

**ADDIS ABABA UNIVERSITY, COLLEGE OF HEALTH  
SCIENCES, SCHOOL OF MEDICINE  
DEPARTMENT OF MICROBIOLOGY, IMMUNOLOGY &  
PARASITOLOGY**



**PREVALENCE AND RISK FACTORS OF PNEUMOCOCCAL COLONIZATION OF THE  
NASOPHARYNX AMONG CHILDREN ATTENDING KINDERGARTEN, BAHIR DAR,  
NORTH WEST ETHIOPIA**

**By: Fetlework Bereded Tefera(BSc.)**

**A Thesis Submitted to the School of Graduate Studies of Addis Ababa University in Partial  
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**April, 2015  
Addis Ababa, Ethiopia**

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**April, 2015**

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## **APPROVED BY THE BOARD OF EXAMINERS**

This thesis by Fetlework Bereded is accepted in its present form by the board of examiners as satisfying thesis requirement for the degree of Masters in Medical Microbiology.

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## List of Abbreviations

AAU-Addis Ababa University

ACIP-Advisory Committee on Immunization Program

ARI-Acute Respiratory Infection

CAP-Community-Acquired Pneumonia

CDC-Centre for Disease Control and Prevention

CHS-Collage of Health Science

CI-Confidence Interval

COPD -Chronic Obstructive Pulmonary Disease

GAPPD-Global Action Plan for the Prevention and Control of Pneumonia

GDC-Group Day Care

HIV-Human Immune Deficiency virus

IMCI -Integrated Management of Childhood Illness

IPD-Invasive Pulmonary Disease

MALDI-TOF-Matrix –Assisted Laser Desorption Ionization time Of Flight

NP-Nasopharyngeal

OPD-Out Patient Department

OR-Odds Ratio

PCV-Pneumococcal Conjugate Vaccine

PPV-Pneumococcal Polysaccharide vaccine

SOP-Standard Operational Procedure

STGG-Skim Milk-Tryptone-Glucose-Glycerol

WHO-World Health Organization

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## Abstract

**Background:** One of the most important potential pathogens found in the microflora of the nasopharynx is *Streptococcus pneumoniae*. *S. pneumoniae* (*pneumococcus*) is a major cause of disease, ranging from uncomplicated respiratory tract infections to severe invasive pneumococcal disease (IPD).

**Objective:** The aim of this study was to determine the prevalence of *Streptococcus pneumoniae* carriage, antimicrobial resistance pattern, and risk factors of pneumococcal colonization of the nasopharynx among children  $\leq 6$  years of age attending kindergartens in Bahir Dar, North West Ethiopia.

**Methods:** A cross-sectional study was conducted from February 2014 to June 2014 in Bahir Dar, Ethiopia. Convenient sampling technique was used to collect the sample. A total of 239 healthy children were enrolled from four governmental and three private kindergartens. A calcium alginate tipped swab on a flexible aluminium shaft was used to collect nasal swab. Standard techniques of culture on blood agar were used to identify *S. pneumoniae*. The antibiotic susceptibilities of isolates assessed by the disc diffusion method. Data was analyzed using the SPSS version 20.0. Chi-square tests were used for analysis was used to determine demographic characteristics and prevalence of each isolated organism. A p-value of  $<0.05$  was regarded as statistically significant.

**Result:** The overall carriage prevalence of *Streptococcus pneumoniae* was 44.8% (n=107) and crowded living (AOR = 0.459; CI, 0.570-2.439), earlier antibiotic use within 2-4 weeks (AOR = 8.004 CI, 1136- 56.409), and presence of siblings  $< 5$  years old at home (AOR = 0.467; CI, 0.234-0.933) were associated with pneumococcal carriage. Pneumococcal carriage was not associated with sex, family size, breast-feeding. Six (5.6%) *S. pneumoniae* isolates were resistant to ceftriaxone, 9 (8.4%) to chloramphenicol, 47 (43.9%) to trimethoprim-sulfamethoxazole (SXT), 17 (15.9%) to erythromycin, 10 (9.3%) to penicillin and 24 (22.4%) to tetracycline. Ninety three (86.9) *S. pneumoniae* isolates were susceptible to Ceftriaxone 93 (86.9%) and 95 (88.8%) were susceptible to Chloramphenicol.

**Conclusions:** Colonization of the nasopharynx in children attending kindergartens in Bahir Dar was high. Half of the isolates of *S. pneumoniae* were resistant to trimethoprim-sulfamethoxazole (SXT).

**Key words:** Nasopharyngeal carriage; *S. pneumoniae*; Antimicrobial susceptibility; Children; Ethiopia

## INTRODUCTION

### Background

The pneumococcus is one of the most important pathogens. Human upper respiratory tract is the reservoir of a diverse community of commensals and potential pathogens (pathobionts), including *S. pneumoniae* (pneumococcus), *H. influenzae*, *M. catarrhalis*, and *S. aureus*. To cause respiratory disease, bacteria first need to colonize the nasopharyngeal niche. Colonization of this niche is a dynamic process: acquisition and elimination of species, interactions among microbes and between microbes and the host, and interference by environmental factors are suggested to cause a dynamic and complex microbial interplay (Bosch *et al.*, 2013).

Thirty to 70% of young children carry *S. pneumoniae* in their nasopharynx, and up to 40% of the carriers are colonized with penicillin-non susceptible *S. pneumonia* (PNSP) but the natural reservoir of pneumococci is the mucosal surface of the upper respiratory tract of humans and may spread locally to cause upper or lower respiratory tract infection. In some circumstances, the organisms are able to enter the bloodstream from the nasopharynx via the cervical lymphatic, leading to bacteraemia and, occasionally infection of other organ systems. The asymptomatic nasopharyngeal carriage of *S. pneumoniae* is widely prevalent in young children and has been related to the development of disease and the spread of the pathogen (Marchisio *et al.*, 2002, Zitta *et al.*, 2012). *S. pneumoniae* can cause a wide variety of clinical symptoms, either by direct extension from the nasopharynx or by invasion and haematogenous spread. High-risk groups children younger than 5 years, particularly aged 2 years or younger; adults and older than 55-65 years, conditions that cause immune deficits (eg, HIV infection, malignancy, or diabetes mellitus) (Bogaert *et al.*, 2006, Monteros *et al.*, 2012).

Pneumococcus is transmitted by direct contact with respiratory secretions from patients and healthy carriers, who may carry pneumococcus in their nose or throat to household contacts or adults, are the major cause of nasopharyngeal carriage and invasive pneumococcal disease (IPD). The incubation period varies by site of infection, and can be as short as 1-3 days. Infection

can occur at any time throughout the year but rates peak during the winter months (Kuo *et al.*, 2011).

Young children are considered to be the most important carrier for horizontal dissemination of bacterial strains within the community. Having siblings and day-care attendance were shown to be independent factors associated with carriage and acute respiratory tract infections. The rates of nasopharyngeal carriage are 44% among all children six years old or younger, 60 to 80 % among children attending child care centres outside the home (Hill *et al.*, 2008, Scotet *et al.*, 2001). Children attending day-care centers (DCCs) are at higher risk of carriage of *S. pneumoniae* in general and antibiotic-resistant *S. pneumoniae* in particular, than are children who are cared for at home (Givon-Lavi *et al.*, 2002).

Pneumonia and pneumococcal infection remains the leading cause of mortality worldwide. Mortality is highest in patients who develop sepsis or meningitis (Vernt *et al.*, 2011). In developing countries, deaths from pneumococcal disease are common in children under 5 years. Case fatality rates may be up to 20% for pneumonia, and as high as 50% for meningitis in developing countries. Lack of exclusive breastfeeding, nutritional deficiencies, and indoor air pollution are risk factors for pneumonia including pneumococcal pneumonia in infants and young children. Pneumonia killed an estimated 935,000 children under the age of five in 2013, accounting for 15% of all deaths of children under five years old. Transmission from person to person is by droplet infection (Greenwood *et al.*, 1999, Mills *et al.*, 2015, Vernt *et al.*, 2011).

Carriage is generally higher in developing countries and among economically deprived populations. Pneumonia affects children and families everywhere, but is most prevalent in South Asia and sub-Saharan Africa (Greenwood *et al.*, 1999, Usuf *et al.*, 2014).

The first vaccine, a seven-valent pneumococcal conjugate vaccine (PCV7), targeting children was first licensed in the United States in 2000. The European Union (EU) authorised PCV7 in 2001 for use in children aged between the age of two months and five years (Decarvalho *et al.*, 2009), PCV7 was introduced in Brazil in 2001, but it was available to a small portion of the population (Pidade *et al.*, 2013), in Greece in October 2004 and formed part of the national

immunization schedule in January 2006(Katarolis *et al.*, 2009) in Taiwan until October 2005(Kuo *et al.*, 2011). The vaccine introduced in Ethiopia in 2011 is called Synflrix™. It is a safe and effective vaccine that protects against 10 common serotypes that cause pneumococcal disease and is therefore referred to as PCV10. Is a liquid vaccine that is provided in a 2-dose vial (Federal Ministry of Health, 2011).

### Basic Characteristics and Identification

Pneumococci are Gram positive, non-motile, non-sporeforming, coccoid bacteria. Like many other streptococci, they are aero tolerant (facultative) anaerobes. They lack the enzyme catalase, which is required to neutralize the large amounts of hydrogen peroxide produced by the bacteria, and they therefore need to be cultured on media with capacity to neutralize hydrogen peroxide, for example blood agar. Hydrogen peroxide is oxidizing haemoglobin to green methaemoglobin, which is observed as greenish haloes around the bacterial colonies on blood agar; a phenomenon called alpha haemolysis (Huang *et al.*, 2008, Klugman, K.P *et al.*, 2009).

Under anaerobic conditions they switch to beta haemolysis caused by an oxygen-labile haemolysin. Growth is enhanced by incubation in 5% carbon dioxide. On Gram stain, pneumococci often appear in pairs, and were therefore formerly named *Diplococcus pneumoniae*, although single cells and small chains also appear. Later, they were found out to be members of the streptococcus family, and the name was changed to *S. pneumoniae* in 1974. In the routine laboratory, the presumptive diagnosis of pneumococci is based on recognition of typical colony morphology on blood agar and sensitivity to optochin(ethylhydrocupreine) and bile (they undergo lysis when sodium deoxycholate is added to the culture. Two different morphologies occur, depending on the amount of capsule which is produced by the strain. Most common are round smooth umbilicated colonies, 0.5-1.0 mm in diameter, whereas heavily encapsulated strains, especially serotype 3, form mucoid dome shaped colonies with a diameter of up to 5 mm. (Backhaus *et al.*, 2011, Facklam R. *et al.*, 2002, Klugman, K.P. *et al.*, 2009).

## Cell wall composition and serotyping

Like other Gram positive organisms, the pneumococcus has a cell wall containing a peptidoglycan layer and teichoic acid in larger amount. The polysaccharide substance (Slime layer) around the cell wall secreted by the bacterium becomes thick and forms a capsule. The cell wall is covered by a polysaccharide capsule that is immunogenic and its polysaccharide composition differs among strains. The capsular antigenic component of the organism is used to divide into serotypes (Huang *et al.*, 2008).

