበምረዳዉቋንቋባግባቡተመልሶልኛል፡፡

ከዚህምበተጨማሪየናሙናዎቹዉጤትባስፈለገኝጊዜማግኘትእንደምችልአዉቀያለሁ፡፡

ስምናቀንፅፈዉይፈርሙ

ስም----------ቀን -------/-----/----------ፊረማ ----------

የተመራማሪዉስምናፊርማ…….

ስም----------ቀን----------ፊረማ

የተሳታፊዉኮድ፡----------

ANNEX V. QUESTIONNAIRE SOCIO-DEMOGRAPHIC CHARACTERISTICS OF THE PARTICIPANTS

This information is very valuable to us. The information you provide is completely voluntary and anonymous. We value any information you will be able to provide for us.

1-Participant code: _ _ _ _ _

2-Date of interview (dd/mm/yyyy) /_ __ /_ __/ _____

3-Age_____

4-Sex: male □ female □

5-Do you have the habit of smoking? Yes□ No □

6. Do you have the habit of sharing utensil in family or elsewhere? Yes □ No □

7. Do you have close contact with some who has been recently been infected with Tonsillopharyngitis (strep-throat)? Yes □ No □

8. Do you discontinue the medication you are given in case you feel some discomfort?

Yes □ No □

9. Do you have the habit of hand washing after sneezing and or coughing into your hands?

Yes □ No □

10. Is there any recurrent tonsillitis (RT) in your family? Yes □ No □

11. Do you have any chronic illness? Yes □ No □

ANNEX VI: STANDARD OPERATING PROCEDURES (SOPS)

SOPs that are required for media preparation, reagent preparation, specimen collection and laboratory investigation are described below. In general manufacturers' instructions should be

SOP for Blood agar media preparation

Purpose: This procedure provides instructions how to prepare Blood agar media.

Principle: Meat extract and Peptone provide nitrogenous compounds, vitamins, carbon, sulphur and amino acids in Blood Agar Base. The medium contains sodium chloride for the osmotic balance. Blood Agar Bases are relatively free of reducing sugars, which have been reported to adversely influence the hemolytic reactions of beta-hemolytic microorganisms. Sheep blood gives best results for S.aureus. Hemolytic patterns may vary with the source of animal blood or type of base medium used. The low pH helps in stabilization of red blood corpuscles and favors the formation of clear hemolysis zone.

Abbreviation:

BA= blood agar

Materials, Supplies and equipment

- Blood agar base powder
- Weighing paper
- Distilled water
- Spatula
- Sterile sheep blood
- Refrigerator
- Balance
- Autoclave

• Hot plate

• Bunsen burner

• Distiller

• Dispenser

• Graduated cylinder

• Flask

• Test tube

• PH meter

Procedure:

1. Weigh and Suspend 40grams of powder in1littr distilled water

2. Mix thoroughly and heat to boiling to dissolve the medium completely with frequent agitation

3. When cool adjust the ph to 7.3

4. Autoclave at 15 lbs pressure at (121°C) for 15 minute

5. Cool the medium at 50°C

6. Add 50ml of sheep blood and mix with gentle rotation.

7. Dispense 20ml of the solution in to sterile Petri dish

8. Allow the medium to solidify label with date and store at 4°C

Limitations: Blood agar is not a selective media so we couldn't differentiate microorganisms from the agar.

Clinical utility: A non-selective medium for the isolation and cultivation of many pathogenic and non-pathogenic microorganisms. The medium is often used to observe the forms of hemolysis from pathogenic microorganisms.

SOP for Catalase

Purpose: This procedure provides instructions to detect the production of catalase by bacteria.

Principle: Catalase is an enzyme that breaks hydrogen peroxide (H2O2), a toxic metabolic by products of aerobic and facultative anaerobic bacteria into non toxic products water (H2O) and oxygen (O2). H2O2 is toxic to cells. It is highly reactive molecule that damage cell components. So the bacteria living in presence of oxygen produces enzyme catalase that breaks down H2O2 into H2O and O2.

