

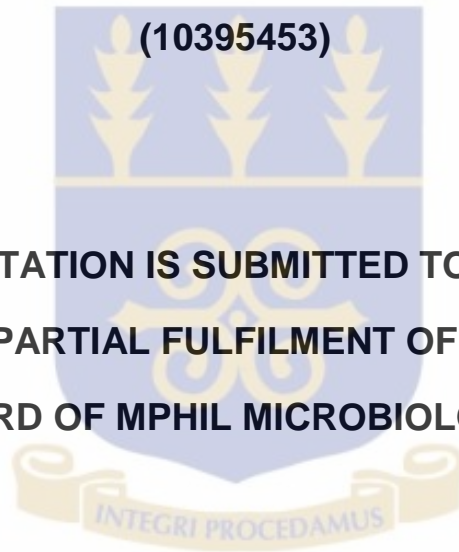
**UNIVERSITY OF GHANA**  
**COLLEGE OF HEALTH SCIENCES**  
**PNEUMOCOCCAL CARRIAGE AMONG HIV POSITIVE CHILDREN**  
**AT THE KORLE-BU TEACHING HOSPITAL**

**BY**

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**(10395453)**

**THIS THESIS/DISSERTATION IS SUBMITTED TO THE UNIVERSITY OF  
GHANA, LEGON IN PARTIAL FULFILMENT OF THE REQUIREMENT  
OF THE AWARD OF MPhil MICROBIOLOGY DEGREE.**



**JULY, 2015.**

## DECLARATION

I, Jennifer Adoley Annan, declare that the work presented in this thesis is the result of my own research work carried out in the Department of Microbiology Research Laboratory, University of Ghana Medical School (UGMS) and under the supervision of Prof. Eric Sampane-Donkor (UGMS) and Dr. Ebenezer Badoe (Child Health, KBTH) and that all references cited in this work have been duly acknowledged.

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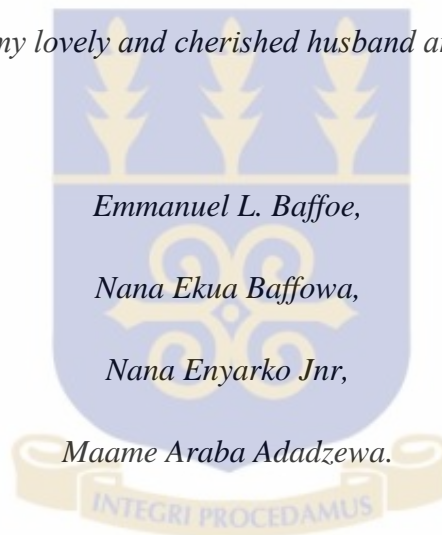


## DEDICATION

*First, to the Lord GOD Almighty*

*Second, to my lovely Parents, Mr. and Mrs. Raymond Annan*

*And to my lovely and cherished husband and kids...*



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**MAY GOD BLESS THEM ALL**



## ABSTRACT

### ***Background:***

The pneumococcus is carried as a normal flora of the upper respiratory tract and carriage is the precursor for development of pneumococcal disease, and is also responsible for pneumococcal transmission from person-to-person. HIV positive children have about forty times greater risk of invasive pneumococcal disease compared to healthy children. In Africa, little is known about the pneumococcus in relation to people with HIV infection.

### ***Aim:***

The aim of the study was to investigate the epidemiology of pneumococcal carriage among HIV positive children at the Korle-Bu Teaching Hospital.

### ***Method:***

One hundred and eighteen HIV positive children were recruited at the Korle-Bu Teaching Hospital in Accra and nasopharyngeal swabs were collected from them. The specimens were cultured for *S. pneumoniae* and other bacteria, and isolates were identified by standard microbiological methods. Antibiotic susceptibility testing was carried out on *S. pneumoniae* isolates by the Kirby Bauer method. Epidemiological data on demographic, household and clinical features of the study participant were collected.

### ***Results:***

Bacterial agents isolated from the study subjects included *Moraxella catarrhalis* (39.8%), coagulase negative staphylococci (33.1%), *Streptococcus pneumoniae* (30.5%), viridans streptococci (27.1%),

diphtheroids (29.7%), *Staphylococcus aureus* (22.0%), *Citrobacter* spp. (4.2%) and *Neisseria meningitidis* (0.9%). Pneumococcal carriage among different age groups were 33.3% (<5 years), 33.3% (5-9 years) and 28.9% (> 9 years). The only determinant of pneumococcal carriage among the study participants was the presence of respiratory symptoms ( $p=0.008$ ). Pneumococcal resistance, to the various antibiotics tested, were to cotrimoxazole (58.3%), tetracycline (33.3%), erythromycin (33.3%), oxacillin (27.8%) and ceftriaxone (5.6%).

***Conclusion:***

About a third of the HIV positive children carried the pneumococcus as normal flora in the nasopharynx and carriage appears to be unaffected by demographic and household characteristics. The presence of respiratory symptoms may probably be the main risk factor of pneumococcal carriage among HIV positive children. Ceftriaxone may be a relatively suitable antibiotic for treating pneumococcal infections among HIV positive children in Accra.

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## LIST OF ABBREVIATIONS

AOM.....Acute Otitis Media

CAP.....Community Acquired Pneumoniae

IPD.....Invasive Pneumococcal Disease

PRSP.....Penicillin - resistant *Streptococcus pneumoniae*

RTI.....Respiratory Tract Infection

SMH.....Sheep blood Mueller-Hinton

## CHAPTER ONE

### 1.0 INTRODUCTION

#### 1.1 BACKGROUND

*Streptococcus pneumoniae* also referred to as the pneumococcus, is a Gram-positive, lancet-shaped bacteria usually occurring in pairs (Todar, 2004). *S. pneumoniae* is part of the normal bacterial flora of the upper respiratory tract of humans, and is mainly found in the nasopharynx of 15-60% individuals (Todar, 2004) depending on local epidemiology (Brugger *et al.*, 2009). Colonization of the nasopharynx is transient and typically asymptomatic and occurs shortly after birth (Reingold *et al.*, 2006). Carriage of *S. pneumoniae* is higher in children than adults, and the colonization rate peaks around the age of 1-2years (Aniasson *et al.*, 1992; Appelbaum *et al.*, 1996). For unknown reason, pneumococcal carriage rate appears to be relatively higher in the developing world compared to the developed world (Obaro and Adegbola, 2002). In most colonized healthy host, it does not result in disease but promotes the continuous presence of *S. pneumoniae* within the populace and thus, the level of transmission is maintained at a low-level among contacts (Muench and Rajnik 2008; Kim *et al.*, 2011).

The pneumococcus is one of the most virulent human pathogens and causes a wide range of infections, including invasive and non-invasive diseases. There are about one million new pneumococcal infections every year, majority (50%) of which occur among children <5 years and responsible for 10–20% of all deaths in this age group (O'Brien *et al.*, 2009) as compared with carriage rates of only 4 to 12% in adults (Regev-Yochay *et al.*, 2004; Hussain *et al.*, 2005; Greenberg *et al.*, 2006) and 8.2% in adolescents (Cardozo *et al.*, 2008). Children are the major

carriers (Regev-Yochay *et al.*, 2003; Hussain *et al.*, 2005; Regev-Yochay *et al.*, 2004) and most of the children have at least one pneumococcal infection (typically of the middle ear) within the first 5 years of life (Tuomanen *et al.*, 1995; Flannery *et al.*, 2006; Hjuler *et al.*, 2008). Carriage is more prolonged among children than in adults (Reingold *et al.*, 2006) and healthy carriers are the major disseminators of the infection (Brooks *et al.*, 2007) although high isolation rates are recorded during respiratory tract infections (RTI's) (Syrjänen *et al.*, 2001). Transmission usually occur in crowded areas by droplet infection during other RTI's by direct contact with secretions or aerosols created by sneezing and coughing (O' Brien *et al.*, 2003; Champoux *et al.*, 2004). As humans are the main host for *S. pneumoniae*, successful transmission of *S. pneumoniae* among humans is crucial for survival of the organism, and without this the pneumococcus is likely to be eliminated. It attaches to nasopharyngeal cells through interaction of bacterial surface and with this, normal colonization can become infectious if the organism is carried into areas such as the eustachian tube or nasal sinuses, where it can cause otitis media and sinusitis, respectively (Aguiar *et al.*, 2006). Pneumonia occurs if the organism is inhaled into the lungs and not cleared again (Reingold *et al.*, 2006). Once the organism makes its way to a site where it is not normally found, it spreads to the blood stream (bacteremia) and is carried to the meninges, joint spaces, bones, and peritoneal cavity (Schuchat *et al.*, 1991), and may result in deep seated Invasive Pneumococcal Disease (IPD) such as pneumonia, meningitis, brain abscess, septic arthritis or osteomyelitis (Reingold *et al.*, 2006; Chen *et al.*, 2009). Invasive Pneumococcal Diseases most frequently affects young children (particularly age 6 to 24 months), older adults (age  $\geq$  65 years), and immunocompromised individuals (children or adults) (Ortqvist *et al.*, 2005; Lynch *et al.*, 2005).

An important characteristic of the pneumococcus is the presence of a polysaccharide capsule which defines over 90 capsular types, commonly referred to as serotypes (Henrichsen, 1995; Vestheim

*et al.*, 2008; Zartler *et al.*, 2009; Calix *et al.*, 2010; Hausdorff *et al.*, 2000) and is considered the main virulence determinant of *S. pneumoniae*. The capsule is bound covalently to their peptidoglycan cell wall (Brooks *et al.*, 2007) and consist of monosaccharides, oligosaccharides and other components of different unique chemical structure (Champoux *et al.*, 2004). The serotypes are further placed into 46 serogroups based on their heterologous structure, chemical composition and antigenic properties of their capsular polysaccharides (Barocchi *et al.*, 2007) and they vary in distribution and among isolates and geographical regions. It is, therefore, likely to find other serotypes not found in other parts of the world such as in Ghana.

The pneumococcal capsule is the basis of current pneumococcal vaccines. Two types of pneumococcal vaccines are available, namely the polysaccharide (unconjugated) vaccine and the recently developed conjugate vaccines. The polysaccharide vaccine has 23 serotypes, and has an average good protective efficacy of the serotypes included in about 60-70% (WHO 1999). Its usefulness is however, limited in the elderly and immunocompromised individuals as the duration of protection is short-lived (WHO 1999). Additionally, infants respond poorly to the vaccine and it induces no herd effect (WHO 1999). Most of these limitations are overcome by the newly introduced conjugate vaccines, in which purified capsular polysaccharides are conjugated to a non-toxic variant of diphtheria toxin. Pneumococcal conjugate vaccines consist of 7-13 capsular serotypes of *S. pneumoniae* and have been shown to have a high efficacy against infections in children (WHO 1999; Selman *et al.*, 2000; Van Bambeke *et al.*, 2007 O'Brien *et al.*, 2009). In addition, the conjugate vaccines have a relatively higher potential to control serotypes most commonly associated with multiple drug resistance (WHO 1999). Following the availability of pneumococcal conjugate vaccines, there has been a global interest in the epidemiology of the

pneumococcus, especially, in the developing world where the brunt of pneumococcal disease is borne.