Over 93 capsular antigenic types (serotypes) have been recognised, each of which elicits type-specific immunity in the host. Serotypes are capable of causing serious disease in humans. Three recent studies illustrate this fact. Of 5619 strains isolated from patients with serious pneumococcal infections in Belgium, 64 different types were identified. All but four of 46 serogroups were identified. Finally, 298 strains from mostly European patients with invasive pneumococcal disease, 77 of them known 84 types were identified. Moreover, all of the six recently described types were found in patients with bacteraemia or meningitis. Although all capsular types may thus be involved in pneumococcal disease at some time, the frequency with which different types are isolated is different (Kalin *et al.*, 1998).

Including the polysaccharide capsule mentioned earlier, that help it evade a host's immune system, it has pneumococcal surface proteins that inhibit complement-mediated opsonization, and it secretes IgA1 protease that will destroy secretory IgA produced by the body and mediates its attachment to respiratory mucosa (Abdullahi *et al.*, 2012).

## Development of disease

The Gram-positive bacterium *S. pneumoniae* (pneumococcus) is the main cause of multiple disease conditions such as community acquired pneumonia, meningitis, otitis media, endocarditis, osteomyelitis, pericarditis, pyogenic arthritis, soft tissue infection, early-onset neonatal septicemia, lower respiratory infection and bacteremia (O'Brien *et al.*, 2009).

Development of pneumococcal disease is from the nasopharynx, pneumococci may spread via existing anatomical channels to other parts of the upper airways and cause mucosal disease. Most

common are infections in the middle ear, otitis media, and in the paranasal sinuses, sinusitis. Pharyngitis, tonsillitis and epiglottitis also occur. Pneumococci may also be inhaled in the lower airways, where they may cause bronchitis or pneumonia. In 20-30% of all culture verified cases of pneumococcal pneumonia, pneumococci are also found in the blood (Backhaus *et al.*2011).

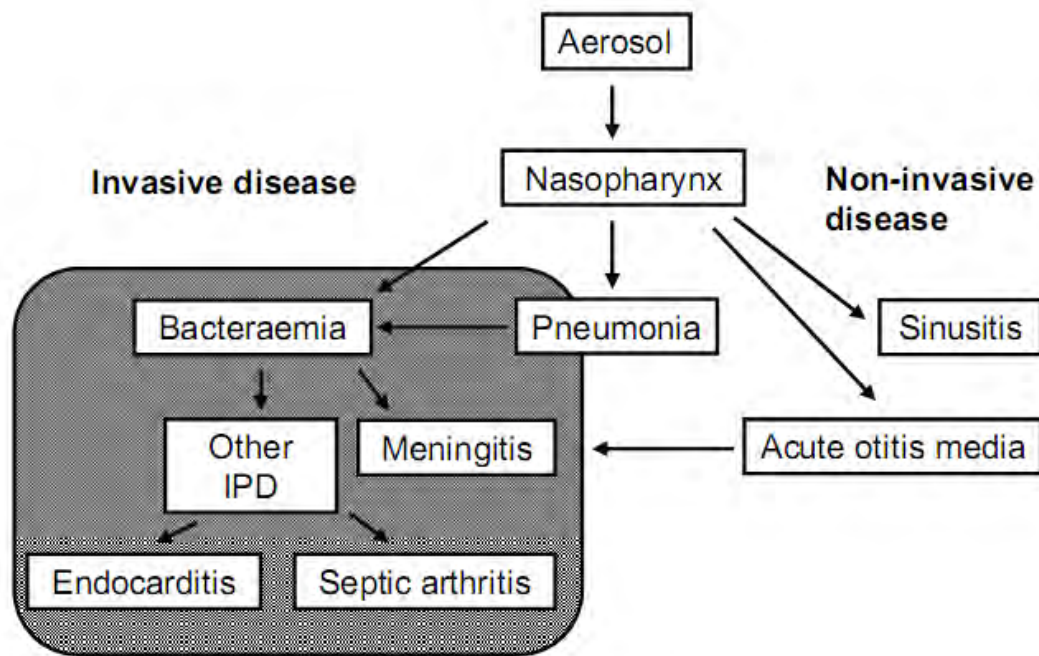


Figure 1: Development of invasive and non-invasive pneumococcal disease (Backhaus *et al.*2011).

Carriage is the first step in disease development and allows horizontal spreading within the community. The outcome of nasopharyngeal colonization is the composition of microflora in these niches, severe pneumococcal infection and its complications result partly from the direct actions of pneumococcal virulence determinants and partly from the host immune responses produce four key effects: adhesion, invasion, inflammation and shock (Gillespie *et al.*, 2000).

The nasopharyngeal (NP) carriage of pneumococcus is highly prevalent among young children and predisposes the carrier, his or her siblings, and others in close contact with the carrier to pneumococcal infection. Thirty to 70% of young children carry *S. pneumoniae* in their



nasopharynx, and up to 40% of the carriers are colonized with penicillin-nonsusceptible *S. pneumonia* (PNSP) (Regev-yochay *et al.*, 2004).

Specific clones are selected with either an invasive pneumococcal disease phenotype or a persistent colonization phenotype with low risk of tissue invasion. Success of the phenotype of invasive pneumococcal disease depends on its capacity for rapid disease induction and efficient person-to-person spread by coughing. By contrast, the non-invasive phenotype uses various surface adhesions, immune evasion strategies, and secretory defenses such as IgA1 protease and inhibitors of antibacterial peptides to help with long-term carriage within the nasopharynx (Gillespie *et al.*, 2000).

Out-of-home group childcare is associated with an increased risk of infectious illnesses including invasive pneumococcal disease (IPD). Carriage studies in child day care centers (CDCCs) have shown increased risk of pneumococcal nasopharyngeal (NP) colonization. CDCCs may act as foci for the emergence and transmission of antibiotic resistant organisms (Roche *et al.*, 2007).

The pneumococcal nasopharyngeal carriage rate in children in developing countries is generally two to three times higher than those found in children from industrialized countries. Crowding, close contacts with large number of siblings and frequent upper respiratory tract infection are likely to be important risk factors for disease but it is less clear if they play any role in carriage. High pneumococcal carriage rate are frequent in developing countries and are often associated with carriage more than one serotype in the Gambia 22% of the children carry pneumococci of more than one serotype (Abdullahi *et al.*, 2012). Duration of carriage also varies largely between different serotypes. Pneumococcal disease, both invasive and non-invasive, is thought to be preceded by a carrier state (Backhaus *et al.*, 2011). Children are the source of transmission to adults in the family (Regev-yochay *et al.*, 2004).

The incidence of bacterial pneumonia and associated mortality was already high in non-industrialised countries prior to the HIV epidemic and the commonest cause was *S. pneumoniae* accounts for at least one-third of these infections. Similarly, the commonest isolate from blood culture of HIV-infected African children hospitalised with severe pneumonia is *S. pneumoniae*.

A recent study from South Africa estimated the risk of invasive pneumococcal disease to be 40 times greater in HIV-infected children than in HIV-uninfected children. Among HIV-1 infected children, bacterial pneumonia occurs in 19% to 63% and is associated with bacteremia in 15% to 25%. Primary pneumococcal bacteremia is more common in HIV-1 infected children than in HIV-1 infected adults. In a population-based study of children with HIV-1 infection, risk factors for invasive pneumococcal disease included an AIDS diagnosis and high levels of total serum IgG and IgM compared with HIV-1 seropositive controls (Graham *et al.*, 2002).

### **Pathogenesis of pneumococcal disease**

The nasopharynx of children has resident microbial flora that do not usually harm the child but, in some cases, constitute a reservoir of pathogens implicated in respiratory tract infections and invasive diseases. The bacteria carried in the nasopharynx of healthy children reflect the infection-causing strains currently circulating in the community (Marchisio *et al.*, 2002).

Nasopharyngeal colonization by certain bacteria is an important risk factor for specific infections. In the case of *S. pneumoniae*, colonization of the nasopharyngeal mucosa, it is believed to precede the development of certain infections such as otitis media and pneumonia. Pneumococci colonize most of the upper respiratory tract but are recovered most frequently from the nasopharynx (Immergluck *et al.*, 2004).

The pathogenesis of *S. pneumoniae* is mediated by the host response to infection by the organism. The process begins with colonization of the nasopharynx and the oropharynx to a lesser extent. Colonization occurs when the organism gains access into the upper respiratory tract and binds to the host epithelial cells. This binding process is mediated by certain proteins called 'surface protein adhesins' released by the organism which aids the organism in attaching to host epithelial cells. Host defense against pneumococcal infection is mediated by both immunological and non-immunological mechanisms (Gillespie *et al.*, 2000, Jeena *et al.*, 2006).

Pneumococci are spread via direct contact with secretions from carriers, via saliva or are inhaled via an aerosol, where after they colonize the nasopharynx. They usually cause an influx of neutrophils, often resulting in a mild rhinorrhea without other symptoms, which promotes spread

to other hosts, but they are most often not cleared by the immune system until days to months later. The main reason for this is that the capsule protects the pneumococcus against killing by neutrophils. The length of the carriage period depends both on bacterial and host factors (Backhaus *et al.*, 2011). Certain proteins or enzymes displayed on the surface of pneumococci (e.g., hyaluronatase, autolysin, neuraminidases, PsaA and other choline-binding proteins) may contribute to the pathogenesis and disease symptoms (Gillespie *et al.*, 2000).

It has been proposed that attachment of pneumococci to the respiratory epithelium is mediated by a disaccharide receptor on fibronectin, which is present on all epithelial cells. Additionally, adherence of pneumococci to tracheal epithelial cells may be enhanced by prior influenza or parainfluenza virus infection, most likely mediated by viral neuraminidase. This enzyme cleaves sialic acid from glycosphingolipids, which are found in substantial amounts in human lung tissue). Thus, neuraminidase is thought to expose other structures that function as receptors to which pneumococci can adhere (Gillespie *et al.*, 2000).

### **Risk factors for pneumococcal carriage**

Certain environmental and host factors have been explored as possible risk factors for pneumococcal carriage in children. Factors identified to be associated with colonization such as age, ethnicity, immunosuppression including HIV-1 infection, socioeconomic factors like crowding, and temporal association to respiratory viral diseases widely affect the risk of IPD (Ercibengoa *et al.*, 2012, Granat *et al.*, 2007, Immorgluck *et al.*, 2004, Otsuka *et al.*, 2013).

NP colonization is common and usually asymptomatic. Pneumococci are often part of the nasopharyngeal flora; probably all humans are colonized with this organism at least once early in life. The risk of pneumococcal colonization is high, especially under conditions with crowding, such as day-care centres (DCCs), nursing homes, hospitals, and jails (Borgaert *et al.*, 2006, 2011). High carriage rates have been reported from several countries: 89.5% of healthy children attending at DCC in Northern Spain (Ercibengoa *et al.*, 2012), 75.3% in Peruvian children (Graniel *et al.*, 2011), 64% of children's attending at DCC in Netherlands (Borgaert *et al.*, 2001), 60.9% children in Japan (Otsuka *et al.*, 2013) and in Kumasi, Ghana 51.4% (Austrian *et al.*, 1981) and low carriage rate also reported in studies like in Taiwan 14.1% (Kauo *et al.*, 2011), 16.2% in U.S

healthy children (Immorgluck *et al.*, 2004), in Sao Paulo, Brazil 16.4%(Berezinet *et al.*, 2007), in Belgian infants attending at DCC 21%(Malfroot *et al.*, 2004), 27.9% in India(Ravi *et al.*, 2014), in Greece 29.4%(Katsarolis *et al.*, 2009), healthy children attending at DCC in 12 states in Mexico 29.9%(Espinosa *et al.*, 2007). It is believed that factors such as crowding, large number of siblings and frequent upper respiratory tract infections as seen in developing countries could contribute to the higher carriage rates observed in developing countries compared to the industrialized countries.

