ASYMPTOMATIC PHARYNGEAL CARRIAGE RATE OF *Streptococcus pyogenes* AND ITS ANTIMICROBIAL SUSCEPTIBILITY PATTERN AMONG SCHOOL CHILDREN IN HAWASSA TOWN, SOUTH ETHIOPIA

BY

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ABSTRACT

Background: *S.pyogenes or Group A Streptococcus (GAS) causes each year hundreds of thousands mainly morbidity in developing countries. GAS differs from the other pyogenic bacteria in its potential to produce delayed, nonsuppurative sequelae. Asymptomatic pharyngeal carriage rate of GAS is important in transmission of infections. Report on prevalence, related factors, and antimicrobial susceptibility test of GAS among school children in Ethiopia were very limited.*

Objectives*: To determine the asymptomatic pharyngeal carriage rate of S.pyogenes, to assess associated risk factors and to determine its antimicrobial susceptibility pattern among school children in Hawassa town, South Ethiopia*

Methods*: A total of 287 school children aged 5-15years were enrolled in this study during May to October2014. Ethical clearance and informed consents were obtained before data collection. Demographic data were collected by structured questionnaire which was administered by a trained nurse. Throat swabs were collected and inoculated in to Amies transport media. S.pyogenes were identified by colony appearance, Gram stain, Catalase test, 0.04U Bacitracin disks and PYR tests. Antibiotic susceptibility test was performed using disc diffusion method. Results were interpreted as per the recommendations of National Committee for the Clinical laboratory standard institute guideline. Descriptive and Logistic regression modal were used for analysis.*

Results: *Out of 287school children screened,140 (48.8%)were female and 147(51.2%) were male. Overall, S.pyogenes was isolated from 12.2% (35/287)of the children. Higher carriage rate was observed in females 23(16.4%) than males 12(8.2%)($P<0.05$). Low income of family was significantly associated with carriage rate ($p<0.05$).The higher susceptible group were; age 5-8yrs 12(17.1%), those with illiterate parents 19(15.8%), grade one 9(15.8%), with past history of recurrences of URTI 6(14.6%), and family size of more than 8 members 5(15.6%) observed. High level resistance to Tetracycline and low level resistance to Vancomycin was observed, while isolates were sensitive to Penicillin, Amoxacline, Erythromycin, Chloramphenicol, and Ceftriaxone. Resistance against one or more antibiotics was 68.6% (24/35).Out of a total, 29.2% (7/24) showed multiple drug resistances*

Conclusion and Recommendations: *The present study showed that the carriage rates of S.pyognes among school children in Hawassa not different from others few findings in Ethiopia. Although, the further large scale study in the area should be undertaken; we recommend that emphasis has to be given to the female children, improvement of income of family as well as implementation of rational empirical usage of antibiotic therapy in the area should be mandatory.*

Keywords:- *asymptomatic, antibiotic, pharyngeal carriage, S.pyogenes, school children*

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Abbreviations/Acronyms

APSGN	Acute Poststreptococcal Glomerulonephritis
ARF	Acute Rheumatic Fever
ATCC	American Type Culture Collection
BHS	Beta-hemolytic Streptococci
CDC	Centers for Disease Control and Prevention (USA)
CLSI	Clinical Laboratory Standards Institute
GAS	Group A Streptococci
HBP	Heparin Binding Protein
IDSA	Infectious Diseases Society of America
MHA	Muller Hinton Agar
MIC	Minimum Inhibitory Concentration
NGOs	Non Governmental Organizations
PMNs	Polymorphonuclic cells
PYR	Pyrrolidonylarylamidase
RHD	Rheumatic Heart Disease
SD	Standard deviation
SLO	Streptolysin O
SLS	Streptolysin S
SNNPR	Southern Nation Nationalities and People's Region
SOPs	Standard Operational Procedures
STSS	Streptococcal Toxic Shock Syndrome
URTI	Upper Respiratory Tract Infections
W H O	World Health Organization

Operational Definitions of the terms

Asymptomatic Pharyngeal Carriage defined as children who harbor *Streptococcus pyogenes* on their oropharynax without any sign and symptoms(1) .

School age children defined as children whose age lay in between 5 and 15yrs, and attending in primary schools during study period.

Recurrent means if the child had three or more GAS sore throats within the last twelve months.

Multiple drug resistances means GAS isolated strain is resistant to two or more antibiotics.

High level resistant means the isolated GAS strain/s is/are fifty percent or more resistant to the antimicrobial agent/s

Low level resistant means the isolated GAS strain/s is/are at most thirty percent resistant to the antimicrobial agent/s

Sequela means a pathological condition resulting from a prior infection

Chapter 1: Introduction

1.1 Back ground

Group A *Streptococcus* is a human-specific pathogen, first characterized by Lancefield, which is associated with a wide spectrum of diseases ranging from uncomplicated infections to severe invasive diseases and debilitating sequelae such as rheumatic fever and acute glomerulonephritis(2).

Infection begins with colonization of the upper respiratory tract or injured skin surfaces. All age group may carry GAS on their throat and epidermis of skin, however, children aged 5-15 years old are a major reservoir of pharyngeal carriage of *S.pyogenes* colonization(3-5).

Asymptomatic pharyngeal carriage of GAS continues to be challenging due to its complex pathogenic mechanisms (6); for instance, it has potential to form intratissue microcolonies and participate in multispecies biofilms in which, high bacterial burden and horizontal gene transfer can occur (7).

GAS colonization is commonly followed by horizontal dissemination of the pathogens usually between individuals with droplets of saliva or nasal secretions during coughing, sneezing, or even conversation (5). The highest transmission and carriage rates have been reported in populations where people live in close contact with one another such as schools, and families (8).

Socioeconomic, environmental and genetic factors such as poverty, under-nutrition, overcrowding, early socialization, and poor housing, lack of awareness of disease transmission ways in the community are some of determinants of the pharyngeal carriage of GAS colonization (1, 9-11).

Indeed, antibiotic treatment should not be done for the GAS carriers (12). Although, there are unique situations in which eradication may be desirable, including outbreak of RF and GAS pharyngitis in a community such as schools ; in the presence of a family

or personal history of acute rheumatic fever; in a family with excessive GAS infections(12).

GAS has not been developed resistance to any of the Penicillins over the last decades. Nowadays, antimicrobial drug resistant strains are starting to appear among asymptomatic children (13). Most frightening would be resistance to the older, most effective and less expensive drugs such as Penicillin (14). Moreover, the treatment failure to Penicillin account as many as 30% in temperate climate when children frequently retain streptococci in their tonsils (15,16). The periodic surveillance and appropriate antibiotics treatment information in the local area with regard to prevention and controlling strategies against the sequela of bacterium is very crucial.

1.2. Statement of the problem

S.pyogenes has been estimated to be among the ten most common causes of death due to individual pathogens globally. It causes more long-term morbidity than most of pathogens like Tuberculosis(TB),Human Immunodeficiency virus (HIV), and Malaria. RF and RHD are major medical and public health problems among the young worldwide (17).

Globally, about 15 million people were affected by RHD, with approximately 492,000 deaths annually. Among these,468,000 deaths occurring in less developed countries. Incidence of RHD in Africa is estimated at 17-43% of all cardiovascular disease. It is also posing economic problem by costing the precious productive years of many adolescents and young adults (17,18).

Due to periodic and good surveillance systems, clinical information availability, as well as improved socio-economic conditions, the prevalence of rheumatic fever related with GAS decreased dramatically in developed countries (19).

In Africa, roughly 2.4 million school aged children are severely affected with RHD. One million of them live in sub-Saharan Africa. An estimates based on echocardiography suggest that the actual prevalence of RHD is 3–10 times higher than previous judgment (17,20).

In Ethiopia, it is common complication in children as about 50-64 % of all cases of morbidity due to heart diseases is *S.pyogenes*(rheumatognic) origin(21) and the overall mortality rate in patients with RHD is 125·3 per 1000 person-years, and the mean age during death was 22·0 years(22, 23).

The epidemiology of rheumatic fever is identical to that of repeated GAS upper respiratory infection(17); subsequently without intervention it may progress to rheumatic heart disease(24). The detection rate of S.pyogenes from throat culture of patients with URTI or strep sore throat is similar with healthy or asymptomatic carriers (25).

Carriers can be prevented from spreading of GAS infections through screening and adequate knowledge of appropriate antibiotics treatment. This would ultimately reduce the incidence of life- threatening post-infectious sequelae which are debilitating and difficult to treat (26). The findings of the present study would contribute to devise measures of RF/RHD prevention.

Since antibiotics are the most commonly prescribed drugs in health facilities and their irrational use is one of the important factors for the development and spread of resistances an audit of the susceptibility of *S.pyogenes* to antibiotic resistant patterns is important for implementation of rational empirical antibiotic usage strategy in the study area. Thus, the present study is designed in a way to investigate the pharyngeal carriage rate, its related risk factors and antimicrobial susceptibility pattern of *S.pyogenes* among school children in Hawassa town, South Ethiopia.

Chapter 2: Literature review

2.1. General description of *Streptococcus pyogenes*

2.1.1. Microbiology of *Streptococcus pyogenes*

Streptococcus pyogenes or Lancefield group A *Streptococcus* (GAS) is the most pathogenic species in the genus. GAS is gram positive, spherical or ovoid shape cells, 0.6-1.0 μm in diameter. It is non-motile and normally arranged in pairs or straight chains(27). It is a facultative anaerobic, catalase and oxidase negative, nutritionally fastidious and cultivated in complex media, often supplemented with blood or serum(28). When cultured on blood agar plates, GAS appear as white to grey colonies 1-2 mm in diameter surrounded by zones of complete (beta)- hemolysis (28).

2.1.2 Virulence factors

GAS elaborates a number of cell surface components and extracellular products that are important in the pathogenesis of infection and in the immune response of the human host(29)as illustrated in figure 1. GAS employs four main mechanisms; antiphagocytosis, adherence, colonization and toxin production (see figure 2.) One of the primary human defense mechanisms involves phagocytosis, destruction and clearance of foreign invasion by polymorphonuclear leucocytes (29, 30).

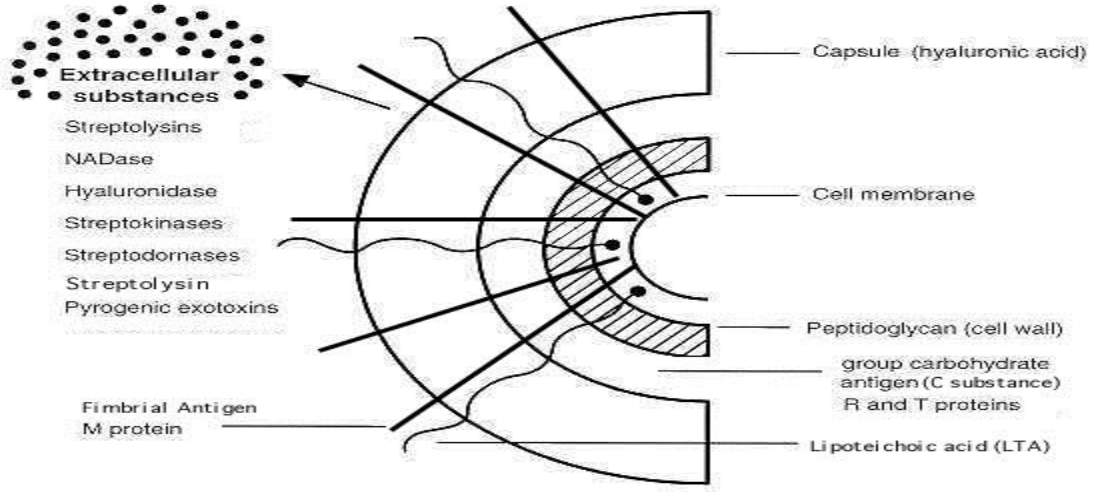


Figure 1 Schematic representation *S.pyogenes* virulence factors as adopted from Todar's Online Textbook of Bacteriology (31).

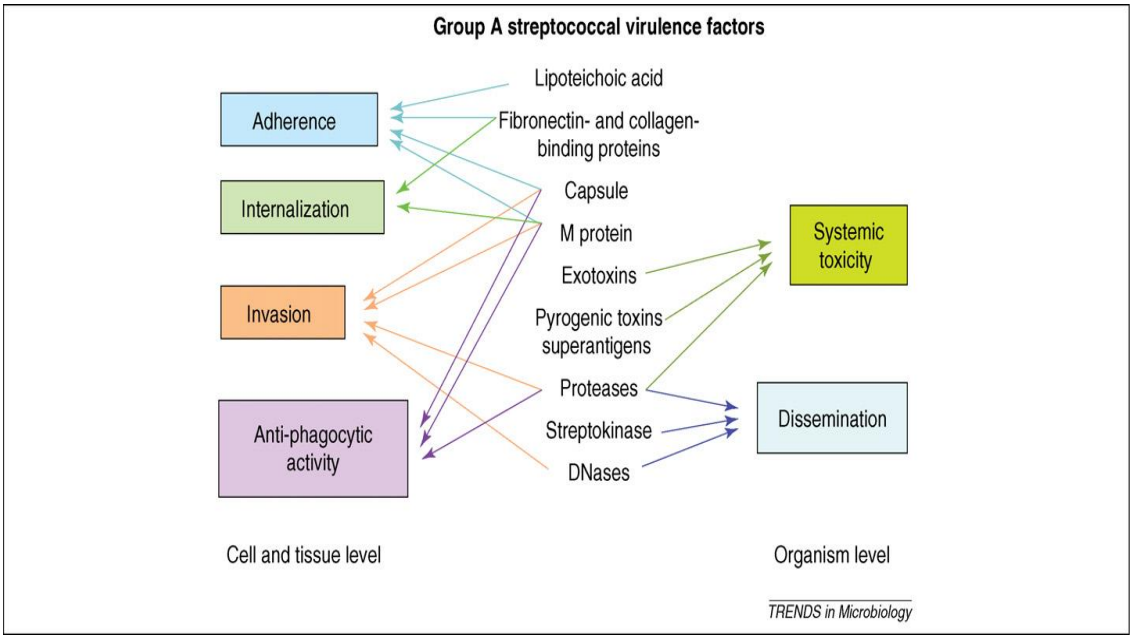


Figure 2. GAS virulence factors at different level of interactions in the host. At the cell and tissue level, these factors contribute to the pathogenicity of GAS by mediating adherence to host cells, by promoting internalization and invasion, and by evading phagocytosis. At the organism level, these virulence factors are involved in the dissemination throughout the host and can induce systemic toxicity. Note, many of these virulence factors are function at several stages during infection processes as adopted from Tart AH et al., 2007 (32).

