UNIVERSITY OF GHANA

COLLEGE OF HEALTH SCIENCES

POST VACCINATION SURVEILLANCE OF PNEUMOCOCCAL SEROTYPES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PROFILE AMONG CHILDREN UNDER FIVE IN ACCRA, GHANA

BY

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DECLARATION

I declare that this thesis is the result of my own research work carried out in the Department of Medical Microbiology, School of Biomedical and Allied Health Sciences, University of Ghana, under the supervision of Dr. Nicholas T.K.D Dayie and Prof. Mercy J. Newman.

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ABSTRACT

Background: In May 2012, Ghana introduced the PCV-13 as part of the routine childhood immunization programme with a view to mitigate the burden of pneumococcal disease among children under five. Vaccination though effective, creates a selection pressure that may lead to serotype replacement or capsular switching. This necessitates the need to perform post vaccination surveillance studies in areas where the PCV-13 has been introduced in order to ascertain current knowledge of serotype distribution necessary to evaluate vaccine efficacy.

Aim: The aim of the study was to determine the antimicrobial susceptibility patterns and serotype distribution post PCV-13 vaccination in Accra, Ghana.

Methodology: The research was a cross-sectional study involving seven randomly selected schools and 410 respondents in the Accra Metropolis of the Greater Accra region of Ghana. Informed consent was obtained from parents of the under-five year old children before a nasopharyngeal swab was taken. Identification and characterisation of *S. pneumoniae* was done based on methods described by the WHO.

Results: Pneumococcal carriage prevalence among the study participants was 64.9%. Penicillin non-susceptibility prevalence was 26.7% with an intermediate resistance of 24.8% and full resistance of 1.9%. Trimethoprim-Sulphamethozaxole showed the highest resistance prevalence of 78.6% and Erythromycin showed the lowest prevalence of 16.7%. All isolates were susceptible to ceftriaxone and levofloxacin. Out of the 111/266 serotyping results, the three most dominant serotypes were; 23B (16.1%), 23F (9.7%) and 19F (7.5%).

Conclusion: Pneumococcal carriage was observed in more than half of the study population characterised by reduction in the prevalence of penicillin non-susceptible *Streptococcus pneumoniae.* Non-PCV-13 serotype (23B) predominates in carriage and this serotype is associated with high MDR. PCV-13 failed to eliminate serotype 23F and 19F.

DEDICATION

This thesis is dedicated to YAHWEH who is my strength and hiding place.

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LIST OF ABBREVIATIONS

MDR:	Multidrug Resistance
PCV:	Pneumococcal Conjugate Vaccine
PPV:	Pure Polysaccharide Vaccine
IPD:	Invasive Pneumococcal Disease
NVT:	Non-vaccine serotypes
NT:	Non-typeable
E-test:	Epsilometer test
MIC:	Minimum Inhibitory Concentration
ORID	Office of Research and Innovative Studies
ADMER	Antibiotic Drug use, Monitoring, Evaluation of Resistance
CLSI	Clinical Laboratory and Standards Institute
ATCC	American Type Culture Collection

CHAPTER ONE

1.0 Introduction

Pneumonia is the major cause of death in children under five both in the developed and developing countries and *Streptococcus pneumoniae* (pneumococcus) is known to be the leading bacterial causative agent (CDC, 2010; UNICEF, 2012).

S. pneumoniae is a Gram-positive bacterium that is carried as a commensal in the nasopharynx of humans most especially young children (Högberg *et al.*, 2007). The bacterium possesses a polysaccharide capsule that facilitates evasion of the host immune system (Hyams *et al.*, 2010). Based on the immunochemistry of the capsular polysaccharide, about 97 serotypes have been identified of which only a limited number are associated with invasive pneumococcal disease (Pilishvili, Noggle and Moore, 2012).

The advent of penicillin in 1943 helped to reduce pneumococcal morbidity and mortality until the first penicillin resistant strains of pneumococci were reported in the 1960s in Papua New Guinea and Australia (Hansman and Bullen, 1967). Since that time, the emergence and spread of multidrug resistance among pneumococcal strains became common on the global scene (Pilishvili, Noggle and Moore, 2012).

The introduction of the Pneumococcal conjugate vaccine (PCV-7) in 2000 led to a considerable decrease in invasive pneumococcal disease in children under five years (CDC, 2010). Few years after the introduction of PCV-7, research has shown that, non-PCV-7 vaccine serotypes have emerged as important causative agents of invasive pneumococcal disease (Mook-Kanamori et al. 2011) hence, the introduction of PCV-13. In Africa, the major serotypes of invasive pneumococcal disease are; serotypes 1, 5, 6A, 6B, 14, 19A, 19F and 23F. In West Africa, the major invasive serotypes are 6A, 5, 14, 1,

and 3 (Donkor et al., 2013). The predominant carriage serotypes circulating in Ghana are; 19F, 6B, 23F and 6A (Dayie *et al.*, 2013). Based on the most current carriage study performed by Dayie *et al.*, (2013), PCV-13 vaccine coverage was determined to be 50% and suggested the possibility of serotype replacement/capsular switching to be a concern in the near future with respect to the efficacy of the PCV-13 introduced in Ghana.

In addition, despite the various advantages PCV-13 vaccine offers to recipients, studies have shown that vaccine pressure led to serotype replacement/capsular switching which subsequently resulted in a marginal increase in invasive disease. This however, has not eroded the gains made by the introduction of PCVs (Chiu & Mcintyre 2013; Mook-Kanamori et al. 2011).

1.1 Problem statement

More than 1.6 million deaths are associated with pneumococcal disease among children under five years worldwide. Pneumococcal disease kills more than AIDS, measles and malaria combined (Adegbola, 2012). Vaccination with PCVs is the major means of reducing the burden of pneumococcal disease among this age group nonetheless, vaccination is saddled with the phenomenon of pneumococcal serotype replacement/capsular switching due to vaccine pressure; leading to non-vaccine types taking over as major causes of invasive pneumococcal disease. For instance, in countries where the vaccine was introduced, studies have shown that, pneumococcal serotypes which were not included in the vaccine have emerged as important causes of invasive pneumococcal disease. Alexandre *et al.*, (2010) reported a rebound effect in the incidence of pneumococcal meningitis in northern France after the introduction of pneumococcal conjugate vaccine and this was attributed to the emergence of non-vaccine serotypes in the PCV-7 era. Furthermore, serotype 19A which was not included

in the vaccine emerged as a major causative agent of invasive pneumococcal disease (Hsu *et al.*, 2010). Carriage studies in other countries indicated that, non PCV-13 serotypes are gradually taking over as colonizers (Camilli *et al.*, 2013). A typical example is the serotype 35B which appears to be taking grounds as an important causative agent of pneumococcal disease (Domenech *et al.*, 2015). In May 2012, Ghana introduced the PCV-13 as part of the routine childhood immunization programme with a view to mitigate the burden of pneumococcal disease among children under five (MOH, 2014). Dayie *et al.*, (2013) reported low PCV-13 vaccine coverage among healthy children under six and predicted that there is the possibility of increased carriage of non-vaccine serotypes post PCV-13 introduction in Ghana hence the need for continues surveillance of carriage serotypes.

There is currently no data in Ghana indicating the prevailing serotypes in healthy children in the post vaccination era. This necessitates the need to perform post vaccination surveillance studies in areas where the PCV-13 vaccine has been introduced in order to ascertain current knowledge of serotype distribution necessary to evaluate vaccine efficacy.

1.2 Rationale

Four years after the introduction of the pneumococcal conjugate vaccine in Ghana, there is no data on prevailing circulating serotypes in healthy children in the country. There is therefore the need to investigate and determine type of serotypes circulating in the healthy population. Findings of this study will enable policy makers to analyse the effectiveness of the vaccine. In addition, previous studies by Dayie *et al.*, (2015), showed increasing incidence of multidrug resistant pneumococci among carriage isolates

hence, antimicrobial susceptibility testing will be performed to determine the current trend of resistance in pneumococci among children under five post PCV vaccination.

1.3 Aim

The aim of this study is to determine the pneumococcal serotype distribution and antimicrobial susceptibility patterns of carriage isolates post PCV-13 vaccination in Accra, Ghana.

1.4 Specific objectives

1. Determine the nasopharyngeal carriage of *S. pneumoniae* post PCV-13 vaccination in a population of healthy children under five years.
2. To determine the antimicrobial susceptibility profile of *S. pneumoniae* carriage isolates among children under five years.
3. To determine the distribution of serotypes among healthy children under five years.

