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Bacterial etiologies, antimicrobial resistance profiles and associated factors among children under the age of five with tonsillitis at Hargeisa Group of Hospital, Somaliland.

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Bacterial etiologies, antimicrobial resistance profiles and associated factors among children under the age of five with tonsillitis at Hargeisa Group of Hospital, Somaliland.

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ACRONYMS

Acute tonsillitis: when symptoms last anywhere from three days to about two weeks

AST: Antimicrobial Sensitivity Testing

BA: Blood Agar

CA: Chocolate Agar

CDC: Centre for Disease Control and prevention

CFU: Colony Forming Units

Chronic tonsillitis: have symptoms that persist beyond two weeks.

CSLI: Clinical and Laboratory Standard Institute

ENT: Ear Nose and Throat

HGH: Hargeisa Group of Hospital

MHA: Mueller Hinton Agar

MRSA: Methicillin Resistant *Staphylococcus aureus*

MSA: Manitol Salt Agar

Recurrent tonsillitis: occurs when a person suffers from multiple episodes of tonsillitis in a year

SPSS: Statistical Software Package of Social Science

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ABSTRACT

Background: Tonsillitis is the third most frequently diagnosed case in pediatrics medicine arround the world. It cuases significant morbidity and loss of school work in children. The emergence of drug resistance in tonsillitis causing tonsillitis is getting higher every year as reproted by the new studies being done. Little is known about the prevalence and the resistance profiles of tonsillitis causing bacterial pathogens among children under the age of five at Hargeisa Group of Hospital.

Objective: To determine the bacterial etiologic agents, antimicrobial resistance profiles and associated factors of tonsilitis among children under the age of five at Hargeisa Group of Hospital.

Methods: A cross-sectional study was conducted at Hargeisa Group of Hospital, Hargeisa, Somaliland from March 13th to July 25th 2020. A total of 374 children under the age of five were included using convenient method. Data on demographic variables and clinical profiles were collected using structured questionnaires. Throat swabs were collected from participants, processed and bacterial species were identified using standard bacteriological methods. Antimicrobial suseptibility was done using disc diffusion method. Logistic regression analysis was calculated to determine the association between variables. P.value < 0.05 was considered as statistical significant.

Results: The mean age of the participants was 4.1years old. Overall, 120(32%) of children had culture confirmed bacterial tonsillitis. Most frequent bacterial isolates were *S. pyogenes* 78(55%) followed by *S. aureus* 42(29%) and *S.pneumoniae* 10(7%). Majority of the bacterial isolates were resistant to ampicillin (100%). *S. aureus* was resistant to clarithromycin (38%). *S.pneumoniae* was resistant to gentamicin (60%). The overall MDR was (50.4%). All isolates of *P. aeruginosa* (100%), 52.6% of *S. pyogenes* and 60% of *S. pneumoniae* were MDR. History of tonsillitis (AOR=0.12 CI=0.06-0.21, and attending school (AOR=2.98 CI=1.64-5.42) were found to be predictors of tonsillitis in children under the age of five.

Conclusion: Bacterial tonsillitis in children under the age of five is a major health problem at Hargeisa. Culture based bacterial isolation and associated antibiotic sensitivity testing is recommended to prevent disease consequence and critical drug resistance.

Key words: Children under the age of five, tonsillitis, bacteria, drug resistance, Hargeisa group Hospital, Somaliland

1. INTRODUCTION

1.1 Background

Tonsillitis is the inflammation of tonsils. The inflammation can also affect other areas at back of the throat including the lingual tonsils and adenoids, and may also be called pharyngitis **(Nuha Saad, 2017)**.Tonsillar infections may be contagious, and can spread from person to person by close contact with infected person, sharing food or drinks and, utensils. Tonsillitis has significant impact on health status and quality of life as it causes significant morbidity and loss of time for school or work in children **(Zephanial, 2019)**.

Tonsillitis can be acute, recurrent or chronic. Acute tonsillitis is defined as a medically documented condition with pyrexia (>38.3°C), and tonsillar exudates, which indicates antibiotic treatment is needed. Recurrent tonsillitis (RT) is recognized for five episodes of tonsillitis in a year which diminishes the activity of daily needs. Chronic tonsillitis (CT) shows structural changes in the tonsillar tissue with episodes of acute tonsillitis accompanied by permanent tissue alterations Pre tonsillar abscess is another type of tonsillitis, with an abscess on only one side typically, indicated with acute tonsillitis with sudden puss filling one tonsil (Pignataro, 2018).

Clinically, sore throat, red swollen tonsils, difficulty of swallowing, fever, cough, headache, chills, malaise, white pus -filled spots on the tonsils, swollen lymph nodes (glands) in the neck, pain in the ears or neck, and weight loss are the common signs and symptoms of tonsillitis. On the other hand, nausea, fatigue, stomach ache, vomiting, furry tongue, bad breath (halitosis), voice changes, difficulty of opening the mouth, loss of appetite, anxiety or fear of choking are less common symptoms of tonsillitis (Campis, 2003, Okoye , 2016).

Bacterial tonsillitis is mainly caused by Gram positive bacteria such as *Streptococcus pyogenes* (*S.pyogenes*), *Staphylococcus aureus* (*S. aureus*) (including methicillin resistant *S. aureus*), *Streptococcus pneumoniae (S. pneumoniae)* and Gram negative bacteria such as *Pseudomonas aeruginosa* (*P. aeruginosa*), *Klebsiella pneumoniae* (*K. pneumoniae*), *Haemophilus influenzae* (*H. influenzae*), and *Morraxella catarrrhalis (M. catarrhalis)* (Agrawal, 2014).

The emergence of drug resistant bacteria pathogens in tonsillitis is getting higher every year as reported by the new studies being done, where formation of biofilm is one of the resistance

causing sources while some other bacteria produce enzymes like beta-lactamase. The irrational use of antibiotics by the patients is also one of the main reasons for emerging drug resistance. Methicillin resistant *S. aureus* (MRSA) is said to be the most frequent drug resistant bacteria to cause tonsillitis in children. (Mayur H. I., 2018).

Unfavorable exposure to environmental pollutants such as use of biofuel for cooking can lead to increased airway inflammation, poor living conditions of the children such as non-supervised hygienic practices of the children, indoor air pollution such as present smoker in the house setting, are associated factors of tonsillitis among children under the age of five (Bhalla et al P. B., 2019).

Little is known about the prevalence and the resistance profile of tonsillitis causing bacterial pathogens in the current study area. Therefore, the etiology and resistance profile of bacterial tonsillitis and its associated factors was assessed on children under the age of five attending at Hargeisa Group of Hospital.

1.2 Statement of the Problem

Tonsillitis is linked with complications including growth retardation (loss of weight and height). Its clinical impacts on children results suppurative and non suppurative complications. The non-suppurative complications include, scarlet fever, acute rheumatic fever, and post streptococcal glomerulonephritis. On the other hand, peritonsillar, para pharyngeal and retropharyngeal abscess formations are the common suppurative complications seen in children with infected tonsils (Campis, 2003).

The global epidemiology of tonsillitis is not known, however, previous studies in Europe reported that 12.1-11.7% prevalence of bacterial tonsillitis in children under the age of five (Shah, 2018). Studies in Asia reported 65.3 - 79% of bacterial tonsillitis in children under the age of five (Shishegar, 2014). Moreover, findings in Africa showed 72- 93% of bacterial tonsillitis in children under the age of five (Abraham, 2019).

The high magnitude of the spread of antimicrobial resistance among patients with tonsillitis has led to failed treatment of the infection and recurrence, in worst scenario mortality, especially in children with low standard of sanitation and public health in under developed countries like Somaliland, where antimicrobials are vastly and frequently used irrationally. There might be an increasing incidence of microbial resistance to commonly used antibiotics for the treatment of tonsillitis (Rebnord, 2017).

In Hargeisa Group of Hospital, same as other similar health settings in Somaliland, routine culture and antibiotic susceptibility testing are not usually performed as an essential part of patient care and the most treatment regime is based on empirical treatment. There are no any studies conducted and published on the prevalence of tonsillitis and antimicrobial susceptibility profile as well as the extent of MRSA and other bacterial pathogens causing tonsillitis in children under the age of five at Somaliland. Moreover, asymptomatic carrier children are a source of dissemination of tonsillitis causative agents to non-infected children whether at home or at school settings which can lead to uncontrollable wide range of tonsillar infections. Therefore, this study aimed at determining the prevalence of bacterial causes of tonsillitis and antimicrobial resistance profiles of the isolates in children under the age of five at Hargeisa Group of hospital.

1.3 Significance of the Study

This study provided data on the magnitude of bacterial tonsillitis among children under the age of five and tracks the antimicrobial resistance profiles of the isolates in children under the age of five at Hargeisa Group of Hospital. The results of the study are useful for management of children with tonsillitis and its prevention of bacterial tonsillitis.

The finding of this study is relevant to Pediatricians as a guideline for empirical treatment of bacterial tonsillitis. Moreover, other public health institutions can use this finding as a preliminary reference for further studies on bacterial tonsillitis in the study area.

The tonsillar bacteria and their Antimicrobial resistance profiles vary in geographical location and through time. Thus, the knowledge of the local bacterial etiology and their antimicrobial resistance profile is important for tracing any change that might have occurred in time so that timely updated reference for optimal empirical therapy of tonsillitis can be made.