2.1.3. Group A *Streptococcus* (GAS) pathogenesis

Streptococcal adhesion, invasion, intracellular trafficking, dissemination and persistence in eukaryotic cells have a variety of implications in the infection pathogenesis (32). A mechanism that enables GAS to persist in saliva at relatively high viable cell numbers for prolonged periods of time has been evolved. The relatively high density achieved in saliva by certain serotypes enhances their likelihood of being transmitted to a new host (2,33).

After causing superficial infections, GAS is exposed to plasma at sites of inflammation, which is mediated by the interaction of M protein and fibrinogen with the β_2-integrin adhesion molecule on the surface of neutrophils (PMNs). This complex induces vascular leakage (34). Many plasma protein-GAS protein interactions interfere with the proper function of host defenses (2, 35).

The precise mechanisms of GAS induced ARF are still not understood, but it is generally accepted that M proteins of the bacterium share structural homology with cardiac myosin, tropomyosin, keratin and laminin. The humoral and cell mediated immune response against streptococcal antigens, and antibodies are generated during cross-reacting with human tissue proteins and eventually evoke autoimmune reaction(36).

The anti-phagocytic mechanism of M protein of GAS has remained unclear, due to the extensive sequence variability in the HVR has made it difficult to understand how this region could have the specific function to prevent phagocytosis(37).

Asymptomatic carriage of GAS; in principle, could arise as a consequence of (i) mutations in the pathogen that down-regulate virulence, (ii) a productive immune response by the host that constrains pathogen proliferation, (iii) internalization in to host cells, or (iv) some combination thereof (32, 37).

2.2. Epidemiology of *Streptococcus pyogenes*

The ecological niche of *S.pyogenes* is quite narrow; their only known hosts are the humans. The primary sites for colonization are the nasal and oropharyngeal mucosal epithelium and the superficial layers of the epidermis(38). Asymptomatic throat carriage of GAS varies by country, age group, type and study setting. Carriage is a highly dynamic process, so that it can be carried for a period of weeks or months (10).

2.2.1. Asymptomatic pharyngeal carriage rate of *Streptococcus pyogenes*

GAS is highly communicable and can cause disease in individuals of all ages. School-age children (5-15 years) are considered as the major reservoir of GAS, with a prevalence of 2.5-25% or more depending on the study setting (39). A study conducted in Pennsylvania, reported that carrier rate of GAS among school going children were 15.5%(10). Similar study in New York among well children the prevalence of *S.pyogenes* carriers was 2.5%(40).

Higher rates found in a prospective study from Turkey were 26% GAS carriers (41). A study were reveled among healthy children attending in three different day care

units in Kayseri, Turkey the prevalence of *S.pyogenes* were 8%(42). In Pleven, Bulgaria the carriage rate of GAS among preschool children were 16.20%(8).

In Aboriginal, Australia the prevalence of throat carriage of GAS among children were 5.6%(43). In Coimbatore, South India the reported carriage rate were 5.09(44). In Chennai, India from overcrowded government or charity- aided schools children harbored by GAS were 8.4% (1), while in a rural community in Northern India, 1.3 %(45). The prevalence of asymptomatic pharyngeal carriage of *S.pyogenes* from 3 schools in Nepal were 14.5% detected (46).

S.pyogenes carriage rate from few African countries reported. In Tunisia 9.0% (47), in Nigeria 10 % (48) and in Pemba (Zair or D.R.C) 8.6% among school children were observed(49).
In Ethiopia, the asymptomatic carriage rate of GAS among healthy school children were 9.7% (50). Another similar previous study reported that the carrier rate of GAS were 16.9 %(21).

2.2.2 Factor associated with asymptomatic pharyngeal carriage rate of *S.pyogenes*

Several factors affecting the asymptomatic pharyngeal carriage of *S.pyogenes*, including host and environmental factors have been identified as potential role in the severity and outcome of infection(40).

Age is one of an important host factor and certain age groups (5 to 15 years old) are at a higher risk for the asymptomatic throat carriage of GAS. Although, within this age group the carriage rate were vary, it was observed that higher carriage rate in New York in between 5-8 years old (40), in Aboriginal, Australia10-14 years (43),in Chitradurga 6-10 years(51),and in Mangalore >14yrs were seen among school age children(26). A cross sectional study conducted in Nepal from different elementary schools, the age group 8-10 years were the most susceptible group for throat carriage (46).

Sex is another host factor for asymptomatic pharyngeal carriage of GAS (5). Study conducted in Combatore city, South India female children were more harbor 71% GAS than male children 10.2% on their throats (44). Another study conducted in Ankara, Turkey showed that girls were more GAS carriage than boys(52). Similarly,

in Bharatpur, Nepal, female children were more *S.pyogenes* carrier than male(46, 53). It was prevalent in male than female in Iraqi (54), and in Mangalore (26).

Another possible risk factor reported in Kerman, Iran that carrier students mostly associated with illiterate, retired, jobless or dead fathers and whose mothers also were house-wives(14). In Iraqi most carrier students were associated with low socioeconomic state of their parents, while carriage of GAS were less in children's with high socioeconomic level of their parents and parent with low educational level was highly associated when compared with parent with high educational level(54). Similar study conducted in Mangalore, India the carrier rate of GAS among children was associated with low socio-economic (26, 55).

Children living in families with more members in house were higher carriage rate of GAS ($P<0.05$) than children living in families with less members in house (3, 49, 56).

Low-level awareness of the disease transmission in the community, water scarcity(1, 55) not having separate kitchen facilities and from there being a smoker in the family(14), residence or geographical location(51, 54) shortage of accesses and misuse of antibiotic(46, 57) are factors affecting GAS carriage.

In family someone exposed to have a primary case of GAS pharyngitis for a long period of time, the risk for secondary infection was 1.8 times greater than that of primary infection (58) this seems that pharyngeal carriage of *S.pyogenes* associated with previous history of pharyngitis. Another prospective study conducted in New York, in *S.pyogenes* carriers were observed following antibiotic treatment ($p<.003$) (40, 58).

S.pyogenes occurrence is cyclical in the community with acute rheumatic fever(59). It was supported by others findings indicated that patients with RF/RHD remain infected for weeks after symptomatic resolution of pharyngitis and may serve as a reservoir for infecting others(60).

2.3. Diseases caused by *Streptococcus pyogenes*

GAS has long been recognized as a human pathogen causing an exceptionally broad range of infections. These infections cause significant morbidity and mortality Worldwide. All diseases are most common in settings of poverty, where living conditions promote transmission of the organism, and prevention and treatment programs are less likely to be present or effective (61).

The most common superficial infections caused by GAS are URTI, including acute tonsillitis ("strep-throat") or pharyngitis, a wide variety of skin and soft-tissue infections, meningitis, peritonitis, pneumonia, septic arthritis, and puerperal sepsis are caused by *S.pyogenes* (62).

The non-suppurative complications of *S.pyogenes* followed by an inappropriate immunologically-mediated response and tissue-specific damage include acute rheumatic fever (ARF) and acute glomerulonephritis(AGN)(17, 61).

2.3.1 Pharyngitis

Pharyngitis is the most common clinical manifestation of GAS infection. It has certain characteristic epidemiological and clinical features. The infection is primarily a disease of children 5–15 years of age with accounting approximately 15-30% in children, and in temperate climates, it usually occurs in the winter and early spring. Whereas, in tropical regions the peak incidence of GAS pharyngitis were occur in March and April (65).

Patients with GAS pharyngitis commonly present with sore throat, severe pain on swallowing, and fever. Headache, nausea, vomiting, and abdominal pain in children were seen. On examination, patients have tonsillopharyngeal erythema, with or without exudates, and tender, enlarged anterior cervical lymph nodes (lymphadenitis)(39). Other findings may include a beefy, red, swollen uvula; petechiae on the palate; excoriated nares (especially in infants); and a scarlatiniform rash. However, none of these findings is specific for group A beta-hemolytic streptococcal pharyngitis. Conversely, the absence of fever or the presence of clinical features such as conjunctivitis, cough, hoarseness, coryza, anterior stomatitis, discrete ulcerative lesions, viral exanthem, and diarrhea strongly suggest a viral rather than a streptococcal etiology(66).

Laboratory confirmation is essential in making a precise diagnosis. Throat culture should be performed, and rapid antigen tests are important when the culture was negative. Treatment of GAS pharyngitis leads to a more rapid resolution of symptoms and signs, decreases transmission of GAS to other children, and prevents the development of acute rheumatic fever (ARF) (66, 67).

2.3.2. Pyoderma

In general, this term is used most commonly to refer to streptococcal impetigo and ecthyma. The infection commonly presents as a small pimple, which evolves to a purulent lesion covered by a honey-colored crust. Lesions are most commonly found on the arms or legs and at the sites of minor trauma, which are invariably needed for the organism to establish an infection (68).The organism is highly transmissible, so affected children may develop lesions elsewhere on their bodies, and multiple cases within the household or classroom are common(39).

2.3.3. Streptococcal toxic shock syndrome (STSS)

In recent years, STSS has been a concern worldwide. It is a severe illness associated with invasive or noninvasive *Streptococcus pyogenes* infection. The disease may result from an infection at any site but most often in association with infection of a cutaneous lesion. The outstanding features of these infections are their multiorgan involvement suggesting a toxin and rapid invasiveness with spread to the bloodstream and distant organs. The toxic features together with the discovery that almost all the isolates produce one of the SPEs have caused this syndrome to be labeled streptococcal toxic shock syndrome (STSS). In some cases, a traumatic injury without any external breaks has been the focus, suggesting that organisms circulating in the blood of carriers could be the cause. Toxicity and a rapidly progressive clinical course (24 to 48 hr) are characteristic, having a case-fatality rate of greater than 50%. In most instances, patients present with shock-like symptoms and a necrotic lesion or painful abscess. Treatment is usually management of the shock, debridement of the lesion, and antibiotic. In some severe cases, amputation may be necessary (2, 17, 19).

2.3.4. Acute Rheumatic Fever

Rheumatic fever is sequelae of pharyngeal infection/carriage with GAS and a latent period of 2 to 3 weeks occurs when the time of the first signs of GAS infection noted

and the symptoms of ARF appear. The major manifestations are polyarthritis, carditis, chorea, erythema marginatum, and subcutaneous nodules. The minor manifestations are arthralgias, fever, and supportive laboratory finding (60,61).

The treatment of patients with ARF is generally directed toward reducing acute inflammation, decreasing fever and toxicity, controlling cardiac failure, preventing episodes of re current ARF after significant streptococcal upper respiratory tract infections, and preventing rheumatic heart diseases(63).

2.3.5. Acute Glomerulonephritis

Unlike rheumatic fever, AGN definitely occurs after either pharyngeal or skin infection with GAS(21). Acute glomerulonephritis follows a Streptococcus pyogenes infection by about 1 week, resulting in the malfunction of the glomeruli in the kidneys. Strategies for the treatment of poststreptococcal AGN are directed toward management of acute problems. All patients diagnosed with poststreptococcal AGN should be treated with a full 10-day course of Penicillin to eradicate the pathogen. Penicillin-allergic patients can be treated with Erythromycin in doses adequate for treatment of streptococcal pharyngitis(64).

2.4. Laboratory Identification

Culture is the gold standard method for the laboratory diagnosis. If it is properly processed and interpreted about 90-95% sensitive(69). A wide variety of rapid methods have been developed, Some of rapid direct tests are Immunochromatographic(IC), Enzyme immunoassay (EIA), Latex agglutination technique and also Biochemical methods like Catalase tests(27).

The *S.pyogenes* identification starts with the observation of beta-hemolytic colonies, when grown in 5% blood agar plates supplemented with 5% co_2 or candle jar.
Presumptive identification can be made using the Bacitracin susceptibility test(69). Other test for the identification of *S.pyogenes* include Pyrrolidonylarylamidase activity, since only this species among streptococci produces the enzyme (27, 28).

2.5. Treatment

A number of antibiotics have been effective in treating GAS infections. These include Penicillin and its congeners (such as Ampicillin and Amoxicillin)(70). Erythromycin is a suitable alternative for patients allergic to Penicillin. Alternative antibiotics include Clindamycin, Cephalosporins, Carbapenems, Vancomycin and Linezolid(71).

2.5.1 Drug Resistance Mechanisms

GAS isolates for the beta-lactam antibiotics (penicillins and cephalosporins) is not typically necessary due to the universal susceptibility of GAS to these drugs, but there are reports of treatment failure. Penicillin failure mechanisms to eradicate GAS might be co-existence of beta-lactamase - producing bacteria within the tonsillar crypts that inactivate penicillin decreased bactericidal effect of beta-lactam antibiotics, normal oral flora especially the 'viridans group' that usually compete with GAS and prevent colonization(42) Since treatment failure, asymptomatic GAS carriers represent the main reservoir, from which the bacteria are spread in the general population(72).