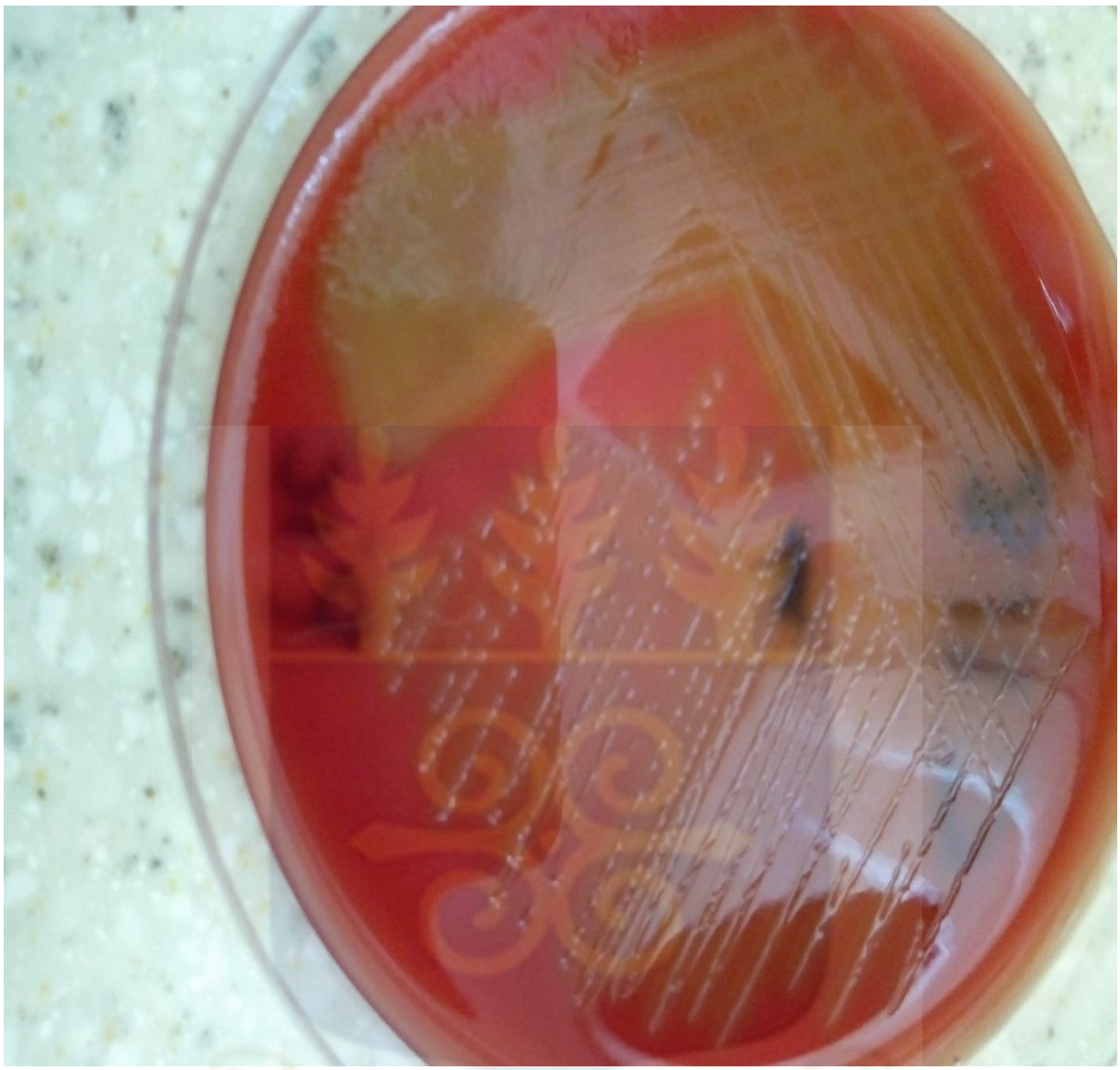
CHAPTER TWO

2.0 Literature Review

2.1 Microbiology of Streptococcus pneumoniae

S. pneumoniae is a Gram-positive, non-motile lanceolate bacterium that can be found in pairs, singly or in chains. The bacterium is nutritionally fastidious, lactic acid fermenter and has a genome size of 2.16Mbps which is highly plastic with a G+C content of 40% (Henriques-Normark and Tuomanen, 2013a). The organism is non-spore forming, facultative anaerobe and exhibits alpha-haemolysis on blood agar. They display small, shiny, round, moist and grey colonies. Colonies older than 18-24hrs may appear flat and centrally depressed; earning it the classical name "draughtsman-shape" colonial morphology. Pneumococci are susceptible to optochin and soluble in bile salt (Tille *et al.,* 2013; Satzke *et al.,* 2013). The cell wall of the bacterium is surrounded by a polysaccharide capsule which is the main antigenic determinant of the organism (Hyams *et al.,* 2010).

Figure one: alpha-haemolytic colonies on 5% sheep blood agar



The cell wall of the pneumococcus as with other Gram positive bacteria comprises of peptidoglycan and teichoic acid. Although the structural composition of the peptidoglycan (N-acetyl glucosamine, N-acetyl muramic and a lysine-containing stem peptide) is the same as for all other Gram positives, what makes the cell wall of the pneumococcus unique is the structural composition of the teichoic acid. It contains a ribitol phosphate backbone covalently attached to a phosphorylcholine (PCho) (Bean and Tomasz, 1977). The PCho is very essential to the pneumococcus because it serves as a docking site for the linkage of pneumococcal proteins to the surface of the bacterium (Rosenow *et al.*, 1997).

Based on the appearance of an illuminated colony, the bacterium could be described as being in the opaque or the transparent phase (phase variation). Phase variation is an adaptive mechanism of the bacterium which helps it to evade the host defence mechanism such as the C-reactive proteins of the host. The transparent phase is adapted for colonization whiles the opaque phase is adapted for invasion (Weiser *et al.*, 1994; Arai *et al.*, 2011).

The organism is transmitted from person-to-person through aerosols. The diseases caused by the pneumococcus ranges from invasive diseases such as bacteraemic community-acquired pneumonia, meningitis and bacteraemia to non-invasive conditions like otitis media and sinusitis (WHO, 2014).

2.2 Nasopharyngeal carriage and serotype distribution of *S. pneumoniae*

The pneumococcus is known to be a natural transient colonizer of the human nasopharynx. Carriage prevalence of the bacterium varies from one geographical location to the other and differs from age-group to age-group (Bogaert, De Groot and Hermans, 2004). Nasopharyngeal carriage and transmission of pneumococcus is predominant in children below the age of five years (Usonis *et al.*, 2015). By the first month of life, the nasopharynx

of most children is known to be colonised by *S. pneumoniae*; most especially children from developing countries (Bogaert, De Groot and Hermans, 2004; Ba *et al.*, 2014). In Gambia, infants were colonised as early as 12 hours post-partum (Darboe *et al.*, 2010). Carriage prevalence is higher in children from developing countries as compared to developed countries (Adegbola *et al.*, 2014). The reported carriage prevalence in the UK is 30% (Gladstone *et al.*, 2015a). In Kenya, Abdullahi *et al.*, (2012) reported a high carriage prevalence of 65.8% among children; with younger children (6-11 months) showing the highest prevalence of 79% and older children (54-59 months) showing the lowest prevalence of 51%. Morocco reported a carriage prevalence of 45.8% in children under two prior to the introduction of PCV13 (Bouskraoui *et al.*, 2011). The carriage prevalence among children less than five years in Senegal is 50% (Ba *et al.*, 2014) and in Nigeria; 52.5% in a study population that include adults with 74.4% in children under two (Adetifa *et al.*, 2012). In Ghana the carriage prevalence among risk groups are; 16.4% Sickle Cell Disease children aged ≤5years (Baffoe-Bonnie *et al.*, 2000) and 28.6% in HIV children <5years (Donkor *et al.*, 2017). Dayie *et al.*, (2013) reported a carriage prevalence of 31%-34% in children under 6 as compared to the carriage prevalence of 27.2% reported in previous studies in children <5years (Donkor *et al.*, 2010). Mills *et al.*, (2015) reported a carriage prevalence of 48.9% in children below the age of five years.

Certain conditions such as crowding, age, previous antibiotic use, viral infection of the upper respiratory tract and older siblings have been shown to be some of the risk factors to pneumococcal colonization of the nasopharynx (Abdullahi *et al.*, 2012; Kumar *et al.*, 2014; Wren *et al.*, 2014). Due to the high prevailing risk factors in developing countries, the carriage prevalence is very high in such countries as compared to developed countries (Donkor *et al.*, 2013).

Carriage is known to precede disease (Kumar *et al.*, 2014; Usuf., 2014). Morbidity in under-fives due to pneumococci is quite common in west Africa as a result of high pneumococcal carriage prevalence and prevailing predisposing factors that favour the transmission of the bacterium (Donkor, 2013). Carriage is more common and prolonged in children as compared to adults (Högberg *et al.*, 2007).

Colonization in itself may seem as a harmless phenomenon in the healthy population and serves as a means of acquiring immunity against the capsular polysaccharide surface associated proteins of the carried strain. However, this colonized individual becomes a source of horizontal transmission to others in the population which may lead to a disease state in susceptible individuals. Furthermore, acquisition of antimicrobial resistance genes is known to occur during the period of asymptomatic colonisation especially in individuals receiving antibiotics for minor ailments (Obaro and Adegbola, 2002; Hiller *et al.*, 2010).

Serotype distribution is known to vary across various geographical location and age-group (Bogaert, De Groot and Hermans, 2004). Serotypes also differ from each other in relation to their ability to colonize, cause invasive disease and their case-fatality rate. For instance, serotype 1 is frequently isolated in invasive diseases but rarely found in carriage (Hausdorff *et al.*, 2000). According to Brueggemann *et al.*, (2003), serotype 1, 5 and 7F are hardly found in carriage studies but frequently isolated in invasive studies. These serotypes however, have low case-fatality rate as compared to serotype 3, 6A, 6B, 19F and 23F which are common nasopharyngeal colonizers; have low invasive potential yet, associated with high case-fatality rate. In bacteraemic pneumonia, serotypes 3, 6A, and B, 9N and 19F are associated with a higher risk of mortality in adult population (Weinberger *et al.*, 2010; Luja *et al.*, 2010). Globally, serotypes 14, 4, 1, 6A, 6B, 3, 8, 7F, 23F, 18C, 19F and 9V are the key agents for invasive pneumococcal disease with serotype 1, 5, 6A, 6B, 14, 19F and 23F being the

dominant serotypes causing IPD in the paediatric population (Cillóniz and Torres, 2014). In West Africa, serotype 1 ranks first (32%) as the isolated organism in invasive pneumococcal disease followed by serotype 5 (15%). In the pre-vaccination era, the leading serotypes causing invasive disease in children under five in the sub-region were; 5, 6A, 1, 3 and 14 (Donkor, Dayie and Badoe, 2013). The predominant serotypes responsible for invasive disease in Ghana were 1, 3 and 8 (Donkor, Dayie and Badoe, 2013). Serotypes 6B, 14, 19F, 18C, 23F, 4 and 9V are reported to be the predominant paediatric serotypes in Europe and the USA (Imöhl *et al.*, 2010; Tan, 2012). In sub-Saharan Africa, the commonest carriage serotypes are 19F, 6B, 6A, 14 and 23F (Usuf *et al.*, 2014). The predominant carriage serotypes in Ghana are 19F, 6B, 23F, and 6A (Dayie *et al.*, 2013).

2.4 Pathogenesis and virulence factors of *S. pneumoniae*

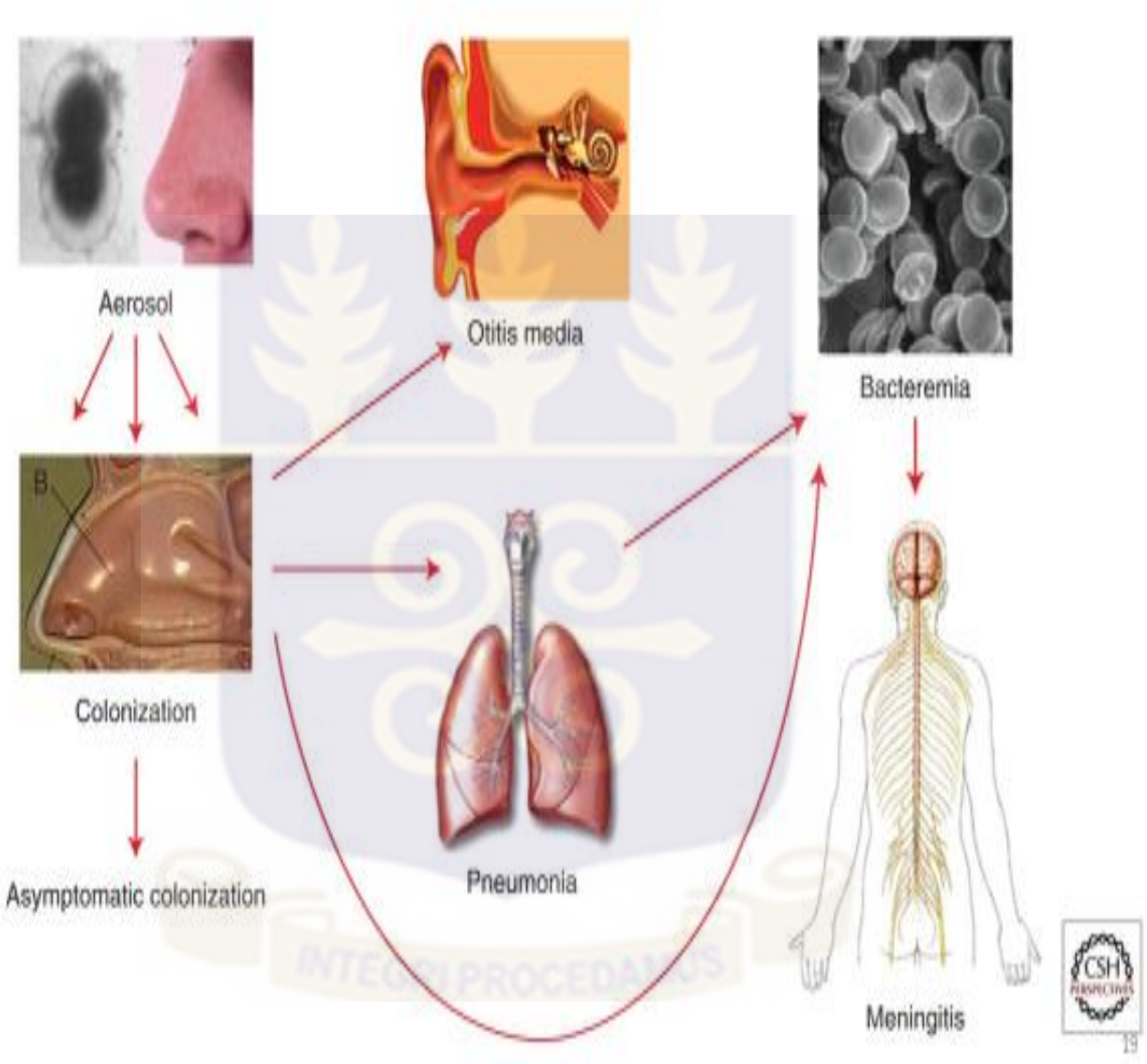
The capsule is the main virulence determinant of the pneumococcus and based on the immunochemistry of the capsular polysaccharide, there are more than 93 serotypes (Pilishvili, Noggle and Moore, 2012). The capsule confers protection on the bacterium by aiding it to escape phagocytosis and also, it inhibits the activation of the complement system (AlonsoDeVelasco *et al.*, 1995; Hyams *et al.*, 2010).

