Streptococcus pneumoniae SEROTYPE PREVALENCE, ANTIBIOTIC SUSCEPTIBILITY AND ASSOCIATED RISK FACTORS AMONG CHILDREN ATTENDING GERTRUDES CHILDREN'S HOSPITAL IN NAIROBI CITY COUNTY-KENYA

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DECLARATION

This thesis is my original work and has not been presented for a degree in any other university or any other award.

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DEDICATION

This thesis is dedicated to my lovely wife and amazing children.

I love you so much

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ABBREVIATIONS AND ACRONYMS

AMR	Antimicrobial resistance
AOM	Acute otitis media
AST	Antibiotic susceptibility testing
APCs	Antigen presenting cells
BA	Blood agar
BHI	Brain heart infusion
CBA	Chocolate blood agar
Cbp	Choline binding protein
CDC	Centers for Disease Control
CLSI	Clinical and laboratory standards institute
CSF	Cerebrospinal fluid
CO_2	Carbon-dioxide gas
DNA	Deoxyribonucleic acid
ECDC	European Center for Disease Prevention and Control
EARSS	European Antimicrobial Resistance Surveillance System
et al	and others
FAO	Food and Agriculture Organization
GAVI	Global Alliance of Vaccines and Immunization
GBA	Gentamicin blood agar
GCH	Gertrude's Childrens Hospital
GCH-RC	Gertrude's Childrens Hospital-Research Committee

H_0	Null hypothesis
IgA	Immunoglobulin A
IgE	Immunoglobulin E
IPD	Invasive pneumococcal disease
JCI	Joint Commission Institute
KEPI	Kenya Expanded Program on Immunization
KEMRI	Kenya Medical Research Institute
Kg	Kilo-grams
KNBS	Kenya National Bureau of Statistics
LMICs	Low and middle income countries
MH-BA	Muller Hinton with blood
MIC	Minimum inhibitory concentration
Mg	Milligrams
NAUP	National Antibiotic Use Plan
NP	Nasopharyngeal
Non-vT	Non-vaccine type
NK-cells	Natural killer cells
OIE	Office International des Epizooties
PBP	Penicillin binding protein
PBS	Phosphate buffered saline
PCR	Polymerase chain reaction
PCV	Pneumococcal conjugate vaccine

PCV-10	10-Valent pneumococcal conjugate vaccine
PCV-7	7-Valent pneumococcal conjugate vaccine
PD	Pneumococcal disease
PspA	Pneumococcal surface protein A
PPV	Pneumococcal polysaccharide vaccine
RTIs	Respiratory tract infections
RBCs	Red blood cells
SPSS	Statistical Package for Social Sciences
USA	United States of America
µl	Micro-liters
vT	Vaccine type
WHO	World Health Organization
WBC	White blood cells

ABSTRACT

Pneumococcal disease remains the biggest killer of children living in Kenya. This is true despite inclusion of the 10-valent pneumococcal conjugate vaccine in the Kenya Expanded Program on Immunization. Serotype replacement, emergence of antibiotic resistance, inaccurate laboratory diagnosis due to optochin resistant bacterial types and a range of environmental and host related risk factors have been touted to be the cause of these statistics elsewhere. This study sought to establish prevalence of *Streptococcus pneumoniae* serotypes, antibiotic susceptibility patterns and associated risk factors among PCV-10 vaccinated and unvaccinated children attending Gertrude's Childrens Hospital. A total of 206 children were recruited for this study. Nasopharyngeal swabs were the main specimen used. Culturing and isolation of the bacteria was done on blood agar with gentamicin and plain blood agar plates respectively. Optochin and bile solubility (where necessary) tests were done as confirmatory assays for the bacteria. Pneumococci serotyping was done using the Gold Standard Quellung Reaction test while the disk diffusion method was used to asses antibiotic susceptibility profiles. Out of the 206 subjects sampled, 20.39% (*n=42*) were found to be carriers of the bacteria. About 52% (*n*=22) of the carriers had received the recommended dose of PCV-10, while 48% (*n*=20) had not. Almost all (*n*=41; 19.90% of subjects) isolates contained non-vaccine type serotypes, while *n*=1 of the isolates (0.49% of subjects) were both optochin resistant and untypeable. Serotypes 28F, 6A, 11A, 3 and 7C were prevalent in both vaccinated and unvaccinated children, whereas serotypes 23A, 17F, 35F, 48, 13 and 35B, and 23B, 20, 19B, 21, untypeable, 15B and 39 were found among unvaccinated and vaccinated cohorts, respectively. Thirty nine (92.86%) of pneumococci isolates were susceptible to erythromycin, 39 (92.86%) were susceptible to vancomycin, 8 (19.86%) were susceptible to oxacillin; 40 (95.24%) were susceptible to clindamycin, 24 (57.86%) were susceptible to ceftriaxone while 18 (42.86%) were non-susceptible. The risk of nasopharyngeal carriage decreased insignificantly when the subject was female (odds ratio [OR]: 0.766, 95% CI: 0.388, 1.511, p-value=0.442). Children between the age of 25-36 months (OR: 1.147 (95% I: 483, 2.722) and 37-48 months (OR: 1, 95% CI: 0.286, 3.501) had an insignificant elevated risk of nasopharyngeal carriage of the bacteria. Children whose mothers were non-cigarrate smokers exhibited low odds of carriage (OR: 0.764 (95% CI: 0.077, 7.537; p=0.818). Serotype replacement, resistance to penicillins and exposure to smoke were correlated with incresaed risk of nasopharyngeal carriage. Continuous and broader epidemiological surveys should be carried out in the entire country to accurately determine the degree of serotype replacement and; people should be sensitised on judicious use and/or consumption of antibiotics. Optochin test should be introduced as a routine assay in diagnosis of *Streptococcus pneumoniae* in hospitals.

CHAPTER ONE
1.0 INTRODUCTION

1.1 Background information

Streptococcus pneumoniae also called pneumococcus is the bacterial causative agent of pneumococcal disease (PD) (Weiser *et al.,* 2018). It is spread horizontally through inhalation of respiratory droplets from an infected person (Henriques & Tuomanen, 2013). The infections affect persons of all ages but are especially prevalent among children and adults below and above the ages of two and 65 years respectively (Tan, 2012). Researchers have reported that PD causes 1.6 million preventable deaths every year across the world (Brooks & Mias, 2018). Intriguingly, the bulk of the fatalities occur in Africa and Asia (Wahl *et al.,* 2018). For instance, Kenya was classified among the top 15 countries with highest mortalities due to pneumonia in 2012 (Dickens *et al.,* 2012). Pneumococcal infections therefore pose a major and urgent threat for Kenya.

In view of the burden of PD and the success of pneumococcal vaccines in industrialized countries (Rodgers & Klugman, 2016); WHO recommended that governments incorporate pneumococcal vaccines in their national immunization programs. Following this policy advisory, and with support from the Global Alliance on Vaccines and Immunization (GAVI), Kenya included the 10-valent pneumococcal conjugate vaccine (PCV-10) in her expanded program on immunization (KEPI) in 2011 (Mbithe, 2012).

The choice of PCV-10 was informed by two major factors, that: lower valence conjugate vaccines had been associated with remarkable declines in cases of PD in industrialized countries (Hammitt *et al.,* 2014) and; PCV-10 contained 42% of pneumococcus non-invasive serotypes and over 74% known invasive serotypes found in Kilifi, Kenya (Brueggemann *et al.,* 2013). Upon introduction of the vaccine into KEPI, the vaccine coverage and uptake was ≥80% (Hammitt *et al.,* 2019), which is above the recommended average vaccine coverage to achieve both direct and herd immunity (Fine *et al.,* 2011). Against this backdrop, it would almost be natural to expect a sharp decline in the burden of all variants of PD. Unfortunately, these infections continue to ravage majority of the children living in Kenya.

A number of reasons suffice: *Streptococcus pneumoniae* has ≥90 serotypes (Geno *et al.,* 2015). The distribution of these serotypes vary on the premise of disease burden, geographical area, age, crowding index and climatic conditions (Schrag, 2007). Epidemiological studies have demonstrated occurrence of vaccine serotype replacement by non-vaccine serotypes (Mehr & Wood, 2012). This phenomenon has been documented to be associated with low efficacy levels of conjugate vaccines as protection offered by the vaccine is only against antigenic material included in its formulation. Replacement of serotypes included in the vaccine (vT) by serotypes not included in the vaccine (nVT) is likely to render the vaccine ineffective.

Escalation in cases of *Streptococcus pneumoniae* resistance to both first line and broad-spectrum antibiotics poses a mammoth threat to the success of conjugate vaccines (Stephanie *et al.*, 2001). Susceptibility of *Streptococcus pneumoniae* to novel antibiotics is serotype specific (Maianskiĭ *et al.*, 2014). Genetic material containing resistant antigenic correlates are likely to be shared among pneumococci serotypes found within the same group (Reinert, 2009a). This trend is especially true in resource poor countries where laws governing use of antibiotics are either lacking or are poorly enforced. Inappropriate use of antibiotics is a perfect precursor to antimicrobial resistance and a possible cause of escalating levels of child mortality due to PD.

Successful treatment of PD is dependent on accurate, timely and reliable laboratory isolation of the causative bacteria (CDC, 2017). Emergence of optochin resistant *Streptococcus pneumoniae* strains have been demonstrated to interfere with accurate diagnosis and isolation of pneumococci in the laboratory (Nagata *et al.*, 2012). Considering the tendency of *Streptococcus pneumoniae* serotypes to share resistant genes among members of the same group, there is a high likelihood of existence of optochin resistant serotypes in circulation among children in Kenya. This may largely impinge on the accuracy in laboratory diagnosis, provide a basis for wrong prescriptions and ultimately escalate cases of child morbidity and mortality due to PD.

Dissemination and adhesion of pneumococci on the epithelial surface of the human nasopharynx depends on several factors: day-care attendance, child's nutritional status, age, exposure to smoke, breast-feeding frequency among others (Ujunwa & Ezeonu, 2014). Age of the child has been correlated with ability of the child to release both cellular and humoral immune defenses while; exposure to active and passive smoke disables proper functioning of antigen presenting cells (APCs) and causes increased production of Immunoglobulin E (IgE) which exacerbates occurrence of type-1 hypersensitive reaction (Bagaitkar *et al.,* 2008). Breast-feeding frequency determines the nutrional status of the child and consequently the ability to produce mucosal immune defenses (IgA) (Ásbjörnsdóttir *et al.,* 2013). Considering the nature of social demographics of most children living in Kenya, these factors may be playing a key role in the sustained high prevalence of PD.

This study sought to find out: whether *Streptococcus pneumoniae* serotypes included in PCV-10 are the ones circulating among children attending Gertrude's Childrens hospital (GCH); assess *Streptococcus pneumoniae* susceptibility patterns to the commonly prescribed antibiotics among children ≤5 years attending GCH; establish prevalence of optochin resistant *Streptococcus pneumoniae* serotypes among children ≤5 years attending GCH and; determine risk factors associated with the nasopharyngeal (NP) carriage of *Streptococcus pneumoniae* among children ≤5 years attending GCH.

1.2 Statement of the problem

Kenya adopted the use of PCV-10 in to her National Program on Immunization in February, 2011. More than 10 years prior to the implementation of PCV-10 in Kenya, the use of lower valence pneumococcal conjugate vaccines in industrialized settings had been associated with tremendous reductions in both *Streptococcus pneumoniae* non-invasive and invasive disease. According to (Hammitt *et al.,* 2019), the uptake of PCV-10 by children in Kenya has consistently been ≥80%. In view of these statistics, it would be expected that the burden of PD among children in Kenya reduces. However, 8 years post implementation of the vaccine in Kenya, pneumococcal disease remains one of the leading causes of child morbidity and mortality. This study therefore sought to find out why pneumococcal infections have remained consistently high among children in Kenya despite the prevalent use of PCV-10.

1.3 Justification of the study

Use of conjugate vaccines is the most promising approach for reducing the prevalence of PD across the world. This is with respect to tremendous reductions in cases of both non-invasive and invasive disease following the use of lower conjugate vaccines in industrialized countries.

As such, continuous and targeted studies meant to establish the performance and general effect of PCVs on the burden of PD in areas where they have been implemented cannot be over-emphasized. PCV-10 has been used in Kenya since 2011 yet; pneumococcal infections remain a major driver of child morbidity and mortality in the country. This study therefore sought to establish the reason behind the high prevalence of PD in Kenya despite inclusion of PCV-10 in KEPI.

1.4 Hypotheses

$H_0$1 *Streptococcus pneumoniae* serotypes included in PCV-10 are not consistent with those circulating among children below 5 years of age and attending Gertrude's Childrens Hospital (GCH);

$H_0$2 *Streptococcus pneumoniae* has not developed resistance to the antibiotic agents prescribed for different pneumococcal disease forms affecting PCV-10 vaccinated and unvaccinated children below 5 years of age and attending GCH;

$H_0$3 The prevalence of *Streptococcus pneumoniae* serotypes that are non-susceptible to optochin is not high among PCV-10 vaccinated and unvaccinated children below 5 years of age and attending GCH and;

$H_0$4 Nasopharyngeal carriage of *Streptococcus pneumoniae* serotypes among PCV-10 vaccinated and unvaccinated children below 5 years of age and attending GCH does not depend on risk factors for PD.

1.5 Study objectives

1.5.1 General objective

To determine prevalence of *Streptococcus pneumoniae* serotypes, antibiotic susceptibility patterns and associated risk factors among children attending Gertrude's Childrens Hospital (GCH) in Nairobi City County-Kenya.

1.5.2 Specific objectives

i. To determine *Streptococcus pneumoniae* polysaccharide capsular serotypes circulating among PCV-10 vaccinated and unvaccinated children attending GCH;
ii. To determine *Streptococcus pneumoniae* antibiotic susceptibility patterns among PCV-10 vaccinated and unvaccinated children attending GCH;
iii. To determine prevalence of optochin resistant *Streptococcus pneumoniae* serotypes among PCV-10 vaccinated and unvaccinated children attending GCH and;
iv. To document risk factors associated with occurrence of nasopharyngeal *Streptococcus pneumoniae* carriage among PCV-10 vaccinated and unvaccinated children attending GCH.

1.6 Significance of the study

Policy makers and the biomedical research fraternity may use information generated by this study to make decisions on the continued use of PCV-10 in Kenya and develop improved strategies in the effort towards effective management and containment of PD.

CHAPTER TWO
2.0 LITERATURE REVIEW

2.1 *Streptococcus pneumoniae*

Streptococcus pneumoniae is the bacterial causative agent of pneumococcal disease both in adults and children (Henriques & Tuomanen, 2013). It is a sociable bacterium of the nasopharyngeal microbiota of hominids but can get intrusive and cause disease under certain varying environments (Shak *et al.*, 2013). On a typical blood agar (BA) preparation, it appears as lancet shaped diplococci but can also present as single cocci or even in chains. The bacteria are gram-positive, non-motile, do not form spores and grow well in anaerobic conditions.

It is a highly assiduous organism growing best at 35-37^{o}C in 5% CO_2 conditions; it is optochin (ethylhydrocuprein hydrochloride) susceptible and soluble in bile salts (sodium desoxycholate). The pneumococcus cell was undergoes spontaneous lysis when exposed to sodium-desoxycholate salts at 35^{0}C-37^{0}C (CDC, 2016). The bacterial growth on blood agar medium is inhibited by ethylhydrocuprein hydrochloride which is quinine trite at very minimal concentrations.

2.1.1 History of *Streptococcus pneumoniae*

The bacteria was first described in 1881 by two scientists working separately; Louis Pasteur in France and George Sternberg in USA (Zivich *et al.,* 2018). They both isolated the bacteria in rabbit blood after Louis Pasteur injected the rabbit with saliva isolated from a child who had died of rabies and George Sternberg had used his own saliva. The bacteria isolated in blood appeared to be diplococci in shape. It was named *microbe septicemique du salive* and *microbe pasteuri* by Pasteur and Sternberg respectively (Watson *et al.,* 1993).

Although there had been some vague information about the bacteria in the 1870's, it is Louis Pasteur and Sternberg George who linked the bacteria to etiology of pulmonary infections in 1880's. Later on, Carl Friedlander classified the pneumococcus as having etiological relationship with lobar (Watson *et al.,* 1993). In the early 19th century, Neufeld discovered the tendency of the pneumococcus capsule to swell when it came into contact with specific antisera; this revelation marked a major breakthrough for the aptitude of scientists to serotype *Streptococcus pneumoniae* (Habib, 2014). According to (Grabenstein & Klugman, 2012), the bacteria was named as *Diplococcus pneumoniae* in 1920 and as *Streptococcus pneumoniae* in 1974 after they established that it formed pairs of cells that appeared chain-like when viewed under objective x400 of a phase contrast microscope respectively.

2.1.2 Laboratory diagnostic features of pneumococci

Streptococcus pneumoniae retains the primary stain (crystal violet) during gram stain because it has a cell wall that contains peptidoglycan layer. As a result, it is classified as gram positive bacteria in shapes of cocci (diplococci or short chains). It is catalase negative because it does not form oxygen bubbles when mixed with hydrogen peroxide on a microscopic slide. The bacteria partially hemolyses red blood cells (RBCs) and are therefore described as ά-hemolytic; on Blood Agar, colonies appear tiny, greyish, moist, mucoidal and sometimes exhibit draughtsman's features. *Streptococcus pneumoniae* measures 0.5-1.25mm in diameter, they are non-motile and contains Pilli which aids the bacteria in adhering to the respiratory epithelial surfaces (Aryal, 2018).

2.1.3 Structural features and pathogenesis of *Streptococcus pneumoniae*

Streptococcus pneumoniae is known to be a commensal and friendly bacteria of the nasopharyngeal microbiota (Shak *et al.*, 2013). The bacterium has several cell surface structures which have also been demonstrated to facilitate the process of disease occurrence (pathogenesis). The structures include: capsule, cell wall, Pili and a number of cell surface proteins (Weiser *et al.,* 2018). At different stages of pneumococcal disease pathogenesis, these various structures have been documented to aid certain patterns that lead to the initial bacterial adhesion to the nasopharyngeal region and eventual invasion to otherwise unwelcome parts of the human body.

2.1.3.1 Pneumococcus capsule

According to Hyams *et al.* (2010), pneumococci polysaccharide capsule fully encloses the cell of all feral serotypes of the bacteria. This capsule is known to be the principal carrier of the *Streptococcus pneumoniae* virulence factors as it highly interferes with the action of the host's complement system and the antigen engulfing capacity of relevant white blood cells (WBCs). It has been documented that the polysaccharide capsule prevents the action of the complement protein C3b to opsonize the bacteria and also specifically impedes the phagocytic capacity of host neutrophils hence enabling the bacteria to freely gain entry to unauthorized regions of the body Hyams *et al.* (2010). Various serotypes of *Streptococcus pneumoniae* are established on the basis of the polysaccharide capsule as this is the main carrier of its antigenic correlates (Geno *et al.*, 2015).

Pneumococci are highly transformable bacteria, vulnerable to exchange of antigenic correlates from one strain to the other with the bacterial DNA being at the center of it all (Cornick *et al.*, 2017). This phenomenon is especially true with regard to members of the *Streptococcus pneumoniae* fraternity that share groups. The genes encoding for various virulence factors as contained in one cassette of a given strain of the pneumococcus polysaccharide capsule can be horizontally transferred to another serotype within the same group (Andam & Hanage, 2015).

2.1.3.2 *Streptococcus pneumoniae* cell wall

Pneumococci cell wall which contains peptidoglycan and teichoic acid adhering to every third N-acetylmuramic acid is about six layers thick (Todar, 2020). It also contains lipoteichoic acid which chemically resembles the teichoic acid and both have phosphorylcholine. The choline residue of the phosphorylcholine is imperative in the pathogenesis of *Streptococcus pneumoniae* as it peculiarly binds to choline binding receptors that are located in most human cells (Maestro & Sanz, 2016). The bacterial cell wall has been associated with some actions of the immune system. The C-reactive protein (CRP) for example, binds to the phosphorylcholine located in the pneumococci cell wall and trigger compliment activation which eventually facilitates the removal of the bacteria from the system (Paterson & Mitchell, 2006).

2.1.3.3 Pili

The earliest stage in the pathogenesis of pneumococcal disease is the attachment of *Streptococcus pneumoniae* on mucosal surfaces of the human nasopharynx. This adherence is made possible by the Pili of *Streptococcus pneumoniae* (Novick *et al.,* 2017). These long bacterial accessory structures are capable of elongating beyond the polysaccharide capsule, the pilus-1 is encoded by pilus islet-1 (PI-1) whose expression is regulated by R/rA and it is often found in about 30% of all pneumococci isolates (de-Angelis *et al.,* 2011).

There are therefore two pili with which *Streptococcus pneumoniae* adheres to the epithelial surfaces of the upper respiratory surfaces of human hosts; PI-1 and PI-2 (Basset *et al.,* 2011).

2.1.3.4 Pneumococcus surface proteins

According to Todar (2020), *Streptococcus pneumoniae* contains a blend of >500 membrane and cell wall associated surface proteins. The report further elucidates that the cell wall contains about five penicillin binding proteins (PBPs) which are majorly involved in its synthesis, ≥2 neuraminidases and an Immunoglobulin-A (IgA) proteolysis enzyme. Further, pneumococci cell surface also contains choline binding proteins (CBPs) which bear the microbe's prime virulence correlates. These factors include: PspA, LytA, LytB, LytC and CbpA (Mitchell & Mitchell, 2010).

Pneumolysin (PLY) is another major virulent factor for the bacteria. It is a toxin found in all clinical serotypes of *Streptococcus pneumoniae.* For this reason, the protein is classified as one of the major virulent correlates of this bacterium (Hupp *et al.,* 2019). The concentration of the toxin in the system determines the level and type of effect in the host; a low concentration for instance have been associated with partial activation of the complement system and release of inflammatory mediators while a higher concentration have been associated with direct cell lysis (Hirst *et al.,* 2004).

As *Streptococcus pneumoniae* is prone to self-lysis due to the hydrolysis of the cell wall caused by LytA autolysin, research has associated this self-lysis with release of pneumolysin. Other researchers like Andre *et al.* (2017) for instance, found out that the activation of LytA autolysin caused production of pneumolysin which further activated major immunological defenses by the host organism. Autolysin, mainly LytA, LytB and LytC which is a cell wall degrading enzyme which has been demonstrated to facilitate the hydrolysis of the peptidoglycan layer of the pneumococcal cell wall (Mitchell & Mitchell, 2010). It has been further reported to be responsible for breaking up of glycosidic bonds and peptide cross-bridges between the various chemical constituents of peptidoglycan in order to give a leeway for introduction of new ones during the process of bacterial development (Jedrzejas, 2001).

This phenomenon was further substantiated on in a study done by Mellroth *et al.* (2012), that LytA causes disintegration by splitting the lactyl-amide connections between the peptide and glycan domains. The production of pneumolysin (PLY) which is responsible for initiation of various immunological responses from the host has been observed to be dependent on the availability of autolysin (AL). However, a study by Balachandran *et al.* (2001), opposed the postulate that release of PLY to the pneumococcus extracellular space is dependent on availability of autolysin.

The researchers argue that PLY has been found in the log-phase of pneumococcus growth a level at which AL would not yet have been released.

Neuraminidase (NanA) is involved in the acceleration of rates of reaction during the production of sialic acid residues in the bacteria. Research has further observed that NanA is involved in host subjugation which is a major component of the pathogenesis of pneumococcal disease (Parker *et al.*, 2009). A study by Pettigrew *et al.* (2006), demonstrated that pneumococcus produces three distinct neuraminidases on the least (NanA, NanB and NanC). NanA which is reserved among most pneumococcal strains is the most expressed at transliteration level. *Streptococcus pneumoniae* neuraminidase modifies host's immune defense faculties and glycans providing a perfect platform for the pneumococcus to excitedly colonize the host (Brooks & Mias, 2018).

NanA also creates a source of carbohydrates for the pneumococcus as it cleaves host's mucosal sugars to utilizable states; whether the sugars are vital in the development of *Streptococcus pneumoniae* is yet to be well profiled (Parker *et al.*, 2009)). IgA is the principle immune protein involved in provision of upper respiratory surface immunity (Corthesy, 2013). A study by Janoff *et al*. (2014), reported existence of pneumococcus specific IgA-1 protease which cleaves IgA-1 antibody (Ab) at the hinge region in to antigen (Ag) binding fragment (Fab) and fragment crytallizable fragment (Fc) segments.

The hydrolysis of IgA-1 in to constituent fragments (Fc & Fab) has been shown to interfere with the effector functions of the antibody and hence allowing the pneumococcus a leeway to burgeon and begin to cause pneumococcal illnesses (Janoff *et al*., 2014).

2.2 Serotypes and serotyping

A serotype or serovar is an organism which can be serologically placed or classified as belonging to a certain strain. Further, it can be described as an unambiguous distinction within a species of a given organism or among immune cells of various individuals. These microorganisms or cells are classified jointly on the basis of their cell surface antigenic correlates, hence giving a leeway for the epidemiologic classification of organisms to the sub-species level (Shiel, 2020). Serotyping therefore, is an act of assigning micro-organisms in to their species or sub-species.

Pneumococci have so far been classified into ≥90 serotypes belonging to 46 distinct groups (Geno *et al.,* 2015). The distribution of the various serotypes varies greatly on the basis of geographical location and a series of other factors. The antigenic capacity of diverse serotypes also varies (Geno *et al.,* 2015). As such, not all the serotypes so far classified have capacity to cause disease. What is more is that there have been reports of vaccine serotypes being replaced by non-vaccine serotypes.

For instance, a study by Heath *et al.* (2018), reported that there was no difference in nasopharyngeal carriage before and after PCV-10 vaccination in their study cohort. However, what was notable is a reduction in vaccine serotypes from the initial 42% to 19% after vaccination and an escalation in non-vaccine serotypes after vaccination. This phenomenon could explain the reason behind sustained high prevalence of PD despite the use of PCV-10 in Kenya.

2.2.1 Laboratory methods for *Streptococcus pneumoniae* serotyping

The pneumococcus has up to present been classified in to ≥90 serotypes placed in 46 serogroups; each serotype elicits unique antigenic correlates and can therefore only be countered by serotype specific immune responses. Serotype citizenry of a given serogroup elicit closely correlated antigenic correlates and thus may to some extent also share immunological responses, for example serotypes 6A & 6B, 9L, 9N & 9V belong to serogroups 6 and 9 respectively (Geno *et al.*, 2015).

Having a flawless understanding of the taxonomy of serotypes and how they are placed in their respective groups plays a major role in understanding not only the science of vaccine efficacy and designing vaccine impact studies but also the terrestrial distribution of various serotypes (Ngocho *et al.*, 2019). There are several laboratory methods for serotyping pneumococci. They include: latex agglutination method, Quellung reaction method, PCR based methods and pneumococcal genotyping (Jauneikaite *et al*., 2015).

2.2.1.1 Quellung reaction method

Quellung reaction also known as the Neufeld method is a biological reaction in which the host's serotype specific Abs bind to the bacterium capsule antigens, alters its cell-surface refractive index and causes it to appear opaque and swollen (Habib *et al.,* 2014). Profiled first in the year 1902 by Fred Neufeld, the method which only had capacity to profile up to three serotypes was extensively applied to antiserum treatment of Pneumococcal disease (Habib *et al.,* 2014). This was especially important because of the fact that successful treatment of pneumococcal disease then was reliant on accurate placement of the infecting pneumococcus to one of the three serotypes, and the Quelling reaction was at the center of it all. Later on in the early 19th century, Dr. Albert Sabin and Fred Griffin, following tremendous research activities, made it possible for the Quellung reaction assay to be done a little more rapidly (Griffith, 1928 and Sabin, 1933).

Together with other key scientists of the time, they discovered 29 more pneumococcal serotypes and; established that the bacteria had capacity to transfer and/or exchange its antigenic correlates especially with members of the same serogroup by use of the deoxyribonucleic acid (DNA) molecule (Mavroidi *et al.,* 2007). Following this incredible innovation, the assay was extensively espoused and taken-up by most if not all industry players around the world.

For instance, during the World War II, Eddy, a distinguished American scientist, was able to profile and classify 75 *Streptococcus pneumoniae* serotypes using the Quellung reaction assay and simply named them in the order of discovery (Geno *et al.*, 2015). Present-day and wide-ranging studies done have gradually led to the distinction between serotypes and serogroups; serotypes were described as *Streptococcus pneumoniae* strains that produced PLY with an inimitable biological assembly and immunologic chattels while a serogroup was defined as two or more serotypes whose PLY structure and immunologic properties were fundamentally analogous (Hirst *et al.*, 2004). The serum intervention as a way of treating various bacterial infections was widely if not fully supplanted by use of antimicrobials in the dawns of 1940's but identification of bacterial serotypes for different purposes still rendered the Quellung assay very vital to the story of infectious diseases globally (Barbaros and Akdis 2003).

The relevance of the assay was especially augmented by various reasons: to understand clear topographical dissemination of *Streptococcus pneumoniae* serotypes, to establish the variance in the invasiveness of the serotypes and to provide relevant guidance with regard to development and monitoring of vaccines (Habib, 2014). So far, over 90 capsular pneumococcal serotypes have been profiled using the Quellung reaction method.