### **a. Age**

The nasopharynx of preschool children is the ecological niche of *S. pneumoniae* (Katsarolis *et al.*, 2009). The incidence of pneumococcal carriage is highest in young children, peaking during the first 2 years of life and starting to decrease gradually after the age of 3–5 years in most developed country populations (Birgit Simell *et al.*, 2012). High prevalence (85%) in children were recorded in Ethiopia, Mozambique and Gambia (Usuf *et al.*, 2014). In Mexico, Children under five years of age who attend day-care centers are at a greater risk of being asymptomatic nasopharyngeal carriers and consequently have a greater risk of developing pathological processes related to *S. pneumoniae*. Sixty-five percent of pneumococcal infections occur in children under two years of age. This rises to 85% in children under four years of age (Espinosa-de los Monteros LE *et al.*, 2007).

Young children who have older siblings attending DCCs tend to have more respiratory diseases than do those who do not have older siblings in DCCs (Givon-Lavi *et al.*, 2002).

### **b. Crowding**

The risk of pneumococcal colonization is high, especially under conditions with crowding, such as day care centers (DCCs), nursing homes, hospitals, jails, military camps and prisons, is associated with an increase in the transmission of pneumococci among individuals (Hoge *et al.*, 1994, Bogaert *et al.*, 2001, 2006). Daycare or school attendance or having a sibling that attends day-care has been strongly associated with IPD. Day-care center (DCC) attendance has

specifically been cited as a predisposing factor for IPD due to resistant pneumococci (Roche *et al.*, 2007).

In a longitudinal study of nasopharyngeal carriage in children attending day care in Sweden, carriage was higher in children attending day-care centers with over 45 children in the class compared to those with less than 45 children in the class (Paiet *et al.*, 2005). This further emphasizes the importance of crowding as a risk factor for pneumococcal carriage in children.

### **c. Respiratory tract infections**

Acute respiratory infection (ARI) is the main cause of child morbidity and mortality in developing countries. In Brazil, ARI causes 8% of deaths among children younger than five years. National and international data from developing countries confirm pneumococcus as the main etiologic agent of pneumonia and acute otitis media in children, especially in those younger than five years. Human nasopharynx is the main site of acquisition of pathogenic bacteria in the respiratory tract. Most individuals maintain a commensal relation, establishing a status of asymptomatic colonization (Rosen *et al.*, 1984).

Pneumococcal carriage has been shown to increase in the presence of respiratory infection. Respiratory infections are known to undermine the integrity of the mucosal epithelium thereby enhancing the ability of the pneumococcus to adhere to the epithelium (Korona *et al.*, 2012).

This could then lead to increased carriage rates. The nasopharynx of children has resident microbial flora that do not usually harm the child but, in some cases, constitute a reservoir of pathogens implicated in respiratory tract infections and invasive diseases (Berezin *et al.*, 2007).

### **d. Siblings**

The presence of a colonized sibling has been documented as one of the strongest risk factors for pneumococcal carriage in infancy (Dunais *et al.*, 2003). This has been observed in both the developed and developing country settings. In separate studies conducted in Israel and Finland, infants have been shown to acquire pneumococci from their older siblings. A study in Israel study compared pneumococcal carriage in adults with that of children aged 6 years and below

and identified young age and having young siblings as important risk factors for carriage in children (Syrjanen *et al.*, 2001). In a second southern Israeli study (Givon-Lavi *et al.*, 2002), pneumococcal strains acquired by younger siblings were compared with those present in day care centers in order to determine the association between carriages among infants cared for at home and carriage among their older siblings who attended day care centers. One or more strains identical by serotype and antibiotic susceptibility were isolated in the older sibling's day care centre in 76% of cases compared to 32% - 63% in all other day care centers. In the last study, nasopharyngeal swabs were collected on 10 occasions from 100 children and their family members (Regev-yochay *et al.*, 2004).

#### **e. Breast –feeding**

Breastfeeding has been shown to be protective against otitis media and some respiratory tract infections (Levino *et al.*, 2001). Protection against invasive pneumococcal disease has also been demonstrated in a case control study to identify risk factors for invasive pneumococcal disease in children aged 2 -59 months (Chantry *et al.*, 2006). But studies investigating the role of breastfeeding in pneumococcal carriage have shown conflicting results. Rosen et al analyzed antibodies to pneumococcal polysaccharides in human milk obtained from mothers and their effect on nasopharyngeal colonization of their infants (Levine *et al.*, 1999). This study showed that pneumococcal capsular antibodies in human milk did not protect against carriage. Similarly in a study of nasopharyngeal colonization of respiratory pathogens among infants aged 1 – 2 months, Kaleida et al did not demonstrate any difference in rates of colonization between exclusively breastfed and exclusively bottle fed infants (Rosen *et al.*, 1996).

On the other hand, Duffy et al. observed lower rates of pneumococcal carriage in exclusively breastfed compared to formula fed infants in a longitudinal follow up of new born infants to assess the relationship of exclusive breastfeeding to episodes of acute otitis media (Kaleida *et al.*, 1993).

## **f. Previous antibiotic use**

Pneumococcal resistance to antibiotics is increasing globally. In Belgium in 2002, the Pneumococcal Reference Laboratory in Leuven recorded resistance rates for invasive *S. pneumonia* isolates of 15.1% for penicillin (MIC 0.12– 2 mg/ L), 30.7% for tetracycline and 36.1% for erythromycin, with this resistance being more important in children than in the elderly. Most studies investigating risk factors for antibiotic resistance in pneumococci have identified antimicrobial use as a major determinant. Other risk factors for carriage of resistant pneumococci are young age, attendance at day care centres, and previous hospitalization (Malfroot *et al.*, 2004).

Exposure to antibiotic treatment during the previous 3 months was similar in both groups. Unlike other studies, in this survey recent antibiotic treatment was not associated with carriage of a resistant strain, even in the child minder setting where horizontal transmission has less chance of occurring (Backhaus *et al.*, 2011, Espinosa *et al.*, 2007). This may be related to the high carriage rates which can mask the effect of antibiotic use (Rosen *et al.*, 1996).

Finally, previous antibiotic usage has been associated with an increased risk of IPD caused by a resistant strain of pneumococcus. Specifically, prior treatment with  $\beta$ -lactam antibiotics has been associated with IPD due to a drug-resistant strain. In a case-control study of children 2-59 months old, IPD due to a penicillin-resistant pneumococcal strain was independently associated with at least one course of antibiotics in the previous three months (Kaleida *et al.*, 1993). Prior antibiotic usage may alter an individual's nasopharyngeal flora and increase the likelihood of carrying resistant pneumococci that can cause IPD under opportunistic conditions (Gary *et al.*, 2000).

## **Diagnostic Methods**

Pneumococcal infection and disease can affect a variety of organ systems resulting in a number of disease syndromes. Diseases caused by pneumococcus include severe diseases such as pneumonia, meningitis and bacteraemia (presence of bacteria in the blood), and milder diseases such as middle ear infection (otitis media), sinusitis and bronchitis (WHO, 2013).

Conventional diagnosis of *S. pneumoniae* consists of two stages: first, determining the syndrome by history, clinical examination, and chest radiology; and second, determining the etiology by microbiological, serological, and molecular tests (Scott *et al.*, 2008).

Laboratories may receive nasopharyngeal (NP) swabs in the course of prevalence surveys and carriage studies of respiratory organisms. Culture methods for this type of specimen are included below. Swabs taken from the upper respiratory tract (*e.g.*, the nasopharynx) to inoculate the primary culture medium; the nasopharyngeal swab should be rolled over one-fourth of the plate (*i.e.*, one quadrant). For *S. pneumoniae*, the selective medium is a tryptone soy agar plate containing 5% sheep or horse blood and 5 µg/ml of gentamicin sulfate. If one swab is being collected for recovery of both *S. pneumoniae* and *H. influenzae*, the blood agar and gentamicin plate should be inoculated first, followed by the inoculation of the chocolate agar and bacitracin plate (because *S. pneumoniae* is more susceptible to the antibacterial activity of the bacitracin than *H. influenzae* is to the antibacterial activity of gentamicin)(CDC,2002).

Pneumococcus can be identified by different types of laboratory techniques. These include the simple and rapid technique of Gram stain to the complicated method of identification of the organism at the gene level. The simplest method is observing Gram positive cocci in pair under the microscope from a clinical sample. The gold standard is the culture method, isolating live organisms and doing further identification as described above and antimicrobial susceptibility test (Cheesbrough *et al.*, 2006, Todar *et al.*, 2012).

A major problem with cultures in both severe and non-severe disease is the low sensitivity. Sometimes the cultures are negative because the patient has received antibiotics prior to culture. On some occasions, the cultures remain negative despite optimal sampling of specimens. One reason for this is that pneumococci are fastidious, in the sense that they have complicated nutritional requirements (Ruiz-Gonzalez *et al.*, 1999).

Recently, an abundant mass of studies have been published using various polymerase chain reaction (PCR) tests, either pneumococcal specific or multiplex methods on various specimens, for example sputum, broncho alveolar lavage (Strålin *et al.*,2006), nasopharyngeal



specimens (Strålin *et al.*, 2006), trans thoracic lung aspirates (Carrol *et al.*, 2011) blood (Resti *et al.*, 2010) and CSF (Abdeladim *et al.*, 2010). New diagnostic tools, especially Matrix-assisted laser desorption/ionization – time of flight (MALDI-TOF) mass spectrophotometry, with capacity to analyze large molecules, such as DNA, proteins, peptides and polysaccharides, is now entering the routine diagnostics. It provides reliable species determination for a vast number of clinically relevant species much faster than the old phenotypic methods (Bizzini *et al.*, 2010).

Serological analysis of paired sera is another useful complement method. Serotype determination of invasive pneumococcal strains has no value in the clinical routine diagnostics, but it is crucial in surveillance for evaluation of pneumococcal vaccine effects. The Quellung method, based on a method from the 19<sup>th</sup> century, has remained the gold standard (Lund *et al.*, 1978). Although latex agglutination kits (Slotved *et al.*, 2004) have simplified the process to a certain extent, serotyping is still expensive and time consuming. Therefore new PCR-based serotyping methods have been developed (Mc Ellistrem *et al.*, 2009).

### **Management of Pneumococcal Diseases**

The use of antibiotics has dramatically reduced mortality due to pneumococcal disease. Penicillin is the treatment of choice for both severe and non-severe pneumococcal infections, unless resistance or hypersensitivity forces the physicians to choose other alternatives. Cephalosporins and carbapenems are usually highly active alternatives in severe disease. Pneumococci are naturally resistant to aminoglycosides, such as gentamycin, but when administered together with penicillin, a synergistic effect exists in vitro at least in penicillin non-susceptible *Streptococcus pneumoniae* (PNSP). Fluoroquinolones, tetracycline and co-trimoxazole are also usually effective, but should be regarded as second or third line drugs in the treatment of pneumococcal infections with known etiology (Anadiotis *et al.*, 2002).