2. LITERATURE REVIEW

2.1. Overview of Bacterial Tonsillitis

Tonsillitis is seen in winter and early spring which can occur occasionally or reoccur frequently (Vijayashree, 2014).Children are vulnerable for catching of tonsillitis, since tonsils are located at the front of the oro-digestive tract and are an easy target for the pathogenic bacteria (Muhamad, 2018).

There are different types of tonsillitis, and are divided to acute tonsillitis, recurrent tonsillitis, and chronic tonsillitis. Acute tonsillitis has symptoms including odynophagia (severe pain in the mouth and esophagus in attempt of swallowing). Bacterial pathogens such as *S. aureus*, *S. pneumoniae*, and *P. aeruginosa* are the major causes of acute tonsillitis. The condition is characterized by swelling and redness of the tonsils, with exudates, cervical lymphadenopathy and fever (Alotaibi, 2017).

Recurrent tonsillitis relates to recurrences of acute tonsillitis. This type of tonsillitis is defined if episodes of tonsillitis in one year evidenced in seven bacterial cultures, or five in two sequential years or three in three repeated years. In such conditions, the pause of the antibiotic leads to another bout of the bacterial infection within a few weeks, thus triggering it to return once more. On the other hand, a single violence of acute tonsillitis can be caused by several diverse bacterial organisms and flash up again a few weeks after termination of an antibiotic treatment (Stelter, 2014).

Chronic tonsillitis is associated with chronic sore throat, in which the infection causes recurring tonsillitis. Chronic tonsillitis is usually associated with bad breath and persistent tender cervical nodes. Chronic tonsillitis describes the most common lesions contained by pharynx inflammatory with multiple complications both local-regional and at the distance (glomerulonephritis, joint rheumatism, endocarditis, enteritis, etc.). Chronic tonsillitis usually described as focal tonsillitis, hypertrophic or scleroatrophic caseous cryptic tonsillitis as recurrent type, and simple hypertrophic tonsillitis soft type in children and hard type in adults (Pignataro, 2018).

Allergies like hay fever and those affecting the nose are a source of infection as allergies that cause itching of pharyngeal area, if the child itches and scratches that may allow bacteria to settle in the pharyngeal area and cause infection. On the other hand, irritants such as smoke can damage the pharyngeal area making the tissues an easy target for bacteria (Pambuk, 2018).

The surroundings of the children are a potential source of infection, where food chewed and as well as air breathed can bring pathogens to the children. Direct contact of mouth while kissing the child can as well cause infection of the child's tonsils (Okoye E. O., 2016).

2.2. Epidemiology of tonsillitis in children under the age of five

There is no global epidemiological survey done, but studies showed that in the US, from the total ear nose and throat (ENT) diseases encountered, 40 million (5%) were tonsillitis. In Spain, 4 million (15%) ENT cases had tonsillitis in pediatrics. On the other hand, in France 9 million of ENT diseases had tonsillitis (Abdul wahab haidara, 2019). Furthermore, a study done in the UK documented 79% of bacterial caused tonsillitis (Thors et al., 2016). A study done in Trinidad, reported 62.5% of culture confirmed bacterial tonsillitis in children under the age of five (Maureen et all, 2008).

A study done in India showed that 72% of the processed samples had tonsillitis. Of them, 10% occurred in children under the age of five (Mahanjan et all, 2018). On the other hand, a study conducted at Saudi Arabia on 52 study subjects showed that 37 (65%) of the subjects were positive for pathogenic bacteria tonsillitis (Mohammed et all, 2013).

A study done in Egypt conducted on 35 children showed that 34% (12) of the cases had showed growth of bacterial pathogens (Sarah Yousef A. E., 2014). A study done in Nigeria conducted on 73 children showed that 73% of the subjects had tonsillitis, of these, 39 (53%) had bacterial pathogens (Okoye E. O., 2016). A study done in Cameroon on 512 children under the age five showed 54(19%) of the study subjects were detected for bacterial pathogens causing tonsillitis (Tazinya et al., 2018).

A study done in Tripoli, Libya showed 71% of the study participants tested for tonsillar bacterial isolate (Ati et al., 2018). A study done in Jimma, Ethiopia showed that tonsillitis caused by *S. pyogenes* accounted 40 (11.3%) out of the 355 children (Tesfaw et al, 2016).

2.3 Bacterial isolates from tonsillitis

A study done in the UK showed 4.4% for *Streptococcus pyogenes*, 86.1% for *Morraxella catarrrhalis*, 85.3% for *Haemophilus influenzae* and 6.6% for *Staphylococcus aureus* (Ati et al., 2018)

A study done in Trinidad on 102 children, 90 children had bacterial isolates, and from these 62.5% of the tonsillitis was due to *S. aureus*, 79.2% were *Streptococcus* spp., *Proteus* spp., *K. pneumoniae* and *Pseudomonas* spp. counted 4%, 6%, and 4% isolation percentage respectively (Maureen et all, 2008).

In India *S. pyogenes* (67%), *S.aureus* (38%), *H. influenzae* (21%), *P. aeruginosa* (3%), and *E. coli* (2%), respectively were isolated from children under the age of five with tonsillitis (Kalaiarasi, 2018). In a study done at Nigeria 39 (53%) of the children had bacterial isolates with 19 (48%) *S. pyogenes*, 5(12%), *S. aureus*, 5 (12%) where *K. pneumoniae*, 3(7.69%) *S. pneumoniae*, 3(7.69%) *Pseudomonas* spp., 3(7.69%) *Proteus* spp., and 1(5.1%) *E. coli* (Okoye et., 2016). In a study conducted at Egypt, 34%(12) of the cases showed growth of pathogens where *S. aureus* was the most common bacteria isolated with 6 out 34 samples. Only one *Streptococcus pyogenes* was isolated. However, 3 (8.5%) *Escherichia coli* and 2 (5.7%) and *Klebsiella pneumoniae* were isolated (Sarah Yousef S. A., 2014). A study done in Tripoli, Libya conducted on 1110 study participants showed that 34% of the isolates were Streptococci, 22.8% were *Pseudomonas aeruginosa*, 13.8% were *Escherichia coli* and 2.2% were *Klebsiella pneumoniae*. (Ati et al., 2018)

Unfortunately there is no information or studies done on Somaliland, which indicates that there is no exact information about the prevalence or the epidemiological state of tonsillitis in under five children of Somaliland.

2.4. Antibiotic resistance of bacterial isolated in tonsillitis

The surfacing of antimicrobial resistance has put a tremendous amount of pressure in the management of infectious diseases like tonsillitis. The irrational use of antimicrobials costs 700,000 deaths per year throughout the world. If there are no appropriate managements not taken against antimicrobial resistance, it will cost US$ 100 trillion and most importantly 10 million deaths per year (Tadesse, 2017)).

A study done in London, England and Ontario, Canada reported Streptococci were resistant to clindamycin (32%), and erythromycin (41%) and *Staphylococcus* spp. were resistant to clindamycin (60%) and erythromycin (80%). *Staphylococcus aureus* showed a global sensitive to oxacillin, vancomycin and sulphamethoxazole (Sowerby, 2013).

A study done in India showed that *Proteus* spp. was 100% resistant to erythromycin, gentamicin, cefeclor, tobramycin and vancomycin. Other pathogenic bacteria isolated were mildly resistant to majority of the drugs (Mayur I. a., 2018).

A study done in Benin showed *S. pyogenes, S. aureus, K. pneumoniae, P. aeruginosa*, and *Proteus mirabilis* isolates from children with tonsillitis were 100% resistant to ampicillin. *Pseudomonas aeruginosa* isolates was resistant to cefuroxine (33.3%). *S. pyogenes*, *S. aureus*, *K. pneumoniae* and *P. aeruginosa* isolates showed 35.3%, 25%, 50% and 66.7% of resistance to cephalexin, respectively. Moreover, these isolates revealed 88.9%, 75%, 100%, 100%, 33.3% resistance to cotrimoxazole, respectively (Sadoh et al., 2007). A study done in Jimma, Ethiopia showed that *S. pyogenes* isolates were 50% resistant to tetracycline (Tesfaw et al, 2016, p. 9). So far, antimicrobial resistance pattern of bacterial tonsillitis in Somaliland was not known. Thus, it is difficult to show the picture of antimicrobial resistance in the study area.

2.5. Factors Associated with tonsillitis

Tonsillitis is highly related to age and children are more frequent of being infected by tonsillitis than adults. A close contact with infected people is another factor causing tonsillitis. The hygiene of the child can be a risk factor, because poor hygiene can predispose the children to infection. In the meantime, mothers' smoking behavior during pregnancy affects the children's' exposure to tonsillitis. Children's weight at birth and after birth also affects tonsillitis infection of the children under the age of five (Gahleitner, 2016).

Socio-economic status of the families is one of the main factors associated with tonsillitis in children under the age of five because high socioeconomic status families are more likely to seek medical attention, in addition to this, child's age to acknowledge the pain is also one of the main factors especially if they are infants. Furthermore, sex of the child is also one of the main factors affecting the tonsillitis in children under the age of five where males children more likely to have infected with tonsils than female children (Sadoh et al., 2007). Other factors including residence of the family, living in a crowded house, attending schools, and HIV status are significantly associated with tonsillitis among children under the age of five (Fadlyana *et al*, 2018).

2.6 Diagnosis and treatment of tonsillitis

Looking for exudates, redness, and cough are initial indicators of bacterial tonsillitis. Rapid antigen tests and nucleic acid testing are frequently under taken in detection of bacterial tonsillitis. Microscopic identification of bacteria from the sample is another way detection of bacterial infection. Throat swab culture taken from the tonsils is an effective way and gold standard for the detection of bacterial pathogens from tonsils (Bourbeau, 2003).