In addition to the capsule, other virulence factors of the pneumococcus includes, pneumococcal surface antigen A (PsaA), Choline-Binding protein A (CbpA), hyaluronidase, neuraminidase, pneumolysin and autolysin. (Mitchell and Mitchell, 2010).

The first step to pathogenesis is the colonization of the nasopharynx through inhalation leading to the carriage stage. Colonization is not always followed by disease, however, studies have shown that, recent colonizers are often the culprit in disease causation. The process by which the bacterium transits from a colonizer to cause disease is poorly

understood but an imbalance between host, agent and environmental factors could favour transition from colonisation to symptomatic disease stage (AlonsoDeVelasco *et al.*, 1995; Bogaert, De Groot and Hermans, 2004; Cillóniz and Torres, 2014).

Figure 2: Route of transmission and pneumococcal disease



Source: Cold Spring Harbour Perspectives in Medicine 2013; a01021

The pneumococcus evades/breaches the immune system by the help of the polysaccharide capsule and it's numerous surface proteins and enzymes (pneumolysin, neuraminidase A, etc.) (Henriques-Normark & Tuomanen, 2013). The capsule is negatively charged thereby helping it to avoid mucous entrapment through the process of repulsion. The spatial arrangement of its atoms prevents binding of leucocytes to complements fixed onto the cell wall beneath the capsule. Repulsion of the mucous brings the organism close to the receptors on the surface of the epithelial cell of the host. At this stage, the pneumococcus decreases the expression of its capsule (transparent variant) leading to the uncovering of its co-receptors e.g. the CbpA and consequently binds to the epithelial cells of the host; establishing a state of colonization. Binding to the receptors of the host favours the translocation of the bacterium at the site of infection across the various barriers leading to invasion of the host tissues (Nelson *et al.*, 2007; Henriques-Normark and Tuomanen, 2013b).

The host however, engages both the innate and the adaptive immune system to help combat the invading microbe. The innate arm of the host's immune system acts to prevent colonization through the actions of respiratory mucous, lysozymes, secretary IgA (sIgA), lactoferrin and components of the complement system (Actor, Hwang and Kruzel, 2009; Bogaert *et al.*, 2010; Corthésy, 2013). The respiratory mucous acts as a physical barrier to the adherence of the bacterium to the epithelial surface whilst the lysozyme exerts a bactericidal effect by catalysing the breakdown of the bacterial cell wall (Nelson *et al.*, 2007).

The pneumococcus in effect counteracts the host's defences by activating a plethora of events. The negatively charged capsule instigates repulsion of the sialic acid residue present in the mucous and this aids the bacterium to escape the entrapment effect off the mucous. The enzyme neuraminidase helps to decrease the viscosity of the mucous by cleaving of mucin thereby making bare the receptors on the host cell for adhesion. Pneumolysin which is a pore-

forming-toxin paralyses the cilia of the respiratory epithelium thereby decreasing their beating activity leading to inability to clear the organism and also, the pneumolysin causes separation of the tight junctions of the cells (Mook-Kanamori *et al.,* 2011). It also produces a protease (IgA1) which neutralises the effect of the sIgA by cleaving it off the surface of the pneumococcus thereby changing the surface charge and consequently, increasing the proximity of the pneumococcal cell-wall choline to the platelet- activating-factor receptor (Weiser *et al.*, 2003; Attali *et al.*, 2008). The binding of the various cell-wall choline induces internalization and trans cellular movement of the pneumococcus leading to invasion of the cells of the host (Bogaert, De Groot and Hermans, 2004).

2.4 Pneumococcal diseases

S. pneumoniae causes a plethora of clinical disease conditions that are grouped into two categories; invasive pneumococcal disease and non-invasive pneumococcal disease (Henriques-Normark and Tuomanen, 2013). Bacteraemic pneumonia, sepsis, and meningitis constitute invasive pneumococcal disease and this occurs as a result of invasion of sterile sites such as the blood and the cerebrospinal fluid by this bacterium. Non-invasive diseases includes otitis media, sinusitis and non-bacteraemic pneumonia. The elderly, 65 years and above and children below the ages of two years are especially at high risk of pneumococcal disease (Bruce *et al.*, 2015; Linden *et al.*, 2015). Other risk factors to pneumococcal disease includes; immunodeficiency state or other underlying illnesses such as HIV/AIDS, sickle cell disease, asplenia, kidney disease (nephrotic syndrome) and cancer. Day care attendance, lack of breastfeeding and exposure to smoke can also predispose one to pneumococcal disease (WHO, 2006). The WHO (2011), estimated that, pneumococcal infection alone accounts for about 14.5million cases of severe diseases, and out of this number, more than 820,000 children below the age of five died from the disease. The highest number of death in this age

group occurred in developing countries. The frequently isolated bacterial pathogen in invasive conditions (meningitis) in children under 5years is *S. pneumoniae* (Donkor *et al.*, 2010). It was estimated by the WHO that, in Ghana, morbidity and mortality in children under five years due to pneumococcal infections was 71,933 cases and 4,216 deaths respectively as at the end of 2008 (MOH, 2014).

Amongst the diseases caused by the pneumococcal organism, pneumonia ranks high as the frequently encountered pneumococcal disease; accounting for about 95% of all documented global cases of pneumococcal diseases (WHO, 2012a). About 98% of global community-acquired-pneumonia (CAP) cases occurs in developing countries; making it the leading cause of morbidity and mortality in children below five years in these countries (Rudan *et al.*, 2013). Among the identified risk factors for development of CAP, the key risk factors includes; malnutrition, low birth weight (≤ 2.5kg), non-exclusive breastfeeding in the first 4 months of life, use of solid fuel (fire wood and charcoal) and crowding (≥ 7 individuals sharing the same household) (Rudan *et al.*, 2013).

In Ghana, close to 800,000 (795,448) new cases of community-acquired-pneumonia (CAP) was documented in 2010 among children below the age of five. *Streptococcus pneumoniae* was the leading bacterial cause; accounting for 57,857 of the cases. 17,354 of these diagnosed CAP cases were severe resulting in the children being admitted of which 2,573 of the children lost their lives (Rudan *et al.*, 2013).

Studies have shown that, the high morbidity and mortality rate as a result of pneumonia during the influenza season occurs as a result of co-infection with the pneumococcus. This phenomenon leads to a synergistic effect resulting in a quick and effective binding of the pneumococcus to the receptors on the host's cell and a decrease in the clearance of bacterium

from the nasopharynx culminating in increase in pneumonia cases (Nakamura, Davis and Weiser, 2011; Wren *et al.*, 2014).

Pneumonia occurs when the bacterium gains access to the lower respiratory tract via aspiration or the haematogenous route and proliferates; resulting in the garnering of proinflammatory and anti-inflammatory cytokines by the host's immune system which also results in an extravasation of fluids into the alveoli leading to a collateral damage to the host tissue (Canvin *et al.*, 1995). The above process leads to a consolidation of the lung which is detected on an x-ray film (Gillespie and Balakrishnan, 2000; Poll and Opal, 2009).

Destruction of the cell junction by pneumolysin aid the bacterium to gain access into the blood stream. Bacteraemia results in close proximity of the pneumococcus to the blood-brain barrier. The bacterium then attaches itself to the vascular endothelium and finally enters the cerebrospinal fluid after which it binds to the parenchymal cells of the brain resulting in meningitis. Inflammatory response by the immune system consequently leads to an increased intracranial pressure characterised by cerebral oedema, increased cerebral blood volume and alteration in CSF drainage. The above activity coupled with defective perfusion of the brain results in the neurological sequelae such as hearing loss and other neurological defects seen in meningitis survivors (Ring *et al.*, 1998; Mook-Kanamori *et al.*, 2011).

Meningitis is not a common manifestation of pneumococcal infection but when it does occur, it is associated with a high case-fatality rate (Donkor, 2013). The case-fatality rate ranges from 27% to 80% globally. Developing countries have the highest case-fatality rate as a result of challenges with availability of medical resources (WHO, 2012a, 2013).

2.5 Treatment of pneumococcal diseases

The WHO recommends aminopenicillins and penicillins as the first line of treatment for acute otitis media and community-acquired pneumonia (WHO, 2014). Other classes of drugs such as the cephalosporins, the macrolides, and fluoroquinolones are used for the treatment of penicillin resistant strains.

In Ghana, the Standard Treatment Guideline (2010) recommends the penicillins as the first line of treatment and the use of erythromycin for those who have allergy to penicillins.

2.6 Prevention of pneumococcal disease

Vaccination helps to reduce the burden of pneumococcal infections among children under two years of age (Cillóniz and Torres, 2014). Pneumococcal vaccine was introduce into the vaccination program in Ghana in 2012 with the aim to mitigate pneumococcal disease morbidity and mortality cases in the country. A vaccination schedule of 6weeks, 10weeks and 14weeks was adopted (MOH, 2014) as shown in figure three below. However, any child below the age of five years who has not had the pneumococcal vaccine upon first contact is started on the pneumococcal vaccine schedule (catch up campaign).

Figure 3: A chart showing the immunization schedules in Ghana.