There are two main acquired macrolide resistance mechanisms in *S.pyogenes,* posttranscriptional target site modification (methylation) and efflux. Target site modification is mediated by erythromycin resistance methylase (*erm*) genes and macrolide efflux, is mediated by *mef*(A) resistance genes(73)

Tetracycline is bind reversibly to the 30S ribosomal subunit, blocking the binding of the tRNA, inhibiting protein synthesis. Resistance to Tetracycline arises upon genetic acquisition of *tet* genes. Four variants of this gene were already reported in *S. pyogenes, tet*(M) and *tet*(O), that encode ribosomal protection proteins and less frequently, *tet*(K) and *tet*(L), encoding efflux pumps(74) (fig. 3)

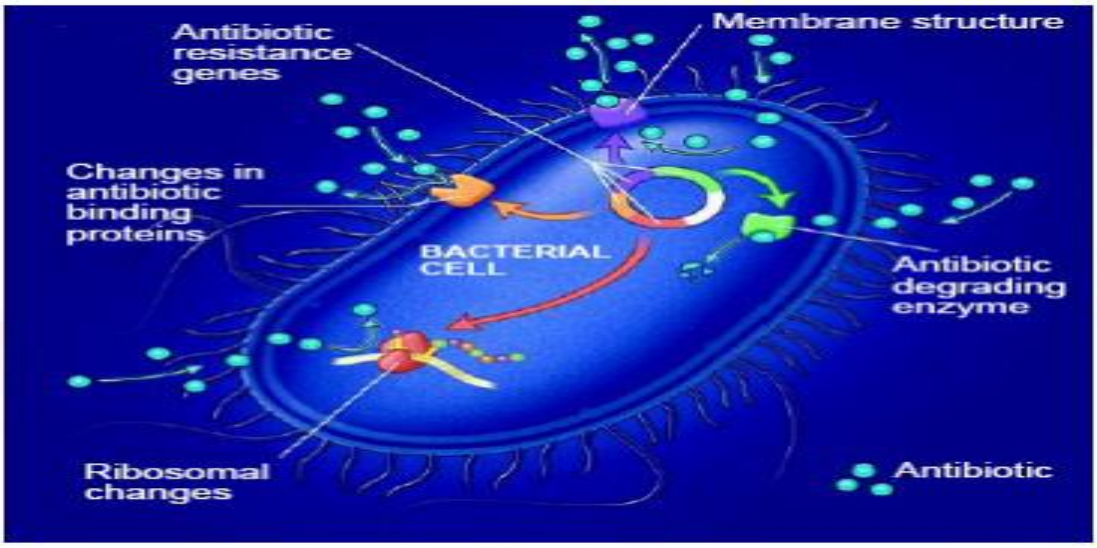


Figure 3. diagrammatic representation of mechanism of antibiotic resistance
As Adapted from: Conly J. et al., 2002

2.5.2. Antimicrobial Resistance pattern

Since antibiotic resistance of bacterial pathogens may vary according to geographic location, and these bacteria can quickly spread to family members, schoolmates; it might cause significant danger and suffering for children due to their common infections(75).

Resistance to Erythromycin in *S.pyogenes* isolated from school children were 48% in Pittsburgh(76). Similar study conducted in Boston area were 7.7%(77),in Santiago, Chile,7.2%(78),in Span 32.8%(79) and among Italian children the isolates of *S.pyogenes* were resistant to Macrolides 38.3%(80).
The antibiotic test were performed in Kerman, Iran on GAS carriers the reported resistant rate to Penicillin were 100%, to Cotrimoxazole 91.2% and to Amoxicillin 87.7% (14). The Indian observation, showed that the resistance rate to Penicillin, Amoxicillin and Erythromycin were 76.4%, 79.1% and 30.4% respectively,while,100% sensitive to Vancomycin (51).

In Kathmandu, Nepal the reported resistance rate to Chloramphenicol 7.8% ,and Erythromycin 5.2% (13). It was found in similar country that the GAS isolates were resistant to Erythromycin 5.8% and Ampicillin7.8% (56). Also, in Pokhara, Nepal the *S.pyogenes* isolate resistant to Erythromycin 15.6% and Tetracycline 6.6% had been reported(81).

In Chennai, India resistance rate of Erythromycin, Tetracycline, Clindamycin, Chloramphenicol and Ciprofloxacin were16.2%,27.4%,6.8%,4.3% and2.5% respectively encountered, while, no resistance was observed to Penicillin, and Amoxycillin(82). In similar country the isolates of *S.pyogenes* strain were resistant to Erythromycin 29.4% (83). In north India the rate of resistance to Erythromycin 10.2%, Tetracycline 24.4%, and Co-trimoxazole 12.2% were reported (84). It was observed in Coimbatore, elsewhere 23.07% to Erythromycin and 15.4% to Amikacin were seen (44). A cross sectional study enrolled in Assam, India reported that the group A streptococcal isolates were resistant to Erythromycin 5.6%, and to Cefotaxime 27.8% seen from throats of school-children(85).

In Benin, Nigeria it was reported that Ampicillin and Cotrimoxazole drugs ineffective to pharygitis treatment(86). In Dakar, Senegal the isolates of *S. pyogenes* were 100% resistant to Tetracycline (87). In Tunisia, a study reveled that the resistant rate to Tetracycline 43.9% and to Erythromycin 4% were observed(88), in Egypt resistance rate to Erythromycin 21.3%, and to Tetracycline were 32%(89) and 100% sensitive to Vancomycin (89). In Pemba among school children who harbor *S.pyogenes* on their throats, the isolates were 83.2% resistant to Tetracycline (49).

In Ethiopia, antimicrobial resistance pattern to the GAS isolated from throat/phyernax were not adequately explicated. However, an earlier study showed that the GAS isolates were 68 % resistance to Tetracycline among school children (21). Before five years ago a study report showed that the resistant rate to Tetracycline 47% and Vanocmycin 7.7% (50) .

2.6. Prevention and Control

In Ethiopia GAS infections and its sequelae might be challenging to control and prevent due to highly diversified nature of the serotypes (90,91). Active surveillance for asymptomatic carriage rate among high risk group(school age children), early treatment with appropriate antibiotics, which will reduce the risk of non-suppurative sequelae (92) and hand hygiene, droplet and contact precautions, proper maintenance of environmental hygiene are some of important measure to take (93).

2.7. Significance of the study:

To the knowledge of the investigators this is the first paper to study asymptomatic pharyngeal carriage of *S.pyogenes*, associated risk factors and its antimicrobial susceptibility pattern in Hawassa town, south Ethiopia. The findings of this study will be used to take intervention to target groups and to deliver information for program planners, policy makers, local decision makers, health care providers, and local Non Governmental Organizations (NGOs) working on primary school age children. Also, it will give insight to drug prescribers, suppliers and venders in implementation of rational empirical use of antibiotic strategy in the study area. It will be base line data to undertake further risk assessment and providing necessary information in designing, monitoring and surveillance programs throughout the country. Over all, it gives clue information for control and prevention activity against non- suppurative sequelae of this organism.

Conceptual frame work

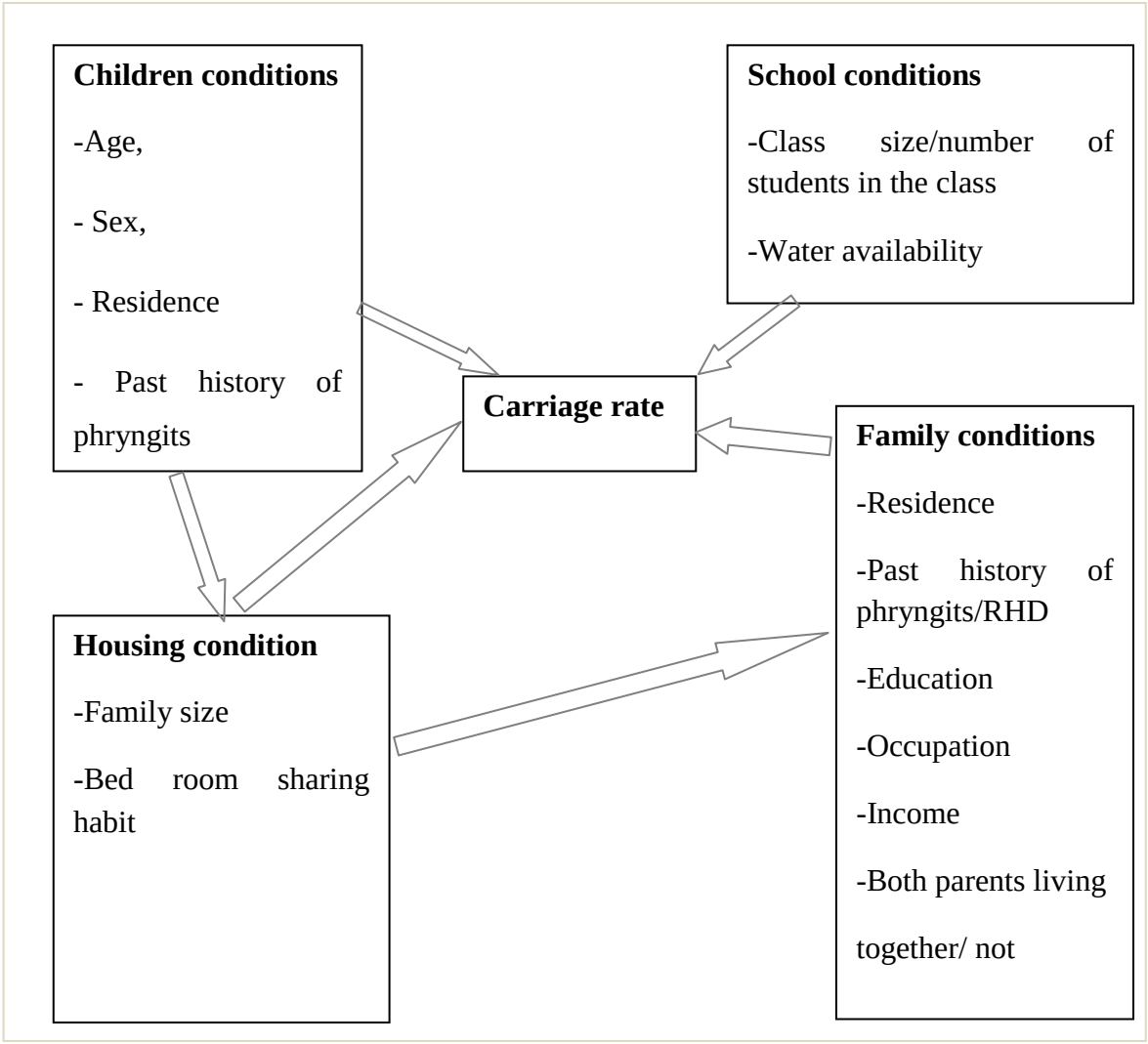


Figure 4. Conceptual frame work for the study of *S.pyogenes* carriage among school children in Hawassa town, South Ethiopia from May-October 2014.

Chapter 3: Objectives

3.1 General Objective

The present study was aimed to determine asymptomatic pharyngeal carriage rate of *S.pyogenes*, to elucidate its associated risk factors and to evaluate its antimicrobial susceptibility pattern among school children in Hawassa, South Ethiopia, 2014.

3.2 Specific Objectives

- To determine the prevalence of *S.pyogenes.*
- To determine the antimicrobial susceptibility pattern of isolated *S.pyogenes*.
- To assess risk factors associated with the carriage rate of *S.pyogenes*.

Chapter 4: Materials and Methods

4.1 Study Area

Hawassa is the capital town of Southern Nation Nationalities and people's Government. It is located 275 kilometers South of Addis Ababa, and has an altitude of 1665 m above sea level with mean annual temperature and rainfall of 20.9^{O}C and 997.6 mm, respectively. The town links Ethio-Kenya international road and based on Central statistical agency (CSA) report in 2014 Hawassa town has about 399,461 total populations. Male and female accounts 199,768 and 199,693 respectively. According to the data collected from Hawassa town Administrative Education Bureau in 2013/2014 academic year, 75,440 children have been registered for elementary education in 108 primary schools. Among a total, in 19 governmental primary schools a total of 38580 students with 17951 males and 20629 females have been registered for grade 1-8.

4.2 Study Design and period

A school based cross-sectional study design was undertaken to conduct the research from May-October 2014 in Hawassa, South Ethiopia.

4.3 Population:

4.3.1 Source population

All school children in Hawassa town during the study period.

4.3.2 Study population

All systematically sampled governmental primary school children who fulfill the inclusion criteria were included in this study.

4.4 Eligibility Criteria

4.4.1 Inclusion Criteria

- All children aged 5-15years, and who were attended the class in selected schools during the study period.
- Those children with parental/guardians consent and assent from themselves were eligible for the study.

4.4.2. Exclusion Criteria

- All children who were taking or had received antibiotics within the last two week
- All children who had streptococcal sore throat such as fever, soreness of throat, cough and watery nasal discharge were excluded as recommended by WHO for pharyngitis.

4.5 Sample size and sampling technique/sampling procedure

4.5.1 Sample size determination

Considering a previous estimated prevalence (p) among healthy Ethiopian school children of 9.7% (50), and single population proportion formula was applied. The estimated margin of error was 5%, and 95% confident interval.

$$n = \frac{(Z\alpha/2)^2 \times P(1-P)}{d^2} = \frac{(1.96)^2 \times 0.097(1-0.097)}{(0.05)^2} = 134$$

n = sample size required.