## 1.2 PROBLEM STATEMENT

*S. pneumoniae* is associated with severe invasive diseases, including meningitis, pneumonia and septicaemia as well as non-invasive diseases such as otitis media. Children with HIV infection have about 40 times greater risk of invasive pneumococcal disease compared to healthy children; these infections are more likely to be fatal, and the incidence does not decline with age as in healthy children (Santoro *et al.*, 2013; Madhi *et al.*, 2000; Heffernan *et al.*, 2005). Globally, the annual incidence of pneumococcal infections is estimated to be one million every year, majority (50%) of which occur among children <5 years and responsible for 10–20% of all deaths in this age group (O'Brien *et al.*, 2009). Though a global problem, the public health impact of pneumococcal disease is higher in the developing world, where children <5 years of age are most affected, and the organism is responsible for 10–20% of all deaths in this age group (WHO 1999; O'Brien *et al.*, 2009). The burden of pneumococcal disease is exacerbated by the increasing resistance of the organism to essential antimicrobial drugs, particularly penicillin, cephalosporins and macrolides. Resistant strains of the organism have been reported in both developing and developed countries, and contribute to the high mortality of its diseases (Van Bambeke *et al.*, 2007). Pneumococcal antibiotic resistance carry more significance in HIV positive people, as HIV infection is known to be a risk factor for colonization with resistant strains (Cardoso *et al.*, 2006). The enormous burden of *S. pneumoniae*, especially as a result of its increasing and widespread drug resistance, underscores the importance of control through vaccination. In many developing countries, however, there is lack of epidemiological data on the pneumococcus which is a major hindrance to the establishment of an effective pneumococcal vaccination program.

### 1.3 JUSTIFICATION

Though majority of pneumococcal infections occur in the developing world (Greenwood 2006), pneumococcal epidemiology in the developing world is poorly understood. In Ghana, the few pneumococcal studies that have been carried out focused on invasive disease (Obaro *et al.*, 2002; Hill *et al.*, 2006; Hill *et al.*, 2008; Donkor *et al.*, 2010; Dayie *et al.*, 2013). Consequently, very little is known about epidemiology of pneumococcal carriage in the country, particularly in relation to HIV positive people. Understanding the epidemiology of pneumococcal carriage is important, as carriage of the organism is a precursor for the development of pneumococcal disease, and is responsible for person-to-person transmission rather than pneumococcal disease (Bogaert *et al.*, 2004). Additionally, recent pneumococcal vaccines are based on reducing pneumococcal carriage, hence incidence of pneumococcal disease. Thus, pneumococcal carriage studies represent a suitable model for understanding host-pathogen interaction of the pneumococcus as well as understanding the epidemiology of this important human pathogen (Donkor *et al.*, 2010).

Epidemiological information from this study including pneumococcal carriage risk factors and antibiotic susceptibility would go a long way to improve the prevention and management of pneumococcal diseases among HIV positive children in Ghana.

#### **1.4 AIM**

The aim of the study was to investigate the epidemiology of pneumococcal carriage among HIV positive children at the Korle-Bu Teaching Hospital.

#### **1.5 SPECIFIC OBJECTIVES**

The specific objectives of the study were to;

1. determine the prevalence of pneumococcal carriage and other bacteria among HIV positive children at the Korle-Bu Teaching Hospital.
2. determine the risk factors of pneumococcal carriage among the HIV positive children.
3. evaluate antibiotic susceptibility patterns of pneumococci and other bacteria commonly isolated from the HIV positive children.

## CHAPTER TWO

### 2.0 LITERATURE REVIEW

#### 2.1 *STREPTOCOCCUS PNEUMONIAE* INFECTIONS

*S. pneumoniae* is the most common cause of community-acquired pneumonia (CAP) in adults, accounting for 30 to 70% of cases requiring hospitalization (Fang *et al.*, 1990; Bartlett *et al.*, 1995; Kalin *et al.*, 2000; Ortqvist *et al.*, 2005). The major diseases caused by *S. pneumoniae* include pneumonia, meningitis, septicaemia, and otitis media. There are two types of pneumonia, namely, bronchial pneumonia and lobar pneumonia, and *S. pneumoniae* is a major cause of both types. In an infection of pneumonia, *S. pneumoniae* stimulates the immune system and causes migration of white blood cells to the lungs. The interaction of white blood cells, proliferating bacteria and excessive fluid define the presence of pneumonia (Tuomanen *et al.*, 1995), which can be detected by a chest X-ray. Bacteraemia and septicaemia can occur in 20–30% of cases with pneumococcal pneumonia (Musher, 2004). *S. pneumoniae* meningitis perhaps has the highest case fatality rate (~40%) among the various pneumococcal infections and up to 50% of survivors suffer from debilitating sequelae such as mental retardation and motor deficiency (Bohr *et al.*, 1984; Leimkugel *et al.*, 2005). The clinical presentations of meningitis caused by *S. pneumoniae* are similar to other bacterial causes of acute meningitis and include severe headache, photophobia, neck stiffness, and fever. Otitis media is the most common of the pneumococcal diseases. The condition is an inflammation of the middle ear and up to 50% of the cases are caused by *S. pneumoniae* (Musher, 2004). The major clinical signs of the infection include limited mobility and enlarged tympanic membrane. It is reported that successful treatment with antibiotics can still lead to recurrent otitis media due to proximity of the middle ear to the nasopharynx where *S.*

*pneumoniae* resides (Libson *et al.*, 2005). Other *S. pneumoniae* diseases include conjunctivitis, acute tracheobronchitis, endometritis, peritonitis, endocarditis, arthritis, and osteomyelitis. These infections are, however, relatively uncommon.

A meta-analysis of 122 reports of CAP between 1966 and 1995 implicated *S. pneumoniae* in 66% of nearly 7000 cases (Kalin *et al.*, 2000). In addition, *S. pneumoniae* is the leading cause of bacteremia (Musher *et al.*, 2000; Yu *et al.*, 2003; Shah *et al.*, 2006), meningitis (Schuchat *et al.*, 1995), upper respiratory tract infections, and otitis media (Klein, 1994) worldwide. Bacteremia is present in ~20% of pneumococcal pneumonias in adults, with case-fatality rates of 10 to 30% (Watanakunakorn *et al.*, 1993; Ortqvist, 1999; Kalin *et al.*, 2000; Musher *et al.*, 2000; Alanee *et al.*, 2007). Mortality rates are much lower in children (< 3%) (Mufson *et al.*, 1999; Robinson *et al.*, 2001; Kaplan *et al.*, 2002). Invasive pneumococcal disease (IPD), defined as isolation of *S. pneumoniae* from a normally sterile site [e.g., blood; cerebrospinal fluid (CSF); surgical aspirate; pleural, pericardial, peritoneal, bone, or joint fluid] (Schuchat *et al.*, 1991) most frequently affects young children (particularly age 6 to 24 months), older adults (age  $\geq$  65 years), and immunocompromised individuals (children or adults) (Lynch *et al.*, 2005). The World Health Organization (WHO) estimates that 1.6 million people, including up to 1 million children < 5 years old, die of IPD annually (WHO, 2007), with developing countries bearing the greatest burden (Scott, 2006).

In North America and Europe, the annual incidence of pneumococcal bacteremia is 15 to 40/100,000 individuals (Mufson *et al.*, 1999; Robinson *et al.*, 2001; Ortqvist *et al.*, 2005; Shah *et al.*, 2006). In the United States in 2003, 35,000 cases of IPD in adults  $\geq$  age 18 led to 5600 deaths; 44% of cases and 60% of deaths were in adults  $\geq$  65 years of age (Shah and Ratner, 2006). In the United States also, the annual incidence of IPD in children < 2 years old was 160 to 180

cases/100,000 compared with 45 to 90 cases/100,000 in adults > 65 years of age (Mufson *et al.*, 1999; Whitney *et al.*, 2003).

Regional differences in the incidence of IPD have been noted (Robinson *et al.*, 2001; Ortqvist *et al.*, 2005; Jefferson *et al.*, 2006; Shah *et al.*, 2006). In Europe, the annual incidence of IPD in children < 2 years old is much lower than in the United States, ranging from 16 to 35 cases/100,000 (Flamaing *et al.*, 2002; Diez-Domingo *et al.*, 2002; Konradsen and Kaltoft, 2002). In the early to mid-1990s the reported incidence of pneumococcal bacteremia increased in several countries, including the United States (Flamaing *et al.*, 2002). *S. pneumoniae* has been linked to over 4 million cases and almost half a million deaths per annum among African children under 5 years old (Feikin *et al.*, 2001). Following the introduction of pediatric heptavalent pneumococcal conjugate vaccine (PCV7) in the United States in February 2000, the incidence of IPD declined substantially in both children and nonvaccinated adults (by herd immunity) (Talbot *et al.*, 2004; Hsu K *et al.*, 2005; Shah *et al.*, 2006). Unfortunately, the incidence of IPD due to non-PCV7 serotypes is increasing globally (Gonzalez *et al.*, 2006; Bender *et al.*, 2008).

## **2.2 STREPTOCOCCUS PNEUMONIAE CARRIAGE AND TRANSMISSION**

*S. pneumoniae* is part of the normal bacterial flora of the upper respiratory tract of humans, and is mainly found in the nasopharynx. Carriage of the organism is more prevalent in children than adults. This is more frequent especially in young children in the developing countries where the point prevalence for the first 5 years of life may be up to 90% and the age of first acquisition as low as 1-2 months (Granat *et al.*, 2001) ; the colonization rate rises from birth until it peaks around the age of 1–2 years, and thereafter an age related decline is observed (Lloyd-Evans *et al.*, 1996; Hussain *et al.*, 2005). *S. pneumoniae* carriage normally lasts for a couple of weeks, and

duration periods of more than 30 weeks are observed (Sleeman *et al.*, 2008). A seasonal carriage trend has also been described, with peak rates occurring during January–March (Gray *et al.*, 1980). Children acquire several different strains over time, and less immunogenic serotypes tend to be carried in the nasopharynx for a much longer period of time than the more immunogenic serotypes (Obaro and Adegbola, 2002).