2.2.2 *Streptococcus pneumoniae* serotypes

Streptococcus pneumoniae bacteria is described and classified according to the antigenic correlates exhibited by their capsular polysaccharide. Different pneumococcal strains demonstrate diverse antigenic physiognomies as carried by their capsular polysaccharides which provides a basis for serotyping (Habib *et al.*, 2014). However, some serotypes exhibits close antigenic kinship and are therefore grouped together to form serogroups (Mostowy *et al.,* 2017). The concept of serogrouping and serotyping of pneumococcus is a fundamental pillar in the development of vaccines, determining the epidemiology and burden of pneumococcal disease and measuring the impact of vaccine and antimicrobial interventions. So far, *Streptococcus pneumoniae* has been classified in to 46 antigenically distinct serogroups and nearly 100 individual serotypes (Kawaguchiya *et al.*, 2017).

While all pneumococci capsular polysaccharide serotypes are capable of causing disease, the severity of disease caused by the various serotypes vary; only a handful of serotypes have so far been associated with invasive pneumococcal disease globally (Clarke *et al.,* 2004). Fascinatingly, the various pneumococcal strains are able to horizontally exchange antigenic correlates as they interact while at their ecological dwelling place (Hiller & Sá-Leão, 2018).

These marvels are coordinated by the bacterial DNA where genes associated with virulence and perhaps antimicrobial resistance are passed from one serotype to another but mostly among types of the same serogroup (Chaguza *et al.,* 2015). Against this backdrop, *Streptococcus pneumoniae* serotypes which would have been less associated with causation of disease gradually become pathologic and further expand the epidemiologic importance of the bacteria.

2.2.3 *Streptococcus pneumoniae* serotype distribution

Different *Streptococcus pneumoniae* serotypes possess dissimilar capacities of intrusiveness, antimicrobial resistance (AMR) and antigenicity. Research done in settings where vaccination programs have been implemented are therefore highly essential to monitor the impact of pneumococcal conjugate and polysaccharide vaccine interventions from time to time (Pan *et al.,* 2015).

All capsular polysaccharide *Streptococcus pneumoniae* serotypes have ability to cause disease, the only disparity is that the degree of virulence varies from one serotype to another with only a limited number causing >70% of PD worldwide (Nurse-Lucas *et al.,* 2016). What is more is that Cui *et al.,* (2017) supported the postulate above that *Streptococcus pneumoniae* serotype epidemiology around the globe is well balanced on the basis of age, disease burden and geographical location. Some serotypes have been found to be more associated with nasopharyngeal (NP) colonization than invasiveness.

2.2.3.1 Introduction of vaccines and emergence of antimicrobial resistant *Streptococcus pneumoniae* serotypes

The introduction of pneumococcal conjugate and polysaccharide vaccines has been directly and in some cases indirectly associated with tremendous declines in cases of vaccine type (vT) PD among vaccinated children around the world. Pneumococcal conjugate vaccine impact studies carried out in various parts of the world, have reported cases where pneumococcal disease due to serotypes included in vaccines has reduced significantly while; disease due to serotypes not included in the vaccine emerged (Levy *et al.,* 2019). Along with the phenomena of non-vaccine type serotypes replacing vaccine-type serotypes, has emerged rampant cases of antimicrobial resistant *Streptococcus pneumoniae* strains.

In Europe, about three *Streptococcus pneumoniae* serotypes have been associated with cases of multi-drug AMR; serotypes 15A, 19A, 14, (Yahiaoui *et al.*, 2018). A study done and documented by (Tan, 2012), in the United States of America (USA) stated that 6A, 6B, 9V, 14, 19A, 19F, and 23F were associated with most cases of AMR. However, with an exception of 19A all the rest are included in PCV-13 and therefore the effect of AMR significantly mitigated. Unfortunately serotype 19A continues to wreak havoc due to its tremendous propensity for resistance to penicillin and erythromycin.

Further, Liñares *et al.* (2010) assessed pneumococci isolates over a 30-year period; the study documented serotypes 6B, 6A, 9V, 14, 15A, 19F, 19A, and 23F to be associated with rampant cases of AMR. The trend of antimicrobial resistance however began to gradually and steadily decline during early 2000. This trajectory was attributed to introduction of especially conjugate vaccines. According to Buchy *et al.,* (2020) the introduction of PCV-7 (Heptavalent Vaccine) vaccine in USA in early 2000 was accompanied by an enormous decrease in cases of vaccine type pneumococcal disease. The diminution in cases of PCV-7 vT pneumococcal infections was accompanied by a commensurate decline in cases of penicillin and erythromycin resistance.

However, while cases of vaccine type penicillin and erythromycin resistance decreased, there was remarkable increase in cases of non-vaccine type penicillin and erythromycin non-susceptibility especially by serotype 19A (Liñares *et al.,* 2010). Another study done among children at the Italian Hospital of Desio, Lombardy reported that serotypes 3, 7F, 8, 19F, 23F and 24A were responsible for over 70% of all reported antimicrobial resistance incidences while serotype 19A was responsible for 78% of cases of AMR (Intra *et al*., 2017).

Serotypes 19F, 15A and 6B have also been linked to high level cases of AMR both in children and adults according to a study done in India (Nagaraj *et al*., 2017). Studies done in Kenya after introduction of PCV-10 have reported remarkable decrease in cases of vT pneumococcal infections. However, prevalence of the disease continues to be high, most likely due to replacement of vT by non-vaccine (non-vT) serotypes. As some serotypes are more associated with cases of AMR, it is highly likely that the non-vT serotypes are resistant to common antibiotics hence the sustained high prevalence in Kenya.

2.2.3.2 Distribution of *Streptococcus pneumoniae* serotypes according to age

There exists a nexus between age and the risk of harboring various pneumococci serotypes. For instance, while serotypes 3 and 14 are among the frequently isolated types around the globe, they are conspicuously absent in isolates from children below 5 years of age and adults above the age of 64 years respectively (Inostroza *et al.,* 2002). In equal measure, both serotypes 3 and 14 have been occasionally associated with episodes of severe forms of pneumococcal disease. Serotype 14 being reported to be responsible for a more severe form of disease episodes (Alanee *et al.,* 2007).

Remarkably, *Streptococcal pneumoniae* serotypes ranked between number 28F and 38 were found in high frequency among children the age of 5 years and adults above the of 65 years in Temuco (Inostroza *et al.,* 2002).

Serotypes 28F, 31, 33F, 34, 35F were found to be responsible for about 11% of pneumococcal disease incidents among children below the age of five years in Temuco (Inostroza *et al.,* 2002). Of note is that while these serotypes are massively causing severe forms of IPD among children, none of them is included in the current formulations of PCV vaccines. Serotype 33F is included in PPV23 but unfortunately the vaccine is not administered to children below the age of 2 years because of low immunogenicity.

Another study done by Imöhl *et al.* (2010), to establish distribution of *Streptococcus pneumoniae* serotypes from isolates collected between 1992 and 2006 in German, reported the most frequently isolated serotypes as: 7F, 14, 23F, 1 and 6B. Serotype 7F was found to be most frequent among infants below 4 months of age; serotype 19F was more ubiquitous among infants between the age of 4 months and 12 months while; serotypes 6B, 14 and 18C were reported to be more predominant among children amid the age of one and five years. Further, Harboe *et al.* (2009) who carried out a study to measure the association between various pneumococcal serotypes and incidences of death in Denmark using isolates collected between 1977 and 2007, reported the most frequently serotypes as: 18C, 7F, 1, 6A and 4. These five serotypes were part of ten serotypes known to cause well over 80% of child pneumococcal disease epidemics around the world.

Not all pneumococcal serogroups contain serotypes that cause severe forms of disease among neonates. Serogroups 19, 9, 3, 18, 1, 6, 14, 5 and 12 for instance, have been found to be strongly linked with pneumococcal disease occurrence among neonates in Denmark (Imöhl *et al.,* 2010). Some serotypes though not frequently isolated and associated with disease, whenever found, seemed to be stuck within certain age groups; serotype 7F for example, was found among children below the age of 6 months than it was found among those outside the age of 6 months in Denmark (Imöhl *et al.,* 2010).

A study done in Mexico reported serotype 3 as containing high degree of virulence and associated it with increased fatality rates. This was attributed to its ability to increased levels of encapsulation and mucus (Gabriela *et al.,* 2020). In Denmark, serotype 3 was reported to be most common among adults above the age of 65 years (Fjeldhøj *et al.,* 2018). Elsewhere, serotype 3 has been associated with all age groups but more notably with occurrence of pneumococcal disease among neonate cohorts (Imöhl *et al.,* 2010). Serogroup 19 having serotypes 19A and 19F has been well described and strongly linked to most episodes of PD among infants below the age of 4 months (Imöhl *et al.,* 2010). However, only serotype 19F and not 19A has been linked to occurrence of most episodes of pneumococcal disease among natives of all age groups globally.

In Latin America for example, serotypes found in serogroup 19 have been linked to occurrence of invasive forms of disease among neonates (Soto *et al.*, 2016); a unique scenario which fails to resonate well with findings from most other parts of the world.

2.2.3.3 Distribution of *Streptococcus pneumoniae* serotypes according to geographical location

According to Nurse *et al.* (2016), the distribution of *Streptococcus pneumoniae* around the world vary extensively in respect of the region and time period. Findings from a systematic and meta-analysis study carried out by Johnson *et al.* (2010) and aimed at establishing the global epidemiology of *Streptococcus pneumoniae* serotypes demonstrated that between 6-11 serotypes accounted for over 70% of PD cases globally. The study further reported that serotypes: 1, 5, 6A, 6B, 14, 19F and 23F were the most common across the world and were responsible for over 300,000 and 200,000 mortality cases among children below the age of five years in Africa and Asia respectively (Johnson *et al.*, 2010).

Geographical variances in terms of numbers and types of pneumococcal strains were vividly described in this study; for instance serotypes 4, 6B, 9V, 14, 18C, 19F, 23 were found to be at the core of invasive pneumococcal disease cases in North America, Africa and Asia respectively (Johnson *et al.*, 2010).

They concluded based on their study findings that only a limited number of over 90 profiled pneumococcal serotypes are responsible for the world pneumococcal disease burden; they further opined that while their study did not provide country level pneumococcal serotype epidemiology, continuous vaccine impact assessments be done to generate the data.

Serotype 14 is most prevalent in all regions of the world representing a significant amount of all IPD cases; serotype 6A and 6B combined accounted for about 14% of world IPD cases; serotype 1 which has been vastly correlated with manifestation of meningitis in Africa was one of the serotypes greatly isolated from Africa and Asia; serotype 5 was also one of the serotypes highly isolated in Africa and Asia (Johnson *et al.,* 2010). Further, serotypes 19F and 23F are responsible for up to 18% of all world IPD cases; serotypes 18C is the most common in highly industrialized countries like Europe, USA and China while; serotype 19A which has been broadly associated with AMR has also been unexpectedly found to be most prevalent in high income countries (Johnson *et al.,* 2010). Serotype 1 which was first recovered in 1913 has been well profiled and expansively linked to occurrence of cases of complicated pneumonia around the world over-time (Kirkham *et al.,* 2006). It has often been associated with pneumococcal pneumonia among children living in closed up environments.

2.2.3.4 Distribution of *Streptococcus pneumoniae* serotypes according to disease burden

The global, regional and local distribution of *Streptococcus pneumoniae* serotypes has been extensively correlated with disease burden (Kanungo, 2015). According to a study done in Spain to establish the most predominant serotype among hospitalized patients diagnosed with pneumococcal pneumonia over a period of two years, concluded that all serotypes included in PCV-13 (1, 3,4,5,6A, 6B, 7F, 9V, 14, 18C, 19A, 19F and 23F) were often recovered (Menéndez *et al.*, 2017). Another study conducted by Caierão *et al.* (2014), in Brazil subsequent to introduction of conjugate vaccines recovered serotypes: 14, 3, 4, 23F, 7F, 9V, 12F, 20, 19F, 8, 19A, and 5 from hospitalized patients diagnosed with pneumococcal pneumonia. Another study done in Sub-Saharan Africa, reported serotypes 19F, 6B, 6A, 14 and 23F as being most prevalent across the region (Usuf *et al.*, 2014).

2.2.3.5 Non-typeable *Streptococcus pneumoniae*

Non-typeable *Streptococcus pneumoniae* also known as non-capsulated pneumococcus has often been isolated from hitherto young patients diagnosed with severe cases of conjunctivitis and otitis media (Andrade *et al.*, 2010). While the non-typeable *Streptococcus pneumoniae* bacteria is infrequently isolated from the NP region, it is always associated with disease outbreaks most of the time it is recovered (Xu *et al.*, 2011).

So far, a number of etiologies have been described and associated with the non-capsulation of the *Streptococcus pneumoniae*; interruption of the organisms' *cps* area which harbors the gene responsible for encrypting proteins necessary for the capsular polysaccharide generation; destruction of the capsular polysaccharide and; *Streptococcus pneumoniae* expresses a capsular polysaccharide that is yet to be identified and well described (Keller *et al.*, 2016).

Before serotyping, precise and accurate identification of *Streptococcus pneumoniae* should be done using the following elaborate steps: correct colony morphology as described under the *Streptococcus pneumoniae* identification criteria above, optochin susceptibility and where necessary, bile solubility analysis. *Streptococci* from the *mitis* group are inhabitants of the same NP microbiota as *Streptococcus pneumoniae; Streptococcus pseudopneumoniae* is not capsulated, it is optochin non-susceptible when cultured under CO_2 environments while susceptible when cultured under O_2 conditions and are bile insoluble and; existence of *Streptococcus viridans* which are optochin susceptible but bile insoluble, makes it pretty difficult to irrefutably and out rightly conclude that the organism isolated which would have failed the serotyping test is a non-typeable *Streptococcus pneumoniae.*

According to Keller *et al.* (2016)**,** non-typeable *Streptococcus pneumoniae* is not capsulated and therefore not associated with etiology of acute otitis media (AOM) and sinusitis; it is not known to manifest with formation of pus but have been often recovered in many epidemics of acute conjunctivitis. Further, Xu *et al.* (2011), in their study done in USA among children below the age of five years to establish the etiology of acute otitis media, reported that out of 320 non-typeable *Streptococcus pneumoniae* isolates assayed, 27 of them were recovered from patients diagnosed with AOM. What is more is that majority of the isolates associated with AOM and conjunctivitis were also found to be resistant to penicillins (Casey *et al*., 2013).

Other researchers including Andrade *et al.* (2010), demonstrated genetic resemblance of different nontypeable *Streptococcus pneumoniae* isolates that cause AOM. These findings were consistent with those of Carvalho *et al*. (2003) who reported that all nontypeable *Streptococcus pneumoniae* are optochin susceptible, bile soluble and Gen-Probe AccuProbe test positive. This study also reported that the site of collection of the sample meant for isolation and serotyping of pneumococcus has got somewhat a correlation with the frequency of occurrence of nontypeable *Streptococcus pneumoniae* (Carvalho *et al.*, 2003). For instance, samples collected from normally sterile sites had a highly reduced frequency of recovery of nontypeable *Streptococcus pneumoniae* as compared to samples like sputum, swabs etc collected from possible unsterile sites.

2.3 Antimicrobial resistance (AMR)

According to WHO (2017), AMR refers to the aptitude of illness causing micro-organisms (bacteria, parasites, viruses, fungi etc) to render antimicrobial agents ineffective against them. Consequently, disease treatment regimens which would have been previously effective against them become ineffective, infections blowout liberally and prospects of sequale and mortality escalate. Further, Tenover & McGowan (2008), describe AMR as a situation where disease causing microorganisms become immune to antimicrobial agents. It currently poses a major public health threat to the global citizenry as the general AMR related mortality stands at 700,000 annually and estimated to reach 10,000 million people/annually by the year 2050 if no effective, targeted and deliberate intervention is taken (O'Neill, 2014).

Multi-drug resistant bacteria (MDR) are particularly of more epidemiological concern as they are associated with remarkable incidences of morbidity and mortality around the world (Van *et al.*, 2007). The public health plight that is AMR has no borders as bacteria which would have developed resistance are capable of transferring the resistant genes horizontally across the resident microbiota. What is more is that the population dissemination of AMR is multisectoral; the environment, wild animals, pet animals, livestock and humans are all players on the AMR stadium, constituting the concept of “One Health” (Zinsstag *et al.,* 2011).

Streptococcus pneumoniae which happens to be one of the top causes of child morbidity and mortality globally has become exceedingly impervious to novel antimicrobial preparations (Kim *et al.,* 2016). The organism has exhibited colossal resistance trends to penicillins from as long as 1967 and it continues to wreak havoc especially to the economically malnourished nations around the world today (Charpentier & Tuomanen, 2000). Two major interventions are the only available sources of hope: antimicrobial use and vaccine administration (Schrag *et al.,* 2000). With the skyrocketing cases of *Streptococcus pneumoniae* AMR, the world our children live in today gazes at a major catastrophe. The socio-economic burden wielded by the occurrence of AMR is gargantuan. For instance, the unit cost of treating antimicrobial resistant pneumococcal pneumonia among children below the age of 5 years is over fivefold more expensive compared to the unit cost of treating non antimicrobial resistant pneumococcal pneumonia in the same cluster (Shrestha *et al.,* 2018). Extended hospital stays, the need to perform repeated clinical and laboratory examinations and the accompanying social drainage make AMR a major cause of concern to all stakeholders around the globe.

The degree of *Streptococcus pneumoniae* resistance to various antimicrobial agents varies extensively from one region and/or county to another both among industrialized and low and middle level income countries (LMICs). A study done in Europe and America using data from prospective tracking epidemiology device, reported varying resistance patterns in different regions as follows: "25-50% in Greece, France, Spain and Israel, 10-25% in Portugal, Finland, Turkey and Ireland and 1-5% in Germany, UK, Norway, Sweden and Austria" (Reinert, 2009b).

The degree of pneumococcus resistance also varies from one antimicrobial agent to another; for instance, some pneumococcus serotypes exhibit more resistance tendencies to penicillins than to macrolides, some are more non-susceptible to cephalosporin than they are non-susceptible to penicillins (Cillóniz *et al.*, 2018). Antimicrobial alteration is one of the major techniques used by microbial agents to evade the action of the antimicrobial agents. A variety of enzymes strategically produced by certain target bacterial cells are known to amplify the tendency of bacteria to incapacitate the action of certain antibiotics (Peterson & Kaur, 2018). Aminoglycosides, B-lactams and Chloramphenicol are highly implicated antimicrobial agents by this bacterial strategy.

Interestingly, *Streptomyces* bacteria which are known to produce aminoglycoside antibiotics are also known to produce enzymes that disable the action of the antibiotics; these enzymes have been reported to be physiologically synonymous with those found in aminoglycoside resistant bacteria (Peterson & Kaur, 2018). Antimicrobial target sites for most therapeutic agents are located on the inside of the target bacterial cells; as such, for such antimicrobial agents to exert their effect, they must permeate the external membranes of the bacteria. To evade the effect of this class of antimicrobials, bacteria develop robust machineries to prevaricate the permeability of such agents; without which there won't be action (Munita & Arias, 2016).

The outer membrane acts as a primary barrier to the entrance of certain external molecules including antimicrobial molecules. Some of the antimicrobial agents affected by this mechanism include: beta-lactams, tetracycline and fluoroquilones (Munita & Arias, 2016). To achieve their ultimate and absolute effect on bacteria and other microbial cells, antimicrobial agents must gain unencumbered access to target sites located on and/or in the bacterial cell. On the flip side, bacterial cells deviously continue to develop mechanisms that interfere with the intend of antimicrobial agent's. A very fascinating competition.

Defacing of the target sites also serve to diminish affinity of the antimicrobial agent to various target sites located on bacteria; a very well documented mechanism of resistance among multiple species of bacteria It can be accomplished by use of various routes: mutations in the genes that encode for the appearance and/or structure of the target sites, enzymatic amendments of the target sites and interchange or circumventing of the initial target site (Kapoor *et al.*, 2017).

2.3.1 Commonly prescribed pneumococcal disease antimicrobial agents

Until 1970's most pneumococcal isolates were greatly susceptible to very low doses of most antimicrobial agents available then; antibiotics like: macrolides, penicillins, clindamycin, rifampin, vancomycin, cephalosporin's and sulfamethoxazole were considered to be highly effective against pneumococcal infections (Brusch, 2020a). However, resistance to most antimicrobial agents listed above, which had earlier been reported to be very lethal against pneumococcal infections began to appear around 1990's. Resistance to penicillins and cephalosporin's for instance is via modification of the cell wall targets called penicillin binding proteins (PBPs); any minor adjustment in the arrangement of the PBP molecules located in the bacterial cell congruently affects the tendency of antimicrobial agents to bind to them effectively conferring resistance (Livermore, 2012).

Increasing the concentration of the drug, above the known target bacteria minimum inhibitory concentration (MIC) has been shown to also improve susceptibility levels (Li *et al.,* 2017). Resistance of pneumococci to penicillins seems to confer analogous effects on most other antibiotics. Resistance in most other antibiotics following resistance among penicillins is mediated by a gene that encodes for resistance in multiple antibiotics (Nuermberger & Bishai, 2004).

Some of the antibiotics that conform to these phenomena include: macrolides, cephalosporins, sulfonamides, quinolones, chloramphenicol, trimethoprim-sulfamethoxazole and other penicillin derivatives. Relatively elevated resistance levels to tetracycline and macrolides have been reported not just in developing but also in industrialized countries (Yayan, 2014). Pneumococcal isolates that demonstrate resistance to macrolides also show escalated resistance levels to clindamycin (Leclercq & Courvalin, 2002). Resistance to vancomycin has remained relatively low in some countries, both in LIMs and remarkably industrialized countries; interestingly there have been pneumococcal isolates that survive the effect of vancomycin but do not grow in the presence of vancomycin, a concept called tolerance (Wang *et al.,* 2019). Fluoroquilones have remained very effective against most pneumococcal isolates worldwide (Brusch, 2020b). This may be largely due to the fact that they are rarely prescribed, safe for certain countries and care homes where they are mostly given to patients.

2.3.2 Factors that augment *Streptococcus pneumoniae* resistance to various antimicrobial agents

As the degree of *Streptococcus pneumoniae* resistance to various antimicrobial agents increase, the need to fathom the dynamics of resistance both in terms of etiology and pathogenesis also surge. For a period spanning over several decades, antimicrobial therapy has demonstrated remarkable effectiveness against pneumococci; thousands of millions of people around the world have successfully been treated and levels of morbidity and mortality due to pneumococcal disease reduced significantly. Fear and anxiety over possible occurrence of cases of resistance to novel antimicrobials by pneumococci have silently been ravaging key industry players over time. Now that it is here with us, killing thousands of children below the age of 5 years, players on the global, regional and local stadia tasked with ensuring a healthy world populace, are working around the clock to contain it.

Frantic efforts, all relevant arsenals let loose, the world is in a dash; the war against pneumococci AMR must be won or “no one wants to imagine the consequences”, 10,000 million demises annually by the year 2050 (O'Neill, 2014). Key among possible interventions to containment of Pneumococci AMR cases is to comprehend the underlying etiologies and subtleties. Research has reported the causes of AMR in different ways: biological based causes, epidemiologically related causes, judicious antimicrobial use and rational antimicrobial prescription trends (Kim *et al.*, 2016).

2.3.2.1 Biological causes of antimicrobial resistance

2.3.2.1.1 The ecological niche and transfer of resistant genes

The human NP region is the natural reservoir of the *Streptococcus pneumoniae* bacteria; it provides an impeccable podium where pneumococci cohabit with other adherents of the NP microbiota without necessarily getting invasive. This also provides a route via which the bacteria gets transmitted from the carrier to vulnerable persons; carrier because adhesion of *Streptococcus pneumoniae* to the NP mucosal surface is the preliminary phase in the pathogenesis of pneumococcal disease and though colonization precedes development of pneumococcal disease, it does not always lead to invasiveness (Bou *et al.,* 2018).

Nasopharyngeal carriage of pneumococci may vary on the basis of age where young children are more vulnerable to carriage than adults, general living conditions such as household size, attendance at daycare centers, crowding index and perhaps genetic platforms which are yet to be well described. The duration of NP carriage of *Streptococcus pneumoniae* also varies on the tenets of the serotype carried and also immunological capacity at the time. Not all carriage cases result in invasiveness; scholars are yet to perfectly describe and report the actual factors that mediate transition from colonization to invasinessness (Wada *et al.,* 2019).

Some of the hypothesized dynamics of transmission which are fundamentally under investigation comprise but not limited to: infection by the influenza virus which is thought to meddle with the integrity of the natural barriers of the NP sub-mucosa predisposing carriers to possible invasion and; a range of cytokines (cell to cell signaling molecules) which have been demonstrated to have capacity to cause inflammation invitro using animal models are also assumed to exert a similar effect invivo in humans (Randle *et al.,* 2011); augmenting chances of disease progression. According to Heath *et al.* (2018), the human NP region asymptomatically wharfs both antimicrobial susceptible and non-susceptible pneumococci. Bacteria that share an ecological niche and have close genetic kinship, for instance those that may belong to one serogroup, have been demonstrated to have capacity to not only share defensive correlates but also antigenic correlates. *"They are generous, they care about their siblings' wellbeing; they create safe living conditions for themselves".*

As such, *Streptococcus pneumoniae* serotypes that develop resistance to given antimicrobial agents, may be due to mutation, horizontally and munificently share the genes responsible for the resistance with members of the same group and living within the same NP microbiota (transformation). The genes are then incorporated in the new hosts by homologous recombination (Hiller *et al.,* 2010).

These bacterial resistant genes can also be passed from one person to another via transfer of NP droplets containing bacterial strains that wield resistant genes may be through coughing, sneezing, kissing and more.

2.3.2.1.2 Pneumococcal serotypes and antimicrobial resistance

Pneumococci have since been profiled and classified into ≥90 serotypes. Different serotypes have been reported to entertain different abilities of AMR to different antimicrobial agents. For instance, serotypes 6B, 6A, 9V, 14, 15A, 19F, 19A, and 23F have high levels of AMR trends to penicillin and erythromycin (Liñares *et al.*, 2010). Much as every pneumococcal serotype has a unique genetic composition, AMR trends have been demonstrated to be prevalent only with certain serogroups.

The main reason why certain *Streptococcus pneumoniae* are more prone to AMR tendencies more than others is yet to be clearly profiled. However, possibility of higher carriage levels at the NP region is thought to play a critical role (Wada *et al.*, 2019). The higher the carriage rate, the more the serotype is likely to be exposed to a raft of antimicrobial agents and the more therefore they are likely to derive survival mechanisms which end up rendering them resistant to novel antimicrobial agents. This however, is hypothetical, a subject largely within confines research.

2.3.2.2 Recent antimicrobial use and occurrence of antimicrobial resistance

Inapt antimicrobial use and/or ingestion have been expansively correlated with occurrence of AMR both in the elderly and children the world over (Malik & Bhattacharyya, 2019). Because of its connotation with the manifestation of AOM, pneumonia and even meningitis, as well as emergence of *Streptococcus pneumoniae* serotypes that are resistant to penicillins and; its high ranking among organisms ravaging communities, *Streptococcus pneumoniae* is classified as one of the important organisms by the European Antimicrobial Resistance Surveillance (EARSS, 2018).

Resistance to antibiotics like penicillins by *Streptococcus pneumoniae* is pretty much related to misuse or overuse. Reports have indicated that misuse of antibiotics other than those from the beta-lactam family can as well cause emergence of resistant *Streptococcus pneumoniae.* The use sulphamethoxazole (co-trimoxazole) and macrolides like erythromycin for instance, have also been strongly associated with occurrence of penicillin resistant *Streptococcus pneumoniae* (Mobarki *et al.,* 2019). In general therefore, any unregulated and injudicious sale of antibiotics in any given community terrifically upsurge chances of emergence of penicillin resistant *Streptococcus pneumoniae.*

2.3.2.3 The concept of "One Health" and occurrence of antimicrobial resistance

Antimicrobial resistance emergence due to recent antimicrobial use does not only affect the use of antibiotics by the affected subjects but also have a link with animals, plants and the general environment where the subjects live (Frank, 2008). Plants, animals and the environment harbor strong connections with AMR and can determine to a large extent whether or not the subject is classified as having used and/or not consumed antibiotics recently. This is especially important because some of the antimicrobial agents used for treatment of infections in animals and plants have the same composition as those used to treat infections in humans.

As such, consumption of animal, plant and general environmental products is likely to provide a perfect dais for transfer of AMR correlates over to naïve humans (Frank, 2008). Animals and plants are continuously exposed to a range of infections that have similarities with those that affect humans; as such, they are treated with the same antibiotics used to treat similar human infections. Farmers also administer antibiotics to animals and plants as preventive measures just in case of occurrence of disease; they also give certain drugs to promote rapid growth. Because of these "not so good" practices, humans innocently acquire and spread resistant correlates amongst each other when they also use the same antimicrobial agents and whenever they consume affected animal and plant products (Mills, 2014).

Key international organizations tasked with management of diseases of superior public health reputation like: WHO, United Nations Food and Agricultural Organization (FAO) and the World Organization for Animal Health (OIE) coined a multi-sectoral approach to management of AMR globally (FAO/OIE/WHO, 2010). The flow of microbial resistant correlates from animals, plants and the environment to humans must be regulated if the war on global AMR is to be won. While not so many studies have documented the association between animals, plants and the environment with the emergence of pneumococcal AMR, it is not a hidden fact that it must be one of the key routes to occurrence of resistance.