$Z_{\alpha/2}$=the standard normal distribution at 1-α% confidence level and α=5% (1.96).

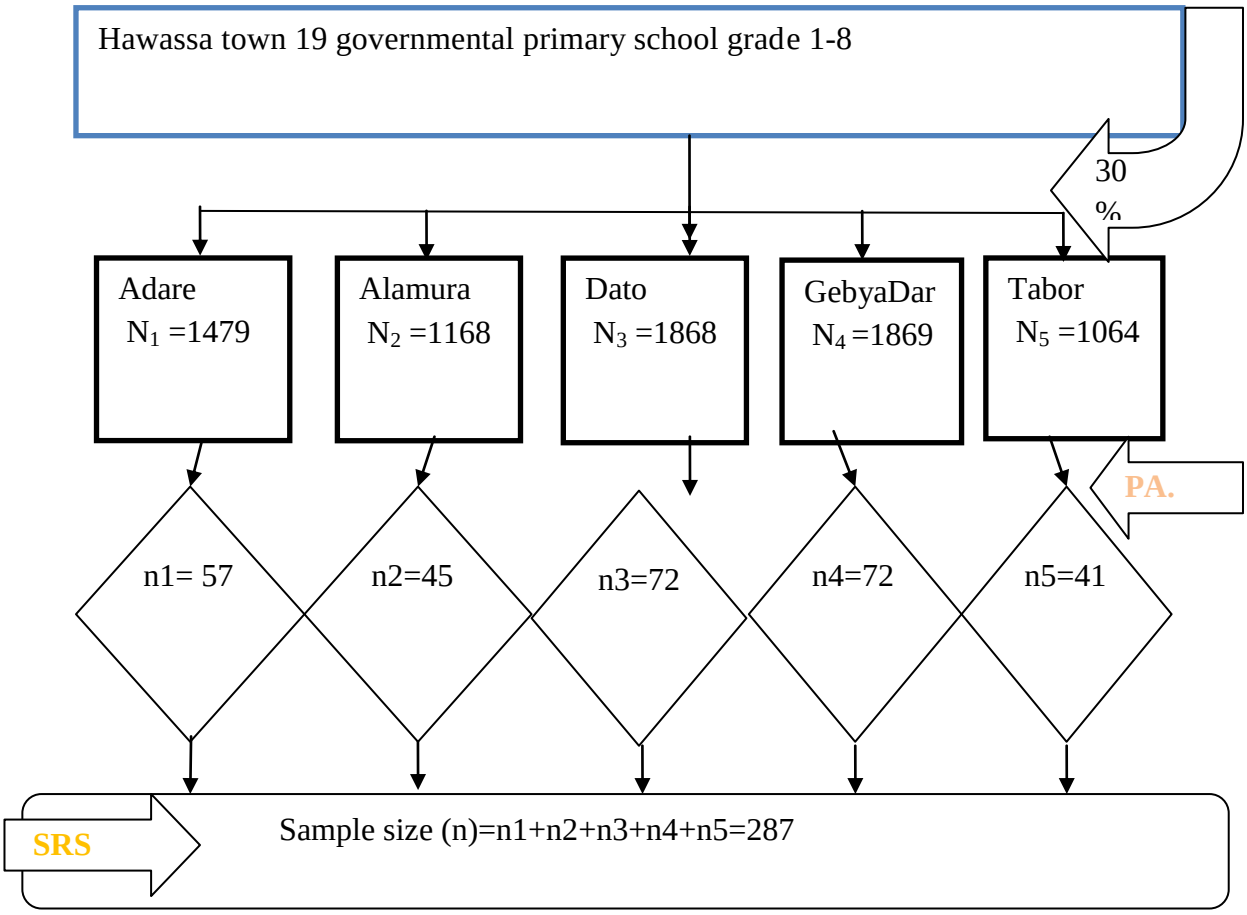
P = prevalence of carriage rate among isolates of similar study which is 9.7%.

d^2 = margin of sampling error tolerated (0.05).

Considering the design effect, n=134x2=268 +Non response rate 10%. A total sample size (n) of 295 school children were proposed to be conducted. But due to not willing to participate, 287 (97.3%) school children were included in our study.

4.5.2 Sampling technique and Sampling procedure

The study participants were selected by using a multi-stage stratified sampling technique. For the consideration of representativeness, from the total of 19 governmental primary schools 26 % (5 of the schools) were included. Simple random sampling technique was used to select the schools. Proportionate amount of samples were assigned to each selected schools. Then study participants were identified by the lists of students using simple random sampling technique. This sampling technique is diagrammatically shown in the following figure.



Note: SRS= Simple random sampling, PA= Proportional allocation

Figure 5: Schematic presentation of sampling technique and procedure for data collection among school children in Hawassa town, South Ethiopia from May-October, 2014.

4.6 Study Variables

4.6.1 Dependent Variables

- *S.pyogenes* carriage rate
- Antimicrobial susceptibility test

4.6.2 Independent Variables

- Age
- Sex
- Residence
- Parents/guardian occupation
- Children with/without parents/guardians
- Person per bed room
- Family size
- Educational status of parents/guardians
- Income of parents/guardians
- Past history of RF/URTIs or recurrences of sore throat
- Schools
- Grade of students

4.7. Data Collection and procedures

Before data collection permission was obtained from SNNPR government Education bureau, Hawassa town Education and school directors. A written informed consent explained to the study participants who fulfill eligibility criteria, freely given written informed consent (Annex II) to parents/guardians or their care takers with legal custody for signature.

4.7.1 Demographic data

Data were collected from each child who had informed and consented parents/guardians and verbal assent from participants; with semi-structured questionnaire which were carried out by qualified BSc nurse. The questionnaire has Demographic and socio-economic factors, and history of sore throat and acute

rheumatic fever (ARF) (annex III). Pretest was carried out in one of the school in which 5% of respondents, and later on not included in the actual study.

4.7.2 Sample collection

For the collection of throat swab the children was asked to tilt the head back and open the mouth wide. Under direct visualization with the good illumination, the swab was rubbed over each tonsillar area with sterile cotton tipped. Care was taken to make sure that the swab does not touch the tongue, lips, cheeks (buccal mucosa), teeth and uvula (see figure below).

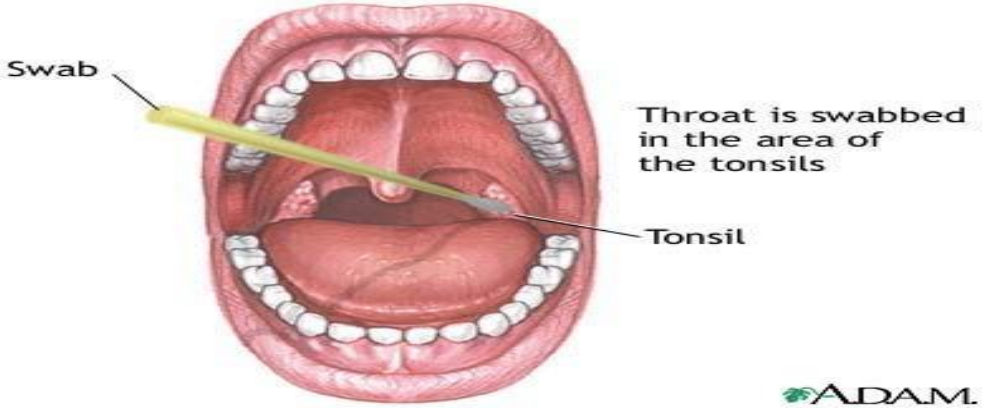


Figure 6: Anatomical site for the throat swab collection. Available at: http://nursingcrib.com/medical-laboratory-diagnostic-test/throat-culture-procedure/

All the swabs were immediately transferred to Amies transport medium (Oxiod, UK). Each sample was labeled with code number, age, sex, location, time and date. Within 2 hours the collected swab were transported to the laboratory of the Department of Microbiology in Hawassa University where isolation and identification was done. (41, 46, 51)

4.8 Isolation and Identification of *S.pyogenes*

The throat swabs were directly inoculated to 5% sheep blood agar plates (Blood agar base, Oxiod UK) by rolling the swab over a small area of the plate and streaking the sample using a sterile loop and the plates were incubated at 37ºC with candle jar to maintain 5% Co_2 atmosphere and examined for beta-haemolytic colonies after 24hrs and 48 hrs. It was identified by their typical fine translucent, dry, shiny or mucoid colony morphology and beta (complete)-haemolysis on a 5% sheep-blood agar plate.

Colonies from plates with beta-haemolytic reaction were sub-cultured on 5% sheep blood agar plates with 0.04U Bacitracin disks(Oxoid, Uk) and incubated in similar manner as an earlier. Those beta-hemolytic plates were parallel subject to gram stain and catalase tests. All gram positive, catalase negative and any zone of inhibition around the disk were candidate for Pyrrolidonyl arylamidase (PYR-amnopeptidase) tests (Becton, Dickinson and Company, Mexico). All isolates which gave purple color in PYR tests were identified as *S.pyogenes* according to publications(94) and WHO and UK Standards for Microbiology Investigations(28, 95).

4.9 Antimicrobial susceptibility testing

Antibiotic susceptibility test (AST) was performed using disc diffusion method. The suspension of bacterium was prepared by picking pure colony with a sterile wire loop, suspends in sterile nutrient broth and incubated 2-5hrs to allow bacterium to reach their log phase. Then the suspension was adjusted to a 0.5 McFarland's turbidity standard. Sterile cotton swab was dipped in to suspension of the isolate in broth, squeezed free from excess fluid against the surface of bottle. The swab was then evenly distributed to entire surface on Muller Hinton agar supplemented with 5% sheep blood agar. The plates were left at room temperature for 3-5 minute to dry up (Annex V). Later on, antibiotic discs (Oxiod, UK) with the following drug contents: Penicillin (10U), Erythromycin (15µg), Amoxicillin (10µg), Chloramphenicol (30µg),Ceftriaxone (30µg),Vancomycin (30 µg*)* and Tetracycline(10µg) were applied on the plates by using forceps, and properly spaced to prevent any overlap. After incubating the plates at 35^0C-37^0C for 24 hours in candle jar, the diameters of Zones of inhibition was interpreted as susceptible, intermediate, or resistant per the recommendation of Clinical Laboratory Standards Institute (CLSI) guideline(96).

4.10: Quality assurance

Pre-test was done on 5% of the sample size before actual work. The data collectors were trained by the principal investigator. The data collections were administered by experienced a senior clinical BSc nurse. The collected data were checked for their completeness at the end of every day.

It was also guaranteed by implementing quality control throughout all laboratory processes. All materials, equipment, and procedures in each stage of quality assurances strictly followed standard operational procedures (SOPs)(Annex V)of medical microbiology. Culture media were tested for sterility by incubating 5% of the batches and gram stain, catalase test, and PYR tests were tested for performances. All plates and disks were stored in 4^{o}c. The reference strains *Streptococcus pyogenes* (ATCC 19615) were used for the entire procedures. The reference strain was obtained from Ethiopian Public Health Institute (Pasteur institute).

4.11. Data processing and analysis procedure

Data entry and analysis was performed by using Statistical Package for Social Sciences (SPSS) version16.0 computer software. Frequency distribution and series of cross tabulation were used to describe socio-demographic variables and prevalence of *S.pyogenes.* Bivariate and multivariate analyses in binary logistic regression methods were performed to identify the existence of association between socio-demographic variables and prevalence of *S.pyogenes*. First, the data were analyzed by series of bivariate analysis then those variables at a cut-off point P-value less than 0.25 were a candidate for multivariate analysis. Odds ratio (OR) and 95% confidant interval (CI) were used to estimate the risk of being *S.pyogenes* carriage. P-value < 0.05 in multivariate analysis was used as for the presence of statistical significance. The result was displayed using graphs and tables.

4.12 Ethical Considerations

The study was ethically approved and cleared by ethical review committee of Jimma University College of Public Health and Medical Sciences Graduate Studies and SNNPR Education Bureau. Letter of permission were obtained from, Hawassa town Education office and director of the schools and verbal permission from Hawassa University college of Health Science, Department of Laboratory(Microbiology). The aim and purpose of the study , right to participate or not, right to withdraw, right to ask questions, benefit and risk of participating in this study and keeping confidentiality of the information they provide were explained to the study participants and informed written consent(Annex II) were obtained from each child's parents or guardian. Verbal assent was obtained from all children.

4.13 Dissemination plan of the study finding

The results will be presented to Jimma university scientific community during thesis defense. After that the final report will be submitted to Hawassa University College of Medicine and Health Sciences, SNNPR and Hawassa town Administrative health bureau plus Educational bureaus, and NGOs working on related area as well as to school where the study participant selected. Further attempt will be made to publish it on national and international scientific journals.

Chapter 5: Result

5.1. Socio-demographic characteristics of study participants

A total of 287 school children were examined for *S.pyogenes* carriage rate and its associated risk factors .The median and mean age of students were 10 (5-15 range) and 10.02 respectively with SD of 2.301.The predominant age group was 9-12 years 180(62.7%). 140(48.8%) of study participants were female and the remaining is male. Age and sex distribution of study subjects were illustrated in figure 7. The male to female ration was 1.05:1.The proportion of students participated in this study from Gebyadar, Adare, Alamura, Dato and Tabor primary schools were 72(25.1%), 57(19.9%), 45(15.7%), 72(25.1%) and 41(14.3%) respectively.