Bacterial tonsillitis can be treated using antibiotics. Analgesics like aspuprophen, is also given to children in minimizing symptoms of tonsillitis. Mouth washes such as nasturtium and horseradish which is composed of antibacterial are given to both children and adults. For uncontrollable tonsillitis, tonsillectomy is a way to remove the tonsils completely, whereas cryptolysis is removal of tonsillar ring and replacing with a bare crypt superficially and the natural crypt shrinks finally. Prophylactic drugs, hand washing, avoiding of sharing food with infected patients, and avoiding prolonged close contact with the infected are the main ways to prevent tonsillitis in children (Alotaibi, 2017). Amoxicillin and penicillin V are the first line drugs for children with tonsillitis as reported by the Centre for Disease Control (CDC, 2017).

3. OBJECTIVES OF THE STUDY

3.1 General Objectives

- To determine the bacterial etiologies of tonsillitis, antimicrobial resistance profiles of the isolates and associated factors in children under the age of five at Hargeisa Group of Hospital.

3.2 Specific Objectives

- To isolate the bacterial etiologic agents of tonsillitis in children under the age of five.
- To determine the antimicrobial resistance profiles of the bacterial isolates from tonsillitis.
- To identify factors associated with bacterial tonsillitis in children under the age of five.

4. METHODS AND MATERIALS

4.1 Study Design, Setting and Period

A hospital based cross-sectional study was conducted from 13thMarch to July 25th 2020 in Hargeisa Group of Hospital (HGH), Hargeisa, Somaliland. Hargeisa Group of Hospital is located in Maroodi Jeex Region, the capital city of Somaliland known as Hargeisa (9.5624° N, 44.0770° E). According to the 2019 census report from Central Statistics Department of Somaliland, Hargeisa has a total population of 1.2 million.

Hargeisa Group of Hospital is a public hospital with vast diversity of health care services, carried out by more than 200 healthcare professionals. Hargeisa Group of Hospital is the largest referral hospital in Somaliland. The institution was built long before the civil war in 1988 and now is one of the health hubs in Somaliland. Currently, HGH has different departments including administration, surgery department, orthopedic department, laboratory department, pediatric department, obstetric department, gynecology department, abdominal ultrasound, and dentistry unit. The pediatric department of HGH has outpatient and inpatient departments. It has 10 inpatient Pediatricians serving at the wards, with twenty three Nurses for 24 hours rounds. The outpatient department has also four Pediatricians, and two Nurses serving for daily hours from 8 O'clock to 2 O'clock Eastern time. Daily 50 outpatients followed by 1 to 4 hospitalized children attending the Pediatric department of the hospital for different medical conditions such as tonsillitis, diarrhea, pneumonia, rare heart conditions, gastritis, and skin diseases.

4.2 Source Population

The source population composed of all children under the age of five attended the department of Ear Nose and Throat (ENT) of HGH.

4.3 Study Population

Study population was children under the age of five with tonsillitis at Ear Nose and Throat(ENT) of HGH during study period.

4.4 Study participants

Study participants were children under the age of five with bacterial tonsillitis at Ear Nose and Throat (ENT) and actually enrolled in the study.

4.5 Inclusion and Exclusion Criteria

Children under the age of five with signs and symptoms of tonsillitis(sore throat, red swollen tonsils, difficulty of swallowing, fever, cough, headache, tiredness, chills, malaise, white pus - filled spots on the tonsils, swollen lymph nodes, pain in the ears or neck, and weight loss) were included in the study. On the other hand, those children who were on antibiotic treatment within the previous two weeks of sample collection, and had tonsillectomy were excluded from the study.

4.6 Sample size Determination and Sampling Technique

Since there was no previous study related to the subject in the study area, 50% prevalence of tonsillitis had been taken to calculate the sample size: Accordingly the sample size was calculated using a single population proportion formula (**n=** Z^2 P (1- P)/d^2), by considering 95% confidence interval, 5% margin of error and 0.5 proportion of bacterial tonsillitis. Therefore, the calculated sample size was 384. However, due to the lack of sufficient throat swab and incomplete questionnaire based data, only 374 children under the age of five with tonsillitis were included in the study.

A convenient sampling technique was used to include the study participants. All children under the age of five with tonsillitis attending at ENT department of HGH and who fulfilled the inclusion criteria were included consequently until the required study participants were reached.

4.7 Variables

4.7.1 Dependent Variables

Bacterial causes of tonsillitis (prevalence of bacterial tonsillitis).

4.7.2 Independent variables

Demographic variables: Child's age, mother's age, father's age, gender, residence, maternal education, paternal education, and parental occupation.

Clinical variables: History of tonsillitis, current type of tonsillitis, number of previous tonsillitis, body temperature, sore throat, swollen tonsils, headache, swollen lymphnodes, difficulty in swallowing, white exudates on the throat, weight loss, tonsillar structural change, and history of drug use.

Patient related variables: History of participants, contact with someone who had cough, type of breast feeding, attending day care, and attending school.

Environmental variables: Overcrowding and use of wood biofuel.

4.8 Throat Swab Sample Collection and processing

Throat swabs were taken by the attending pediatricians from each patient using a sterile swab stick. The swab stick was introduced into a well exposed mouth with the tongue depressed by a wooden spatula where necessary and visible exudates or hyperemic areas on tonsillar walls were swabbed. The swab stick was carefully replaced in its sheath and sent for microbiological analysis. Then, all samples were immediately transported to the Microbiology department of Hargeisa Group of Hospital using Amies transport media (Oxoid, England) for culture and antimicrobial susceptibility testing (AST).

Swabs were simultaneously plated onto a blood agar (BA), chocolate agar (CA), and MacConkey (MAC) media and incubated at 37ºC for 48h. Chocolate Agar was incubated in a candle jar for 48h in 37ºC to get 5% CO2. Bacterial colonies were further sub-cultured on to BA to identify species. Cultures were considered negative if there was no significant bacterial growth (10^6 CFU/ml) after 48h (Sadoh et al, 2008).

Cultured pure colonies were further identified by Gram staining and biochemical tests including oxidase, coagulase, catalase, and urease test. *Streptococcus pyogenes* were identified as Gram positive chain forming small colonies that formed complete hemolysis on BA and coagulase negative and catalase negative. *Streptococcus pneumoniae* isolates were identified by gram positive alpha-hemolytic small colonies on BA, which were optochin susceptible. *Staphylococcus aureus* were identified by gram positive cluster forming glistering golden yellow colonies on blood agar and Manitol Salt Agar (MSA) which were coagulase, catalase and oxidase positive. *Morraxella catarrrhalis* were identified by large kidney shaped diplococcus gram negative grey to white hemispheric colonies on BA with both oxidase and catalase positive.

4.9 Screening of Methicillin Resistant *Staphylococcus aureus* (MRSA)

Methicillin resistant *Staphylococcus aureus* was screened using oxacillin disk diffusion sensitivity testing. Pure colonies of *Staphylococcus aureus* were inoculated on MSA and 30 µg of oxacillin discs were impregnated on the plate media and incubated for 16 -18 h. The zone of inhibiton was measured by caliper. Measurements from the CLSI 2019 standard was followed as reference for interpretation. Accordingly, a zone of inhibition of ≤ 21 mm of oxacillin disk against *Staphylococcus aureus* isolates were considered as mecA positive and reported as methcillin resistant while if the zone of inhibition value of oxacillin disk against *Staphylococcus aureus* is ≥ 25mm, it was considered as mecA negative, and reported as methicillin sensitive (CLSI, 2019).

4.10 Antimicrobial Susceptibility Testing

The antimicrobial susceptibilities of all the identified bacterial isolates were performed according to the criteria of Clinical and Laboratory Standards Institute (CLSI, 2019) using the Kirby-Bauer disc diffusion method on Mueller-Hinton Agar (MHA) (HiMedia, India). A loop full of bacteria was taken from a pure culture colony and transferred to a tube containing 5ml of normal saline and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was then adjusted to the turbidity of McFarland 0.5 (which carries 10^8 CFU/ml) in order to

standardize the inoculums size and was swabbed on a dry surface of MHA plate (150 mm) using a sterile cotton swab. Antibiotic discs were dispensed using a single disc dispenser.

The plates were then incubated at 37^{o}C for 24h. Diameters of the zone of inhibition around the discs were measured using a digital caliper. The results of the zone of antibiotics were interpreted based on the CLSI guideline (CSLI, 2019). The following antibiotic discs were tested: ampicillin (10 µg), amoxicillin-clavulanic acid (20/10 µg), gentamicin (10 µg), clarithromycin (15 µg), erythromycin (15 µg), vancomycin (30 µg), oflaxacin (5 µg) and ciprofloxacin (5 µg). These antimicrobial drug disks were selected based on the frequent prescriptions of these drugs for the treatment of tonsillitis infection in the study area and using the 2019 CLSI (CLSI, 2019).

4.11 Data Collection Procedures

Structured questionnaire was used to collect data on demographic characteristics of the study participants. Demographic profiles of the children were collected with face- to- face interviewing of the study participants' guardians/parents. Clinical profiles of tonsillitis among children under the age of five were screened by attending pediatricians in charge.

4.12 Quality Control

Proper specimen collection was followed through explaining for the child's guardian/parent, and swabbing on the surface of tonsils. All the throat swab specimens were analyzed within two hours of collection in order to prevent contamination, and any specimen delaying to this time was stored < 48 hours at room temperature.

