IMMUNOGENICITY OF 10-VALENT PNEUMOCOCCAL CONJUGATE VACCINE AMONG INFANTS AT MBAGATHI DISTRICT HOSPITA, KENYA

Walekwa Michael Nyongesa (BSc. UEAB)
Department of Medical Laboratory Science

A research thesis submitted in partial fulfilment for the award of the degree of Master of Science in Infectious Diseases in the School of Medicine of Kenyatta University

AUGUST, 2015
DECLARATION

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

Name: Walekhwa Michael Nyongesa

Signature:………………… Date:……………………

Supervisors:

This thesis has been submitted for examination with our approval as supervisors

1. Dr. Margaret W. Muturi
   Department of Medical Laboratory Science
   Kenyatta University, Kenya

   Signature: ……………… Date: …………………

2. Prof. Elizabeth Bukusi (MBChB, M.Med, MPH, PhD, PGD (Research Ethics))
   Chief Research Officer and Deputy Director (Research and Training) KEMRI
   Co-Director Research Care Training Program, (RCTP)

   Signature:……………………… Date:…………………………
DEDICATION

I dedicate this work to wife Nancy Siboe, my daughter Hina Walekhwa and son Bradley Walekhwa.
ACKNOWLEDGEMENT

I sincerely thank Dr. Margaret Muturi for her professional input that has seen this thesis reach this level, not to mention her unmatched patience and motherly treatment. Dr. Elizabeth Bukusi’s courtesy, organization and professionalism were incredible. It is the concoction of her powerful virtues that got this thesis fine-tuned to this level. I thank National Council of Science and Technology (NCST) for the funding. I would have had a lot of financial hiccups had you not funded this research work. God bless you. Mbagathi Hospital staff offered me incredible support and advice.

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My son Bradley Walekhwa and daughter Hinah Walekhwa are the reason I always work hard. I owe my success and life to them. The completion of this thesis is their success. I will always do my best to ensure that you succeed in life.

My parents (Ben Walekhwa & Emily Walekhwa), you are the best a child can ever have. You have been there for me in good and bad times. You prayed, you paid fees, you toiled for me and you were prejudiced. You didn’t give up on me. I can never compensate you for these. God bless you for me.

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## Abbreviations and Acronyms

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<th>Abbreviation</th>
<th>Description</th>
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<tbody>
<tr>
<td>23vPPS</td>
<td>23-valent pneumococcal polysaccharide vaccine</td>
</tr>
<tr>
<td>6BTT</td>
<td>Monovalent serotype 6B conjugated with tetanus toxoid</td>
</tr>
<tr>
<td>ANOVA</td>
<td>Analysis of variance</td>
</tr>
<tr>
<td>AOM</td>
<td>Acute Otitis Media</td>
</tr>
<tr>
<td>aP</td>
<td>Acellular pertussis</td>
</tr>
<tr>
<td>CC</td>
<td>Clonal complex</td>
</tr>
<tr>
<td>CD4</td>
<td>Surface glycoprotein on T-helper cells</td>
</tr>
<tr>
<td>CFR</td>
<td>Case fatality rate</td>
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<tr>
<td>CI</td>
<td>Confidential interval</td>
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<tr>
<td>COPD</td>
<td>Chronic obstructive pulmonary disease</td>
</tr>
<tr>
<td>CPS</td>
<td>Cell wall polysaccharide</td>
</tr>
<tr>
<td>CRP</td>
<td>C-reactive protein</td>
</tr>
<tr>
<td>CSF</td>
<td>Cerebrospinal fluid</td>
</tr>
<tr>
<td>DT</td>
<td>Diptheria toxoid</td>
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<tr>
<td>ELISA</td>
<td>Enzyme-linked immunosorbent assay</td>
</tr>
<tr>
<td>ermB</td>
<td>Erythromycin resistance methylase B</td>
</tr>
<tr>
<td>GMC</td>
<td>Geometric mean concentration</td>
</tr>
<tr>
<td>Hi</td>
<td>Haemophilus influenzae</td>
</tr>
<tr>
<td>Hib</td>
<td>Haemophilus influenzae type B</td>
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<tr>
<td>IFN-γ</td>
<td>Interferon gamma</td>
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<tr>
<td>IgA</td>
<td>Immunoglobulin A</td>
</tr>
<tr>
<td>Abbreviation</td>
<td>Description</td>
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<tr>
<td>IgG</td>
<td>Immunoglobulin G</td>
</tr>
<tr>
<td>IgM</td>
<td>Immunoglobulin M</td>
</tr>
<tr>
<td>IL-10</td>
<td>Interleukin 10</td>
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<tr>
<td>IPD</td>
<td>Invasive pneumococcal disease</td>
</tr>
<tr>
<td>IPV</td>
<td>Inactivated polio vaccine</td>
</tr>
<tr>
<td>KEPI</td>
<td>Kenya Expanded Program on Immunization</td>
</tr>
<tr>
<td>LAC</td>
<td>Latin America and Caribbean</td>
</tr>
<tr>
<td>MALDI-TOF</td>
<td>Matrix assisted laser desorption/ionization - time of flight</td>
</tr>
<tr>
<td>MBL</td>
<td>Mannose binding lectin</td>
</tr>
<tr>
<td>MDH</td>
<td>Mbagathi District Hospital</td>
</tr>
<tr>
<td>mefA</td>
<td>Macrolide efflux A</td>
</tr>
<tr>
<td>MGUS-</td>
<td>Monoclonal gammopathy of unknown significance</td>
</tr>
<tr>
<td>MHC</td>
<td>Major histocompatibility complex</td>
</tr>
<tr>
<td>MIC</td>
<td>Minimal inhibitory concentration</td>
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<tr>
<td>MLST</td>
<td>Multi-locus sequence typing</td>
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<tr>
<td>MM</td>
<td>Multiple myeloma</td>
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<tr>
<td>MMR</td>
<td>Measles, mumps and rubella vaccine</td>
</tr>
<tr>
<td>NA</td>
<td>North America</td>
</tr>
<tr>
<td>NVT</td>
<td>Non-vaccine serotypes</td>
</tr>
<tr>
<td>OD</td>
<td>Optical density</td>
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<tr>
<td>OPA</td>
<td>Opsonophagocytic assay</td>
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<tr>
<td>OPA</td>
<td>Opsonophagocytic activity</td>
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OPSI - Overwhelming post-splenectomy infection
PBS - Phosphate-buffered saline (a medium)
PBS - Phosphate buffered saline
PCR - Polymerase chain reaction
PCV - Pneumococcal Conjugate Vaccine
PFGE - Pulsed field gel electrophoresis
PNSP - Penicillin non-susceptible *Streptococcus pneumoniae*
PPS - Pneumococcal polysaccharide vaccine
PPV - Pneumococcal polysaccharide vaccine
PRP-T - Polyribosyl ribitol phosphate (Haemophilus influenzae antigen)
PRR - Pattern recognition receptors
PS - Polysaccharide
PsaA - Pneumococcal surface adhesion A
PspA - Pneumococcal surface protein A
RA - Rheumatoid arthritis
RIA - Radio immune assay
SLE - Systemic lupus erythematosus
SP - *Streptococcus Pneumoniae*
ST - Sequence type
T cell - Thymus derived lymphocytes
TLR - Toll-like receptor
TPP - Target Programme Profile
TT - Tetanus toxoids
<table>
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<th>Abbreviation</th>
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<tbody>
<tr>
<td>Ug/ml</td>
<td>Microgram/Millilitre</td>
</tr>
<tr>
<td>VRS</td>
<td>Vaccine related serotypes</td>
</tr>
<tr>
<td>VRT</td>
<td>Vaccine related serotypes</td>
</tr>
<tr>
<td>VT</td>
<td>Vaccine serotypes</td>
</tr>
<tr>
<td>WHO</td>
<td>World Health Organization</td>
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ABSTRACT

Pneumococcal diseases are responsible for killing at least one million children under the age of five every year: over 70% of these deaths are in developing countries. This total is greater than that due to malaria, AIDS and measles combined. *Streptococcus pneumoniae* has over 90 serotypes, out of which eight are included in the 10-valent Pneumococcal conjugate vaccine (Synflorix) currently in use in Kenya. Pneumococci are highly diverse and serotype prevalence is dynamic based on age, time period and geographical area. There is no published data on serotypes found in Nairobi and other areas in Kenya except the coastal region. It is therefore possible that these serotypes are different from those included in the vaccine, subjecting the immunogenicity of the vaccine among Kenyan infants to doubt. Since its launch for free public use in February, 2011 increasing number of children has been immunized, yet, it was not clear to what extent the vaccine is protective. This study therefore evaluated the immunogenicity of the vaccine by measuring serum concentration of IgG antibodies among infants immunized with PCV-10 at the Mbagathi District Hospital (MDH). It also investigated; the vaccine herd effect on study infants biological mothers and factors that affect the vaccine immunogenicity. It was a cross-sectional study where infants that completed 3-doses of PCV-10 had IgG antibodies to serotype-specific capsular polysaccharide measured by enzyme-linked immunosorbent assay. The majority (76.1%) of the infants who had completed the required dose of the pneumococcal conjugate vaccine had IgG serum titres of between 0.34mg/dl to 0.36mg/dl. Thirteen percent of infants had 0.30mg/dl to 0.33mg/dl serum concentration of PD specific IgG antibodies. The remainder of the studied infants had IgG antibody titres ranging between 0.25mg/dl to 0.29mg/dl and ≤0.25mg/dl respectively. Also, the total score for serum concentration of PD specific IgG among study infants biological mothers was between 0.34 – 0.36 (61.6%), which meets the threshold set by WHO. This study also found out that there was multi-collinearity between IgG antibody levels (mg/dl) for infants and vaccinated infant’s biological mothers and several variables. These variables included: Use of alcoholic drinks by infants’ biological mother during pregnancy (r =.595, p ≤ 0.05); Maternal diet during pregnancy (r =.137, p ≤ 0.05); Breast feeding frequency (r =.220, p ≤ 0.05); Gap with other children (r =.133, p ≤ 0.05); Child hospitalization (r =.131, p ≤ 0.05); Chronic illness (r =.154, p ≤0.01); and IgG antibody levels (mg/dl)for infants (r =.675, p > 0.05). The 10-valent pneumococcal conjugate vaccine is immunogenic against Pneumococcal Disease four weeks after completion of three doses among infants attending child welfare clinic at Mbagathi District Hospital, Kenya.
CHAPTER ONE: INTRODUCTION

1.1 Background

*Streptococcus pneumoniae* (pneumococcus) remains a leading cause of serious illness, including bacteraemia, meningitis, and pneumonia among children and adults worldwide (Pekka and Cynthia, 2010). It is also a major cause of sinusitis and acute otitis media (AOM). The World Health Organization (WHO, 2010) estimates that there are 150.7 million cases of pneumonia each year in children younger than five years, with as many as 20 million cases severe enough to require hospital admission. In developing countries, pneumonia infections are not only more prevalent but more severe, accounting for more than 4 million deaths annually (Gupta and Sarosi, 2001). The World Health Organization (WHO) estimates that in 2008, out of the about 8.8 million global annual deaths amongst children aged <5 years, 476 000 (333 000 – 529 000) were caused by pneumococcal infections (WHO, 2008). In developing countries case-fatality rates among younger infants may reach 20% for pneumococcal septicemia and 50% for meningitis (Park *et al*., 2007). Pneumonia contributes 20 per cent or 39,760 children deaths in Kenya every year (Casey, 2011). Despite the availability of antibiotic therapies, mortality caused by pneumococcal disease remains high.

*Streptococcus pneumoniae* has more than 90 serotypes (WHO, 2009). In the pre-vaccination era, 6 – 11 of these serotypes accounted for ≥70% of all invasive pneumococcal disease worldwide (Wuorimaa, 2007). The continuing emergence of penicillin-resistant and multidrug-resistant pneumococcal strains is an increasing global threat posing serious therapeutic challenges (WHO, 2010). In view of the considerable public health impact of successful vaccines against pneumococcal disease, The World Health Organization (WHO) has stated that the development of safe, effective vaccines that offer broad protection against pneumococcal disease should be a
high priority (Johansson et al., 2011). The most effective way to prevent Pneumococcal deaths is
to ensure that all children have access to a safe and affordable vaccine (Nuorti, 2007). In 2007,
The World Health Organization (WHO) recommended that all countries incorporate
pneumococcal conjugate vaccines in their national infant immunization programs (Whitney et
al., 2010) yet, while a vaccine against pneumococcal disease has been widely used in Europe and
the United States since 2000, it was not optimal for developing countries. This is because it lacks
serotypes or different strains of Pneumococcal bacteria, known to be common in developing
countries (Santosham, 2004) and the cost of implementing it was prohibitive. In 2009, a 10-
valent pneumococcal conjugate vaccine (Synflorix) was approved by the European Commission
for prevention of IPD caused among infants and young children by the 10 serotypes in the
vaccine (Dagan et al., 2002).