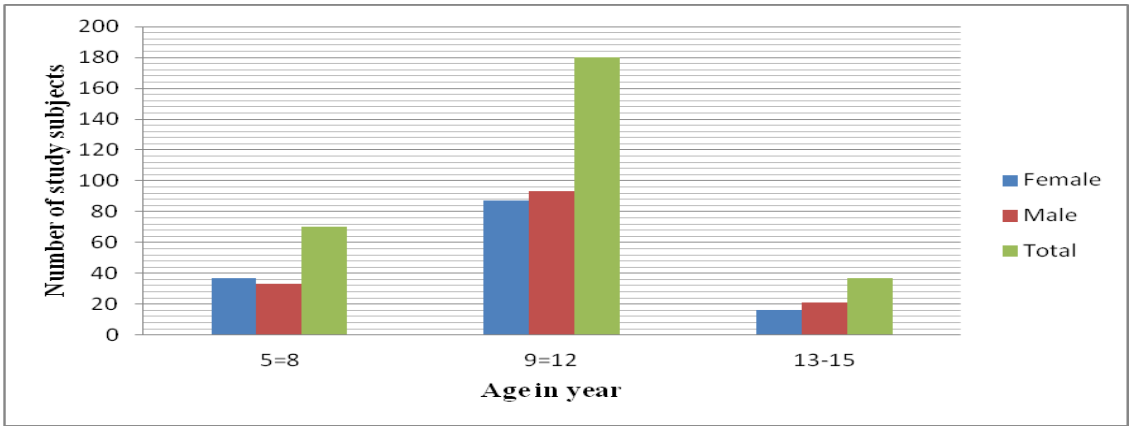


Figure 7: Age and sex distribution of students investigated for pharyngeal carriage of *S.pyogenes* in Hawassa town, South Ethiopia from May-October 2014.

About seventy-three percent of study participant's living status was with parents. The majority of study participants found to have illiterate parents. About fifty percent of students' mothers were house-wife and daily labor, merchant, and student by father occupations. From the total 287 children, 162(56.4%) parents /guardian had a monthly income between 500-900ETB, 33(11.5%) had a monthly income 1000-1500 ETB and 92(32.1%) had greater than 1500ETB per month (Table 1)

All study participants were assessed for the history of cardiac illness and past history of upper respiratory tract infection (URTI) and 246 (85.7%) children had no evidence of history of cardiac illness and absence of recurrences' of throat infections(tonsillitis) as illustrated in Table 1.

5.2. Distribution/ prevalence of *S.pyogenes*:

Among 287 school children 35(12.2%) were confirmed to have *S.pyogenes* in throat swab culture. The pharyngeal carriage rates of *S.pyogenes* were highly distributed in females 23(16.4%) than males 12(8.2%). Similarly,12(17.1%),5(13.5%) and 18(10.0%) of *S.pyogenes* were recovered from their age group of 5-8, 13-15 and 9-12 years old respectively. The distribution of *S.pyogenes* isolated from Gebyadar, Adare, Alamura, Dato and Tabor were 10(13.9%), 7(12.3%), 3(6.7%), 12(16.7%) and 3(7.3%) respectively. Accordingly, 9(15.8%), 11(12.8%), 9(11.4%) and 6(9.2%) were distributed in grade one, two, four and three.

Regarding to living status of children, the prevalence of *S.pyogenes* was 21(10.1%) with both parents, 7(25.0%) only mothers, and 7(14.9%) other than the 3 groups were observed. It was observed that most of *S.pyogenes* isolated from children who have had illiterate parents 19(15.8%) and low carriage rate were identified from 1-4 grade complete parents.

The prevalence of *S.pyogenes* higher among children whose mother's occupation were employed 8(17.0%) followed by house wife 17(12.0%) and other kind of occupations like daily labor, merchant, student 10(10.2%). Similarly, it was prevalent in those children with fathers' occupational status were like daily labor, merchant, and student 21(14.8%) followed by farmer 7(10.8%) and employed 7(8.8%).

Highest (16.0%) carriage rate was detected of parents who earns from 500-900ETB per month, followed by the rate of 12.1% of parents who earns 1000-1500ETB per month, and least (5.4%) rate was seen among families who gets >1500ETB per month.(Table1)
Among a total of 35(12.2%) *S.pyogenes* isolates, the highest carriage rate was observed in student's family more than 5 person per bed room sharing practices16 (14.8%) and the low carriage rate was found in 3-5 person per bed room sharing 4(5.6%). Students family holds more than 8 person per house 5(15.6%) higher in carriage rate than <5 person per house 11(12.8%) and 5-8 person per house 19(11.2%)(Table 2)

The carriage rate of *S.pyogenes* with respect to past history /recurrences of URTI was found to be 6(14.6%) and absences of the cases the carriage rate was 29(11.8%) (Table.1)

Table 1. Distribution of *S. pyogenes* with different variables among school children in Hawassa from May-October 2014

Variables		*S.pyogenes*		
		Positive(N=35)	Negative(N=252)	Total(N=287)
		No (%)	No (%)	No (%)
Sex	Female	23(16.4%)	117(83.6%)	140(48.8%)
	Male	12(8.2%)	135(91.8%)	147(51.2%)
Age	5-8	12(17.1%)	58(82.9%)	70(24.4%)
	9-12	18(10.0%)	162(90.0%)	180(62.7%)
	13-15	5(13.5%)	32(86.5%)	37(12.9%)
Grades of the students	1	9(15.8%)	48(84.2%)	57(19.9%)
	2	11(12.8%)	75 (87.2%)	86(30.0%)
	3	6(9.2%)	59(90.8%)	65(22.6%)
	4	9(11.4%)	70(88.6%)	79(27.53%)
Living situation of children	With others	7(14.9%)	40(85.1%)	47(16.4%)
	Mother only	7(25%)	21(75.0%)	28(9.8%)
	Father Only	0(.0%)	4(100.0%)	4(1.4%)
	Father and Mother	21(10.1%)	187(89.9%)	208(72.5%)
Educational status of parent	Illiterate	19(15.8%)	101(84.2%)	120(41.8%)
	1-4	1(5.0%)	19(95.0%)	20(6.7%)
	5-12	10(8.9%)	102(91.1%)	112(39.0%)
	>12	5(14.3%)	30(85.7%)	35(12.2%)
Occupational status of Mothers	Others	10(10.2%)	88(89.8%)	98(34.1%)
	House Wife	17(12.0%)	125(88.0%)	142(49.5%)
	Employed	8(17.0%)	39(83.0%)	47(16.4%)
Occupational status of Fathers	Others	21(14.8%)	121(85.2%)	142(49.5%)
	Farmer	7(10.8%)	58(89.2%)	65(22.6%)
	Employed	7(8.8%)	73(91.2%)	80(27.9%)
Family income	500-900ETB	26(16.0%)	136(84.0%)	162(56.4%)
	1000-1500ETB	4(12.1%)	29(87.9%)	33(11.5%)
	>1500ETB	5(5.4%)	87(94.6%)	92(32%)
Past history of re- current of URTI/RHD	Yes	6(14.6%)	35(85.4%)	41(14.3%)
	No	29(11.8%)	217(88.2%)	246(85.7%)

Note, With others=guardians or care givers, With both= both mother and father, others=daily labor, merchant, students…URTI=Upper respiratory tract infection, RHD=Rheumatic heart disease

5.3. Risk factor analysis for pharyngeal carriage

Possible risk factors such as age, sex, children living status, parents/guardians occupation, parents/guardians education, income of parents, family size, person per bed room sharing habit, and past history of recurrences of URTI were evaluated for the association with pharyngeal carriage of *S.pyogenes* (Table 2.)

It was observed in bivariate analysis that, female children (COR =2.212; 95%CI= 1.055-4.638; p =.036), low income of parents (COR =3.326; 95%CI= 1.231-8.990; p=0.018), Occupational status of mothers (COR =0.554; 95%CI=.203-1.511; p=0.249), schools where they attended (COR=2.533; 95%CI .671-9.567;p=0.17), Father Occupation(COR=1.810; 95%CI=.733-4.467;p=0.198), and person per bed room sharing habit (COR=.370; 95%CI=.118-1.165;p=0.089) were met our cutoff criteria of $p<0.25$.

Further examination was made by using multivariate analysis to control the possible confounding effects of the risk variables on the carriage rate. It was showed that the female children (AOR=2.730; 95%CI=1.24-6.037; p=0.013), and low income of parents (AOR=11.917; 95%CI= 2.729-52.032; p=0.001) were associated with *S.pyogenes* carriage. Conversely, Occupational status of Mothers (AOR=.100; 95%CI= .023-.437; p=0.002) was associated with reduced likelihood of risk for asymptomatic pharyngeal carriage of *S.pyogenes* .

However, age group, schools where they attended, living status, father occupation, parents/guardians education, family size, person per bed room sharing habit, and past history of recurrences of URTI were not statistically significant with the asymptomatic pharyngeal carriage rate of *S.pyogenes* (P>0.05)

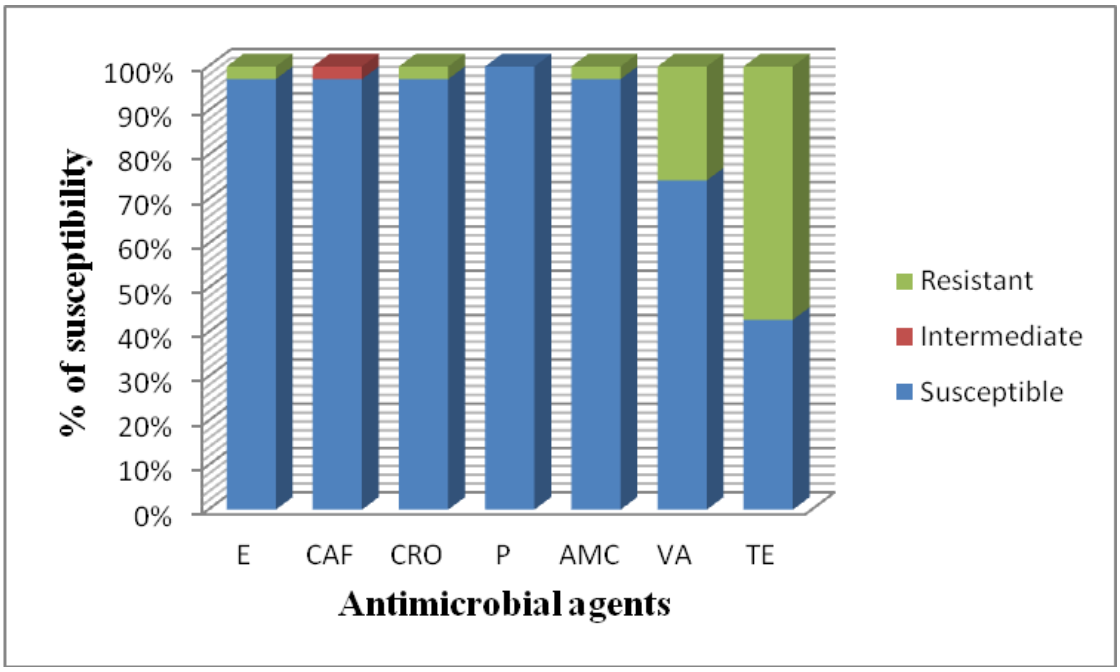
Table 2. Associated factors for acquisition of pharyngeal carriage rate of *S.pyogenes* among school children in Hawassa town from May-October 2014

Associated factors		***S.pyogenes* carriage rate**		**Odds ratio With 95%CI**			
		Yes No (%)	No No (%)	COR	P value	AOR	P value
Sex	F	23(16.4%)	117(83.6%)	2.212(1.055-4.638)	0.036	2.730(1.24-6.037	0.013
	M	12(8.2%)	135(91.8%)	1		1	
Age	5-8	12(17.1%)	58(82.9%)	1.324(.428-4.095)	0.626		
	9-12	18(10.0%)	162(90.0%)	.711(.246-2.054)	0.529		
	13-15	5(13.5%)	32(86.5%)	1		1	
School	Gebeyadare	10(13.9%)	62(86.1%)	2.043(.529-7.896)	0.300	1.204(.240-6.055)	0.821
	Adare	7(12.3%)	50(87.7%)	1.773(.430-7.313)	0.428	2.051(.371-11.349)	0.411
	Alamura	3(6.7%)	42(93.3%)	.905(.172-4.755)	0.906	.253(.028-2.257)	0.218
	Dato	12(16.7%)	60(83.3%)	2.533(.671-9.567)	0.17	.717(.104-4.948)	0.736
	Tabor	3(7.3%)	38(92.7%)	1		1	
Educational status of parents	Illiterate	19(15.8%)	101(84.2%)	1.500(.053-4.739)	0.546		
	1-4	1(5.0%)	19(95.0%)	1.316(.034-2.915)	0.309		
	5-12	10(8.9%)	102(91.1%)	1.008(.187-2.854)	0.365		
	>12	5(14.3%)	30(85.7%)	1		1	
Mother Occupation	Others	10(10.2%)	88(89.8%)	.554(.203-1.511)	0.249	.100(.023-.437)	0.002
	House Wife	17(12.0%)	125(88.0%)	.663(.266-1.654)	0.378	.112(.027-.458)	0.002
	Employed	8(17.0%)	39(83.0%)	1			
Father Occupation	Others	21(14.8%)	121(85.2%)	1.810(.733-4.467)	0.198	.597(.159-2.250)	0.446
	Farmer	7(10.8%)	58(89.2%)	1.259(.418-3.792)	0.683	.640(.120-3.417)	0.602
	Employed	7(8.8%)	73(91.2%)	1		1	
Family income	500-900	26(16.0%)	136(84.0%)	3.326(1.231-8.990)	0.018	11.917(2.729-52.032)	0.001
	1000-1500	4(12.1%)	29(87.9%)	2.400(.604-9.543)	0.214	5.575(1.065-29.176)	0.042
	>1500	5(5.4%)	87(94.6%)	1		1	
Family size	>8	5(15.6%)	27(84.4%)	1.263(.402-3.968)	0.690		
	5-8	19(11.2%)	150(88.8%)	.864(.391-1.908)	0.717		
	<5	11(12.8%)	75(87.2%)	1			
Person per bed Room sharing	>5	16(14.8%)	92(85.2%)	1.078(.504-2.308)	0.846	.635(.231-1.744)	0.379
	3-5	4(5.6%)	67(94.4%)	.370(.118-1.165)	0.089	.231(.065-.823)	0.324
	<3	15(13.9%)	93(86.1%)	1		1	
Past history of re- current of URTI/RHD	Yes	6(14.6%)	35(85.4%)	1.283(.497-3.313)	0.607		
	No	29(11.8%)	217(88.2%)	1			