Culture media were checked for sterility and their normal shelf life. The media was tested every time after preparation for sterility checking by incubating a plate of each media overnight in a different incubator than one used for culture. Antibiotics discs were tested for date of expiry. Efficiency of antibiotic discs were tested by using bacterial standard strains of *S.aureus* ATCC29213, *S. pneumoniae*, ATCC49618, and *P. aeruginosa* ATCC27853 as positive controls.

4.13 Data analysis

Data was entered, cleaned and analyzed by using Statistical Software for Statistical Package for Social Sciences (SPSS) version 25. Univariate analysis was made to generate summary values for the most important variables. Logistic regression analysis was made to determine the association between dependent and independent variables. Generated data was compiled by frequency tables and other statistical summary measures. Stepwise logistic regression model was used to find factors associated with culture positive tonsillitis and statistical significance was set at $p<0.05$.

4.14 Ethical Considerations

An ethical clearance letter was obtained from the Institutional Review Board (IRB) of College of Medicine and Health Science (CMHS), Bahir Dar University. A permission letter was obtained from the Ministry of Health, Somaliland, and Hargeisa Group of Hospital (HGH). Following well-versed about the purpose and importance of the study, written informed consent was obtained from children parents/guardians before collecting data. Information obtained during this study was kept confidential and used only for the study purpose. Bacteriological positive results were communicated to health pediatricians managing children for treatment purposes.

5 RESULTS

5.1 Characteristics of the study participants and prevalence of bacterial tonsillitis

Due to the lack of throat swab and incomplete data, 374 children under the age of five actually participated in the study. Among 374 children, 200 (53.5%) were males. Most (81.6%) of the participants were urban dwellers. The age range of children was 2 to 5 years. Majority (37.7%) of the children were five years old (mean = 4.1, median = 4). The children mother's age ranged from 20- 45 years. Most (69%) of the parents were employed (Table 1).

5.1.1 Prevalence of bacterial tonsillitis

Overall, the prevalence of culture confirmed bacterial tonsillitis was 120 (32.1%). The proportion of bacterial tonsillitis was higher in males 76 (36.5%) than females 47 (27%). It was higher in urban 101 (33.1%) than rural 19 (27.5%) residents. The percentage of bacterial tonsillitis was higher (41.9%) in children from mother's unable to read and write than other groups (5.9- 26.7%). Children under the age of five from fathers who were not able to read and write and only able to read and write had higher percentage of culture confirmed bacterial tonsillitis (32.7 and 51.8%) than those children from a father of better educational status (23.5%, 36.6% and 24%) (Table 1).

Table1: Socio-demographic characteristics of study participants and prevalence of culture confirmed bacterial tonsillitis among children under the age of five at Hargeisa Group of Hospital, March 13, to July 25, 2020.

Variables	Total	Culture result		COR (95% CI)	P- value
		Positive	Negative		
		N (%)	N (%)		
Children age (in years)					
2	2 (0.5)	2 (100)	0	NA	0.199
3	110 (29.4)	36 (32.7)	74 (67.3)		
4	121 (32.4)	40 (33.1)	81 (66.9)		
5	141 (37.7)	42 (29.8)	99 (70.2)		
Gender					
Male	200 (53.5)	73 (36.5)	127 (63.5)	0.64 (0.41-1.00)	0.05
Female	174 (46.5)	47 (27)	127 (73)		
Residence					
Rural	69 (18.4)	19 (27.5)	50 (72.5)		0.37
Urban	305 (100)	101 (33.1)	204 (66.9)	0.78 (0.43 -1.37)	
Father's education					
Not able to read and write	55 (14.7)	18 (32.7)	37 (67.3)	3.46 (0.6717.72)	0.002
Able to read and write	56 (15)	29 (51.8)	27 (48.2)	2.53 (0.46 -13.79)	
Primary school	81 (21.7)	19 (23.5)	62 (76.5)	1.01 (0.20-5.57)	
High school	82 (21.9)	30 (36.6)	52 (63.4)	0.9 (0.16 -5.04)	
Higher education	100 (26.7)	24 (24)	76 (76)		
Mother's education					
Not able to read and write	186 (49.7)	78 (41.9)	108 (58.1)	NA	0.001
Able to read and write	105 (28.1)	28 (26.7)	77 (73.3)		
Primary school	36 (9.6)	7 (19.4)	29 (80.6)		
High school	30 (8)	6 (20)	24 (80)		
Higher education	17 (4.5)	1 (5.9)	16 (94.1)		

Parental occupation					
Unemployed	101 (31)	26 (22.4)	90 (77.6)	1.98(1.19-3.29)	0.020
Employed	258 (69)	94 (36.4)	164 (63.6)		
student	15 (4)	2 (13)	13 (87)		
Mother's age					
20-25	98 (26.2)	26 (26.5)	72 (73.5)	NA	0.14
26-30	169 (45.2)	59 (34.9)	110 (65.1)		
31-35	90 (24.1)	27 (30)	63 (70)		
36-40	15 (4)	6 (40)	9 (60)		
41-45	2 (0.5)	2	0		
Total	**374 (100)**	**120 (32.1)**	**254 (77.9)**		

Key: NA: Not applicable

Prevalence of bacterial tonsillitis in relation to clinical profiles

Table 2 depicts the clinical profiles versus bacterial tonsillitis among children under the age of five. From the total, 172 (46%) of children under the age of five had history of tonsillitis. The majority of children had presented with acute tonsillitis (54%) and with sore throat 343 (91.7%). Swollen tonsils was presented in 367 (98.1%) of study participants. On the other hand, cervical lymphadenopathy was presented in 151 (40.4%) of study participants. Moreover, 147 (39.3%) and 69 (18.4%) of children had difficulty of swallowing and white exudates, respectively.

The percentage of tonsillitis was higher in those children with history of tonsillitis (55.2%) than the others (12.4%). The percentage of tonsillitis was highest (55.7%) in those children with symptoms of chronic tonsillitis. Moreover, the prevalence of culture confirmed tonsillitis was higher in those children with tonsillar structural change (57.7%) than those without (28%). Children under the age of five with swollen tonsils had higher percentage of culture confirmed tonsillitis (32.4%) than those without swollen tonsils (14.3%). The proportion of culture confirmed bacterial tonsillitis was higher in those children who had weight loss (46.9%) than the counters (22.9%) (Table 2).

Table2: Prevalence and clinical characteristics profiles of children under the age of five with tonsillitis at Hargeisa group of hospital, March 13, to July 25, 2020.

Variables	Culture results			COR (95% CI)	P- value
	Total N (%)	Positive N (%)	Negative N (%)		
History of tonsillitis					
Yes	172 (46)	95 (55.2)	77 (44.8)	8.72 (5.22 -14.63)	< 0.001
No	202 (54)	25 (12.4)	177 (87.6)		
Number of previous tonsillitis					
None	202 ()	26 (12.9)	176 (87.1)		
One	15 (4)	9 (60)	6 (40)	8 (4.63-13.82)	< 0.001
Two	24 (6.4)	14 (58.3)	10 (41.7)	1.38 (0.44-4.35)	0.58
Three	13 (3.5)	6 (46.2)	7 (58.3)	0.84 (0.35-2.1)	0.71
>Four	120 (32.1)	65 (54.2)	55 (45.8)	0.79 (0.26-2.35)	0.67
Type of tonsillitis					
Acute	202 (54)	25 (12.4)	177 (87.6)		
Chronic	52 (13.9)	30 (57.7)	22 (42.3)	0.11 (0.68-0.19)	< 0.001
Recurrent	120 (32.1)	65 (54.2)	55 (45.8)		
Weight loss					
Yes	143 (38.2)	67 (46.9)	76 (53.1)	2.96 (1.89- 4.64)	< 0.001
No	231 (61.8)	53 (22.9)	178 (77.1)		
Tonsillar structural change					
Yes	52 (13.9)	30 (57.7)	22 (42.3)	3.52 (1.93-6.42)	< 0.001
No	322 (86.1)	90 (28)	232 (72)		
Temperature					
37 C °	42 (11.2)	12 (28.6)	30 (71.4)	1.21 (0.59-)	0.61
> 38^{o}c	301 (88.8)	108 (32.5)	224 (67.5)		
Sore throat					
Yes	343 (91.7)	111 (32.4)	232 (63.6)	0.86 (0.38-1.92)	0.704

No	31 (8.3)	9 (29)	22 (71)		
Swollen tonsils					
Yes	367 (98.1)	119 (32.4)	248 (67.6)	0.35 (0.04 - 2.92)	0.308
No	7 (1.9)	1 (14.3)	6 (85.7)		
Headache					
Yes	105 (28.1)	33 (31.4)	72 (68.6)	1.04 (0.64-1.69)	0.865
No	269 (71.9)	87 (32.3)	182 (67.7)		
Swollen lymphnodes					
Yes	151 (40.4)	53 (35.1)	98 (64.9)	1.26 (0.81-1.96)	0.304
No	223 (59.6)	67 (30)	156 (70)		
White exudates					
Yes	69 (18.4)	19 (27.5)	50 (72.5)	0.77 (0.43-1.37)	0.370
No	305 (81.6)	101 (33.1)	204 (66.9)		
Difficulty of swallowing					
Yes	147 (39.3)	27 (18.4)	120(81.6)	0.33 (0.19 - 0.53)	<0.001
No	227 (60.7)	93 (41)	134 (59)		

Prevalence of bacterial tonsillitis in relation to other variables

Overall, 228 (61%), 96 (25.7%) of children under the age of five had weight loss, history of contact with cough patients and exclusive breast feeding, respectively. On the other hand, 86 (23%) of children under the age of five were day care center attenders while 282 (75.4%) of participants were school attenders. The majority of participants lived in a crowded house (71.7%) and had exposure to biofuel (88.5%) (Table 3).