2.3.2.4 Injudicious use of antibiotics and emergence of antimicrobial resistance

As early as the 1940's, a caution was raised by the then globally celebrated AMR stalwart Sir. Alexander Fleming. He stated that as there would always be human and animal infections, the demand for antibiotics could only increase and the more the antibiotic use, the more the likelihood of emergence of resistance (Kodish, 2018). There exists direct relationship between the use, whether judicious or otherwise and/or ingestion of antibiotics and the level of AMR. Bacteria are always looking for ways to nurture their progeny and as obviously, the more they interact with antimicrobial agents, the more they are likely to develop machineries to escape their action.

One such dubious technique is where closely related bacteria generously share antimicrobial resistant genes horizontally, a phenomena that is believed to be mediated by plasmids and transposons (Reinert, 2009b). It is also possible, as it has been reported expansively that AMR can simply emerge due to mutations which can be spontaneous and/or caused by prolonged exposure to radiations. Countries classified in the low and middle income (LMIC) strut, face special AMR challenges the world over (Ayukekbong *et al.*, 2017).

For instance, antibiotics are the only available and relatively most affordable intervention option in case of infections in such regions; a strong factor that provides an impeccable platform for misappropriation by the illiterate and even literate populace (Kumar *et al.*, 2013). What is more is most dispensing chemists in developing countries do not ask patients for prescriptions before selling out drugs (Bebell & Muiru, 2014). The challenge of patients not conscientiously complying with antimicrobial consumption guidelines and most of them deciding to buy medications over the counter without prescriptions is a major set-back in the fight against AMR in low and middle income countries (LIMCs).

Any antimicrobial agent meant for treatment of humans, plants or animals and found in any individuals' hands without a doctor's prescription is considered illicit. This is because it provides a seamless conduit via which antimicrobials wreck the community. However, in Kenya for example, major antibiotics like penicillins, tetracycline and chloramphenicol are actually hawked to uninformed citizens at public bus-stops (Mutua *et al.,* 2017).

No appropriate guidelines are given at these points and worse still they are not enforced. The result is that subjects abandon the antimicrobial agents as soon as they have felt better, in some circumstances, they go ahead and give them out to other members of the family who may be feeling unwell and they also discard them improperly to the environment. Consequently, resistance levels surge, as the bacterial cells would have been well accustomed to these agents and developed mechanisms to dodge their action. Appallingly, nationals in most developing countries believe more in the use of antimicrobials to manage infections as opposed to upholding appropriate hygiene levels. The endpoint of all these illegalities and ignorance bouts is that AMR levels skyrocket as levels of morbidity and mortality escalate.

The risk of hypersensitive reactions to some of these antimicrobial agents by subjects is pretty likely; it is even more unsafe when the subject is not aware that they have active allergies to certain molecules in the drug (Sánchez *et al.*, 2013). Chances of sequale and even demise of the subject are exceptionally amplified. Effects of antimicrobial overuse whether legitimately or otherwise can only be worse. Over-prescribing of these agents sometimes even for ailments that are caused by viruses is massive.

2.3.3 Antibiotic treatment plan for different pneumococcal infections

As pneumococcal disease present in various forms, antibiotic treatment for the different forms of PD may also vary. For instance, the first line of treatment for otitis media and sinusitis is amoxicillin in doses of 80-90 mg/kg/day (Gotthelf, 2005). In the event that no notable improvement has been observed within a maximum of 72 hours of antibiotic consumption, then the subject should be given amoxicillin-clavulunate, 3rd generation cephalosporin or better still ceftriaxone (Batard *et al.*, 2018). Amoxicillin 90 mg/kg/day in two doses of 45 mg/kg/day is the recommended regimen for most previously health subjects who are currently having pneumonia infection for the first time (Gotthelf, 2005).

The recommended antibiotic plan for meningitis is with meningeal doses of vancomycin with a cephalosporin like ceftriaxone or cefotaxime or in cases where the subject is allergic to beta-lactams, vancomycin with rifampin should be given; there should never be an attempt for mono-therapy with one of the antibiotics above as they cannot achieve the required threshold to penetrate the CSF (CPS, 2001).

2.4 Optochin

Optochin which is an imitative form of hydro-quinine, was first coined in the year 1911 by two scientists: Levy and Morgenroth, as a noble therapeutic plan for pneumococcal infections (Burckhardt *et al.,* 2017). This is because it had demonstrated mammoth effect on the four strains of pneumococci known as at then. While it was originally meant for treatment of pneumococcal disease complications, massive side effects laced with wide spread treatment inconsistencies occasioned WHO to terminate it as a noble therapeutic drug (Pikis *et al.,* 2002). Optochin distinctly deters growth of pneumococci at very minute concentrations and as such, it has over the decades been the most effective test used for differentiation and accurate identification of the bacteria from other alpha-hemolytic streptococci like viridans.

While optochin can hinder growth of other alpha-hemolytic streptococci species albeit at higher concentrations, another study demonstrated that concentrations at as low as 5 mg/ml, optochin can effectively impede the growth of *Streptococcus pneumoniae* (Tankeshwar, 2013). As ethylhydrocuprein hydrochloride is greatly soluble in water and freely diffuses in laboratory culture media, filter paper, aptly imbued with ethylhydrocuprein hydrochloride can be used in a disk diffusion model to precisely identify isolates of *Streptococcus pneumoniae*. Pneumococci cells surrounding the filter paper disk which would have been saturated with optochin lyse because of the alteration in the surface tension and consequently form a zone of inhibition around it (Aryal, 2015).

The standard diffusion disk meant for identification of *Streptococcus pneumoniae* on blood agar with gentamicin (GBA) should measure about 6mm and impregnated with 5g of the ethylhydrocuprein hydrochloride solvent. Any positive result, meaning the isolate is *Streptococcus pneumoniae* is expected to create a zone of inhibition of at least 14mm on a 6mm disk on GBA. Any zone of inhibition of less than 14mm on a 6mm disk and had previously demonstrated other features akin to pneumococci are further queried for bile solubility. Bile soluble isolates, coupled with other distinct features of pneumococci (formation of a depression on the center of flattened colonies on GBA or BA aged 18-24 hours with alpha-hemolysis), will be presumptly confirmed as *Streptococci pneumoniae* (Aryal, 2015).

2.4.1 Optochin resistant *Streptococcus pneumoniae*

Considering the enormous pressure exerted by the various derivatives of pneumococcal disease on the global public health tenets, especially in the developing continents; both in terms of massive pneumococcal antibiotic resistance and emergence of lethal forms of pneumococcal strains, accurate, timely and precise identification of the pneumococcus is no longer a subject of deliberation (Burckhardt *et al.,* 2017). Key to this is the accuracy and reliability of the noble optochin test results. Although optochin was profiled and strongly recommended for identification of *Streptococcus pneumoniae* in the laboratory during the early 19th century, it only became popular almost 30 years later in the 1950's. The initial strain which demonstrated remarkable resistance to optochin was isolated in the year 1987 in Finland and since then, there have been very sporadic cases of optochin resistant *Streptococcus.*

Genetical alteration in the *c* subunit and not the *a* subunit of the *atpCAB* operon which encodes for the target site of optochin may be responsible for the occurrence of optochin resistance (Pikis *et al.,* 2002). The same study reported that prolonged use of antimalarial chemotherapy and consumption and/or use of penicillins may also have a nexus with occurrence of optochin resistance by the *pneumococcus*.

Later, Pinto *et al.* (2013) reported about 26 optochin resistant *Streptococcus pneumoniae* all recovered from both clinically relevant sites of both healthy carriers and subjects who were already presenting with clinical symptoms. All the isolates were: catalase negative, gram positive diplococci, they were alpha-hemolytic, bile soluble and had *lytA*, *ply* and *psaA* genes. However, they were all optochin non-susceptible; a clear confirmation that an isolate can satisfy all requirements for classification as *Streptococcus pneumoniae* but still be resistant to optochin.

Also, Kellogg *et al.* (2001) in an attempt to investigate the relevance of optochin testing in the identification and characterization of *Streptococcus pneumoniae*, concluded that the degree of sensitivity and specificity of the optochin test was at 98% and 99% respectively for the two parameters; this explicitly spelled out the importance of the test. Another study by Ing *et al.* (2012) regarding characterization of the *Streptococcus pneumoniae*, most of the bacterium's isolates which turn out to be optochin resistant also turn out to be bile insoluble, non-encapsulated and non-typeable.

2.5 Risk factors associated with nasopharyngeal (NP) carriage of *Streptococcus Pneumoniae*

Adhesion of the tenacious pneumococci to the nasopharyngeal epithelium of humans and other relevant hominids is not only considered crucial to the development of PD but also in the spread of the bacterium from one person to another (Neill *et al.,* 2014). Understanding the nasopharyngeal carriage profile of pneumococci is therefore essential for the epidemiologic management of the disease. Isolation of unalike pneumococci serotypes from the nasopharyngeal microbiota is therefore considered a rich reflection of PD type and/or encumbrance in a given community (Løvlie *et al.,* 2020). Presence of the bacteria in the nasopharyngeal region has been extensively described as the pre-condition for the development of pneumococcal disease.

The prevalence of carriage of the *Streptococcus pneumoniae* has been described to vary largely on the basis of factors like: the subjects' age, immune status, ecological location among others (Weiser *et al.,* 2018). According to Simell *et al.* (2012), pneumococci can colonize the nasopharyngeal epithelium for varying periods of time before becoming invasive; the immunological status, recent viral infections, acute respiratory tract infection and young age of the host having been described to play a fundamental role in the development of disease from carriage to invasiveness.

Further, Abaye *et al.,* (2019), reported that children living in Africa have a slightly elevated risk of suffering from the various forms of PD as compared to their counterparts in developed countries; they are also likely to contract the disease at a young age. This could perhaps be due to the conservative social demographics in Africa; largely, a developing continent. While pneumococcus is a very belligerent organism capable of thriving by use of its own virulence entitlements, it also to a very large extent relies on other factors to expedite its dissemination in the community. These factors include: host and environmental related.

2.5.1 Environmental related risk factors

Environmental risk factors refer to a state, substance or event in the surrounding that carries enormous probability of causing harm to the milieu and/or human health. Environmental factors have overtime been reported to play a fundamental role in the pathogenesis and transmission of the pneumococcus especially among children below the age of 5 years. They include: attendance at day-care centers, recent consumption/use of antibiotics, smoke, recent or current viral infections, acute respiratory infections, household crowding, hand contamination (poor hygiene), among others (Fadlyana *et al.*, 2018).

2.5.1.1 Attendance at day-care centers

Attendance at day-care centers (preschool education center, nursery, baby-care center) has been designated as one of the major platforms for dissemination of especially respiratory tract infections (RTIs) among children (Schuez *et al.*, 2017). Because of inescapable inflexible demands of life, a significant number of children begin to attend day-care centers from averagely 2 years of age. As RTIs constitute an alarming public health problem among children, daycare centers provide unfettered podia for horizontal transmission of the pneumococcus and worse still, transmission of AMR genes.

According to Nafstad *et al.* (2005) early exposure of children to daycare centers is associated with recurrent cases of acute otitis media (AOM), high possibility of AMR, high probabilities of asthma occurrence and a grander risk of invasive pneumococcal disease. However, other researchers have reported a converse relationship between the period of time spent at daycare versus risk of developing respiratory tract infections; for instance, Kamper *et al.* (2006) opines that the period of time spent at the daycare center by a given child and age do not pose a commensurate relationship with occurrence of respiratory tract infections. In other words, children exclusively undergoing homecare have no advantage over those attending daycare in terms of development of RTIs.

A study by Oliveira *et al*. (2019) reported increased risk of acquiring RTIs among children undergoing out of home care. The study attributed the amplified risk to a number of factors: poor hand washing trends, touching fomites and putting their hands in the mouth directly, free passage of stool and general poor hygiene practices. Moreover, poorly developed immune systems in children were highlighted as a key pre-disposing factor to occurrence of RTIs at the day-care centers. One's a single child is infected, chances of infecting those in their play-groups becomes quite increased. Often, infectious agents that find way into daycare centers are those which are prevalent in the general community where the daycare is functioning.

This is because most children below the age of 5 years are asymptomatic carriers of most infectious agents who act as reservoirs for these organisms. Chances of transferring them to the daycare centers are higher because asymptomatic carriers would not have undergone any requisite treatment. Ones the infectious agent finds way to the daycare center, its further dissemination is reliant on the age of the children, their immune status, their gender, among other factors (Tahoun *et al.*, 2019). Parental literacy levels, family house hold size, number of careers per child at the center and overcrowding index also play a role.

2.5.1.2 Recent consumption and/or use of antibiotics versus nasopharyngeal pneumococcus carriage

Adaptation of *Streptococcus pneumoniae* to frequently used antimicrobial agents has been comprehensively reported to pose a very heavy-duty nexus with enlarged carriage of *Streptococcus pneumoniae* at the NP microbiota (Angoulvant *et al.,* 2015). The more the antimicrobial agents' one uses/consumes, the more the rates of *Streptococcus pneumoniae* carriage and vice versa. The model of global antibiotic driven AMR applies to a very large extent in this case; as bacteria are constantly looking for ways to survive the effect of antimicrobial agents, there is a lot of exchange of resistant genes among the various pneumococcus serotypes at the NP microbiota which then get to be transmitted among children living in the same vicinity.

2.5.1.3 Smoking

Exposure to smoke, whether active or passive has been largely linked to causation of various illnesses through inflammation, oxidative tension and damage to DNA (mutation). Moreover, there exists a direct contributory relationship between smoke and occurrence of most respiratory tract infections (UDHHS, 2010). Wide-ranging modification in the structure of the immune system constitutes the mechanism of how smoking causes RTIs.

Smoking has an effect on the general white blood cell (WBC) counts in peripheral blood (PBF) and their general physiology; smoke has also been reported to hamper with the effective and/or normal production of Natural Killer Cells (NK-cells) and immunoglobulin E (IgE) which are directly responsible for protection against viral infections (pneumococcus carriage pre-disposing factor) and coordination of allergic reactions that constitute the upper respiratory tract (UDHHS, 2010). Generally, exposure of smoke to the nasopharyngeal microbiota interferes with the feasibility of the floral community at the region, consequently exposing the host to pathogenic bacteria. Pneumococci serotypes which would not have been associated with disease etiology may die due to smoke and therefore offer uncontested space to the serotypes that may cause disease to the host (Vaart *et al.*, 2004).

There also exists a commensurate relationship between exposure to smoke and stimulation of the natural immune system; research has discoursed that individuals who smoke, run a significantly decreased capacity of initiating natural immunity against respiratory infections. Further, Jaspers (2016), sought to provide evidential data to the above postulate; the scholar reported after carrying out a longitudinal study among adult males, that all in individuals who at one point or another was diagnosed with invasive pneumococcal disease (IPD) had at some point been a cigarette smoker or had cohabited with a smoking partner.

2.5.1.4 Viral-upper respiratory tract infections and nasopharyngeal carriage of *Streptococcus Pneumoniae*

According to Morpeth *et al.* (2018), viral infections of the upper respiratory tract of humans pose a strong pre-disposition to thriving of bacterial infections. This phenomena has been described vividly by Stark *et al*. (2006) on the strength that during active viral infections, bacterial clearance capacity in hosts is significantly abridged and there is increased up-regulation of receptors necessary for bacterial adherence at the NP region. Moreover, Diavatopoulos *et al.* (2010), to investigate the relationship between viral infections and occurrence of pneumococcal disease using animal models completely supported the above postulate.

Other studies, Marks *et al.* (2013) and Chao *et al.* (2015) separately and on distinct study cohorts reported possibility of either the actual virus or immunologic responses to viral infections dispersing the pneumococcus from biofilms thereby effectively augmenting invasion. Other studies have reported an increase in the incidences of pneumococcal pneumonia following an infection with influenza or Para-influenza viruses (Grijalva *et al*., 2014).

2.5.1.5 Over-crowding and nasopharyngeal carriage of *Streptococcus pneumoniae*

Overcrowding is calculated by dividing the number of persons occupying a given household by the total number of rooms in that particular house. On this basis, the number of persons per room should be within the following precincts: one room should have a maximum of two persons, two rooms should have a maximum of three persons, three rooms should have a maximum of five persons, four rooms should have a maximum of seven persons, five rooms should have a maximum of ten persons and any more room should have an additional two persons (Melki *et al.,* 2004).

Overcrowding exerts unnecessary pressure on common human amenities in a population including proper ventilation and hygiene levels. As *Streptococcus pneumoniae* mode of transmission is from person to person via the respiratory route, over-crowding offers a very easy platform for the bacteria to blowout. More so, house-holds that host more members are mostly low economically; as such, they have limited access to healthcare facilities and therefore are more prone to infectious diseases (Howie *et al.,* 2016). These individuals act as spread points for most infectious diseases including pneumococcal disease.

In the recent past, there has been increased levels of person to person transmission of pneumococci since the bacteria are capable of horizontal movement among humans living in close proximity with each other (Donkor, 2013). A study done by Kwak *et al*. (2015) among children attending a daycare center in Korea, opined that overcrowding lessens chances of getting asthma but increased chances of acquiring pneumococcal disease.

2.5.2 Host related risk factors

While the host's environment plays a very fundamental role in the acquirement and dissemination of pneumococci, there are other factors related to the host of interest which have largely been associated with the NP carriage of *Streptococcus pneumoniae.* For instance, Inostroza *et al.* (2002), documented that the host's age, immune status and gender may to some extent influence the tendency of an individual to carry *Streptococcus pneumoniae* in the NP region.

2.5.2.1 Age, host's immune system and gender

Often, some communicable diseases occur among children below the age of five years and the elderly above the age of 65 years. For instance, pneumococcal disease incidences are reported more among children below the age of 2 years and among the elderly who are 65 years and above (Tan, 2012).

Certain behavioral features common to these age groups could be playing a key role towards this position. For example Mody (2007) and Biezen *et al.* (2019) opine that children under the age of five years and the elderly people have challenges with hygiene practices like proper hand washing techniques and as such are likely to carry a lot of infections from fomites. Poorly developed and waning immune systems among young children and the elderly respectively makes up another major contributing factor (Brooks & Mias, 2018).

Both humoral (antibody mediated) and cellular (T-cell mediated) forms of immunity are yet to be fully developed and functional in young children. However, among the elderly, immunity (both antibody & cell mediated) has started to wane-off (Berical *et al.*, 2016). While the issue of gender is yet to be comprehensively described, some report have linked being male to increased vulnerability to invasive pneumococcal disease (Maurice *et al.*, 2016). Environmental risk factors concomitant with carriage and eventual occurrence of invasive pneumococcal disease affect more male than female children.

2.6 Pneumococcal vaccines

Other than antimicrobial therapy, vaccine administration is the only other dependable intervention available following the grave public health threat posed by the various forms of PD (Stephanie *et al.,* 2001). Overtime, several vaccines have been developed across the globe with a view of protecting both children and adults from the sharps of the dreaded pneumococcal disease.

They include: pneumococcal polysaccharide vaccines (PPV), pneumococcal conjugate vaccines (PCV) and the proposed multi-valent pneumococcal protein vaccine (although it is still largely under development) (Feldman & Anderson, 2014). The successful invention and implementation of both pneumococcal polysaccharide vaccines and pneumococcal conjugate vaccines has significantly reduced incidences and prevalence of PD in both developing and industrial continents around the world.

2.6.1 Pneumococcal polysaccharide vaccines (PPVs)

The PPV is made up of well purified pneumococcal capsular polysaccharides (Latifi *et al.,* 2018). The initial PPV which contained 14 serotypes of *Streptococcus pneumoniae* was first licensed and adopted for use in the United States of America in the year 1977; in 1983, the 14-valent PPV was replaced by a 23-valent PPV which contained 23 serotypes from *Streptococcus pneumoniae* (Kim *et al.,* 2019).

The 23-valent PPV covers a significant proportion of pneumococci serotypes affecting mostly adults around the world (Falkenhorst *et al.,* 2017). The PPV23 is recommended for use among individuals of different age groups: the elderly above 65 years of age, any person between the of 2 and 64 years and has a compromised immune system, and any adult at 18-64 years and smokes cigarettes (Berild *et al.,* 2020). However, the vaccine is contraindicated for use among children below 2 years of age as it does not elicit sufficient immunological correlates of protection (Licciardi *et al.,* 2012). Other than the capsular polysaccharide antigens, the PPV also contains small amounts of sodium chloride and phenol (which is used as a preservative).

2.6.2 Pneumococcal conjugate vaccines (PCVs)

The foremost series of PCV was first licensed for use in USA in the year 2000 (DeStefano *et al.,* 2008). The vaccine constituted serotypes: 4, 9V, 14, 19F, 23F, 18C, and 6B and was therefore called PCV-7. The serotypes were linked to a diphtheria variant called CRM 197. In 2010, PCV-10 and PCV-13 were licensed for use in USA. The PCV-13 had all PCV-7 serotypes in addition to 1, 3, 5, 6A, 7F and 19A serotypes while PCV-10 had all the PCV-7 serotypes in addition to serotypes 1, 5 and 7F. All the additional serotypes were also yoked to CRM 197 (Daniels *et al.,* 2016).

According to the WHO (2013), the choice of the vaccine to be used is dependent on: the prevailing serotypes, the cost effectiveness and the vaccine supply capacity. While the multi-valent protein based vaccines have capacity to cover against more serotypes, they may not be as effective as the PCV series vaccines (Daniels *et al.*, 2016).

CHAPTER THREE
3.0 METHODOLOGY

3.1 Study location

This study was conducted at Gertrude's Children's Hospital (GCH). It is the largest and most established stand-alone pediatric health-care facility in East and Central Africa. The hospital, which was established in 1947, was the first in Sub-Saharan Africa to be accredited by the Joint Commission International (JCI), USA. It has more than 10 branches within Nairobi and its environs and operates a number of free clinics. The hospital attends to over 300,000 out-patients annually with a wide catchment area of East African region, and 9000 at its Muthaiga. It also works with a range of partners and donors to provide quality healthcare to about 5000 children from not so privileged family backgrounds (HIC, 2019). This study was conducted at the GCH-Githongoro clinic which receives children from not so economically established backgrounds. Given their stringent social demographics, these children are highly vulnerable to respiratory infections including pneumococcal disease (PD).

3.2 Study design

This was a descriptive cross-sectional study (Kesmodel, 2018). It was conducted on a scientifically selected subset of the population of interest and findings from the sampled population inferred on the general population from which samples were drawn.

3.3 Study population

The study population comprised children below five years of age who qualified to be vaccinated with the 10-valent pneumococcal conjugate vaccine (PCV-10). Children living in either Nairobi or Kiambu Counties and satisfied other parameters as set out in the inclusion and exclusion criteria constituted the sample population.

Those subjects who would have been clinically diagnosed with any variant of pneumococcal disease and satisfied other inclusion rationale were main targets for the study. The researcher requested for a brief session with each them (one on one) and vividly explained the main objective of the study to them. Those who understood and voluntarily accepted to have their children participate in the study were requested to sign a study assent form on behalf of their children before filling the questionnaire. On completion of filling the questionnaire, the researcher collected a nasopharyngeal swab from the subjects for examination in the laboratory.

3.4 Inclusion and exclusion criteria

For inclusion to or exclusion from this study, the researcher used the following criteria:

3.4.1 Inclusion

i. The subject needed to be between the age of 6 months and 5 years;

ii. If vaccinated, the subject should have received three doses of PVC-10 at-most four weeks preceding collection of the sample;

iii. If unvaccinated, the subject needed not to have ever received a dose of PCV-10 before;

iv. Be clinically diagnosed by the resident clinician as having a variant of pneumococcal disease;

v. Needed to be a resident of either Kiambu or Nairobi Counties at the time of sample collection and;

vi. The parent or legal guardian needed to sign an assent form.

3.4.2 Exclusion

i. Any subject who had a known immune-suppressive condition at the time of the study;

ii. Any subject whose biological parent or legal guardian declined to sign an informed assent form;

iii. Any subject who had received less than three doses of PCV-10 at most four weeks preceding sample collection and;

iv. Any subject who at the time of the study or for a period of at-most two weeks preceding sample collection, had consumed any antibiotics agents.

3.5 Sampling technique

This study employed purposive sampling as the most relevant and ideal system of sampling study subjects. This is because the researcher had set out strict criteria which prospective subjects needed to satisfy before being recruited. Subjects who did not satisfy the inclusion rationale as set out in 3.4.1 above, were not recruited. Prospective study subjects visiting the clinic for treatment and other healthcare needs were approached by the researcher after examination by the clinician on site.

3.6 Sample size determination

To determine the minimum sample size, the formula for Fisher *et al*. (1991) was used, with a prevalence rate of 17% (Githii *et al.*, 2013).

$$n = \frac{z^2 \hat{p}(1-\hat{p})}{m^2}$$

Where:

n= Desired minimal sample size (where population is ≥10,000);

z= Standard normal deviation = 1.96;

p= Prevalence rate;

m= the desired degree of accuracy @ 95% confidence level= 0.05 and;

n=1.96^2 x 0.17(1-0.17) / 0.05^2=217

Sample size (*n*) = 217

Since the target population is ≤10000, the value of *n* was further adjusted as follows:

nf= *n*/1+ {*n*/N}

Where:

nf= Desired minimum sample size (where population is ≤10,000)

n= Calculated sample size

N= Total population

nf= 217÷ [1+ (217/5,000)]

nf= 207 subjects

3.7 Ethical considerations

Ethics approval for this study was given by the Kenyatta University Ethics Review Committee (Annex V) and permission to carry out the study obtained from the National Commission of Science Technology and Innovation (Annex VI). Gertrude's Children's Hospital research committee (GCH-RC) issued a permit for the study to be conducted at the GCH outpatient clinic (Annex VII). The study mainly entailed use of nasopharyngeal swabs as the main sample. Collection of the specimen was done professionally by the researcher and all used samples and reagents were safely stored, discarded and incinerated, as appropriate to avoid spread of potential infections.

Subjects participated in the study on voluntary terms after signing an informed assent form (Annex I); this was after the study had been properly explained to them after qualifying the inclusion criteria. All data obtained from the parents or legal guardians regarding themselves and/or their children were handled in confidence only by the researcher. The nasopharyngeal swab was collected with at most care and professionalism as the researcher had undergone thorough training at the KEMRI-Wellcome Trust, Kilifi prior to the onset of sample collection. Laboratory findings about the subjects were given to the GCH Head of Laboratory Operations to facilitate issuance to the subject's parents or legal guardians in case of need. Any undesired reactions noted during the sample procurement process were immediately reported to the site clinician or nurse for requisite attention.

3.8 Risk factors associated with nasopharyngeal carriage of the pneumococcus

A field-tested and standardized questionnaire was administered to parents or legal guardians to study subjects. This was done to collect information on a range of demographics which may have a direct or indirect association with transmission of pneumococci. The risk factors studied are as listed in Annex II.

3.9 Collection and storage of study samples

Samples were collected according to the procedure of (Lambert *et al.,* 2008). Copan flocked swabs inserted through the nostrils were used to gently swab the nasopharyngeal region of study subjects. The depth of swab insertion was estimated to be half the distance between the tip of the child's nose and the anterior portion of the ear. After collection, nasopharyngeal swabs were dipped in Amies media, stored in a cold box and transported to the GCH main laboratory within 3 hours of collection. On delivery to the laboratory, the swabs were either plated immediately or incubated at 37^0C and plated later but within 24 hours of collection.

3.9.1 Laboratory identification of pneumococci

The laboratory isolation of pneumococci was conducted with respect to (Kellogg *et al.*, 2001). Within 24 hours, all collected swabs were inoculated on to 5% sheep blood agar plates with gentamicin (GBA). Incubation was then done at 37^oC in 5% CO_2 and assessed not later than 48 hours for growth of pneumococci. Isolates with colonies that were mucoid, with draughtsman's appearance, α-haemolytic were presumably identified as pneumococci. Further definitive tests like optochin susceptibility and bile solubility where appropriate were also done. Carriage was defined as isolation of a sole confirmed pneumococci colony and definitively confirmed on optochin. Fresh isolates were obtained from pure cultures and stored in brain heart infusion agar enriched with 5% sheep blood.

The isolates were rejuvenated in regularities of 14 days in order to preserve their feasibility before serotyping.

3.9.2 Optochin sensitivity test (ethyl hydrocupreine hydrochloride)

Optochin (ethyl hydrocupreine hydrochloride) sensitivity test is used for the presumptive identification of alpha-haemolytic streptococci as *Streptococcus pneumoniae* (Robson *et al.,* 2007). All colonies on blood agar (BA) with Gentamicin demonstrating *Streptococcus pneumoniae* characteristics after at least 24 hours of culturing were evenly inoculated on BA plates. This was done using a sterile inoculating loop. An Optochin disk was aseptically placed on the preparation using a sterile antibiotic dispenser and incubated for 24-48 hours at $37^{o}C$ in 5% CO_2 atmosphere. After 24-48 hours, a clearance zone of $\geq$ 14mm around the Optochin disk was a confirmation that the organism was *Streptococcus pneumoniae.* Plates with clearance zones < 14mm but $\geq$ 6mm were further subjected to bile solubility test. Bile soluble stocks confirmed that the organism was *Streptococcus pneumoniae.*

3.9.3 Bile solubility test

Where appropriate, the researcher used a sterile cotton inoculating swab to add fresh colonies of *Streptococcus pneumoniae* from blood agar plate to 1.0 ml of 0.85% saline solution to achieve turbidity of 1.0 McFarland standard. The cell suspension was divided into two equal volumes of 0.5ml/tube.