Requirements:

- Pure culture of staphylococcus and streptococcus spp
- 3% Hydrogen peroxide
- A glass slide/test tube
- Glass rod

Procedure

Slide test:

1. Prepare a fresh hydrogen peroxide 3% solution

2. Place a drop of 3% H2O2 on opposite ends of a clean grease free glass slide with the help of dropper

3. Transfer a small portion of both cultures onto drop of 3% H2O2 with the help of a sterile glass rod

4. Examine for immediate bubbling of O2

Note: slide test is not recommended because of risk of contamination from active bubbling

Tube test:

1. Pour 2-3ml of 3% hydrogen peroxide into a test tube

2. Transfer the culture with the help of glass rod and immense into H2O2 solution

3. Observe for immediate bubbling

Result interpretation:

- ✓ Staphylococcus spp: give bubbling of gas, catalase positive
- ✓ Streptococcus spp: give no bubbling of gas, catalase negative

Limitation: Do not use wire loop to transfer the culture as H2O2 may reacts with iron to give false positive; H2O2 is toxic to human so handle carefully; observe the result within 30 seconds; culture should not be more than 24 hours old

Bacitracin Test

I. Principle

The bacitracin disk is sensitivity test used to differentiate the beta- hemolytic *Streptococcus*.

II. Inoculum

An overnight culture grown on 5% sheep blood agar incubated 35°C in CO_2.

III. Reagents and Materials

1. bacitracin "A" disk (BBL)

IV. Procedure

1. Select a beta-hemolytic colony and heavily inoculate a quadrant of a 5% sheep blood agar plate.
2. Drop an "A" disk in the heaviest zone of inoculation.
3. Tap disk lightly to ensure that it adheres to the agar.
3. Incubate plate overnight in CO_2 at 35°C.

V. Reading and Interpretation

Any zone of inhibition is considered a positive test or sensitive test.

Growth to the edge of the disk is interpreted as a negative test or resistant test.

VI. Limitations; False negative reactions may result if too little inoculum is used.

VII.Quality Control

Quality Control is performed on each shipment and lot of bacitracin disk. *Streptococcus pyogenes* is the positive (sensitive control) and *Enterococcus faecalis*SS1273 is the resistant or negative control. Results are recorded in the QC log book.

Pyrrolidonylarylamidase Test (PYR)

I.Principle

Some bacteria produce pyrrolidonylarylamidase which hydrolyzes the substrate L- pyrrolidonyl - β-naphthylamide to form β-naphthylamine. A pink to red color forms when p-dimethylaminocinnam-aldehyde (PYR reagent) is added to β-naphthylamine.

II. Inoculum

Strains are grown on blood agar plates overnight at 35°C in CO_2. More than 1 day of incubation may be necessary for more fastidious genera such as the gemellae, alloiococci, and helcococci. The strains to be tested are grown on a blood agar plate until sufficient growth is seen to heavily inoculate the disks.

III. Reagents and Materials

PYR disk (Remel)

PYR reagent

Loops

Deionized Sterile water

IV. Procedure

The procedure that is used in the *Streptococcus* laboratory is modified from the package insert. The LAP test is usually done simultaneously.

1. Place the disks on blood agar plate in an area of little or no growth or on a slide. The moisture from the plate is usually sufficient to rehydrate the disk. If the disk is placed on a slide, then a tiny drop of sterile deionized water is added. (DO NOT OVERSATURATE THE DISK).
2. Using a loop or wooden stick, inoculate the disks heavily. Using two or more loop-full of culture is necessary for satisfactory results.
3. Leave the plates with the disks on the bench at room temperature for 10 minutes.

4. Add the detection reagent and read after 3 minutes.

V. Reading and Interpretation

The development of a red color within 3 minutes is positive. No change in color or a yellow color is negative. The color develops immediately. Discard the test after 10 minutes.

VI. Limitations

False negative reactions may result if too little inoculum is used.

VII. Quality Control

Each lot and shipment of PYR disks are tested for positive and negative reactions. *Enterococcus faecalis*strain SS-498 is used for positive reaction and *Streptococcus sanguinis*strain SS-910 is used for a negative reaction

SOP for Muller Hinton media preparation

Principle And Interpretation

Mueller Hinton Agar is recommended for the diffusion of antimicrobial agents impregnated on paper disc through an agar gelas described in CLSI Approved Standard. The Kirby-Bauer procedure is based on agar diffusion of antimicrobial substances impregnated on paper discs. This method employs disc with a single concentration of antimicrobial agent and the zone diameters observed are correlated with minimum inhibitory concentration (MIC) values.