PCV10 contains the seven serotypes included in PCV-7 (serotypes 4, 6B, 9V, 14, 18C, 19F, and
23F) and three additional serotypes (serotypes 1, 5 and 7F). PCV-10 is approved for use among
children aged 6 weeks to 71 months and supersedes PCV-7, which was licensed by FDA in 2000
(Lynch, 2007). Pneumococcal infections, irrespective of serotype, can be successfully treated
with antibiotics, but the increasing resistance among Pneumococci to antibiotics has highlighted
the need for prevention, which can be achieved by vaccination. PPV is not immunogenic to all
serotypes in children less than two years of age and the PCVs have limited serotype coverage.
This has raised interest in a new type of vaccine, specifically the pneumococcal protein vaccines.
The protein vaccines have the potential to protect against all serotypes and they are immunogenic
already in infants (Park et al., 2007). Since protection against pneumococcal infections early in
life is essential, several prevention strategies should be considered, including maternal and early
infant immunization. In clinical trials of new PCVs, placebo controlled studies on the prevention
of IPD are neither practical nor ethical. The WHO, 2008 recommends that the immunogenicity of new pneumococcal vaccine formulations be evaluated by comparison of serotype-specific immunoglobulin G (IgG). Enzyme-linked immunosorbent assays (ELISAs) have been standardized, and levels of IgG associated with IPD protection have been proposed. A threshold level of 0.35g/mL after the infant immunization series has been recommended by a World Health Organization working group as a reference value, applicable on a global basis, for assessing the potential efficacy against IPD of novel PCVs (WHO, 2009).

On 14th February 2011, Kenya rolled-out this pneumococcal conjugate vaccine (Casey, 2011), which has been specially tailored to meet the needs of children in developing countries. Since the launch of the vaccine in February, 2011, there is a rising number of children enrolling, yet, it is not clear to what extent the vaccine is protective. Studies have not been conducted on the effectiveness of the pneumococcal vaccine on Kenyan children. Therefore there is need to evaluate the immunogenicity to determine the effectiveness of the vaccine in Kenyan children.

1.2 Statement of the Problem

*Streptococcus pneumoniae* has over 90 serotypes, out of which eight are included in the 10-valent Pneumococcal conjugate vaccine (Synflorix) currently included in the Kenya Division of Vaccine and Immunization (KDVI) programme. Pneumococci are highly diverse and serotype prevalence is dynamic based on age, time period and geographical area (Wuorimaa, 2010). There is no information on the pneumococcal serotypes found in Nairobi and other pneumococcal disease endemic areas in Kenya. It is possible that they are different from those included in the vaccine, putting the effectiveness of the vaccine in question. Pneumococcal diseases are responsible for killing at least one million children under the age of five every year: over 70% of
these deaths are in developing countries (Avery and Goebel, 1999). This total is greater than that due to malaria, AIDS and measles combined. Since its launch for free public use in February, 2011, there is an increase in the number of children being immunized, yet, it is not clear to what extent the vaccine is protective. No data have been published on the immunogenicity after each dose in a series, or on the relative immunogenicity at various ages in Kenyan children. This study therefore evaluated the concentration of pneumococcal disease immune antibodies as indicators of immunogenicity.

1.3 Justification of the Study

Pneumococcal disease in young children has not been as well characterized in terms of serotype distribution in East Africa as it has been in industrialized countries. The 10-valent pneumococcal conjugate vaccine (Synflorix), covers Serotypes 4, 6B, 9V, 14, 18C, 19F, 23F 1, 5 and 7F. Out of these serotypes, eight, i.e. 1, 4, 5, 6A, 6B, 14, 18C, 19A, 19F and 23F were found to be affecting children in Kilifi, Kenya (Ndiritu et al., 2010). Chances of serotype variation are high in other parts of Kenya, since serotypes vary with regard to geographical areas and it is likely that the vaccine may not be immunogenic. It is possible that replacement disease from non-vaccine serotypes may have varying effects in different settings, including reports of emerging drug resistance as documented by Harish et al., 2011. A study done in 2009 in East African countries, recommended that countries such as Kenya, Uganda and Tanzania conduct appropriate surveillance to establish a baseline measure and monitor the impact of vaccination, including the occurrence and magnitude of possible replacement disease (Mudhune and Wamae, 2009).
1.4 Hypothesis

The 10-valent pneumococcal conjugate vaccine (Synflorix) does not elicit adequate IgG antibodies to confer protection against pneumococcal disease in infants (below 12 months of age) at Mbagathi District Hospital, Nairobi.

1.5 Objectives

1.5.1 General Objective

To measure immunogenicity of 10-valent pneumococcal conjugate vaccine against Pneumococcal disease (PD) among infants attending Mbagathi District Hospital (MDH), Kenya.

1.5.2 Specific Objectives

1. To determine serum concentration of Pneumococcal disease specific IgG among infants with completed 3-doses of PCV-10 at MDH

2. To determine serum concentration of PD specific IgG among biological mothers with study infants who would have completed 3-doses of PCV-10 at MDH

3. To evaluate factors that affect effectiveness of PCV-10 among children attending child welfare clinic at MDH

1.6 Study Significance

The results of this study will be shared with various interest groups who include: National Council for Science, Technology and Innovation, MDH, Kenyatta University and other research institutions to help inform policy formulation and further research on safe and more immunogenic PCV formulations in the country and beyond. Further, good practices identified on the PCV-10 immunization at Mbagathi District Hospital can be duplicated in other parts of the country to improve PCV-10 vaccination uptake, safety and effectiveness.
CHAPTER TWO: LITERATURE REVIEW

2.1 Microbiology and Patho-Physiology

Pneumococci are Gram positive, non-motile, non-spore forming, coccoid bacteria. Like many other streptococci, they are aerotolerant (facultative) anaerobes (Ghaffar, 2001). They lack the enzyme catalase, which is required to neutralize the large amounts of hydrogen peroxide produced by the bacteria, and they therefore need to be cultured on media with capacity to neutralize hydrogen peroxide, for example blood agar (Berg et al., 2006). Hydrogen peroxide is oxidizing hemoglobin to green methaemoglobin, which is observed as greenish haloes around the bacterial colonies on blood agar; a phenomenon called alpha hemolysis (Peltola, 2004).

Under anaerobic conditions they switch to beta hemolysis caused by an oxygen-labile haemolysin. Growth is enhanced by incubation in 5% carbon dioxide. On Gram stain, pneumococci often appear in pairs, and were therefore formerly named Diplococcus pneumoniae, although single cells and small chains also appear (Musher, 2003). Later, they were found out to be members of the streptococcus family, and the name was changed to Streptococcus pneumoniae in 1974. In the routine laboratory, the presumptive diagnosis of pneumococci is based on recognition of typical colony morphology on blood agar (Backhaus, 2012). Two different morphologies occur, depending on the amount of capsule which is produced by the strain. Most common are round smooth umbilicated colonies, 0.5-1.0 mm in diameter, whereas heavily encapsulated strains, especially serotype 3, form mucoid dome shaped colonies with a diameter of up to 5 mm. Pneumococci are distinguished from other alpha
haemolytic streptococci based on colony morphology and sensitivity to optochin (ethylhydrocupreine) and bile (Karlsson et al., 2007).

2.2 The Capsule

The most important virulence factor of *S. pneumoniae* is the polysaccharide capsule providing protection against both antibody dependent and independent immunity, especially opsonisation and phagocytosis by neutrophils. Nearly all Pneumococci are surrounded by a polysaccharide capsule, but un-encapsulated strains occur occasionally (Ghaffar, 2001). They are known to cause outbreaks of conjunctivitis, but are rarely found in nasopharyngeal colonization and invasive disease. Based on immunological properties of the capsule, pneumococci are divided into 46 serogroups (Daniels, 2010). Of those, 20 are further divided into 2 – 4 serotypes, while 26 serogroups have only one serotype. Ninety-three immunologically unique serotypes have so far been described (Backhaus (2012) and McCullers (2010).

2.3 Genetic Properties of Pneumococci

Pneumococci are highly promiscuous, in the sense that they are easily transformed and may incorporate foreign DNA from other pneumococcal strains and from other species. Genes are not only transferred between different pneumococci; for example, resistance genes seem to have been transferred from closely related alpha streptococci, such as *S. oralis* and *S. mitis* (Whatmore, 1999). Croucher et al., (2011), performed a whole genome sequencing of 240 isolates from the same clone, and they found that the high degree of genetic heterogeneity could be explained by a high frequency of recombinational events, especially in resistance genes and among genes coding for capsular polysaccharides. This is the major reason why pneumococci are
successful in evading our attempts to defeat them with antibiotics, and this may also help them to circumvent the effects of vaccination in a long term perspective.

Therefore, not only serotype distribution and antimicrobial resistance have to be monitored in order to understand the epidemiology of pneumococcal infections but also the genetic content (the genotype) needs to be characterized by molecular methods (Whatmore et al., 1999).

2.4 Non-capsular Virulence Factors

If not only serotype affects virulence, which non-capsular factors may also influence virulence? The capsule is surrounding a thick cell wall composed of peptidoglycans, teichoic acids and lipoteichoic acid, which are important during induction of the innate immune response (Zou et al., 2010). Both pili and several different classes of surface proteins that promote adherence to the respiratory epithelium are attached to the cell wall (Shakhnovich, 2002). One of them, pneumococcal surface protein A (PspA), is required for full virulence. It is thought to reduce complement activation, both the classical and the alternative pathway.

Pneumococcal surface protein C (PspC) is also a major virulence factor. Teichoic acid of the pneumococcus is also called the c-polysaccharide (c-ps). Despite the resembling name, it is not the same as the capsular polysaccharide. It is almost invariably expressed by isolates of Streptococcus pneumoniae, and it is involved in pathogenesis, mainly by inducing innate immunity (Zou et al., 2010). Antibodies against cps are commonly detected in patients with pneumococcal pneumonia, and therefore, this polysaccharide early gained interest as a potential target for a serotype independent vaccine, but antibodies against it did not turn out to be protective. C-ps react strongly with one of the major proteins which is produced by the host during the acute phase of inflammation (Croucher, 2011). This protein is therefore named the C-
reactive protein (CRP). The presence of CRP has been shown to reduce lethality among mice infected by *S. pneumoniae*, but this protective function does not seem to require binding of CRP to the c-polysaccharide, so the mechanism of that interaction seems to be more complex than that. The c-polysaccharide is used as a target for antigen detection tests (Croucher *et al.*, 2011).

Autolysin, LytA, is an enzyme involved in remodeling of the cell wall structure during cell division (Kadioglu *et al.*, 2007). It can also induce lysis of bacteria in the stationary phase, and when they are exposed to antibiotics, for example penicillin. During lysis, both cell wall fragments and several virulence factors are released. Most important of them is pneumolysin, a cytotoxin which is stored in the cytoplasm and is released when pneumococci undergo lysis and die. It induces pores in cholesterol rich membranes and leads to lysis of human cells, induction of pro-inflammatory cytokines as well as activation of complement, thereby promoting invasion.

It also influences evasion of human dendritic cell responses. Maus, (2005), showed that administration of purified pneumolysin in the lungs of mice gave a lung injury similar to pneumonia, and that this injury was the result of direct toxic effects of pneumolysin on the alveolar-capillary barrier rather than effects of resident and recruited phagocytic cells. It is therefore intriguing, that serotype 1 strains belonging to one of the most common sequence types (ST 306) according to Murphy, (2009), have been shown to express a non-hemolytic and therefore non-functioning pneumolysin causing less apoptosis but a stronger inflammatory response in dendritic cells than haemolytic alleles. A range of other toxins are also produced. Hydrogen peroxide, further discussed in the colonization section, can be regarded as a toxin because of its toxic effect on respiratory epithelium (Henrichsen, 1995). Hyaluronidase is another important virulence factor degrading connective tissue, involved in the crossing of the
blood-brain barrier during the pathogenesis of meningitis. Pneumococci also produce an enzyme degrading NET’s produced by neutrophils.