Added 0.5 ml of 2% sodium desoxycholate solution (bile salts) to one tube and 0.5ml of 0.85% saline solution to another tube. Gently mixed the two tubes and incubated for up to 2 hours at 37^{o}C in 5% CO_2. Vortexed the tubes while observing for any clearance of turbidity within 10 minutes. Any clearance in the bile tube and not in the saline tube indicated a positive test. Bile solubility test was only done on one sample which had all other features of *Streptococcus pneumoniae* but was optochin non-susceptible.

3.9.4 Antibiotic susceptibility testing

Antibiotic susceptibility testing (AST) was performed using the Kirby–Bauer (disc diffusion) method according to Clinical and Laboratory Standards Institute guidelines (CLSI, 2016). A 0.5 McFarland standard of freshly subcultured organisms was inoculated on a 150mm Mueller–Hinton plate with 5% sheep blood (Annex IV). A standard disk dispenser was used to dispense the various antibiotic disks on the MH-BA agar. Incubation was done overnight (20-24hours) at 37^{o}C in 5% CO_2. Antibiotics tested included: erythromycin (15µg), vancomycin (30µg), ceftriaxone (25µg)), clindamycin (2µg) oxacillin (1µg). The antimicrobials were regarded as sensitive or resistant on the basis of the following reference ranges (CLSI, 2016): erythromycin, vancomycin (17mm), ceftriaxone (30mm), clindamycin (19mm), oxacillin (15mm).

3.9.5 Capsular serotyping of *Streptococcus pneumoniae*

Capsular serotyping was done using the Quellung Reaction method according to (Habib *et al.*, 2014) (Annex III). Frozen vials containing *Streptococcus pneumoniae* stocks stored at -80^{0}C were thawed at room temperature for about 30 minutes. About 10µl of the stored *Streptococcus pneumoniae* cells were obtained and suspended in 50µl of phosphate buffered saline (PBS) and gently vortexed. The researcher added 10µl of the suspended cells/PBS on to a glass slide and mixed with 10µl of pooled antisera.

The glass slide was gently swirled while observing for any agglutination reaction. This was done successfully until a positive reaction was observed with various pooled antisera. The researcher then processed individual groups under various pools. Ten µl of the suspended cells in PBS was added to a glass slide and mixed with various *Streptococcus pneumoniae* serotype specific antisera included in the antisera pools that would have initially given a positive reaction. This was done until a positive reaction with the particular serotype specific antisera was observed. Those serotypes which didn't belong to any pool were typed directly until a positive reaction was observed. The cells/PBS/serotype specific antisera mixture on the glass slide were covered with a cover slip and observed under a phase contrast microscope at ×400 objective lens with oil emulsion.

3.10 Statistical analysis

The statistical package for social scientists (SPSS) version. 22 was used for data entry, cleaning and analysis. To summarize socio-demographic features of the subjects, nasopharyngeal (NP) carriage levels, bacterial serotypes and antimicrobial susceptibility patterns, descriptive statistics were used. In order to profile risk factors for pneumococcal infections, bivariate and multi-variate logistic regression statistics were conducted. To assess associations between risk factors and NP carriage, adjusted odds ratios (ORs) were conducted. This was done at 95% confidence interval. Any scores with *p values* of less than 0.05 were deemed significant.

CHAPTER FOUR
4.0 RESULTS

4.1 Socio-demographic factors of study subjects

Various socio-demographic characteristics of the study subjects were described and summarized as presented in Tables 4.1a, 4.1b and 4.1c. The characteristics analysed in the order of tables 4.1a, 4.1b and 4.1c include: child's age in months, child's gender, age of mother in years, net family income/month (USD); mother's level of education, mother's smoking status, presence of alcoholic in the house, size of residential house, type of cooking fuel, method of waste disposal, source of lighting in the house and; consumption of antibiotics two weeks prior to collection of the sample, child's attendance at daycare center, number of household occupants, breast-feeding type and PCV-10 immunization status.

Table 4.1a: Socio-demographic data of PCV-10 vaccinated and unvaccinated children attending GCH

Factor	Description	*n*=206	Valid percent (%)
Child's age (months)	6-12	68	33.0
	13-24	47	22.8
	25-36	46	22.3
	37-48	17	8.3
	49-60	28	13.6
Child's gender	Male	97	47.1
	Female	109	52.9
Age of mother (years)	18-24	47	22.8
	25-29	69	33.5
	30-34	54	26.2
	35-39	22	10.7
	40-45	14	6.8
Family's income per month (USD)	70-140	115	56.1
	150-250	59	28.8
	260-350	15	7.3
	360-450	6	2.9
	>460	10	4.9
	Total	205	100.0
Missing	System	1	

n: Total number of subjects per category
%: Percentage of subjects per category
USD: United States Dollars

Table 4.1b: Socio-economic demographic data of PCV-10 vaccinated and unvaccinated children attending GCH

Characteristic	Description	*n*=206	Percent (%)
Education level of the Mother	Primary School	61	29.61
	Secondary School	88	42.72
	Tertiary College	38	18.45
	University	19	9.22
Mother's smoking Status	Smoker	4	1.94
	Non smoker	202	98.06
Presence of alcoholic in the house	Yes	9	4.37
	No	197	95.63
Size of the house of Residence	Single room	113	54.85
	One bedroom	42	20.39
	Two bedroom	46	22.33
	Three bedrooms	5	2.43
Type of cooking fuel	Firewood	9	4.37
	Stove	62	30.1
	Charcoal	38	18.45
	Gas	94	45.63
	Electricity	3	1.46
Method of waste disposal	Public sewerage system	154	74.76
	Private septic tank	47	22.82
	Other	5	2.43
Source of lighting in the house	Candle	5	2.43
	Traditional lamp	11	5.34
	Lantern	1	0.49
	Solar	3	1.46
	Electricity	185	89.81
	Missing	1	0.49

n: Total number of subjects per category
%: Percentage of subjects per category

Table 4.1c: Socio-economic demographic data of PCV-10 vaccinated and unvaccinated children attending GCH

Factor	Description	*n*=206	Percent (%)
Consumption of antibiotics two weeks prior to collection of sample			
	Yes	112	54.37
	No	94	45.63
Child's attendance at day care center			
	Yes	39	18.93
	No	167	81.07
No of household occupants			
	One	37	17.96
	Two	52	25.24
	Three	61	29.61
	Four	33	16.02
	Five	12	5.83
	≥five	10	4.85
Breast feeding type	none	1	0.49
	Moderate	72	34.95
	Exclusive	133	64.56
Child immunization			
	Yes	106	51.46
	No	100	48.54

n: Total number of subjects per category
%: Percentage of subjects per category

4.2 *Streptococcus pneumoniae* serotype distribution

4.2.1 *Streptococcus pneumoniae* carriage among study subjects

Table 4.2 below is a summary of *Streptococcus pneumoniae* carriage profile among PCV-10 vaccinated and unvaccinated children. Out of the 206 subjects studied, 42 (20.39%) were found to be carriers of the bacteria in their nasopharyngeal region. Out of the 42 (20.39%) *Streptococcus pneumoniae* carriers, 22 (10.68%) had received PCV-10 vaccination and 20 (9.71%) had not been immunized by PCV-10. Forty one (97.6%) of the 42 *Streptococcus pneumoniae* serotypes isolated were non-PCV-10 and one (0.49%) was untypeable.

Table 4.2: *Streptococcus pneumoniae* NP carriage profile among PCV-10 vaccinated and unvaccinated children attending GCH

	All children		**PCV-10 vaccinated children**		**PCV-10 unvaccinated children**	
	n	**%**	***n***	**%**	***n***	**%**
Overall *Streptococcus pneumoniae* carriage	42	20.39	22	10.68	20	9.71
Proportion of *Streptococcus pneumoniae* Serotypes	*n*	%	*n*	%	*n*	%
PCV-10	0	0.00	0	0.00	0	0.00
Non PCV-10 serotypes	41	19.90	41	19.90	41	19.90
Non typeable	1	0.49	1	0.49	1	0.49

PCV-10: 10-valent pneumococcal conjugate vaccine
n: Total number of Pneumococci isolated
%: Percentage of Pneumococci isolates recovered

4.2.2 *Streptococcus pneumoniae* serotype distribution among PCV-10 vaccinated and unvaccinated children of varying age groups

Table 4.3 below represents the distribution of various *Streptococcus pneumoniae* serotypes among study subjects on the basis of age. Nine serotypes which include: 28F (2), 11A (2), 23A (2), 3 (2), 6A (2), 17F (1), 35F (1), 7C (1) and untypeable (1) were found among children within the age of 6-12 months. Serotypes: 20 (2), 21 (1), 39 (1), 28 (F), 35B (1), 17F (1) and 13(1) were found among children between 13-24 months old; serotypes: 23B (3), 19B (2), 3 (2), 20 (1), 28F (1), 7C (1), 23A (1) and 48 (1) were found among children within 25-36 months old. Serotypes: 6A (2), 15B (1) and 28F (1) were found among children aged 37-48 months and serotypes: 6A (1) and 28F (1) were isolated from children aged 49-60 months old.

Table 4.3: Distribution of *Streptococcus pneumoniae* serotypes among PCV-10 vaccinated and unvaccinated children attending GCH on the basis of age

	All	6-12 Months	13-24 Months	25-36 Months	37-48 Months	49-60 Months
Subjects with NP carriage (*n*)	42	16	8.00	12	4	2
Carriage (%)	20.39	23.53	17.02	26.09	23.53	7.14
Number of different serotypes seen	18	9	7	8	3	2
Serotypes seen	28F (8)	28F (4)	20 (2)	23B (3)	6A (2)	6A (1)
	6A (5)	11A (2)	21 (1)	19B (2)	15B (1)	28F (1)
	3 (4)	23A (2)	39 (1)	3 (2)	28F (1)	
	23B (3)	3 (2)	28F (1)	20 (1)		
	20 (3)	6A (2)	35B (1)	28F (1)		
	23A (3)	17F (1)	17F (1)	7C (1)		
	19B (2)	35F (1)	13 (1)	23A (1)		
	17F (2)	7C (1)		48 (1)		
		untypeable				
	7C (2)	(1)				
	11A (2)					
	35F (1)					
	15B (1)					
	untypeable (1)					
	48 (1)					
	35B (1)					
	21 (1)					
	39 (1)					
	13 (1)					

n: Total number of Pneumococci isolates recovered from study subjects
%: Percent of Pneumococci isolates recovered from study subjects

4.2.3 *Streptococcus pneumoniae* serotype distribution among PCV-10 vaccinated and unvaccinated children attending GCH according to PCV-10 vaccination status

Table 4.4 below represents *Streptococcus pneumoniae* serotype distribution among study children on the basis of PCV-10 immunization status. Serotypes 28F (5), 23A (3), 6A (3), 17F (2), 11A (1), 3(1), 35F (1), 48(1), 13(1), 35B (1) and 7C (1) were isolated from children who had received the recommended dose of PCV-10 vaccine. Serotypes: 3(3), 28F (3), 23B (3), 20(3), 19B (2), 6A (2), 21(1), 11A (1), 7C (1), untypeable (1), 15B (1) and 39(1) were isolated from children who had not received PCV-10 vaccination.

Table 4.4: Serotype distribution among PCV-10 vaccinated and unvaccinated children attending GCH According to PCV-10 vaccination status

PCV-10 unvaccinated children (100/206)			PCV-10 vaccinated children (106/206)		
Serotype	***n***	**%**	**Serotype**	***n***	**%**
28F	5	5	3	3	2.83
23A	3	3	28F	3	2.83
6A	3	3	23B	3	2.83
17F	2	2	20	3	2.83
11A	1	1	19B	2	1.89
3	1	1	6A	2	1.89
35F	1	1	21	1	0.94
48	1	1	11A	1	0.94
13	1	1	7C	1	0.94
35B	1	1	Untypeable	1	0.94
7C	1	1	15B	1	0.94
			39	1	0.94

n: Number of specific pneumococci serotypes isolated
%: Percentage of specific serotypes isolated

4.2.4 *Streptococcus pneumoniae* serotype distribution among PCV-10 vaccinated and unvaccinated children attending GCH according to gender

Table 4.5 shows the distribution of *Streptococcus pneumoniae* serotypes on the basis of gender (male and female). Serotype 28F was most prevalent among male subjects than their female counterparts. Serotypes 6A, 3, 23B and 20 were more prevalent in female subjects than they were in male subjects. Overall, female subjects harbored more serotypes than their male counterparts.

Table 4.5: *Streptococcus pneumoniae* serotype distribution among PCV-10 vaccinated and unvaccinated children attending GCH according to subject gender

Serotype	Frequency (*n*)	Male	Female
28F	8	5	3
6A	5	2	3
3	4	1	3
23B	3	0	3
20	3	1	2
23A	3	2	1
19B	2	0	2
17F	2	1	1
7C	2	0	2
11A	2	0	2
35F	1	1	0
15B	1	1	0
untypeable	1	0	1
48	1	0	1
35B	1	0	1
21	1	0	1
39	1	0	1
13	1	1	0
TOTAL	**42**	**15**	**27**

n: Percentage of pneumococci serotypes isolated from various subjects according to gender

4.3 Antimicrobial resistance

4.3.1 *Streptococcus pneumoniae* serotypes antimicrobial susceptibility patterns

Table 4.6 represents susceptibility patterns of *Streptococcus pneumoniae* to various antibiotic agents. The antibiotics had different disc concentrations. They include: Vancomycin (30µg, ≥17mm); erythromycin (15µg, ≥21mm); clindamycin (2µg, ≥19mm); Oxacillin (1µg, ≥19mm) and Cetriaxone (1µg, ≥30mm). These disk zone diameter interpretive standards are equivalent of minimal inhibitory concentrations (MIC) breakpoints as recommended by CLSi (McAdam, 2019). The highest level of resistance was exhibited to oxacillin (penicillin) followed by ceftriaxone (cephalosporin) while; the bacteria was most susceptible to clindamycin.

Table 4.6: *Streptococcus pneumoniae* susceptibility patterns to selected antibiotic agents among PCV-10 vaccinated and unvaccinated children attending GCH

Antibiotic agent	*n*=42	%
Erythromycin		
Sensitive	39	92.86
Resistant	3	7.14
Vancomycin		
Sensitive	39	92.86
Resistant	3	7.14
Oxacillin		
Sensitive	8	19.05
Resistant	34	80.95
Clindamycin		
Sensitive	40	95.24
Resistant	2	4.76
Ceftriaxone		
Sensitive	24	57.86
Resistant	18	42.86

n~number of *Streptococcus* pneumoniae isolates
%~percent of *Streptococcus* pneumoniae isolates that was susceptible to the antibiotic

4.3.2 Susceptibility profiles of *Streptococcus pneumoniae* serotypes to various antibiotic agents among PCV-10 vaccinated and unvaccinated children attending GCH

Table 4.7 represents susceptibilities of *Streptococcus pneumoniae* serotypes to various antimicrobial concentrations. The antibiotic disk concentrations and zones used are as described in table 4.6 above.

All the 8 (19.05%) isolates of serotype 28F were susceptible to erythromycin, vancomycin and clindamycin; one (2.38%) was susceptible to oxacillin and 7 (16.67%) were non-susceptible; three (7.14%) were susceptible to ceftriaxone while 5 (11.9%) were non-susceptible. All the 5 (11.9%) isolates of serotype 6A were susceptible to erythromycin; four (9.52%) isolates of serotype 6A were susceptible to vancomycin while 1(2.38%) was non-susceptible; one (2.38%) 6A was susceptible to oxacillin while 4 (9.52%) 6As were non-susceptible; four (9.52%) 6As were susceptible to clindamycin while 1 (2.38%) was non-susceptible.

Two (4.76%) 6As were susceptible to ceftriaxone while 3 (7.14%) were non-susceptible. Isolates from serotypes: 13, 15B, 21, 35B, 35F, 39, 48 and untypeable were susceptible to: erythromycin, vancomycin, clindamycin and ceftriaxone but were non-susceptible to oxacillin except serotype 21 and untypeable. Isolates from serotypes: 3, 23A, 23B, 20, 11A, 17F, 19B and 7C were susceptible to: erythromycin, vancomycin and clindamycin. Serotypes: 3, 23A and 11A were resistant to erythromycin, 11A and 7C were resistant to vancomycin, 3, 23A, 23B, 20, 17F, 19B and 7C were non-susceptible to oxacillin. Serotype 17F was resistant to clindamycin while serotypes: 3, 23B, 11A, 17F and 7C were resistant to ceftriaxone.

Table 4.7: *Streptococcus pneumoniae* serotype susceptibilities to various antimicrobial agents among PCV-10 vaccinated and unvaccinated children attending GCH

	Erythromycin		Vancomycin		Oxacillin		Clindamycin		Ceftriaxone	
Serotype	**Resistant**	**Sensitive**	**Resistant**	**Sensitive**	**Resistant**	**Sensitive**	**Resistant**	**Sensitive**	**Resistant**	**Sensitive**
28F	0	8 (19.05)	0	8 (19.05)	7 (16.67)	1 (2.38)	0	8 (19.05)	5 (11.9)	3 (7.14)
6A	0	5 (11.90)	1 (2.38)	4 (9.52)	4 (9.52)	1 (2.38)	1 (2.38)	4 (9.52)	3 (7.14)	2 (4.76)
3	1 (2.38)	3 (7.14)	0	4 (9.52)	4 (9.52)	0	0	4 (9.52)	2 (4.76)	2 (4.76)
23A	1 (2.38)	2 (4.76)	0	3 (7.14	3 (7.14	0	0	3 (7.14	0	3 (7.14
23B	0	3 (7.14)	0	3 (7.14	2 (4.76)	1 (2.38)	0	3 (7.14	2 (4.76)	1 (2.38)
20	0	3 (7.14)	0	3 (7.14	2 (4.76)	1 (2.38)	0	3 (7.14	0	3 (7.14
11A	1 (2.38)	1 (2.38)	1 (2.38)	1 (2.38)	2 (4.76)	0	0	2 (4.76)	1 (2.38)	1 (2.38)
17F	0	2 (4.76)	0	2 (4.76)	1 (2.38)	1 (2.38)	1 (2.38)	1 (2.38)	2 (4.76)	0
19B	0	2 (4.76)	0	2 (4.76)	1 (2.38)	1 (2.38)	0	2 (4.76)	1 (2.38)	1 (2.38)
7C	0	2 (4.76)	1 (2.38)	1 (2.38)	2 (4.76)	0	0	2 (4.76)	1 (2.38)	1 (2.38)
13	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
15B	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
21	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)
35B	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
35F	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
39	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	0	1 (2.38)
48	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)	0	1 (2.38)
Untypeable	0	1 (2.38)	0	1 (2.38)	1 (2.38)	0	0	1 (2.38)	1 (2.38)	0

Susceptibility zones: Vancomycin (30µg, ≥17mm); erythromycin (15µg, ≥21mm); clindamycin (2µg, ≥19mm); Oxacillin (1µg, ≥19mm) and Cetriaxone (1µg, ≥30mm)

4.3.3 Effect of selected risk factors on susceptibility patterns of *Streptococcus pneumoniae* to various antibiotic agents

Table 4.8 represents the association between selected risk factors and susceptibility of *Streptococcus pneumoniae* to various antibiotic agents. The analysis was done using odds ratios (ORs) at 95% confidence interval (95% CIs); significance levels were determined by computation of P-Values. Odds ratios were done to determine whether the various selected risk factors (predictors) had any association with susceptibility of the bacteria to the various antibiotic agents (outcome). P-values were done to establish the strength of association between the predictors and outcomes.

An odds ratio less than 1 (OR<1) meant that there was a negative association between the selected risk factor and antibiotic susceptibility of *Streptococcus pneumoniae* while; OR≥1 meant that there existed no association at all. Any figure greater than one (OR>1) was interpreted to mean that the selected risk factor had a positive association with the antibiotic susceptibility of *Streptococcus pneumoniae.*

Table 4.8: Effect of selected risk factors on *Streptococcus pneumoniae* susceptibility to various antimicrobial agents among PCV-10 vaccinated and unvaccinated children attending GCH

	Erythromycin		Vancomycin		Oxacillin		Clindamycin		Ceftriaxone	
Risk factors	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**	**OR (95% CI)**	**p-value**
Gender										
Male	1		1		1		1		1	
Female	1.94 (0.23, 16.10)	0.541	0.14 (0.01,2.81)	0.196	0.51 (0.11,2.26)	0.372	0.2 (0.01,4.43)	0.308	0.56 (0.17,1.86)	0.341
Age (Months)										
6-12	1		1		1		1		1	
13-24	0.34 (0.01, 7.98)	0.504	0.34 (0.01,7.98)	0.504	0.25 (0.03,2.32)	0.224	2.07 (0.18,23.36)	0.557	0.3 (0.05,1.74)	0.181
25-36	0.76 (0.09, 6.61)	0.801	0.76 (0.09,6.61)	0.801	0.26 (0.03,2.09)	0.207	0.41 (0.02,11.05)	0.598	0.58 (0.13,2.49)	0.463
37-48	0.64 (0.03, 16.05)	0.789	0.64 (0.03,16.05)	0.789	0.23 (0.02,2.87)	0.251	1.15 (0.04,33.33)	0.936	0.34 (0.04,2.87)	0.321
49-60	1.16 (0.04, 32.08)	0.930	1.16 (0.04,32.08)	0.93	0.1 (0.01,1.62)	0.104	2.07 (0.06,66.3)	0.682	0.79 (0.07,9.22)	0.85

OR=1: Exposure does not affect odds of outcome, OR>1: Exposure associated with higher odds of outcome, OR<1: Exposure associated with lower rates of outcome. CI is used to estimate the precision of the OR. The higher the CI the lower the precision of the OR and the lower the CI the higher the precision of the OR. P-Value is a measure of significance levels of the exposure against the outcome.

Risk Factors	Erythromycin OR (95% CI)	P-Value	Vancomycin OR (95% CI)	P-Value	Oxacillin OR (95% CI)	P-Value	Clindamycin OR (95% CI)	P-Value	Ceftriaxone OR (95% CI)	P Value
Recent use of antibiotics (2 weeks)										
No	1		1		1		1		1	
Yes	1.30 (0.16, 10.78)	0.810	1.3 (0.16,10.78)	0.81	0.8 (0.18,3.58)	0.771	4.11 (0.19,91.08)	0.371	0.89 (0.27,2.98)	0.856
Breast feeding type										
None	1		1		1		1		0.42 (0.01, 11.92)	0.613
Moderate	0.09 (0.00, 6.45)	0.270	0.78 (0.03, 23.53)	0.885	1.93 (0.06, 62.17)	0.71	0.29 (0.01, 10.76)	0.502	0.16 (0.01, 4.41)	0.28
Exclusive	0.47 (0.02, 13.89)	0.660	0.06 (0, 4.13)	0.192	1 (0.04, 27.7)	1	0.18 (0.01, 6.72)	0.356		
PCV-10 immunization										
No	1		1		1		1		1	
Yes	0.52 (0.06, 4.29)	0.541	1.59 (0.19,13.17)	0.67	0.64 (0.14,2.84)	0.553	0.16 (0.01,3.64)	0.253	1.24 (0.37,4.09)	0.729
Day care attendance										
No	1		1		1		1		1	
Yes	1.69 (0.20, 14.37)	0.633	5.35 (0.62,45.99)	0.126	2.14 (0.32,14.27)	0.431	2.9 (0.27,31.05)	0.378	4.97 (1.17,21.14)	0.03

OR=1: Exposure does not affect odds of outcome, OR>1: Exposure associated with higher odds of outcome, OR<1: Exposure associated with lower rates of outcome. CI is used to estimate the precision of the OR. The higher the CI the lower the precision of the OR and the lower the CI the higher the precision of the OR. P-Value is a measure of significance levels of the exposure vis a vis the outcome.

4.4 Prevalence of optochin resistant *Streptococcus pneumoniae* serotypes among children ≤5 years attending GCH

Table 4.9 below shows different *Streptococcus pneumoniae* serotypes isolated and their susceptibility profiles to Optochin. Serotypes: 11A, 13, 15B, 17F, 19B, 20, 21, 23A, 23B, 28F, 3, 35B, 35F, 39, 48, 6A and 7C were all susceptible to Optochin with disc clearance zones of >14mm. However, the untypeable *Streptococcus pneumoniae* serotype was non-susceptible with a disc clearance zone of 6mm.

Table 4.9: Susceptibility patterns of various *Streptococcus pneumoniae* serotypes to optochin among PCV-10 vaccinated and unvaccinated children attending GCH

	Optochin non-susceptible		Optochin susceptible		Total	
Serotype	***n***	**%**	***n***	**%**	***n***	**%**
11A	0	0	2	4.88	2	4.76
13	0	0	1	2.44	1	2.38
15B	0	0	1	2.44	1	2.38
17F	0	0	2	4.88	2	4.76
19B	0	0	2	4.88	2	4.76
20	0	0	3	7.32	3	7.14
21	0	0	1	2.44	1	2.38
23A	0	0	3	7.32	3	7.14
23B	0	0	3	7.32	3	7.14
28F	0	0	8	19.51	8	19.05
3	0	0	4	9.76	4	9.52
35B	0	0	1	2.440	1	2.38
35F	0	0	1	2.44	1	2.38
39	0	0	1	2.44	1	2.38
48	0	0	1	2.44	1	2.38
6A	0	0	5	12.2	5	11.9
7C	0	0	2	4.88	2	4.76
untypeable	1	100	0	0	1	2.38
Total	**1**	**100**	**41**	**100**	**42**	**100**

n: number of both optochin susceptible and non-susceptible pneumococci
%: percent of both optochin susceptible and non-susceptible pneumococci

4.5 Risk factors associated with nasopharyngeal *Streptococcus pneumoniae* carriage among PCV-10 vaccinated and unvaccinated children attending GCH

Tables 4.10a and 4.10b below shows the association between nasopharyngeal carriage of *Streptococcus pneumoniae* (outcome) with various selected risk factors (predictors). Odds ratios were done to demonstrate the nature of this association. Where: OR=1 meant that the selected risk factor had no effect on nasopharyngeal carriage of the bacteria (no risk); OR>1 meant that the selected risk factor was positively associated with nasopharyngeal carriage of the bacteria (increased risk) and; OR<1 meant that the selected risk factor was negatively associated with the nasopharyngeal carriage of antibiotic susceptibility of *Streptococcus pneumoniae (*reduced risk). P-values were done to demonstrate the degree of significance of the relationships at 95% CI.

Table 4.10a: Analysis of the risk factors associated with nasopharyngeal carriage of *Streptococcus pneumoniae* among PCV-10 vaccinated and unvaccinated children attending GCH

Risk factors	*Streptococcus pneumoniae* colonization		Univariate analysis	
	No	Yes		
	n(%)	*n*(%)	OR (95% CI)	P-value
Gender				
Male	75 (77.32)	22 (22.68)	1	
Female	89 (81.65)	20 (18.35)	0.766 (0.388, 1.511)	0.442
Age (months)				
6-12	52 (76.47)	16 (23.53)	1	
13-24	39 (82.98)	8 (17.02)	0.667 (0.259, 1.715)	0.4
25-36	34 (73.91)	12 (26.09)	1.147 (0.483, 2.722)	0.756
37-48	13 (76.47)	4 (23.53)	1 (0.286, 3.501)	1
49-60	26 (92.86)	2 (7.14)	0.25 (0.053, 1.170)	0.078
Mother's smoking status				
Smoker	3 (75)	1 (25)	1	
Non smoker	161 (79.7)	41 (20.3)	0.764 (0.077, 7.537)	0.818

This was done at 95% CI. OR=1: Exposure does not affect odds of outcome. OR>1: Exposure associated with higher odds of outcome, OR<1: Exposure associated with lower rates of outcome

Table 4.10b: Analysis of the risk factors associated with nasopharyngeal carriage of *Streptococcus pneumoniae* among PCV-10 vaccinated and unvaccinated children attending GCH

Risk factors	*Streptococcus pneumoniae* colonization		Univariate analysis	
	No	Yes		p-value
	n(%)	*n*(%)	OR (95% CI)	
Cooking method				
Gas	74 (78.72)	20 (47.62)	1	
Charcoal	31 (81.58)	7(16.67))	0.835(0.321, 2.176)	0.713
Stove	48 (77.42)	14 (22.58)	1.079(0.498, 2.339)	0.847
Electricity	3(7.14)	0(.00)		0.675
Firewood	8(19.05)	1(2.38)		0.546
Recent antibiotics use (two weeks)				
Yes	88 (78.57)	24 (21.43)	1	
No	76 (80.85)	18 (19.15)	0.868(0.438, 1.721)	0.686
Day care attendance				
Yes	28 (71.79)	11 (28.21)	1	
No	136 (81.44)	31 (18.56)	0.58(0.261, 1.290)	0.182
Breast feeding type				
Moderate	56 (77.78)	17 (22.22)	1	
Exclusive	108 (81.20)	25 (18.80)	0.81 (0.40, 1.64)	0.559
Child immunization				
Yes	84 (79.25)	22 (20.75)	1	
No	80 (80)	20 (20)	0.955(0.484, 1.881)	0.893
Overcrowding index				
2 or less	68 (77.27)	20 (22.73)	1	
3+	55 (83.33)	11 (16.67)	0.70(0.326, 1.491)	0.352

At 95% CI. OR=1: Exposure does not affect odds of outcome, OR>1: Exposure associated with higher odds of outcome, OR<1: Exposure associated with lower rates of outcome. Overcrowding index=household members / household rooms

CHAPTER FIVE

5.0 DISCUSSION, CONCLUSIONS AND RECOMENDATIONS

5.1 Discussion

5.1.1 Socio-demographic characteristics of the study subjects

Results show that twenty three 23% (*n*=47) of the biological mothers to study children were between the ages of 18-24 years, 34% (*n*=69) were between the ages of 25-29 years, 26% (*n*=54) were between the age of 30-34 years, 11% (*n*=22) were between the age of 35-39 years and 7% (*n*=14) were between the age of 40-45 years. The age classification of the mothers was done according to (Kato *et al.*, 2017). Women between the ages of 25-29 years formed majority of the biological mothers with those between the ages of 40-45 years being the minority. This trend can be attributed to the fact that most women prefer getting children when between 25-29 years and the trend systematically reduces as years advance towards menopause. This may be due to the fact that at the age of 25-29 years most women have cleared college and not yet very active in their various careers. These results are coherent with those of Mathews & Hamilton (2016) who also reported that most women get children when they are in their mid-twenties mainly because at this age the ovulation cycle for most women would have regularized and with all other factors constant, conception can almost be predictable.