MINISTRY OF HEALTH, GHANA HEALTH SERVICE

EXPANDED PROGRAMME ON IMMUNIZATION

NATIONAL IMMUNIZATION AND VITAMIN A SCHEDULE FOR CHILDREN

AGE	PICTURE	VACCINES	DOSE	ROUTE AND SITE OF ADMINISTRATION
At Birth		BCG	0.05ml	Intra-dermal, right upper arm
		OPV 0	2 drops	Oral
6 weeks		OPV 1	2 drops	Oral
		DPT-HepB-Hib 1	0.5ml	Intra-muscular, left thigh
		Pneumococcal 1	0.5ml	Intra-muscular, right thigh
		Rotavirus 1	1.5ml	Oral
10 weeks		OPV 2	2 drops	Oral
		DPT-HepB-Hib 2	0.5ml	Intra-muscular, left thigh
		Pneumococcal 2	0.5ml	Intra-muscular, right thigh
		Rotavirus 2	1.5ml	Oral
14 weeks		OPV 3	2 drops	Oral
		DPT-HepB-Hib 3	0.5ml	Intra-muscular, left thigh
		Pneumococcal 3	0.5ml	Intra-muscular, right thigh
6 months		Vitamin A	100,000 I.U	Oral
9 months		Measles 1	0.5ml	Sub-cutaneous, left upper arm
		Yellow Fever	0.5ml	Sub-cutaneous, right upper arm
12 months		Vitamin A	200,000 I.U	Oral
18 months		Measles 2	0.5ml	Sub-cutaneous, left upper arm
		Vitamin A	200,000 I.U	Oral

NB: After 18 months vitamin A will be given every six months till child is five years old

There are two main types of pneumococcal vaccines as indicated in table one below; the pneumococcal polysaccharide vaccine (PPV) and the pneumococcal conjugate vaccine (PCV). PPV was the first vaccine formulated in the 1970's in response to the widespread antimicrobial resistance developed by the pneumococcus (CDC 2011). The pneumococcal polysaccharide vaccine (Pneumovax 23, Merck) currently in use contains a preparation of 25µg each of purified capsular polysaccharide antigen from 23 different serotypes with 0.25% phenol as a preservative. It acts by inducing a B-cell dependent immune response resulting in the production of immunoglobulin M (IgM) (Cillóniz and Torres, 2014). It is recommended for use in adults who are 65 years and above and for individuals between the ages of 2 years through to 64 years who are at a high risk of pneumococcal disease (Chiu and Mcintyre, 2013). Even though it affords some level (60%-70%) of protection against invasive pneumococcal disease, the immunity induced by this vaccine is short-lived hence requires periodic re-vaccination (Cillóniz and Torres, 2014). In addition, it lacks the capacity to mount a T cell-independent immune response in children below the age of 2yrs and does not induce immunological memory; consequently, it is not recommended for use in children below the age of two years (WHO, 2006).

Children below the age of 2years form the bulk of the population that bears the brunt of the pneumococcal disease and this culminated in the development of the pneumococcal conjugate vaccine. The PCV (Prevenar 13) is used for children below the age of 2years, all adults 65years and above, and individuals who are above 2years and with certain risk factors (CDC, 2010; Esposito *et al.*, 2010; Pilishvili, Noggle and Moore, 2012; Chiu and Mcintyre, 2013). To increase the immunogenicity of the vaccine, the pure capsular polysaccharide was conjugated with a mutant (non-toxic) toxin of the diphtheria toxin (CRM_{198}) resulting in the induction of a T cell-dependent immune response in children below the age of 2years This

type of immunological response has an added advantage of inducing a life-long immunological memory characterised by affinity maturation and booster response on subsequent exposure to the antigen. In addition, it confers both mucosal and systemic immunity to the recipient (WHO, 2006).

The mucosal immune response contributes importantly to protection against nasopharyngeal colonization with serotypes included in the vaccine thereby leading to a decrease in carriage of vaccine type serotypes. It also induces a herd immunity as compared to the PPV23. Other benefits that this vaccine provide include; a decrease in disease caused by antibiotic resistant strains and a reduction in the use of antibiotics (Obaro and Adegbola, 2002; Miller *et al.*, 2011).

Table 1: Types of vaccines developed and serotypes included

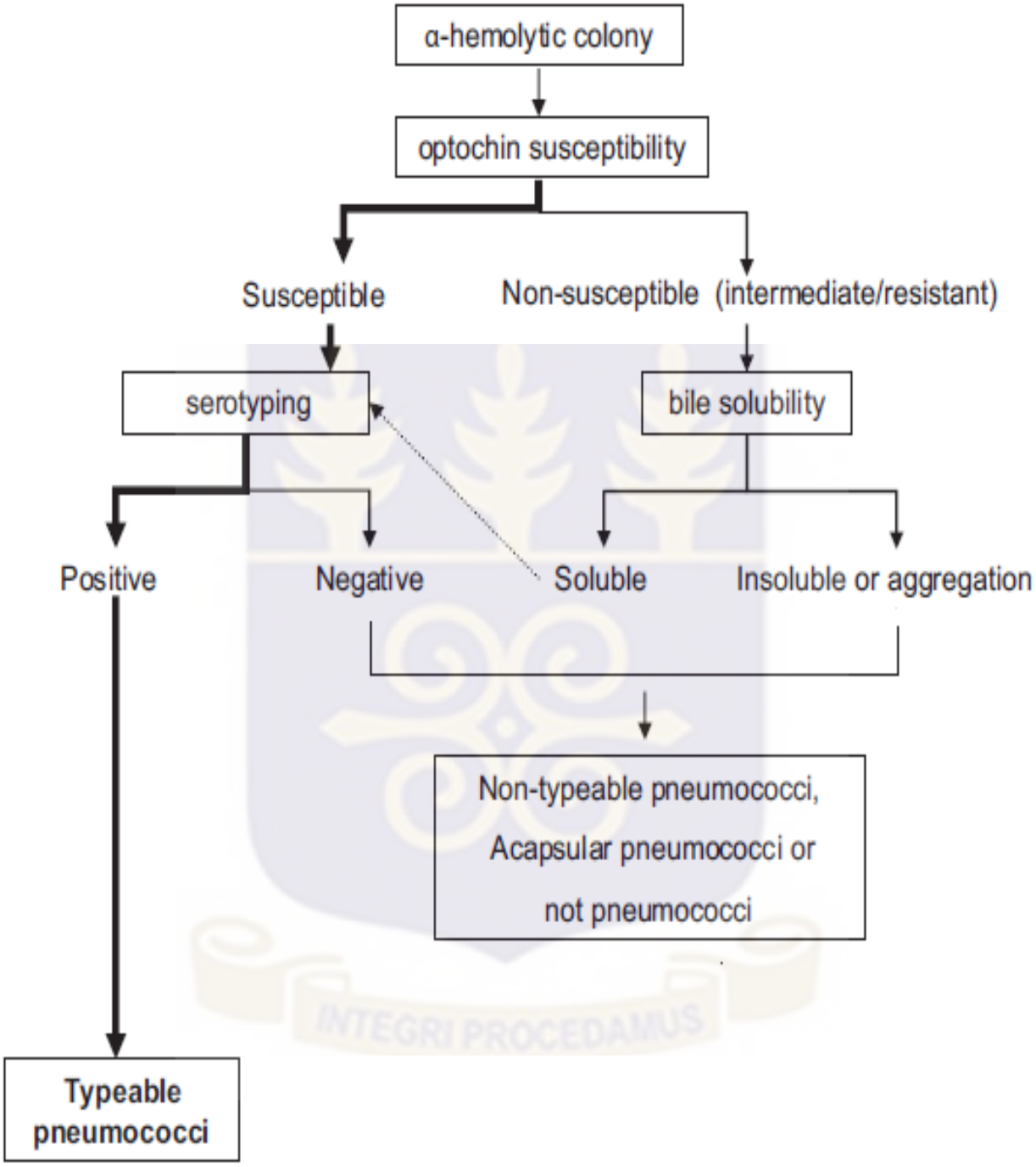
Vaccine type	Serotypes
1. PPV	
• **PPV-23**	1, 2, 3, 4, 5, 6B, 7F, 8, 9N, 9V, 10A, 11A, 12F, 14, 15B, 17F, 18C, 19A, 19F, 20, 22F, 23F, and 33F (WHO, 2012b)
2. PCV	
• **PCV-7***	4, 6B, 9V, 14, 18C, 19F and 23F (WHO, 2012b)
• **PCV-10**	1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F and 23F
• **PCV-13**	1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F (Chiu and Mcintyre, 2013).

*PCV7 is no longer in use.

2.5 Identification and typing of *S. pneumoniae*

Pneumococcus carriage is determined by taking a nasopharyngeal swab. The sample is then transported in a skimmed milk, tryptone, glucose and glycerol transport medium (STGG media) into the laboratory. The swab is inoculated onto a 5% sheep's blood agar and incubated under microaerophilic (5%-10% CO_2) conditions over 18-24hrs. Colonies showing alpha-haemolysis are subjected to optochin susceptibility and bile solubility test to confirm the presence of *S. pneumoniae* (Satzke *et al.*, 2013). The Quellung reaction is used to identify the various serotypes based on the type of capsule that they express (Dayie *et al.*, 2013).

Figure 4: Flowchart indicating the laboratory test used to identify *S. pneumoniae*



Source: (Satzke *et al.*, 2014)

2.6: **Antibiotic resistance in the pneumococci**

The pneumococcus is a highly competent organism; having the ability to take up foreign genetic material from it environment. This kind of ingenuity places the pneumococcus amongst the class of bacteria that have high resistance tendencies (Tomasz, 1997; Schrag, Beall and Dowell, 2001; Marks, Reddinger and Hakansson, 2012; Henriques-Normark and Tuomanen, 2013). Resistance of the pneumococcus to an antimicrobial drug (Penicillin) was first reported in 1967 in Australia from a clinical isolate of a patient from Papua New Guinea, and then subsequently in South Africa in 1977 (Hansman and Bullen, 1967; Jacobs *et al.*, 1978; Tomasz, 1997). Penicillin-resistant pneumococci has the potential of being resistant to other β-lactams since resistance is not achieved by the secretion of β-lactamases but by the alteration of its penicillin-binding protein (Chiou, 2006; Johnsborg and Håvarstein, 2009). Resistance to β-lactams is more often than not associated with resistance to other classes of antibiotics such as aminoglycosides, chloramphenicol, fluoroquinolones, tetracycline, macrolides, etc.(Tomasz, 1997). In the pneumococcus, acquisition and spread of antibiotic resistance is known to occur during colonisation (Marks, Reddinger and Hakansson, 2012).

The rate of penicillin resistance varies from one geographical location to another and also differs from season to season (Albanese *et al.*, 2002) besides, different serotypes differ in their resistance as compared to others. The rate and duration of carriage of penicillin resistant serotypes is higher and more prolonged in children below the age of five years as compared to older children (Högberg *et al.*, 2007). Serogroups 6, 9, 14, 19, and 23 proved to have a very high resistance capacity (Schrag, Beall and Dowell, 2001). The CDC (2013) reported a resistant rate of 30% in severe pneumococcal infections in the USA. Carriage studies in the Middle East reported that, 70% of the isolates were penicillin-nonsusceptible with 13% being fully resistant and 30% being multiple drug resistant strains (Regev-Yochay *et al.*, 2012). In

sub-Saharan Africa, countries such as Tanzania, (Moyo *et al.*, 2012) reported a prevalence rate of 67.8%. In Ghana Donkor *et al* (2010) reported penicillin resistant rate of 19.6% in both invasive and non-invasive isolates with a MDR of 56.6% in children below the age of five years. The most recent carriage study in children less than 6years indicated an intermediate penicillin resistant rate of 45% and a MDR of 72.2% (Dayie *et al.*, 2013, 2015).