2.5 Colonization of the Nasopharynx

Pneumococci are spread via direct contact with secretions from carriers, via saliva or are inhaled via an aerosol, where they colonize the nasopharynx (Murphy et al., 2009). They usually cause an influx of neutrophils, often resulting in a mild rhinorrhea without other symptoms, which promotes spread to other hosts, but they are most often not cleared by the immune system until days to months later (Klugman, K. 2001). The main reason for this is that the capsule protects the pneumococcus against killing by neutrophils. The length of the carriage period depends both on bacterial and host factors. Some serotypes are generally carried for long periods whereas others usually persist only for a short time (Slinger, 2010). Both age and immune status of the host influence carriage duration; especially children below two years of age carry pneumococci for much longer periods than adults (Kadioglu, 2008).

Also the risk of a subsequent development of disease varies greatly depending on both host and bacterial factors (Murphy, 2007). Because of its dual nature, being a commensal and a pathogen, pneumococci can be classified as commensal pathogens (Klugman and Feldman, 2009). The pneumococcus is highly adapted to survive in the nasopharynx in many ways: although catalase negative, this anaerobe tolerates the oxygen rich environment because both exported and cytosolic proteins contain less amino acids (cysteine) that are vulnerable to erroneous oxidization. Furthermore, its production of hydrogen peroxide is high enough to inhibit growth of more sensitive competing bacteria, like Staphylococcus aureus and also to cause damage to host tissues (O'Brien and Santosham, 2009). The high amounts of hydrogen peroxide also cause
oxidative damage to the DNA of the pneumococcus itself, which is partly overcome by its high ability to take up and integrate DNA from the environment. It also produces an array of enzymes facilitating its nasopharyngeal life style: one enzyme makes it less vulnerable to degradation by the lysozyme of the mucus; other enzymes reduce the activity of complement components, C-reactive protein and secretory IgA, thereby reducing opsonisation and hence inhibiting clearance by neutrophils (O'Brien and Santosham, 2009). Other enzymes allow the pneumococcus to retrieve nutrients from the environment and to expose adhesive molecules on the epithelium (Sleeman et al., 2009).

Adhesion to the respiratory epithelium is promoted by a number of molecules, for example pili. It has been shown that a gene coding for a pilus made certain pneumococci more successful in an animal model of carriage, and also that a certain clone expressing pili had been spreading very successfully among humans (Slinger, 2010). Furthermore, pneumococci also form biofilms, consisting of a layer of bacteria and extracellular matrix, making them less vulnerable to attacks from the immune system. And finally, they adapt to the environment by displaying two different phenotypic variants, by changing the thickness of the polysaccharide capsule: the transparent colony variant, with a smaller amount of capsular polysaccharide per cell, is dominating in the colonizing state, thereby promoting adhesion, whereas the opaque variant, with a larger amount of capsular polysaccharide, is more commonly seen when they are in the blood stream, when they need to be protected against opsonophagocytic killing.

2.6 Interactions with Other Pathogens

In the nasopharynx, pneumococcal strains compete with other Pneumococci with other serotypes occurring there at the same time, and with other species, like alpha streptococci, Staphylococcus
and Haemophilus influenza (Darboe, M. 2010). The interactions are not only the question of a fight about food and accommodation; Pneumococci also benefit from their capacity to internalize genes from other species. Furthermore, several viruses have a profound effect on development of the disease. There is firm evidence that much of the morbidity and mortality during influenza pandemics, for example in 1918 and in 2009, was due to pneumococcal pneumonia. Also in the absence of pandemics, peak incidence of invasive pneumococcal disease usually follows the peak incidence of seasonal influenza (Brueggemann, 2004).

Influenza virus facilitates pneumococcal adherence and invasion in the lungs through several mechanisms: both unspecific epithelial damage and specific effects of viral neuraminidase facilitate the binding of the pneumococcus to receptors in the epithelium. Furthermore, the virus induces the expression of such receptors. The viral infection also leads to a modified immune response, partly through interferon gamma, leading to a decreased capacity of alveolar macrophages to clear Pneumococci (Hill et al., 2008). There are also interactions with other viruses, for example respiratory syncytial virus. It has been shown that pneumonia patients co-infected with virus and bacteria seem to develop more severe disease than those with a bacterial aetiology alone (Brueggemann et al., 2004).

2.7 Development of the Disease

Development of pneumococcal disease is a rather rare event compared to carriage. Severe infections, leading to death of the host, are not promoting spread of the organism, and could therefore be regarded as a mistake by the bacteria from an evolutionary point of view. In contrast, in cases where pneumococci cause less severe disease, leading to cough or nasal secretions, development of disease promotes spread of the organism and is therefore “motivated”
also regarded from an evolutionary perspective (Granat et al., 2007). It is easier to understand the various virulence factors, when keeping in mind that they have evolved as adaptations to survival in the hostile environment of the nasopharynx (Park et al., 2007).

An overview of the development of pneumococcal disease show that from the nasopharynx, pneumococci may spread via existing anatomical channels to other parts of the upper airways and cause mucosal disease (Granat et al., 2007). Most common are infections in the middle ear, otitis media, and in the paranasal sinuses, sinusitis. Pharyngitis, tonsillitis and epiglottitis also occur. Pneumococci may also be inhaled in the lower airways, where they may cause bronchitis or pneumonia. In 20-30% of all culture verified cases of pneumococcal pneumonia. Pneumococci are also found in the blood (Weinberger, 2008).

Infectious episodes where pneumococci are isolated from blood or other normally sterile body sites are defined as invasive pneumococcal disease (IPD). The most common IPD manifestation is bacteraemic pneumonia, where pneumococci have managed to cross the respiratory epithelium of the lower respiratory tract (Murphy et al., 2008). Sometimes bacteraemia is found without a detectable primary focus. In those cases, the bacteria are believed to have crossed the mucosal barrier in the nasopharynx instead (Darboe, 2010). Both the upper and lower respiratory epithelium is crossed through a process called transmigration (Weinberger et al., 2008). One of the mechanisms involved is the binding of pneumococci to the receptor for one of the key mediators in the inflammatory response, the platelet activating factor (PAF). When Pneumococci multiply on the mucosal surface, neutrophils migrate into the alveolus (Wuorimaa, 2010).

During the attempts of alveolar macrophages and neutrophils to kill the pneumococci on the luminal side of the mucosal surface, large amounts of cytokines are released (Harboe et al.,
2009). These cytokines activate epithelial cells to express platelet activating factor (PAF receptors on their surface (Sleeman et al., 2009). The phosphorylcholine and lipoteichoic acid on the pneumococcal surface have the capability to attach to the PAF-receptor (File, et al., 2006). The epithelial cell internalizes the receptor-bound pneumococcus, which is subsequently transferred across the stroma and through the endothelium of the blood vessel, to finally be released in the blood in the capillary on the other side of the mucosal barrier (Harboe et al., 2009).

In most cases, the bacteria are then rapidly killed by the defense forces waiting for them in the blood, mainly opsonisation by complement factors and antibodies, followed by phagocytosis by myeloid cells. If the bacteria are not recognized by the immune defense, or manage to grow fast enough to overwhelm its capacity, either because they are extra virulent, or because the immune system of the host is impaired, they multiply and give rise to a blood stream infection (Harboe et al., 2009). Pneumococci may then spread to distant locations in the body though the bloodstream, causing various secondary manifestations, for example osteitis, septic arthritis, endocarditis or abdominal infections. When transition to further compartments occurs, this process has similarities with the transmigration over the respiratory epithelium (Marriott et al., 2009).

If the Pneumococci manage to cross the blood-brain barrier and multiply within the cerebrospinal fluid (CSF), they give rise to meningitis. Spread to the CSF occurs either via the bloodstream, most commonly from a pulmonary focus, or by extension of an infection in the middle ear or in the paranasal sinuses (Backhaus, 2012).
2.8 Pneumococcal Disease Epidemiology

Pneumococcus is an extra cellular pathogen, which spreads by aerosols (Wuorimaa and Kayhty, 2002). The World Health Organization (WHO) has estimated that 1.2 million children under 5 years of age die annually because of pneumococcal disease (Kyaw et al., 2001). More than 70% of pneumococcal diseases in children occur before the age of 2 years (Jones et al., 2001).

Asymptomatic nasopharyngeal carriage of *S. pneumoniae* is widely prevalent among young children in both developed and developing countries, which is important since it is related both to development of disease and to spread of the pathogen to other individuals (Tugba, 2010). Data from the US and Europe indicate that the estimated annual incidence rates per 100 000 young children are 700 for pneumococcal pneumonia, 10 for pneumococcal meningitis, 20 to 140 for pneumococcal bacteraemia and 40 000 for pneumococcal otitis media (CDC, 1997). In developing countries, the rate of infection can be extremely high already approaching 100% during the first years of life (Wuorimaa and Kayhty, 2002). Pneumococcal pneumonia is therefore a particular problem in developing countries.

Approximately 800,000 children die each year due to pneumococcal disease and >90% of these deaths occur in developing countries where few children have access to life-saving serotype-based vaccines. In addition, the emergence of antibiotic resistant strains necessitates the development of an effective vaccine with large serotype coverage. Understanding the serotype epidemiology of invasive pneumococcal disease (IPD) among children is necessary for vaccine development and introduction of health policies (Hope et al., 2010). In Kenya, Pneumonia contributes 20 per cent or 39,760 children deaths in every year (Casey, 2011). This great children mortality rate can be hypothetically attributed to lack of an effective and affordable vaccine. In
2007, The World Health Organization (WHO) recommended that all countries incorporate pneumococcal conjugate vaccines in their national infant immunization programs (Whitney et al., 2010). Yet, while a vaccine against Pneumococcal disease has been widely used in Europe and the United States since 2000, it was not optimal for developing countries. This is because it lacks serotypes or different strains of Pneumococcal bacteria, known to be common in developing countries (Santosham et al., 2004) and the expenses involved in getting the vaccine.

2.8.1 Risk Factors to Pneumonia Infection

Several risk factors such as age below 5 years, attendance at day care centres, frequent occurrence of viral respiratory tract infections, living in a crowded family, lower socioeconomic status, use of antibiotics, exposure to tobacco smoke which favour the nasopharyngeal carriage of *S. pneumoniae* have been thought to contribute to the acquisition of this disease (Tugba et al., 2010).

2.8.1 Pneumococcal Serotype Distribution

*S. Pneumoniae* exhibits 90 capsular polysaccharide serogroups/types (Kadioglu et al, 2008). The serotype distribution varies with age, disease and geographical region (Wuorimaa et al., 2002). The serotypes most frequently involved in pneumococcal AOM in infants are 6A, 6B, 14, 19F and 23F. This distribution resembles that of pneumonia and invasive disease (Kayhty et al., 2002). A wider range of disease types appears in developing countries than in developed countries. Types 4, 6A, 6B, 7F, 14, 19A, 19F and 23F prevail worldwide (Wuorimaa and Kayhty, 2002), but serogroup 18 is common only in developed countries. Types 1 and 5 are common in developing populations and in a few European countries. The virulent serotype 3 is often associated with fatal diseases dominates in Spain and USA (Wuorimaa et al., 2002). The
serotypes mostly associated with anti-microbial resistance includes 6A, 6B, 9V, 14, 19F and 23F.

2.8.2 Regional Serotype Distribution

Regional estimates of the proportion of IPD among young children show that a limited number of serotypes seem to cause most IPD worldwide (Hope et al., 2010). The number of serotypes accounting for more than 70% of IPD range from 6 in North America to 9 in Africa and 11 in Asia. Seven serotypes (1, 5, 6A, 6B, 14, 19F, and 23F) are the most common globally, the seven most common in both Africa and Asia, and account for 58%–66% of IPD in every region (Maria, 2010). Each region has a single country with a significantly greater number of isolates than any other country in that region (i.e., Asia, Israel; Africa, South Africa; Latin America and Caribbean (LAC), Brazil; NA, US; Europe, United Kingdom; and Oceania, Australia).