According to this results, majority of the subjects *n*=113 (54.9%) lived in single rooms, *n*=42 (20.4%) lived in one bed roomed houses, *n*=46 (22.3%) lived in two bed roomed houses and *n*=5 (2.4%) lived in three bed roomed houses. Studies have reported on relationships between the sizes of residential housing versus the burden of infectious diseases. For instance, WHO (2018), postulated that limited access to clean water, proper ventilation and inadequate social distancing increase the risk of transmission of infectious diseases. Since people's economic status is likely to dictate the size of their residential house, low income earners, who form the largest stratum of Kenya's populace are likely to live in relatively smaller houses (KNBS, 2020). This trend steadily improves with high income earners living in ≥3 roomed houses; the burden of infectious diseases accordingly decreasing.

These results are in congruence with the report made by the Habitat for Humanity, (2017) which documented a proliferation of informal settlements in urban areas with 60% of the population living in slums or congested homes typically with only one room and no tolerable aeration. Consequently, families are at an increased risk of ailments such as malaria, respiratory infections, and worse still, jigger infestation. The results also demonstrated that the smaller the size of the house of residence, the more the number of people sharing it with the study subjects.

According to (Lu *et al.*, 2016), low income is often associated with vulnerability to poverty and interestingly, large family sizes. On the contrary, results have also shown that the bigger the size of the house of residence, the smaller the family size. People who live in somewhat bigger houses often have a higher income. According to GoK & NCAPD (2010), there exists a linear relationship between family income level, access to information and chances of ideal and effective family planning. Thus, households with higher income are more likely to understand the metrics of family planning as compared to their peers from the flipside (Potrich *et al*., 2015). With regard to transmission of *Streptococcus pneumoniae*, the higher the number of household members, the poorer the prospects of sufficient social distancing and therefore the higher the risk of *Streptococcus pneumoniae* transmission. The relationship that seems to cut across all cohorts studied is that: the less the level of income one had, the smaller the house they will live in and the more likely they are to have more number of children.

People who have less income are more likely to engage in frequent sexual activities as they may not afford other leisurely activities available. As a result, they end up having larger families which impinges on the quality of indoor social distancing and therefore enhancing transmission of the subject bacteria. These results also show that the younger the child, the more they are likely to pick up infections and vise-versa.

Since the researcher sampled and recruited subjects at the hospitals when they presented with various respiratory complications, odds are that younger children would be most recruited because they are still within the most vulnerable window of growth hence the need to seek for frequent healthcare attention. These findings are consistent with those of Tazinya *et al.*, (2018), who opined that the younger the child, the more vulnerable they are to acquisition of infections because of their poorly developed immune systems. There were more female subjects than there were males in the study. A scenario that was consistent with the national housing and population census which showed that the population of females slightly out-number males in Kenya (WDA, 2015); whether gender of the subject has an association with transmission of *Streptococcus pneumoniae* is a concept that is discussed elsewhere under selected risk factors and how they relate with the bacterial nasopharyngeal carriage.

5.1.2 Epidemiology of *Streptococcus pneumoniae* serotypes

This study found that 20% of both PCV-10 vaccinated and unvaccinated children ≤5 years of age were carriers of *Streptococcus pneumoniae* in their nasopharyngeal region (NP). This is therefore a 4% rise from the 16% that was reported before inclusion of PCV-10 in the Kenya expanded program on immunization (KEPI) (GAVI, 2011). Interestingly, all the 18 serotypes isolated from the 42 isolates were non-PCV-10 types.

This is a very important finding as it may be used to justify the sustained high level of child morbidity and mortality due to pneumococcal infections in Kenya despite proper coverage and uptake of the vaccine. The findings perfectly mirrors those of Heath *et al.* (2018) which documented that PCV-10 vaccination did not reduce nasopharyngeal (NP) pneumococci carriage in their cohorts.

A study done by Githii *et al.* (2013) among children attending Thika District Hospital reported serotypes: 6A, 23F, 19F, 13, 6B, 14A, 20, 7C, 1, 15B, 35B, 19A, 11A, 34, 5, 3 and 23A. These serotypes constituted only about 50% of the serotypes included in PCV-10 and effectively represented a remarkable shift from Brueggemann *et al.*, (2013) which had reported that PCV-10 contained 42% of non-invasive and over 70% of all known invasive serotypes before introduction of the vaccine in KEPI in 2011. That the current study is reporting a 97% disparity between PCV-10 and circulating serotypes points to a steady yet incredible trend of serotype replacement. This therefore partly justifies why morbidity and mortality due to pneumococcal infections among children below the age of 5 years in Kenya remains largely uncontested. The tremendous occurrence of vaccine serotype (vT) replacement by non-vaccine serotypes(non-vT) may be credited to the increased level of antimicrobial misuse by a greater percentage of the study population; an illicit practice which has been extensively correlated with massive occurrence of serotype replacement elsewhere.

Such a scenario was also reported by Gladstone *et al*. (2015) which indicated that significant *Streptococcus pneumoniae* serotype replacement occurred among children vaccinated with PCV-7 and living in the United Kingdom (UK) between 2001 and 2011. The shift in the profile of serotypes was attributed to the pressure exerted on the pneumococci community by the vaccine which caused an alteration in the genetic anatomy of the bacteria.

Ten valent Pneumococcal conjugate vaccines protect against 10 different serotypes of *Streptococcus pneumoniae.* These serotypes are: 1, 4, 5, 6B, 7F, 9V, 14, 18C, 19F, 23F (Sime *et al.,* 2019). None of these 10 serotypes was found in the study population yet this is the vaccine currently included in KEPI, targeting the same population. The vaccine only protects against those serotypes included in its formulation; sero-protection does not exist for those serotypes not included in the vaccine. Some studies have however argued that serotypes found within the same group may share antigenic correlates and correspondingly therefore may also share immunological correlates; in such case, protection against one serotype may confer protection against another (Westen *et al.,* 2018). Serotypes 6A/6B and serotypes 19A/19F for instance, may most likely be exuding such a conniving yet very harmful relationship to human health.

Results show that *Streptococcus pneumoniae* carriage decreased with increasing age as the majority of the isolates (11.65%) were obtained from children at 6–24 months old and 8.74% from children >24 months.

The study demonstrated a linear relationship between child age and *Streptococcus pneumoniae* carriage. Similar studies done elsewhere reported findings that partly agree with this (Dunne *et al.,* 2019). The former being attributable to development of *Streptococcus pneumoniae*-specific IgG antibodies due to vaccination and during that window before most children start attending school. Unlike findings from other studies serotype 28F was the most prevalent as it was present in all five age groups profiled. This is a clear scenario of rampant serotype replacement due to the pathogen's efforts to evade the action of the immune system and eventual sharing of the resistant genes within the neighboring microbial community; especially in the nasopharyngeal region (Calatayud *et al*., 2010).

Serotypes 28F, 6A, 11A, 3 and 7C were found to be prevalent in both vaccinated and unvaccinated children, whereas serotypes 23A, 17F, 35F, 48, 13, 35B and 23B, 20, 19B, 21, untypeable, 15B, 39 were found among unvaccinated and vaccinated groups respectively. There exist different antigenic features between and within various strains of *Streptococcus pneumoniae* (Geno *et al*., 2015). While the most of pneumococci serotypes are capable of causing disease, the frequency with which they are isolated varies (Hjálmarsdóttir *et al.,* 2017).

In this case, vaccination would only be partially effective and, if so, due to inter-strain antigenic characteristics. While trying to evade the action of the immune system, *Streptococcus pneumoniae* has a tendency to exchange resistant genes and other important antigenic correlates at the nasopharyngeal region (Chaguza *et al.,* 2015). Resistance to antimicrobial agents is occasioned by among other factors, misuse of the antibiotics (Reinert, 2009a). This is largely due to poorly enforced antibiotic use regulations by the authorities. The 99.9% variation in the isolated serotypes with regard to the PCV-10 serotypes is evidence of possible serotype replacement or implementation of a vaccine that is completely irrelevant to the epidemiological needs of children living in Nairobi and Kiambu counties.

5.1.3 *Streptococcus pneumoniae* antimicrobial resistance profiling

The WHO (2014) recommends the use of penicillins (amoxicillin) or erythromycin as the first line antibiotics for treatment of pneumococcal infections and; cephalosporins (ceftriaxone) as alternative interventions. In the current study, most (93%, *n*=39) of the *Streptococcus pneumoniae* isolates were sensitive to erythromycin and vancomycin. Erythromycin and vancomycin act by inhibiting protein synthesis and development of the cell wall in the target bacteria (Kapoor *et al.,* 2017).

Streptococcus pneumoniae resistance to erythromycin and vancomycin can be attributed to the likelihood of previous irregular exposure to the drug agents. However, in the current study, the number of erythromycin and vancomycin resistant *Streptococcus pneumoniae* is almost medically insignificant. The low levels of resistance exhibited in this study can probably be attributed to the fact that vancomycin and erythromycin are less commonly dispensed over the counter as they are prescription only antibiotics (Mukokinya *et al.,* 2018).

About 19% (*n*=8) of the *Streptococcus pneumoniae* isolates were susceptible to oxacillin. Being an antibiotic in the penicillin group of drugs it is expected that it will be one of the most prescribed and irregularly used on the local market. Elsewhere, there seems to be a downward trend in penicillin resistant *Streptococcus pneumoniae* strains. For instance, between 1995 to 2000 Malaysia recorded a 26% decrease which was quite encouraging. Further, the same study reported a 40% decrease in Singapore between 1997 and 2008 (Mamishi *et al.,* 2014). It will therefore be interesting to elucidate the susceptibility behavior of pneumococci to penicillin over a period of time in Kenya. Moreover, failure to consume the correct regimen ends up in antibiotic selection pressure which consequently brings forth resistant strains that could then be horizontally spread across the population. The remarkably high resistance levels of *Streptococcus pneumoniae* serotypes to oxacillin in this study can be explained by the above scenario.

These results mirrors those of Bronzwaer *et al*. (2002) who reported increased antibiotic resistance of *Streptococcus pneumoniae* especially to different derivatives of penicillins as a consequence of previous irregular exposure. High levels of sensitivity to clindamycin unarguably agree with findings by St. Jude Children's Research Hospital, (2009) who reported that clindamycin and azithromycin work more effectively against pneumococci than a typical first-line regimen with the "beta-lactam" antibiotic ampicillin.

Clindamycin is especially recommended for *Streptococcus pneumoniae* treatment because of its high spectrum to bacteria in group A streptococci (Smieja, 1998). Usually, it is reserved for cases of *Streptococcus pneumoniae* high resistance to penicillins and/or cases of subjects being allergic to first line antibiotic therapies (Montagnani *et al.,* 2007). High level of Ceftriaxone resistance *Streptococcus pneumoniae* observed in this study is appalling to say the least. This is because high spectrum cephalosporins like Ceftriaxone are recommended for invasive pneumococcal disease in cases where other first line antibiotics have failed (Molyneux *et al*., 2011).

Resistance would only be expected in cases where the subjects had been inappropriately exposed to other cephalosporins (Mollendorf *et al.,* 2014). Irregular and non-prescribed purchase of antibiotics at local pharmacies and shops may be the main culprits behind these unfortunate findings.

These results are in congruent with those of Chiu *et al.*, (2007) done in Taiwan which reported increased cases of penicillin resistant *Streptococcus pneumoniae* isolates being resistant to ceftriaxone. The resistance which mainly affected serotypes 6B, 14, 19F, and 23F was attributed to an alteration of seven amino acids that are located on penicillin binding protein 2B. The bacteria has over 90 serotypes which belong to different groups on the basis of their capsular polysaccharides and the degree of cross-reactivity (Geno *et al.,* 2015). The various serotypes behave differently when exposed to antibiotics.

In this study, *n*=7 strains out of *n*=8 of serotype 28F were resistant to oxacillin while only *n*=3 were sensitive to ceftriaxone. Oxacillin, which is a penicillin, is normally given as a first line of treatment for the various forms of PD and ceftriaxone is a broad spectrum antibiotic normally given after penicillin has failed (Peterson, 2006). The mode of resistance to the two antimicrobial agents is shared to some extent as both involve the use of a four atom beta-lactam ring (Kong *et al*., 2010). As such, resistance in one is likely to influence resistance in another. Possible inappropriate exposure to one or both of these agents prior to the collection of the study samples may explain the high resistance levels in both. Most strains of serotypes 6A, 3, 23A, 23B, 20, 11A and 17 were oxacillin and ceftriaxone resistant but highly susceptible to erythromycin, vancomycin and clindamycin.

Of interest is that the behaviour of *Streptococcus pneumoniae* isolates to ceftriaxone in the current study has notable coherence with those reported by (Lee *et al.,* 2018). According to the study, 38% of the invasive pneumococcal disease (IPD) isolates in Taiwan exhibited resistance to ceftriaxone. Consequently, most of the cases died of meningitis related ailments. If this trend is anything to go by, then Kenya must implement stringent antibiotic use regulations to avert the possible negative effects of AMR.

Serotypes 19A, 7C, 13, 15B, 21, 35B, 35F, 39, 48, and the untypeable *Streptococcus pneumoniae* were highly susceptible to erythromycin, clindamycin, vancomycin, oxacillin and ceftriaxone. While most *Streptococcus pneumoniae* serotypes in this study are susceptible to majority of the antibiotic agents studied, resistance to oxacillin and ceftriaxone is clinically significant and largely worrying.

A similar study, examined susceptibility profiles of invasive *Streptococcus pneumoniae* to penicillin and ceftriaxone among children diagnosed with meningitis in Israel (Waisbourd *et al.,* 2010). The study reported higher resistance levels to penicillins especially among children who had taken oral antibiotics prior to being admitted to the hospital. While resistance to ceftriaxone was not significant, the rates could not be neglected for purposes of clinical decisions.

The slightly lower rates of *Streptococcus pneumoniae* resistance between the two cohorts may be due to the enrolment of both vaccinated unvaccinated children and differences in the enactment and implementation of the antibiotic use/consumption policies. Although none of the risk factors had a significant influence on the *Streptococcus pneumoniae* nasopharyngeal carriage, odds demonstrated that age, gender, recent consumption of antibiotics by subjects, attendance at daycare centers, breast-feeding type and vaccination status had a bearing on the effectiveness of antibiotics. For instance, being female and young increased the subject's resistance to erythromycin while it reduced susceptibility to vancomycin, clindamycin, ceftriaxone and oxacillin.

Children exposed to antibiotics within two weeks prior to sample collection and the PCV-10 unvaccinated ones had increased odds of being resistant to all the antibiotics studied. Gender and age disparities can influence antibiotic susceptibility patterns (Lee *et al.,* 2016). For instance, the study reports that males are much more likely to expose themselves to risk factors that augment horizontal transmission of the bacteria while; females carry an estrogen receptor on some of their key immune cells (T-cells, B-cells, dendritic cells and macrophages), a structural orientation that is likely to provide privileged protection to female subjects as compared to their male counterparts (Laffont *et al.,* 2017).

Breastfeeding type, attendance at day-care centers and PCV-10 vaccination status play a major role in antibiotic susceptibility according to findings in this study. With the exception of oxacillin, odds of *Streptococcus pneumoniae* being resistant to clindamycin, vancomycin, erythromycin, ceftriaxone decreased when subjects had been moderately or exclusively breastfed. According to Gómez *et al.*, (2002), the likelihood of horizontal transfer of *Streptococcus pneumoniae* resistant genes within a community increases.

5.1.4 Prevalence of optochin resistant *Streptococcus pneumoniae*

In this study, only one *Streptococcus pneumoniae* isolate was optochin resistant. The isolate qualified all other preliminary tests necessary for identification of *Streptococcus pneumoniae*, including bile-solubility test but was resistant to optochin. What is notable and perhaps a subject of concern is that the same isolate could not be serotyped by both Quellung Reaction and the Multiplex-Polymerase Chain Reaction (PCR) methods. It was also resistant to penicillin (a recommended first line of treatment for *Streptococcus pneumoniae*) and ceftriaxone (a broad spectrum antibiotic recommended especially for pneumococcal meningitis). It is not yet clear whether there exists a relationship between *Streptococcus pneumoniae* optochin resistance; non-encapsulation; non-sensitivity to the gold standard Quellung reaction and; and resistance to penicillins.

The *Streptococcus pneumoniae* polysaccharide capsule is the source of virulence for the various serotypes (Paton & Trappetti, 2019). The loss or complete lack of the polysaccharide capsule makes it difficult to identify the various *Streptococcus pneumoniae* serotypes. Earlier, a study reported that the loss or lack of the polysaccharide capsule may greatly wane the chance of IPD but does not eradicate the possibility of occurrence (Hammerschmidt *et al.*, 2005).

More frequently, non-invasive diseases such as otitis media and conjunctivitis have been reported to be caused by non-encapsulated *Streptococcus pneumoniae* (Murrah *et al.*, 2015). The overall number of clinical cases instigated by non-encapsulated *Streptococcus pneumoniae* is undoubtedly underrated because serotyping is not routinely done. Because of this, serotype-nontypeable pneumococci may not be further classified. A concept that underscores the need to understand prevalence of optochin resistant *Streptococcus pneumoniae*. Further, the statistics of nasopharyngeal pneumococcal carriage isolates that do not have the polysaccharide capsule have been massively undervalued; pneumococci may in some occasions fail to encapsulate due to mutations, disruption and even deletion of genes responsible for capsule synthesis from the *cps* locus (Bradshaw & McDaniel, 2019). Considering the epidemiological significance of non-capsulated pneumococci serotypes, deliberate and targeted efforts must be made to understand its distribution across Kenya and regionally. Obviously, this would have a nexus with child morbidity and mortality.

According to Dias *et al.* (2007) and Pikis *et al.* (2002), attribute point genetical alterations in the *c* and not the *a* subunits of the *atpCAB* operon which encodes for the optochin's target site in the *Streptococcus pneumoniae* and prolonged consumption and/or use of antimalarial drugs to optochin resistance. Given the high prevalence of malaria in some parts of Kenya and therefore a possibility of prolonged use of anti-malarial drugs, its link with optochin resistance should be a cause for concern. It will also be interesting to find out whether the tendency of *Streptococcus pneumoniae* to be resistant to optochin is a dispensation that unfolds along specific genetical lines or whether it is a random concept.

5.1.5 Risk Factors Associated with Nasopharyngeal Carriage of *Streptococcus Pneumoniae*

The risk factors studied include: PCV-10 immunization status, age, gender, breastfeeding type, attendance at day-care center, recent consumption of antibiotics (two weeks prior to study), type of cooking fuel, whether mother smokes or not and overcrowding index (number of household occupants against number of rooms in that household). This study results show that children who were between the ages of 25-36 months had elevated odds of nasopharyngeal *Streptococcus pneumoniae* carriage. This could be because maternal antibodies would have started waning from the system and the subjects were yet to develop robust cellular and humoral mucosal immunities necessary to protect them against attack by *Streptococcus pneumoniae*.

These results are consistent with those of Koliou *et al.* (2018), which reported that age, though not an independent factor, as having a link to both carriage of *Streptococcus pneumoniae* and occurrence of IPD. It would naturally be expected that study subjects who had not received three doses of PCV-10 vaccination would be more vulnerable to nasopharyngeal *Streptococcus pneumoniae* carriage. However, this is not the case according to results from the current study as even subjects who had received the full dose were found to be *Streptococcus pneumoniae* carriers. Vaccine serotype replacement by non-vaccine serotypes due to selective vaccine pressure is obviously to blame. This situation strongly underscores the need for a vaccine with broader serotype coverage.

Surprisingly, all serotypes found in these cohorts were non-vaccine type. This is a clear case of vaccine type serotype replacement by non-vaccine type serotypes mostly instigated by abuse of antibiotics and cases of inadequate social distancing. Although the association may be statistically insignificant, results from this study demonstrate that gender, breastfeeding type, attendance at daycare center, recent consumption of antibiotics (two weeks prior to sample collection), type of cooking fuel, whether mother smokes or not and overcrowding have a linear relationship with nasopharyngeal *Streptococcus pneumoniae* carriage.

Elsewhere, Greenberg *et al*. (2006) reported direct relationships between: exposure to smoke and attendance at daycare center with nasopharyngeal *Streptococcus pneumoniae* carriage. However, the current study only reports very minimal relationships. Minimal is relative especially if the consequences include increased child morbidity and mortality.

5.2 Conclusions

i. Children receiving PCV-10 in Kenya may not be protected against pneumococcal infections due to serotype replacement;

ii. Most pneumococci isolates that exhibit resistance to oxacillin (penicillin) also exhibit resistance to ceftriaxone (cephalosporin);

iii. Resistance to optochin which is meant for definitive diagnosis of *Streptococcus pneumoniae* is low in the study population and;

iv. Vaccination with PCV-10, attendance at daycare center and exposure to both passive and active smoke augments nasopharyngeal carriage of *Streptococcus pneumoniae.*

5.3 Recommendations

i. The government of Kenya should consider introducing a vaccine with a broader *Streptococcus pneumoniae* serotype coverage and more effectively one with locally circulating *Streptococcus pneumoniae* serotypes;

ii. Relevant authorities including: Pharmacy and Poisons Board (PPB), Research Institutions and Learning Institutions should be more assertive to curb injudicious use of antibiotics by people living in Kenya. In fact, there should be deliberate legislation to punish individuals who knowingly or otherwise abuse antibiotics;

iii. Continuous surveys meant to take early note of *Streptococcus pneumoniae* resistance to optochin be done within short frequencies as this is a very important *Streptococcus pneumoniae* diagnostic tool. Further, optochin susceptibility test should be introduced as one of the routine tests in the process of isolation and identification of *Streptococcus pneumoniae* and;

iv. Parents and/or caregivers should be advised to avoid exposing children to smoke whether actively or passively. Daycare attendance by young children should be discouraged as it provides a platform for horizontal transfer of pneumococcal infections.

REFERENCES

Abaye, G., Fekadu, H., Haji, K., Alemu, D., Anjulo, A. A., & Yadate, D. T. (2019). Prevalence and risk factors of pneumococcal nasopharyngeal carriage in healthy children attending kindergarten, in district of Arsi Zone, South East, Ethiopia. *BMC Research Notes*, *12*(1). https://doi.org/10.1186/s13104-019-4283-3

Alanee, S. R. J., McGee, L., Jackson, D., Chiou, C. C., Feldman, C., Morris, A. J., … Klugman, K. P. (2007). Association of Serotypes of *Streptococcus pneumoniae* with Disease Severity and Outcome in Adults: An International Study. *Clinical Infectious Diseases*, *45*(1), 46–51. https://doi.org/10.1086/518538

Andam, C. P., & Hanage, W. P. (2015). Mechanisms of genome evolution of Streptococcus. *Infection, Genetics and Evolution*, *33*, 334–342. https://doi.org/10.1016/j.meegid.2014.11.007

Andrade, A. L. S., Franco, C. M., Lamaro-Cardoso, J., André, M. C. D. P. B., Oliveira, L. L. G., Kipnis, A., … Brandileone, M. C. C. (2010). Non-typeable *Streptococcus pneumoniae* carriage isolates genetically similar to invasive and carriage isolates expressing capsular type 14 in Brazilian infants. *Journal of Infection*, *61*(4), 314–322. https://doi.org/10.1016/j.jinf.2010.07.003

Andre, G. O., Converso, T. R., Politano, W. R., Ferraz, L. F. C., Ribeiro, M. L., Leite, L. C. C., & Darrieux, M. (2017). Role of *Streptococcus Pneumoniae* proteins in evasion of complement-mediated immunity. *Frontiers in Microbiology*, *8*(FEB), 1–20. https://doi.org/10.3389/fmicb.2017.00224

Angoulvant, F., Cohen, R., Doit, C., Elbez, A., Werner, A., Béchet, S., … Levy, C. (2015). Trends in antibiotic resistance of *Streptococcus pneumoniae* and *Haemophilus influenzae* isolated from nasopharyngeal flora in children with acute otitis media in France before and after 13 valent pneumococcal conjugate vaccine introduction. *BMC Infectious Diseases,* 21; 15:236. https://doi.org/10.1186/s12879-015-0978-9

Aryal, S. (2018). Habitat and Morphology of *Streptococcus pneumoniae.* https://microbenotes.com/habitat-and-morphology-of-*Streptococcus-pneumoniae*/ Accessed: June 13th, 2020

Aryal, S. (2015). Optochin Susceptibility Test for the identification of *Streptococcus pneumoniae*.
https://microbenotes.com/optochin-susceptibility-test-principle-procedure-and-results/
Accessed: May 14th, 2020

Ásbjörnsdóttir, K. H., Slyker, J. A., Weiss, N. S., Mbori-Ngacha, D., Maleche-Obimbo, E., Wamalwa, D., & John-Stewart, G. (2013). Breastfeeding is associated with decreased pneumonia incidence among HIV-exposed, uninfected Kenyan infants. *AIDS*, *27*(17), 2809–2815.
https://doi.org/10.1097/01.aids.0000432540.59786.6d

Ayukekbong, J. A., Ntemgwa, M., & Atabe, A. N. (2017). The threat of antimicrobial resistance in developing countries: Causes and control strategies. *Antimicrobial Resistance and Infection Control,* 6:47.
https://doi.org/10.1186/s13756-017-0208-x

Bagaitkar, J., Demuth, D. R., & Scott, D. A. (2008). Tobacco use increases susceptibility to bacterial infection. *Tobacco Induced Diseases,* 18; 4(1):12.
https://doi.org/10.1186/1617-9625-4-12

Balachandran, P., Hollingshead, S. K., Paton, J. C., & Briles, D. E. (2001). The autolytic enzyme lytA of *Streptococcus pneumoniae* is not responsible for releasing pneumolysin. *Journal of Bacteriology,* 183(10): 3108–3116.
https://doi.org/10.1128/JB.183.10.3108-3116.2001

Bandettini, R., & Melioli, G. (2012). Laboratory diagnosis of *Streptococcus pneumoniae* infections: Past and future. *Journal of Preventive Medicine and Hygiene,* 53(2):85-8.
https://doi.org/10.15167/2421-4248/jpmh2012.53.2.321

Basset, A., Turner, K. H., Boush, E., Sayeed, S., Dove, S. L., & Malley, R. (2011). Expression of the type 1 pneumococcal pilus is bistable and negatively regulated by the structural component RrgA. *Infection and Immunity*, *79*(8), 2974–2983.
https://doi.org/10.1128/IAI.05117-11

Batard, E., Javaudin, F., Kervagoret, E., Caruana, E., Le Bastard, Q., Chapelet, G., … Montassier, E. (2018). Are third-generation cephalosporins associated with a better prognosis than amoxicillin–clavulanate in patients hospitalized in the medical ward for community-onset pneumonia? *Clinical Microbiology and Infection*, *24*(11), 1171–1176.
https://doi.org/10.1016/j.cmi.2018.06.021

Bebell, L. M., & Muiru, A. N. (2014). Antibiotic use and emerging resistance: How can resource-limited countries turn the tide? *Global Heart*, 9(3):347-58. https://doi.org/10.1016/j.gheart.2014.08.009

Berical, A. C., Harris, D., Dela Cruz, C. S., & Possick, J. D. (2016). Pneumococcal vaccination strategies: An update and perspective. *Annals of the American Thoracic Society*, 13(6):933-44. https://doi.org/10.1513/AnnalsATS.201511-778FR