Rates of resistance to cotrimoxazole among strains of *S. pneumoniae* and *H. influenza* are variable but generally very high amoxicillin is now preferred as a first-line treatment for pneumonia, based in part on the finding that more severe cases do better with amoxicillin (Scott *et al.*, 2008). Currently, the burden of antimicrobial resistance is very high all over the world including the developing countries. Antimicrobial resistance is specific for some strains of

pneumococcus and serotypes 6A, 6B, 9V, 14, 19F, 23F are responsible for most of the resistance to penicillin and other related antibiotics (Martín-Galiano *et al.*, 2003, CLSI, 2012). Multi drug resistant pneumococci are spreading all over the world and become a great public health concern (WHO, 2013). During the era of vaccine, another challenge is that strains not covered by the vaccine may replace vaccine strains become more resistant to antibiotics that are primary choices for the treatment of pneumococcal infection. (Martín-Galiano *et al.*, 2003, CLSI, 2012). The proportion of children with pneumonia caused by resistant strains might fall with the introduction PCVs (Scott *et al.*, 2008).

The recent finding that oral amoxicillin is equivalent to parenteral antibiotics for the management of severe pneumonia has prompted some agencies to recommend home treatment of severe pneumonia (Scott *et al.*, 2008).

The effective management of cases of pneumonia relies on appropriate antibiotic therapy and supportive care, particularly with oxygen in more severe cases. The WHO has promoted case management at the level of community health worker and first-level health facility. Since 1992, the management of cases of childhood pneumonia has been incorporated into a broader strategy known as Integrated Management of Childhood Illness (IMCI), which was developed by the WHO and is now the standard of care for sick children in most developing countries. So far, IMCI has only been introduced into communities that already have access to health care, thus excluding children whose risk of mortality is highest (Scott *et al.*, 2008).

### **Pneumococcal vaccines**

The first preventive strategy against pneumococcal disease was introduced by Sir Almroth Wright in 1911 when he suggested that the inoculation of killed whole pneumococci might protect against pneumococcal infections. Prevention of infections caused by *S. pneumoniae* and of spread of this pathogen is an important goal of an effective vaccine. Therefore, new vaccines have been developed that are also immunogenic in risk groups, such as young children, the elderly, and immunocompromised patients (Bogaert *et al.*, 2001).

Immunization has enormous potential to reduce the burden of childhood deaths from pneumonia in developing countries. Vaccines are in development against several pathogens that cause pneumonia, including *S. pneumoniae*, parainfluenza virus, *S. aureus*, and *M. tuberculosis*. Immunoepidemiological studies in developing countries suggest that childhood exposure through nasopharyngeal carriage and intermittent pneumococcal invasion generates immune responses to non capsular antigens (Scott *et al.*, 2008).

There are two different types of vaccines currently available against *S. pneumoniae*, the polysaccharide vaccine and the conjugate vaccines (Pittet *et al.*, 2012).

#### **a. Pneumococcal Polysaccharide Vaccines**

Pneumococcal polysaccharide vaccine (PPV-23) which is available today, it contains purified capsular polysaccharides from 23 of the most common serotypes isolated from patients with invasive disease. Several observational studies have indicated good protective effectiveness against both IPD and pneumococcal pneumonia, especially among certain risk groups and the elderly, and the vaccine is therefore recommended by WHO and CDC. PPV gives rise to a T-cell independent immune response, and is therefore not evoking memory B-cells and as a result, there is no booster response on repeated vaccination. Another consequence of the T-cell independent mechanism of action is that it is not immunogenic in children younger than 2 years, and the immunogenicity in elderly individuals and immunodeficient patients is variable (Huss *et al.*, 2009).

#### **b. Pneumococcal Conjugate Vaccines**

The conjugate vaccine is available in four forms, PCV7, PCV9, PCV10 and PCV13 covering seven, nine, ten and thirteen serotypes respectively. PCV13 is the newest of them all and it covers for serotype 1,3,4,5,6A,6B,7F,9V,14,18C,19A, 19F and 23F. First licensed in 2000, the 7-valent pneumococcal conjugate vaccine (PCV7), Prevnar® (Wyeth Vaccines), is currently the only pneumococcal conjugate vaccine intended for use in infants and young children. Recent trials using a 9-valent pneumococcal conjugate vaccine (PCV9), covers for serotype 1, 4, 5, 6B, 9V, 14, 18C, 19F, 23F (Wyeth Vaccines) in The Gambia and in South Africa have demonstrated

efficacy against pneumonia and invasive pneumococcal diseases in developing country settings. Since then, vaccines of higher valence—a 10-valent vaccine (PCV10), Synflorix® (Glaxo Smith Kline) and a 13-valent vaccine (PCV13) (Wyeth Vaccines) have replaced the PCV9 in the pipeline (Kim *et al.*, 2010).

The first PCV, Prevenar®, contained polysaccharides from 7 serotypes (4, 6B, 9V, 14, 18C, 19F and 23F) known to be common causes of IPD among children in North America. It was introduced in the childhood vaccination program in the United States in 2000, and later in several other countries (Bogaert *et al.*, 2006). The pneumococcal conjugate vaccines (PCV) were developed to circumvent the inability of PPV to evoke an immune response in children below two years of age. The basic principle is that the capsular polysaccharide is conjugated to a carrier protein CRM197, which will be recognized by T-helper cells that will orchestrate the subsequent immunological response, including to production of antibodies also directed to the polysaccharide (Scott *et al.*, 2008). The introduction of the conjugate vaccines underscores the need for detailed and long-term epidemiological surveillance of *S. pneumoniae* (Bogaert *et al.*, 2001). The conjugation technique has been also successful in creating more immunogenic vaccines with higher protection against many other diseases e.g. *Haemophilus influenzae* type b, *Neisseria meningitidis* groups A, C, W-135 Y and *Salmonella typhi* (Scott *et al.*, 2008).

In January 2013, the FDA approved the pneumococcal vaccine Prevnar 13, or PCV13, for the prevention of invasive pneumococcal disease in children and adolescents between 6 and 17 years of age. In February, the CDC's Advisory Committee on Immunization Practices (ACIP) voted for the use of the vaccine in children with immune deficiencies. The panel recommends routine use of a single dose of PCV13 for children aged 6 to 18 years who have an immuno compromising condition (e.g., sickle cell disease, HIV infection) and have not previously received the vaccine (Katsarolis *et al.*, 2009).

PCV13, Prevenar13; Pfizer Inc. (formerly Wyeth) has replaced PCV7 and includes new serotypes in addition to the previous serotypes. PCV13 is as immunogenic as PCV7 for both the common serotypes and additional serotype.

In conclusion, PCV-13 might be a good alternative to PPV-23 in some adult risk patients with well known poor response to PPV-23, considering the advantages and shortages of both PCVs and PPV-23(Scott *et al.*, 2008).

Synflorix (GlaxoSmithKline, London, UK) is a ten-valent PCV also used in Europe. Based on the ELISA threshold, non-inferiority of this PCV10 compared with PCV7 was demonstrated for eight out of ten serotypes (it was not reached for 6B and 23F).

### **Pneumococcal disease in Ethiopia**

In Ethiopia communicable diseases are most common due to climatic variation, poor sanitary, and inaccessibility of health facilities, in spite of awareness (Federal Ministry of Health, 2011).

The major health problems of the country are largely preventable communicable diseases and nutritional disorders. More than 90% of child deaths are due to pneumonia, diarrhea, malaria, neonatal problems, malnutrition and HIV/AIDS. According to the WHO and United nation Children Fund(UNICEF)Global action plan for prevention and control of pneumonia, pneumonia kills more children than any other illness in the world. Given the high burden of under five mortality associated with pneumonia, pneumonia control efforts are critical to achieving the millennium Development Goal 4(MDG4). In Ethiopia an estimated 1 in every 4 deaths among children under-five years of age, is caused by pneumonia every year (Federal Democratic Republic of Ethiopia Ministry of Health, 2010).

Ethiopia is one of the countries severely affected by pneumococcal infections. However, although there are reports of clinically suspected cases of pneumococcal disease there are no laboratory- confirmed cases reported officially to the World Health Organization. Knowledge of serotype distribution and antimicrobial susceptibility patterns are important in relation to the treatment of pneumococcal disease and vaccination programs (WHO, 2012).

## Statement of the problem

Pneumococcal disease remains a leading cause of death among children worldwide. Pneumonia and diarrhea caused over 1.5 million under-five children deaths, respectively accounting for 15% and 9% of the 6.3 million under-five deaths that occurred globally in 2013. Colonization begins very soon after birth and intrapartum colonization has even been described. Most studies have found asymptomatic carriage rates of between 30% and 62% in children fewer than 2 years of age (Backhaus *et al.* 2011, CDC, 2013, Ercibegoa *et al.*, 2012).

The nasopharynx is the major ecological niche of *S. pneumoniae*; spread from the nasopharynx to the lower respiratory tract or other sites may cause IPD. Children attending kindergartens are at increased risk for infectious diseases. The nasopharyngeal carriage rate is highest in children, mainly during the first years of life.

Pneumococcal disease is preceded by asymptomatic colonization, which is especially high in children. Colonization with pneumococci is mostly symptomless; it can progress to respiratory or even systemic disease. Pneumococcal disease will not occur without preceding nasopharyngeal colonization with the homologous strain. In addition, pneumococcal carriage is believed to be an important source of horizontal spread of this pathogen within the community. Therefore, part of the strategy to prevent pneumococcal disease focuses on prevention of nasopharyngeal colonization, especially in children (Borgaert *et al.*, 2011).

Risk factors for nasopharyngeal carriage in children include age less than 5 years, having young siblings and attendance in day care centres. *S. pneumoniae* remains a major cause of childhood illness and death. It kills at least one million children under the age of five every year, >70 % of these deaths are in developing countries. The burden of the disease in youngest and oldest sections of the population is more, in both developed and less developed countries. Incidence and case fatality rate due to invasive pneumococcal disease varies widely between different areas (Backhaus *et al.* 2011, Ercibegoa *et al.*, 2012).

Pneumonia and diarrhea are the major killer disease of children in developing countries. Like in other countries, children in Ethiopia are seriously affected by communicable diseases among which acute respiratory infections play the major role . More than 90% of child deaths in Ethiopia are due to pneumonia, diarrhea, malaria, neonatal problems, malnutrition and HIV/AIDS (HSDP, Mohammed *et al.*, 2000).

## Significance of the study

This study is aiming at describing prevalence, antimicrobial resistance and risk factors of pneumococcal colonization of the nasopharynx among children attending in kindergarten in Bahir Dar, Ethiopia.

Pneumococcal carriage is a predisposing factor and first step in causal chain of pneumococcal pathogenesis. It is important to study the carriage rate to measure the effect of vaccine on colonization density and/or clearance, acquisition, duration of carriage, development of effective control strategy and in public health decision-making.

Furthermore, it will be an initial data or preliminary study for further studies of pneumococcal colonization at kindergarten in Bahir Dar, Ethiopia since there is no such kind of study that has been conducted in the area before.



## OBJECTIVE

### General objective

To determine the prevalence of *S. pneumoniae* carriage of the nasopharynx; identify risk factors associated with pneumococcal colonization of the nasopharynx and antimicrobial susceptibility pattern of isolates of pneumococci among children attending Kindergarten in Bahir Dar, North West, Ethiopia.

### Specific objectives

- To determine the prevalence of *S. pneumoniae* carriage of the nasopharynx of children in Bahir Dar.
- To determine risk factors associated with pneumococcal colonization of the nasopharynx among children in Bahir Dar.
- To identify the antimicrobial susceptibility pattern of isolates of pneumococci isolated from children aged  $\leq 6$  years in Bahir Dar.