Serotype 14 is the most common serotype accounting for 12%–29% of IPD in each region (Maria et al., 2010). Serotype 6B ranks second in every region, except Africa (ranked fifth); when combined with serotype 6A, this sero-group account for 14%–18% of IPD across regions (Hope et al., 2010). Serotype 1, a known cause of meningitis outbreaks in the African meningitis rank among the top four serotypes in those regions with the highest IPD burden (Africa, Asia, and LAC). Serotype 5 ranks third in Africa and LAC, and fifth in Asia. Serotypes 1, 5, and 14 together account for 28%–43% of IPD across African regions (Laura et al., 2010). Serotypes 23F and 19F are responsible for 9%–18% of IPD overall. Serotype 18C is common (ranked fourth or fifth) in regions with a large proportion of high-income countries (i.e., Europe, NA, and Oceania). Serotype 19A, which is frequently associated with antibiotic resistance, is relatively more important in Europe (6%) than other regions (Richard, 2010).
2.8.3 Serotype Distribution by GAVI Alliance Eligibility

Serotypes 14, 5, and 1 are the most common serotypes accounting for more than 30% of IPD in GAVI alliance-eligible countries (Laura et al., 2010). Serotype 14 cause most (24%) IPD, and serotypes 1 and 5 ranked fifth and eighth, respectively in non GAVI alliance-eligible countries. Serotypes 6B, 19F, and 23F are the most commonly observed serotypes for both GAVI Alliance- and non-GAVI Alliance–eligible countries (Richard et al., 2010). Serotype 14 dominates the global serotype distribution for children below 2 years and those above this age. Serotype 1 is less common among children below 2 years of age (ranks ninth) than among 2–4 year olds (Hope et al., 2010). This is across the World. Serotypes (1, 5, 6A, 6B, 14, 19F, and 23F) account for approximately 9 million cases and 500,000 deaths due to PD in children below 5 years of age globally (Laura et al., 2010).

2.9 Pneumococcal Conjugate Vaccines

The initial vaccine was a 4 valent vaccine. In the search for effective vaccines to prevent pneumococcal disease, a capsular 14-valent polysaccharide vaccine was introduced in the USA in 1977 and subsequently a 23-valent pneumococcal polysaccharide vaccine (23vPn) in 1983 (Daniel et al., 2008). Serotypes in the 23-valent polysaccharide vaccine (PPV23) but not in PCV7 include 1, 2, 3, 5, 7F, 8, 9N, 10A, 11A, 12F, 15B, 17F, 19A, 20, 22F, and 33F. Three pneumococcal conjugate vaccines (PCVs) are currently available and are protective in young children. A 7-valent vaccine (PCV7, Prevnar, Pfizer) was licensed in 2000 and contains SP serotypes 4, 6B, 9V, 14, 18C, 19F, 23F—the serotypes most commonly causing invasive PD (IPD) among young children in North America (NA). PCV10 (Synflorix, GlaxoSmithKline) was licensed for use in Canada, Australia, and Europe in late 2008 to early 2009 and contains PCV7 serotypes plus serotypes 1, 5, and 7F. PCV13 (Prevnar13, Pfizer) added serotypes 3, 6A, and
19A to the PCV10 serotypes and was licensed in Chile and by the European Medicines Agency in 2009.

The 13vPnC vaccine consists of saccharides from pneumococcal serotypes 1, 3, 4, 5, 6A, 6B, 7F, 9V, 14, 18C, 19A, 19F, and 23F individually conjugated to CRM. The final formulation contains 5mmol/L succinate buffer, with 0.125mg elemental aluminum as aluminum phosphate per 0.5mL dose. PCV7 is currently being replaced by PCV13 vaccine as manufacturing and supply are scaled up (Johnsons et al., 2010). Despite the availability of these vaccines since 2000, few countries in geographic regions with the highest burden of pneumococcal disease have introduced the vaccine into national immunization programs (Murphy et al., 2007). In October 2006, WHO convened an expert consultation meeting to evaluate the minimum or optimal serotype composition of PCVs for use in resource-poor countries. Hausdorff et al., (2010), published estimates of sero-group distributions in year 2000, but since then, little additional data have become available on serotypes for countries in regions where disease burden is the highest. After reviewing IPD epidemiology and existing literature on the global distribution of serotypes, it was concluded that updated estimates of serotype distribution are needed (Maria et al., 2010).

### 2.10 Impact of PCV-10 Vaccination

Different impacts of the PCV vaccination programme have been observed in different countries. In the USA, a dramatic reduction in the invasive pneumococcal disease rates in both vaccinated and non-vaccinated population was observed shortly after the introduction of PCV-7 into their routine. Herd protection exceeded the effects of direct protection by the PCV-7 (Wyeth, 2006). On the other hand, in some European countries (France, Netherlands, Spain, UK), no reduction
in IPD incidence among non-vaccinated adults was observed 2-3 years after the introduction of PCV-7 in universal infant immunization (Wysocki et al., 2008).

In these countries, the reduction in vaccine-serotypes IPD rates was partially offset by the increase in non-vaccine serotypes IPD. The differences in the results of the vaccination programme may be explained at least in part, by country-specific differences in PD rates, circulating serotypes causing disease, the adopted vaccine schedule (three or four doses, with or without catch-up vaccination of older children) and vaccine coverage (Sartori, 2010).

In Kenya, the circulating serotypes may be slightly different from those causing disease in other countries and the actual rates of PD are still difficult to obtain, and especially for the less severe diseases treated at the outpatient healthcare facilities (Scott, 2010). PCV-10 not previously used in routine immunization programme has been introduced, with cost-effectiveness studies having been part of decision process for the inclusion of new vaccines into the national immunization programme. However, an active nationwide surveillance on PD is necessary in order to evaluate the impact and long-term benefits of the PCV-10 immunization programme. The PCV-10 is given in all government vaccination centers to children aged 6, 10, and 14 weeks as part of the routine immunization schedule, with catch-up vaccination for under-1 children in the first year of the programme. The 13-valent pneumococcal conjugate vaccine (PCV-13) was recently introduced in the country but only in the private healthcare system, with low population coverage.

Pneumococcal disease mainly affects young children and the aged, perhaps due to poor immunity, but adults also carry the bacteria in the nose. Previous studies have shown that vaccinating children with PCV-10 protects adults through induction of "herd immunity"
(Iannelli, 2008). Vaccinating a few individuals in the population protects most of the people in the population. This is because the bacterium lives in the nose, and it is transmitted through coughing, picking the nose and touching others. Therefore, if you vaccinate children, they will not transmit to the parents. Clinical trials revealed that the vaccine was also effective in children with HIV (Scott et al., 2010).

2.11 Immune Response to PCV Vaccine

Immunization with bacterial polysaccharide (PS) antigens typically induces a T-cell-independent type 2 antibody response characterized by high levels of immunoglobulin M (IgM), IgG antibodies primarily of the IgG3 subclass in mice and IgG2 in humans, an absent or blunted memory response, and no requirement for the direct involvement of T cells (Melegaro and Edmunds, 2004).

2.11.1 Mucosal Immune Responses

The natural infection by pneumococcus originates in the nasopharynx. It starts with asymptomatic carriage which in some cases leads to invasion and clinical disease (Uchiyama et al, 2009). The balance between local defence mechanisms, competition with other organisms and the invasiveness of the serotype determines whether the organism will successfully invade the body (Uchiyama et al., 2009). Secretory IgA and locally leaked IgG against pneumococcal polysaccharides and surface associated proteins have been found in children who are colonized with pneumococcus. The mucosal protection however, involves other immune mechanisms under natural exposure as demonstrated in a mouse model showing the role of CD4+ T-cells and IL17. Factors enhancing the elimination of the pneumococcus from the nasopharyngeal mucosa involve local innate immune responses in the nasopharynx which also plays a role in initiating
adaptive immune responses. The recruitment of neutrophils and functional pneumococcal pneumolysin enhance antigen uptake in the nasal associated lymph nodes which is an important step in adaptive immunity. Toll like receptors 2 and 4 on antigen presenting cells and phagocytes and nucleotide binding oligomerization domain 1 (NOD 1) in the cytoplasm have been found to play a critical role in the mucosal defense against polysaccharide encapsulated *Haemophilus influenza*, whereas the protection against non-capsulated HI (*Haemophilus Influenza*) did not.

At the mucosal surface the pneumococcus can decrease the viscosity of the mucus with neuraminidases leading to the exposure of surface receptors that can interact with the pneumococcal surface-associated protein. Secondary to cytokine stimulation the epithelial cell upregulates PAF receptors to which the pneumococcus increases the affinity through its cell-wall phosphocholine. In addition the CbpA/PspC, can bind directly to the polymeric Ig receptor (pIgR) on the epithelial cell and pneumococcal IgA1 protease can cleave opsonising IgA which increases migration through the mucosa. Altogether, the interactions between the pneumococcus and the epithelium can facilitate pneumococcal invasion and lead to systemic infection. The increased invasiveness of pneumococci following influenza infection may be explained by neuraminidase activity of the viruses contributing to enhanced adherence of the pneumococci. Surface associated pneumococcal proteins are now increasingly investigated as possible vaccine candidates. Some of the pneumococcal proteins are present on a vast majority of pneumococcal strains belonging to most of the known serotypes and such a vaccine would have the potential of decreasing colonization as well as systemic disease across all serotypes.

**2.1.1.2 Systemic Immune Responses**
If the pneumococcus succeeds in evading the mucosal defence the systemic immune response takes over with opsonization and phagocytosis of the pathogen (Nelson and Laurell, 2007). The host defense against encapsulated bacteria depends on humoral immunity with antigenspecific antibodies, complement activation and phagocytic killing or lyses of gram-positive or gram negative bacteria, respectively (Balachandran et al, 2002). The humoral immune responses are divided into thymus dependent (TD) or thymus independent (TI) antibody responses depending on whether the antigen is presented in the major histocompatibility complex class II (MHC-II) on antigen presenting cells (GSK, 2009). TD proteins antigens and peptides derived from them associate with the MHC-II, leading to activation of T-helper cells inducing isotype switching and generation of immunological memory. Purified polysaccharides such as PPS stimulate B-cells in a T-cell independent manner. In infants and young children this leads to IgM antibody production that does not progress to isotype switching, somatic hyper mutation, affinity maturation or memory generation. In adults, a polysaccharide-specific IgM+ memory B cells, originating in the marginal zone of the spleen, have been described as a responsive cell type to the PPS and are thought to take part in the natural immunity to the pneumococcus (Zhang and Finn 2004). Children start to respond to PPS around 2 years of age, coinciding with the occurrence of the marginal zone B cells in the spleen. (Henrichsen, 1995).

Studies have also indicated that besides being not presented in the MHC II the polysaccharide immune response may differ due to the nature of the B cell antigen receptor signaling of the responding B cell subpopulations (Kadioglu, 2007). In a study on SCID mice transplanted with human B lymphocyte subsets and then immunized with heat-inactivated streptococcus or with PPV23, IgM anti-polysaccharide and anti-protein antibody responses from IgM memory B lymphocytes were observed (Holmes et al., 2006). IgG anti-polysaccharide and anti-protein
responses were also observed from switched memory B lymphocytes. In addition, IgM memory B cells elicited an IgG anti-polysaccharide and anti-protein response indicating versatile role of IgM memory B cells in T-independent and T-dependent immune responses (Jedrzejas et al., 2002). Mouse studies have further shown that immunization with PCV induces carrier-specific T cell responses that increase with age and determine the levels of PPS11 specific Ab elicited. A weak and Th2-biased response was observed in neonatal mice while infant mice showed a mixed Th1-Th2 response, as observed in adults (Sleeman et al., 2009).

Serotype polysaccharide specific IgG and complement confer protection against the given pneumococcal serotype. Newborns and infants up to the age of 2 years are unable to produce IgG antibodies to bacterial capsular polysaccharides (Holmes et al., 2006). Consequently, children up to the age of 2 years have an increased susceptibility for infections with encapsulated bacteria such as pneumococcus and Haemophilus influenzae type b and Neisseria meningitidis. The polysaccharides have been rendered immunogenic by direct covalent binding to immunogenic proteins. These polysaccharide-protein conjugates are taken up by antigen presenting cells that present the protein moiety in the MHC II molecule and induce clonal expansion of protein specific T helper cells (Zhang, 2000).

2.12 Pneumococcal Conjugate Vaccine Immunogenicity

PCV10 and PCV13 are licensed for the prevention of invasive disease, pneumonia and acute otitis media caused by the respective vaccine serotypes in children from 6 weeks to 5 years of age. In addition, PCV13 is licensed for the prevention of pneumococcal disease in adults aged ≥50 years (Murphy et al 2007). Recent reviews of randomized, controlled trials of PCV7 and PCV9 have shown vaccine efficacies (VEs) against invasive pneumococcal disease of 71% and
93%, respectively, following 3 primary doses or 3 primary plus a booster dose (Wuorimaa et al., 2007). For radiologically confirmed pneumonia (first episode), the estimated VE for 3 primary doses was 24%. Based on the immunogenicity data, PCV10 and PCV13 show comparable VEs for serotypes contained in the vaccines (WHO, 2012).