Berild, J. D., Winje, B. A., Vestrheim, D. F., Slotved, H. C., Valentiner-Branth, P., Roth, A., & Storsäter, J. (2020). A systematic review of studies published between 2016 and 2019 on the effectiveness and efficacy of pneumococcal vaccination on pneumonia and invasive pneumococcal disease in an elderly population. *Pathogens*, 3; 9(4):259. https://doi.org/10.3390/pathogens9040259

Biezen, R., Grando, D., Mazza, D., & Brijnath, B. (2019). Visibility and transmission: Complexities around promoting hand hygiene in young children - A qualitative study. *BMC Public Health*, *19*(1). https://doi.org/10.1186/s12889-019-6729-x

Bou Ghanem, E. N., Maung, N. H. T., Siwapornchai, N., Goodwin, A. E., Clark, S., Muñoz-Elías, E. J., … Leong, J. M. (2018). Nasopharyngeal Exposure to *Streptococcus pneumoniae* Induces Extended Age-Dependent Protection against Pulmonary Infection Mediated by Antibodies and CD138 + Cells. *The Journal of Immunology*, *200*(11), 3739–3751. https://doi.org/10.4049/jimmunol.1701065

Bradshaw, J. L., & McDaniel, L. S. (2019). Selective pressure: Rise of the nonencapsulated pneumococcus. *PLoS Pathogens*, *15*(8). https://doi.org/10.1371/journal.ppat.1007911

Bronzwaer, S. L. A. M., Cars, O., Buchholz, U., Mölstad, S., Goettsch, W., Veldhuijzen, I. K., … Degener, J. E. (2002). The relationship between antimicrobial use and antimicrobial resistance in Europe. *Emerging Infectious Diseases*, *8*(3), 278–282. https://doi.org/10.3201/eid0803.010192

Brooks, L. R. K., & Mias, G. I. (2018). *Streptococcus pneumoniae's* virulence and host immunity: Aging, diagnostics, and prevention. *Frontiers in Immunology*, 22; 9:1366. https://doi.org/10.3389/fimmu.2018.01366

Brueggemann, A. B., Muroki, B. M., Kulohoma, B. W., Karani, A., Wanjiru, E., Morpeth, S., … Scott, J. A. G. (2013). Population genetic structure of *Streptococcus pneumoniae* in Kilifi, Kenya, prior to the introduction of pneumococcal conjugate vaccine. *PLoS ONE*, *8*(11). https://doi.org/10.1371/journal.pone.0081539

Brusch, J. L. (2020a). Pneumococcal Infections (*Streptococcus pneumoniae*) Medication. https://emedicine.medscape.com/article/225811-medication Accessed: May 15th, 2020

Brusch, J. L. (2020b). Pneumococcal Infections (*Streptococcus pneumoniae*) Medication. https://emedicine.medscape.com/article/225811-medication Accessed: June 05th, 2020

Buchy, P., Ascioglu, S., Buisson, Y., Datta, S., Nissen, M., Tambyah, P. A., & Vong, S. (2020). Impact of vaccines on antimicrobial resistance. *International Journal of Infectious Diseases*, (90) 188-196. https://doi.org/10.1016/j.ijid.2019.10.005

Burckhardt, I., Panitz, J., Burckhardt, F., & Zimmermann, S. (2017). Identification of *Streptococcus pneumoniae:* Development of a Standardized Protocol for Optochin Susceptibility Testing Using Total Lab Automation. *BioMed Research International*, 2017:4174168. https://doi.org/10.1155/2017/4174168

Caierão, J., Hawkins, P., Sant'anna, F. H., Da Cunha, G. R., D'Azevedo, P. A., McGee, L., & Dias, C. (2014). Serotypes and genotypes of invasive *Streptococcus pneumoniae* before and after PCV10 implementation in southern Brazil. *PLoS ONE*, *9*(10). https://doi.org/10.1371/journal.pone.0111129

Calatayud, L., Ardanuy, C., Tubau, F., Rolo, D., Grau, I., Pallarés, R., … Linares, J. (2010). Serotype and genotype replacement among macrolide-resistant invasive pneumococci in adults: Mechanisms of resistance and association with different transposons. *Journal of Clinical Microbiology*, *48*(4), 1310–1316. https://doi.org/10.1128/JCM.01868-09

Canadian Paediatric Society. (2001). Therapy of suspected bacterial meningitis in Canadian children six weeks of age and older. *Paediatrics & Child Health*, *6*(3), 147–152. https://doi.org/10.1093/pch/6.3.147

Carvalho, M. G. S., Steigerwalt, A. G., Thompson, T., Jackson, D., & Facklam, R. R. (2003). Confirmation of nontypeable *Streptococcus pneumoniae*-like organisms isolated from outbreaks of epidemic conjunctivitis as *Streptococcus pneumoniae*. *Journal of Clinical Microbiology,* 41(9): 4415–441.
https://doi.org/10.1128/JCM.41.9.4415-4417.2003

Casey, J. R., Kaur, R., Friedel, V. C., & Pichichero, M. E. (2013). Acute otitis media otopathogens during 2008 to 2010 in Rochester, New York. *Pediatric Infectious Disease Journal,* 32(8):805-809.
https://doi.org/10.1097/INF.0b013e31828d9acc

Center for Disease Control & Preventition. (2016). Identification and Characterization of *Streptococcus pneumoniae*.
https://www.cdc.gov/meningitis/lab-manual/chpt08-id-characterization-streppneumo.html
Accessed: June 09th, 2020

Center for Disease Control & Preventition. (2017). Pneumococcal disease, diagnosis and treatment. *National Center for Immunization and Respiratory Diseases*.
https://www.cdc.gov/pneumococcal/about/diagnosis-treatment.html
Accessed: June 13th, 2020

Centers for Disease Control and Prevention (US). (2010b). How Tobacco Smoke Causes Disease: The Biology and Behavioral Basis for Smoking-Attributable Disease. How Tobacco Smoke Causes Disease:
https://pubmed.ncbi.nlm.nih.gov/21452462/
Accessed: June 13th, 2020

Chaguza, C., Cornick, J. E., & Everett, D. B. (2015). Mechanisms and impact of genetic recombination in the evolution of *Streptococcus pneumoniae. Computational and Structural Biotechnology Journal,* 13: 241–247.
https://doi.org/10.1016/j.csbj.2015.03.007

Chao, Y., Marks, L. R., Pettigrew, M. M., & Hakansson, A. P. (2015). *Streptococcus pneumoniae* biofilm formation and dispersion during colonization and disease. *Frontiers in Cellular and Infection Microbiology,* 4: 194.
https://doi.org/10.3389/fcimb.2014.00194

Charpentier, E., & Tuomanen, E. (2000). Mechanisms of antibiotic resistance and tolerance in *Streptococcus pneumoniae*. *Microbes and Infection*, 2(15):1855-64.
https://doi.org/10.1016/S1286-4579(00)01345-9

Chiu, C. H., Su, L. H., Huang, Y. C., Lai, J. C., Chen, H. L., Wu, T. L., & Lin, T. Y. (2007). Increasing ceftriaxone resistance and multiple alterations of penicillin-binding proteins among penicillin-resistant *Streptococcus pneumoniae* isolates in Taiwan. *Antimicrobial Agents and Chemotherapy*, *51*(9), 3404–3406.
https://doi.org/10.1128/AAC.01563-06

Cillóniz, C., Garcia-Vidal, C., Ceccato, A., & Torres, A. (2018). Antimicrobial Resistance Among Streptococcus pneumoniae. In *Antimicrobial Resistance in the 21st Century* (pp. 13–38).
https://doi.org/10.1007/978-3-319-78538-7_2

Clarke, S. C., Scott, K. J., & McChlery, S. M. (2004). Serotypes and sequence types of pneumococci causing invasive disease in Scotland prior to the introduction of pneumococcal conjugate polysaccharide vaccines. *Journal of Clinical Microbiology*, *42*(10), 4449–4452.
https://doi.org/10.1128/JCM.42.10.4449-4452.2004

Cornick, J. E., Tastan Bishop, Ö., Yalcin, F., Kiran, A. M., Kumwenda, B., Chaguza, C., … Everett, D. B. (2017). The global distribution and diversity of protein vaccine candidate antigens in the highly virulent *Streptococcus pnuemoniae* serotype 1. *Vaccine*, *35*(6), 972–980.
https://doi.org/10.1016/j.vaccine.2016.12.037

Corthesy, B. (2013). Multi-faceted functions of secretory IgA at mucosal surfaces. *Frontiers in Immunology*, *4:185*, 1–2.
https://doi.org/https://doi.org/10.3389/fimmu.2013.00185

Cui, Y. A., Patel, H., O'Neil, W. M., Li, S., & Saddier, P. (2017). Pneumococcal serotype distribution: A snapshot of recent data in pediatric and adult populations around the world. *Human Vaccines and Immunotherapeutics*, *13*(6), 1229–1241.
https://doi.org/10.1080/21645515.2016.1277300

Daniels, C. C., Rogers, P. D., & Shelton, C. M. (2016). A review of pneumococcal vaccines: Current polysaccharide vaccine recommendations and future protein antigens. *Journal of Pediatric Pharmacology and Therapeutics*, 21(1):27-35.
https://doi.org/10.5863/1551-6776-21.1.27

de Angelis, G., Moschioni, M., Muzzi, A., Pezzicoli, A., Censini, S., Delany, I., … Barocchi, M. A. (2011). The S*treptococcus pneumoniae* Pilus-1 displays a biphasic expression pattern. *PLoS ONE*, *6*(6). https://doi.org/10.1371/journal.pone.0021269

DeStefano, F., Pfeifer, D., & Nohynek, H. (2008). Safety profile of pneumococcal conjugate vaccines: Systematic review of pre- and post-licensure data. *Bulletin of the World Health Organization*, *86*(5), 373–380. https://doi.org/10.2471/BLT.07.048025

Dias, C. A., Agnes, G., Frazzon, A. P. G., Kruger, F. D., D'Azevedo, P. A., Carvalho, M. D. G. S., … Teixeira, L. M. (2007). Diversity of mutations in the atpC gene coding for the c subunit of F 0F1 ATPase in clinical isolates of optochin-resistant *Streptococcus pneumoniae* from Brazil. *Journal of Clinical Microbiology*, *45*(9), 3065–3067. https://doi.org/10.1128/JCM.00891-07

Diavatopoulos, D. A., Short, K. R., Price, J. T., Wilksch, J. J., Brown, L. E., Briles, D. E., … Wijburg, O. L. (2010). Influenza A virus facilitates *Streptococcus pneumoniae* transmission and disease. *The FASEB Journal*, Vol. 24, pp. 1789-1798. https://doi.org/10.1096/fj.09-146779

Dickens, O., Gideon, K., Evans, A., & Jared, O. (2012). Risk factors of severe pneumonia among children aged 2-59 months in western Kenya: a case control study. *Pan African Medical Journal*, *13*(13), 1–13. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3542783/pdf/PAMJ-13-45.pdf

Donkor, E. S. (2013). Understanding the pneumococcus: transmission and evolution. *Frontiers in Cellular and Infection Microbiology*, 3(7). https://doi.org/10.3389/fcimb.2013.00007

Dunne, E. M., Choummanivong, M., Neal, E. F. G., Stanhope, K., Nguyen, C. D., Xeuatvongsa, A., … Russell, F. M. (2019). Factors associated with pneumococcal carriage and density in infants and young children in Laos PDR. *PLoS ONE*, *14*(10). https://doi.org/10.1371/journal.pone.0224392

European Antimicrobial Resistance Surveillance. (2018). Surveillance of antimicrobial resistance in Europe. *Stockholm, ECDC*, 47–49. https://www.ecdc.europa.eu/en/publications-data/surveillance-antimicrobial-resistance-europe-2018 Accessed: May 12th, 2020

Fadlyana, E., Dunne, E. M., Rusmil, K., Tarigan, R., Sudigdoadi, S., Murad, C., … Kartasasmita, C. (2018). Risk factors associated with nasopharyngeal carriage and density of *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*, and *Staphylococcus aureus* in young children living in Indonesia. *Pneumonia*, *10*(1). https://doi.org/10.1186/s41479-018-0058-1

Falkenhorst, G., Remschmidt, C., Harder, T., Hummers-Pradier, E., Wichmann, O., & Bogdan, C. (2017). Effectiveness of the 23-valent pneumococcal polysaccharide vaccine (ppv23) against pneumococcal disease in the elderly: Systematic review and meta-analysis. *PLoS ONE*, *12*(1). https://doi.org/10.1371/journal.pone.0169368

Food and Agricultural organization/Office International des Epizooties/World Health Organization. (2010). The FAO-OIE-WHO Collaboration. *A Tipartite Concept (Note).* https://www.who.int/foodsafety/areas_work/zoonose/concept-note/en/#:~:text=FAO%2FOIE%2FWHO%20Collaboration%20(Tripartite),the%20environments%20we%20all%20share. Accessed: May 20th, 2020

Feldman, C., & Anderson, R. (2014). Review: Current and new generation pneumococcal vaccines. *Journal of Infection*, 69(4):309-25. https://doi.org/10.1016/j.jinf.2014.06.006

Fine, P., Eames, K., & Heymann, D. L. (2011). "Herd immunity": A rough guide. *Clinical Infectious Diseases*, *52*(7), 911–916. https://doi.org/10.1093/cid/cir007

Fjeldhøj, S., Laursen, R. P., Larnkjær, A., Mølgaard, C., Fuursted, K., Krogfelt, K. A., & Slotved, H. C. (2018). Probiotics and carriage of *Streptococcus pneumoniae* serotypes in Danish children, a double-blind randomized controlled trial. *Scientific Reports*, *8*(1). https://doi.org/10.1038/s41598-018-33583-9

Frank, D. (2008). One world, one health, one medicine. *The Canadian Veterinary Journal = La Revue Veterinaire Canadienne*, *49*(11), 1063–5. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC2572090/ Accessed: June 17th, 2020

Gabriela, E; Soraia, I. G; Catarina, S. C; Catarina I. M; João A. C; María N. C; Barajas, A. S; Maria, E. V; José M. C; Antonio, L. V; Lucía, M. M; María, R. V; & Mário, R. C. (2020). *Streptococcus pneumoniae* Serotype 3 in Mexico (1994 to 2017): Decrease of the Unusual Clonal Complex 4909 Lineage following PCV13 Introduction. *Journal of Clinical Microbiology*, *57*(1), 1–2.
https://doi: 10.1128/JCM.01354-18

Geno, K. A., Gilbert, G. L., Song, J. Y., Skovsted, I. C., Klugman, K. P., Jones, C., … Nahm, M. H. (2015). Pneumococcal capsules and their types: Past, present, and future. *Clinical Microbiology Reviews*, 28(3):871-99.
https://doi.org/10.1128/CMR.00024-15

Global Alliance on Vaccines and Immunization. (2011). Kenya : pneumonia and pneumococcal disease, 1–2.
https://www.gavi.org/sites/default/files/document/kenya---pneumonia-and-pneumococcal-diseasepdf.pdf
Accessed: June 13th, 2020

Barbaros, H. Oral; & Cezmi, A. Akdis. (2003). Antibody-Based Therapies in Infectious Diseases. In *Diagnostic and Therapeutic Antibodies*, Vol 40 (pp. 157–178).
https://doi.org/10.1385/159259-076-4:157

Gladstone, R. A., Jefferies, J. M., Tocheva, A. S., Beard, K. R., Garley, D., Chong, W. W., … Clarke, S. C. (2015). Five winters of pneumococcal serotype replacement in UK carriage following PCV introduction. *Vaccine*, *33*(17), 2015–2021.
https://doi.org/10.1016/j.vaccine.2015.03.012

Government of Kenya & National Cordinating Agency for Population and Development. (2010). Equalizing Access to Family Planning Can Reduce Poverty and Improve Health. *Policy Brief*, *1*(10), 1–8.
http://www.ncpd.go.ke/wp-content/uploads/2016/11/Policy-Brief-10-Family-Planning-Inequality-Poverty-6Jul.pdf
Accessed: June 17th, 2020

Githii, S., Gunturu, R., Ann, Muigai., & Samuel, K (2013). Carriage rate and serotypes of *Streptococcus pneumoniae* amongst children in thika hospital, Kenya. *African Journal of Laboratory Medicine*, 2(1): 45.
https://doi: 10.4102/ajlm.v2i1.45

Gómez-Barreto, D., Calderón-Jaimes, E., Rodríguez, R. S., Espinosa, L. E., Viña-Flores, L., & Jiménez-Rojas, V. (2002). Carriage of antibiotic-resistant pneumococci in a cohort of a daycare center. *Salud Publica de Mexico*, *44*(1), 26–32. https://doi.org/10.1590/S0036-36342002000100004

Gotthelf, L. N. (2005). Diagnosis and treatment of otitis media. *Small Animal Ear Diseases*, 275–303. https://doi.org/10.1016/B0-72-160137-5/50017-9

Grabenstein, J. D., & Klugman, K. P. (2012). A century of pneumococcal vaccination research in humans. *Clinical Microbiology and Infection*, Suppl 5:15-24. https://doi.org/10.1111/j.1469-0691.2012.03943.x

Greenberg, D., Givon-Lavi, N., Broides, A., Blancovich, I., Peled, N., & Dagan, R. (2006). The Contribution of Smoking and Exposure to Tobacco Smoke to *Streptococcus pneumoniae* and Haemophilus influenzae Carriage in Children and Their Mothers. *Clinical Infectious Diseases*, 1; 42(7):897-903. https://doi.org/10.1086/500935

Griffith, F. (1928). The significance of penumococcal types. *Journal of Hygiene*, *64*(2), 129–175. https://doi.org/10.1017/S0022172400040420

Grijalva, C. G., Griffin, M. R., Edwards, K. M., Williams, J. V., Gil, A. I., Verastegui, H., … Lanata, C. F. (2014). The role of influenza and parainfluenza infections in nasopharyngeal pneumococcal acquisition among young children. *Clinical Infectious Diseases*, 58(10):1369-76. https://doi.org/10.1093/cid/ciu148

Habib, M., Porter, B. D., & Satzke, C. (2014). Capsular Serotyping of *Streptococcus pneumoniae* Using the Quellung Reaction. *Journal of Visualized Experiments*, (84), e51208. https://doi.org/10.3791/51208

Habitat for Humanity. (2017). Our Kenyan office helps communities in need to build homes and build hope to alleviate housing poverty in Kenya. https://www.habitatforhumanity.org.uk/country/kenya/ Accessed: June 14th, 2020

Hammerschmidt, S., Wolff, S., Hocke, A., Rosseau, S., Müller, E., & Rohde, M. (2005). Illustration of pneumococcal polysaccharide capsule during adherence and invasion of epithelial cells. *Infection and Immunity*, *73*(8), 4653–4667. https://doi.org/10.1128/IAI.73.8.4653-4667.2005

Hammitt, L. L., Akech, D. O., Morpeth, S. C., Karani, A., Kihuha, N., Nyongesa, S., … Scott, J. A. G. (2014). Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of *Streptococcus pneumoniae* and non-typeable *Haemophilus influenzae* in Kilifi, Kenya: Findings from cross-sectional carriage studies. *The Lancet Global Health*, *2*(7), e397–e405. https://doi.org/10.1016/S2214-109X(14)70224-4

Hammitt, L. L., Etyang, A. O., Morpeth, S. C., Ojal, J., Mutuku, A., Mturi, N., … Scott, J. A. G. (2019). Effect of ten-valent pneumococcal conjugate vaccine on invasive pneumococcal disease and nasopharyngeal carriage in Kenya : a longitudinal surveillance study. *The Lancet*, *393*(10186), 2146–2154. https://doi.org/10.1016/S0140-6736(18)33005-8

Harboe, Z. B., Thomsen, R. W., Riis, A., Valentiner-Branth, P., Christensen, J. J., Lambertsen, L., … Benfield, T. L. (2009). Pneumococcal serotypes and mortality following invasive pneumococcal disease: A population-based cohort study. *PLoS Medicine*, *6*(5). https://doi.org/10.1371/journal.pmed.1000081

Heath, C. J., Nayakwadi-Singer, M., King, C. H., Malhotra, I., Mutuku, F., Mukoko, D., & LaBeaud, A. D. (2018). Nasopharyngeal carriage of *Streptococcus pneumoniae* in children in Coastal Kenya. *American Journal of Tropical Medicine and Hygiene*, *98*(4), 1046–1050. https://doi.org/10.4269/ajtmh.16-0813

Henriques-Normark, B., & Tuomanen, E. I. (2013). The pneumococcus: Epidemiology, microbiology, and pathogenesis. *Cold Spring Harbor Perspectives in Medicine*, *3*(7), 1–15. https://doi.org/10.1101/cshperspect.a010215

Heritage Insurance Company. (2019). Heritage Partners with Gertrude's Hospital to Improve Health Access. https://heritageinsurance.co.ke/content/heritage-partners-gertrude's-hospital-improve-health-access Accessed: June 10th, 2020

Hiller, N. L., Ahmed, A., Powell, E., Martin, D. P., Eutsey, R., Earl, J., … Ehrlich, G. D. (2010). Generation of genic diversity among *Streptococcus pneumoniae* strains via horizontal gene transfer during a chronic polyclonal pediatric infection. *PLoS Pathogens*, *6*(9). https://doi.org/10.1371/journal.ppat.1001108

Hiller, N. L., & Sá-Leão, R. (2018). Puzzling Over the Pneumococcal Pangenome. *Frontiers in Microbiology*, *9*. https://doi.org/10.3389/fmicb.2018.02580

Hirst, R. A., Kadioglu, A., O'Callaghan, C., & Andrew, P. W. (2004). The role of pneumolysin in pneumococcal pneumonia and meningitis. *Clinical and Experimental Immunology,* 138(2):195-201. https://doi.org/10.1111/j.1365-2249.2004.02611.x

Hjálmarsdóttir, M., Quirk, S. J., Haraldsson, G., Erlendsdóttir, H., Haraldsson, Á., & Kristinsson, K. G. (2017). Comparison of serotype prevalence of pneumococci isolated from middle ear, lower respiratory tract and invasive disease prior to vaccination in Iceland. *PLoS ONE*, *12*(1). https://doi.org/10.1371/journal.pone.0169210

Howie, S. R. C., Schellenberg, J., Chimah, O., Ideh, R. C., Ebruke, B. E., Oluwalana, C., … Mulholland, K. (2016). Childhood pneumonia and crowding, bed-sharing and nutrition: A case-control study from The Gambia. *International Journal of Tuberculosis and Lung Disease*, *20*(10), 1405–1415. https://doi.org/10.5588/ijtld.15.0993

Hupp, S., Grandgirard, D., Mitchell, T. J., Leib, S. L., Hathaway, L. J., & Iliev, A. I. (2019). Pneumolysin and the bacterial capsule of *Streptococcus pneumoniae* cooperatively inhibit taxis and motility of microglia. *Journal of Neuroinflammation*, *16*(1). https://doi.org/10.1186/s12974-019-1491-7

Hyams, C., Camberlein, E., Cohen, J. M., Bax, K., & Brown, J. S. (2010). The *Streptococcus pneumoniae* capsule inhibits complement activity and neutrophil phagocytosis by multiple mechanisms. *Infection and Immunity*, Vol. 78, No. 2, p. 704–715. https://doi.org/10.1128/IAI.00881-09

Imöhl, M., Reinert, R. R., Ocklenburg, C., & Van Linden, M. Der. (2010). Association of Serotypes of *Streptococcus pneumoniae* with Age in Invasive Pneumococcal Disease. *Journal of Clinical Microbiology,* 48(4):1291-6. https://doi.org/10.1128/JCM.01937-09

Ing, J., Mason, E. O., Kaplan, S. L., Lamberth, L. B., Revell, P. A., Luna, R. A., & Hulten, K. G. (2012). Characterization of nontypeable and atypical *Streptococcus pneumoniae* pediatric isolates from 1994 to 2010. *Journal of Clinical Microbiology*, 50(4). https://doi.org/10.1128/JCM.05182-11

Inostroza, J., Vinet, A. M., Retamal, G., Lorca, P., Ossa, G., Facklam, R. R., & Sorensen, R. U. (2002). Influence of Patient Age on *Streptococcus pneumoniae* Serotypes Causing Invasive Disease. *Clinical and Vaccine Immunolog*, 8(3): 556–559. https://doi.org/10.1128/cdli.8.3.556-559.2001

Intra, J., Besana, S., Savarino, C., & Brambilla, P. (2017). Serotype Distribution and Antimicrobial Resistance of *Streptococcus pneumoniae* Invasive Isolates Collected at the Italian Hospital of Desio, Lombardy, from 2008 to 2016. *Frontiers in Public Health*, 14; 5:169. https://doi.org/10.3389/fpubh.2017.00169

Janoff, E. N., Rubins, J. B., Fasching, C., Charboneau, D., Rahkola, J. T., Plaut, A. G., & Weiser, J. N. (2014). Pneumococcal IgA1 protease subverts specific protection by human IgA1. *British Dental Journal*, **7**, 249–25. https://doi.org/10.1038/mi.2013.41

Jaspers, I. (2016). Abstract Pulmonary Effects of Exposure to Tobacco Smoke and New Tobacco Products. In *2016 AAAS Annual Meeting (February 11-15, 2016)*. https://aaas.confex.com/aaas/2016/webprogram/Paper16208.html Accessed: April 16th, 2020

Jauneikaite, E., Tocheva, A. S., Jefferies, J. M. C., Gladstone, R. A., Faust, S. N., Christodoulides, M., … Clarke, S. C. (2015). Current methods for capsular typing of *Streptococcus pneumoniae*. *Journal of Microbiological Methods*, 113:41-9. https://doi.org/10.1016/j.mimet.2015.03.006

Jedrzejas, M. J. (2001). Pneumococcal Virulence Factors: Structure and Function. *Microbiology and Molecular Biology Reviews*, *65*(2), 187–207. https://doi.org/10.1128/mmbr.65.2.187-207.2001

Johnson, H. L., Deloria-Knoll, M., Levine, O. S., Stoszek, S. K., Hance, L. F., Reithinger, R., … O'Brien, K. L. (2010). Systematic evaluation of serotypes causing invasive pneumococcal disease among children under five: The pneumococcal global serotype project. *PLoS Medicine*, *7*(10). https://doi.org/10.1371/journal.pmed.1000348

Fisher, A; John, W; Laing, J; & Stoeckel, J. (1991). Handbook for family planning operations research design. 2nd ed. TT, Pg. 1-75. https://file:///C:/Users/User/Desktop/Fisher%20et%20al%20-sample%20Size%20Determination%20Formulae.pdf Accessed: April 16th, 2020

Kamper-Jorgensen, M., Wohlfahrt, J., Simonsen, J., Gronbaek, M., & Benn, C. S. (2006). Population-Based Study of the Impact of Childcare Attendance on Hospitalizations for Acute Respiratory Infections. *Pediatrics*, 118(4):1439-46. https://doi.org/10.1542/peds.2006-0373

Kanungo, R. (2015). Knowledge of serotype prevalence & burden of invasive pneumococcal disease: A prerequisite to vaccine introduction in the country. *Indian Journal of Medical Research,* 142(3): 241–244. https://doi.org/10.4103/0971-5916.166528

Kapoor, G., Saigal, S., & Elongavan, A. (2017). Action and resistance mechanisms of antibiotics: A guide for clinicians. *Journal of Anaesthesiology Clinical Pharmacology*, 33(3):300-305. https://doi.org/10.4103/joacp.JOACP_349_15

Kato, T., Yorifuji, T., Yamakawa, M., Inoue, S., Doi, H., Eboshida, A., & Kawachi, I. (2017). Association of maternal age with child health: A Japanese longitudinal study. *PLoS ONE*, *12*(2), 1–11. https://doi.org/10.1371/journal.pone.0172544

Kawaguchiya, M., Urushibara, N., Aung, M. S., Shinagawa, M., Takahashi, S., & Kobayashi, N. (2017). Serotype distribution, antimicrobial resistance and prevalence of pilus islets in pneumococci following the use of conjugate vaccines. *Journal of Medical Microbiology*, *66*(5), 643–650. https://doi.org/10.1099/jmm.0.000479

Keller, L. E., Robinson, D. A., & McDaniel, L. S. (2016). Nonencapsulated *Streptococcus pneumoniae* : Emergence and Pathogenesis . *mBio,* 22; 7(2):e01792. https://doi.org/10.1128/mbio.01792-15

Kellogg, J. A., Bankert, D. A., Elder, C. J., Gibbs, J. L., & Smith, M. C. (2001). Identification of *Streptococcus pneumoniae* revisited. *Journal of Clinical Microbiology*, *39*(9), 3373–3375. https://doi.org/10.1128/JCM.39.9.3373-3375.2001

Kesmodel, U. S. (2018). Cross-sectional studies – what are they good for? *Acta Obstetricia et Gynecologica Scandinavica*, *97*(4), 388–393. https://doi.org/10.1111/aogs.13331

Kim, J. H., Chun, B. C., Song, J. Y., Kim, H. Y., Bae, I. G., Kim, D. M., … Kim, M. J. (2019). Direct effectiveness of pneumococcal polysaccharide vaccine against invasive pneumococcal disease and non-bacteremic pneumococcal pneumonia in elderly population in the era of pneumococcal conjugate vaccine: A case-control study. *Vaccine*, *37*(21), 2797–2804. https://doi.org/10.1016/j.vaccine.2019.04.017