Few studies have investigated pneumococcal carriage among HIV positive people. In Tanzania, a study reported pneumococcal carriage of 81% among HIV positive children less than 12 years and identified two risk factors for carriage of the organism including household members who have respiratory symptoms and with an increase in the child's weight prior to swabbing (Anthony *et al.*, 2012). In Cambodia, a study reported pneumococcal carriage of 17.6% among HIV positive children, and also several other bacteria of *Staphylococcus aureus* (30.4%) was the most prevalent (Krcmery *et al.*, 2013).

In developing countries, carriage rates are relatively higher especially, in children. In the Gambia, Lloyd-Evans *et al.* (1996) reported a carriage rate of 80% among children under five years of age, and a lower rate of 20% in adults. In a study by Gratten *et al.* (1986), it was found that 60% of infants in Papua New Guinea acquired *S. pneumoniae* already during the neonatal period, and all infants were colonized within the first 3 months of life (Gratten *et al.*, 1986). Similarly, high *S. pneumoniae* carriage rates in the developing world have been reported in several other countries including Zambia (Frederiksen and Henrichsen, 1988), Pakistan (Mastro *et al.*, 1993), The Philippines (Lankinen *et al.*, 1994), Papua New Guinea (Gratten *et al.*, 1986), and Bangladesh (Granat *et al.*, 2007). In the developed world, *S. pneumoniae* carriage appears to be lower than rates found in the developing world. Aniasson *et al.* (1992) reported that in Sweden, only 12% of infants were colonized with *S. pneumoniae* at 3 months, 30% at 7 months, and 32%

at 12–18 months. In the UK, a longitudinal study by Goldblatt *et al.* (2005) showed an overall prevalence rate of 25%, with carriage rates of 52 and 8% in children under 2 years and adults over 18 years of age, respectively. Labout *et al.* (2008) studied *S. pneumoniae* carriage in infants in the Netherlands and observed carriage rates of 8.3% at age 1.5 months, 31.3% at 6 months, and 44.5% at 14 months. The high pneumococcal carriage rates in the developing world appear to provide more opportunities for multiple carriage, as relatively higher multiple carriage rates have also been reported in the developing countries compared to the developing world (Gratten *et al.*, 1994; Obaro *et al.*, 1996; Brugger *et al.*, 2010).

As humans are the main host for *S. pneumoniae*, successful transmission of *S. pneumoniae* among humans is crucial for survival of the organism, and without this the pneumococcus is likely to be eliminated. Transmission of *S. pneumoniae* occurs through respiratory droplets from people with pneumococcal disease or more commonly healthy individuals who carry the organism in the nasopharynx (Bogaert *et al.*, 2004; Sleeman *et al.*, 2005). Little is known about the risk factors of pneumococcal transmission, though certain risk factors including number of siblings and visits to general practitioners for mild upper respiratory disease have been identified (Sleeman *et al.*, 2005). Additionally, higher rates of pneumococcal transmission are known to occur at certain sites including day care centers, military camps, and prisons (Givon-Lavi *et al.*, 2002; Bogaert *et al.*, 2004). Givon-Lavi *et al.* (2002) compared pneumococcal isolates recovered from children in day care centers with isolates recovered from younger siblings not attending day care by pulsed-field gel electrophoresis. This showed a high level of genetic similarity among isolates from the specific day care center the older sibling was attending and those isolated from younger siblings, which indicate that pneumococcal transmission may be a highly efficient process.

Following acquisition, the pneumococcus may establish in the nasopharynx of the new host, and in most cases this leads to asymptomatic colonization (Sleeman *et al.*, 2008). Occasionally, however, the newly acquired pneumococcus moves from the nasopharynx to other parts of the human host such as the lungs where it evades the host defence mechanisms and causes disease (Bogaert *et al.*, 2004). Because asymptomatic carriers far exceed symptomatic individuals, most of the links in the transmission chain of person-to-person are not visible. In contrast, a respiratory disease such as measles is also transmitted from person-to-person through the same route as pneumococcus but asymptomatic colonization does not happen, and each link in the transmission chain is evident as disease (Mrozek-Budzyn, 2010). There is evidence that the risk for progression of pneumococcus from asymptomatic colonization to disease seems to be greatest soon after acquisition and a complex interplay of factors are involved (Sleeman *et al.*, 2005). Colonizing pneumococci strains may elicit an immune response that may eliminate them (Obaro and Adegbola, 2002). Additionally, the composition of the microflora of the nasopharynx, which is thought to contain more than 700 diverse species, may support or hinder colonization and invasion by symbiosis and/or competition (Aniasson *et al.*, 1992; Harputluoglu *et al.*, 2005). There are significant differences in the attack rate of different serotypes, where attack rates refer to the incidence of invasive pneumococcal disease per the incidence of pneumococcal acquisition. Pneumococcal serotypes such as 1, 4, 5, and 9A have high attack rates, while serotypes such as 9N, 16F, 20, and 38 have low attack rates; generally, attack rates are higher for serotypes which are carried for short time periods (Sleeman *et al.*, 2005). Phase variation where pneumococcal variants have the same serotype but vary from opaque to transparent colonies, is thought to be important in the progression of *S. pneumoniae* from carriage to invasive disease (Weiser *et al.*, 1994; Arai *et al.*, 2011). This is because the opaque form has been commonly isolated from patient

samples, while the transparent form is adapted to colonization of the nasopharynx. According to Ring *et al.* (1998), phase variation of the transparent type increases pneumococcal invasion into human brain microvascular endothelial cells as much as six-fold.

### **2.3 PNEUMOCOCCAL VACCINES**

At present, two types of pneumococcal vaccines are available, namely the polysaccharide (unconjugated) vaccine and the recently developed conjugate vaccines. The polysaccharide vaccine has 23 serotypes, and has an average good protective efficacy of the serotypes included in about 60-70% (WHO 1999). Its usefulness is however, limited in the elderly and immunocompromised individuals as the duration of protection is short-lived (WHO 1999). Additionally, infants respond poorly to the vaccine and it induces no herd effect (WHO 1999). Most of these limitations are overcome by the newly introduced conjugate vaccines, in which purified capsular polysaccharides are conjugated to a non-toxic variant of diphtheria toxin. Pneumococcal conjugate vaccines consist of 7-13 capsular serotypes of *S. pneumoniae* and have been shown to have a high efficacy against infections in children (WHO 1999; Selman *et al.*, 2000; Van Bambeke *et al.*, 2007; O'Brien *et al.*, 2009). In addition, the conjugate vaccines have a relatively higher potential to control serotypes most commonly associated with multiple drug resistance (WHO 1999).

## 2.4 ANTIBIOTIC RESISTANCE OF *STREPTOCOCCUS PNEUMONIAE*

*Streptococcus pneumoniae* remained uniformly susceptible to all classes of antibiotics that had been active against the organism since the beginning of the antibiotic era to the mid-1970s, with the possible exception of tetracycline. Mortality associated with pneumococcal pneumonia in the prepenicillin era was about 20%; with pneumococcal meningitis, 80% -100% and with pneumococcal bacteremia, 50% (Heffron 1979). These mortality figures, however, dropped to 5%, 20%, and 30%, respectively with the introduction of penicillin, which has become the mainstay of chemotherapy for pneumococcal diseases (Breiman *et al.*, 1990). In the ensuing decades, resistance of pneumococci to a variety of antimicrobial agents has been well documented (Okeke *et al.*, 2005) and has evolved from an ominous medical curiosity to a worldwide health problem. Pneumococcal resistance has increased to a point that it is clinically relevant in the following classes of antibiotics: beta-lactams (penicillins, cephalosporins, and carbapenems), macrolides (erythromycin, azithromycin, clarithromycin, and lincosamides [clindamycin]) and tetracyclines and folate inhibitors (trimethoprim-sulfamethoxazole [TMP-SMX] (Oduyebo *et al.*, 2006). The prevalence of antimicrobial resistant *S. pneumoniae* among the invasive isolates has increased over the years in many countries and hence accounts for an increase in low response to treatment (Sadowy *et al.*, 2006). In a study by Liu *et al.* (1998), a high prevalence of resistance in different geographic locations was attributed to due to rapid clonal selection of a few resistant strains. The mechanism of acquisition of drug resistant genes has been attributed to transfer of genes from conjugate transposons or transformation, that is, incorporation of free DNA from related species in nasopharyngeal flora (Nuermberger *et al.*, 2004). According to Chen *et al.* (2009), an increase in penicillin resistance among invasive isolates and specific serotypes has been identified as an important determinant of fatality and have been proposed to be suitable markers of the resulting

sequel due to IPD (Okeke *et al.*, 2005). In a study to assess the carriage of *S. pneumoniae* in children in Finland, it was observed that antimicrobials temporarily reduced carriage rates and thus the overall proportion was similar disregarding the usage of oral antimicrobial treatment within 28 days but were lower if treatment was ongoing or had ended within 7 days (Syrjänen, *et al.*, 2001). Kim *et al.* (2011), reported a resistance rate of *S. pneumoniae* ranging from 15% in 1999 to 71.4% in 2009 among healthy children in Korea. In a study in Switzerland by Mühlemann *et al.* (2003), risk factors for carriage of penicillin-resistant *S. pneumoniae* (PRSP) were found to be young age, previous antibiotic treatment attendance to day care centers and AOM. In Ghana, a recent study by Mills *et al.* (2015) reported MDR (multidrug resistance) prevalence of 87% and resistance were particularly high for cotrimoxazole (100%), ampicillin (88%), tetracycline (78%) and penicillin (63%). Majority of antibiotic resistant *S. pneumoniae* in developed countries have been found among a few serogroups (6, 9, 14, 19 and 23) with serotypes 23F, 19F, 14 and 6A more often reported to be resistant to antimicrobials than the others (Oduyebo *et al.*, 2006; Katsarolis *et al.*, 2009). However serotypes 3, 6A, 6B, 9N, 9V, 14, 19A and 19F have been associated with multiple drug resistant *S. pneumoniae* (Okeke *et al.*, 2005).

Increase in serotype 19A has been reported in countries such as Israel Canada, France, Korea and USA with extensive use of antimicrobials (Amrine-Madsen *et al.*, 2008; Choi *et al.*, 2008; Dagan *et al.*, 2009).