A standardized suspension of the organism is swabbed over the entire surface of the medium. Paper discs impregnated with specific amounts of antimicrobial agents are then placed on the surface of the medium, incubated and zones of inhibition around each disc are measured. The susceptibility is determined by comparing with CLSI standard. The various factors, which influence disc diffusion susceptibility tests, are ***agar depth, disc potency, inoculums concentration, pH of the medium and beta-lactamase production by test organisms***.

Quality Control

Appearance

Sterile Mueller Hinton Agar + 5% Sheep Blood in 90 mm disposable plates.

Colour

Red colored medium

Quantity of medium

25ml of medium in disposable plate

Reaction

7.10- 7.50

Cultural Response

Cultural characteristics observed after an incubation at 35-37°C for 18-24 hours with added 5% w/v sterile sheep blood.

Sterility test

Passes release criteria

Organism	**Growth**	**Recovery**	**Hemolysis**	**Clindamycin CD 2 mcg**	**Erythromycin E 15mcg**	**Vancomycin VA 30 mcg**
Streptococcus pyogenes *ATCC 19615*	luxuriant>=70%		beta	19 -25 mm	25 -30 mm	20 -28 mm
H.influenza *ATCC 49766*	luxuriant>=70%					

Limitations

1. This medium is recommended for susceptibility testing of pure cultures only.
2. Inoculum density may affect the zone size. Heavy inoculums; may result in smaller zones or too less inoculums may results in bigger zones.
3. As antimicrobial susceptibility is carried with antibiotic disc, proper storage of the disc is desired which may affect the potency of the disc.
4. Under certain circumstances, the in vitro results of antibiotic susceptibility may not show the same in vivo.

Abbreviation:

MHA= Mueller Hinton agar

RT= Room temperature

Minimum inhibitory concentration= MIC

Clinical Laboratory Standard Institute=CLSI

Materials, Supplies and equipment

• MHA powder

• Distilled water

• Flask

• Petri dish

• Graduated cylinder

• Balance

• Distiller

• Bunsen burner

• Autoclave

• Hot plate

• PH meter

Procedure:

1. Suspend 38 gm of MHA powder & transfer in to a flask containing 1000 ml of distilled water.

2. Boil until the powder completely dissolved

3. Autoclave at 1210c for 15 minute.

4. Adjust PH at 25oc is 7.3 +/- 0.2.

5. Mix well and dispense aseptically in to sterile Petri dish.

Clinical Utility: MHA is used in antimicrobial susceptibility testing by the disk diffusion method.