Kim, L., McGee, L., Tomczyk, S., & Beall, B. (2016). Biological and epidemiological features of antibiotic-resistant *Streptococcus pneumoniae* in pre- and post-conjugate vaccine eras: A United States perspective. *Clinical Microbiology Reviews*, 29(3):525-52. https://doi.org/10.1128/CMR.00058-15

Kirkham, L. A. S., Jefferies, J. M. C., Kerr, A. R., Jing, Y., Clarke, S. C., Smith, A., & Mitchell, T. J. (2006). Identification of invasive serotype 1 pneumococcal isolates that express nonhemolytic pneumolysin. *Journal of Clinical Microbiology*, *44*(1), 151–159. https://doi.org/10.1128/JCM.44.1.151-159.2006

Kodish, S. (2018). The power of narratives: A new understanding of antibiotic resistance. *International Journal of Communication*, *12*, 745–765. https://ijoc.org/index.php/ijoc/article/view/7413 Accessed: April 16th, 2020

Koliou, M. G., Andreou, K., Lamnisos, D., Lavranos, G., Iakovides, P., Economou, C., & Soteriades, E. S. (2018). Risk factors for carriage of *Streptococcus pneumoniae* in children. *BMC Pediatrics*, 26; 18(1):144. https://doi.org/10.1186/s12887-018-1119-6

Kong, K. F., Schneper, L., & Mathee, K. (2010). Beta-lactam antibiotics: From antibiosis to resistance and bacteriology. *APMIS*, 118(1):1-36. https://doi.org/10.1111/j.1600-0463.2009.02563.x

Kumar, Sg., Adithan, C., Harish, B., Roy, G., Malini, A., & Sujatha, S. (2013). Antimicrobial resistance in India: A review. *Journal of Natural Science, Biology and Medicine*, 4(2): 286–291. https://doi.org/10.4103/0976-9668.116970

Kwak, B. O., Choung, J. T., & Park, Y. M. (2015). The association between asthma and invasive pneumococcal disease: A nationwide study in Korea. *Journal of Korean Medical Science*, *30*(1), 60–65. https://doi.org/10.3346/jkms.2015.30.1.60

Laffont, S., Seillet, C., & Guéry, J. C. (2017). Estrogen receptor-dependent regulation of dendritic cell development and function. *Frontiers in Immunology,* 8: 108. https://doi.org/10.3389/fimmu.2017.00108

Lambert, S. B., Whiley, D. M., O'Neill, N. T., Andrews, E. C., Canavan, F. M., Bletchly, C., … Nissen, M. D. (2008). Comparing Nose-Throat Swabs and Nasopharyngeal Aspirates Collected From Children With Symptoms for Respiratory Virus Identification Using Real-Time Polymerase Chain Reaction. *Pediatrics*, *122*(3), e615–e620. https://doi.org/10.1542/peds.2008-0691

Latifi-Navid, H., Latifi-Navid, S., Mostafaiy, B., Jamalkandi, S. A., & Ahmadi, A. (2018). Pneumococcal Disease and the Effectiveness of the PPV23 Vaccine in Adults: A Two-Stage Bayesian Meta-Analysis of Observational and RCT Reports. *Scientific Reports*, *8*(1), 11051. https://doi.org/10.1038/s41598-018-29280-2

Leclercq, R., & Courvalin, P. (2002). Resistance to macrolides and related antibiotics in *Streptococcus pneumoniae*. *Antimicrobial Agents and Chemotherapy,* 46(9):2727-34. https://doi.org/10.1128/AAC.46.9.2727-2734.2002

Lee, D. S., Choe, H. S., Kim, H. Y., Yoo, J. M., Bae, W. J., Cho, Y. H., … Lee, S. ju. (2016). Role of age and sex in determining antibiotic resistance in febrile urinary tract infections. *International Journal of Infectious Diseases*, *51*, 89–96. https://doi.org/10.1016/j.ijid.2016.08.015

Lee, H. Y., Wu, T. L., Su, L. H., Li, H. C., Janapatla, R. P., Chen, C. L., & Chiu, C. H. (2018). Invasive pneumococcal disease caused by ceftriaxone-resistant *Streptococcus pneumoniae* in Taiwan. *Journal of Microbiology, Immunology and Infection*, *51*(4), 500–509. https://doi.org/10.1016/j.jmii.2016.12.004

Levy, C., Ouldali, N., Caeymaex, L., Angoulvant, F., Varon, E., & Cohen, R. (2019). Diversity of Serotype Replacement After Pneumococcal Conjugate Vaccine Implementation in Europe. *Journal of Pediatrics,* 213(252-253). https://doi.org/10.1016/j.jpeds.2019.07.057

Li, J., Xie, S., Ahmed, S., Wang, F., Gu, Y., Zhang, C., … Cheng, G. (2017). Antimicrobial activity and resistance: Influencing factors. *Frontiers in Pharmacology*, 13; 8:364.
https://doi.org/10.3389/fphar.2017.00364

Licciardi, P. V., Balloch, A., Russell, F. M., Burton, R. L., Lin, J., Nahm, M. H., … Tang, M. L. K. (2012). Pneumococcal polysaccharide vaccine at 12 months of age produces functional immune responses. *Journal of Allergy and Clinical Immunology*, *129*(3).
https://doi.org/10.1016/j.jaci.2011.11.043

Liñares, J., Ardanuy, C., Pallares, R., & Fenoll, A. (2010). Changes in antimicrobial resistance, serotypes and genotypes in *Streptococcus pneumoniae* over a 30-year period. *Clinical Microbiology and Infection*, 16(5):402-10.
https://doi.org/10.1111/j.1469-0691.2010.03182.x

Livermore, D. M. (2012). Mechanisms of Resistance to Cephalosporin Antibiotics. *Springer Link*, *34*, 64–88.
https://link.s pringer.com/article/10.2165/00003495-198700342-00007
Accessed: April 16th, 2020

Løvlie, A., Vestrheim, D. F., Aaberge, I. S., & Steens, A. (2020). Changes in pneumococcal carriage prevalence and factors associated with carriage in Norwegian children, four years after introduction of PCV13. *BMC Infectious Diseases*, *20*(1).
https://doi.org/10.1186/s12879-019-4754-0

Lu, C., Black, M. M., & Richter, L. M. (2016). Risk of poor development in young children in low-income and middle-income countries: an estimation and analysis at the global, regional, and country level. *The Lancet Global Health*, 4(12):e916-e922.
https://doi.org/10.1016/S2214-109X(16)30266-2

Maestro, B., & Sanz, J. M. (2016). Choline binding proteins from *Streptococcus pneumoniae*: A dual role as enzybiotics and targets for the design of new antimicrobials. *Antibiotics*, 14; 5(2):21.
https://doi.org/10.3390/antibiotics5020021

Maianskiĭ, N. A., Aliab'eva, N. M., Lazareva, A. V., & Katosova, L. K. (2014). Serotype diversity and antimicrobial resistance of *Streptococcus pneumoniae. Vestn Ross Akad Med Nauk*, (7-8):38-45
https://doi.org/10.15690/vramn.v69i7-8.1108

Malik, B., & Bhattacharyya, S. (2019). Antibiotic drug-resistance as a complex system driven by socio-economic growth and antibiotic misuse. *Scientific Reports*, *9*(1). https://doi.org/10.1038/s41598-019-46078-y

Mamishi, S., Moradkhani, S., Mahmoudi, S., Hosseinpour - Sadeghi, R., & Pourakbari, B. (2014). Penicillin-Resistant trend of *Streptococcus pneumoniae* in Asia: A systematic review. *Iranian Journal of Microbiology*, *6*(4), 198–210. https://www.ncbi.nlm.nih.gov/pmc/articles/PMC4367934/pdf/IJM-6-198.pdf Accessed: April 16th, 2020

Maurice, D; A., Schaffner, W., Griffin, M. R., Halasa, N., & Grijalva, C. G. (2016). Persistent sex disparities in invasive pneumococcal diseases in the conjugate vaccine era. *Journal of Infectious Diseases*, *214*(5), 792–797. https://doi.org/10.1093/infdis/jiw222

Marks, L. R., Davidson, B. A., Knight, P. R., & Hakansson, A. P. (2013). Interkingdom signaling induces *Streptococcus pneumoniae* biofilm dispersion and transition from asymptomatic colonization to disease. *mBio*, 23;4(4):e00438-13. https://doi.org/10.1128/mBio.00438-13

Mathews, T. J., & Hamilton, B. E. (2016). Mean Age of Mothers is on the Rise: United States, 2000-2014. *NCHS Data Brief*, no 232. Hyattsville, MD: National Center for Health Statistics (232), 1–8. https://www.cdc.gov/nchs/data/databriefs/db232.pdf Accessed: April 14th, 2020

Mavroidi, A., Aanensen, D. M., Godoy, D., Skovsted, I. C., Kaltoft, M. S., Reeves, P. R., … Spratt, B. G. (2007). Genetic relatedness of the *Streptococcus pneumoniae* capsular biosynthetic loci. *Journal of Bacteriology*, 189(21):7841-55. https://doi.org/10.1128/JB.00836-07

Mbithe, F. (2012). Determinants Of The Uptake Of Pneumococcal Conjugate Vaccine In Makindu District Kenya. *Kenyatta University Repository*. http://ir-library.ku.ac.ke/handle/123456789/6864 Accessed: March 16th, 2020

Mehr, S., & Wood, N. (2012). *Streptococcus pneumoniae* - a review of carriage, infection, serotype replacement and vaccination. *Paediatric Respiratory Reviews*, 13(4):258-64. https://doi.org/10.1016/j.prrv.2011.12.001

Melki, I. S., Beydoun, H. A., Khogali, M., Tamim, H., & Yunis, K. A. (2004). Household crowding index: A correlate of socioeconomic status and inter-pregnancy spacing in an urban setting. *Journal of Epidemiology and Community Health*, *58*(6), 476–480. https://doi.org/10.1136/jech.2003.012690

Mellroth, P., Daniels, R., Eberhardt, A., Rönnlund, D., Blom, H., Widengren, J., … Henriques-Normark, B. (2012). LytA, major autolysin of *Streptococcus pneumoniae*, requires access to nascent peptidoglycan. *Journal of Biological Chemistry*, *287*(14), 11018–11029. https://doi.org/10.1074/jbc.M111.318584

Menéndez, R., España, P. P., Pérez-Trallero, E., Uranga, A., Méndez, R., Cilloniz, C., … Torres, A. (2017). The burden of PCV13 serotypes in hospitalized pneumococcal pneumonia in Spain using a novel urinary antigen detection test. CAPA study. *Vaccine*, *35*(39), 5264–5270. https://doi.org/10.1016/j.vaccine.2017.08.007

Mills, G. (2014). One Health: Antimicrobial resistance and disease control: making One Health work. *The Veterinary Record*, 15; 175(19):473-4. https://doi.org/10.1136/vr.g6458

Mitchell, A. M., & Mitchell, T. J. (2010). *Streptococcus pneumoniae*: Virulence factors and variation. *Clinical Microbiology and Infection*, 16(5), 411-418. https://doi.org/10.1111/j.1469-0691.2010.03183.x

Mobarki, N., Almerabi, B., & Hattan, A. (2019). Antibiotic Resistance Crisis. *International Journal of Medicine in Developing Countries*, *40*(4), 561–564. https://doi.org/10.24911/ijmdc.51-1549060699

Mody, L. (2007). Infection Control Issues in Older Adults. *Clinics in Geriatric Medicine*, 23(3):499-514. https://doi.org/10.1016/j.cger.2007.02.001

Mollendorf, C. Von; Cohen, C., De Gouveia, L., Quan, V., Meiring, S., Feldman, C., … Von Gottberg, A. (2014). Factors associated with ceftriaxone nonsusceptibility of *Streptococcus pneumoniae:* Analysis of south african national surveillance data, 2003 to 2010. *Antimicrobial Agents and Chemotherapy*, 58(6):3293-305. https://doi.org/10.1128/AAC.02580-13

Molyneux, E., Nizami, S. Q., Saha, S., Huu, K. T., Azam, M., Bhutta, Z. A., … Qazi, S. A. (2011). 5 versus 10 days of treatment with ceftriaxone for bacterial meningitis in children: A double-blind randomised equivalence study. *The Lancet*, 28; 377(9780):1837-45. https://doi.org/10.1016/S0140-6736(11)60580-1

Montagnani, F., Zanchi, A., Stolzuoli, L., Croci, L., & Cellesi, C. (2007). Clindamycin-resistant *Streptococcus pneumoniae*. *Emerging Infectious Diseases*, 13(5): 801–802. https://doi.org/10.3201/eid1305.060699

Morpeth, S. C., Munywoki, P., Hammitt, L. L., Bett, A., Bottomley, C., Onyango, C. O., … Scott, J. A. G. (2018). Impact of viral upper respiratory tract infection on the concentration of nasopharyngeal pneumococcal carriage among Kenyan children. *Scientific Reports*, 8:11030. https://doi.org/10.1038/s41598-018-29119

Mostowy, R. J., Croucher, N. J., De Maio, N., Chewapreecha, C., Salter, S. J., Turner, P., … Fraser, C. (2017). Pneumococcal Capsule Synthesis Locus cps as Evolutionary Hotspot with Potential to Generate Novel Serotypes by Recombination. *Molecular Biology and Evolution*, *34*(10), 2537–2554. https://doi.org/10.1093/molbev/msx173

Mukokinya, M. A., Opanga, S., Oluka, M., & Godman, B. (2018). Dispensing of antimicrobials in Kenya: A cross-sectional pilot study and its implications. *Journal of Research in Pharmacy Practice*, *7*(2), 77. https://doi.org/10.4103/jrpp.jrpp_17_88

Munita, J. M., & Arias, C. A. (2016). Mechanisms of Antibiotic Resistance. In *Virulence Mechanisms of Bacterial Pathogens* (pp. 481–511). https://doi.org/10.1128/9781555819286.ch17

Murrah, K. A., Pang, B., Richardson, S., Perez, A., Reimche, J., King, L., … Swords, W. E. (2015). Nonencapsulated *Streptococcus pneumoniae* causes otitis media during single-species infection and during polymicrobial infection with nontypeable Haemophilus influenzae. *Pathogens and Disease*, *73*(5). https://doi.org/10.1093/femspd/ftu011

Mutua, J. M., Gitao, C. G., Bebora, L. C., & Mutua, F. K. (2017). Antimicrobial Resistance Profiles of Bacteria Isolated from the Nasal Cavity of Camels in Samburu, Nakuru, and Isiolo Counties of Kenya. *Journal of Veterinary Medicine*, 1–6. https://doi.org/10.1155/2017/1216283

Nafstad, P., Jaakkola, J. J. K., Skrondal, A., & Magnus, P. (2005). Day care center characteristics and children's respiratory health. *Indoor Air*, 15(2):69-75. https://doi.org/10.1111/j.1600-0668.2004.00310.x

Nagaraj, S., Kalal, B. S., Manoharan, A., & Shet, A. (2017). *Streptococcus pneumoniae* serotype prevalence and antibiotic resistance among young children with invasive pneumococcal disease: experience from a tertiary care center in South India. *GERMS*, 1; 7(2):78-85. https://doi.org/10.18683/germs.2017.1112

Nagata, M., Ueda, O., Shobuike, T., Muratani, T., Aoki, Y., & Miyamoto, H. (2012). Emergence of Optochin Resistance among *Streptococcus pneumoniae* in Japan. *Open Journal of Medical Microbiology*, *2*(1), 8–15. https://doi.org/10.4236/ojmm.2012.21002

Neill, D. R., Coward, W. R., Gritzfeld, J. F., Richards, L., Garcia-Garcia, F. J., Dotor, J., … Kadioglu, A. (2014). Density and duration of pneumococcal carriage is maintained by transforming growth factor β1 and T regulatory cells. *American Journal of Respiratory and Critical Care Medicine*, *189*(10), 1250–1259. https://doi.org/10.1164/rccm.201401-0128OC

Ngocho, J. S., Magoma, B., Olomi, G. A., Mahande, M. J., Msuya, S. E., de Jonge, M. I., & Mmbaga, B. T. (2019). Effectiveness of pneumococcal conjugate vaccines against invasive pneumococcal disease among children under five years of age in Africa: A systematic review. *PLoS ONE*, *14*(2). https://doi.org/10.1371/journal.pone.0212295

Novick, S., Shagan, M., Blau, K., Lifshitz, S., Givon-Lavi, N., Grossman, N., … Nebenzahl, Y. M. (2017). Adhesion and invasion of *Streptococcus pneumoniae* to primary and secondary respiratory epithelial cells. *Molecular Medicine Reports*, *15*(1), 65–74. https://doi.org/10.3892/mmr.2016.5996

Nuermberger, E. L., & Bishai, W. R. (2004). Antibiotic Resistance in *Streptococcus pneumoniae*: What Does the Future Hold? *Clinical Infectious Diseases*, *38*(Suppl_4), S363–S371. https://doi.org/10.1086/382696

Nurse-Lucas, M., McGee, L., Hawkins, P. A., Swanston, W. H., & Akpaka, P. E. (2016). Serotypes and genotypes of *Streptococcus pneumoniae* isolates from Trinidad and Tobago. *International Journal of Infectious Diseases*, 46:100-6. https://doi.org/10.1016/j.ijid.2016.04.005

O'Neill, J. (2014). Antimicrobial Resistance : Tackling a crisis for the health and wealth of nations. *The Review on Antimicrobial Resistance*. https://amr-review.org/sites/default/files/AMR%20Review%20Paper%20-%20Tackling%20a%20crisis%20for%20the%20health%20and%20wealth%20of%20nations_1.pdf
Accessed: April 08th, 2020

Oliveira, P. D. de, Bertoldi, A. D., Silva, B. G. C. d., Rodrigues Domingues, M., Neumann, N. A., & Silveira, M. F. da. (2019). Day care attendance during the first 12 months of life and occurrence of infectious morbidities and symptoms. *Jornal de Pediatria*, *95*(6), 657–666. https://doi.org/10.1016/j.jped.2018.05.012

Pan, F., Han, L., Huang, W., Tang, J., Xiao, S., Wang, C., … Zhang, H. (2015). Serotype distribution, antimicrobial susceptibility, and molecular epidemiology of *Streptococcus pneumoniae* isolated from children in Shanghai, China. *PLoS ONE*, *10*(11). https://doi.org/10.1371/journal.pone.0142892

Parker, D., Soong, G., Planet, P., Brower, J., Ratner, A. J., & Prince, A. (2009). The NanA neuraminidase of *Streptococcus pneumoniae* is involved in biofilm formation. *Infection and Immunity*, 77(9): 3722–3730. https://doi.org/10.1128/IAI.00228-09

Paterson, G. K., & Mitchell, T. J. (2006). Innate immunity and the pneumococcus. *Microbiology,* 152(Pt. 2):285-293. https://doi.org/10.1099/mic.0.28551-0

Paton, J. C., & Trappetti, C. (2019). *Streptococcus pneumoniae* Capsular Polysaccharide . In *Gram-Positive Pathogens* (pp. 304–315). https://doi.org/10.1128/9781683670131.ch19

Peterson, E., & Kaur, P. (2018). Antibiotic resistance mechanisms in bacteria: Relationships between resistance determinants of antibiotic producers, environmental bacteria, and clinical pathogens. *Frontiers in Microbiology*, 30; 9:2928. https://doi.org/10.3389/fmicb.2018.02928

Peterson, L. R. (2006). Penicillins for Treatment of Pneumococcal Pneumonia: Does In Vitro Resistance Really Matter? *Clinical Infectious Diseases*, *42*(2), 224–233. https://doi.org/10.1086/497594

Pettigrew, M. M., Fennie, K. P., York, M. P., Daniels, J., & Ghaffar, F. (2006). Variation in the presence of neuraminidase genes among *Streptococcus pneumoniae* isolates with identical sequence types. *Infection and Immunity*, 74(6):3360-5. https://doi.org/10.1128/IAI.01442-05

Pikis, A., Campos, J. M., Rodriguez, W. J., & Keith, J. M. (2002). Optochin Resistance in Streptococcus pneumoniae: Mechanism, Significance, and Clinical Implications. *The Journal of Infectious Diseases*, 1; 184(5):582-90. https://doi.org/10.1086/322803

Pinto, T. C. A., Souza, A. R. V., De Pina, S. E. C. M., Costa, N. S., Neto, A. A. B., Neves, F. P. G., … Teixeira, L. M. (2013). Phenotypic and molecular characterization of optochin-resistant *Streptococcus pneumoniae* isolates from Brazil, with description of five novel mutations in the *atpC* gene. *Journal of Clinical Microbiology*, 1(10):3242-9. https://doi.org/10.1128/JCM.01168-13

Potrich, A. C. G., Vieira, K. M., & Kirch, G. (2015). Determinants of financial literacy: Analysis of the influence of socioeconomic and demographic variables. *Revista Contabilidade E Financas*, 26(69). https://doi.org/10.1590/1808-057x201501040

Randle, E., Ninis, N., & Inwald, D. (2011). Invasive pneumococcal disease. *Archives of Disease in Childhood: Education and Practice Edition*, 96(5):183-90. https://doi.org/10.1136/adc.2010.191718

Reinert, R. R. (2009a). The antimicrobial resistance profile of *Streptococcus pneumoniae*. *Clinical Microbiology and Infection*, Suppl 3:7-11. https://doi.org/10.1111/j.1469-0691.2009.02724.x

Reinert, R. R. (2009b). The antimicrobial resistance profile of *Streptococcus pneumoniae*. *Clinical Microbiology and Infection*, Suppl 3:7-11. https://doi.org/10.1111/j.1469-0691.2009.02724.x

Robson, R. L., Essengue, S., Reed, N. A., & Horvat, R. T. (2007). Optochin resistance in *Streptococcus pneumoniae* induced by frozen storage in glycerol. *Diagnostic Microbiology and Infectious Disease*, *58*(2), 185–190. https://doi.org/10.1016/j.diagmicrobio.2007.01.006

Rodgers, G. L., & Klugman, K. P. (2016). Surveillance of the impact of pneumococcal conjugate vaccines in developing countries. *Human Vaccines and Immunotherapeutics*, *12*(2), 417–420. https://doi.org/10.1080/21645515.2015.1057671

Sabin, A. B. (1933). Immediate pneumococcus typing directly from sputum by the neufeld reaction. *Journal of the American Medical Association*, *100*(20), 1584–1586. https://doi.org/10.1001/jama.1933.02740200018004

Sánchez-Borges, M., Thong, B., Blanca, M., Ensina, L. F. C., González-Díaz, S., Greenberger, P. A., … Jaén, M. J. T. (2013). Hypersensitivity reactions to non beta-lactam antimicrobial agents, a statement of the WAO special committee on drug allergy. *World Allergy Organization Journal*, 6(1):18. https://doi.org/10.1186/1939-4551-6-18

Schrag, S. (2007). Pneumococcal disease : Global burden , epidemiology , scope for vaccine prevention Pneumococcal Carriage and Disease, (August). https://www.sabin.org/sites/sabin.org/files/%282%2920_9_0900_stephanie_schrag.pdf Accessed: May 1st, 2020

Schrag, S. J., Beall, B., & Dowell, S. F. (2000). Limiting the spread of resistant pneumococci: Biological and epidemiologic evidence for the effectiveness of alternative interventions. *Clinical Microbiology Reviews*, 13(4):588-601. https://doi.org/10.1128/CMR.13.4.588-601.2000

Schuez-Havupalo, L., Toivonen, L., Karppinen, S., Kaljonen, A., & Peltola, V. (2017). Daycare attendance and respiratory tract infections: A prospective birth cohort study. *BMJ Open*, 7:e014635. https://doi.org/10.1136/bmjopen-2016-014635

Shak, J. R., Vidal, J. E., & Klugman, K. P. (2013). Influence of bacterial interactions on pneumococcal colonization of the nasopharynx. *Trends in Microbiology*, 21(3):129-35. https://doi.org/10.1016/j.tim.2012.11.005

Shiel, W. C. (2020). Medical Definition of Serotype. https://www.medicinenet.com/script/main/art.asp?articlekey=5469 Accessed: May 1st, 2020

Shrestha, P., Cooper, B. S., Coast, J., Oppong, R., Do Thi Thuy, N., Phodha, T., … Lubell, Y. (2018). Enumerating the economic cost of antimicrobial resistance per antibiotic consumed to inform the evaluation of interventions affecting their use. *Antimicrobial Resistance and Infection Control*, *7*(1). https://doi.org/10.1186/s13756-018-0384-3

Sime, W. T., Aseffa, A., Woldeamanuel, Y., Brovall, S., Morfeldt, E., & Henriques-Normark, B. (2019). Serotype and molecular diversity of nasopharyngeal Streptococcus pneumoniae isolates from children before and after vaccination with the ten-valent pneumococcal conjugate vaccine (PCV10) in Ethiopia. *BMC Infectious Diseases*, *19*(1). https://doi.org/10.1186/s12879-019-4024-1

Simell, B., Auranen, K., Käyhty, H., Goldblatt, D., Dagan, R., & O'Brien, K. L. (2012). The fundamental link between pneumococcal carriage and disease. *Expert Review of Vaccines*, *11*(7), 841–855. https://doi.org/10.1586/erv.12.53

Smieja, M. (1998). Current indications for the use of clindamycin: A critical review. *Canadian Journal of Infectious Diseases,* 9(1): 22–28. https://doi.org/10.1155/1998/538090

Soto-Noguerón, A., Carnalla-Barajas, M. N., Solórzano-Santos, F., Arrendondo-García, J. L., Arzate-Barbosa, P., Tinoco-Favila, J. C., … Echániz-Aviles, G. (2016). Streptococcus pneumoniae as cause of infection in infants less than 60 days of age: Serotypes and antimicrobial susceptibility. *International Journal of Infectious Diseases*, *42*, 69–73. https://doi.org/10.1016/j.ijid.2015.12.001

St. Jude Children's Research Hospital. (2009). More Effective Treatment For Pneumonia Following Influenza Found, Study Shows. https://www.sciencedaily.com/releases/2009/01/090108171636.htm Accessed: June 13th, 2020

Stephanie, J. S; Bernard, B; & Scott, Dowell. (2001). Resistant pneumococcal infections. *Comprehensive Therapy*, *5*(5), 69–74. https://www.who.int/drugresistance/technicalguidance/en/resistantinfection.pdf Accessed: May 3rd, 2020

Stark, J. M., Stark, M. A., Colasurdo, G. N., & LeVine, A. M. (2006). Decreased bacterial clearance from the lungs of mice following primary respiratory syncytial virus infection. *Journal of Medical Virology,* 78(6):829-38. https://doi.org/10.1002/jmv.20631

Kenya National Bureau of Statistics. (2020). 2019 Kenya Population and Housing Census Reports.
http://housingfinanceafrica.org/documents/2019-kenya-population-and-housing-census-reports/
Accessed: May 3rd, 2020

Tahoun, M. M., Hasab, A. A. H., & El-Nimr, N. A. (2019). Infection control in child daycare centers: logistics, knowledge, and practices of caregivers. *Journal of the Egyptian Public Health Association*, *94*(1).
https://doi.org/10.1186/s42506-019-0016-7

Tan, T. Q. (2012). Pediatric invasive pneumococcal disease in the United States in the era of pneumococcal conjugate vaccines. *Clinical Microbiology Reviews,* 25(3):409-19.
https://doi.org/10.1128/CMR.00018-12

Tankeshwar, A. (2013). Optochin Sensitivity Test: Principle, Procedure, Results and Quality Control, 1–2.
https://microbeonline.com/optochin-Test-Principle-Procedure-Expected-Results-and-Quality-Control/.
Accessed: May 3rd, 2020

Tazinya, A. A., Halle-Ekane, G. E., Mbuagbaw, L. T., Abanda, M., Atashili, J., & Obama, M. T. (2018). Risk factors for acute respiratory infections in children under five years attending the Bamenda Regional Hospital in Cameroon. *BMC Pulmonary Medicine*, *18*(1).
https://doi.org/10.1186/s12890-018-0579-7

Tenover, F. C., & McGowan, J. E. (2008). Antimicrobial resistance. In *International Encyclopedia of Public Health*, Pg.211-219.
https://doi.org/10.1016/B978-012373960-5.00452-4

The Clinical and Laboratory Standards Institute. (2016). Performance Standards for Antimicrobial Susceptibility Testing CLSI supplement M100S. *Clinical and Laboratory Standards Institute, Wayne, PA*, M100, 29th ed.
https://clsi.org/media/2663/m100ed29_sample.pdf
Accessed: May 3rd, 2020

Todar, K. (2020). *Streptococcus pneumoniae* and pneumococcal pneumonia.
http://textbookofbacteriology.net/S.pneumoniae_2.html
Accessed: May 3rd, 2020

Ujunwa, F., & Ezeonu, C. (2014). Risk factors for acute respiratory tract infections in under-five children in Enugu Southeast Nigeria. *Annals of Medical and Health Sciences Research*, *4*(1), 95. https://doi.org/10.4103/2141-9248.126610

Usuf, E., Bottomley, C., Adegbola, R. A., & Hall, A. (2014). Pneumococcal carriage in sub-Saharan Africa - A systematic review. *PLoS ONE*, *9*(1). https://doi.org/10.1371/journal.pone.0085001