Vaccination strategies in both children and adults will continue to evolve. Serotypes affecting adults and children differ, mandating different strategies for specific populations. Conjugate vaccines containing up to 13 serotypes have been introduced in Ghana in 2012. The vaccine, however, covers only 50% of the prevalent serotypes found in healthy child carriers and that the risk for serotype replacement is high. (Dayie *et al.*, 2013). Thus, regular monitoring of

pneumococcal carriage is essential to identify newly dominant serotypes for future vaccine formulations and to determine prevalence of antimicrobial resistance (Dayie *et al.*, 2013). It should also be emphasized that vaccinating elderly adults against influenza has reduced the risk of all-cause pneumonia (Nichol *et al.*, 1994) and may protect against IPD (Christenson *et al.*, 2004).

Following the availability of pneumococcal conjugate vaccines, there has been a global interest in the epidemiology of the pneumococcus, especially, in the developing world where the brunt of pneumococcal disease is borne.

## **2.5 HUMAN IMMUNODEFICIENCY VIRUS INFECTION AND PNEUMOCOCCAL DISEASE.**

During the era prior to highly active antiretroviral therapy (HAART), the rate of IPD among adults with HIV infection or acquired immunodeficiency syndrome (AIDS) in the United States or Europe was > 40 times higher than age-matched populations (Boschini *et al.*, 1996; Nuorti *et al.*, 2000; Jordano *et al.*, 2004). The incidence of IPD was highest among injection drug users with HIV (Jordano *et al.*, 2004; Nuorti *et al.*, 2000). In HIV-infected children, the incidence rates of IPD are exceptionally high (ranging from 183 to 18,500 episodes per 100,000 child year (Laufer *et al.*, 2006; Bliss *et al.*, 2008). A study of Gambian women with HIV/AIDS noted increased rates of NP colonization, often with pediatric serotypes (Gill *et al.*, 2008) Surveillance data from the ABC Study in the United States noted that HIV-infected persons accounted for 15 to 20% of cases of IPD from 1998 to 1999 (Fry *et al.*, 2003).

Since the introduction of HAART, marked declines in the incidence of IPD have been noted in HIV-infected adults in developed countries (Jordano *et al.*, 2004; Dworkin *et al.*, 2001; Heffernan *et al.*, 2005). Fewer data are available in children, but epidemiological studies cited several fold

reductions in the incidence of bacteremias or pneumonias (Viani *et al.*, 2004) in children between the pre-HAART and HAART eras (Nachman *et al.*, 2005). Unfortunately, in areas of the world with a large burden of HIV infection, the incidence of IPD may be increasing (Karstaedt *et al.*, 2001). Some studies reported lower mortality rates for IPD, including meningitis, among HIV-infected patients (Almirante *et al.*, 1998). This lower mortality rate may in part reflect a blunted inflammatory response to *S. pneumoniae* (Janoff *et al.*, 1993) and younger age of HIV-infected patients (Jordano *et al.*, 2004).

In the ABC surveillance study from 1998 to 1999, the distribution of serotypes causing IPD differed among adults with HIV/AIDS compared with adults with no underlying disease (Fry *et al.*, 2003). The serotype distribution among HIV/AIDS patients was similar to those with hematogenous cancers (Fry *et al.*, 2003). Similarly, a series of IPD in South Africa noted differences in serotypes and antimicrobial resistance patterns among HIV-infected and non-HIV-infected persons (Madhi *et al.*, 2000; Karstaedt *et al.*, 2001).

Pneumococcal vaccination is recommended for HIV-infected adults and adolescents with CD4+ lymphocyte counts > 200 cells/ $\mu$ L, (Masur *et al.*, 2002) but data supporting efficacy are limited (Lopez-Palomo *et al.*, 2004). Immune deficits associated with HIV infection may dampen the antibody response to 23PPV (Janoff *et al.*, 1993; 2007; French *et al.*, 2008). The use of 23PPV did not reduce the incidence of IPD, pneumonia, or mortality in a cohort of HIV-infected adults in Uganda (French *et al.*, 2000). Retrospective studies in the United States suggested that 23PPV protects against IPD among certain groups of HIV-infected patients, specifically those with CD4 counts  $\geq$  200 cells/ $\mu$ L or  $\geq$  500 cells/ $\mu$ L at the time of vaccination or those receiving HAART (Gebo *et al.*, 1996; Dworkin *et al.*, 2001; Lopez-Palomo *et al.*, 2004). These studies were not controlled for comorbidities. A recent prospective study in the United States (the Veterans Aging

Cohort 5-Site Study) found that vaccination with 23PPV reduced the risk of pneumonia in HIV-infected adults; current smoking, low haemoglobin level, and low CD4 cell count significantly increased the risk (Rodriguez-Barradas *et al.*, 2008). Among non-HIV infected patients, vaccination with 23PPV did not confer protection (possibly because of lack of statistical power) (Rodriguez-Barradas *et al.*, 2008).

Benefits associated with HAART may reflect its effects on improving B cell function (Moir *et al.*, 2003) and qualitative and quantitative responses to pneumococcal antigens (Rodriguez-Barradas *et al.*, 2003). One randomized trial in HIV-infected adults found that vaccination with two injections of either PCV7 or 23PPV 2 months apart elicited higher antibody responses compared with placebo/23PPV (Feikin *et al.*, 2001). Vaccination with 23PPV after previous vaccination with PCV7 enhanced antibody response in HIV-infected adults (Kroon *et al.*, 2000). PCV7 is immunogenic in HIV-infected children, but less so than in HIV-uninfected children (Spoulou *et al.*, 2005 Abzug *et al.*, 2006). In children with HIV-infection, a positive correlation between antibody concentration elicited by PCV7 and duration of HAART was found (Abzug *et al.*, 2006). However, the functional activity of pneumococcal antibodies elicited by PCV7 was lower in HIV-infected compared with non-infected children (Madhi *et al.*, 2007). Clinical efficacy of PCV7 in HIV-infected patients remains uncertain, however, a South African trial noted significant reductions in IPD and pneumonia in HIV-infected children with a nine-valent vaccine (Klugman *et al.*, 2003). This effect was attenuated at 5 years compared with non-HIV-infected children (Madhi *et al.*, 2007).

The optimal vaccination policy for HIV-infected persons has not been elucidated; however, universal vaccination of HIV-infected patients is reasonable to reduce colonization and infection (Bliss *et al.*, 2008).



## **CHAPTER THREE**

### **3.0 MATERIALS AND METHODS**

#### **3.1 STUDY AREA**

The study was carried out at the Child Health Department of the Korle-Bu Teaching Hospital (KBTH) located in Accra, the capital city of Ghana. Accra has a population of about two million people and there are 27 hospitals (Ghana Statistical Service, 2010). The Korle-Bu Teaching Hospital is the premier health care facility in Ghana and the only tertiary hospital in the southern part of Ghana. The hospital is affiliated with the medical school of the University of Ghana and has a 2000 bed capacity. The Child health Department of the Hospital has a HIV/AIDS clinic which runs once a week.

#### **3.2 STUDY DESIGN AND SAMPLING**

This was a cross sectional study involving HIV positive children recruited at the Child Health Department of the Korle-Bu Teaching Hospital from February to April, 2015. All HIV positive children less than 15 years were eligible for the study. Children whose HIV status could not be confirmed were excluded as well as those who had been on antibiotics (except Cotrimoxazole) two weeks before the sampling. The study recruited children on cotrimoxazole as previous reports in Ghana showed that pneumococci strains are 100% resistant to this antibiotic (Mills *et al.*, 2015).

Based on 95% confidence limits with an allowable error of 5%, one hundred and eighteen consecutive HIV positive children visiting the Outpatient Department of KBTH were recruited in the study. The sample size was determined as follows:

$$n = \frac{t^2 \times p(1-p)}{m^2}$$

*Description:*

n = required sample size

t = confidence level at 95% (standard value of 1.96)

m = margin of error at 5% (standard value of 0.05)

p = estimated prevalence of pneumococcal carriage = 81% or 0.81 [Anthony *et al.* (2012). Nasopharyngeal carriage of *Streptococcus pneumoniae*: prevalence and risk factors in HIV-positive children in Tanzania (Anthony *et al.*, 2012).

This gives a minimum sample size of 118.

### 3.3 SPECIMEN COLLECTION

Specimen collection was done from February to April 2015. The WHO working group standard method (<http://www.vaccine.uab.edu>) for detecting carriage of *S. pneumoniae* in the upper respiratory tract was used in the collection of specimen and isolation of pure culture (O'Brien *et al.*, 2003). Collection of nasopharyngeal specimen was done by a trained paediatrician with nylon-tipped paediatric size nasopharyngeal swabs. The subject's head was tilted slightly backward to straighten the passage from the front of the nose to the nasopharynx to make insertion of the swab easier. The swab passed directly backwards, parallel to the floor of the nasopharynx. The swab was passed through one nostril until it reached the posterior pharynx

which is approximately one-half to two-thirds the distance from the nostril to the ear lobe. If resistance was encountered, the swab was removed, and the other nostril is tried, since the patient may have a deviated septum. The swab was allowed to sit in the place for 5-10 seconds and was rotated at 180° to saturate the tip before removing it slowly. Swab specimen was placed in a labeled vial containing 1 ml skim milk-tryptone-glucose-glycerin (STGG) medium. Using a scissors disinfected with 70% alcohol wipe, the swab was cut in a length 4cm into aseptically cut off from the handle of the swab, leaving the swab itself in the transport medium and the cap tightened. The specimen was maintained and transported on ice packs within 4 hours to the Department of Microbiology Research Laboratory at the School of Biomedical and Allied Health Sciences, University of Ghana. The transport medium containing the swabs were vortexed for 1-2 minutes and stored at -80°C.

### **3.4 DATA COLLECTION ON RISK FACTORS OF *STREPTOCOCCUS PNEUMONIAE* COLONISATION**

A structured questionnaire was used to collect data on risk factors of pneumococcal carriage from the study participants. The questionnaire covers three areas including demographic features, clinical features and household characteristics, and is shown in Appendix 1.

### **3.5 ANALYSIS OF SPECIMEN**

The nasopharyngeal specimens were inoculated onto chocolate agar and two types of blood agar plates made with 5% sheep blood using a sterile calibrated loop; one of the blood agar plates was made selective for pneumococci by adding gentamicin at a concentration of 5 ug/ml. The inoculated plates were incubated at 37°C in 5% CO<sub>2</sub> (Bowers and Jeffries, 1995). The plates were

examined for growth after 18-24 hours. Bacterial colonies were identified according to the Manual of Clinical Microbiology (Murray *et al.*, 2003).