The proportion of culture confirmed bacterial tonsillitis was higher among participants who had history of contact with cough patients (61%) than the counters (24%). Day care center attending children had higher (39.5%) percentage of bacterial tonsillitis than others (29.9%). Moreover, Children who are attending school had higher (52.2%) percentage of bacterial tonsillitis than the counters (25.5%).The proportion of bacterial tonsillitis was higher in those children who had exposure to biofuel (35.3%) than others (7%) (Table 3).

Table 3: Prevalence of tonsillitis and patient related factors of children under the age of five with symptoms of tonsillitis at Hargeisa Group of hospital.

Variables		Culture result		COR (95% CI)	P-value
		Positive N (%)	Negative N (%)		
Contact with cough patients					
Yes	228 (61)	85 (37.3)	143 (62.7)	1.89 (1.18 - 3.00)	0.008
No	146 (39)	35 (24)	111 (76)		
Breast feeding					
Mixed	278 (74.3)	91 (32.7)	187 (67.3)	1.12 (0.68-1.86)	0.65
Exclusive	96 (25.7)	29 (30.2)	67 (69.8)		
Attending day care center					
Yes	86 (23)	34 (39.5)	52 (60.5)	1.54 (0.93-2.53)	0.092
No	288 (77)	86 (29.9)	202 (70.1)		
Over crowding					
Yes	268 (71.7)	76 (28.4)	192 (71.6)	0.56 (0.35-0.89)	0.014
No	106 (28.3)	44 (41.5)	62 (58.5)		
Wood biofuel					
Yes	331 (88.5)	117 (35.3)	214 (64.7)	7.29 (2.21-24.1)	0.001
No	43 (11.5)	3 (7)	40 (93)		
Attending school					
Yes	92 (24.6)	48 (52.2)	44 (47.8)	0.31 (0.19-0.51)	< 0.001
No	282 (75.4)	72 (25.5)	210 (74.5)		
Total	**374 (100)**	**120 (32.1)**	**254 (67.9)**		

5.2 Distribution of bacteria isolates

A total of 143 (32%) bacterial pathogens were isolated from 120 culture positive samples. Of them, 25 (20.8%) of the samples had mixed bacterial isolations. The most common bacteria isolated were *Streptococcus pyogenes* 78 (55%), followed by *Staphylococcus aureus* 42 (29%),

*Streptococcus pneumoniae*10 (7%), and *Klebsiella pneumoniae* 6 (4%). The total isolation of MRSA (Methicillin resistant *Staphylococcus aureus*) was 19 (15.8), and 11 (57.9%) of MRSA had mixed bacterial isolations (Table 4).

Table 4: Distribution of Bacterial isolates from children under the age of five with tonsillitis at Hargeisa Group of Hospital (n=120).

Bacteria isolations	**Number (Percent)**
Streptococcus pyogenes	78 (55%)
Staphylococcus aureus	28 (23%)
Streptococcus pneumoniae	10 (7%)
Klebsiella pneumoniae	2 (3.3%)
Morraxella catarrrhalis	4 (3%)
Pseudomonas aeruginosa	3 (2%)
S.pyogenes + S.aureus	10 (8%)
S.pyogenes + P.aeruginosa	3 (2.5%)
S.pneumoniae + S.aureus	4 (3.3%)
S.pneumoniae + K.pneumoniae	2 (1.7%)
S.aureus + M. catarrrhalis	4 (3.3%)
MRSA	19 (15.8%)
Total *S.pyogenes*	78 (55%)
Total *S.aureus*	42 (29%)
Total *S.pneumoniae*	10 (7%)
Total *K. pneumoniae*	6 (4%)
Total *M. catarrrhalis*	4 (3%)
Total *P.aeruginosa*	3 (2%)
Total isolates	**143 (32%)**

5.1Antibiotic resistance profile of bacterial isolates

Overall, 131 bacterial isolates were resistant to ampicillin (92%). Relatively, higher resistance percentages were found against gentamicin (41%), ofloxacin (34%) and erythromycin (31%). *Streptococcus pyogenes, Staphylococcus aureus, Streptococcus pneumoniae* and *Morraxella catarrrhalis* isolates revealed an overall resistance of 33%, 34%, 29% and 50%, respectively.

Streptococcus pyogenes revealed resistant to ofloxacin (43%), and gentamicin (42%). *Staphylococcus aureus* isolates showed resistance to clarithromycin (38%), and ciprofloxacin (35%). The percentage of *Streptococcus pneumoniae* isolates resistance to gentamicin clarithromycin and erythromycin was 60%, 30%, and 30% respectively. *Klebsiella pneumoniae* isolates were resistant to ampicillin (83%) and erythromycin (67.7%) (Table 5).

Table 5: Antibiotic resistance profile of bacterial isolates from children under the age of five with tonsillitis at Hargeisa Group of Hospital

Antibiotics tested	Bacterial isolates													
	***S. pyogenes* (n = 78)**		***S.pneumoniae*(n = 10)**		***S. aureus* (n = 42)**		***M.catarrrhalis* (n = 4)**		**P.aeruginosa (n = 3)**		***K.pneumoniae* (n = 6)**		**Total (n =143)**	
	#T	**R%**	**#T**	**R%**	**#T**	**R%**	**#T**	**R%**	**#T**	**R%**	**#T**	**R%**	**#T**	**R%**
Amoxicillin-clavulanic acid	78	6 (77)	10	0	42	10(23.8)	4	2 (50)	3	1 (33.3)	6	2(33.3)	143	21 (14.6)
Ciprofloxacin	78	8 (10)	10	0	42	15 (35)	4	2 50)	3	3 (100)	6	2(33.3)	143	30 (20.9)
Clarithromycin	78	22 (28)	10	3 (30)	42	16 (38)	4	2 (50)	3	1 (33.3)	6	2(33.3)	143	46 (32)
Gentamicin	78	33 (42)	10	6 (60)	42	13 (31)	4	3 (75)	3	2 (6.7)	6	2(33.3)	143	59 (41)
Vancomycin	78	10(12.8)	10	0	42	14(33.3)	4	0	3	1 (33.3)	6	2(33.3)	143	27 (18.8)
Ofloxacin	78	34 (43)	10	1(10)	42	11 (26)	4	0	3	2 (67.7)	6	1(16.7)	143	49 (34)
Erythromycin	78	22(28.2)	10	3 (30)	42	11(26.2)	4	3 (75)	3	2 (67.7)	6	4(67.7)	143	45 (31)
Ampicillin	78	74(94.9)	10	10(100)	42	35 (88)	4	4 (100)	3	3 (100)	6	5(83.3)	143	131 (92)
Total	**624**	**209 (33)**	**80**	**23 (29)**	**336**	**115 (34)**	**32**	**16 (50)**	**24**	**15 (23)**	**48**	**18 (36)**	**1144**	**408 (35.6)**

#T: number of isolates tested, R%: percentage of isolates resistant to antimicrobials

Profiles of MDR bacterial isolates

Overall, 72 (50.4%) of the bacterial species were resistant to three or more classes of antibiotics. The overall MDR of *Streptococcus pyogenes* was 41(52.6%). The overall MDR of S*taphylococcus aureus, Streptococcus pneumoniae* and *Klebsiella pneumoniae* isolates were 18 (42.9%), 6 (60%) and 3 (50%), respectively (Table 6).

Table 6: Multidrug resistance profiles of bacterial isolates from children under the age of five with tonsilitis at Hargeisa Group of Hospital

Bacterial species	**R0**	**R1**	**R2**	**R3**	**R4**	**R5**	**R6**	**Over all MDR**
	N (%)	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N (%)**	**N(%)**	**N (%)**
S. pyogenes (78)	0	16(20.5)	21(26.9)	24 (30.3)	14(17.9)	3(3.8)	0	41(52.6)
S. aureus (42)	0	8(19)	16(38)	2 (4.8)	7(16.7)	5(11.9)	4(9.5)	18 (42.9)
S. pneumoniae (10)	0	4(40)		5 (50)	1(10)	0	0	6 (60)
K. pneumoniae (6)	0	1(16.7)	2(33.3)	1 (16.7)	2(33.3)	0	0	3 (50)
M. catarrrhalis (4)	0	1(25)	2(50)	1 (25)	0	0	0	1 (25)
P. aeruginosa (3)	0	0	0	2 (66.7)	1(33.3)	0	0	3 (100)
Total (143)	**0**	**30 (20.9)**	**41(28.7)**	**35 (24.5)**	**25(17.5)**	**8 (5.6)**	**4(2.8)**	**72(50.4)**

R0: resistant all classes of antibiotics, R1, R2, R3, R4, R5, R6: resistant to 1, 2, 3, 4, 5, and 6 antibiotic classes MDR: Resistance of an isolate to three or more antibiotics taken from different classes

5.2 Multivariable analysis

On multivariable analysis, bacterial tonsillitis was significantly associated with difficulty of swallowing (AOR= 6.99, CI= 3.56-13.13), weight loss (AOR= 0.33, CI= 0.186-0.597), attending school (AOR= 2.98, CI= 1.64-5.42), history of tonsillitis (AOR= 0.12, CI= 0.06-0.21) and exposure to biofuel (AOR= 0.19, CI = 0.04-0.84). Children who had difficulty of swallowing were 7 times more likely to become culture positive for bacterial tonsillitis, compared to children who did not have difficulty of swallowing. Likewise, participants who attended school were 3 times more likely to had culture confirmed bacterial tonsillitis compared to participants who did not attend school. Children under the age of five with history of tonsillitis are more likely to had bacterial tonsillitis than those without history of tonsillitis. Similarly, participants who had

weight loss and exposure to biofuel were more likely to become culture confirmed tonsillitis compared to those who did not have weight loss and exposure to biofuel (Table 7).