The immunogenicity and reactogenicity of the involved antigens are shown not to be significantly altered when PCVs are given concomitantly with other childhood vaccines. VE with PCV9 remained significant (78%) against IPD 6 years after PCV immunization. PCVs are considered safe in all target groups for vaccination, including immunocompromised individuals (WHO, 2012). The World Health Organization (WHO) recommends that inclusion of PCVs be given priority in childhood immunization programmes world-wide, especially in countries with under-5- mortality of >50/1000 live births. For administration to infants, 3 primary doses (3p+0 schedules) or, as an alternative, 2 primary doses plus a booster (2p+1 schedule) are recommended. Primary vaccination can be initiated as early as at 6 weeks of age. In choosing between the 3p+0 and 2p+1 schedule, countries should take into consideration local disease epidemiology, particularly the peak age of disease. If the 3p+0 schedule is used, the minimum interval between doses should be 4 weeks, with vaccinations scheduled at 6, 10, and 14 weeks of age or at 2, 4, and 6 months of age, depending on programmatic convenience (WHO, 2012).

2.13 Side Effects and Contra-indications of Pneumococcal Vaccine

Any medicine, including a vaccine, could possibly cause a serious problem, such as severe allergic reaction. However, the risk of any vaccine causing serious harm, or death, is extremely small. Pneumococcal vaccine uncommonly causes side effects; however, in clinical studies the most frequently reported adverse events included soreness and/or redness at the injection site,
fever, irritability, drowsiness, restless sleep, decreased appetite, vomiting, rash, and diarrhoea (Wysocki et al., 2008). Risks are associated with all vaccines, including PCV-10. Hypersensitivity to any vaccine component, including diphtheria toxoid, is a contraindication to its use. Pneumococcal vaccine is not indicated for use in adults. The decision to administer PCV-10 should be based on its efficacy in preventing invasive pneumococcal disease (Sartori et al., 2010).

2.14 Administration of PCV-10 Vaccine

The 10-valent pneumococcal conjugate vaccine is administered in three doses at age 6, 10, and 14 weeks, with catch-up vaccination for under-1 children in the first year of the programme. PCV-10 is co-administered with routine childhood vaccines (pentavalent and OPV) (Knuf, 2010). PCV-10 injection is administered at the right thigh while the pentavalent injection at the left thigh. This approach avoids additional visits, reduces administration costs and enhances compliance and vaccination coverage (Clark & Sanderson 2009).

2.15 Herd Immunity

Herd immunity has long been recognized as an important benefit of vaccines. Vaccines against diphtheria, poliovirus, varicella, rubella, measles, hepatitis B virus, Bordetella pertussis, and a variety of veterinary pathogens have important herd immunity effects, and herd immunity is being emphasized as an important strategy against influenza (Treanor, 2007). Largely unanticipated at the time of vaccine introduction, the herd immunity effect of the bacterial polysaccharide conjugate vaccines has been a major contributor to the successful control of invasive and noninvasive disease due to Haemophilus influenzae type b (Hib), major serotypes of Streptococcus pneumoniae, and Neisseria meningitidis serogroup C (Ramsay, 2010). Maiden
et al., (2009) in their study and other recent studies provide additional lessons about how herd immunity and bacterial polysaccharide conjugate vaccines work together, as well as information about the limitations of herd immunity. However, many questions remain to be answered. How should these vaccines be introduced to maximize and maintain effectiveness through individual and herd immunity, for example; should we use an infant or toddler strategy, other limited cohorts, catch-up campaigns, or focus on individuals with highest carriage and transmission dynamics? How is agent acquisition, and thus transmission, prevented? How will these vaccines alter microbial biology, which can have short term or long term influences on vaccine effectiveness and/or population susceptibility? Does each bacterial conjugate vaccine have similar herd immunity effects? How can herd immunity be maintained? What is the role of herd immunity in the protection of populations with waning individual protection? (McVernon, 2005).

The immune basis for herd immunity is not well defined. The bacterial polysaccharide-protein conjugate vaccines produce higher levels of mucosal IgG antibody, presumably due to transudation of IgG from serum and possibly higher-avidity antibodies (Zhang and Finn, 2004).

Immunization of infants using pneumococcal conjugate vaccines has reduced invasive pneumococcal disease (IPD) and hospitalization for pneumonia in children in randomized trials (WHO, 2004). Since its introduction, 10-valent conjugate pneumococcal vaccine (PCV10) has reduced IPD dramatically, including in unvaccinated age groups, through induction of herd immunity (Pelton, 2004). Pneumococcal conjugate vaccination of infants has previously been shown to reduce invasive pneumococcal disease (IPD) among seniors through prevention of pneumococcal transmission from infants to adults (herd immunity), (Simonsen, 2011). De Wals et al., 2008, have reported a significant decline in pneumococcal (lobar) pneumonia in Canadian children <5 years of age after Canada introduced the vaccine for all children in December 2004.
Protecting the unvaccinated through herd immunity is a remarkable and very powerful effect of vaccines and should be an important consideration in strategies for vaccine introduction, implementation, evaluation, and monitoring, rather than an afterthought (WHO, 2011).

A better understanding of the dynamics of herd immunity and the length of protection it provides, as well as the impact of herd immunity on microbial biology, the mechanisms of microbial replacement, and natural immunity and biology are clearly needed to use this genie wisely (Zou, 2010).

### 2.16 Factors that Affect Effectiveness of Pneumococcal Conjugate Vaccines

The success of introduction of a new vaccine into the National immunization program depends on the knowledge about the vaccine, practices by the health workers and the sociodemographic factors of the community involved in the immunization (WHO, 2002). The incorporation and implementation of a new vaccine into national program is not recommended in the absence of specific trainings of health workers and community sensitization (WHO, 2002). Health care providers who care for children should have a thorough grasp of these potential complications and be prepared to educate parents/caregivers appropriately so that barriers to adherence can be minimized (Tenreiro, 2005).

Although the benefits of vaccines are overwhelmingly positive from economic and medical science perspectives, complex issues – such as safety, adherence, and scheduling hinder the implementation of immunization program (Wuorimaa, 2007). In order to reach Millennium Development Goal (MDG) for child survival, Global action plan for the prevention and control of pneumonia (GAPP) recommends a 90% coverage rate of pneumonia immunization (WHO,
2009). Many of the countries that do not meet pneumonia prevention targets are expected to introduce new pneumonia vaccines, expand community-based case management and strengthen health systems (IVAC, 2010). Assuring that these plans are implemented will require funding and continued public attention to pneumonia, which will help contribute to a substantial decline in childhood pneumonia deaths (IVAC, 2010). Factors such as: infants age, use of alcohol by caregiver, maternal diet during pregnancy, breast feeding frequency, gap with other children, child hospitalization, type of hospital, name of estate, size of residential house, number of house mates, chronic illness, duration of chronic illness if any, whether child is on medication, and child is been on medication for how long are key to serum antibody levels (WHO, 2002).

**2.17 PCV-10 Introduction in Kenya**

In February 2007, the Global Alliance for Vaccine and Immunization (GAVI) announced a US$1.5 billion Pneumococcal to accelerate the development and introduction of pneumococcal vaccines for the world's poorest countries (GAVI Alliance–eligible: countries with a gross national income per capita <US$1,000 in year 2003). Essential to the Pneumococcal AMC was the establishment of minimum vaccine product specifications, also known as the target product profile (TPP), for a vaccine to be eligible for AMC funds (Johnson et al., 2010). Most developing countries have no access to these conjugates yet. However, Kenya launched and included the 10-PCV vaccine in the National Child Immunization Program in February 2011. Since then the immunogenicity of the vaccine has not been assessed to determine the effectiveness of the vaccine. Hence the aim of this study is to determine whether the vaccine elicits protective humoral immunity to vaccinated children.
CHAPTER THREE: MATERIALS AND METHODS

3.1 Study Site

This study was done at the Child Welfare Clinic of Mbagathi District Hospital, Nairobi Kenya. The hospital serves mainly Kibra district residents with >60% coming from the neighbouring Kibra estate (KNBS, 2009). Most of these children present with pneumococcal disease complications. Kibra is situated in Nairobi’s South Western Peri-urban zone approximately seven kilometres from the Nairobi City Centre. It is an informal settlement comprising of ten villages covering approximately 250 hectares of land with an estimated population of about 500,000 people. That gives an average population density of 2000 people per hectare although some villages are more crowded than others (KNBS, 2009).

3.2 Study Design

A descriptive cross-sectional study was used.

3.3 Study Population

Infants aged between 3-12 months and who had completed PCV-10 immunization and their biological mothers were targeted for this study.

3.3.1 Inclusion Criteria List

Children who attend the Child Welfare Clinic of Mbagathi District Hospital for vaccination and who had finished three doses of PCV-10 at least four weeks earlier, were recruited. Participating children were aged between 3 to 12 months. Another target population was biological mothers of study infants aged 3-12 months and who were eligible for PCV-10 immunization at MDH.
3.3.2 Exclusion Criteria

Any infant with a medical condition (for example; one that requires frequent or long-term referrals to health-care facilities) and which would interfere with the assessment of the study objectives were excluded from the study. All subjects not meeting the general inclusion criteria or refused to sign the consent form were excluded.

3.4 Sample Size

To determine the minimum sample, the formula of Daniel et al., (2009) was to be used, with a prevalence rate of 50% (WHO, 2010).

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

Sample Size (n) = 384,

Where, \( \hat{p} \) is the assumed prevalence of immunogenicity among vaccinated children, m is the desired margin of error around the estimated prevalence herein taken to be 5% and for 95% confidence \( z=1.96 \). The sample size was 384.

3.5 Sampling Procedure

Purposive sampling method was used where the targeted study subjects were recruited as they came. A total 318 infants were recruited on first come, first sampled basis. An equivalent number of mothers were enrolled along with their infants to study herd immunity effects. This represented a response rate of 82.8%.
3.6 Informed Consent

Biological mothers of study infants were approached at the Child Welfare Clinic of Mbagathi District Hospital as they brought their infants for weight check and vitamin A administration, to sign a written informed consent document. This was done after reading the document and having the study explained to them in Kiswahili as appropriate (Appendix I). They also had opportunity to ask all questions which facilitated their informed participation in the study.

3.7 Research Instruments

A study questionnaire was formulated by the main researcher and moderated by the research supervisors. Copies of the standardized questionnaire were produced and used to collect data from all the sampled subjects.

3.8 Pre-Testing Study Tools

Pre-testing of the study instruments was carried out at Kiambu District Hospital whereby 40 infant biological mothers and 40 infants were recruited. This was important in operationalizing the study tools and the conceptual framework. Corrections and amendments on the instruments were then made before the actual study commenced. Kiambu district hospital has almost similar conditions as the study hospital, Mbagathi. Thus it was appropriate for the pilot study. Reliability was ensured through use of well-designed questionnaires and daily checking and correction of completed questionnaires.

3.9 Collection and Storage of Study Samples

Samples were collected by qualified and government registered phlebotomists working at the Hospital. Serum samples were required for this study. Coagulant-free vials were used to store the
collected sample. After child preparation (entailing presence of mother to ease tension), a 5ml capillary blood sample (heelstick) was collected aseptically in to the vials for infants and venous blood sample for study infant’s biological mothers.

**3.10 Sample Processing**

After collection, specimen was stored at room temperature until a clot formed (usually 15-45 minutes), then was centrifuged to obtain serum specimen for assay. Serum to be analyzed within 24 hours was stored at 4°C and samples to be analyzed out of this range of time was stored frozen at -20°C. Samples were then transported Human Diagnostics World Laboratory on dry ice.

**3.10.1 Sample Analysis**

All serum samples were analyzed using standard Enzyme-Linked Immunosorbent Assay (ELISA) technique (Relevant to the sero-parameter being tested, i.e. IgG antibodies). The pneumococcal antibody threshold concentration limit of 0.35μg/ml was considered sero-protective (WHO, 2008). Samples with antibody levels below this value were considered as lacking sero-protection.