### **3.6 IDENTIFICATION OF *STREPTOCOCCUS PNEUMONIAE* AND OTHER BACTERIA**

From the primary plate, pre-sumptive *S. pneumoniae* colonies, based on colonial morphology and  $\alpha$ -haemolysis, were picked and streaked out on a sheep blood agar plate. This was followed by application of an optochin disc and over- night incubation at 37°C in 5% carbon dioxide. (O'Brien, *et al.*, 2003). After incubation, the zone of inhibition around the optochin disc was measured. Zone size  $\geq$  14mm was interpreted as Optochin sensitive and a zone size of <13mm was interpreted as Optochin resistant. Isolates that were Optochin sensitive were recorded as *S. pneumoniae*. Optochin-resistant isolates were considered to be viridian streptococci.

Other bacteria that grew on the agar plates were identified based on colonial morphology, Gram stain and a battery of biochemical reactions such as catalase test, coagulase test and oxidase test

### **3.7 ANTIBIOTIC SUSCEPTIBILITY TESTING**

Antibiotic susceptibility testing of *S. pneumoniae* isolates was done by Kirby Bauer method using *S. pneumoniae* (NCTC 10319) as control (NCCL, 2005). The antimicrobial drugs that were tested include ampicillin-10 $\mu$ g, tetracycline-30 $\mu$ g, erythromycin-15 $\mu$ g, ceftriaxone-30 $\mu$ g and cotrimoxazole-25 $\mu$ g. The pneumococcus test isolate was emulsified in peptone until the turbidity was similar to that of 0.5% McFarland's standard. A sterile cotton swab was dipped into the suspension and the excess fluid removed by turning the swab against the inside of the container. Using the dipped cotton swab, SMH agar was swabbed evenly across the whole surface in three different dimensions in order to obtain a semi confluent growth after incubation. After incubation, the zones of inhibition around the antibiotic discs were measured and

interpreted based on the breakpoint criteria of the Clinical and Laboratory Standards Institute (CLSI, 2012).

Antibiotic susceptibility testing was also carried out on other bacterial pathogens isolated from the study participants using the Kirby Bauer method described above. For Gram positive organisms, *S. aureus* (ATCC 25923) was used as a control strain while for Gram negative organisms, *Escherichia coli* (ATCC 25922) was used as a control strain.

### **3.8 DATA ANALYSIS**

Data was entered into Microsoft Excel and exported into SPSS version 16 and Epi Info version 3.5.1 for all statistical analyses. Frequency tables were generated for the various variables and data described according to person, place, and time. The prevalence of nasopharyngeal carriage of *S. pneumoniae* was presented as proportions of individuals in different age groups and gender. Antibiotic resistance rates were estimated. A logistic regression model was used to analyse exposures associated with carriage and the results were presented as *p* values and 95% confidence interval.

## CHAPTER FOUR

### 4.0 RESULTS

#### 4.1 DEMOGRAPHIC, HOUSEHOLD AND CLINICAL FEATURES OF THE STUDY

##### PARTICIPANTS

A total of one hundred and eighteen (118) HIV positive children were recruited in the study and their demographic and household features are reported in Table 1. The gender distributions of the study participants were similar and included 51.7% males and 48.3% females. Their mean age was  $5.8 \pm 3.3$  years and majority of them were in the age group  $\geq 9$ -15years (59.3%). Majority of them were Christians (83.9%), lived in compound houses (73.7%) and attended school (86.4%). The study participants lived in houses with an average of 17 people and 23% were exposed to passive smoking.

As shown in Table 2, the mean CD4 counts of the study participants were 1088.9 cells/mm<sup>3</sup> and 82.2% of them were on antiretroviral drugs. Only 11% had received the pneumococcal vaccine (PCV13). A proportion of 56.8% had respiratory symptoms and the most common symptom was cough (38.5%) followed by runny nose (25.6%). Otitis media occurred in 23.9% of the study participants while 4.3% were asthmatic.

##### 4.2 BACTERIA ISOLATED FROM THE STUDY PARTICIPANTS

Culture of nasopharyngeal swabs collected from the study participants yielded several different organisms (Table 3). The most prevalent organism was *Moraxella catarrhalis* (39.8%), followed by coagulase negative staphylococci (33.1%), *Streptococcus pneumoniae* (30.5%), diphtheroids (29.7%), viridian streptococci (27.1%) and *Staphylococcus aureus* (22%).

**Table 1: Demographic and household characteristics of the study participants**

<b>Parameter</b>	<b>N</b>	<b>%</b>
<b>Age (mean= 5.8±3.3 yrs)</b>		
<i>&lt;5yrs</i>	21	17.8
<i>5-9 yrs</i>	27	22.9
<i>≥9yrs</i>	70	59.3
<b>Gender</b>		
<i>Male</i>	61	51.7
<i>Female</i>	57	48.3
<b>Current school attendance</b>	102	86.4
<b>Religion</b>		
<i>Christian</i>	99	83.9
<i>Moslem</i>	19	16.1
<b>Resident type</b>		
<i>Compound house</i>	87	73.7
<i>Self contained</i>	31	26.3
<b>No. of persons in house (mean=17±13)</b>		
<i>&lt;5</i>	9	7.6
<i>5-10</i>	40	33.9
<i>11-20</i>	34	28.8
<i>21-30</i>	15	12.7
<i>31-40</i>	10	8.5
<i>&gt;40</i>	8	6.8
<b>Exposure to passive smoking</b>	23	19.5

**Table 2: Clinical features of the study participants**

<b>Parameter</b>	<b>N</b>	<b>%</b>
<b>Respiratory symptoms</b>		
<i>Difficulty in breathing</i>	10	8.6
<i>Cough</i>	45	38.5
<i>Runny nose</i>	30	25.6
<i>Sore throat</i>	12	10.3
<b>Asthma</b>	5	4.3
<b>Otitis media</b>	28	23.9
<b>Antiretrovirals taken</b>	97	82.2
<b>Pneumococcal vaccination (PCV13)</b>	13	11

Mean CD4 counts of study participants=1088.9 cells/mm<sup>3</sup>.

PCV 13- Pneumococcal conjugate vaccine 13-valent.

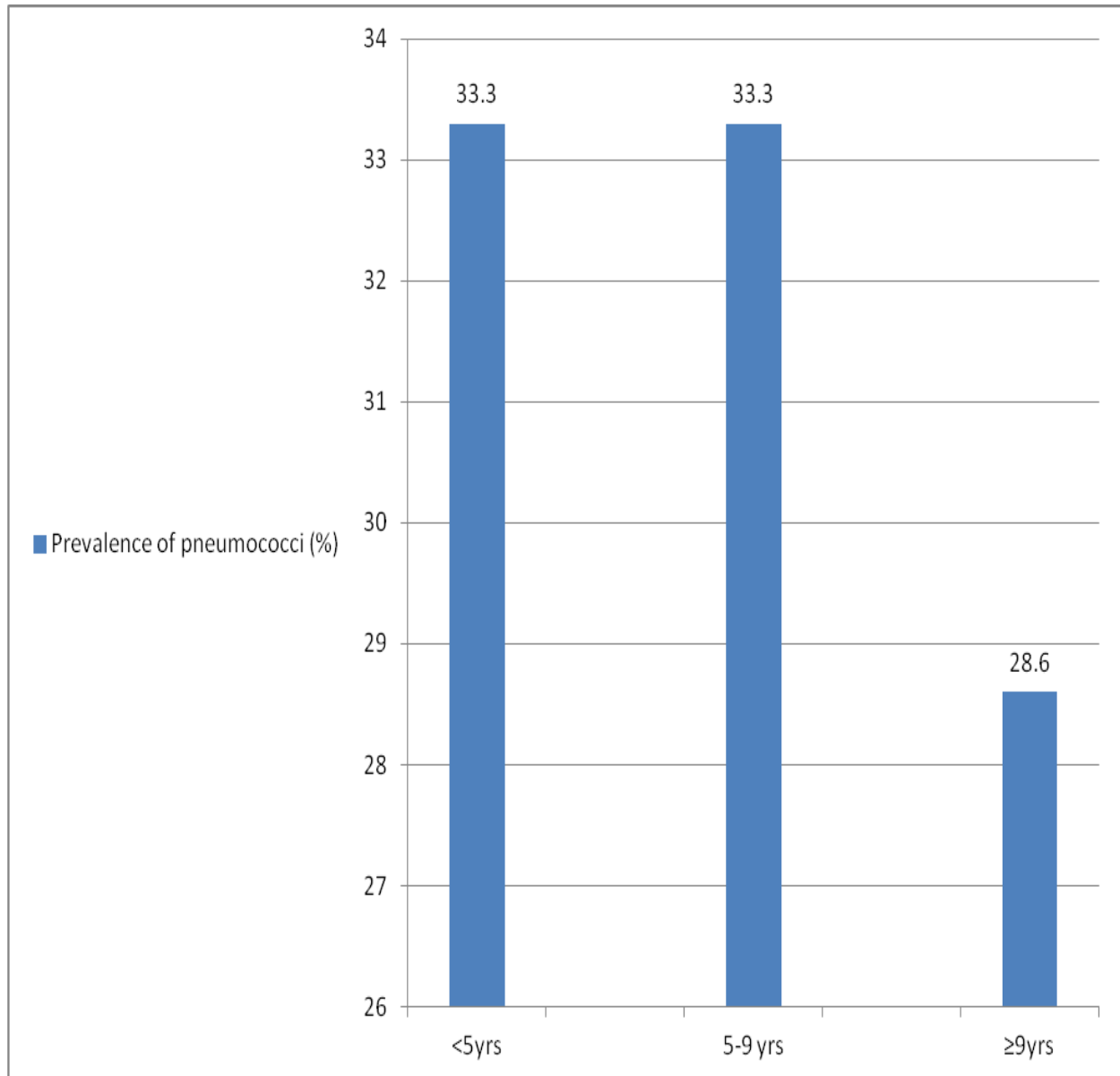
**Table 3: Bacterial colonization of the nasopharynx of HIV positive children <15 years**

<b>Bacteria</b>	<b>N</b>	<b>%</b>
<i>Moraxella catarrhalis</i>	47	39.8
Coagulase negative staphylococci	39	33.1
<i>Streptococcus pneumoniae</i>	36	30.5
Diphtheroids	35	29.7
Viridan streptococci	32	27.1
<i>Staphylococcus aureus</i>	26	22
<i>Citrobacter spp.</i>	5	4.2
<i>Neisseria meningitides</i>	1	0.85

### **4.3 CARRIAGE OF PNEUMOCOCCUS AND ITS RISK FACTORS**

As shown in Figure 1, pneumococcal carriage among the different age groups were 33% (<5 years), 33% (5-9 years), 28.9 years ( $\geq$ 9-12years).