Figure 2 Pathogenesis of Group A Streptococcus

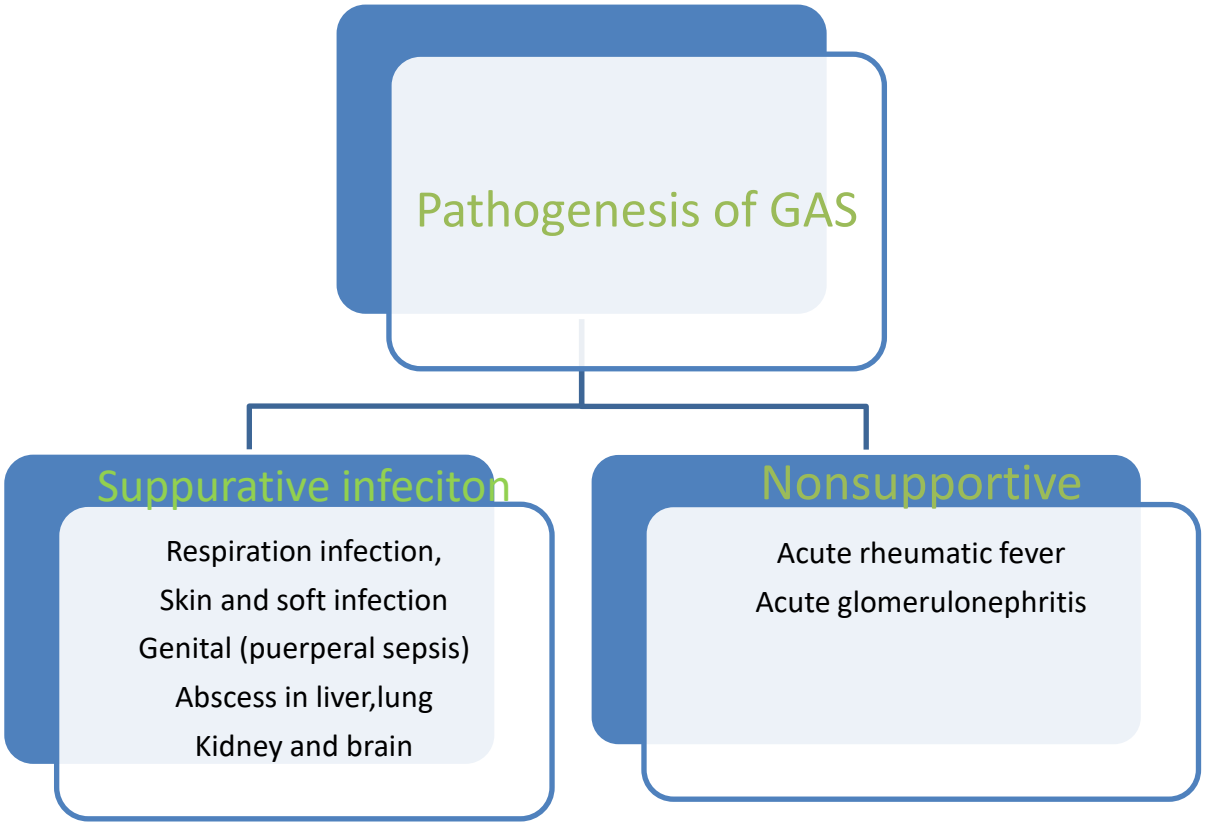
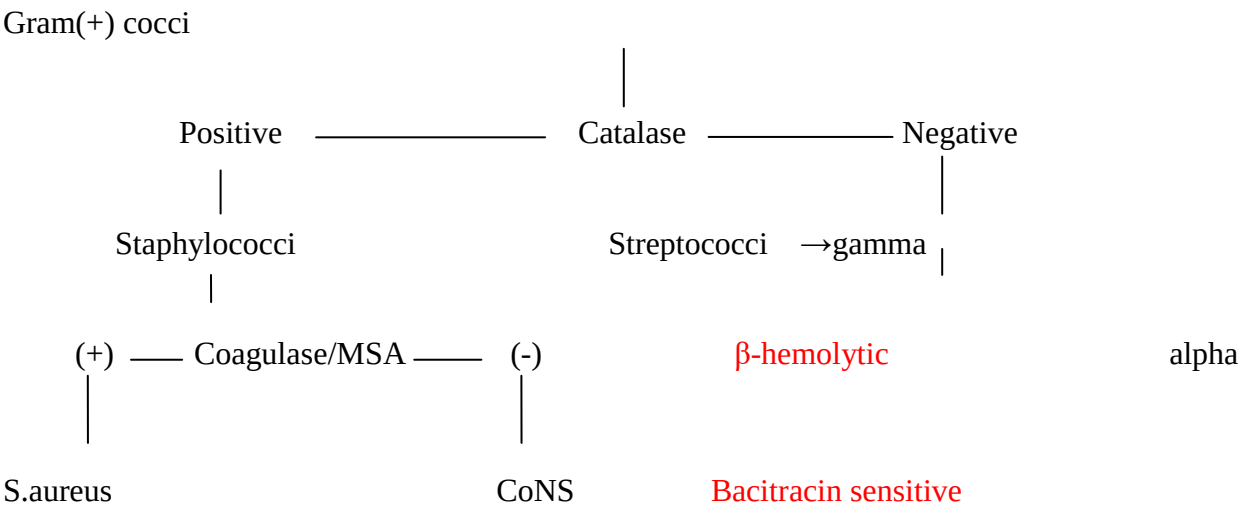


Figure 3: flow chart for identification of GAS



ANNEX VII: DECLARATION

The undersigned declares that this thesis complies with the regulations of the University and meets the accepted standards with respect to originality and quality. Principal investigator also agrees to accept responsibility for the scientific ethical and technical conduct of the research project and for provision of required progress reports.

M.Sc. Candidate: Terefe Selbane (B.Sc.)

Signature:_____

Date of submission: _____

This thesis has been submitted with our approval as advisors.

Advisors: Abraham Tesfaye (MSc, PhD)

Regasa Diriba (MSc.)

Signature: _____

Date: _____

Place: Addis Ababa, Ethiopia.