**3.10.2 ELISA Procedure**

The researcher set incubator to 37±1°C. All reagents were brought to room temperature and shook well before use (approximately 1 hour), without removing the plate from the bag. The researcher then removed plate 1 from the package, determined the numbers of wells to be employed by counting in four wells for the controls: two for the cut off serum and one each for the negative and positive sera. Thereafter, 100 μl of serum diluent 2 was added to all wells. Added 5 μl of each sample, 5 μl of positive control 3G, 5 μl of cut off control 4G (in duplicate)
and 5 μl of negative control 5G into the corresponding wells. Shook the plate in a plate shaker (2 min) in order to achieve a homogenous mixture of the reagents. Mixed homogenously with the pipette and dispensed 105 μl of each diluted sample to the wells 1. The preparation was the covered with a sealing sheet and incubated at 37±1°C for 45 min. The researcher then removed the seal, aspirated liquid from all wells and washed five times with 0.3 ml of washing solution 9 per well and drained off any remaining liquid. Immediately, 100 μl of IgG conjugate solution 6G was added into each well, covered with a sealing sheet and incubated in incubator/water bath at 37±1°C for 30 min. Thereafter, the seal was removed, aspirated liquid from all wells and washed five times with 0.3 ml of washing solution 9 per well and drained off any remaining liquid. Immediately after, 100 μl of substrate solution 7 was added into each well, incubated at room temperature for 20 minutes protected from light then added 50 μl of stopping solution 8 into all wells immediately then read OD’s with a spectrophotometer at 450/620 nm within 1 hour of stopping (Harlow and Lane, 2010).

3.11 Ethical Approval

Scientific and ethical approval was given by Ethics Review Committee (ERC) and authorization of the study was sought from Kenyatta University Graduate School and Mbagathi District Hospital. Permission and funding was sought from NACOSTI. Signed informed consent was sought from study infant’s biological mothers after a clear explanation of the study and its purpose. Barcodes were used in this study and these codes were not linked to the personal identification or names. The procedure entailed some little pain which lasted just for some minutes. Results from this study may be useful in informing policy formulation.
3.12 Data Analysis

Univariate analysis was done to determine the most significant variable that affects the PCV-10 vaccine immunogenicity. Cross-tabulations and correlation analysis were used to generate the relationship between dependent and independent variables respectively. Ninety-five percent confidence intervals were used. Graphs, pie-charts and tables were used to analyze the participant’s demographic characteristics.
CHAPTER FOUR: RESULTS

4.1 Introduction

The purpose of this study was to determine serum levels of IgG antibodies as indicators of immunogenicity against PD among infants vaccinated with PCV-10 (Synflorix) that attended Mbagathi District hospital from September 2013 to November 2013.

4.2 Sample Population

The initial sample population comprised of 384 infants below 12 months of age attending Child welfare clinic at Mbagathi District Hospital, and who had completed three doses of PCV-10 at least four weeks prior to the study. Three hundred and eighty four biological mothers to the study infants were also sampled to participate in the study. However, a total of sixty six (66) participants (both study infants’ biological mothers and study infants), initially targeted for the study, could not be reached. This was largely due to some of the study infant’s mother’s failing to attend clinic on the appointment date. As a result, the sample size reduced from 384 to 318 for both study infants and study infant’s biological mothers, and the response rate was therefore 82.8%. According to Garry L. (2010), a response rate of ≥60% is representative enough.

4.3 Socio-Demographic Factors

The demographic characteristics of the respondents assessed in this study included: infant’s age, use of alcohol by mother, maternal diet during pregnancy, breast feeding frequency and gap with other children. Others were child hospitalization; number of house mates, chronic illness, and child is on medication for how long.
4.3.1 Infants Age

The infants were from the age of three to twelve months with the youngest being four months and oldest twelve months. Most of the infants (30.8%) were between 6 to 8 months followed by infants between 3 to 5 months (24.5%) as shown in figure 4.1.

Figure 4.1 The age of infants vaccinated with PCV-10 that attended child welfare clinic at Mbagathi District Hospital.

Graph showing various age brackets of study infants and their percentages. The classification is as per WHO, 2009 policy paper on PCV-10 (Synflorix) vaccine administration.
4.3.2 Use of Alcoholic Drinks by Mother

Data shows that an overwhelming majority of the mothers (90.6%) did not use alcohol. However, a small percentage 9.4% drank alcohol during the time they were pregnant (Figure 4.2).

![Graph showing percentage of mothers who used alcoholic drinks during pregnancy](image)

Graph shows percent of infant's biological mothers who used any form of alcoholic drinks during the time they were pregnant with the study infants.

Figure 4.2 Percentage of mothers who used alcoholic drinks during pregnancy

4.3.3 Maternal Diet during Pregnancy

Eighty six percent of the mothers’ diet had all the required food nutrients with the remaining percentage (14%) taking a diet that did not contain all the required nutrients (Figure 4.3).
4.3.4 Infant’s Daily Breast Feeding Frequency

The majority of the mothers (77.4%) breast fed their babies more than five times a day, while 1.26%, 2.52%, 8.1% and 10.69% of the mothers breast fed their babies one per day, twice per day, three times per day and four times per day respectively (Figure 4.4).
4.3.5 Study Infant’s Gap with other Biological Siblings

Most of the infants (64.2%) had more than a two year gap between them with other children and 14.5% had a gap of less than one year (Figure 4.5).
4.3.6 Child Hospitalization since Birth

The majority of the infants (89.9%) had not been hospitalized since birth. However, only 10.1% infants had been hospitalized (Figure 4.6).
Figure 4.6 Whether the study infant had ever been hospitalized since birth or not

The graph shows the number of infants' (in form of percentage) whose mothers said they had been hospitalized before commencement of the study.
4.3.7 Type of Hospital attended by the Various Study Infants

The majority of the infants (89.3%) attended government District hospitals with only 3.1% infants attending Private hospitals (Figure 4.7)

The chart shows the type hospital where infants who had ever been sick and hospitalized since birth attended. The options range from Government dispensaries, Government District Hospital, Government Provincial Hospital, Government National Hospital and all sets of private hospitals.

Figure 4.7 Type of hospital attended by the study infants
4.3.8 The Estate where Study Infants Resided

The majority of the infants and mothers (91.2%) who attend the welfare clinic at the hospital reside in Kibra (Figure 4.8).

![Graph showing estate distribution](image)

The graph shows estates where various study infants resided from. The categories included: Eastlands, Kibera, Highrise/Hurlingham, and Street children.

**Figure 4.8 The estate where study infants resided**

4.3.9 Number of people that share the same house as the study infant

Most of the mothers (54.1%) lived with three to five household members followed by mothers (19.5%) who lived with more than ten household members (Figure 4.9).
Graph shows the number of household members who shared the same house with the study infants. The options range from less than two (<2), three to five, six to seven, eight to ten and more than ten (>10). This classification was adopted WHO, 2009 policy paper on PCV-10 administration.

Figure 4.9 Number of members that shared household with the study infant
4.3.10 Chronic Illness

The majority of the mothers (95.6%) did not have any chronic illness (Figure 4.10).

Figure 4.10 Percent of infants who suffered from a chronic illness

Graph shows various chronic illness that study infants suffered from. They include HIV/AIDS, Cancer, Sickle cell anaemia and none. The graph also shows the percent of infants who suffered from the mentioned illness.
4.4 Laboratory Investigation

This section shows serum concentration of pneumococcal disease specific IgG among infants with completed 3-doses of PCV-10 at MDH and cross tabulation of IgG antibody levels against socio-demographic factors both among infants and mothers. The socio-demographic characteristics include: Age of the infant, daily breast feeding frequency, presence of any chronic illness, type of diet (whether balanced or unbalanced) and use of alcoholic drinks by mother during pregnancy.

4.4.1 IgG Antibody Levels (mg/dl) for Infants

Majority of the infants (74.1%) had antibody levels between 0.34 – 0.36 mg/dl - after completion of 3-doses of PCV-10; this was followed by 10.69% infants representing 0.37-0.39 mg/dl antibody levels (mg/dl) (Figure 4.11).
Graph shows various classifications of serum IgG antibody titers among study infants. It also shows the percentage of infants who fall within the various serum IgG antibody level groupings. The classification range from 0.25-0.29, 0.30-0.33, 0.34-0.36, 0.37-0.39 and <0.25. This classification was adopted from the WHO, 2009 policy paper on PCV-10 (Synflorix) classification.

**Figure 4.11 IgG antibody levels for study infants**

### 4.4.2 Serum IgG Antibody Levels for Infants against Socio-demographic Factors

Data shows that infants between the ages of 6-8 months (81.6%), 9-10 (73.7%) and 11-12 months (81.8%) had the highest score of serum IgG antibody levels of 0.34-0.36 mg/dl compared to infants between those of ages of 3-5 months who had very low IgG antibody levels. At as late
as 11-12 months, some infants (6.1%) still had antibody levels that were below 2.5mg/dl (Figure 4.12).

**Table 4.1 Infants age and IgG antibody levels (mg/dl)**

<table>
<thead>
<tr>
<th>Count</th>
<th>IgG ANTIBODY LEVELS (mg/dl) FOR INFANTS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>3 to 5 months</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>6 to 8 months</td>
<td>3</td>
<td>10</td>
</tr>
<tr>
<td>9 to 10 months</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>11 to 12 months</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>5</td>
<td>13</td>
</tr>
</tbody>
</table>

**4.4.3 Use of Alcoholic drinks by mothers and IgG Antibody Levels (mg/dl) for Infants**

Most of the infants (83.3%) whose mothers did not drink alcohol had a high IgG antibody level of 0.34 - 0.36 mg/dl compared to infants (6.7%) whose mothers used alcohol (Figure 4.13).

**Table 4.2 Use of alcoholic drinks by mother and IgG antibody levels (mg/dl) for infants**

<table>
<thead>
<tr>
<th>Count</th>
<th>IgG ANTIBODY LEVELS (mg/dl) FOR INFANTS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>Use of alcoholic drinks by mother</td>
<td>Yes</td>
<td>3</td>
</tr>
<tr>
<td>No</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>6</td>
<td>6</td>
</tr>
</tbody>
</table>
4.4.4 Maternal Diet during Pregnancy and IgG Antibody Levels (mg/dl) for infants

Data shows that 61% of the infants whose mother’s diet was balanced during pregnancy had IgG antibody levels of 0.34 - 0.36 mg/dl compared to 13.8% of infants whose mother’s diet during pregnancy was not balanced falling in the same margin (0.34-0.36mg/dl). Nine percent of infants whose mothers had all nutrients included in their diet during pregnancy also had high serum IgG antibody levels (0.37-0.39mg/dl) after completing the recommended three doses of the vaccine (Figure 4.14).

Table 4.3 Maternal diet during pregnancy and IgG antibody levels (mg/dl) for infants

<table>
<thead>
<tr>
<th>Count</th>
<th>IgG ANTIBODY LEVELS (mg/dl) FOR INFANTS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>Maternal diet durante</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Balanced diet</td>
<td>11</td>
<td>20</td>
</tr>
<tr>
<td>Unbalanced diet</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>28</td>
</tr>
</tbody>
</table>

4.4.5 Daily breast feeding frequency and IgG Antibody Levels (mg/dl) for Infants

The percentage of infants with IgG antibody level of 0.34 - 0.36 mg/dl increases significantly as the breast feeding frequency increases. Sixty one percent of the infants breast fed more than five times a day had IgG antibody levels between 0.34 to 0.36 mg/dl respectively. Data also shows interesting results i.e. infants who had the lowest IgG antibody level of less than 0.25 mg/dl were breast fed only once a day with the percentage of infants with IgG antibody level of less
than 0.25 mg/dl systematically decreasing as the breast feeding frequency increases (Figure 4.15).

Table 4.4: Breast feeding frequency and IgG antibody levels (mg/dl) for infants

<table>
<thead>
<tr>
<th>Count</th>
<th>IgG ANTIBODY LEVELS (mg/dl) FOR INFANTS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>Breast feeding frequency</td>
<td>Once per day</td>
<td></td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Twice per day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>Three times a day</td>
<td>1</td>
</tr>
<tr>
<td></td>
<td>Five times a day</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>More than five times a day</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>1</td>
<td>6</td>
</tr>
</tbody>
</table>

4.4.6 Gap with other Children and IgG Antibody Levels (mg/dl) for Infants

As the gap between children increases, infants’ IgG antibody levels also increase. Infants’ gap of less than 1 year had <1% infants with IgG antibody level of 0.34 - 0.36 mg/dl, gap of 1-2 years had 32% infants with IgG antibody level of 0.34 - 0.36 mg/dl, and gap of more than 2 years had 47% infants with IgG antibody level of 0.34 - 0.36 mg/dl (Figure 4.16).
4.4.7 Chronic Illness and IgG Antibody Levels for Infants

More infants (28.6%) with chronic illness (HIV/AIDS) had IgG antibody level of less than 0.25 mg/dl compared to infants (2%) without chronic illness (Figure 4.17).