Table 4 shows the association of pneumococcal carriage with demographic, household and clinical features of the study participants. None of the demographic features was significantly associated with pneumococcal carriage ( $p > 0.05$ ). Similarly, none of the household features was significantly associated with pneumococcal carriage ( $p > 0.05$ ). The only clinical feature that was significantly associated with pneumococcal carriage was respiratory symptoms. In this case individual respiratory symptoms including difficulty in breathing, cough, sore throat and cold did not affect pneumococcal carriage significantly. The overall combination of these respiratory symptoms was, however, significantly associated with pneumococcal carriage ( $p=0.008$ ).



**Figure 1: Age specific prevalence of pneumococci carriage among HIV positive children <15 years**

**Table 4: Factors associated with pneumococcal carriage among HIV positive children**

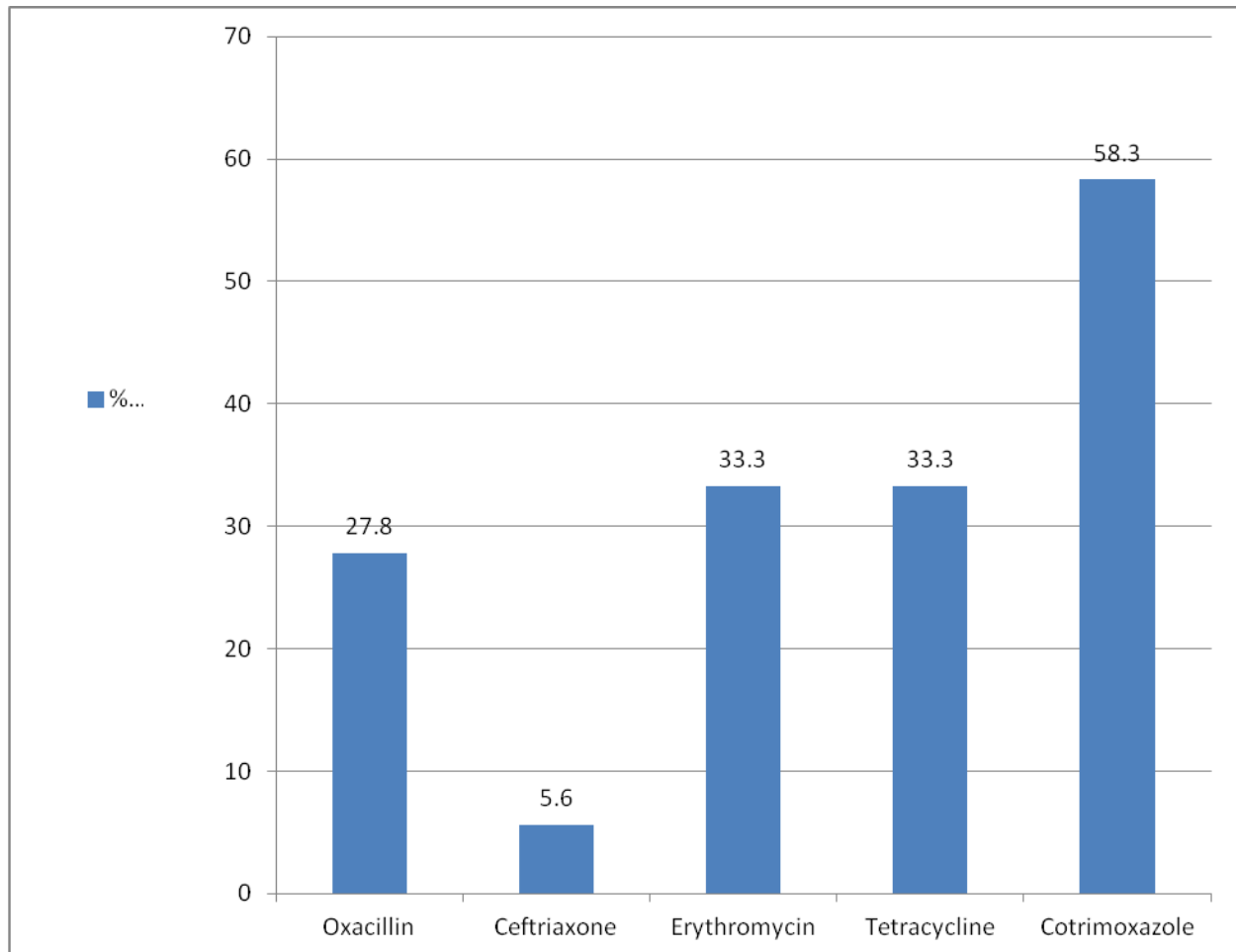
<b>Parameter</b>	<b>P value</b>
<b>Age</b>	>0.05
<b>Gender</b>	>0.05
<b>Current school attendance</b>	>0.05
<b>Religion</b>	>0.05
<b>No. of persons in house</b>	>0.05
<b>Exposure to passive smoking</b>	>0.05
<b>Resident type</b>	>0.05
<b>*Respiratory symptoms</b>	0.008
<i>Difficulty in breathing</i>	>0.05
<i>Cough</i>	>0.05
<i>Runny nose</i>	>0.05
<i>Sore throat</i>	>0.05
<b>Asthma</b>	>0.05
<b>Otitis media</b>	>0.05
<b>Antiretrovirals taken</b>	>0.05
<b>Pneumococcal vaccination</b>	>0.05

\* Significantly associated with pneumococcal carriage

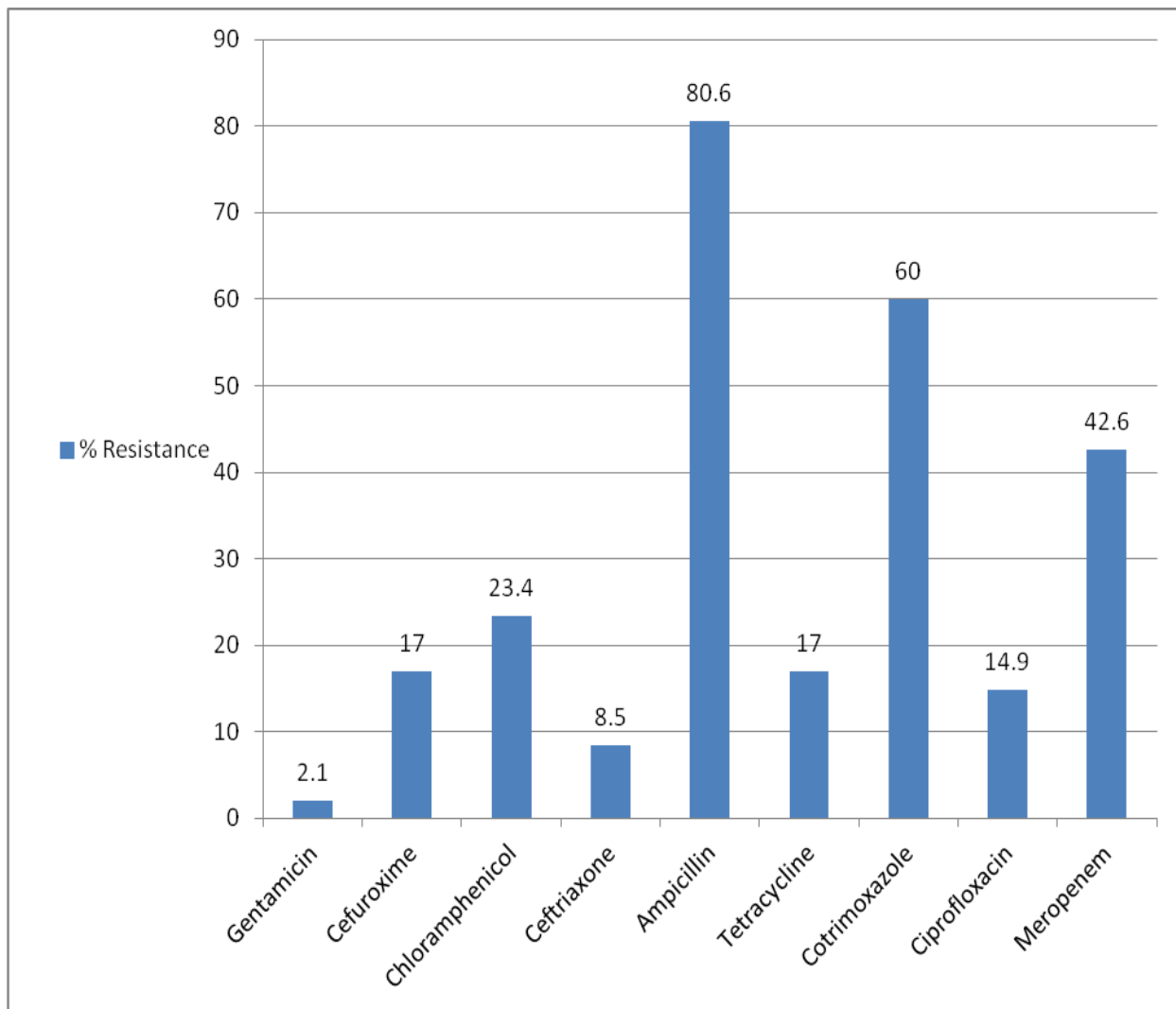
#### 4.4 ANTIBIOTIC RESISTANCE

Antibiotic resistance testing was carried out for the pneumococcus and two other bacteria isolated from the study participants including *S. aureus* and *M. catarrhalis*. Pneumococcal resistance decreased across cotrimoxazole (58.3%), tetracycline (33.3%), erythromycin (33.3%), oxacillin (27.8%), ceftriaxone (5.6%) (Figure 2). *M. catarrhalis* resistance was highest for ampicillin (80.6%), followed by cotrimoxazole (60.0%), meropenem (42.6%), chloramphenicol (23.0%), cefuroxime (17%), tetracycline (17.0%), ciprofloxacin (14.9%), ceftriaxone (8.5%) and gentamicin (2.1%) (Figure 3), *S. aureus* resistance was highest for penicillin (100%), followed by tetracycline (80.8%), cefuroxime (73.1%), erythromycin (38.5%), ciprofloxacin (19.2%), gentamycin (23.8%) and ceftioxin (7.7%) (Figure 4), Four of the twenty six *S. aureus* strains were methicillin resistant indicating a carriage of 3.4% of MRSA.