Van Bambeke, F., Reinert, R. R., Appelbaum, P. C., Tulkens, P. M., & Peetermans, W. E. (2007). Multidrug-resistant *Streptococcus pneumoniae* infections: Current and future therapeutic options. *Drugs*, 67(16):2355-82. https://doi.org/10.2165/00003495-200767160-00005

Van Der Vaart, H., Postma, D. S., Timens, W., & Ten Hacken, N. H. T. (2004). Acute effects of cigarette smoke on inflammation and oxidative stress: A review. *Thorax*, 59(8):713-21. https://doi.org/10.1136/thx.2003.012468

Van Westen, E., Knol, M. J., Wijmenga-Monsuur, A. J., Tcherniaeva, I., Schouls, L. M., Sanders, E. A. M., … Rots, N. Y. (2018). Serotype-specific igg antibody waning after pneumococcal conjugate primary series vaccinations with either the 10-valent or the 13-valent vaccine. *Vaccines*, *6*(4). https://doi.org/10.3390/vaccines6040082

Wada, F. W., Tufa, E. G., Berheto, T. M., & Solomon, F. B. (2019). Nasopharyngeal carriage of *Streptococcus pneumoniae* and antimicrobial susceptibility pattern among school children in South Ethiopia: Post-vaccination era. *BMC Research Notes*, *12*(1). https://doi.org/10.1186/s13104-019-4330-0

Wahl, B., O'Brien, K. L., Greenbaum, A., Majumder, A., Liu, L., Chu, Y., … Knoll, M. D. (2018). Burden of *Streptococcus pneumoniae* and Haemophilus influenzae type b disease in children in the era of conjugate vaccines: global, regional, and national estimates for 2000–15. *The Lancet Global Health*, *6*(7), e744–e757. https://doi.org/10.1016/S2214-109X(18)30247-X

Waisbourd-Zinman, O., Bilavsky, E., Tirosh, N., Samra, Z., & Amir, J. (2010). Penicillin and ceftriaxone susceptibility of *Streptococcus pneumoniae* isolated from cerebrospinal fluid of children with meningitis hospitalized in a tertiary hospital in Israel. *Israel Medical Association Journal*, *12*(4), 225–228. https://pubmed.ncbi.nlm.nih.gov/20803882/

Wang, C., Chen, Y.-H., Fang, C., Zhou, M., Xu, H., Jing, C., … Zhang, C. (2019). Antibiotic resistance profiles and multidrug resistance patterns of *Streptococcus pneumoniae* in pediatrics. *Medicine*, *98*(24), e15942. https://doi.org/10.1097/md.0000000000015942

Watson, D. A., Musher, D. M., Jacobson, J. W., & Verhoef, J. (1993). A Brief History of the Pneumococcus in Biomedical Research: A Panoply of Scientific Discovery. *Clinical Infectious Diseases*, *17*(5), 913–924. https://doi.org/10.1093/clinids/17.5.913

Weiser, J. N., Ferreira, D. M., & Paton, J. C. (2018). *Streptococcus pneumoniae*: Transmission, colonization and invasion. *Nature reviews. Microbiology*, Vol 16, pp. 355-367. https://doi.org/10.1038/s41579-018-0001-8

World Data Atlas. (2015). Kenya - Male to female ratio of the total population. https://knoema.com/atlas/Kenya/topics/Demographics/Population/Male-to-female-ratio
Accessed: May 13th, 2020

World Health Organisation. (2014). Revised WHO Classification and Treatment of Childhood Pneumonia at Health Facilities: Evidence Summaries. *World Health Organization*. https://apps.who.int/iris/bitstream/handle/10665/137319/9789241507813_eng.pdf?sequence=1
Accessed: June 6th, 2020

World Health Organisation. (2013). Introduction of pneumococcal vaccine A handbook for district and health facility staff World Health Organization. *WHO*, *WHO/IVB/13*, 1–20. https://apps.who.int/iris/bitstream/handle/10665/90380/WHO_IVB_13.10_eng.pdf?sequence=1&isAllowed=y
Accessed: June 6th, 2020

Xu, Q., Kaur, R., Casey, J. R., Sabharwal, V., Pelton, S., & Pichichero, M. E. (2011). Nontypeable *Streptococcus pneumoniae* as an otopathogen. *Diagnostic Microbiology and Infectious Disease,* 69(2): 200-204. https://doi.org/10.1016/j.diagmicrobio.2010.09.019

Yahiaoui, R. Y., Bootsma, H. J., den Heijer, C. D. J., Pluister, G. N., John Paget, W., Spreeuwenberg, P., … Stobberingh, E. E. (2018). Distribution of serotypes and patterns of antimicrobial resistance among commensal *Streptococcus pneumoniae* in nine European countries. *BMC Infectious Diseases*, *18*(1). https://doi.org/10.1186/s12879-018-3341-0

Yayan, J. (2014). The comparative development of elevated resistance to macrolides in community-acquired pneumonia caused by *Streptococcus pneumoniae*. *Drug Design, Development and Therapy*, *8*, 1733–1743. https://doi.org/10.2147/DDDT.S71349

Zinsstag, J., Schelling, E., Waltner-Toews, D., & Tanner, M. (2011). From "one medicine" to "one health" and systemic approaches to health and well-being. *Preventive Veterinary Medicine,* 1; 101(3-4):148-56. https://doi.org/10.1016/j.prevetmed.2010.07.003

Zivich, P. N., Grabenstein, J. D., Becker-Dreps, S. I., & Weber, D. J. (2018). *Streptococcus pneumoniae* outbreaks and implications for transmission and control: a systematic review. *Pneumonia*, *10*(1). https://doi.org/10.1186/s41479-018-0055-4

APPENDICES

APPENDIX I: INFORMED ASSENT FORM

My name is **Michael Walekhwa**, a Ph.D student from Kenyatta University. I am conducting a study on "***Streptococcus Pneumoniae*** **Serotype Prevalence, Antibiotic Susceptibility Patterns and Associated Risk Factors among PCV-10 Vaccinated and Unvaccinated Children Attending GCH**". The information will be used by the Ministry of Medical Services and Ministry of Public Health and sanitation to establish whether the pneumonia vaccine being given to our children is protecting them or not.

Benefits

If you participate in this study you will help us to learn how to provide effective screening services that can improve the health of children and reduce the risk of Pneumonia.

Your child will also benefit from being screened for body temperature and if you are found to have a problem you will be advised on the treatment.

Procedures to be followed

Participation in this study will require that I ask you some questions and I also examine your child in order to screen them for the strain of *Streptococcus Pneumoniae* they could be carrying if any, how the particular responds to different antibiotics and factors that pre-dispose your child to such infections.

Nasopharyngeal swabs specimen will be taken from your child in order to be able to carry out the above tests. The researcher will record the information about you and your child in a questionnaire.

You have the right to refuse participation in this study. You will get the same care and medical treatment whether you agree to join the study or not and your decision will not change the care you will receive from the clinic today or that you will get from any other clinic at any other time.

Please remember the participation in this study is voluntary. You may ask questions related to the study at any time.

You may refuse to respond to any questions and you may stop an interview at any time. You may also stop being in the study at any time without any consequences to the services you receive from this clinic or any other organization now or in the future.

Discomforts and Risks

Some of the questions you will be asked are on intimate subject and may be embarrassing or make you uncomfortable. If this happens, you may refuse to answer these questions if you so choose. You may also stop the interview at any time. The interview may add approximately half an hour to the time you wait before you receive your routine services.

Reward

This study shall enable us to establish whether PCV-10 is protecting our children or not.
No incentives shall be given to participants.

Confidentiality

The interviews and examinations will be conducted in a private setting within the clinic. Your name will not be recorded on the questionnaire. The questionnaires will be kept in a locked cabinet for safe keeping at Getrudes Hospital and Agakhan University Hospital respectively. Everything will be kept private.

Contact Information

If you have any questions you may also contact Dr. Margaret Muturi 1. On 0722758523, 2. Prof. Eucharia Kenya on 0722721435 or the Kenyatta University Ethical Review Committee Secretariat on
chairman.kuerc@ku.ac.ke,secretary.kuerc@ku.ac.ke, ercku2008@gmail.com

Participant's statement

The above information regarding my participation in the study is clear to me. I have been given a chance to ask questions and my questions have been answered to my satisfaction. My participation in this study is entirely voluntary. I understand that my records will be kept private and that I can leave the study at any time. I understand that I will still get the same care and medical treatment whether I decide to leave the study or not and my decision will not change the care that I will receive from the clinic today or that I will get from any other clinic at any other time.

Name of Participant…….....

_____ _____

Signature or Thumbprint Date

Investigators statement

I, the undersigned, have explained to the volunteer in a language s/he understands, the procedures to be followed in the study and the risks and benefits involved

Name of Interviewer…….....

_____ _____

Signature or Thumbprint Date

APPENDIX II: STUDY QUESTIONNAIRE

***Streptococcus pneumoniae* SEROTYPE PREVALENCE, ANTIBIOTICSUSCEPTIBILITY PROFILES AND ASSOCIATED RISK FACTORS AMONG PCV-10 VACCINATED AND UNVACCINATED CHILDREN ATTENDING GERTRUDES CHILDREN'S HOSPITAL**

INSTRUCTIONS

1. Please do not write your name anywhere in the questionnaire;
2. Put a tick (√) in box next to your preferred response and;
3. Where no responses/choices are provided please write the response in the spaces provided.

SECTION A: MATERNAL ASSOCIATED DEMOGRAPHICS			
NO	**Item**	**Options**	**Response**
1.	**Maternal age *(years)***	18-24	
		25-29	
		30-34	
		35-39	
		40-44	
		45-49	
		Other	
2.	**Maternal level of education**	Primary school	
		Secondary school	
		Tertiary college	
		University	
		Other	
3.	**Family average income/months *(USD)***	70-140	
		150-250	
		260-350	
		360-450	
		≥ 460	
		Other	
4.	**Maternal smoking status**	Yes	
		No	
5.	**Maternal consumption of alcohol**	Yes	
		No	
6.	**Maternal knowledge on factors that promote transmission of pneumococcal disease**	Dirt	
		Cold conditions	
		Crowding	
		Poor nutrition	
		Other	
7.	**Size of the family's residential house**	Single room	
		One bedroom	
		Two bedroom	
		Three bedroom	
8.	**Method of home waste disposal**	Public sewerage system	
		Private septic tank	
		Other	

9.	**Type of cooking fuel**	Firewood	
		Stove	
		Charcoal	
		Gas	
		Electricity	
10.	**Source of lighting at home**	Candle	
		Traditional lamp	
		Lantern	
		Solar	
		Electricity	
		Other	
SECTION B: CHILD ASSOCIATED DEMOGRAPHICS			
1.	Age of child *(months)*	6-12	
		13-24	
		25-36	
		37-48	
		49-60	
2.	Gender of the child	Female	
		Male	
3.	Child's attendance of daycare	Yes	
		No	
4.	Number of people who share same household as the child	One	
		Two	
		Three	
		Four	
		Five	
		>5	
5.	Type of breastfeeding practiced for the 1st six months	Moderate *(child given other food)*	
		Exclusive *(child entirely feeds on breast milk)*	
6.	Child's weight (Kgs)	1.1-2.0	
		2.1-3.0	
		3.1-4.0	
		4.1-5.0	
7.	Whether child was immunized with the PCV-10 vaccine*(verify from the ANC booklet)*	Yes	
		No	
		If yes, state number of injections	
8.	Whether child had consumed any antibiotic agents two weeks prior to collection of study sample	Yes	
		No	
Thank you for voluntarily allowing your child to participate in this study			

APPENDIX III: ETHICS APPROVAL LETTER

KENYATTA UNIVERSITY
ETHICS REVIEW COMMITTEE
Moi Library 1st Floor, Office No. 25

Fax: 8711242/8711575
Email: chairman.kuerc@ku.ac.ke
secretary.kuerc@ku.ac.ke
secretariat.kuerc@ku.ac.ke
Website: www.ku.ac.ke

P. O. Box 43844,
Nairobi, 00100

Tel: 8710901/12

Our Ref: KU/ERC/APPROVAL/VOL.1 (12)

Date: 12th January, 2017

Michael Walekhwa
Kenyatta University
P.O. Box 43844
NAIROBI

Dear Walekhwa,

APPLICATION NUMBER **PKU/535/I628** – "STREPTOCOCCUS SEROTYPE EPIDEMIOLOGY, ANTI-MICROBIAL RESISTANCE AND SERUM IMMUNOGLOBIN G ANTIBODY LEVEL AMONG PCV-10 VACCINATED INFANTS IN NAIROBI COUNTY, KENYA" – VERSION 3

1. IDENTIFICATION OF PROTOCOL

The application before the committee is with a research topic **"Streptococcus Serotype Epidemiology, Anti-Microbial Resistance and Serum Immunoglobin G Antibody Level among Pvc-10 Vaccinated Infants in Nairobi County, Kenya" Version 3** received on 29th November, 2016 and discussed on 10 January 2017.

2. APPLICANT
Michael Walekhwa

3. SITE
Nairobi County, Kenya

4. DECISION

The committee has considered the research protocol in accordance with the Kenyatta University Research Policy (section 7.2.1.3) and the Kenyatta University Ethics Review Committee Guidelines **AND APPROVED that the research may proceed for a period of ONE year from 12th January, 2017.**

5. ADVICE/CONDITIONS

i. Progress reports are submitted to the KU-ERC every six months and a full report is submitted at the end of the study.
ii. Serious and unexpected adverse events related to the conduct of the study are reported to this committee immediately they occur.
iii. Notify the Kenyatta University Ethics Committee of any amendments to the protocol.
iv. Submit an electronic copy of the protocol to KUERC.

When replying, kindly quote the application number above.
If you accept the decision reached and advice and conditions given please sign in the space provided below and return to KU-ERC a copy of the letter.

DR. TITUS KAHIGA
CHAIRMAN ETHICS REVIEW COMMITTEE

KENYATTA UNIVERSITY
OFFICE OF THE CHAIRMAN
19 JAN 2017
ETHICS REVIEW COMMITTEE
P.O. BOX 43844 - 00100 NAIROBI

I Michael N. Walekhwa ...accept the advice given and will fulfill the conditions therein.

Signature..... Dated this day of 19/01/2017 ..... 2017.

cc. Vice-Chancellor
DVC-Research Innovation and Outreach

APPENDIX IV: NACOSTI RESEARCH AUTHORIZATION LETTER

NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone:+254-20-2213471,
2241349,3310571,2219420
Fax:+254-20-318245,318249
Email:dg@nacosti.go.ke
Website: www.nacosti.go.ke
when replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No.
NACOSTI/P/17/65428/15801

Date:
6th March, 2017

Michael Nyongesa Walekhwa
Kenyatta University
P.O. Box 43844-00100
NAIROBI.

RE: RESEARCH AUTHORIZATION

Following your application for authority to carry out research on ***"Evaluation of streptococcus pneumoniae serotype distribution, anti-microbial non-susceptibility and immunogenicity of PCV-10 among vaccinated infants in Nairobi County,"*** I am pleased to inform you that you have been authorized to undertake research in **Nairobi County** for the period ending **6th March, 2018.**

You are advised to report to **the County Commissioner, the County Director of Education and the County Director of Health Services, Nairobi County** before embarking on the research project.

On completion of the research, you are expected to submit **two hard copies and one soft copy in pdf** of the research report/thesis to our office.

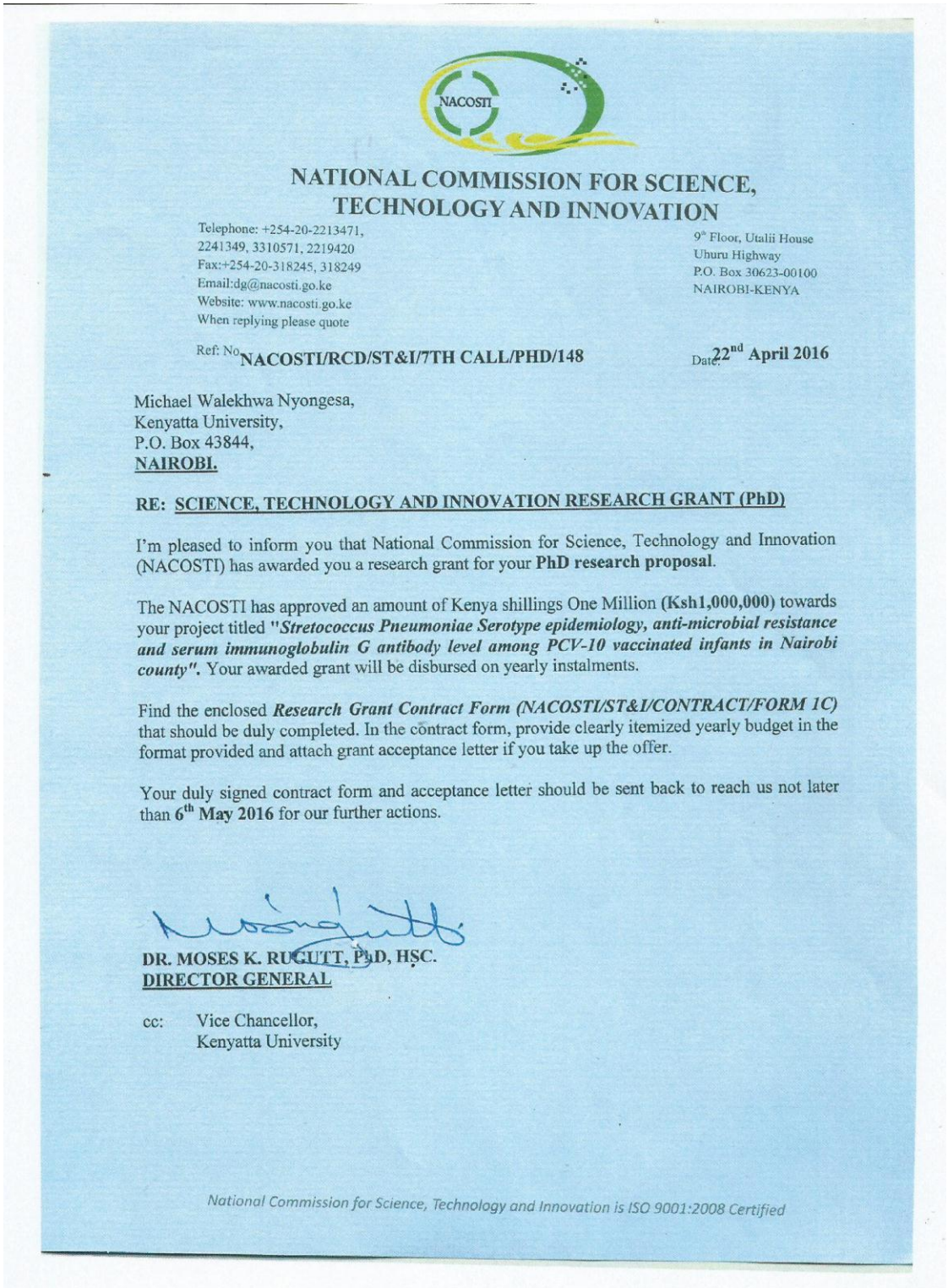
DR. STEPHEN K. KIBIRU, PhD.
FOR: DIRECTOR-GENERAL/CEO

Copy to:

The County Commissioner
Nairobi County.

The County Director of Education
Nairobi County.

APPENDIX V: PH.D GRANT AWARD LETTER



NACOSTI

NATIONAL COMMISSION FOR SCIENCE, TECHNOLOGY AND INNOVATION

Telephone: +254-20-2213471,
2241349, 3310571, 2219420
Fax:+254-20-318245, 318249
Email:dg@nacosti.go.ke
Website: www.nacosti.go.ke
When replying please quote

9th Floor, Utalii House
Uhuru Highway
P.O. Box 30623-00100
NAIROBI-KENYA

Ref: No **NACOSTI/RCD/ST&I/7TH CALL/PHD/148** Date: **22nd April 2016**

Michael Walekhwa Nyongesa,
Kenyatta University,
P.O. Box 43844,
NAIROBI.

RE: SCIENCE, TECHNOLOGY AND INNOVATION RESEARCH GRANT (PhD)

I'm pleased to inform you that National Commission for Science, Technology and Innovation (NACOSTI) has awarded you a research grant for your **PhD research proposal**.

The NACOSTI has approved an amount of Kenya shillings One Million **(Ksh1,000,000)** towards your project titled ***"Stretococcus Pneumoniae Serotype epidemiology, anti-microbial resistance and serum immunoglobulin G antibody level among PCV-10 vaccinated infants in Nairobi county".*** Your awarded grant will be disbursed on yearly instalments.

Find the enclosed ***Research Grant Contract Form (NACOSTI/ST&I/CONTRACT/FORM 1C)*** that should be duly completed. In the contract form, provide clearly itemized yearly budget in the format provided and attach grant acceptance letter if you take up the offer.

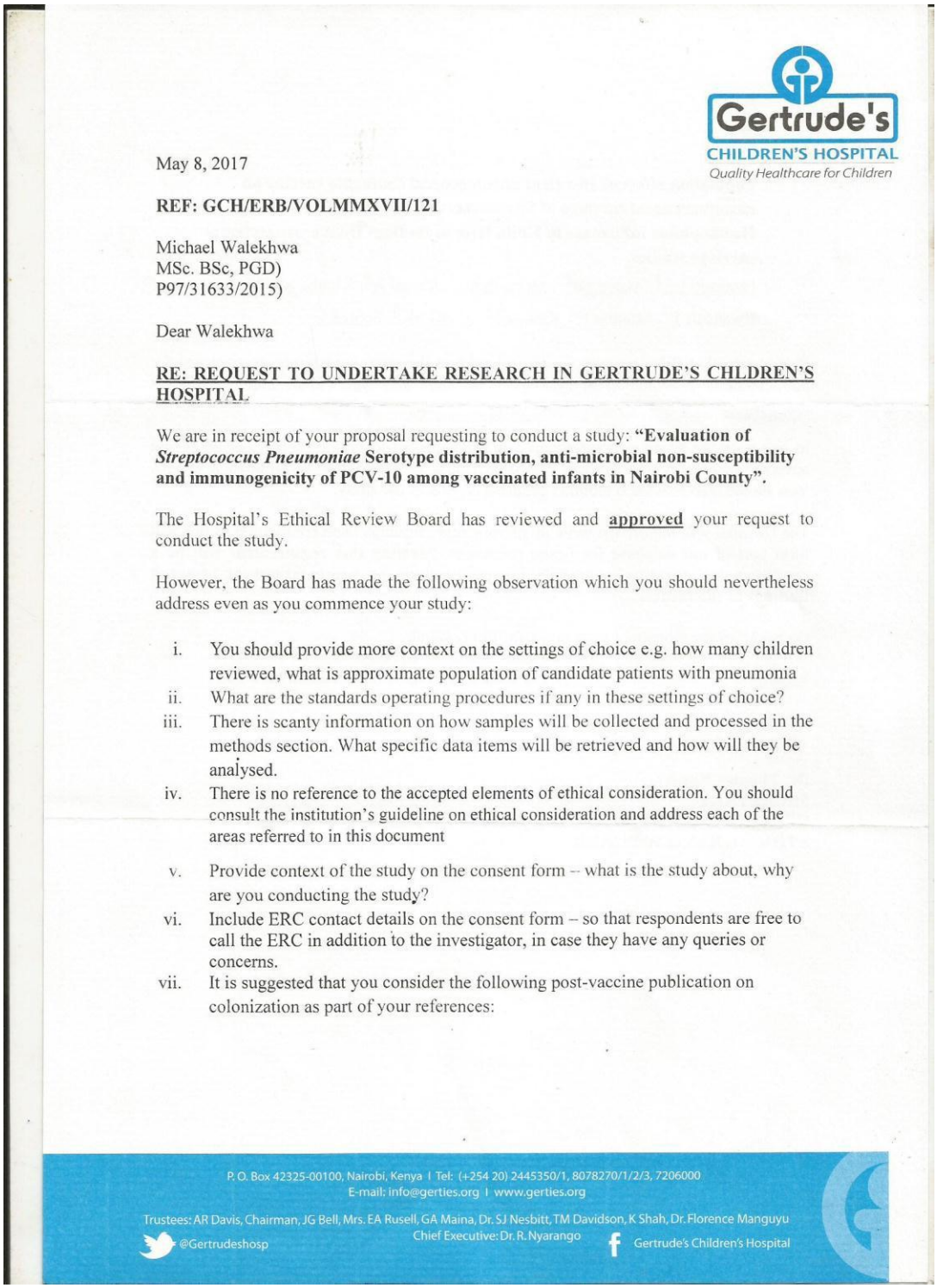
Your duly signed contract form and acceptance letter should be sent back to reach us not later than **6th May 2016** for our further actions.

DR. MOSES K. RUGUTT, PhD, HSC.
DIRECTOR GENERAL

cc: Vice Chancellor,
Kenyatta University

National Commission for Science, Technology and Innovation is ISO 9001:2008 Certified

APPENDIX VI: GCH RESEARCH PERMIT



Gertrude's
CHILDREN'S HOSPITAL
Quality Healthcare for Children

May 8, 2017

REF: GCH/ERB/VOLMMXVII/121

Michael Walekhwa
MSc. BSc, PGD)
P97/31633/2015)

Dear Walekhwa

RE: REQUEST TO UNDERTAKE RESEARCH IN GERTRUDE'S CHLDREN'S HOSPITAL

We are in receipt of your proposal requesting to conduct a study: **"Evaluation of *Streptococcus Pneumoniae* Serotype distribution, anti-microbial non-susceptibility and immunogenicity of PCV-10 among vaccinated infants in Nairobi County".**

The Hospital's Ethical Review Board has reviewed and **approved** your request to conduct the study.

However, the Board has made the following observation which you should nevertheless address even as you commence your study:

i. You should provide more context on the settings of choice e.g. how many children reviewed, what is approximate population of candidate patients with pneumonia
ii. What are the standards operating procedures if any in these settings of choice?
iii. There is scanty information on how samples will be collected and processed in the methods section. What specific data items will be retrieved and how will they be analysed.
iv. There is no reference to the accepted elements of ethical consideration. You should consult the institution's guideline on ethical consideration and address each of the areas referred to in this document
v. Provide context of the study on the consent form -- what is the study about, why are you conducting the study?
vi. Include ERC contact details on the consent form – so that respondents are free to call the ERC in addition to the investigator, in case they have any queries or concerns.
vii. It is suggested that you consider the following post-vaccine publication on colonization as part of your references:

P. O. Box 42325-00100, Nairobi, Kenya I Tel: (+254 20) 2445350/1, 8078270/1/2/3, 7206000
E-mail: info@gerties.org I www.gerties.org
Trustees: AR Davis, Chairman, JG Bell, Mrs. EA Rusell, GA Maina, Dr. SJ Nesbitt, TM Davidson, K Shah, Dr. Florence Manguyu
Chief Executive: Dr. R. Nyarango
@Gertrudeshosp
Gertrude's Children's Hospital

Cont.

Population effect of 10-valent pneumococcal conjugate vaccine on nasopharyngeal carriage of Streptococcus pneumoniae and non-typeable Haemophilus influenzae in Kilifi, Kenya: findings from cross-sectional carriage studies.

Hammitt LL[1], Akech DO[2], Morpeth SC[3], Karani A[2], Kihuha N[2], Nyongesa S[2], Bwanaali T[3], Mumbo E[4], Kamau T[5], Sharif SK[5], Scott JA[6].

Please note that this approval is only to conduct the study and is not an approval for publication or presentation of findings. A separate approval will be required for this purpose.

In line with the board's requirements you are expected to provide reports of all severe adverse reactions within 24hours and to provide a monthly report of all adverse events. You should also provide 6 monthly progress reports of the study.

The Hospital will require the write up of your study findings upon completion as this will form part of our database for future references. **Meeting this requirement will be a condition for granting approvals for publications or presentations of research findings in the future.**

On behalf of the Hospital I wish you a fruitful research.

Regards

Dr. Thomas Ngwiri
SECRETARY
GERTRUDE'S CHILDREN'S HOSPITAL
ETHICAL REVIEW BOARD

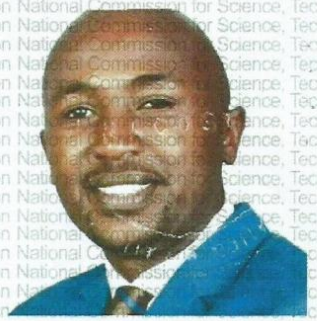
APPENDIX VII: NACOSTI RESEARCH PERMIT RECEIPT

THIS IS TO CERTIFY THAT:
MR. MICHAEL NYONGESA WALEKHWA
of KENYATTA UNIVERSITY, 1285-100 NAIROBI,has been permitted to conduct research in *Nairobi County*

on the topic: *EVALUATION OF STREPTOCOCCUS PNEUMONIAE SEROTYPE DISTRIBUTION, ANTI-MICROBIAL NON-SUSCEPTIBILITY AND IMMUNOGENICITY OF PCV-10 AMONG VACCINATED INFANTS IN NAIROBI COUNTY*

for the period ending:
6th March,2018

Permit No : NACOSTI/P/17/65428/15801
Date Of Issue : 6th March,2017
Fee Recieved :Ksh 2000



.....
Applicant's Signature

.....
Director General
National Commission for Science, Technology & Innovation