Globally, it has been shown that, factors such as the intrinsic ability of the pneumococcus to take up foreign genetic material (transformation) from closely related bacteria species contributes to the development and spread of resistance in the pneumococcus. Other factors such as antibiotic use has also been cited (Schrag, Beall and Dowell, 2001; Henriques-Normark and Tuomanen, 2013). In Ghana, one of the main driving force for antibacterial resistance is the imprudent use of antibiotics and easy accessibility to antibiotics (Donkor *et al.*, 2012; Dayie *et al.*, 2015). Table two below shows the mechanism employed by the pneumococcus in becoming resistant to the antimicrobials used against it.

Table 2: Mechanisms of antimicrobial resistance of *S. pneumoniae*

Class of antimicrobial	Mode of action	Resistance mechanisms
1. Beta-lactams **a. Penicillins** **b. Cephalosporins** **c. Carbapenems** **d. Monobactams**	Inhibit cell wall synthesis by binding to Penicillin Binding Protein (PBP) (Novak *et al.*, 2000)	Alteration in the PBP resulting from mutation in the gene coding for it (Contreras-Martel *et al.*, 2009;Hakenbeck *et al.*, 1999)
2. Macrolides	Inhibit protein synthesis at the 50s ribosomal subunit (Leclercq and Courvalin, 2002)	• Methylation of V domain of the 23rRNA • Mutation resulting in the alteration of the 23rRNA • Up regulation of the efflux pump (Edelstein, 2004)
3. Fluoroquinolones	Stimulates disruption of bacterium DNA (Hooper, 2000)	• Mutation • up regulation of efflux pump (Bambeke, Pages and Lee, 2006)
4. Trimethoprim sulphamethoxazole	Inhibits folic acid synthesis of bacteria (Masters *et al.*, 2003)	Mutation of binding enzymes (Huovinen, 2001;*Toleman et al.*, 2007)
5. Glycopeptides	Inhibits peptidoglycan cell wall synthesis (Yim *et al.*, 2014)	Mutation of binding molecules (Rice, 2012)

2.7 Emergence of non-vaccine serotypes

Vaccination with the pneumococcal conjugate vaccine as a preventive measure helps to mitigate the burden of pneumococcal disease (Dagan and Klugman, 2008; Weil-Olivier *et al.*, 2012). Though vaccination is an excellent tool for controlling this organism, the plethora of pneumococcal serotypes possess a major challenge in the quest to control the pneumococcus (Henriques-Normark and Tuomanen, 2013). The vaccine only covers the few serotypes that causes invasive disease consequently, a selective pressure is introduced into the pneumococcal population (Sheppard *et al.*, 2010; Henriques-Normark and Tuomanen, 2013b), leading to the occupation of the niche left vacant by vaccine types with non-vaccine types through the phenomenon of capsular switching and serotype replacement (Wyres *et al.*, 2013). In the UK, the predominant cause of community-acquired pneumonia in the post vaccination era are non-vaccine serotypes (Elemraid *et al.*, 2013).

Serotype replacement occurs when recessive pre-existing serotypes of *S. pneumoniae* takes over as the major carriage and disease-causing serotypes when vaccination leads to the elimination of dominant serotypes causing invasive disease (Ahn *et al.*, 2015; Alexandre *et al.*, 2010). An example is the emergence of serotype 19A as the major causative agent of invasive disease in the PCV-7 era (Hsu *et al.*, 2010; Isaacman *et al.*, 2010; Pelton *et al.*, 2007). Other serotypes have also been seen to occupy the niche left vacant by the vaccine serotypes (Tan, 2012). The likely driving force for serotype replacement is the competitive interaction between strains (Mehtälä *et al.*, 2013).

The introduction of PCV-13 is gradually seeing a similar pattern in serotype replacement during the PCV-7 era (Linden *et al.*, 2015; Linden *et al.*, 2015). Carriage studies indicate that non-PCV-13 serotypes are gradually taking over as colonizers (Camilli *et al.*, 2013). A

typical example is the serotype 35B which appears to be taking grounds as an important causative agent for pneumococcal disease (Domenech *et al.*, 2015). Other serotypes that are also increasing in proportion includes; 23B and 15A (Linden *et al.*, 2015).

S. pneumoniae has the ability to naturally undergo recombination. In the process of undergoing this recombination, a gene that encodes for a non-vaccine serotype is acquired (Croucher *et al.*, 2015). The process of substituting one capsular gene for another is referred to as capsular switching (Wyres *et al.*, 2013). The pneumococcus ability to receive pathogenic islands from other genus of the streptococcus such as the mitis group through horizontal gene transfer contributes to a large extents in the virulence capacity of the pneumococcus capsule (Andam and Hanage, 2014).

The phenomenon of capsular switching is closely linked to the acquisition of resistance to penicillin. This is due to the close proximity of the penicillin binding protein gene to the capsular locus (Wyres *et al.*, 2013).

CHAPTER THREE

3.0 Materials and methods

3.1 Study area

This study was carried out in Accra metropolis of the Greater Accra region of Ghana. The Accra metropolis is the capital city of Ghana and falls within the coastal belt of Ghana with humid and warm climatic conditions. Accra has the highest population density compared to other districts in Ghana (www.ghanadistrict.com; retrieved 28th August, 2015).

Figure 5: The map of Ghana showing the location of Accra.

3.2 Sampling site

The study was carried out in nurseries and kindergartens within Accra metropolis of the Greater Accra region of Ghana within the months of September through to December 2016.

3.3 Sampling procedure

A list of nurseries and Kindergartens in the Accra metropolis was obtained from the Ghana Education service. A total of 7 schools were randomly selected with recourse to the sample size. Consent was obtained from the parents of the children and children whose parents decline to give consent were excluded from the study and also, children who declined assent after parental consent were also excluded. Children who had active upper respiratory tract infections or have been given antibiotics within the last two weeks prior to sampling were excluded. Postnatal cards were obtained from the parents in order to ascertain the immunization status of the child.

3.4 Microbiological investigation

3.4.1 Specimen collection

Nasopharyngeal specimen was collected using the WHO's recommended methodology (Satzke *et al.*, 2013). Nylon-tipped paediatric sized FlOQSwabs (Copan Flock Technologies, Italy) were used to collect nasopharyngeal specimens. A total of 410 swab samples were obtained. The swab specimens were placed in a labelled cryo-vial containing 1 ml of skim milk-tryptone-glucose-glycerine (STGG) medium and then transported on ice to the laboratory within 3 hours of collection (Satzke *et al.*, 2013). Samples were vortexed and immediately stored at -80^{o}c upon arrival at the laboratory pending further processing.

3.4.2 Specimen processing

The specimen were processed based on the WHO recommendation for characterizing *S. pneumoniae* (Satzke *et al.*, 2013). The sample were inoculated onto a 5% sheep blood agar containing 5µg/ml of Gentamicin and then incubated at 37^{o}C in 5% CO_2 18-24hrs. All alpha-haemolytic colonies were subjected to optochin susceptibility testing. Isolates which are optochin susceptible were identified as *S. pneumoniae*.

Figure 6: A picture of samples being analysed in the laboratory.

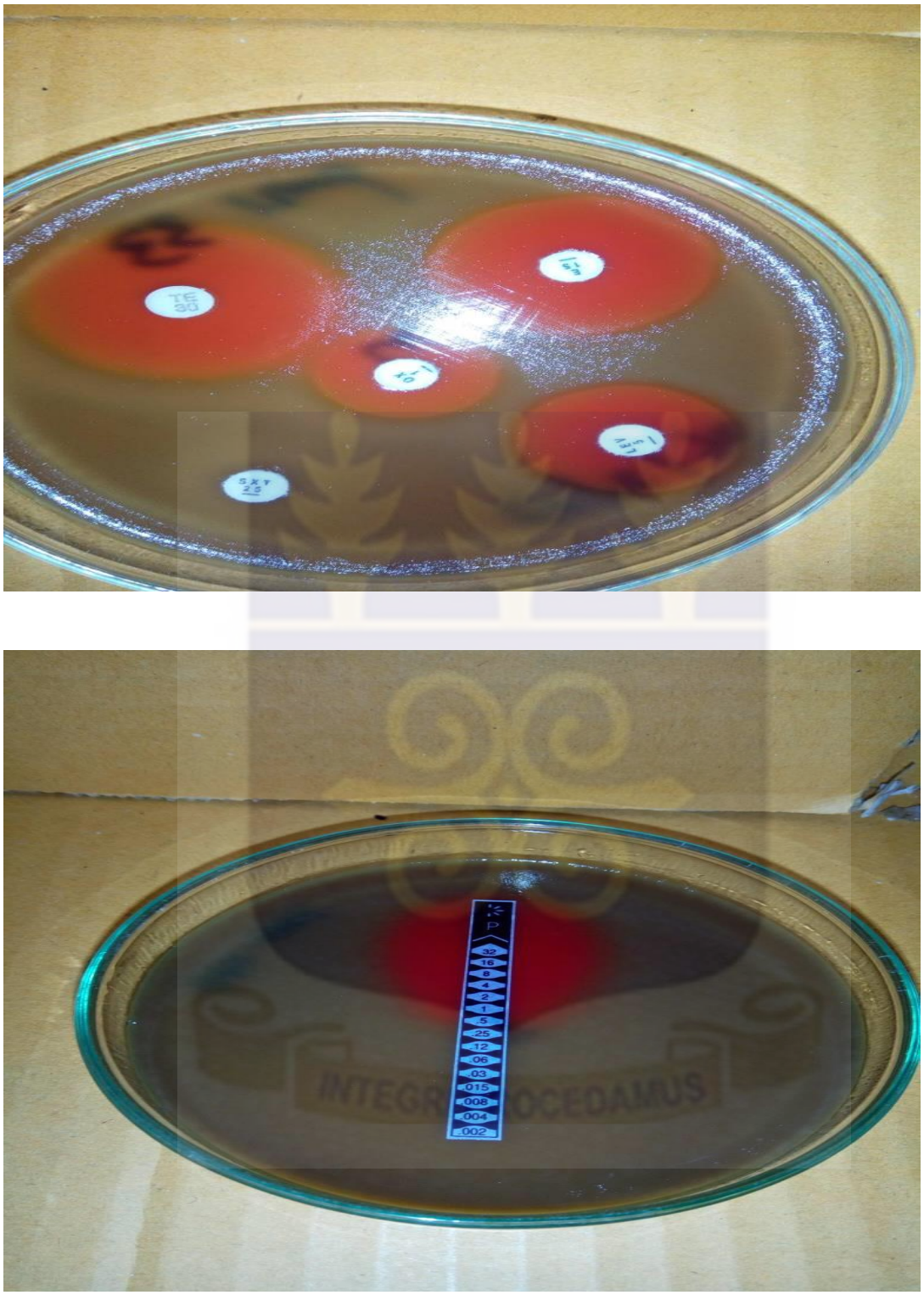
Serotyping/grouping of these isolates were done using the Pneumotest-latex agglutination kit (SSI Diagnostica, Hillerød, Denmark) and the result were confirmed by the Quellung reaction using the serotype specific antisera (SSI Diagnostica). All specimens were screened for multiple serotypes and cultured in serum broth (SSI Diagnostica). Serotyping were directly performed on the culture to determine multiple reactions to the various antisera. Where multiple serotypes were observed, they were isolated, identified and serotyped as described above.