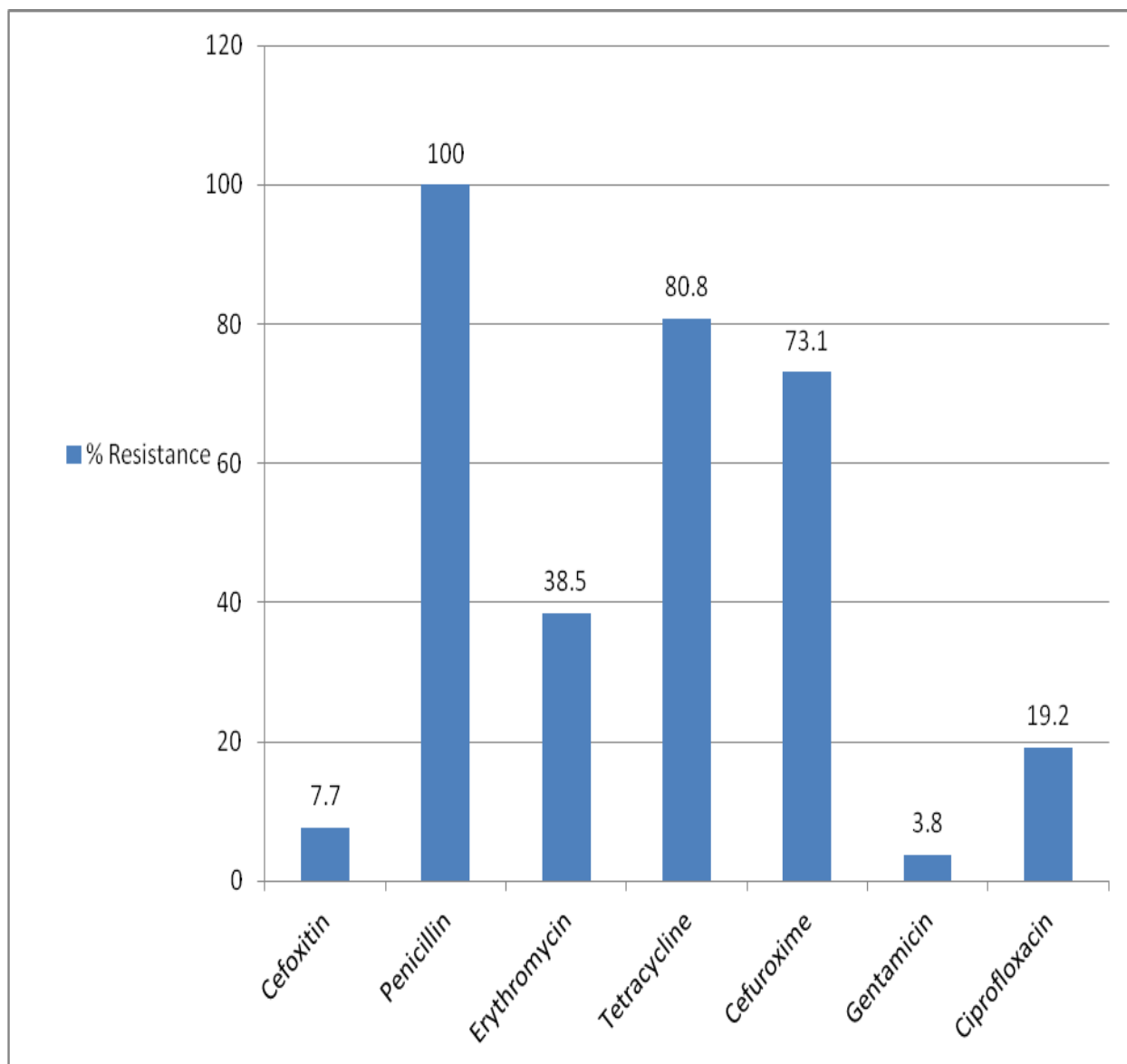
The prevalence of multiple drug resistance (resistance to three or more classes of antibiotics) was 16.7% (9/36) for *S. pneumoniae*, 57.4% (27/47) for *M. catarrhalis* and 84.6% (22/26) for *S. aureus*.



**Figure 2: Resistance of *Streptococcus pneumoniae* to various antibiotics**



**Figure 3: Resistance of *Moraxella catarrhalis* to various antibiotics**



**Figure 4: Resistance of *Staphylococcus aureus* to various antibiotics**



**Figure 5: A blood agar plate with colonies of *Streptococcus pneumoniae* showing alpha haemolysis (greenish colouration)**



**Figure 6: Optochin disc screening of alpha haemolytic Streptococcus colonies**

From left: alpha haemolytic Streptococcus colonies showing resistance to optochin.

From right: alpha haemolytic Streptococcus colonies showing susceptibility to optochin.

## CHAPTER FIVE

### 5.0 DISCUSSION

In this study, the epidemiology of pneumococcal carriage among HIV positive children less than 15 years was investigated among outpatients at the Korle-Bu Teaching Hospital in Ghana. This is probably the first report on pneumococcal carriage among HIV infected people in Ghana, and one of the few in sub-Saharan Africa. The overall prevalence of pneumococcal carriage was 30.5% and carriage was lower among older children (28.9% for age  $\geq 9-12$ ) than carriage among younger children (33.3% for age groups  $<5$  yrs and  $5-9$  yrs), which is consistent with previous studies (Labout *et al.*, 2008; Dayie *et al.*, 2013)

It is important to note that data from this study is post-vaccination data as the pneumococcal vaccine was introduced in Ghana in 2012. Previous pre-vaccination data in Ghana reported pneumococcal carriage rates of 27-51% among children  $<6$  years (Denno *et al.*, 2002; Donkor *et al.*, 2010; Dayie *et al.*, 2013; Mills *et al.*, 2015,). By comparison a study on HIV positive children in Tanzania reported a pneumococcal carriage prevalence of 80% among children  $<12$  years (Anthony *et al.*, 2012). This Tanzanian study was carried out prior to the introduction of pneumococcal conjugate vaccines into the country in 2013, which explains the very high pneumococcal carriage compared to what this study found in Ghana. In Cambodia, Kremery *et al.* (2013) reported a significant decline in pneumococcal carriage among HIV positive children following pneumococcal vaccination.

Apart from the pneumococcus, several other bacterial agents were isolated from the HIV positive children and it may be worthwhile discussing the overall bacterial flora of the nasopharynx of the study subjects. While *M. catarrhalis* was the commonest organism carried by the HIV positive

children in this study (39.8%), in a similar study in Cambodia, *M. catarrhalis* was quite rare (6%) (Kremery *et al.*, 2013). In the Cambodian study, *S. aureus* was the commonest organism isolated (30.4%) but in this study, it was the fourth common organism at a prevalence of (22%). These reflect variations in microbiota composition in different geographical regions and could be due to a wide range of factors. Members of the enterobacteriaceae were rarely isolated from the nasopharynx of the study participants, an observation that agrees with previous studies (Kremery *et al.*, 2013). Like the Cambodian study (Kremery *et al.* (2013), some of the common colonisers of the nasopharynx such as *Neisseria meningitidis* and *Haemophilus influenzae* were rarely isolated which could be due to long-term routine vaccination against these organisms in many countries including Ghana.

The association of respiratory symptoms with pneumococcal carriage in this study has been previously reported for both healthy and HIV positive children (Anthony *et al.*, 2012), and may be related to pneumococcal transmission events. Respiratory droplets transmit the pneumococcus either from healthy or sick people. In this study, the children with pneumococcal colonisation may have contracted respiratory symptoms from other people causing damage to the respiratory tract, which increases the chance of acquiring pneumococcus (Bogaert *et al.*, 2004; Sleeman *et al.*, 2005). This explanation is evident from a Tanzanian study that showed that pneumococcal carriage among HIV positive children was significantly associated with respiratory symptoms in their caregivers (Anthony *et al.*, 2012). Interestingly, data from the current study shows that the relationship between pneumococcal carriage and respiratory symptoms is quantitative as a significant relationship between the two variables was observed only when the various respiratory symptoms were pooled. Further studies are required to throw more light on this, which could provide insights into pneumococcal transmission. Several risk factors of pneumococcal carriage,

such as smoking, history of acute asthma and nutritional status reported by other investigators (Mills *et al.*, 2015) were not observed in our study.

Antibiotic resistance has become a major public health problem especially with important pathogens such as *S. pneumoniae* and *S. aureus*. Much of the recent interest in the epidemiology of pneumococci involves tracing the spread of penicillin resistance, as this has been the main drug of choice for treating pneumococcal infections for a long time. In this study, pneumococcal penicillin resistance was 33.3%, while previous studies have reported wide disparities in pneumococcal resistance to penicillin of 0-66% (Denno *et al.*, 2002; Donkor *et al.*, 2010; Dayie *et al.*, 2013; Mills *et al.*, 2015). The mechanism of resistance to penicillin and other beta-lactam antibiotics in pneumococci is attributed to variations in penicillin binding proteins and is disseminated among pneumococci through intraspecies or interspecies recombination, an event which is known to occur predominantly in the nasopharynx. It is important to note that ceftriaxone showed the lowest pneumococcal resistance, an observation, which concurs with previous studies (Dayie *et al.*, 2013; Mills *et al.*, 2015), and therefore highlights this antibiotic as a suitable choice for empirical treatment of pneumococcal infections in both healthy and HIV positive people. Pneumococcal resistance to cotrimoxazole was quite high (58.3%) but significantly lower than what had been previously reported in Ghana (100%) by several studies. This is interesting as cotrimoxazole is routinely administered in prophylaxis to HIV positive people (WHO 2006). This finding suggests that cotrimoxazole prophylaxis does not necessarily lead to antibiotic resistance of pneumococci carried by HIV positive people. Donkor *et al.* (2013) has reported a similar observation about pneumococcal carriage and penicillin prophylaxis in relation to people with sickle cell disease. In the recruitment of subjects in this study, children on antibiotics were excluded with the exception of those on cotrimoxazole as data in Ghana had showed 100%

pneumococcal resistance to this antibiotic (Mills *et al.*, 2015). However, more than 40% of pneumococci were susceptible to cotrimoxazole in this study, suggesting pneumococcal carriage may be higher than what was observed (30.5%).

The extremely high percentage resistance (>80%) of *S. aureus* and *M. catarrhalis* to penicillin/ampicillin is due to the production of beta-lactamase by most strains of these bacteria that degrade the beta lactam ring of penicillin (Nathwani *et al.*, 1993). Methicillin resistant *S. aureus* is the most important *S. aureus* strain in terms of antibiotic resistance. In this study, MRSA carriage was 3.4%, which is significantly higher than the 0.3% carriage recently reported among healthy people in Ghana (Egyir *et al.*, 2014). HIV infection has been identified as an independent risk factor for determining colonization with MRSA though the reasons for this are not clear. Despite the high levels of resistance to the several antibiotics tested, over 90% of the *S. aureus* isolates were susceptible to cefoxitin which indicates the suitability of this antibiotic for managing *S. aureus* infections among HIV positive children.