Table7: Multivariable analysis on the risk factors of tonsillitis in children under the age of five with tonsillitis.

Variables	COR (95%CI)	P-value	AOR (95%CI)	P-value
Gender				
Male	0.64 (0.41-1.00)	0.05	0.68 (0.38-1.19)	0.18
Female				
History of tonsillitis				
Yes	0.11 (0.68-0.19)	< 0.001	01.2 (0.06-0.21)	< 0.001
No				
Type of tonsillitis				
Acute				
Chronic	8.37 (4.82-14.53)	< 0.001		
Recurrent	0.87 (0.45-1.67)	0.67	0.45 (0.01-17.2)	0.67
Parental occupation				
Unemployed	1.98 (1.19-3.29)	0.008	1.68 (0.89-3.18)	0.11
Employed				
Contact with cough				
Yes	1.89 (1.18-3.0)	0.008	0.71 (0.37-1.33)	0.29
No				
Tonsillar structure				
Yes	3.52 (1.93-6.42)	< 0.001	2.1 (0.05-80.7)	0.69
No				
Over crowded				
Yes	0.56 (0.35-0.89)	0.02	0.93 (0.45-1.91)	0.93
No				
Difficulty of swallowing				
Yes	0.32 (0.198-0.53)	< 0.001	6.99 (3.56-13.73)	< 0.001
No				
Weight loss				
Yes	2.96 (1.89-4.64)	< 0.001	0.33 (0.186-0.597)	< 0.001
No				
Attending school				
Yes	0.31 (0.19-0.51)	< 0.001	2.98 (1.64-5.42)	< 0.001
No				
Attending day care center				
Yes	1.54 (0.93-2.53)	0.09	1 (0.44-2.34)	0.97
No				
Wood biofuel				
Yes	7.29 (2.21-24.1)	0.001	0.19 (0.04-0.84)	0.029
No				

6 DISCUSSION

Although tonsillitis in children under the age of five is a major health problem at Hargeisa and can lead to unprecedented consequences including peritonsillar abscess, tonsillar stones, and rheumatic fever if not early detected and treated, the magnitude of bacterial causes of tonsillitis was unknown in Somaliland so far. Therefore, identification and classification of different bacterial pathogens causing tonsillitis is a necessary step to curtail for the treatment of tonsillitis.

The overall prevalence of culture confirmed bacterial causes of tonsillitis in Hargeisa Group of Hospital was 32%. However, due to the lack of previous data in Somaliland, comparison was unable to be made. On the other hand, the magnitude of tonsillitis in this study is higher than similar studies in Jimma, Ethiopia (11.3%) (Tesfaw *et al.,* 2015), Tanzania (20.6%) *(Zephania at al,* 2019), Norway (21.6%) (Kvestad *et all*, 2005), and Bangladesh (19%) (Rameez *et all*, 2014). However, this study result was lower than studies done in the UK (79%) (all, 2016), Trinidad (62.5%) (Maureen *et all*, 2008), India (72%) (Mahanjan *et all*, 2018), Saudi Arabia (65%) (Mohammed *et all*, 2013), Benin (73.97%) (Sadoh *et all*, 2007), Libya (71%) (Ati *et all*, 2018), and Bahir Dar, Ethiopia (51%) (Mulu *et al,* 2018). The variations of reported prevalence of tonsillitis in children under the age of five from continent to continent, and from country to country might be dictated by geographical differences, community living status and practices, host factor and educational level of parents.

In this study, the prevalence of tonsillitis in children under the age of five was higher in males than in females which is similar to studies done in Iraq (Ali *et al*, 2019), India (Bhalla et al P. B., 2019) and Nigeria (Adegbiji *et al*, 2020). The variations on the percentage of tonsillitis between male and females might be due to males spending more time for outside activity than females.

In this study, the percentage of tonsillitis was higher among children lived in urban than rural areas. This was similar with studies done in India (Bhalla *et al* P. B., 2019), and Bahir Dar, Ethiopia (Mulu *et al,* 2018). This variation might be due to frequent encounter with infected people, contact with air pollution from biofuel use, and school or house crowding.

The prevalence of bacterial isolates was highest in study participants which had chronic tonsillitis (57.7%). However, there were no studies showed the prevalence of chronic tonsillitis in comparison to acute and recurrent tonsillitis.

Streptococcus pyogenes was the most frequent bacterial isolate from children under the age of five with tonsillitis in this study and its percentage (55%) is similar with studies in Ohio (58%) (James Fox, 2006), Italy (69%) (Almadori, 1988), and Trinidad (82.2%) (Maureen et all, 2008). However, it is higher than studies done in Egypt (17%) (Bassilli at al, 2002), Iran (20%) (Jasir, 2002), Iraq (29.7%) (Ayoub Bazzaz, 2018), India (22.25%) (Kalaiarasi, 2018), Saudi Arabia (40%) (Abdulwahab, 2002), and Hawassa, Ethiopia (12.2%) (Asrat Anja, 2019).This variation might be influenced by climate changes, age and geographical inhabitation of the study participants.

The percentage of *Staphylococcus aureus* in this study was 29%, which is comparable with a related study in Ethiopia (Mulu et al 2018).However, it was lower than studies done in Brazil (40%) (Veraluce Cavalcantia, 2019), Trinidad (68.9%) (Maureen et all, 2008) and Nigeria (32.1%) (Adegbiji et al, 2020). This variation might be due to persistence of *Staphylococcus aureus* in the tonsillar tissues and antibiotic resistance.

The percentage (7%) of *Streptococcus pneumoniae* isolates from children with tonsillitis in the present study is lower than studies done from Poland (14%) (Artur Niedzielski, 2013), Belgium (21%) (Malfroot, 2004), Rome (4%) (Petrosillo, 2002) and south Ethiopia (62.5%) (Fiseha Wada, 2019). However, it was higher than studies done in US (3.5%) (principil, 1999), Nepal (4%) (Dharm Bhatta, 2018), and Nigeria (3.3%) (Garba et al, 2017).

The percentage (4%), of *Klebsiella pneumoniae* caused tonsillitis in this study is higher than a study done in Brazil (1.4%) (Lima, 2010), but was lower than studies done in Singapore (6.6%) (Loganathan A. U., 2006), Indonesia (7%) (Farida et al, 2012), and Patil Medical College, India (6%) (Mahajan, 2017). In this study the prevalence of *Morraxella catarrrhalis* was 3% which is different from studies done in USA (22%) (Gober, 2006), Brazil (28.5%) (Mirela Prates, 2018), Denmark (53%) (Navine, 2016), Indonesia (38%) (Fadlyana, 2018), Estonia (16%) (Naaher, 2000), Tanzania (90.8%) (Sopio Chochua, 2018), and Bahir Dar, Ethiopia (12.3%) (Mulu et al, 2018).

In this study, the prevalence of *Pseudomonas aeruginosa* was 2% which is similar to study done in Benin, Nigeria (2%) (Babaiwa, 2013), but higher than studies done in Singapore (1.6%) (Loganathan a. a., 2006), Malaysia (1.9%) (Saad Alasil, 2013). However, this was lower than

study done in Berlin (5%) (Uzeyir Yildizoglu, 2014), and Saudi Arabia (2.94%) (Mohammed et all, 2013). The variation of these isolations might be due to age and living status variations of studies participants.

The percentage of MRSA isolation among children under the age of five with tonsillitis was 15.8% in this study. This is higher than studies done in Germany (0.8%) (Zautner et al, 2010), Lahore (5.5%) (Arslaan Javeed, 2016), Japan (0.8%) (Hirakataa et al, 2005), and Gondar, Ethiopia (2.3%) (Tigabu, 2018), but lower than studies done in the USA (16%) (Itzhak, 2006), Brazil (3.3%) (Veraluce Cavalcantia, 2019), Benin (17.95%) (Sadoh et all, 2007)and Uganda (32%) (Kateete et al, 2019). The variation between the studies might be due to geographical variations, age and child contact to hospitalized patients who could have gotten the MRSA from hospitals.

The majority (92%) of bacterial isolations were resistant to ampicillin in this study as reported in Nigeria (100%) (Sadoh et all, 2007; Babaiwa, 2013) and China (53%-65%) (Wang, 2016). The high resistance of ampicillin by all of the bacterial isolates might be due to production of beta lactamase enzyme as well as abuse and excessive use of cheap drugs, which can be afforded and administered without a physician's guidance.

The percentage of *Streptococcus pyogenes* resistant to gentamicin (42%) and ofloxacin (43%) in the present study was comparable to studies done in Iran (32.2%) (Shirin Sayyahfar, 2015), Nigeria, Benin (40%) (Babaiwa, 2013). The resistance of *Streptococcus pyogenes* to the above drugs might be due to the enzymatic inactivation mediated by aminoglycoside-modifying enzymes (AMEs), and point mutations in the quinolones resistance-determining region (QRDR).