3.4.3 Antimicrobial susceptibility testing

Penicillin susceptibility testing was done by using the Kirby-Bauer disc diffusion method (Bauer *et al.*, 1966) as indicated in figure 7. 1µg oxacillin disc (Oxiod Company, UK) was used for the test. This was followed by the determination of the Minimum Inhibitory Concentrations (MICs) of all oxacillin-resistant isolates using penicillin G MIC strips (Oxoid Company, UK). The Clinical Laboratory Standards Institute (CLSI) guideline on interpreting susceptibility test results was used as an interpretative tool with *S. pneumoniae* ATCC 49619 as a control strain (CLSI, 2016).

All isolates were further subjected to additional antimicrobial susceptibility testing to Erythromycin (5µg), Tetracycline (30µg), Trimethoprim-sulphamethoxazole (1.25/23.75µg) and Levofloxacin (5µg). The susceptibility test using Oxoid disk was performed by spreading an inoculum of 0.5 McFarland standard onto Müller-Hinton (Oxoid, UK) agar plates containing 5% sheep blood. The plates were incubated between 18-24hours at 37^0C in a 5% CO_2 incubator, after which the zones of inhibition were measured with a measuring rule. Antimicrobial susceptibility was determined according to the CLSI guidelines, with S. pneumoniae ATCC 49619 used as a control (CLSI, 2016).

Figure 7: Picture of plates showing antimicrobial susceptibility



A multidrug-resistant (MDR) isolates has been identified as showing resistance to penicillin (intermediate or full resistance) and resistance to at least two other classes of antimicrobials (Richter *et al.,* 2009; Magiorakos *et al.,* 2011).

3.5 Inclusion criteria

Only children five years and below were included in the study.

3.6 Exclusion criteria

Children who have had antimicrobial therapy in the past two weeks were excluded from the study.

3.7 Data analysis

All data collected were entered into Microsoft Excel and descriptive analysis done using STATA version 12. Frequency tables and graphs were generated for the various variables.

3.8 Ethical clearance

Ethical approval for this study was obtained from the Ethics and Protocol Review Committee of the College of Health Sciences, University of Ghana. Nasopharyngeal swab samples as well as demographic data was obtained from the participants after consent was obtained from the parents/guardians of the participants followed by an assent from the children themselves. If a child whose parents/guardian has given consent declines assent, that child is excluded from the study.

CHAPTER FOUR

RESULTS

4.1 Demographic of the study participants

A total of 410 children aged ≤ 5years were sampled from seven schools; out of these seven schools, two were government schools, two were private schools and three of them were mission schools. This number consisted of 51.2% (210) males and 48.8% (200) females. The number of children within each age group is indicated in Table 4.1 below (page: 38). The lowest age sampled was 6 months and the highest age was 60 months. The mean age for the children sampled was 38.8months ± 10.6. All the children sampled have been vaccinated. Most parents and some schools with children in the age group of 0-12 and 13-24months declined consent hence only a few number of this age group 0-12 (n=5) and 13-24 (n=56) was obtained. In total, only 61 out of the total 410 sampled were ≤2years.

Table 4.1: Carriage prevalence and serotype distribution of *Streptococcus pneumoniae* by age group in children ≤5years attending Nurseries and Kindergartens in Accra.

Age group (months)	Number of children			Number of children with carriage of Streptococcus pneumoniae (%)	serotypes	Number of children with multiple serotypes
	M	F	Total (%)			
0-12	2	3	5 (1.2)	2 (0.8)		
13-24	31	25	56 (13.7)	34 (12.8)	15A,6A,23B,3, 18B,23F,10A,6B	
25-36	86	86	172 (42.0)	120 (45.1)	13,15C,8,15B,6B, 23B,23F,18C,19F,3, 4,35F,23,6A,12F,13 19B,16F,14,11A,15A	3
37-48	70	59	129 (31.5)	82 (30.8)	19F,10A,3,13,23B,23F, 34,6B,14,38,6A,20,28F, 11A,40,16F,46,1,16F, 19A,31	4
49-60	21	27	48 (11.7)	28 (10.5)		
Total	210	200	410	266		

4.2 Pneumococcal carriage prevalence

A total of 64.9% (266) out of the 410 children sampled were found to be colonised with *Streptococcus pneumoniae.* The lowest age of carriage was 6 months whiles the highest age was 60 months. Carriage prevalence per age group is as indicated in Table 4.1.

4.3 Pneumococcal Serotype Prevalence

Out of the 266 pneumococcal isolates, 111 were serotyped. Out of the 111, 21 of the isolates were not viable and this could be due to inability of the isolates to survive storage conditions during transport to Statens Serum Institute, Copenhagan where the serotyping was done. Three of the isolates were non-typable (NT). A total of 31 different serotypes were realised. The three most dominant serotypes were 23B (15/93), 23F (9/93) and 19F (7/93) (Figure 4.1 at page 41). These predominant serotypes accounts for 16.1%, 9.7% and 7.5% respectively of the total serotypes realised as indicated in table 4.2 below (page 40). Carriage of non-PCV13 serotypes constitutes 67.7% (21/31) of the total serotypes. Out of the total serotypes, PCV-10, PCV-13 and PPV-23 serotypes makes up 19.4% (6/31), 32.3% (10/31) and 41.9% (13/31) respectively.

Carriage of multiple serotypes was observed in seven children and all the children were carrying two serotypes (table 4.1, page 38). Out of the multiple carriage, two were carrying vaccine serotypes, three carrying both vaccine type and a non-vaccine serotypes and two carrying non-vaccine serotypes.

Table 4.2: Streptococcus pneumoniae Serotypes, MDR and vaccine coverage

Serotypes	Total number	(%)	MDR		Serotype included in vaccine
			(n)	**(%)**	
23B	15	16.1	3	20.0	NVT
23F	9	9.7	1	11.1	PCV7,PCV10,PCV13,PPV23
19F	7	7.5	2	28.6	PCV7,PCV10,PCV13,PPV23
19B	5	5.4	2	40.0	NVT
34	4	4.3	1	25.0	NVT
16F	6	6.5	0	0.0	NVT
6B	4	4.3	0	0.0	PCV7,PCV10,PCV13,PPV23
14	5	5.4	1	20.0	PCV7,PCV10,PCV13,PPV23
6A	3	3.2	1	33.3	PCV13
18C	2	2.2	0	0.0	PCV7,PCV10,PCV13,PPV23
13	3	3.2	0	0.0	NVT
10A	2	2.2	0	0.0	PPV23
3	2	2.2	1	50.0	PCV13, PPV23
15A	2	2.2	0	0.0	NVT
12F	2	2.2	0	0.0	PPV23
18B	1	1.1	0	0.0	NVT
11A	4	4.3	0	0.0	PPV23
35F	1	1.1	0	0.0	NVT
23	1	1.1	0	0.0	NVT
40	2	2.2	0	0.0	NVT
46	1	1.1	0	0.0	NVT
15B	1	1.1	0	0.0	PPV23
1	1	1.1	0	0.0	PCV10,PCV13, PPV23
19A	1	1.1	1	100	PCV13, PPV23
31	2	2.2	0	0.0	NVT
28F	1	1.1	0	0.0	NVT
15C	1	1.1	0	0.0	NVT
8	1	1.1	0	0.0	NVT
6A	2	2.2	1	50.0	PCV13
20	1	1.1	0	0.0	PPV23
38	1	1.1	0	0.0	NVT

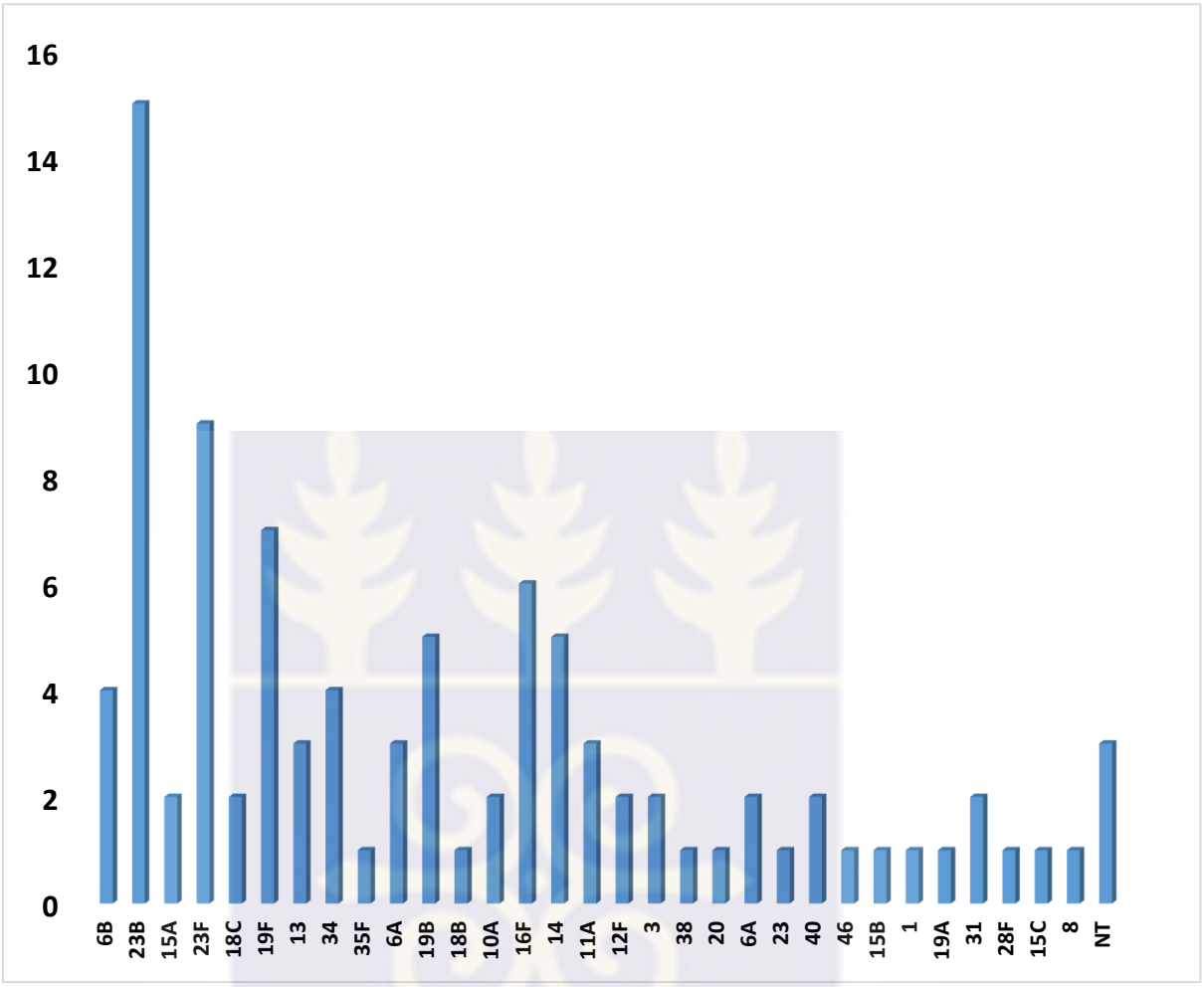
Where;