## METHODS AND MATERIALS

### Study area

This study was carried out in Bahir Dar, capital city of Amhara Regional State, Northwest Ethiopia, which is located at 564 km from Addis Ababa, capital city of Ethiopia. There are 20 Kebeles (12 urban and 8 rural) in the city with total population of the administration in the 2011/2012 was 276,450 of which 231,376 were Urban residents. Among this population 37,321 were <5years age. There are five kindergartens owned by the government, one of them found in Tis Abay but still it is included in Bahir Dar and 30 kindergartens owned by private organizations. In each kindergarten there are 30 children on average with a 1:1 male to female ratio.

### Study design and period

A cross sectional design was conducted on four governmental and three private kindergartens from February 2014 to June 2014 in Bahir Dar.

### Source and study population

The source population was all children attending kindergartens in Bahir Dar. The study population were all children  $\leq 6$  years and attending kindergartens.

### Selection Criteria

**Inclusion criteria:** All apparently healthy children  $\leq 6$  year who are living in Bahir Dar attending kindergartens.

**Exclusion criteria:** All children on antibiotics for the last two weeks; children with any sign of respiratory infection and those above six years of age.

## Sampling Techniques and Sample Size

### Sampling Technique

Convenient sampling technique was used to enrol children. The number of children enrolled from four governmental and three private kindergartens ranged from 30 to 59. From all kindergartens except one, 30 samples were collected from each. In one governmental kindergarten, all the attendees (KG1-KG3) were included for the study (n=59) because of parents and kindergartens staff request to enrol all children.

### Sample Size

To determine the sample size for this particular quantitative study, a single proportion sample size formula was used considering the following assumptions.

**Assumptions:** A 95% confidence level, margin of error (0.05) carriage rate among children attending kindergartens. Since there was a study on prevalence of pneumococcal colonization at age  $\leq 6$  years among children in Kenya 17 % (Githii *et al.*, 2013). The above assumptions was substituted in the following single population proportion formula.

$$\begin{aligned}n &= (Z_{\alpha/2})^2 p (1-p) / d^2 \\&= (1.96)^2 (0.17) (0.83) / (0.05)^2 \\&= 216.81 = \sim \mathbf{217}\end{aligned}$$

Where  $n$  = required sample size for this cross sectional survey

$z$  = Percentiles of the standard normal distribution corresponding to 95% confidence level which equals to 1.96 ( $z$  value at  $\alpha = 0.05$ )

$P$  = Proportion of carriage rate (17%) from previous study

$d$  = 0.05 (5% margin of error)

To compute for non-response rate, 10% of the total sample = 22 was added. Thus, a total of **239** study subjects were included.

## Variables

### Independent Variables

Age, sex, socio-economic living conditions, childcare, respiratory tract infection, over crowdedness and earlier antibiotics use

### Dependent variable/Outcome variables

State of pneumococcal colonization

## Data Collection Methods and Laboratory Diagnosis

### Data Collection Methods

A structured pre-tested questionnaire was used to collect data on socio-demographic characteristics of children's parent and children's, clinical and other history of children's and associated risk factors for *Streptococcus pneumoniae* colonization. Trained data collectors administered the questionnaires at kindergartens.

### Laboratory Diagnosis

#### Sample Collection, Transportation, and Processing

In accordance with the CDC core methodology for pneumococcal carriage studies, a calcium alginate tipped swab on a flexible aluminium shaft (Bibby Sterilin, UK) was used to collect nasopharyngeal swab (WHO, 2011, Ozdemir *et al.*, 2013, Pidade *et al.*, 2013, Roche *et al.*, 2007). Nasal swab collection was carried out by laboratory technician with a previous experience in doing so.

A single nasopharyngeal swab was collected from each participant using a paediatric sized calcium alginate swab inserted through the nares into the posterior according to the recommendation of CDC guide lines (WHO, 2011, Pidade *et al.*, 2013). The swab was rotated 180°, withdrawn and inserted in Skim milk-Tryptone-Glucose Glycerol (STGG) transport medium. The NP STGG specimens were transported from kindergartens to Amhara regional laboratory within 8 hr at room temperature and vortexed for approximately 10-20 second for culture and antimicrobial sensitivity test (Ozdemir *et al.*, 2013).

## ***Microbiological Procedures***

### **Culture**

Fifty µl of the NP samples were cultured on sheep blood agar supplemented with 5 µg/ml gentamicin which was used for selective medium to culture pneumococci from nasopharyngeal samples (CDC, WHO, 2002) and grown overnight at 37°C in a CO<sub>2</sub>-enriched atmosphere by using a candle jar. A typical colony of small, greyish, moist and watery surrounded by a greenish zone of alpha-hemolysis of the medium around the colony was checked. If enough colony growth obtained, we proceed to optochin susceptibility as a confirmatory test.

The suspected alpha-hemolytic colony was sub cultured onto blood agar (with 5 µg optochin disc) and streptococcal selective plates, which were read after overnight incubation in candle-jar at 37°C. Pneumococcal identification was based on colony morphology and optochin sensitivity.

### **Antimicrobial test**

Antimicrobial susceptibility testing was determined using Kirby-Bauer disc diffusion technique for the confirmed *S. Peumoniae* isolates (CLSI, 2012). Direct colony suspension, equivalent to a 0.5 McFarland standard, prepared using colonies from an overnight (18-to 20-hour) sheep blood agar plate. A sterile cotton swab was dipped into the standardised solution and used for evenly inoculating Mueller–Hinton plates. The plates were then allowed to dry. Antibiotics with the following concentrations were placed on the plates: penicillin (15µg), erythromycin (15µg), chloramphenicol (30µg), ceftriaxone (30µg), tetracycline (30µg) and Trimethoprim-sulfamethoxazole (25µg). The antibiotics were well spaced in order to prevent the overlapping of inhibition zones. The plates were incubated at 37°C for 24 hours. Quality control for susceptibility patterns was performed on a daily basis using *S. pneumoniae* ATCC 49619. Susceptibility results were interpreted according to the Clinical Laboratory Standard Institute Guidelines (CLSI) (CLSI, 2012).

## Data Quality Control

The quality of data was controlled starting from the time of questionnaires preparations. The questionnaires were developed by reviewing relevant literatures on the subject to ensure reliability. To verify that susceptibility tests results are accurate, it was included at least one control organism with each test or new set of testing conditions. Standard strain of *S. pneumoniae* ATCC 49619, the NCCLS control strain was used when testing *S. pneumoniae*. Zone diameters obtained for the control strain were compared with CLSI, 2012.

Optochin disks were tested with positive and negative controls. As a positive control we used *S. pneumoniae* ATCC 49619 and *S. Mitis* strain ATCC 49456 was used as a negative control.

## Operational Definitions

**Children** – A person below the age of adulthood.

**Colonization** - Presence of bacteria on nasopharynx without causing disease.

**Multi Drug resistance** -resistance to two or more classes of antibiotics.

## Statistical Analysis

All recorded data was transferred from excel to SPSS version 20.0. Data was summarized by using descriptive statistics, frequency tables, and figures. The magnitude of association between the different variables in relation to the outcome variable was measured by odds ratio with 95% confidence interval. Binary logistic regression analysis was made to obtain odds ratio and the confidence interval of statistical associations. The strength of statistical association was measured by adjusted odds ratios and 95% confidence intervals. Statistical significance was declared at  $p < 0.05$  and variables which showed statistical significant association ( $p < 0.05$ ) in the bivariate analysis were included in the final model.

## Ethical Considerations

The study was conducted after getting ethical clearance from Department of Microbiology Immunology and Parasitology research and ethics committee, School of Medicine, CHS, and AAU. Each child's parent was adequately informed about the purpose of the study and the

importance of their participation. Written Consent was taken from all parents/guardians before commencing the study.

### **Dissemination of result**

The finding of this study will be submitted to the school of Medicine, Department of Microbiology, Immunology and Parasitology, Addis Ababa University as a partial fulfilment of M.Sc. in Medical Microbiology. Finally, the finding will be disseminated to Amhara Regional State Health Bureau and Kindergartens. It will be also communicated to the concerned bodies and presented through publication, seminars and workshops as well as further effort will be made to publish the findings on national and international peer reviewed journal.

## RESULTS

### Socio-demographic characteristics

A total of 239 children were enrolled in the study. The gender ratio was 1:1 with 113 (47.3%) males and 126(52.7%) females. The age distribution was from 4 to 6 years old ([table 1](#)).

**Table1:** Socio-demographic characteristics of children and parents.

| Variables                   | Frequency (N = 239) | Percent |
|-----------------------------|---------------------|---------|
| <b>Address</b>              |                     |         |
| Bahir Dar                   | 239                 | 100     |
| Outside Bahir Dar           | 0                   | 0       |
| <b>Age</b>                  |                     |         |
| 4years                      | 70                  | 29.3    |
| 5years                      | 100                 | 41.8    |
| 6years                      | 69                  | 28.9    |
| <b>Sex</b>                  |                     |         |
| Male                        | 113                 | 47.3    |
| Female                      | 126                 | 52.7    |
| <b>Ethnicity of parents</b> |                     |         |
| Amhara                      | 193                 | 80.8    |
| Tigray                      | 32                  | 13.4    |
| Others                      | 14                  | 5.9     |



|                                      |     |      |
|--------------------------------------|-----|------|
| <b>Religion of parents</b>           |     |      |
| Orthodox Christian                   | 175 | 73.2 |
| Muslim                               | 44  | 18.4 |
| Protestant                           | 17  | 7.1  |
| Others                               | 3   | 1.3  |
| <b>Educational status of parents</b> |     |      |
| Unable to read and write             | 51  | 21.3 |
| Primary(1-8)                         | 104 | 43.5 |
| Secondary(9-12)                      | 50  | 20.9 |
| Tertiary(diploma and above)          | 34  | 14.2 |
| <b>Marital status of parents</b>     |     |      |
| Single                               | 29  | 12.1 |
| Married                              | 163 | 68.2 |
| Divorced                             | 38  | 15.9 |
| Widowed                              | 9   | 3.8  |
| <b>Occupation of parents</b>         |     |      |
| Merchant                             | 20  | 8.4  |
| House wife                           | 34  | 14.2 |
| Student                              | 36  | 15.1 |
| GOV employee                         | 1   | 4    |
| NGO employee                         | 65  | 27.2 |
| Un employed                          | 59  | 24.7 |
| Others                               | 24  | 10.0 |
| <b>Monthly income of parents</b>     |     |      |
| 250                                  | 9   | 3.8  |
| 250-500                              | 33  | 13.8 |
| 500-1000                             | 70  | 29.3 |
| 1000-2000                            | 52  | 21.8 |
| 2000                                 | 18  | 7.5  |
| >2000                                | 56  | 23.4 |
| Don't know                           | 1   | 0.4  |

## Pneumococcal carriage rate and risk factors

Out of the 239 samples, by  $\alpha$ -haemolysis and susceptibility to optochin was 118/239(49.4%) and 107/239(44.8%) respectively (Table 2). The highest *S. pneumoniae* carriage rate (56.5%) was observed in children aged 6 years old. About 162(67.8%) had got breastfeeding for 2 years; the rest 77(32.2%) of them did not get breast-feeding. One hundred and fifty six (65.3%) children were living in crowded condition. Of all 239 children, 135(56.6%) attended kindergarten for  $\leq 12$  month and 179(74.9%) were full time attendants. All the parents provided information on earlier antibiotic use of their children if any and the majority 92 (38.5%) were taken within the last 6-12 month. The majority of the children 156 (65.3%) were living with <5year siblings. Out of 239 children, 201 had no respiratory infection during the last 30 days ([table 2](#)).