Note, 1=Constant/reference, With others=guardians or care givers, With both= both mother and father, others =daily labor, merchant, students…, CI= confident interval, COR=crude odds ratio, AOR =adjusted odds ratio

5.4. Antimicrobial susceptibility pattern of *S.pyogenes*

The antimicrobial susceptibility tests were performed for all 35 *S.pyogenes* isolates to commonly prescribed drugs. It was observed that all (100%) *S.pyogenes* isolates were susceptible to Penicillin. The isolates also showed 97.1% susceptible to Erythromycin Chloramphenicol, Ceftriaxone, and Amoxacline, and 74.3% to Vancomycin. Relatively least 42.9% susceptibility was observed to Tetracycline. There was one(2.9%) *S.pyogenes* isolates which developed intermediate resistant to Chloramphenicol (Fig. 8)



Note; E= Erythromycin, CAF= Chloramphenicol, CRO= Ceftriaxone, P= Penicillin, AMC= Amoxacline, VA= Vancomycin, TE= Tetracycline

Figure 8: Susceptibility pattern of *S.pyogenes* isolated from school children in Hawassa from May-October 2014

Antibiogram of *S.pyogenes* isolates showed that resistance against one or more antibiotics were 68.6%(24/35), and 29.2%(7/24) of the isolates were multiple drug resistances. Of 29.2%(7/24) MDR, 6(25.00%) and 1(4.2%) were resistant to two and three tested drugs respectively. Two multiple drug resistances patterns were noted. Table 3

Table 3. Antibiogram of *S.pyogenes* isolates resistant to two and/or three antibiotics among school children in Hawassa town from May-October 2014

Phenotype resistant pattern	Number observed	Percentage
TE,AMC	1	4.2
TE, CRO	1	4.2
TE,VA	4	16.7
TE,VA,E	1	4.2
Total	7	29.2

Note: AMC= Amoxacline; E=Erythromycin; CRO=Ceftriaxone; TE=Tetracycline ; VA=Vancomycin;

Chapter 6: Discussion

S.pyogenes throat carriage is an important public health issue, which causes to post streptococcal sequelae and the healthy carriers of the GAS are the sources for bacterial dissemination which lead to disease and even to severe epidemics(97). The overall asymptomatic pharyngeal carriage rate of *S.pyogenes* among school children in this study was 12.2%. The demographic characteristics, particularly female children and low income of parents were significantly associated with the carriage rate. Similarly, the isolates showed no resistant to Penicillin. However, resistance against Vancomycin and Tetracycline were 9(25.7%), and 20(57.1%) respectively.

The prevalence of *S.pyogenes* in this study 12.2% which was comparable with study findings reported in Ethiopia 9.7%(50), in Tunisia 9.0%(47), in Nigeria 10%(48) and in India 13.9%(25). It was lower than those observed in previous study among school children in Ethiopia16.9%(21), and also lower than reports from Pittsburgh, Pennsylvania15.9% (10),Australia19.5% (43) and Duzce, Turkey 26%(41). However, comparatively higher than studies reported from Pemba, Zair or D.R.C 8.6%(49), Chennai, India 8.4%(1), and Mangalore, India 5%(26).The Carrier rate variation might be influenced by several factors such as methodology of study, geography, season, and socioeconomic differences are few of examples(10, 45, 49, 50).

Our result revealed that higher carriage in females 23(16.4%) than males 12(8.2%). Similar reports were reported in India where higher prevalence seen among females (2.14%) than in boys (1.76%) (53), and similar report was observed in Ankara, Turkey where the rate of isolation of the pathogens were higher in girls (2.46%) than boys (2.39%) (52) and in Nepal higher among girls (12.5%) than boys (9.4%) were seen(13). However, it was inconsistent with many studies conducted in the different parts of the world including Ethiopia (13, 39, 50, 51). Presumably these difference rates could be factors like degree of social interaction that is female children might have more interaction with family members and school classmates than the male. Although, in theory both sex equally affected.

The prevalence of *S.pyogenes* was higher 12(17.1%) in 5-8years age group in our study. It was similar with others report (25, 46, 51). However, inconsistent with study

done in India, reported that higher in higher age group (>11years age group)(55), and in Iraqi 8-12 years old age group higher carriage rate were observed(54). Our study finding might be confirmed that *S.pyogenes* carriage rate increase in lower school age group than the older children. Immature immune system or decline of the maternal immunity, the presence of genetic and environmental factors and high exposure to contact with the others in this age group might make high susceptible to the carriage.

Higher prevalence were found in Dato 12(16.7%) followed by Gebyadar 10(13.9%), Adare 7(12.3%), Tabor 3(7.3%) and Alamura 3(6.7%) primary schools. Similar study conducted in Pemba(Zair orD.R.C) prevalence of *S.pyogenes* among school children from different schools resulted that 17(3.4%) in Konde I to 15(12.5%) in Mkanyageni (49), in Nepal 8(9.4%) in Maitidevi and 12(13.3%) in Kirtipur(13), in India 230(23.7%) in Urban, and 51(37.5%) in minority residence *S.pyogenes* carriage rate were observed (51).This might be due to the variation in the living standards (including the socioeconomic levels). In our study, Dato had been overcrowding in the class room as well as most of families were farmers and in Gebyadar most of parents were daily labor, merchants and no labor at all.

In this study higher carriage rate observed among study participants living with only mother, others/guardians and both parents were 7(25%), 7(14.9%) and 21(10.1%) respectively. The higher prevalence was detected in children living with mother 7 (25%). This might be mothers were more likely to subsequently acquire the bacteria. The possible explanation might be exposure to children and duration of exposure to a GAS-infected person, mothers spent more time within households than the others.

The detection rate of *S.pyogenes*, were high in children who had illiterate parents 19(15.8%). It was in-lined with study revealed in India 44.9%(51), and in Iraqi 66.7%(54). This might reflects the literate parents had high level of awareness of the disease transmission in the community than the illiterate parents. For example, literate parents need to understand the importance of immunization for their children and decide to have the small number of family size.

It was observed in the present study that *S.pyogenes* isolated from children whose mother's occupation were employed 8(17.0%) followed by housewife 17(12.0%) and

other kind like daily labor, merchant, and student 10(10.2%). Similarly, whose fathers' occupational status were other kind like daily labor, merchant, and student 21(14.8%) followed by farmer 7(10.8%) and employed 7(8.8%). It was incomparable with study done in India which showed that occupation of parents were manual unskilled 26(31.7%), manual skilled 26(32%), semi-professional 11(39.2%) and professional 2(22.2%) were observed (98). This might be the socioeconomic and cultural difference, in our country most of a time children spent their time with similar age group in a community than their family.

The carrier group of *S.pyogenes* was higher 15.6% among children living within families of more than 8 members than less family members in our study. Accordingly, *S.pyogenes* carriage was significantly higher in large family size as reported in different authors (3, 43, 56). This might be an increasing the number of family members lead in most occasions to increase the rate of prevalence of many infectious diseases including *S.pyogenes*.

Our study findings revealed that more (14.6%) carriage rate was seen among children who had pervious history of recurrences of pharyngitis, and it was comparable with the other study findings reported elsewhere (54, 58). Exposure to and duration of contacts with the GAS pharyngitis cases, and also related with host immune response to the infection might influence the carriage rate.

In present study the socio-demographic characteristics like age group, schools where they attended, living status, occupational status of parents/guardians, educational status of parents/guardians, family size, person per bed room sharing habit, and past history of recurrences of URTI were not statistically significant. However, studies revealed that the demographic variables like low income of family(26, 54), age(3, 55, 58), past history of URTI(54, 58), gender(3), large family size(3, 56), person per one bed room sharing habits(14), occupational status of parents (14, 54), and Educational status of parents(14, 54) were significantly associated to the asymptomatic pharyngeal carriage rate of *S.pyogenes*. The discrepancies might be geography, study settings, methodology of the study, genetic and socio-economic difference.

There was clear association of pharyngeal carriage rate of *S.pyogenes* and female children were seen in this study(AOR=2.730; 95%CI=1.24-6.037; p=0.013) and similar results was reported in Iraqi(3). Another statistically significant association was seen among children's family which has low income carries more *S.pyogenes* (AOR=11.917; 95%CI= 2.729-52.032; p=0.001). This was in-lined with the earlier finding in Ethiopia(21) and other studies carried out in Mangalore(26), in Kerman-Iran(14), and in Iraqi (54). This might be the influx of people to the urban area, causing crowding and lower standards of living conditions might be the possible indicator.

The results of antimicrobial susceptibility tests of this study was showed that all isolates sensitive to Penicillin. The finding is similar as compared with the report found in Ethiopia(50). It was also agreed with studies conducted in different parts in the world (13, 46, 99) which showed that Pencillin and its derivatives remained drug of chose to threat the GAS infection. However, it was disagreed with study findings in India where the resistance rate to Penicillin, and Amoxicillin were 76.4%, 79.1%respectively(51) and Penicillin resistant strains 4% in MIC have been published (89). The complicated pathogenic mechanism of GAS, easily available of the Penicillin drug in the community, and absence of periodic surveillance program might be emergence resistance toward this drug in the future in our.

In our finding the rate of bacterial isolates which were resistant to Tetracycline was 57.1% which was almost comparable with the other study done in Ethiopia 47% (50),Tunisia 43.9% (88) and Egypt 32% (89). However, it was much higher compared with studies conducted in Span (79), Nepal (81) and India (82) where the resistant rates were 6.8%, 6.6%, and 27.4% respectively. But lower compared to study results conducted in Dakar, Senegal 100%(87) and In Pemba (Zair, D.R.C) 83.2% (49). Higher usage over a period of time as its broad spectrum agents, more commonly available or cheaper than the other drugs and its consumption for animal treatment can promote the emergence of resistance continuing in Ethiopia.

In this study 25.7% *S.pyogenes* were developed resistant to Vancomycin which was higher than the other studies conducted in Ethiopia where the resistance rate was 7.7%(50), in Egypt and in India where all(100%) isolates were susceptible (51, 89).The discrepancy of the result might be the geographical distribution of phenotype, misuse of

antibiotics, and absence of adequate surveillance program may contribute to the emergence of resistance bacteria.

The rate of antimicrobial resistance especially to Erythromycin in this study was low (2.9%), and was similar with study finding in Ethiopia where the resistant rate was nil (50). Higher resistant rate were also reported from Span 32.8% (79), Nepal 5.2% to15.6% (13, 51, 81) ,India 5.6% to 30.4% (44, 51, 82, 84, 85), Egypt 21.3% (89), and Tunisia 4%(88). The discrepancy might be the geographical distribution of GAS strain and also the usage of specific class of Macrolids.

The present study showed that the resistant rate to Chloramphenicol was 2.9%, and this is in agreement with other studies undertaken (44), and in Nepal where Chloramphenicol resistance was 6.7%- 7.8%(13,46), and in Chennai, India 4.3% (82). The resistant rate to Ceftriaxon 2.9% was observed in our study, and it was lower than study reports from India where resistance rate was 26%-27.8% (51, 85). The discrepancy of the result might be the geographical distribution of phenotype, study setting, and methodology.

The overall findings of our result showed most of *S.pyogenes* isolates were susceptible to most tested antibiotics. However, 7(29.2%) of isolated GAS strains were showed multiple drug resistant (two or more drugs). Such a pattern of co-resistance or multiple-drug resistance amongst GAS strains has not been reported. The multiple-drug resistance found from few isolated strains in the present study might be due to the ease of availability of antibiotics and misuse of the drugs.

Limitation of the study

- The study included children who are attending only public/government schools and the findings may not be wholly representative of all school age children in Hawassa town.

7. Conclusion and Recommendation

7.1. Conclusion

The findings of present study have shown an overall *S.pyogenes* throat carriage of 12.2% among school children. The figure considered as significant when compared to other few studies done in Ethiopia. The high evidence of socio demographic variables especially low income of parents is as a prime risk factor associated with the carriage rate. Another, the most striking differences is the significantly high carriage rate among female children than male; this difference could not be accounted in few published studies in Ethiopia. Although, the other socio-demographic factors are not associated with the throat carriage rate, but the frequencies distribution was vary with each of studied variables. The antimicrobial testing results showed that all *S.pyogenes* isolates were susceptible to Penicillin, and most isolates were also susceptible to Amoxacline, Chloramphenicol, Erythromycin, and Ceftriaxone. Low and high rate of resistance was observed against Vancomycin and Tetracycline respectively. The isolated *S.pyogenes* strains were showed multiple drug resistant (two or more drugs).

7.2. Recommendation

- To get more comprehensive information on the magnitudes of the problem and the contribution of risk factors, further large scale study should be conducted

- Improvement of the family income is very important to decrease the spread of the disease.

- Penicillin and its derivatives should be used in situations where children need to be treated.