NVT: Non-Vaccine Serotypes

PCV: Pneumococcal Conjugate Vaccine

PPV: Pure Polysaccharide Vaccine

Figure 4.1: Serotype distribution in 111/266 of *S. pneumoniae* isolates in healthy children ≤5years

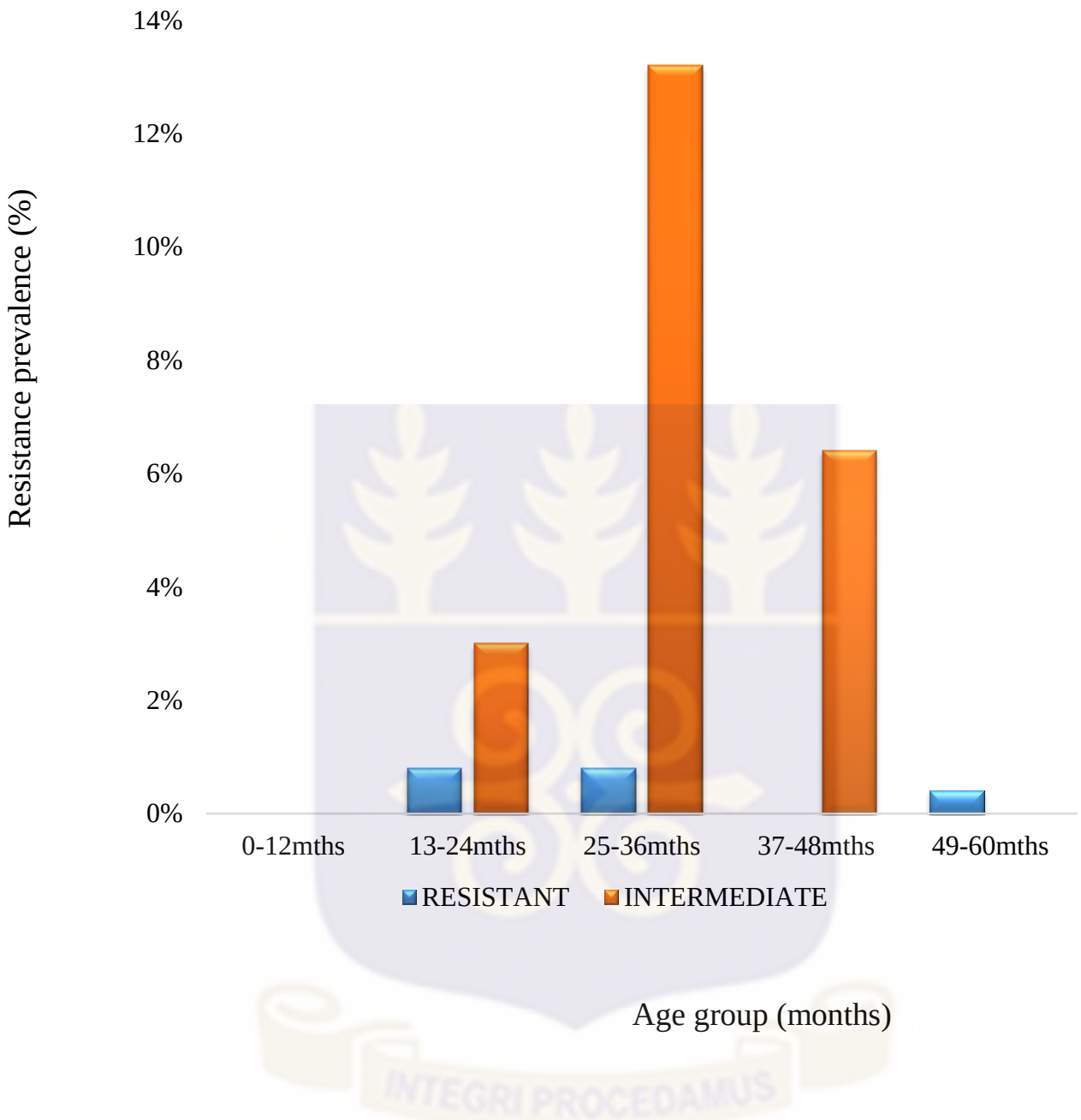


NT: Non-typeable

4.3: Penicillin Resistance

All the 266 isolates were tested for Penicillin susceptibility. Using the 2016 Clinical and Laboratory Institute Standards interpretative criteria, 25% (66/266) had intermediate resistance (breakpoint: 0.12-1µg/ml) to Penicillin with 1.9% (5/266) showing full resistance (breakpoint: ≥2µg/ml). The highest MIC recorded for Penicillin G was 8µg/ml. This isolate with the highest MIC value was carried by a child who was within the 13-24months age group. No child within the 0-12months age group showed carriage of Penicillin Non-Susceptible (intermediate or full resistance) *Streptococcus pneumoniae*. The 13-24 and 25-36 months age group showed equal carriage prevalence of 0.8% (2/266) of fully resistant *Streptococcus pneumoniae* isolates. However, children within the age group of 25-36months had the highest carriage prevalence [13.2% (35/266)] of intermediate resistant pneumococcal isolates. Figure 4.2 below (page 43) depicts the penicillin resistant prevalence in the various age groups.

Figure 4.2: Penicillin G Resistance by Age Group



4.4: Resistance to other Antimicrobials

The 266 isolates were also tested against 30µg Tetracycline (Oxoid, UK), 1.25/23.75µg Trimethoprim-Sulphamethoxazole (Oxoid, UK), 5µg Levofloxacin Oxoid, UK) and 15µg Erythromycin (Oxoid, UK) disk. The resistance profile for these antimicrobials were as follows; for Trimethoprim-Sulphamethoxazole, 9.8% (26) showed intermediate resistance and 68.4% (182) was fully resistant. For Erythromycin, 11.7% (31) showed full resistance and 4.5% (12) showed intermediate resistance. Twenty two (22) i.e. 8.3% out of the 266 isolates showed an intermediate resistance to Tetracycline whiles 66.7% (176) were fully resistant. All the isolates were susceptible to Levofloxacin and Ceftriaxone. Table 4.3 (page 45) indicates the susceptibility profile of the various antimicrobials.

4.5: Multidrug Resistance

Multidrug resistance (MDR) was defined as non-susceptibility to Penicillin G and two or more other antimicrobials (Lalitha *et al.*, 2002). 20.4% (54/266) were resistant to Penicillin G and two other antimicrobials.

The prevalence of MDR in the three predominant serotypes were 20.0% (23B), 11.1% (23F) and 28.6% (19F).

Table 4.3: Antibiotic Susceptibility Profile of *Streptococcus pneumoniae* Isolates

Antibiotics	Susceptible		Intermediate		Resistance		Non-susceptible	
	n	(%)	n	(%)	n	(%)	n	(%)
***Penicillin G (µg/ml)**	195	(73.3)	66	(24.8)	5	(1.9)	71	(26.7)
Erythromycin (mm)	223	(83.8)	12	(4.5)	31	(11.6)	43	(16.2)
Tetracycline (mm)	67	(25.2)	22	(8.3)	177	(66.8)	199	(74.8)
Trimethoprim-Sulphamethoxazole (mm)	58	(21.8)	26	(9.8)	182	(68.4)	208	(78.6)
Levofloxacin (mm)	266	(100)	0	(0)	0	(0)	0	(0)
Ceftriaxone (µg/ml)	266	(100)	0	(0)	0	(0)	0	(0)

*Breakpoint for penicillin (Susceptible: MIC= ≤0.06µg/ml, Intermediate resistance: MIC= 0.12-1µg/ml and Resistance: MIC= ≥2µg/ml).

CHAPTER FIVE

DISCUSSION AND CONCLUSION

5.1: Discussion

In May 2012, Ghana introduced PCV13 into the Expanded Program on Immunization as parts of the efforts to lessen the burden of pneumococcal disease in children ≤ 5years (MOH, 2014). As with any other vaccine, it is imperative to monitor pneumococcal serotypes and their antimicrobial susceptibility patterns in order to determine the effectiveness of the vaccine and to ascertain whether there have been serotype replacement in the pneumococcal population.

This study was the first post-vaccination surveillance conducted in Accra (Ghana) to determine the pneumococcal carriage prevalence, antimicrobial susceptibility profile and the prevailing serotypes among healthy pre-school children ≤ 5years.

The overall pneumococcal carriage prevalence was 64.9%. This prevalence was high when compared to the pre-vaccination study by Dayie *et al.,* (2013). This difference could possibly be due to the age difference included in the study i.e. ≤ 5years as against ≤ 6years in the previous study. Other parameters such as risk factors (e.g. crowding index) which were not assessed in this study may have contributed to the high carriage prevalence. The overall carriage prevalence in this study was comparably lower than what was obtained in studies from other African countries such as the Gambia where 85.4% carriage prevalence was reported after PCV-13 vaccination (Roca *et al.,* 2015). The carriage prevalence in this study was however similar to the 65.8% observed in Kenya by Abdullahi *et al.,* (2012). In addition, it has been observed in other geographical settings in Africa that, carriage prevalence ranges from 45.8%-52.5%

(Bouskraoui *et al.*, 2011; Ba *et al.*, 2014; Adetifa *et al.*, 2012). These carriage prevalence were however comparably lower than what was obtained in this study. Even though the overall carriage prevalence in this study appears to be high, carriage of PCV13 serotypes was low (32.3.0%) as compared to the non-PCV13 serotypes (67.7%). This observation is consistent with findings from other countries where the PCV13 have been introduced (Roca *et al.*, 2015; Cohen and Levy, 2017). In the pre-vaccination era, PCV13 serotypes constituted about 50% (Dayie *et al.*, 2013) of the total serotypes isolated. In this study, a reduction in the carriage of PCV13 serotypes were observed.

The three predominant serotypes in this study were 23B, 23F and 19F. The predominant serotypes in the pre-vaccine era were 19F, 6B and 23F (Dayie *et al.*, 2013). Serotype 19F and 23F appears to be the vaccine serotypes which are part of the three predominant serotypes in the vaccine era. Carriage studies in Turkey indicated that, serotype 19F and 23F are the vaccine serotypes that predominates in the PCV-13 vaccine era (Soysal *et al.*, 2016). Carriage studies in the USA and Israel also indicates that, serotype 23F and 19F are among the top ten isolated vaccine serotypes in vaccinated children (Lee *et al.*, 2014; Dagan *et al.*, 2017). This phenomenon may be attributed to the levels of serum concentrations of serotype-specific pneumococcal anticapsular IgG antibody after vaccination, as well as to other factors such as duration of vaccine protection and the number of individuals that comes into contact with the vaccine recipient (crowding) (Flasche *et al.*, 2015). The serum concentration of the serotype-specific IgG antibody required for protection against being colonised by a vaccine serotype after vaccination differs from serotype to serotype hence, if the required serum level of that specific IgG is not attained, a vaccinated individual still stands the chance of being colonised by that particular vaccine serotype (Dagan *et al.*, 2016). This however does not indicate lack of

effectiveness of the vaccine to protect against invasive pneumococcal disease caused by that particular serotype since the serum level of serotype-specific IgG required (0.35µg/ml IgG) to protect against IPD is lower than what is required (≥2µg/ml IgG) to protect against colonisation (Flasche *et al.*, 2015; Dagan *et al.*, 2016). Invasive studies in the country indicating which serotypes are causing invasive disease in vaccinated individuals will be a better indicator of vaccine failure.