**Table 2:** Carriage status of children's by age, sex and breast-feeding.

| Variables             | Prevalence |          |      |             |         |
|-----------------------|------------|----------|------|-------------|---------|
|                       | Sample     | Carriers | (%)  | 95% CI      | p value |
| <b>Sex</b>            |            |          |      |             |         |
| Male                  | 113        | 49       | 43.4 | 0.57-2      | 0.876   |
| Female                | 126        | 58       | 46   | 1.00        |         |
| <b>Age</b>            |            |          |      |             |         |
| 4year                 | 70         | 26       | 37.2 | 1.00        |         |
| 5year                 | 100        | 42       | 42   | 0.128-0.78  | 0.012   |
| 6year                 | 69         | 39       | 56.5 | 0.178-0.945 | 0.036   |
| <b>Breast feeding</b> |            |          |      |             |         |
| Yes                   | 77         | 35       | 45.5 | 0.57-2.43   | 0.656   |
| No                    | 162        | 72       | 44.4 | 1.00        |         |

Being age of 5 and 6years was significantly associated with *S. pneumoniae* carriage rate (AOR = 0.316; 95% CI, 0.128, 0.780;  $p = 0.012$  and AOR=0.411; 95% CI, 0.178, 0.945;  $p = 0.036$ ) respectively. In addition, *S. pneumoniae* carriage was significantly higher in children living in crowded condition (AOR = 0.459; 95% CI, 0.570, 2.439;  $p = 0.022$ ). Moreover, there was a significantly association between *S. pneumoniae* carriage and earlier antibiotic use within 2-4weeks (AOR = 8.004; 95% CI, 1136, 56.409;  $p = 0.037$ ) and in children living with siblings < 5 years old (AOR = 0.467; 95% CI, 0.234, 0.933;  $p = 0.031$ ). However, there was no significant association between, sex, family size, breast-feeding, period of attending time, stay in kindergarten, sharing the same room and carriage by *S. pneumoniae* ([table 3](#)).

**Table 3:** Risk factors for *S. pneumoniae* in 239 attending kindergartens, Bahir Dar, North West Ethiopia, 2014

| Variables               | <u>Carriage status(n=239)</u> |      |           |      |                    |                    |         |
|-------------------------|-------------------------------|------|-----------|------|--------------------|--------------------|---------|
|                         | Yes(n=107)                    |      | No(n=132) |      | COR(95%CI)         | AOR(95%CI)         | P-Value |
|                         | N                             | %    | N         | %    |                    |                    |         |
| Children’s variables    |                               |      |           |      |                    |                    |         |
| Age                     |                               |      |           |      |                    |                    |         |
| 4years                  | 26                            | 24.3 | 44        | 33.3 | 1.00               | 1.00               |         |
| 5years                  | 42                            | 39.2 | 58        | 44   | 0.455(0.230,0.897) | 0.316(0.128,0.780) | 0.012   |
| 6years                  | 39                            | 36.4 | 30        | 22.7 | 0.557(0.300,1.035) | 0.411(0.178,0.945) | 0.036   |
| Family size             |                               |      |           |      |                    |                    |         |
| 1-3                     | 39                            | 36.4 | 49        | 37.1 | 1.114(0.328,3.783) | 1.315(0.278,6.214) | 0.730   |
| 4-6                     | 63                            | 58.9 | 76        | 57.6 | 1.161(0.351,3.835) | 1.159(0.255,5.266) | 0.848   |
| 7-9                     | 5                             | 4.7  | 7         | 5.3  | 1.00               | 1.00               |         |
| Living in Crowded house |                               |      |           |      |                    |                    |         |
| Yes                     | 62                            | 57.9 | 94        | 71.2 | 0.557(0.325,0.954) | 0.459(0.236,0.894) | 0.022   |
| No                      | 45                            | 42.1 | 38        | 28.8 | 1.00               | 1.00               |         |

|                                   |    |      |     |       |                     |                       |              |
|-----------------------------------|----|------|-----|-------|---------------------|-----------------------|--------------|
| <b>Earlier antibiotic use</b>     |    |      |     |       |                     |                       |              |
| Within 2 weeks                    | 3  | 2.8  | 10  | 76..6 | 1.00                | 1.00                  |              |
| Within 2-4 weeks                  | 10 | 9.4  | 19  | 14.4  | 1.754(0.391,7.865)  | 5.482(0.688,43.696)   | 0.108        |
| Within 1-6 months                 | 23 | 21.5 | 32  | 24.2  | 2.396(0.593,9.687)  | 8.004(1136,56.409)    | <b>0.037</b> |
| Within 6-12 months                | 49 | 45.8 | 43  | 32.6  | 3.798(0.981,14.706) | 16.458(2.341,115.685) | <b>0.005</b> |
| No                                | 22 | 20.5 | 28  | 21.2  | 2.619(0.642,10.683) | 9.093(1.249,66.200)   | <b>0.029</b> |
| <b>Period of attending time</b>   |    |      |     |       |                     |                       |              |
| ≤12months                         | 59 | 55.1 | 76  | 57.6  | 1.104(0.660,1.846)  | 1.334(0.687,2.589)    | 0.395        |
| ≤12month                          | 48 | 44.9 | 56  | 42.4  | 1.00                | 1.00                  |              |
| <b>Stay in kindergarten</b>       |    |      |     |       |                     |                       |              |
| Full time                         | 76 | 71   | 103 | 78    | 1.449(0.806,2.605)  | 1.875(0.897,3.918)    | 0.095        |
| Half time                         | 31 | 29   | 29  | 22    | 1.00                | 1.00                  |              |
| <b>Having sibling &lt;5 years</b> |    |      |     |       |                     |                       |              |
| Yes                               | 63 | 58.9 | 93  | 70.4  | 0.600(0.351,1.027)  | 0.467(0.234,0.933)    | <b>0.031</b> |
| No                                | 44 | 41.1 | 39  | 29.5  | 1.00                | 1.00                  |              |

|                                                            |    |      |     |      |                    |                    |       |
|------------------------------------------------------------|----|------|-----|------|--------------------|--------------------|-------|
| <b>Sharing the same room</b>                               |    |      |     |      |                    |                    |       |
| <2                                                         | 51 | 46.6 | 58  | 44   | 0.586(0.94,3.648)  | 0.446(0.055,3,577) | 0.447 |
| 2-3                                                        | 47 | 44   | 57  | 43.2 | 0.550(0.88,3.428)  | 0.317(0.039,2.576) | 0.282 |
| >4                                                         | 6  | 5.6  | 15  | 11.3 | 0.267(0.35,2.019)  | 0.143(0.014,1.492) | 0.104 |
| No                                                         | 3  | 2.8  | 2   | 1.5  | 1.00               | 1.00               |       |
| <b>Respiratory tract infection<br/>during last 30 days</b> |    |      |     |      |                    |                    |       |
| Yes                                                        | 18 | 16.8 | 20  | 15.2 | 0.883(0.441,1.769) | 1.811(0.697,4.708) | 0.223 |
| No                                                         | 89 | 83.2 | 112 | 84.8 | 1.00               | 1.00               |       |

### Antimicrobial susceptibility pattern of bacterial isolates

Of 107 isolates screened for antimicrobial susceptibility by the Kirby-Bauer method, 10(9.3%) were resistant to penicillin and 8(7.5%) isolates were intermediate resistance. The remaining 89 (83.2%) isolates were susceptible to penicillin. The rates of resistance to other drugs were as follows: Trimethoprim-sulfamethoxazole (SXT), 43.9%; tetracycline, 22.4%; Erythromycin, 15.9%; Chloramphenicol, 8.4% and Ceftriaxone, 5.6%. Multidrug resistance was found in 28(26.2%) of isolates.

**Table 4:** Antimicrobial susceptibility patterns of *S. pneumoniae*, isolated from 107 children attending kindergartens, Bahir Dar, North West Ethiopia, 2014.

| Antimicrobials                | <i>S. pneumoniae</i> (n=107) |            |            |
|-------------------------------|------------------------------|------------|------------|
|                               | R<br>n (%)                   | I<br>n (%) | S<br>n (%) |
| Ceftriaxone                   | 6(5.6)                       | 8 (7.5)    | 93 (86.9)  |
| Chloramphenicol               | 9 (8.4)                      | 3 (2.8)    | 95 (88.8)  |
| Erythromycin                  | 17 (15.9)                    | 3 (2.8)    | 87(81.3)   |
| Peniciline                    | 10(9.3)                      | 8 (7.5)    | 89 (83.2)  |
| Tetracycline                  | 24(22.4)                     | 2 (1.9)    | 81 (75.7)  |
| Trimethoprim-sulfamethoxazole | 47 (43.9)                    | 11(10.3)   | 49 (45.8)  |

### Multi Drug Resistant pattern

Of 107 isolates, 28(26.2%) were multi drug resistant: 17(15.9%) were resistant to two drugs, 9 (8.4%) were resistant to three drugs and the remaining 2(1.9%) were resistant to four drugs ([table 5](#)).

**Table 5:** Multidrug resistant pattern of *S. pneumoniae*, isolated from 107 children attending kindergartens, Bahir Dar, North West Ethiopia, 2014.

| Drugs  | Resistant to    | No(28) | %     |
|--------|-----------------|--------|-------|
| 2drugs | CAF ERY         | 2      | 7.14  |
|        | ERY SXT         | 3      | 10.71 |
|        | ERY TTC         | 2      | 7.14  |
|        | PEN SXT         | 2      | 7.14  |
|        | PEN TTC         | 1      | 3.6   |
|        | SXT TTC         | 7      | 25    |
| 3drugs | CAF ERY SXT     | 1      | 3.6   |
|        | CAFSXT TTC      | 2      | 7.14  |
|        | ERY SXT TTC     | 6      | 21.4  |
| 4drugs | CAF ERY PEN TTC | 1      | 3.6   |
|        | ERY PEN SXT TTC | 1      | 3.6   |



## DISCUSSION

Pneumococcus has a great public health importance worldwide due to its medical impact up on the society. The main burden of the bacterium are its multiple infections followed by high mortality rate, complications after cure, resistance to empirical antibiotics, as well as variation among strains circulating in a given community. The fatality and prevalence of the organism is higher relative to other similar agents for a specific disease (Ramakrishnan M, *et al.*, 2009).

The aim of this study was to determine prevalence of pneumococcal colonization of *S. pneumoniae* among children attending kindergartens in Bahir Dar. A total of 239 nasopharyngeal samples were collected and cultured, isolation of *S. pneumoniae* by  $\alpha$ -haemolysis and susceptibility to optochin was 118(49.4%) and 107(44.8%) respectively.