### Table 4.5: Gap with other same mother siblings and IgG antibody levels (mg/dl) for infants

<table>
<thead>
<tr>
<th>Gap with other same mother siblings</th>
<th>IgG ANTIBODY LEVELS (mg/dl) FOR INFANTS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>Less than one year</td>
<td>0</td>
<td>2</td>
</tr>
<tr>
<td>One year</td>
<td>1</td>
<td>2</td>
</tr>
<tr>
<td>More than one year</td>
<td>4</td>
<td>4</td>
</tr>
<tr>
<td>Two years</td>
<td>3</td>
<td>20</td>
</tr>
<tr>
<td>More than two years</td>
<td>4</td>
<td>5</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>33</td>
</tr>
</tbody>
</table>
Table 4.6: Chronic illness and IgG antibody levels (mg/dl) for infants

<table>
<thead>
<tr>
<th>Chronic illness</th>
<th>IgG ANTIBODY LEVELS (mg/dl) FOR INFANTS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>27</td>
<td>4</td>
</tr>
<tr>
<td>Any cancer</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>none</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>39</td>
<td>28</td>
</tr>
</tbody>
</table>

4.5 Measurement of Serum Concentration of PD Specific IgG among mothers

This section shows the measurement of serum concentration of pneumococcal disease specific IgG antibodies among mothers and cross tabulation for IgG antibody levels (mg/dl) for selected caregivers against socio demographic factors.

4.5.1 IgG Antibody Levels (mg/dl) for mothers

The majority of the mothers (74.6%) had an antibody levels (mg/dl) that is, between 0.34 – 0.36 mg/dl after their infants’ completion of 3-doses of PCV-10, this was followed by 10% mothers representing 0.37 – 0.39 mg/dl antibody levels (mg/dl) (Figure 4.18).
4.5.2 IgG Antibody Levels of mothers and Socio-demographic Factors

Mothers whose infants were between the age of 9-10 months (58%) and 11-12 months (26%) had the highest score on IgG antibody levels of 0.34-0.36 mg/dl. (Figure 4.19)
Table 4.7 Infants age and IgG antibody levels for infant’s biological mothers

<table>
<thead>
<tr>
<th>Infants age</th>
<th>IgG ANTIBODY LEVELS FOR INFANTS BIOLOGICAL MOTHERS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>3 to 5 months</td>
<td>2</td>
<td>4</td>
</tr>
<tr>
<td>6 to 8 months</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>9 to 10 months</td>
<td>5</td>
<td>15</td>
</tr>
<tr>
<td>11 to 12 months</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Total</td>
<td>12</td>
<td>27</td>
</tr>
</tbody>
</table>

4.5.3 Use of Alcoholic drinks by mother and IgG Antibody Levels (mg/dl)

The majority of mothers (90%) who do not use alcohol have a high IgG antibody level of 0.34 - 0.36 mg/dl compared to mothers (10%) who use alcohol (Figure 4.20)

Table 4.8 Use of alcoholic drinks by mother and IgG antibody levels for infants biological mothers

<table>
<thead>
<tr>
<th>Use of alcoholic drinks by mother</th>
<th>IgG ANTIBODY LEVELS FOR INFANTS BIOLOGICAL MOTHERS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>Yes</td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td></td>
<td>0</td>
<td>22</td>
</tr>
<tr>
<td>No</td>
<td>0</td>
<td>9</td>
</tr>
<tr>
<td>Total</td>
<td>0</td>
<td>31</td>
</tr>
</tbody>
</table>
4.5.4 Maternal Diet during Pregnancy and IgG Antibody Levels (mg/dl)

Data shows that all of the mothers whose diet was balanced during pregnancy (80%) had high IgG antibody levels of 0.34 - 0.36 mg/dl compared to mothers whose diet was unbalanced showing the least IgG antibody level of 0.34 - 0.36 mg/dl (Figure 4.21)

Table 4.9 Maternal diet during pregnancy and IgG antibody levels for infants biological mothers

<table>
<thead>
<tr>
<th>Count</th>
<th>IgG ANTIBODY LEVELS FOR INFANTS BIOLOGICAL MOTHERS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>Maternal diet during pregnancy</td>
<td>Balanced diet</td>
<td>11</td>
</tr>
<tr>
<td>Unbalanced diet</td>
<td>2</td>
<td>8</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>28</td>
</tr>
</tbody>
</table>

4.5.5 Breast Feeding frequency and IgG Antibody Levels (mg/dl) for mothers

The biological mothers whose infants were breast fed only once per day, had IgG antibody levels of 0.34 - 0.36 mg/dl of (<1%). Thereafter, the percentage of mothers with IgG antibody levels of 0.34 - 0.36 mg/dl increases significantly as the breast feeding frequency increases (Figure 4.22).
Table 4.10 Breast feeding frequency and IgG antibody levels for infants biological mothers

<table>
<thead>
<tr>
<th>Breast feeding frequency</th>
<th>IgG ANTIBODY LEVELS FOR INFANTS BIOLOGICAL MOTHERS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>Once per day</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Twice per day</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Three times a day</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Five times a day</td>
<td>1</td>
<td>17</td>
</tr>
<tr>
<td>More than five times a day</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Total</td>
<td>2</td>
<td>19</td>
</tr>
</tbody>
</table>

4.5.6 Number of Housemates and IgG Antibody Levels for mothers

The mothers with 6-7 housemates and <2 housemates had the least percentage for caregivers (40.0% and 46.4%, respectively) with IgG antibody level of 0.34 - 0.36 mg/dl compared to other categories (Figure 4.23).
4.5.7 Chronic Illness and IgG Antibody Levels for mothers

More mothers (14.3%) with chronic illness (HIV/Aids) had IgG antibody level of less than 0.25 mg/dl compared to mothers (3.9%) without chronic illness (Figure 4.24)

Table 4.12: Chronic illness and IgG antibody levels for infants biological mothers

<table>
<thead>
<tr>
<th>Chronic illness</th>
<th>IgG ANTIBODY LEVELS FOR INFANTS BIOLOGICAL MOTHERS</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>0.25 to 0.29</td>
<td>0.30 to 0.33</td>
</tr>
<tr>
<td>HIV/AIDS</td>
<td>1</td>
<td>4</td>
</tr>
<tr>
<td>Any cancer</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>None</td>
<td>12</td>
<td>24</td>
</tr>
<tr>
<td>Total</td>
<td>13</td>
<td>28</td>
</tr>
</tbody>
</table>
4.6 Factors Affecting Effectiveness of PCV-10 among Children

This section shows factors that affect effectiveness of PCV-10 among children attending child welfare clinic at MDH. Variable cross-tabulation and univariate analysis assisted in determining the factors that affect effectiveness of PCV-10 among children.

4.6.1 Factors Affecting Effectiveness of PCV-10 among study infants

According to univariate analysis of factors affecting effectiveness of PCV-10 among children; use of alcohol by mother (F = 188.740, p<0.01), maternal diet during pregnancy (F = 4.104, p<0.01), breast feeding frequency (F = 3.456, p<0.01), gap with other children (F = 7.604, p<0.01), duration of chronic illness (F = 9.700, p<0.01), affected effectiveness of PCV-10 among children attending child welfare clinic at MDH. (Figure 4.25).

Table 4.13: Factors Affecting Effectiveness of PCV-10 among study infants

Dependent Variable: IgG ANTIBODY LEVELS FOR STUDY INFANTS

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>Df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>2.320a</td>
<td>6</td>
<td>.387</td>
<td>.678</td>
<td>.000</td>
</tr>
<tr>
<td>Intercept</td>
<td>7.273</td>
<td>1</td>
<td>7.273</td>
<td>12.762</td>
<td>.000</td>
</tr>
<tr>
<td>Age</td>
<td>.395</td>
<td>1</td>
<td>.395</td>
<td>.694</td>
<td>.000</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>1.007</td>
<td>1</td>
<td>1.007</td>
<td>1.767</td>
<td>.086</td>
</tr>
<tr>
<td>Diet</td>
<td>.020</td>
<td>1</td>
<td>.020</td>
<td>.035</td>
<td>.053</td>
</tr>
<tr>
<td>Breastfeeding</td>
<td>.047</td>
<td>1</td>
<td>.047</td>
<td>.083</td>
<td>.011</td>
</tr>
<tr>
<td>Family gap</td>
<td>.070</td>
<td>1</td>
<td>.070</td>
<td>.124</td>
<td>.726</td>
</tr>
<tr>
<td>Medical history</td>
<td>.809</td>
<td>1</td>
<td>.809</td>
<td>1.419</td>
<td>.235</td>
</tr>
<tr>
<td>Error</td>
<td>86.624</td>
<td>312</td>
<td>.570</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1502.000</td>
<td>318</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>88.943</td>
<td>317</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .026 (Adjusted R Squared = -.012)
4.6.2 Factors Affecting Effectiveness of PCV-10 among mothers Univariate Analysis

In univariate analysis of factors affecting effectiveness of PCV-10 among mothers; infants’ age (F = 5.428, p≤0.01), use of alcohol by mother (F = 105.429, p≤0.05), maternal diet during pregnancy (F = 3.530, p≤0.01), duration of chronic illness (F = 4.521, p≤0.05), affected effectiveness of PCV-10 among mothers attending child welfare clinic at MDH. Explanatory power was as high as 51.7%, showing that there was independent influence of sociodemographic characteristics on the serum IgG among infant’s biological mothers (Figure 4.26).

Table 4.14: The Statistical Test for Univariate Analysis of Factors Affecting Effectiveness of PCV-10 among mothers

<table>
<thead>
<tr>
<th>Source</th>
<th>Type III Sum of Squares</th>
<th>df</th>
<th>Mean Square</th>
<th>F</th>
<th>Sig.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Corrected Model</td>
<td>12.723(^a)</td>
<td>6</td>
<td>2.120</td>
<td>2.290</td>
<td>.038</td>
</tr>
<tr>
<td>Intercept</td>
<td>6.967</td>
<td>1</td>
<td>6.967</td>
<td>7.523</td>
<td>.007</td>
</tr>
<tr>
<td>Age</td>
<td>1.072</td>
<td>1</td>
<td>1.072</td>
<td>1.158</td>
<td>.284</td>
</tr>
<tr>
<td>Alcohol use</td>
<td>7.174</td>
<td>1</td>
<td>7.174</td>
<td>7.746</td>
<td>.006</td>
</tr>
<tr>
<td>Diet</td>
<td>.531</td>
<td>1</td>
<td>.531</td>
<td>.574</td>
<td>.450</td>
</tr>
<tr>
<td>Breast feeding</td>
<td>.901</td>
<td>1</td>
<td>.901</td>
<td>.973</td>
<td>.325</td>
</tr>
<tr>
<td>Family gap</td>
<td>2.859</td>
<td>1</td>
<td>2.859</td>
<td>3.087</td>
<td>.001</td>
</tr>
<tr>
<td>Medical history</td>
<td>.289</td>
<td>1</td>
<td>.289</td>
<td>.312</td>
<td>.007</td>
</tr>
<tr>
<td>Error</td>
<td>140.761</td>
<td>312</td>
<td>.926</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Total</td>
<td>1115.000</td>
<td>318</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Corrected Total</td>
<td>153.484</td>
<td>317</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

a. R Squared = .083 (Adjusted R Squared = .047)
CHAPTER FIVE: DISCUSSION, CONCLUSION AND RECOMMENDATIONS

5.1 Discussion

The demographic characteristics of the respondents included: study infants age, use of alcohol by study infant’s biological mother, maternal diet during pregnancy with study infant, breast feeding frequency and gap with other children. WHO (2009), in its policy paper on administration of PCV-10 (Synflorix) vaccine, classified the above as possible factors that affect PCV-10 immunogenicity among infants. These characteristics were also studied by Nagpur and Daniel (2007), to establish factors that can potentially affect vaccine immunogenicity. Most of the infants studied were between the age of 3-5 months and 10-12 months. At this age range the infants would have taken a full dose of the PCV-10 vaccine under study and therefore started developing antibodies if any.