- Appropriate antibiotic use, should be crucial to prevent the spread of drug-resistant.

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Annexes

Annex I. Information Sheet (English Version)

Title of the study: asymptomatic pharyngeal carriage rate of *Streptococcus pyogenes* and its antibiotic susceptibility pattern among school children in Hawassa town, south Ethiopia

Name of the investigator: Asrat Anja(BSc, MSc)

Name of the advisors: Getenet Beyene (BSc, MSc, PhD,Associate professor)
Zewdineh H/Mariam (BSc,MSc, Lecture)
Deresse Daka (BSc, MSc, Assistant professor)

Name of the organization: Jimma University, College of Public Health and Medical Sciences, Department of Medical Laboratory Sciences and Pathology.

Sponsored organization: Jimma University

Objective of the study: To determine the prevalence of asymptomatic pharyngeal carriage rate of *Streptococcus pyogenes*, to verify associated risk factors and its antimicrobial susceptibility pattern among school children in Hawassa.

Significance of the study

The finding of this study will help us to determine the prevalence of asymptomatic pharyngeal carriage rate of *Streptococcus pyogenes* and susceptibility to antimicrobial drugs. So, by providing up to date information on this bacterial pathogen was important for the prevention and control strategies against non-supporative sequalae of the bacterium.

Unnecessary discomfort:

In few participants might have been mild anxiety sign and then suddenly disappeared.

Benefits

They were diagnosed by the experienced health professional and also the sample collection was be done by BSc nurse.

Confidentiality

All information collected from the study subjects was kept confidential and stored in a file that was not bear a name on it, but coded

Right to refuse or withdraw

Participation in this study was based on the study participant, parents/guardian's voluntariness or full of their willingness.

Whom to contact

If you have any thing in mind that need explanation please contact

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Mr. Deresse Daka: +251911968912

Annex I Information Sheet (Amharic Version)

የጥናቱ ርእስ፡- "ግሩፕ ኤ ስትሪፕቶኮከሳይ"ተሸከሚዎች ስርጭትና መድሐኒት የመለማመድ ሰርጭት በሐዋሣ አንደኛ ደረጃ ትምህርት ቤት ፡፡

የተመራማሪ ስም፡ አሥራት አንጃ

የ አማካሪዎች ስም፡ ዶ/ር ጌትነት በየነ

አቶ ዘውድነህ

አቶ ደረሠ ዳካ

የድርጅቱ ስም ፡- ጂማ ዩኒቨርሲቲ የሕብረተሰብ ጤናና ሕክምና ሳይንስ ኮሌጅ የላብራቶሪና ፓቶሎጂ ዲፖርትመንት

የእስፓንሰር ስም ፡- ጂማ ዩኒቨርሲቲ

<u>የጥናቱ ዓለማ</u>

የዚህ ጥናት ዓለማ "ግሩፕ ኤ ስትሪፕቶኮከሳይ" በመባል በሚታወቀው እና ለጉሮሮ በሽታ፣ ለልብ ህመም እንዲሁም ለሌሎች የጤና እክሎች መንሰኤ የሆነውን ባክቴሪያ ሐዋሣ ያለውን የተሸከሚዎችን ስርጭት ለመረዳት ተያያዝ ገላጨች እንዲሁም መድሐኒት የመለማመድ ስርጭትን ለማወቅ ነው፡፡

ጥናቱ የሚያስገኘው ጥቅም

ይህ ጥናት የልብ ህመምን ጨምሮ ሌሎች የከፉ የጤና ችግሮች የሚያስከትለውን የባክቴሪያ ተሸከሚዎችን ስርጭትና መድሐኒት የመለማመድ ሰርጭትን በመርዳት የመከላከል እርምጃ ለመውሰድ ከፍተኛ አስተዋፅኦ አለው፡፡

ስጋትና ጉዳት

ናሙና የመውሰዱ ጥረት በጥናቱ ተሳታፊዎች ምናልባትም በጣም በጥቅት ልጆች ላይ ወዲያውኑ የሚጠፉ የማቅለሸለሸ ስሜት ሊያስከትልባቸው ይችላል፡፡

ጥቅሞች

የሕክምና ምርመራ በሚደረግበት ወቅት ሕመም ቢገኝበት /ባት ነፃ የማማከር አገልግሎት የማግኘት መብት አለው/አላት፡፡

ምስጢራዊነት

ከዚህ ምርምር የምንሰበስበው መረጃ በምስጢር ይያዛል፡፡ከልጅዎ እና ከርስዎ የሚገኘው መረጃ የሚከማችበት መዝገብ ውስጥ ከልጅዎ ስም ጋር የተያያዘ ፋይል እንዲኖር አይደረግም፡፡ በምስጢር ቁጥር ማለትም በኮድ መልክ ይመዘገባል፡፡

ያለመቀበል ወይንም ጥሎ የመውጣት መብት የተጠበቀ ነው፡፡ ፍቃደኛ ካልሆኑ ልጅዎ በዚህ ጥናት እንዲሳተፍ/ እንድትሳተፍ አይገደድም/አትገደድም፡፡ ላለማሳተፍ መወሰንዎ በልጅዎ የትምህርት ክትትል ላይ እክል አይፈጥርም፡፡

ልጅዎ በጥናቱ መሳተፍ ከጀመረ/ች በኃላ በማናቸውም ሰዓት ከጥናቱ ጥሎ/ላ እንዲወጣ/እንድትወጣ ሊያደርጉ ይችላሉ፡፡ ይህንን በማድረግዎ የልጅዎ የተማሪነት መብት አይሸራረፍም፡፡

የበለጠ መረጃ ከፈለጉ

አቶ አሥራት አንጃ ስልክ ቁጥር+251913073424

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ይህ የጥናት እቅድ ተግባራውየሚሆነው የጅማ ዩኒቨርሲቲ የስነምግባር ቅኝት ኮሚቴና የደበብ ክልል ትምህርት ቢሮ ተገምግሞ ስፀድቅ ነው፡

Annex II. Consent Form (English Version)

Participant's Code no…….

Participant's Name…….……..

Parents/guardian's Name…….……..

I…….the name signed above student parent's /guardian's had been informed that the objective of this study is to assess the asymptomatic pharyngeal carriage rate of *Streptococcus pyogenes* and susceptibility to antimicrobial drugs. I had been informed, that the principal investigator requested me to achieve the above mentioned study, so as give specimen from tonsilopharyngeal area of my child by aseptic techniques. I agreed voluntarily to provide the requested specimen

from tonsilopharyngeal area with sterile cotton swab of my child. I had been also informed about the confidentiality of the questionnaire. I had been informed that I have the right not to be participate my child in this study, due to this no one affect the teaching –learning process. And, also I had been informed that physical examination, diagnosis and sample collection was done by experienced health professional/ nurse, during this time disease have been found right to get minor medication/consultation. Therefore, with full understanding of the importance of the study, I agreed voluntarily to provide the requested sample from my child for the research purposes.

Parents/guardian's signature ……Principal investigator signature……..

Thank you for your participation!!

Annex II . Consent form(Amharic Version)

የስምምነት ቅፅ

የተሳታፊው ልዩ መለያ ቁጥር _____

የተሳታፊ ተማሪ ሙሉ ስም _____

የወላጅ ወይም የአሳዳጊ ሙሉ ስም _____

እኔ _____ ስሙ/ስሚ ከላይ የተጠቀሰው ተማሪ ወላጅ/አሳዳጊ መሆኔን እያረጋገጥኩ የጎሮሮ እና ሌሎች ህመሞች እንዲሁም የልብ ችግር መንስኤ የሆነውን ግሩፕ ኤ ስትሪፕቶኮከሳይ "*S.pyogenes*(GAS) " በመባል የሚታወቀውን ባክቴርያ ላይ ሊደረግ ስለታሰበው በጥጥ አማካይነት ናሙና መውሰድ እንደሚፈለግ ተረድቻለሁ፡፡ ስለጥናቱ ዓላማ እንዲሁም ናሙና የመውሰዱ ጥረት በልጄ/ በማሳድገው/ ጋት ልጅ ላይ ምናልባትም መጠነኛ የማቅለሽለሽ ስሜት ለሰማው/ት እንደሚችል ተገንዝቤያለሁ፡፡

በተጨማሪም በመጠይቁ ውስጥ በተካተቱት ጥያቄዎች መሰረት የምሰጣቸው መረጃዎች በጠቅላላ በሚስጥር እንደሚጠበቁ ተገልፆልኛል፡፡ እንዲሁም ልጄን/የማሳድገውን ልጅ በተመለከተ የምጠየቀውን መረጃ ያለመስጠትና በጥናቱ ያለመተባበር ከጥናቱ በማናቸውም ወቅት ልጄን /የማሳድገውን/ ጋትን ልጅ የማግለል መብቴ የተጠበቀ መሆኑ የተገለጸልኝ ሲሆን ይህንንም ማድረጌ በልጄ/በማሳድገው/ጋት ልጅ አጠቃላይ የትምህርት ክትትልና የትምህርት ቤት እንቅስቃሴ ላይ ምንም አይነት እክል የማይፈጥርበት /ባት መሆኑን በሚገባ ተረድቻለሁ፡፡ ከዚህ በላይ በልጄ/በማሳድገው/ጋት ልጅ ላይ የህክምና ምርመራ በሚደረግበት ወቅት ህመም ቢገኝበት/ባት ህክምና የማግኘት መብት እንዳለው/ላት ተገንዝቤያለሁ፡፡
ስለዚህ የስምምነት ቃሌን የሰጠሁት በአጠቃላይ ሁኔታውን በመረዳትና በፍፁም ፈቃደኝነት ነው፡፡ ከልጄ/ከማሳድገው/ጋት ልጅ የሚወሰደው ናሙና ለምርምር ዓለማ ይውሉ ዘንድ ተስማምቻለሁ፡፡

የወላጅ ወይም የአሳዳጊ ፊርማ _____

የዋናው ተመራማሪ ፊርማ _____

Annex III. Questionnaire

Questionnaire number _____

Date _____ Sex_____

Name of Participant _____ Grade and Section _____

PART I. Demographic and socio economic variable

S. No	QUESTIONS	ALTERNATIVES	CODE
101	Sex	1. Male 2. Female	
102	Age	_____years	
103	Residence	1. Rural 2. Urban	
104	Grade	____Grade	
105	Are your mother and father currently living together?	1. Yes 2. No	
106	With whom did you live until now?	1. With both of my parents 2. With my mother only 3. With my father only 4.Others,specify_____	
107	What is the educational level of your Mother?	1. Illiterate 2. Grades 1-4 3. Grades 5-12 4. 12+	
108	What is the educational level of your Father?	1. Illiterate 2. Grades 1-4 3. Grades 5-12 4. 12+	
109	What is your mother's occupation?	1.employed 2. House wife 3.Others,specify_____	
110	What is your father's occupation?	1.employed 2. Farmer 3.Others, specify_____	
111	What is the monthly income of your Household?	Per month _____ Birr Don't know _____	
112	How much your family size?	1. < 5 2. 5 - 8 3.>8	
113	With how many people do you share the bed room?	1. <3 2. 3 – 5 3. > 5	

ክፍል 1 አጠቃላይ መረጃ

ተ.ቁ	ዓ□ቁ	አማራጭ	ኮት
101	ፆታ	1. ወንድ 2. ሴት	
102	አድሜ	--------ዓመት	
103	የመኖሪያ አድራሻ	1. □□ር 2. ከተማ	
104	ስንተኛ ክፍል ነህ (ነሽ)?	-------- □አል	
105	በአሁኑ ሰዓት አናትና አባት አብረው ነው የሚኖሩት?	1. አዎ 2. አብረው አይኖሩም(□ለም)	
106	አሁን ከማን ጋር ነው የምትኖረው	1. ከአናትና ከአባቱ ጋር 2. ከአናቱ ጋር ብቻ 3. ከአባቱ ጋር ብቻ 4. ሌላ ካለ ዓ ቀስ/ሽ/------	
107	የአናት የትምህርት ሁኔታ□	1. ያልተማረች 2. ከ1-4 ተምራለች 3. ከ5-12 ተምራለች 4. ከ12ኛክፍል በላይ ተምራለች	
108	የአባት የትምህርት ሁኔታ□	1. አልተማረም 2. 1-4 የተማረ ነው 3. 5-12 የተማረ ነው 4. ከ12ተኛ □አል በላ□ ተምሯል	
109	የአናት የሥራ ሁኔታ□	1.የመንግስት ሠራተኛ(ተቀጣሪ) 2. የቤት □እመቤት 3.ሌላ ካለ □□ቀስ---------	
110	የአባት የሥራ ሁኔታ□	1.የመንግስትሠራተኛ(ተቀጣሪ) 2. □ር 3.ሌላ ካለ □□ቀስ-------	
111	የቤተሰብ የወር ገቢ ስንት ነው?	1.----------ብር በ□ር 2. አላውቅም	
112	□ቤተሰብ ቁዓ ር ገቢ ስንት ነው?	1. < 5 2. 5 - 8 3.>8	

113	መኝታ ክፍላች □· □·ስዓ ስንት ሰው ያደራል?	1.3 2. 3 - 5 3. ከ 5 በላይ	

PART II. HISTORY OF SORE THROAT

Question no	Question	Response	Skip to question no
201	Have you ever had sore throat in the last 3-weeks?	1. Yes 2. No	Yes Q. No203
202	Was the last episode of sore throat accompanied by skin rash?	1. Yes 2. No 3. Don't know	
203	When	1.last week, 2.last two weeks 3.before one month 4 before three month	
204	Had measure been taken for treatment?	1. Yes 2. No	Yes Q. No205
205	Did you receive penicillin injection?	1. Yes 2. No 3. Don't know	
206	Do you have family history of cardiac illness?	1. Yes 2. No 3. Don't know	

ክፍል ሁለት <u>የመተንፈሻ አካል ችግር በተመለከተ</u>

201	ባለፈዉ አንድ ዓመት የመተንፈሻ አካል ችግር አጋጥሞህ/ሽ ነበር	1. አዎን 2.አለጋጠመኝም	Yes Q. No302

202	መቼ ነበር ያጋጠመህ/ሽ	1. ከሳምንት በፊት 2.ከሁለት ሳምንት በፊት 3. ከ ወር በፊት 4.ከ ሶስት ወራት በፊት 5. አላስታዉስም	
203	የመተንፈሻ አካል ችግር በገጠመህ/ሽ ጊዜ የቆዳ ሽፍታ ነበር	1. አዎን 2. የለም 3.አላዉቅም	Yes Q. No305
204	ችግሩን ለመቅረፍ ርምጃ ተወስዶ ነበር	1. አዎን 2. የለም	Yes Q. No306
205	የ ፔንሲሊን መርፌ ወስደህ/ሽ ነበር	1. አዎን 2. የለም 3. አላዉቅም	
206	በቤተሰብህ/ሽ ዉስጥ የልብ ህመም ያለበት ሰዉ አለ	1. አዎን 2. የለም 3. አላዉቅም	

Annex IV Laboratory data

Participant's unique code ---------- Participant's initials……..