The resistance of *Staphylococcus aureus* to ciprofloxacin(38%) in this study, was lower than studies done in Egypt (90.9%) (Nada ElSayed, 2018) and Nepal (100%) (Acharya, 2010), but was higher than studies done in Brazil (24.6%) (Veraluce Cavalcantia, 2019) and Benin, Nigeria (19%) (Babaiwa, 2013). This variation might be due to modification at the drug binding site in rRNA.

Streptococcus pneumoniae was resistant to erythromycin (30%) and gentamicin (60%) which is similar to a study done in Kualampur, Malaysia, erythromycin (30%) and gentamicin (34%),but different from studies done in China (56%, 20%) (Li-min Wang, 2016), Lithuania (78.8%)

(Stacevičienė et al, 2016), and Vilnius (90.4%) (Petraitienė et al, 2009) and Hawassa referral hospital, south Ethiopia (12.4%) (Deresse Daka, 2011).

The percentage of MDR *Streptococcus pneumoniae* was 60% in this study, was higher than studies done in Poland (52.9%) (Artur Niedzielsk i, 2013), Sokoto, Nigeria (27.3%) (Garba, 2017), Lithuania (12.5%) (Stacevičienė et al, 2016) and Vietnam (35%) (Hoa, 2010). The prevalence of MDR of *Pseudomonas aeruginosa* in this study was (100%), which is similar to study in Brazil (100%) (Lima, 2010), however this study was higher than studies done in Israel (22%) (Valerie Aloush, 2006), Spain (70%) (Astrid Pe´rez, 2019) and Nigeria (19%) (Olayinka, 2004).These high proportions of MDR among the isolates might be due to productions of beta-lactamase enzyme by *Pseudomonas aeruginosa* and production of Penicillin binding proteins in *Streptococcus pneumoniae*.

In the present study, difficulty of swallowing is one of the predictor variables for tonsillitis in children under the age of five. Similar studies done in India (Bhalla et al P. B., 2019), and Lithuania (Rūta Pribuišienė a, 2015) also reported this. History of tonsillitis was also a predictor variable in this study which was similar to studies done in China (Xueren ouyang, 2017), Jimma, Ethiopia (Tesfaw et al, 2016), and Yemen (Ba-Saddik, 2014). Weight loss was also another predictor variable for tonsillitis in this study which was similar to studies done in Iran (A.A. Kargoshaie, 2009), and Germany (T.Gehrke, 2019). Furthermore, attending school was a risk factor for tonsillitis in this study which is similar to studies done in Wakiso district Uganda (Irene Nayiga, 2017), and Australia. (Andrew C., 2009). This might be due to overcrowding during schooling among children where carrier children can easily interact with healthy children.

6.1 Limitations of the study

This study provided a data on the profile of bacterial isolates and its resistance from children under the age of five with tonsillitis at Hargeisa Group of Hospital. However, the study has limitation to identifying nonbacterial causes of tonsillitis.

6.2 Conclusions and recommendations

This study documented high prevalence of bacterial tonsillitis in Children under the age of five. *Staphylococcus pyogenes* followed by *Staphylococcus aureus* and *Streptococcus pneumoniae* were the most frequent causative agents. High proportion of tonsillitis with MRSA was found. Most the bacterial isolates were resistant to ampicillin. Difficulty in swallowing, history of tonsillitis, weight loss and attending schools were found to be factors associated with tonsillitis. Therefore, school area classes setting need to be re-evaluated and rational usage of antibiotics is needed to be carried out to significantly control the magnitude and the spread of tonsillitis among children. Further investigation is needed to be carried out throughout the country to draw a picture about the magnitude and geographical variance of tonsillitis in under-five children, and to identify nonbacterial pathogens causing tonsillitis in children under the age of five.

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ANNEXES

Annexes1:

Name of Researching Institution：Bahir Dar University

Title of the project: Bacterial etiologies, antimicrobial resistance profiles and associated factors among children under the age of five with tonsillitis at Hargeisa Group of Hospital, Somaliland.

Participant's Name: _____ Age: ____Address:_____

Study ID: _____

I understand that this informed consent is prepared for a study which aims to determine the prevalence of MARSA and other respiratory pathogens among under-five children with tonsillitis attending Hargeisa Group of Hospital Hargeisa, Somaliland.

My participation is based on volunteer and I can withdraw without any prerequisite at any time. I have been told that all the information will be registered on questionnaire format, and kept confidential. I can ask questions and will get brief explanation in the language of my preference. I agree to participate and give necessary information and examination. I agree to be interviewed about the study. Moreover I would like to confirm my agreement by signing.

Signature _____

Agreed to participate in the study: Yes ☐ No ☐

Date_____

1. Name of the principal investigator_____

Signature _____Date_____

Thank you for your participation!!

CONSENT FORM

Participant Information Sheet (English version)

Title of the project: "Bacterial etiologic agents, antimicrobial resistance profiles and associated factors among children under the age of five with tonsillitis at Hargeisa Group of Hospital"

Principal Investigator: Hamda Hussein

Advisor: Wondemagegn Mulu (Associate Professor) and Addisu Melese (Assistant Professor)

Department: Medical Microbiology Unit, Department of Medical Laboratory science, College of Medicine and Health Sciences, Bahir Dar University.

General information- First, I would like thank you for your willingness for your child to participate in this study, and I would appreciate you to listen every question very carefully and ask me any time if anything is unclear.

Introduction:

My name is Hamda Hussein and I am MSc student in Medical Microbiology at Bahir Dar University College of Medicine and Health sciences, school of Health science, Department of medical laboratory science, unit of Medical Microbiology, Bahir dar, Ethiopia. I am doing a research on "Bacterial etiologic agents and their antimcirobial resistance profiles among under five children with tonsillitis at Hargeisa Group of Hospital"

Purpose of the study:

The purpose of this study is to determine prevalence of Bacterial etiologic agents and their antimicrobial resistance and associated risk factors among throat swabs collected from clients at HGH. In order to design treatment and preventive strategies, the explanation of the prevalence, antimicrobial resistance and associated risk factors of this common bacteria is crucial; particularly since its prevalence in the study area is still remain poorly understood, therefore this study will assess antimicrobial resistance profile, and Bacterial etiology of tonsillitis.

Procedure and Participation: For this study to be successful we need your child's participation. And I am asking you to allow for your child to participate voluntarily in this study. If you are okay with your child to voluntary participate in this study, you are expected to understand and

sign the informed consent. Then Socio demographic and information related to the risk factors for acquisition on etiological and antimicrobial resistance of bacterial tonsillitis will be filled on the questionnaire. Sample will be collected for laboratory analysis. Throat swab will be collected by sample collectors.

Confidentiality: All personal information you give about you and your child and data obtained from laboratory analysis will be kept confidential.

Expected benefits: your child's participation in this study will benefit for the region and the nation as a whole. If there is any positive finding in laboratory examination the result will be reported to your child's pediatrician to aid appropriate treatment and management as a usual law of the hospital.

Risks: there is no any risk for participating in this study except that you and your child will spend a maximum of 30 minutes for interview and you will allow for your child a throat swab to be taken for laboratory analysis.

Incentives: there are no special incentives that your child will be given for participating in this research.

Results Dissemination:

There will be a report which is written about the finding of the study, either through publication or any other means. The result will not bear any information relevant to your child's personality or yours in anyway.

Freedom to withdraw: You have the right to withdraw your child and leave the study.

Person to Contact:

If you have question or problem related with the present study, you can contact the principal investigator at any time using the following address:

Principal Investigator:

Ms. Hamda Hussein
(Candidate of MSc, Medical Microbiology Unit, Medical Laboratory Science Department, College of Medicine and Health Sciences, Bahir Dar University)
Cell phone: +252634155897
E-mail: daaroodhamda@gmail.com

If you want more information and check about this project you can contact the following people

Bahir Dar University, College of Medicine and Health Science,

IRB Office.

Advisor's Name and Address: WondmagegnMulu (Associate Professor)

Medical Microbiology Unit, Medical Laboratory Science Department,

College of Medicine and Health Sciences, Bahir Dar University

Mobile: 251918706921

Email: wondem_32@yahoo.com

SIGNITURE——————————————————————

Warqada warbixinta ka qaybgalaha (Somali version)

Ciwaanka cilmibaadhista: "Bacterial etiologies, antimicrobial resistance profiles and associated factors among children under the age of five with tonsillitis at Hargeisa Group of Hospital, Somaliland"

Cilmi baadhaha : Xamda Xuseen

Kor meeraha cilmi baadhaha : wondemagegn Mulu

Qaybta :Medical Microbiology Unit, Department of Medical Laboratory science, College of Health Sciences, Bahir Dar University

War bixin guud: ugu horayn waxan kuugu mahad celinayaa sida aad u o golaatay in ilmahaagu ka qayb qaato cilmibaadhistan. Waxaaan si xushmad leh kaaga codsanayaa in aad si taxadir leh ugu fiirsato su'aalaha iskuna daydo in aad fahanto wixii su'aal aad fahmi waydo ee madmadow kujiro inaad I weydiiso.

War bixin:magacaygu waa Xamda Xuseen, waxan ahay ardayad dhigata waxbarashada heerka labaad ee caafimaadka jaamacada Bahir dar ee dalka Etoobiya. Qaybteeda sayniska caafimaadka waaxda shay baadhka(microbiology). Waxan samaynayaa cilmibaadhis ku saabsan barashada bakteeriyooyinka iyo u adkaysigooda dawooyinka qaar ee cudurka xoqado xanuunka ku dhaca caruurta shanta sano ka yar.