Carriage studies in a population of HIV children showed that 19F and 16F were the dominant serotypes in the PCV-13 era with serotype 16F being the predominant non-vaccine type in carriage (Donkor *et al.*, 2017). However, in this study, the predominant non-vaccine serotype was 23B indicating that replacement with non-PCV13 serotype is taking place. This serotype is one of the serotypes indicated to be predominating in the PCV-13 era as a carriage serotype in Africa (Cohen & Levy, 2017). In Germany, serotype 23B and 15A were isolated as the non-vaccine serotypes causing invasive pneumococcal disease (Linden, Perniciaro and Imöhl, 2015). This picture is however not conclusive yet as only 111 out of the 266 were serotyped.

Three out of the fifteen 23B serotypes constituting 20.0% were found to be non-susceptible to penicillin. This prevalence is lower as compared to the penicillin non-susceptibility prevalence of serotype 23B isolated in Germany (Linden, Perniciaro and Imöhl, 2015). Serotype 23B has been shown in other studies to have high non-susceptibility to penicillin and also increase in proportion of MDR (Steens *et al.*, 2013; Richter *et al.*, 2014).

The Standard Treatment Guideline (2010) in Ghana recommends Penicillins as the first line of treatment for pneumonia and Erythromycin for those who are allergic to

Penicillin. The penicillin non-susceptibility pneumococcal isolates in this study forms 26.7% of the total isolates. This prevalence was low as compared to (44.4%-90.6%) in the pre-vaccine era (Baffoe-Bonnie *et al.,* 2000; Dayie *et al.,* 2013). Studies in other countries where PCV-13 have been introduced showed a significant reduction in the penicillin non-susceptibility prevalence of pneumococcal isolates (Cornick and Bentley, 2012; Jaiswal *et al.*, 2014; Gladstone *et al.,* 2015b; Rodgers, L Gail and Klugman, 2016) and this was attributed to the clearance of penicillin non-susceptible serotypes that are covered by the vaccine. This indicates that penicillin may still be effective in the treatment of pneumococcal disease in Ghana. Pneumococcal isolates showed the lowest resistance to Erythromycin and this result is comparable to the results obtained by Dayie *et al.,* (2015), however studies in Europe and Asia show a very high resistance to Erythromycin (Chan *et al.,* 2016; Soysal *et al.,* 2016). The low Erythromycin non-susceptibility prevalence was attributed to the limited use of the drug in only hospital settings (Newman *et al.*, 2011).

Two of the antimicrobials tested showed very high prevalence of resistance; Tetracycline (74.8%) and Trimethoprim-sulphamethoxazole (78.6%). This resistance prevalence is within the same range (73%-98.4%) as previous studies carried out in Ghana (Newman *et al.*, 2011; Dayie *et al.*, 2015). The high resistance to these drugs was attributed to the abuse of these drugs in prescription and the ease with which the drug is accessible to the general public (Newman *et al.*, 2011). Resistance to Trimethoprime/Sulphamethoxazole in the pneumococcus has been shown to have a strong association with Penicillin resistance (Mayor, 2009). The high resistance of the pneumococcus to this drug was attributed to the use of Fansidar for the treatment of malaria in Africa and also the prophylactic use of this drug in HIV positive patients (Mayer, 2009). In view of the high

resistance of the pneumococcus to this antimicrobial agent, WHO's recommendation that this drug should be used for the treatment of respiratory infections in developing countries should be reconsidered.

The overall MDR of the 266 isolates was 20.4% (54/266) and the most common pattern observed was resistance to Penicillin, Tetracycline and Trimethoprime/Sulphamethoxazole. The MDR in this study was lower when compared to the pre-vaccine results by Dayie *et al.*, (2015). This observation may be attributed to the reduction in the penicillin non-susceptibility prevalence of the isolates.

5.2: Conclusion

Based on the objectives of this study, which were to determine the nasopharyngeal carriage of *S. pneumoniae*, antimicrobial susceptibility profile and the distribution of serotypes among healthy children under five years, it is concluded that:

- The overall pneumococcal carriage prevalence was 64.9% and out of this, 25% showed intermediate resistance and 1.9% showed full resistance to penicillin.
- The predominant serotype isolated is serotype 23B. Twenty percent of this serotype was MDR.
- Replacement with a non-vaccine type (23B) seems to be taking place but this is not yet conclusive as only 111 out of the 266 pneumococcal isolates have been serotyped so far.
- PCV-13 failed to eradicate serotype 23F and 19F among the vaccinated study subjects and these serotypes harbours MDR phenotypes accounting for 11.1% and 28.6% MDR prevalence respectively.

5.3: Limitations

The limitation of this study is the inability to perform serotyping on all the isolates due to time constraints.

5.4: Recommendations

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

- Carriage studies should be extended to other areas of the country (the northern region) and continues nationwide surveillance should be instituted.

- Invasive studies should be conducted to ascertain which serotypes are involved in invasive pneumococcal disease in the PCV-13 era.

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APPENDIX I

Methodology for selecting schools at the study sites

The Ghana Education service in Accra was contacted and a list of all Kindergarten and Nursery schools within the sub-metro under the metropolitan assembly was obtained. All schools on the list that did not have nurseries and kindergartens were excluded from the study.

The schools were randomly selected by balloting using pieces of paper numbered and the number on the paper were matched to the number of the school listed under the circuit. In addition, provision was made by including other schools to replace those ones for which consent was not approved by the school authorities.

APPENDIX II

Sample collection consent form

Principal investigator: Elizabeth Y. Tettey

Institution: Department of Microbiology, SBAHS

Research title: Post Vaccination Surveillance of Pneumococcal Serotypes and Their Antimicrobial Susceptibility Profile in Ghanaian Children under Five

Invitation to participate in a research study: *Streptococcus pneumoniae* (pneumococci) are bacteria that are normally found in the nasopharynx (deep within the nose) of individuals; most especially children below the ages of five years. These children serve as the main reservoirs of the bacterium and it is spread from person to person through aerosols (sneezing, coughing etc). Crowded environments facilitate the transmission of the bacterium. The bacterium can move to the lungs, brain, blood, sinuses and other parts of the body to cause various diseases. Examples of diseases caused by this bacterium includes; pneumonia, meningitis and otitis media. Pneumonia kills children more than malaria, AIDS and measles combined and every year more than 1.6 million children die because of this disease. There are about 93 different types of this organism and out of this, only a few cause diseases in man. There are drugs that are used to treat the infection caused by this bacterium but the drugs are no longer able to treat the diseases as well as they use to. Vaccination is a better option because; it prevents the occurrence of the disease.

Even though vaccination is the best way out, it also has a few challenges because, those types that were not causing disease can now take over as the major disease causing

agents. It is therefore very important to continue checking which of the types of the bacteria are in circulation and also to find out how effective the vaccine was in eliminating those one's that are known to cause disease.

Risk/Hazards of the study: The bacterium lives in the nasopharynx and it is found in the mucus (phlegm) that is, a jelly-like deep within the nose. This substance will be collected using a flexible object known as a swab and it will be sent to the laboratory so as to obtain the bacterium from the mucus. The swab will be inserted into the nasopharynx to collect the mucus. In the process of collecting the mucus, if the swab is not freshly removed from it container but keeps too long in the open before being used, it can transfer other bacteria in the environment to your child to cause infection. Also, the insertion of the swab can cause a minimal discomfort to your child. In order to prevent the occurrence of these potential hazards, only trained and experienced personnel will take the mucus.

Subject's right to refuse or withdraw: In case you go through this form and you give your consent for your child to take part in this study, please remember that participation is voluntary and you have every right to withdraw your agreement at any point in time without any fear of punishment. You also have the right not to answer any question you are not comfortable with.

Confidentiality: The privacy of your child will be kept and data from this work will be treated with respect and be held confidential.

Questions, Concerns or Complaints: in case you want to find out more about this project, please contact Miss Elizabeth Y. Tettey (student) on 0243135546, Dr. NTK Dayie (supervisor) on 0208449415 and Prof. Mercy J. Newman (supervisor) on 02444329266. Both

supervisors work at the Department of Microbiology, SBAHS, University Of Ghana, Korle-Bu.

Consent for inclusion: In case you agree to permit your child to participate in this study, please fill the form below;

I…… the parent/guardian/caretaker of ……. On this day …… (Day/Month/Year) can prove that, I understand the explanations given in the consent form and therefore agree that, my child should participate in the study titled POST VACCINATION SURVEILLANCE OF PNEUMOCOCCAL SEROTYPES AND THEIR ANTIMICROBIAL SUSCEPTIBILITY PROFILE AMONG CHILDREN UNDER FIVE IN ACCRA, GHANA.

Signature of parent/guardian/caretaker……

Contact Address……

Phone number……

Thumb print (where required)

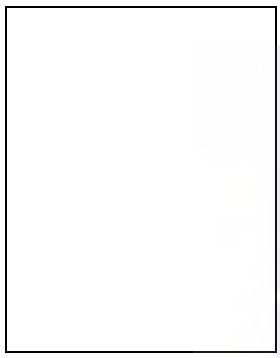
Name of witness: ……

Thumb print (where required)

Name of investigator: ……

Contact address: ……

Phone number: ……



Thumb print (where required)

APPENDIX III

Letter of ethical approval

UNIVERSITY OF GHANA
COLLEGE OF HEALTH SCIENCES
ETHICAL AND PROTOCOL REVIEW COMMITTEE

My Ref. No.....

29th February, 2016.

Elizabeth Yaa Tettey
Department of Medical Microbiology
School of Biomedical and Allied Health Sciences
University of Ghana
Korle-Bu, Accra

ETHICAL CLEARANCE

Protocol Identification Number: **CHS-Et/M.4 – P 4.12/2015-2016**

The Ethical and Protocol Review Committee of the College of Health Sciences on the 25th of February, 2016 unanimously approved your research proposal.

TITLE OF PROTOCOL: **"Post Vaccination Surveillance of Pneumococcal Serotypes and their Antimicrobial Susceptibility Profile in Ghanaian Children under Five"**

PRINCIPAL INVESTIGATOR: **Elizabeth Yaa Tettey**

This approval requires that you submit six-monthly review reports of the protocol to the Committee and a final full review to the Ethical and Protocol Review Committee at the completion of the study. The Committee may observe, or cause to be observed, procedures and records of the study during and after implementation.

Please note that any significant modification of this project must be submitted to the Committee for review and approval before its implementation.

You are required to report all serious adverse events related to this study to the Ethical and Protocol Review Committee within seven (7) days verbally and fourteen (14) days in writing.

As part of the review process, it is the Committee's duty to review the ethical aspects of any manuscript that may be produced from this study. You will therefore be required to furnish the Committee with any manuscript for publication.

This ethical clearance is valid till 30th September, 2016.

Please always quote the protocol identification number in all future correspondence in relation to this protocol.

Signed: .....
PROFESSOR ANDREW A. ADJEI
CHAIRPERSON, ETHICAL AND PROTOCOL REVIEW COMMITTEE

cc: Provost, CHS
Dean, SBAHS
Head of Department

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