The carriage was low when compared to a study reported in Gondar(71%)(Mohammed *et al.*, 2000), Accra, Ghana(48.9%)(Mills *et al.*, 2015),in Nigeria 52.5%(Adetifal *et al.*, 2012),in kilifi district Kenya 51%(Abdullahi *et al.*, 2012), in sub Saharan Africa 63.2%(Usuf *et al.*, 2014),in Gambian infants (51%)(Scott *et al.*,2001), in Peruvian children (75.3%)(Graniel *et al.*,2011) and in London 51%(Roche *et al.*, 2007).This difference might be due to living conditions, seasonal variation of the data collection period, respiratory illness, methodological differences and genetic traits. However, our finding was high as compared to a study done in Gondar University Teaching Hospital, Ethiopia 37(15.8%)(Assefa *et al.*, 2013). This difference might be due to a difference in a study population where their study was conducted on children  $\leq 10$  years age group and pediatrics out patient. In other developing countries the carriage rate was 17% in Kenya (Githii *et al.*, 2013), in India 12.8% (Backhaus *et al.*, 2011), in Italian cities 8.6% (Marchisio *et al.*, 2002), in Brazil 16.4%(Granat *et al.*, 2007) and in Mexico 29.9%(Espinosa *et al.*, 2007). The higher carriage in our study might be due to institutional environment difference, and family size difference.

Among the possible risk factors, Age, day-care attendance, and siblings less than five years living in the house of the child, crowded living, and earlier antibiotic use within two weeks were confirmed as risk factors and showed statistically significant association.

Carriage was highest for children less than 5 years and decreased with age (Usuf *et al.*, 2014). Assessing all colonized children's together, age appeared to be positively associated with carrying *S. pneumoniae*, and this relationship was statistically significant. This was due to sample size ratio from other age group; children's of 5 years enrolled in the study were n=100.

From those 107 children who carried pneumococcus, 42.1% (OR: 0.459, 95%CI: 0.236, 0.894) of them were lived in crowded room. This result was similar with a study conducted in the Netherlands by d. Bogaert *et.al*(Borgaert *et al.*, 2011). This might be *S. pneumoniae* carriage is more frequent in institutional environments such as kindergartens and crowded rooms where crowding facilitates horizontal transfer of bacteria from one child to another.

Unlike the other study by Maria (Maria *et al.*, 2012), in this study recent antibiotic treatment was associated with pneumococcal carriage. Previous antibiotic use was also the other important risk factor. Prior antibiotic usage may alter an individual's nasopharyngeal flora and increase the likelihood of carrying resistant pneumococci that can cause IPD under opportunistic conditions (Jeena *et al.*, 2006).

In this study, we found having sibling <5 years had one of the risk factors for pneumococcal carriage, as also observed in some studies (Berezin *et al.*, 2007, Duanais *et al.*, 2003, Leino *et al.*, 2001, Regev-yochay *et al.*, 2004). It is important to reinforce that the high prevalence of colonization among children who go to kindergartens and have contact with other young children may favour the dissemination of resistant pneumococci.

A significant association of some risk factors like (age, number of room, presence of people affected by ARI in the family, educational status of father, educational status of mother) with nasopharyngeal carriage of *S. pneumoniae* in children was previously reported in Gondar University Teaching Hospital, Ethiopia (Assefa *et al.*, 2013). This was different from our study and this might be study population difference where their study populations were from pediatric outpatient. Other studies have reported similar risk factors. A study in Turkey in healthy children (Duffy *et al.*, 1997) <5 years of age, presence of a child attending a daycare center, recovery from respiratory infection within the last month, low income level of the family and presence of

more children in the family; in Taiwan(Kuo *et al.*,2011), having at least one sibling, attendance at day-care centers and a history of upper respiratory tract infection;in São Paulo(Berezin *et al.*, 2007)day care centres and siblings younger than five years, in North America(Leino *et al.*, 2001), underlying disease and with day care attendance in the previous 3months were associated with carriage rate of *S. pneumoniae* .

In our study, sex, breastfeeding, length of attending year in kindergarten, staying time at kindergarten in a day, sharing the same room and respiratory infection during last 30 days, were not associated with the *S. pneumoniae* carriage.

Highest degree of resistance among the six drugs was seen for SXT, (n=47, 43.9%) ([table 4](#)).Number of resistant isolates to tetracycline, erythromycin, Chloramphenicol and ceftriaxone were 22.4 %, 15.9%, 8.4% and 5.6%, respectively. Thirty six percent of isolates were resistant to one antibiotic, 15.9% to two antibiotics and 10.4 % to three or more antibiotics. None of the isolates were resistant to the entire six drugs. Treatment of infections due to *S. pneumonia* has become a complicated global problem due to antibiotic resistance (Decarvalho *et al.*, 2009).In this study most effective antimicrobial agents for the *S. pneumoniae* isolates were ceftriaxone and Chloramphenicol, to which these isolates were 86.9% and 88.8% susceptible respectively.

In this study, 9.3% resistant and 7.5% isolates were intermediately resistant to penicillin. Unlike other studies, penicillin susceptibility was higher than penicillin resistance (83.2%). Penicillin resistant in a study from India has been reported to be 16.9%, cotrimoxazole resistant 90.6%.In Indian study inappropriate and overuse of penicillin and cotrimoxazole could be the contributory factors for high resistance in our study. Hence the empirical use of penicillin and cotrimoxazole is not justified in this region. The nasopharyngeal isolates also demonstrated resistance to other antimicrobial agents like tetracycline, erythromycin, ciprofloxacin and cefotaxime. The commonly prescribed drugs in Bahir Dar (79%) were penicillin group and chloramphenicol. The increasing use of antibiotics empirically, and the prescription of unnecessary antibiotics has already been reported in our study area (Abula *et al.*,1999), moreover,influence of parents by factors like peer-norms, lack of medicine information could also contribute for the high drug resistance in some of the antimicrobials.

### Limitations of the study

Serotyping test was not performed due to unavailability of antisera in the local market.

### Conclusion

This study was conducted in several kindergartens of Bahir Dar, to ascertain the prevalence of *Streptococcus pneumoniae* carriage rate of the nasopharynx, identify risk factors associated with pneumococcal colonization of the nasopharynx and antimicrobial susceptibility pattern of isolates of pneumococci among children  $\leq 6$  years.

In this study, we observed 44.8% of children  $\leq 6$  years of age attending kindergartens were pneumococcal carriers. Living in crowded setting, having sibling  $< 5$  years, prior antibiotic use were risk factors for pneumococcal carriage.

The findings in this study confirmed that SXT or Tetracycline could not be used for empirical treatment of *S. pneumoniae* in this area. The high resistance rates against SXT and Tetracycline, and the high rate of previous antibiotic treatment of the children indicated that more restrictions on the antibiotic use highly needed.

## Recommendations

Based on the study findings, the following recommendations are forwarded:

- Continuous surveillance at kindergartens using longitudinal study design
- Measures to be taken based on the associated risk factors
- Measures to be taken based on the AST

The findings of this study suggest more qualitative research/further studies that mainly address all areas of associated factors (individual level, group level and societal level factors) that may significantly affect carriage rate to understand the factors that influence carriage rate of children at kindergartens; so that to prevent children from pneumococcal colonization that would better tell to carriers to diagnosed and vaccinated their children.

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## APPENDICES

### Appendix I: Laboratory methods

#### 1. Preparation of skim milk, tryptone, glucose, glycerol transport medium (STGG)

The formula of the Skim-milk tryptone glucose glycerol (STGG) transport medium is:

- Skim milk powder (from Difco or from grocery) 2 g
- Tryptone soya broth (TSB, from Oxoid) 3 g
- Glucose 0.5 g
- Glycerol 10 ml
- Distilled water 100 ml

- a) Mix to dissolve all ingredients.
- b) Dispense in 1.0 ml amounts in screw-capped 1.5-ml vials.
- c) Loosen the screw-cap tops and autoclave for 10 minutes (at 15 pounds).
- d) Tighten caps after autoclaving.
- e) Store STGG frozen at -20°C or refrigerate until use. Use STGG medium within 6 months of preparation (CDC, WHO, 2012).

#### 2. Inoculation of STGG with an NP swab

- a) Thaw frozen tubes of STGG before use.
- b) Label the tube with appropriate patient and specimen information.
- c) Using a calcium alginate, Dacron or flocked swab, collect an NP swab from the children
- d) Insert swab to the bottom of the STGG medium in thawed (room temperature) tube.
- e) Raise the swab slightly and cut the wire portion (i.e., the shaft, or using a disinfected scissor) of the swab at the top level of the container. Allow the bottom portion of the swab (i.e., the tip) to drop into the tube. Discard the remaining shaft into disinfectant solution or a sharps container.
- f) Tighten the screw-cap top securely.
- g) Vortex on high speed for 10–20 seconds.
- h) Freeze specimen immediately in upright position at -70°C, if possible.
- i) Quality control test for sterility of the STGG medium has to be performed by plating a full loop of a homogenized vial from each lot onto trypticase soy agar with 5% sheep blood (BAP) with Gentamicin improves the recovery of *S. pneumonia* from clinical specimens by inhibiting the growth of bacteria that could mask its presence including staphylococci

gramnegative bacilli and incubating the plate at 37°C for 24 h. Any growth should be considered contamination and the lot should be discarded (CDC, WHO, 2012).

### **3. Broth enrichment NP swab culture for enhanced pneumococcal growth**

**a.** Thaw the NP-STGG specimens at room temperature (25°C) and vortex for approximately 10-20 s.

– Re-freeze the specimen (i.e., the STGG) as soon as possible; keep it cool (in an ice water bath if necessary) if the time is extended beyond a few minutes at room temperature.

– Avoid multiple freeze-thaw cycles whenever possible. One way to decrease risk of freeze-thaw cycles within the freezer is to make sure the cryotubes are kept in the back of the freezer shelf and not the front or in the door.

**b.** Transfer 50 µl of the NP-STGG to supplemented 5 ml of Todd Hewitt broth containing 0.5 % yeast extract (THY), and 1 ml of rabbit serum.

**c.** Vortex and incubate for 6 hours at 37°C/CO<sub>2</sub> incubator or candle-jar

**d.** Vortex and inoculate one loop (10 µl) of the THY enriched culture on BAP, streak in four quadrant fashion for colonies isolation and incubate for 18-24 hours at 37°C in CO<sub>2</sub>-incubator or candle-jar (CDC, WHO, 2012).

### **4. Pneumococcal isolate detection and identification**

**a.** Carefully examine the BAP growth, for typical pneumococcal colonies, small, grayish, moist, watery surrounded by a greenish zone of alpha-hemolysis.

**b.** The suspected pneumococcal colonies should be passed to fresh BAP and incubated for 18-24 hours at 37°C in candle-jar. If enough colony growth proceed to optochin susceptibility and bile solubility tests.

**c.** To perform the optochin susceptibility test:

- Streak the suspect alpha-hemolytic colony into BAP in confluent lines
- Place 5 µg optochin disk with 6 mm diameter in the streaked area
- Incubate in CO<sub>2</sub>-incubator or candle-jar at 35-37°C for 18-24 h

If susceptible to optochin (halo diameter >14 mm) it is identified as *S. pneumoniae*



## Appendix II: Consent Form and Information Sheet

### Consent Form

Hello. My name is Fetlework Bereded, a student at Addis Ababa University, School of Medicine, Department of Microbiology, Immunology and parasitology.

I am coming to request about your child. The study is looking at prevalence and risk factors of pneumococcal colonization at kindergartens.

In this study participation is voluntary and you can withdraw to be part of this study at any time, without affecting your right and I guarantee the information which will be provided to me will be confidential and used by members of the study alone. The base of this study used for your child. To fill this questioner it might needs 15-20 minut. Your permission for the child to participate is very important as the success of this study depends much on you, by giving us your sincere answers.

Voluntary agree to participate

☐

Not voluntary agree to participate

☐

Parents name..... Signature.....

Parent's responsibility

1. Mother

3. Other relative

2. Father

4. Not relative

Interviewee name .....date.....