Age _____ Sex----------

Grade---------- section----------

Date of sample collection…… time……

A. Identification and isolation of *S.pyogenes*

A, Culture Result

Positive ☐

Negative ☐

Indeterminate ☐

B. Gram reaction Gram positive……..

C. Catalase test Catalase positive……Catalase negative..……..

D. Bacteracin test susceptible……Resistant……Intermidate……..

E. PYR test PYR positive……PYR negative……

Conclusion of the result…….

B.Antimicrobial susceptibility test result

Antibiotics	Total isolates	Susceptible		Intermediate		Resistant	
		N	%	N	%	N	%
Amoxacillin							
Ceftriaxone							
Erythromycin							
Chloramphenic							
Penicillin							
Vancomycin							
Tetracycline							

Annex V. Principles and procedures (SOPs):-

For swab collection, media and reagent preparations, isolation and identification of *S.pyogenes*, and for drug susceptibility test

1. Collection of Throat Swabs

The purpose of a throat swab culture is to detect the presence of *S.pyogenes* in the throat.

The collection procedures;

- The tonsillar area and/or posterior pharynx was swabbed.
- The swab putted into Amies transport media.
- Students full name, code number, class, age and date and time of collection were labeled
- The swabs were maintained at room temperature and submitted to the laboratory WITH IN 24 hours of collection.

B. Preparation of Media and Reagents

Blood agar base (5%sheep blood agar)

1. Preparation of 5%sheep blood agar media was strictly followed commercially available manufacturer's instructions.
2. 5%sheep blood agar was made selective for *S.pygenes* by the addition of the dye crystal violet**. (**According to crystal violet blood agar preparation instructions)

Amies transport medium

1. The preparation was as instruction of the manufacturer.
2. Sterilize by autoclaving at 121^{o}c for 15min
3. The medium was cooled, and tightened when the bottle caps. The bottles were inverted during cooling, to ensure an even distribution of the charcoal.
4. The medium was dated and given it a batch number.
5. The medium was stored in a cool place away from direct light. Shelf life up to 9 month. PH within the range of 7.1-7.3 at room temperature
6. The specimen was collected on a sterile cotton wool swab and immerses it in the medium, cutting off the swab sticks to allow the bottle top to be replaced tightly. Protect the swab from direct light and heat.

Agar Media (susceptibility)

Mueller-Hinton Agar + 5% Sheep Blood

Steps:

1. Preparation of MHA from a commercially available dehydrated base according to the manufacturer's instructions was done.
2. The agar was immediately after autoclaving; allow cooling in a 45 to 50°C water bath.
3. Add 50 mL of defibrinated sheep blood to 1 L of MHA
4. The freshly prepared and cooled medium was poured into glass or plastic, flat-bottomed Petri dishes on a level, horizontal surface to give a uniform depth of approximately 4 mm.
5. The agar was allow plates to cool further to room temperature and, would be used the same day, unless store in a refrigerator (2 to 8°C).

6. The plates were used within seven days after preparation unless wrapping in plastic, was taken to minimize drying of the agar.
7. A representative sample of each batch of plates was examined for sterility by incubating at 35 ± 2°C for 24 hours or longer.
8. The pH was checked after aseptic addition of the blood to the autoclave and cool medium. The final pH was the same as unsupplemented MHA, pH 7.2 to 7.4.

2.1 Principles and procedures for isolation and identification of *Streptococcus pyogenes*(hemolysis, gram stain, catalase ,bacteracin and PYR tests)

Hemolysis

I. Principle

The hemolytic reaction is particularly useful in the differentiation of the Streptococci. The hemolytic reaction is determined on agar media containing 5% sheep blood. Sheep blood is used because of the convenience in testing throat swabs for ß-hemolytic streptococci.

II. Inoculum

Pure culture on agar media containing 5% sheep blood

III. Reagents

1. Base medium
2. 5% sheep blood agar.

IV. Procedure

1. Streak culture for isolation on 5% sheep blood.
2. Incubate plate at 35ºC-37ºC in CO_2 for 24 h.

V. Reading and Interpretation

A beta-hemolytic reaction was interpreted as complete clearing around the colony.

Gram Stain

I. Principle

The gram stain is used to differentiate gram-positive and gram-negative bacteria. Cellular morphology can also be determined. Gram-positive and gram-negative bacteria are both stained by crystal violet. The addition of iodine forms a complex within the cell

wall. Addition of a decolorizer removes the stain from gram-negative organisms due to their increased lipid content. These cells are stained pink with the counter stain safranin.

II. Specimen

The gram stain can be performed on the growth of any strain grown on any type of media.

III. Reagents and Material

1. Crystal Violet Stain
2. Gram Iodine
3. Decolorizer Solution
4. Methanol
5. Slides
6. Inoculating loop
7. Microscope with Immersion Objective

IV. Procedure

1. Single loop of culture from 5% sheep blood was spread to a microscope slide. Spread the culture over 1/3 to 1/2 to the total area of the slide.
2. The smear was allowed to air dry. It was done up to 1 hour depending on the temperature and humidity of the room.
3. Cover the entire bacterial smear with 3 or 4 drops of methanol to fix the smear and allow to air dry.
4. The bacterial smear was covered with crystal violet stain and allows standing for 1 minute. Gently wash the stain off with cool tap water and drain water from slide.
5. The smear was covered with grams iodine and allows standing for 1 minute. Gently wash the iodine off with water and drain the water from the slide.
6. The smear was rinsed with decolorizer solution for 10 seconds; decolorization was completed when the solution runs clear from the slide. Gently rinse with water and drain the slide.
7. The smear was covered with safranin stain, and allowed to stand for 1 minute, then gently wash the stain from the slide.
8. The slide was blotted dry with absorbent paper and the slide was examined under oil immersion lens.

V. Reading and Interpretation

The gram stain is used to aid in the differentiation of the gram positive cocci. The arrangement of the cells divide on random planes form grape-like clusters of cells is

staphylococci. Streptococci divide on one plane form pairs and eventually form chains if the cells remain attached to each other.

Catalase Test

I. Principal

Hydrogen peroxide is used (H_2O_2) to determine if bacteria produce the enzyme catalase.

II. Specimen

Culture growth on a blood free media or colony growth on blood agar plate carefully transfers in to a slide without carry-over of any of the erythrocytes. Culture growth was typically seen at 35ºC-37 ºC for 24hrs in CO_2.

III. Reagents and Materials

1. Three percent hydrogen peroxide will be obtained from a commercial drug store.
2. Pipette
3. Slides

IV. Procedure

1. The catalase test was best performed by very carefully removing a colony of growth from a blood agar plate with a plastic needle or wooden applicator stick and transferring the colony to a glass slide. A drop of 3% hydrogen peroxide was added to the colony on the slide and observed for effervescence.

V. Reading and Interpretation

Any sign of bubbling will be interpreted as a positive test. The absence of bubbling will be interpreted as negative.

Bacitracin Test

I. Principle

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

II. Inoculum

An overnight culture will be grown on 5% sheep blood agar incubated at 35ºC in CO2.

III. Reagents and Materials

1. bacitracin "A" disk (BBL)

IV. Procedure

1. A beta-hemolytic colony was selected and heavily inoculated on 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.
4. Incubate plate overnight in CO_2 at 35ºC.

V. Reading and Interpretation

Any zone of inhibition will be considered as a positive test or sensitive test.

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

VI. Quality Control

Quality Control was performed on each shipment and lot of bacitracin disk.

Pyrrolidonylarylamidase Test (PYR)

I.Principle

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

II. Inoculum

A bacterium was grown on blood agar plates at 35ºC-37 ºC in CO_2 for 24-48hr incubation. The bacteria to be tested are grown on a blood agar plate until sufficient growth is seen to heavily inoculate the disks.

III. Reagents and Materials

1. PYR disk
2. PYR reagent
3. Loops
4. Deionized Sterile water

IV. Procedure

1. The disks were putted on blood agar plate in an area of little or no growth or on a slide. The moisture from the plate was usually sufficient to rehydrate the disk.

2. The disks were heavily inoculated on culture by using a loop or wooden stick,. Using two or more loop-fulls of culture was necessary for satisfactory results.

3. The plates were left with the disks on the bench at room temperature for 10 minutes.

4. The detection and reading was done after 3 minutes

V. Reading and Interpretation

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

VI. Quality Control

Each lot and shipment of PYR disks was tested for positive and negative reactions

2.2. Principles and procedures for Reagents preparation (for antibiotic tests)

McFarland Turbidity Standard

1. 0.048 mol/L $BaCl_2$ (1.175% w/v $BaCl_2 \cdot 2H_2O$) stock solution was prepared.
2. 0.18 mol/L (0.36 N) H_2SO_4 (1% v/v) stock solution was prepared.
3. With constant stirring to maintain a suspension, add 0.5 mL of the $BaCl_2$ solution to 99.5 mL of the H_2SO_4 stock solution.
4. The correct density was verified for the turbidity standard by measuring absorbance using a spectrophotometer with a 1-cm light path and matched cuvettes. The absorbance at 625 nm was 0.08 to 0.13 for the 0.5 McFarland standards.
5. The barium sulfate suspension was transferred in 4- to 6-mL aliquots into screw-cap tubes of the same size as those used for standardizing the bacterial inoculum.
6. The tubes were tightly sealed and stored in the dark at room temperature.
7. The barium sulfate turbidity was vigorously agitate standards on a vortex mixer before each use and was inspected for a uniformly turbid appearance. Mix latex particle suspensions by inverting gently, not on a vortex mixer.
8. The barium sulfate standards were replaced or their densities verified monthly.

3. SOPs for Inoculum Preparation for Disk Diffusion Tests

3.1. Turbidity Standard for Inoculum Preparation

$BaSO_4$ turbidity standard equivalent to a 0.5 McFarland standard was used to standardize the inoculum density for a susceptibility test.

Inoculum Preparation

1. The inoculum was prepared by making a direct broth suspension of isolated colonies selected from an 18- to 24-hour MHA agar with 5% sheep blood agar plate.
2. The suspension was adjusted to achieve a turbidity equivalent to a 0.5 McFarland standard. The result was performed visually, by using adequate light to compare the inoculum tube and the 0.5 McFarland standards against a card with a white background and contrasting black lines.

3.2. SOPs Procedure for Performing the Disk Diffusion Test

Inoculation of Test Plates

1. The inoculum suspensions, within 15 minutes after adjusting the turbidity was dipped a sterile cotton swab into the adjusted suspension. The swab was rotated several times and press firmly on the inside wall of the tube above the fluid level. This was removes excess fluid from the swab.

2. The dried surface was inoculated on an MHA plate by streaking the swab over the entire sterile agar surface. This procedure was repeated by streaking two more times, rotating the plate approximately 60° each time to ensure an even distribution of inoculum. As a final step, the rim of the agar was swabbed.

3. The lid ajar was left for three to five minutes, but no more than 15 minutes, to allow for any excess surface moisture to be absorbed before applying the drug-impregnated disks.

Test Procedure

1. Selected antimicrobial disks were applied on the surface of the inoculated agar plates by using standard forceps. Each disk was pressed down to ensure complete contact with the agar surface. They were distributed evenly, no closer than 24 mm from center to center. < 9 disks was placed on one 1 50-mm plate,(or <4 disks on a 1 00-mm plate) as its availability.

2. The plates was inverted and placed in an incubator set to 35 ± 2°C within 15 minutes after the disks were applied.

3. The plates were incubated an atmosphere of 5% CO_2 or in candle jar for 20 to 24 hours before measuring the zones of inhibition

Reading Plates and Interpreting Results (CLSI, 2012)

Antibiotics agent	Disk content	Zone Diameter Interpretive Criteria nearest whole		
		Susceptible	Intermediate	Resistant
Amoxycillin	10µg	≥24	–	–
Ceftraxon	30 µg	≥24	–	–
Erythromycin	15 µg	≥21	16-20	≤15
Chloramphenicol	30 µg	≥21	18-20	≤17
Penicillin	10U	≥24	–	–
Vancomicine	30µg	>17	–	–
Tetracycline	10 µg	≥23	19-22	<18

Declaration

I, the undersigned, declare that this thesis is my original work, has not presented for a degree in this or any other university and that all sources of materials used for the thesis have been fully acknowledged.

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