## CHAPTER SIX

### 6.0 CONCLUSIONS, RECOMMENDATIONS AND LIMITATIONS

#### 6.1 CONCLUSIONS

The main conclusions of the study are as follows:

1. Bacteria that commonly colonised the nasopharynx of the HIV positive children were *M. catarrhalis*, coagulase negative staphylococci and *S. pneumoniae*.
2. Pneumococcal carriage among the HIV positive children was 30.5%, which may have been affected by several factors including vaccination and cotrimoxazole prophylaxis.
3. The presence of respiratory symptoms was the main risk factor associated with pneumococcal carriage among the HIV positive children.
4. Antibiotic resistance of nasopharyngeal flora was high especially for *S. aureus*. Ceftriaxone and cefoxitin may be suitable antibiotics for treating *S. pneumoniae* and *S. aureus* infections in the HIV positive children.

#### 6.2 RECOMMENDATIONS

Based on the findings of the study, it is recommended that

1. Infections of the common organisms that colonised the study participants are monitored among HIV positive children
2. Further studies are carried out to serotype pneumococci carried by HIV positive children. This will provide relevant information on HIV positive children with regard to pneumococcal vaccination.

#### 6.3 LIMITATIONS

There are a few limitations of the study. For ethical reasons, a control group of non-HIV positive children could not be recruited in the study. The HIV positive children recruited in the study were

on cotrimoxazole prophylaxis and that could have affected the pneumococcal carriage prevalence observed in the study.

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**APPENDIX 1: PNEUMOCOCCAL CARRIAGE RISK FACTORS QUESTIONNAIRE****SECTION A: IDENTIFICATION/ DEMOGRAPHY**

1. Hospital Registration (Folder) No	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<b>HPIN</b>	
2. Form Number/ child ID :	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<b>PIN</b>	
3.	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>		
4. Name	<i>Family name</i>					<i>Other name</i>						<b>NAME</b>	
5. Sex						<b>1.Male</b>		<b>2.Female</b>					<b>SEX</b>
6. Date of birth	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<b>DOB</b>	
7. Age						<i>Year</i>			<i>Month</i>			<b>AGE</b>	
8. Ethnicity	<input type="text"/>											<b>ETHNIC</b>	
9. Occupation of Guardian	<input type="text"/>											<b>OCUPATION</b>	
10. Religion	<b>1.Traditional</b>			<b>2.Christianity</b>			<b>3.Islam</b>			<b>4.Other</b>			<b>RELIGION</b>
11. Permanent ID/National ID/Voter ID of guardian	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<b>PERM ID</b>	
12. National Health Insurance ID	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<input type="text"/>	<b>NHID</b>	
13. Community of residence	<input type="text"/>											<b>COMMUNIT Y</b>	
14. Sub District of residence	<input type="text"/>											<b>SUBDIST</b>	

**RESIDENTIAL STATUS & MIGRATION RECORD**

15. Do you stay in this district permanently?	<b>1 .yes</b>	<b>2.No</b>	<b>RESIDENT</b>
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15. Do you stay in a residential or compounds house	1.Residential	2.Compound	<b>REIDENTI A</b>
16. How many people live in your house			<b>NUMBHSE</b>
17. How many children in the household are less than 5 years?			<b>LESS5</b>
18. Does any of your household smoke?	1.yes	2.No	<b>SMOKE</b>
19. Does your child attend a day care?	1.yes	2.No	<b>DAY CARE</b>

**VACCINATION & MEDICATION RECORD**

20. Has subject ever taken pneumococcal vaccine?	1.yes	2.No	3.Nk	<b>VACCINE</b>
21. Immunization card seen	1.yes	2.No		<b>IMMUCARD</b>

**RESPONDENT**

22. Name	<i>Family name</i>		<i>Other name</i>			<b>RES_NAME</b>
23. Relationship to subject	1.Father	2.Mother	3.spouse	4.child	5.other (specify)	<b>RELATIVE</b>
24. Educational status of mother	Nil	Basic	Secondary	Tertiary		<b>EDUMOTH</b>
25. Educational status of Father	Nil	Basic	Secondary	Tertiary		<b>EDUMOTH</b>

**SECTION B:****HEALTH FACILITY & SERVICES**

26. Reporting Health Facility		<b>HOSPITAL</b>
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**MEDICAL HISTORY**

27. Is the patient Asthmatic	1.yes	2.No	3.NK	<b>ASTHMA</b>	
28. Is the subject a HIV patient?				<b>HIV</b>	
29. Has subject an immunocompromised disease?				<b>IMMUNO</b>	
30. Has subject ever had otitis media (ear infection)?	1.yes	2.No	3.NK	<b>OTITIS</b>	
31. If Q30 is YES, when did the subject have otitis (month& year)				<b>DATEOTI TI</b>	
32. Has subject sought antibiotic treatment somewhere before coming	1.yes	2.No	3.NK	<b>SOUTRM N</b>	
33. If Q32, is yes where?	1.Hom e	2.Hebalist	3.Health facility	4.other	<b>ORIGIN</b>
34. If Q32 is health facility, what is the name of the health facility?				<b>HEALTFA C</b>	
35. How long was child ill before being sent to the health facility? (Days)				<b>DURATIO N</b>	
36. What were the complaints before subject was sent to the health facility?				<b>COMPLAI N</b>	

**DIAGNOSTIC SERVICES**

37. Clinical diagnosis	1.Malaria	2.ARI	3.Diarrhoea	4. Septicemia	5.other	<b>DIAGNOSIS</b>
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38. Has your child had any of the following signs and symptoms in the past 1 month

A. Fever	1.yes	2.No	3.Nk	<b>FEVER</b>
B. Sore throat	1.yes	2.No	3.Nk	<b>SORETH</b>
C. Runny nose/catarrh	1.yes	2.No	3.Nk	<b>RUN NOSE</b>
D. Blocked nose	1.yes	2.No	3.Nk	<b>BLOK NOSE</b>
E. Headache	1.yes	2.No	3.Nk	<b>HEADACHE</b>
F. Sneezing	1.yes	2.No	3.Nk	<b>SNEEZING</b>
G. Cough	1.yes	2.No	3.Nk	<b>COUGH</b>
H. Difficulty in breathing	1.yes	2.No	3.Nk	<b>BREATHING</b>
I. Middle ear infection/ otitis media	1.yes	2.No	3.Nk	<b>EAR INF</b>

## **APPENDIX 2: INFORMED CONSENT FORM**

I am seeking your consent to enroll your ward in a study in which nasopharyngeal specimen shall be collected from your ward to test for the presence of *Streptococcus pneumoniae*. This activity is part of a study on the epidemiology of pneumococcal carriage among children with HIV in Accra. *Streptococcus pneumoniae* causes infections such as meningitis and pneumonia. Carriage of the organism does not mean that you have an infection but is a risk factor for *Streptococcus pneumoniae* disease. Fortunately, a vaccine for *Streptococcus pneumoniae* has been introduced in Ghana, and this study would help us find out about the performance of the vaccine in the case of children with HIV infection.

### **WILLINGNESS TO PARTICIPATE**

You are to understand that:

1. You allowing your ward to partake in this research is entirely voluntary and that you can decide not to take part in the research and your refusal to participate in the study will not prevent you from being attended to at the health facility.
2. After accepting for your ward to participate in the study, you have the option of changing your mind and withdrawing your ward from the study at any a time without any objection. This will not prevent you from being attended to at the health facility.
3. In the course of the study, you will be made to answer questions about you and your ward's personal life style and health. I will also review your ward's medical records. A swab will be used to take specimen from the nasopharynx of your ward.

## **CONFIDENTIALITY**

The information I shall obtain from you will be kept in the investigator's confidence. In the event of scientific reporting of the findings of the study your ward's name will not be mentioned or identified by name.

## **RISK TO PARTICIPANT**

The possible risks involved in the study may be the fact that you may not be comfortable answering questions about yourself and your ward, in that you may fear that information and findings from the study will be shared with other. You are assured of complete confidentiality. Also your anxiety may be about the nasopharyngeal sample to be taken from your wards. Please understand that the process will not be harmful but your ward may experience some temporary discomfort at the site. In the case of any problem, the doctors at the Child Health Department would attend to your ward.

## **BENEFITS TO PATIENTS**

The study would help us to identify risk factors of *Streptococcus pneumoniae* carriage among children with HIV, which would help us to prevent diseases caused by the organism among this group of children. The study would also help us to identify the antibiotics suitable for treating *Streptococcus pneumoniae* infections among people with HIV infection. Finally, through the study, we would be able to advise the government of the importance of *Streptococcus pneumoniae* vaccination to HIV positive children.

### **Section A: to be completed by researcher.**

I have fully explained to.....the nature and purpose of the above described study procedure and the risk that are involved in its performance.

I have been fully informed of the above described study with its possible benefits and risk my child may face. I have been made to understand that, the study is entirely voluntary and that my child can decide to participate or withdraw from it at any time without any consequences to the questions.

subsequent treatment and care my ward may receive at the health facility. I have been given the opportunity to ask questions that I have in relation to the research. All my questions have been answered to my satisfaction.

Full name of researcher..... Signature..... Date.....

Name..... Signature..... Date.....

**Section C: to be completed by a witness to the consenting process**

Name of witness.....

Signature/ thumbprint..... Date.....

### **APPENDIX 3: PREPARATION AND USE OF 0.5 MCFARLAND STANDARD SOLUTION**

#### **Reagents**

Deionized water, Concentrated H<sub>2</sub>SO<sub>4</sub> and BaCl<sub>2</sub> salt.

#### **Equipment**

3 volumetric flask (100ml), 0.5ml volumetric pipettes and spectrophotometer

#### **Procedure**

- 1.175% (w/v) of BaCl<sub>2</sub> salt was prepared by dissolving 1.175g of the salt in deionized water and made up to the mark in a 100ml volumetric flask.
- 1.0ml of Concentrated H<sub>2</sub>SO<sub>4</sub> was added to 90ml of deionized water in a volumetric flask and made up to the mark with more deionized water.
- 0.5ml of 1.175% BaCl<sub>2</sub>·2H<sub>2</sub>O was added to 85ml of 1% (v/v) H<sub>2</sub>SO<sub>4</sub> in a 100ml volumetric flask. This was made up to the mark with more 1% H<sub>2</sub>SO<sub>4</sub> and swirled gently to facilitate reaction.
- The solution was checked for homogeneity and absence of visible clumps. The optical density was determined at a wavelength of 625nm and found to be approximately 0.09.
- Volumes were dispensed into small sterilized Bijoux bottles, capped and stored in the cupboard at room temperature.

#### **How to use standard**

Turbidity of inoculums were compared to the McFarland Standard in front of a Wickerham card and adjusted until the turbidity appeared to be the same to the unaided eye using the lines of the card as a guide for standard turbidity.