Signature.....

I .....agree to participate in the study having read and understood all the information contained in this consent. The proposed study has been clearly explained to me and I have been given the opportunity to ask questions.

I voluntarily consent to participate in this study and understand that I may withdraw at any time with no penalty.

Signature of participant.....date.....

Certification by study Investigator.

I .....here by certify that I have disclosed the risks and benefits that may be involved, in terms readily understood by the participant.

Signature of Investigator.....date .....

**Address of the principal investigator:**

Fetlework Bereded Tefera

**Cell phone:** +251 932176451/0918814232

**E-mail:** fetleworkyeab@gmail.com

Revocation of Consent by participant.

I hereby wish to WITHDRAW my consent to participate in the research described above and understand that such withdrawal WILL NOT make any difference to the medical care or my social life.

Signature..... date .....

### Appendix III: English version Questionnaire

Addis Ababa University, School of Medicine, Department of Microbiology, Immunology and Parasitology

#### Questionnaire for assessment of risk factors of pneumococcal colonization

001. Questionnaire ID number: \_\_\_\_\_

002. Address: kebele: \_\_\_\_\_

003. Name of health facility: \_\_\_\_\_

Date of interview: \_\_\_\_\_ Time started: \_\_\_\_\_ Time finished: \_\_\_\_\_

Respondent's signature \_\_\_\_\_

Interviewer Name: \_\_\_\_\_ Signature \_\_\_\_\_ Date: \_\_\_\_\_

Supervisor's name: \_\_\_\_\_ Signature \_\_\_\_\_

**Note:** Encircle from the given option and write if any other idea or answer is given.

#### **PART I.** Socio-demographic characteristics of caregivers with their index child (age $\leq 6$ years)

| No  | Question                    | Response                                                                                                  | Skip |
|-----|-----------------------------|-----------------------------------------------------------------------------------------------------------|------|
| 101 | Age of the child            | ----- years                                                                                               |      |
| 102 | Sex of the child            | 1. Boy<br>2. Girl                                                                                         |      |
| 103 | Religion of Parent's        | 1. Orthodox<br>2. Muslim<br>3. Protestant<br>4. Others (specify)_____                                     |      |
| 104 | Ethnicity of Parent's       | 1. Amhara<br>2. Tigray<br>3. Others (specify)_____                                                        |      |
| 105 | Parent's educational status | 1. Unable to read and write<br>2. Primary (1–8)<br>3. Secondary (9–12)<br>4. Tertiary (Diploma and above) |      |

|     |                                       |                                                                                                                                      |  |
|-----|---------------------------------------|--------------------------------------------------------------------------------------------------------------------------------------|--|
| 106 | Marital status of the Parent's        | 1. Single<br>2. Married<br>3. Divorced<br>4. Widowed                                                                                 |  |
| 107 | Parent's occupation                   | 1. Merchant<br>2. House wife<br>3. Student<br>4. Government employee<br>5. NGO employee<br>6. Un employed<br>7. Other (specify)_____ |  |
| 108 | Monthly income of the family (in ETB) | 1. <250<br>2. 250-500<br>3. 500-1000<br>4. 1000-2000<br>5. 2000<br>6. >2000<br>7. Don't know                                         |  |
| 109 | Relation of child- Parent's           | 1. Mother<br>2. Father<br>3. Grandmother/father<br>4. Uncle/aunt<br>5. Other (specify)_____                                          |  |
| 110 | Breast feeding                        | 1.yes<br>2.no                                                                                                                        |  |
| 111 | Living in Crowded house               | 1.yes<br>2.no                                                                                                                        |  |

|     |                                           |                                                                                                   |  |
|-----|-------------------------------------------|---------------------------------------------------------------------------------------------------|--|
| 112 | Earlier antibiotic use                    | 1. within 2 weeks<br>2. within 2–4weeks<br>3. within 1–6 months<br>4. within 6–12 months<br>5. no |  |
| 113 | Having siblings <5 years                  | 1.Yes<br>2.No                                                                                     |  |
| 114 | Period of attending time                  | 1.<12 month<br>2.>12 month                                                                        |  |
| 115 | Stay in kindergarten                      | 1.Full time<br>2.half time                                                                        |  |
| 116 | Sharing the same room                     | 1.<2<br>2,2-3<br>3.<4<br>4. No                                                                    |  |
| 117 | Respiratory tract infection within30 days | 1.Yes<br>2.No                                                                                     |  |

***Thank You for Completing the Interview!!***

**Appendix IV: በአማርኛ የተዘጋጀ የተሳታፊዎች ፈቃደኝነት ማረጋገጫ ቅጽ**

የህክምና ትምህርት ቤት ማይክሮባዮሎጂ ኢሚዮሎጂና ፖራሳይቶሎጂ ትምህርት ክፍል

የሃምሳ ምች ይዘት እና የሚመጣበት መንስኤ መረጃ መሰብሰቢያ መጠይቅ፣

መግቢያ

ጤና ይስጥልኝ፡ ፈትለወርቅ በረደድ እባላለሁ በአዲስ አበባ ዩኒቨርሲቲ ህክምና ት/ቤት የማይክሮባዮሎጂ ኢሚዮሎጂና ፖራሳይቶሎጂ ትምህርት ክፍል የማይክሮባዮሎጂ ተማሪ ነኝ፡፡ ወደዚህ የመጣሁበት አላማ ስለልጅዎ ቃለመጠይቅ ላደርግልዎት ነው፡፡ የመጠይቁ አላማ ምክርፈት ዘመናዊ የሃምሳ ምች ሁኔታና መንስኤውን ለማወቅ መረጃ ለመሰብሰብና አስፈላጊውን ርዕደ ምጃ ለመውሰድ እንዲሁም አስፈላጊውን የመፍትሄ ህሳብ ለሚመለከታቸው ክፍሎች ለመጠቀም ነው፡፡

በዚህ የዳህረ ምሳሌ ጥናት ማንኛውም ወላጅ ልጁን የማሳተፍ ምህንያ ለማሳተፍ መብት ያለው ሲሆን መረጃው በሙሉ ከላይ ከተጠቀሰው አላማው ጭለሌ ላተግባር የማይውል እና መረጃው በሙሉ በሚስጥር የሚቀመጥ መሆኑን ልገልጽለዎት እወዳለሁ፡፡ የዚህ ጥናት መሠረት ለልጅዎም ሆነ ለማህበረሰቡ ጠቃሚ ፋይዳ አለው፡፡ ይህን መጠይቅ ሞልቶ ለመጨረስ ከ15-20 ደቂቃ ሊወስድ ይችላል፡፡ ስለዚህ ከላይ ከገለጽኩልዎ አላማና ጥቅም አንፃር ለቃለ መጠይቁ እንዲተባበሩኝ በትህትና እጠይቃለሁ፡፡

በቃለ መጠይቁ ተስማምቻለሁ

☐

በቃለ መጠይቁ አልተስማማሁም

☐

መጠይቁን የሞላው ወላጅ ስም \_\_\_\_\_ ፊርማ \_\_\_\_\_

የቤተሰብ ኃላፊነት

1. እናት

2. አባት

3. ሌላ ዘመድ

4. ዘመድ ያልሆነ

የጥናቱ ባለቤት ስም \_\_\_\_\_

ቀን \_\_\_\_\_

ፊርማ \_\_\_\_\_

## Appendix V: በአማርኛየተዘጋጀመጠይቅ

### ክፍል 1 የማህበራዊናዲሞግራፊያዊሁኔታ

የሣምባምችመንስኤዎች

የጥያቄቁጥር -----

የት/ቤቱስም -----

ቀበሌ -----

ክፍል -----

መጠይቁየተሞላበትቀን ----- የተጀመረበትሰዓት -----

ያለቀበትሰዓት ----- መጠይቁንየሞላውፊርማ -----

መጠይቁንያደረገውወላጅስም ----- ፊርማ ----- ቀን -----

1. የህፃኑእድሜ -----

2. የህፃኑፆታ:

ወንድ ☐

ሴት ☐

3. የወላጅ ሀይማኖት

1. ኦርቶዶክስ

3. ንግሥታንት

2. ሙስሊም

4. ሌላ /ይጠቀስ/-----

4. የወላጅብሄር

1. አማራ

3. አሮሞ

2. ትግሬ

4. ሌላ /ይጠቀስ/ -----

5.የወላጅየትምህርትመረጃ

1. ማንበብና መጻፍ የማይችል

4. ሦስተኛደረጃ/ዲፕሎማ እና ከዚያ

2. ሁለተኛ ደረጃ /9-12/

በላይ

3. የመጀመሪያ ደረጃ /1-8/

6.የወላጅ የጋብቻ ሁኔታ

1. ያላገባ/ች

3. የፈታ/ች

2. ያገባ/ች

4. የሞተበት/ባት

7.የወላጅ የሥራ ሁኔታ

1.ነጋዴ

2.ስራ ፈላጊ

3.የቤት እመቤት

4.ተማሪ

5. የግል ሠራተኛ

6. መንግስት ሠራተኛ

7.ሌላ /ይጠቀስ/ -----

8.ወርሃዊ የቤተሰብ ገቢ /በኢትዮጵያ ብር/

1. <250

3. 500-1000

5. >2000

2. 250-500

4. 1000-2000

6. አይታወቅም

9.ህጻኑከወላጁጋርያለውዝምድና

1. እናት

4. አጎት/አክስት

2. አባት

5. ሌላ /ይጠቀስ/ -----

3. ሴት አያት/ወንድ አያት

ክፍል 2 የሣምባ ምች መንስኤዎች ሁኔታ

11.የእናት ጡት ወተት?

1. ጠብቷል

2. አልጠባም

12.በተጨማሪ ሁኔታ ነው የሚኖር?

1. አዎ

2. አይደለም

13.በቅርቡ ፀረ-ባክቴሪያ መድሀኒት ወስዷል?

1. በዚህ 2 ሣምንት

3. በዚህ 1-6 ወር

2. በዚህ 2-4 ሣምንት

4. በዚህ 6-12 ወር

14.በአፀደ ህጻናቱ የቆየበት ጊዜ/ወር

1.  $\leq 12$

2.  $\geq 12$

15.በአፀደ ህጻናቱ የቆይታጊዜ

1. ሙሉቀን

2. ግማሽ ቀን

16.ወንድማማችናእህትማማች ከ5 ዓመትበታች

1. አለ

2. የለም



17. ከህጻኑ ጋር አንድ ክፍል የሚጋሩት ቤተሰብ ቁጥር

1.  $< 2$

2. 2-3

3.  $>4$

4. የለም

18. የመተንፈሻ አካላት በሽታዎች በአለፉት 30 ቀናት

1. አለ    2. የለም

## Appendix VI: Declaration

I undersigned here agrees to accept responsibility for scientific ethical and technical conduct of the research project and for provision of required progress reports as per terms and the condition of the research publication office in effect at the time of the grant is forwarded as the result of this application.

**Name of student:** Fetlework Bereded Tefera, BSc.

Signature \_\_\_\_\_

**Place of submission:** Addis Ababa University, School of Medicine, Department of Microbiology, Immunology and Parastology.

Date of submission: \_\_\_\_\_

**Advisor:** This thesis work has been submitted for examination with my approval as University advisor.

Name: Dr. Tamrat Abebe, BSc, MSc, PhD    Signature \_\_\_\_\_

Dr. Adane Mihert, DVM, MSc, PhD    Signature \_\_\_\_\_