U jeedada cilmi baadhistan: waxa weeye inaan ogaano heerka xanuunka, bakteeriyada keenta iyo nooca iyo heerka dawooyinka ay u adkaysan karaan.

Habka iyo kaqaybgelidda cilmibaadhistan: si ay cilmibaadhistu u hirgasho waxaan kaa codsanayaa in aad ilmahaaga u ogolaato inuu ka qaybqaato cilmibaadhista. Haddii aad ogoshahay fadlan faham oo saxeex heshiiskan.Intaa ka dib qodobada la xidhiidha iyo heerka u adkaysiga dawada waxa laagaga buuxin doonaa kuwshineerka.Muunad ilmaha xoqadiihisa laga qaado ayaa loo gudbin doonaa qaybta shaybaadhka dhakhtarka.

Ku kalsoonaanta cilmibaadhistan: war bixinta shakhsiyeed ee adiga iyo ilmahaaga cid dhaafsan cilmibaadhaha iyo shaqaalaha cilmibaadhistu ma arki doonaan.

Maxsuulka cilmibaadhista: ka qaybgalka ilmahaagu wuxuu faa'iido u yahay dhamaan waddanka oo dhan. Wixii jawaab togan ka soo baxa waxa loo istimaali doonaa in si hagar la'aana ilmahaaga loogu daweeyo.

Halista cilmibaadhista: haba yaraatee wax halis ah oo adiga iyo ilmahaaga soogaadhayaa ma jiraan aan ka ahayn 30 daqiiqoood su'aalaha ka jawaabaysaan, iyo muunada xoqadaha ilmaha laga qaadi doono.

Dhiirrigelin: ma jiraan wax dhiirrigelin dheeraada oo ilmahaagu ka heli doono cilmibaadhistan.

Qaybinta maxsuulka cilmibaadhista:waxaa lagu soo ban dhigi doonaa kulamada caafimadwaxana lagu baahin doonaa baraha internet acaafimaadka. Haseyeeshee, wax macluumaad gaara oo adiga iyo ilmahaaga laydinka baahinayaa ma jiraan.

Xorriyad: waxaad xor u tahay in aad xilliga aad doonta ka bixi karto heshiiskan.

Wixii faahfaahina kala xidhiidh:

Xamda Xuseen

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Hadii aad u baahan tahay inaad war bixin dheeraad ah hesho kala xidhiidh shakhsiyaadkan

Bahir Dar University, College of Medicine and Health Science, IRB Office.

Magaca iyo ciwaanka kormeeraha cilmibaadhaha : WondmagegnMulu (Associate Professor)

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College of Medicine and Health Sciences, Bahir Dar University

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SAXEEXA _____

Annexes 2

Questionnaire for the study of Bacterial etiologies, antimicrobial resistance profiles and associated factors among children under the age of five with tonsillitis at Hargeisa Group of Hospital, Somaliland.

Code number: ----------			
I: Socio-demographic characteristics			
S. No.	Questions	Alternative choice for Responses	Remarks
1.	Age	_____ Years	
2.	Mother's age	_____ Years	
3.	Father's age	_____ Years	
4.	Gender	1. Male 2. Female	
5.	Residence	1. Rural 2. Urban	
6.	Maternal education	1. Not able to read and write 2. Read and write 3. Primary school 4. High school 5. Higher education	
7.	Father's education	1. Not able to read and write 2. Read and write 3. Primary school 4. High school 5. Higher education	
8.	Parental occupation	1. Un employed 3. Employed 4. Student	
9.	Monthly income in Dollar	1. $50 2. %50-$100	

		3. $100-$200 4. $200-$300 5. >$300	
II. Tonsillitis related variables			
1.	History of tonsillitis	1. Yes 2. No	
2.	If yes, how many times it occurs for the last one year	_____ Times	
3.	Current type of tonsillitis	1. Acute 2. Chronic 3. Recurrent	
4.	Number of previous tonsils infection	1. One time 2. Two times 3. Three times 4. Four times 5. More than four times	
5.	Temperature	1. 37 ^{o}C 2. 38 ^{o}C 3. > 38 ^{o}C	
6.	Sore throat	1. Yes 2. No	
7.	Swollen tonsils	1. Yes 2. No	
8. 9.	Headache	1. Yes 2. No	
10.	Swollen lymph nodes (adenopathy)	1. Yes 2. No	
11.	Difficulty in swallowing	1. Yes 2. No	
12.	White exudates on the throat	1. Yes 2. No	
13.	Weight loss	1. Yes 2. No	
14.	Tonsillar structural change	1. Yes 2. No	
15.	History of drug use	1. Yes 2. No	

III. Other explanatory variables			
1.	HIV status of the child	1. HIV positive 2. HIV negative	
2.	Contact with someone who had cough	1. Yes 2. No	
3.	Breast feeding	1. Mixed 2. Exclusive	
4.	Is the child currently attending daycare center	1. Yes 2. No	
5.	Is the child currently attending school	1. Does not go to school 2. Goes to school	
6.	Over crowding	1. Not over crowded 2. Over crowded	
7.	Use of wood biofuel	1. Yes 2. No	

Code number: ----------			
I: Deegaanka, iyo dhaqandhaqaale			
S. No.	Su'aalaha	Doorojawaabtakuguhaboon	faahfaahin
10.	Da'da	_____ sanno	
11.	D'ada hooyada	~~Sanno~~	
12.	Da'da aabbaha	~~Sanno~~	
13.	Jinsiga ilmaha	1. Lab 2. Dheddig	
2.	Deggenaanshaha	1. Miyiga	

		2. Magaallada	
3.	Heerka aqoonta hooyada	1. Aan waxbaqorin/akhrin 2. Wax akhrida/ qortana 3. Dugsi hoose/dhexe 4. Dugsi sare 5. Aqoon heerka sare	
6.	Heerka aqoonta aabaha	1. Aan waxbaqorin/akhrin 2. Wax akhrida/ qortana 3. Dugsi hoose/dhexe 4. Dugsi sare 5. Aqoon heerkasare	
6.	Shaqada waalidka	1. Aan shaqayn 3. Shaqeeyo 4. Arday ah	
7.	Dhakhliga billa ha ah (doollar)	1. $50 2. %50-$100 3. $100-$200 4. $200-$300 5. >$300	
II. qodobada la xidhiidhaxoqadaha			
16.	Hore xoqaduhu u xanuuneen	3. Haa 4. Maya	
17.	Haddii jawaabtu tahay haa, imisa jeer sannadkii u dameeyay		
18.	Nooca xoqada haee imika	4. Mid cusub 5. Hore u soo jiray 6. Soo noq-noqda	
19.	Inta jeer ee hore xoqahu ugu dhaceen	6. Hal mar 7. Laba jeer 8. Saddex jeer 9. Afar jeer 10. In ka bdan afar jeer	
20.	Heerkulka	4. 37 ºC 5. 38 ºC 6. > 38 ºC	
21.	Cuno xanuun	3. Haa 4. Maya	

22.	Xoqado bararsan	3. Haa 4. Maya	
23. 24.	Madax xanuun	3. Haa 4. Maya	
25.	Qanjidh sarceedyo bararsan (adenopathy)	3. Haa 4. Maya	
26.	Liqista oo ku adag	3. Haa 4. Maya	
27.	Dheecaan ku yaalla xoqadaha	3. Haa 4. Maya	
28.	Miisaanka oo dhinmay	3. Haa 4. Maya	
29.	Is beddel qaab dhismeedk axoqadaha	3. Haa 4. Maya	
30.	Hore u isticmaalay daawo	3. Haa 4. Maya	

III. <u>godobada kale ee la xidhiidha</u>			
8.	Xaalladda HIV eeimaha	3. HIV leh 4. HIV aanlahayn	
9.	U dhawaadayqofqufacaya	3. Haa 4. Maya	
10.	Naas nuujinta	3. Iskujire 4. Saafi	
11.	Ilmuhu ma tagaa meelaha caruurta lagu xanaaneeyo	3. Haa 4. Maya	
12.	Ilmuhu ma tagaa dugsiga	3. Ma tagaan dugsiga 4. Way tagaan dugsiga	
13.	Caruur ka badan inta loogu talogalay	3. In ku filan intii loogu talogalay 4. In ka bada nintii loogu	

		talogalay	
14.	Ma ku isticmaashaa dhuxul guriga	3. Haa 4. Maya	

BAHIR DAR UNIVERSITY

COLLEGE OF MEDICINE AND HEALTH SCIENCES

SCHOOL OF HEALTH SCIENCES

DEPARTMENT OF MEDICAL LABORATORY SCIENCES

This is to certify that the thesis entitled " Bacterial etiologic agents, their antimicrobial resistance profiles and associated factors among children under the age of five with tonsillitis at Hargeisa Group of Hospital" was carried out by Hamda Hussein under supervision of Mr. Wondemagegn Mulu and Mr. Addisu Melese, College of Medicine and Health Sciences, for the award of MSc Degree in Medical Microbiology.

I declare that this is original and has not been presented or published in this previously submitted in part or full for any degree or diploma of this or any other University.

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Place: Bahir Dar Date of submission:_____

This thesis is submitted for examination with my approval as University advisors.

Advisor(s)

1. Wondemagegn Mulu (MSc, Associate Professor)

   Signature _____ Date _____

2. Addisu Melese (MSc Assistant professor)_____ _____

Signature _____ Date _____