Majority of the study infant’s biological mothers (90%) did not use alcohol during the time they were pregnant. This partly explains why antibody titres among majority of the sampled infants were fairly above the required threshold of >0.35g/dl. Data also shows that most of the mothers (72%) had a balanced diet during pregnancy. This conforms to recommendations from a study done by (Wuorimaa et al., 2007) who found out that a balanced diet has a positive effect on the development of immunity both following vaccination and naturally. Most of the infants who were breast fed more than five times a day and came from households where gap between children is wide, had IgG antibody titres above the WHO recommended threshold.
5.1.1 Serum IgG titres among infants

Data obtained suggest that out of all the study infants who had finished taking the required dose of the pneumococcal conjugate vaccine; majority of them had IgG serum titres of between 0.34mg/dl to 0.36mg/dl which represents 76.1% of the infants studied. This was followed by 13.2% infants with 0.30mg/dl to 0.33mg/dl serum concentration of PD specific IgG antibodies. The remainder of the studied infants had IgG antibody titres ranging between 0.25mg/dl to 0.29mg/dl and ≤0.25mg/dl respectively. The results from a majority of the subjects (76.1%) correlate with the threshold recommended by the World Health Organization (WHO, 2012). The threshold is consistent with available data from passive immunization studies on the level of bacterial polysaccharide immune globulin needed to prevent pneumococcal otitis media and IPD (Maskell and Sefton, 2002).

The findings of this study on the levels of IgG among infants are relatively higher than those found in the study done among Brazilian infants whose IgG antibody titres between 0.30mg/dl to 0.36mg/dl were ≤50% of the total subjects studied (Black et al., 2009). This study reveals that use of alcoholic drinks by study infant’s biological mother, maternal diet during pregnancy; breast feeding frequency and gap with other children affect serum antibody titres. This is because IgG antibody titres for subjects who used alcoholic drinks during pregnancy (16%) were relatively lower (<0.35g/dl) as compared to those who did not. Also, most of the study infants who were breast-fed more than five times a day and whose maternal diet was balanced during pregnancy had their IgG levels higher or equal to the recommended threshold.
It should be noted that the total score for serum concentration of PD specific IgG among infants between 0.34 – 0.36 was 76.1%, relatively higher than the score of 24% reduction in the risk visits within a six month period in a study done among infants in South Africa (Black et al., 1995). This is probably because of the streptococcus bacteria strains included in the vaccine formation. Out of the ten strains included in the vaccine, more than half are found in Kenya and this is not the case in S. Africa. Socio-demographic patterns may have also played a role in causing the disparity in immunogenicity of the vaccine.

5.1.2 Serum IgG antibody titres among biological mothers (Herd immunity)

In this study, the total score for serum concentration of PD specific IgG among mothers was between 0.34 – 0.36 (61.6%), this meets the threshold set by WHO. This is relatively higher than the score reported by a study on IgG serum concentration of PD conducted by Pomat et al, 2013), in Ghana, who found out that only 49% of biological mothers had serum concentration of PD specific IgG having the WHO recommended threshold.

This study also found out that there was multi-co linearity between IgG antibody levels (mg/dl) for mothers and several variables. These variables were: Use of alcoholic drinks by study infants’ biological mother (r =.595, p > 0.01); Maternal diet during pregnancy (r =.137, p > 0.05); Breast feeding frequency (r =.220, p > 0.01); Gap with other children (r =.133, p > 0.05); Child hospitalization (r =.131, p > 0.05); Chronic illness (r =.154, p > 0.01); and IgG antibody levels (mg/dl) for infants (r =.675, p > 0.01). Study by CDC (2010) shared similar results with this study particularly when comparing IgG antibody levels (mg/dl) for study infant’s biological mothers and IgG antibody levels (mg/dl) for infants. The study found out that there was a relationship between levels of reactive IgG among mothers and IgG antibody levels (mg/dl) for infants. Other
studies however, did not find a significant correlation between levels of IgG among mothers and socio-demographic factors. Since its introduction, 10-valent conjugate pneumococcal vaccine (PCV10) has reduced IPD dramatically, including in unvaccinated age groups, through induction of herd immunity (Pelton, 2004).

Pneumococcal conjugate vaccination of infants has previously been shown to reduce invasive pneumococcal disease (IPD) among seniors through prevention of pneumococcal transmission from infants to adults (Simonsen, 2011). De-Wals et al., (2008), have reported a significant decline in pneumococcal (lobar) pneumonia in Canadian children <5 years of age after Canada introduced the vaccine for all children in December 2004, and Jardine et al (2010), very recently reported significant reductions in pneumococcal pneumonia in all age groups in Australia since the vaccine was introduced there in 2005 among infants. Findings from these studies conform to findings from this study since in both; there is a demonstration of herd immunity. Protecting the unvaccinated through herd immunity is a remarkable and very powerful effect of vaccines and should be an important consideration in strategies for vaccine introduction, implementation, evaluation, and monitoring, rather than an afterthought (WHO, 2011).

5.1.3 Factors Affecting the Effectiveness of PCV-10

In this study, the Univariate analysis of factors related to effectiveness of PCV-10 among children, scores for serum concentration of PD specific IgG were higher when respondents had use of alcoholic drinks by study infant’s biological mother, maternal diet during pregnancy, breast feeding frequency, gap with other children and duration of child medication. The relationship between serum concentration of PD specific IgG among infants and socio-demographic factors support the results of existing studies (Croucher, 2012; and Pilishvili, 2010)
and also correlates with the results of studies conducted by Backhaus (2012) and McCullers (2010) that socio-demographic factors affect serum concentration of PD specific IgG. Another study by Ndiritu et al (2010) conducted in Kenya found out that the uptake and effectiveness of PCV-10 was influenced by a variety of factors. The age of the mother had some influence over the uptake of PCV-10. There was a positive Pearson correlation between the two variables, \( r = 0.656, p = 0.078 \). The older the mother, the more likely it was for their child to be protected by the vaccine (Mbithe, 2013). However, the study supported the findings of the current study by stipulating that there was a positive relationship between the uptake of all other Kenya Expanded Program on Immunization (KEPI) vaccinations and immunogenicity.

5.2 Conclusions

i. The 10-valent pneumococcal conjugate vaccine (Synflorix) elicits IgG antibody threshold level sufficient enough to protect Kenyan infants against pneumoniae.

ii. The 10-valent pneumococcal conjugate vaccine (Synflorix) elicits IgG antibody threshold level sufficient enough to protect vaccinated infants biological mothers against pneumoniae.

iii. Socio-demographic factors which include: use of alcoholic drinks by infant’s biological mother during pregnancy and study infant chronic illness negatively affect the development of pneumococcal disease specific IgG.
5.3 Recommendations

i. Parents and other caregivers should be encouraged to continue availing their children for uptake of PCV-10 vaccine since the study has established that it is immunogenic against pneumococcal disease among infants

ii. Expectant mothers should be sensitized about the effects of consuming alcoholic drinks during pregnancy because this study has demonstrated that alcoholic drinks affect attainment of IgG antibody levels sufficient to protect children against pneumonia.

5.3.1 Further studies

i. Further studies should be conducted with a focus on establishing specific streptococcus pneumoniae strains found in all parts of Kenya.

ii. A better understanding of the dynamics of herd immunity and the length of protection it provides, as well as the impact of herd immunity on microbial biology, the mechanisms of microbial replacement, and natural immunity and biology are clearly needed to understand this vaccine more.
REFERENCES


GlaxoSmithKline. (GSK, 2009). Pneumococcal vaccine. Synflorix SPC.


Clinic of infectious and 63 Parasitic Diseases, Hospital das Clinicas, Faculty of Medicine, University of Sao Paulo, Brazil


Appendix I

Consent Form

DETERMINATION OF SERUM IgG CONCENTRATION AS INDICATOR OF IMMUNOGENICITY AMONG INFANTS IMMUNIZED WITH PCV-10 AT MBAGATHI DISTRICT HOSPITAL

Investigators Statement
This is an educational as well as a research study which will be carried out by a researcher from Kenyatta University. This consent form provides information that helps parents or guardians decide whether their children participate in the study or not. Please read it carefully. You may ask any question concerning the purpose of the research, procedures that will be followed, your rights as a participant in the study, risks and benefits of the study.

The following information outlines details of this research. Thank you for taking your time to read the information.

Purpose
This project seeks to:

- Establish whether the pneumonia vaccine your child is taking is effective against pneumonia or not.

Voluntary Participation
This is a voluntary study so you must not take part, but if you do, it will be of great help to us and to the health of your child. The process should take about 10 to 20 minutes to complete. Please note that you will not be paid for your participation in this study. No known disadvantages or risks are associated with taking part in this research.

Confidentiality
Any information given to the study will be kept private. Your name will not be used in any report coming from this study. The consent form will be safely kept where only the study staff will have access to the information.

Results of the study
The results (findings) of the survey for each participating child will be made available at Mbagathi District Hospital. At the end of the research project the overall research results will be published in a report. The results will be presented at meetings to discuss possible intervention in the event that the vaccine is not protective. Your child will not be identifiable from any report or publication.

Procedure
The procedure will involve a small puncture of the child’s vein to obtain 5mls of blood. It may involve some little pain which lasts just for some minutes. Samples shall then be processed later to determine if the children are protected against pneumococcal disease or not. Samples shall be obtained once and safely discarded after analysis.
Benefits
Data generated by this study will be used to provide a basis on how the vaccine could be improved, inform on the usefulness of the vaccine and the value of its continued use. The information may also be used by scientists to develop a suitable vaccine for Kenyan children.

Researchers
If you have any questions or queries about taking part you can contact researchers:

Contacts
Walekhwa Michael N, Dr. Margaret Muturi, Prof. Elizabeth Bukusi
Principal Investigator, Department of MLS, DDRT, Medical laboratory Science DPT,
Kenyatta University, KEMRI, KENYA Kenyatta University
0733-617503
Address: P.O. Box 43844 GPO 00100 Nairobi, KENYA
Tel: +254-721-746493 Tel: +254-722-758523
Email: walekhwam@gmail.com Email: margaretmuturi@yahoo.com

Name and Signature of investigator

.............................................................. ..............................................................
Signature.................................................. Date..................................................

Subject Statement and Signature
The study has been explained to me. I agree to let my child take part in it. I have had a chance to ask questions. If I have more questions, I can ask one of the investigators listed.

Name of Parent/Guardian.................................................................

Signature or fingerprint of Parent/Guardian...........................................

Thank you for voluntarily accepting to participate in this study
Appendix II

Fomu ya kutoa Idhini

UBAINISHI WA KUKOLEA KWA SERUM IgG KAMA DHIHIRISHO LA IMMUNOGENECITY KWA WATOTO WACHANGA WALIOCHANJWA NA PCV-10 KATIKA HOSPITALI YA WILAYA YA MBAGATHI.

TAARIFA YA WACHUNGUZI
Hii ni stadi ya kielimu na utafiti ambao utafanywa na mtafiti kutoka Chuo Kikuu cha Kenyatta. Fomu hii ya idhini inatoa ujumbe unaoweza kuwasaidia wazazi na walezi kuamua iwaliko wao wanaweza kuhusika katika utafiti huu au la. Tafadhali soma fomu hii ya idhini kwa umakinifu. Unaweza ukaauliza swali lolote kuhusu azma/dhamira ya utafiti, mbinu mbinu zitakazofuatua, haki zako kama mihuksika katika stadi hii, hatari zote na faida zinazojitokeza kwenye utafiti huu.
Ujumbe ufuatao unaorodhesha uchunguzi/utafiti huu kwa tafsili. Nashukuru kwa kuchukua muda wako kusoma ujumbe huu.

DHAMIRA
Azma ya mradi huu ni:
Kubainishia iwapo chanjo ya Niumonya ambayo mtoto wako anapewa ina athari kwa gonjwa lenyewe la Niumonya au la. .

KUHUSIKA KWA HIARI
Huu ni uchunguzi wa kielimu unaofanywa kwa hiari na hivyo si lazima uhusika, ingawa kuhusika kwako kutatufaa pakubwa pamoja na kwa afya ya mtoto wako. Utafiti huu utachukua takriban dakika 10 hadi 20 kumalizika. Tafadhali faamu kuwa hakuna malipo kwa kuhusika katika utafiti huu. Hata hivyo, hakuna ubaya wala madhara yanayotokana kwa kuhusika na kuhusika utafiti huu.

USIRI
Ujumbe wowote unaotolewa kwenye utafiti huu utawekwa kama siri. Jana lako halitatumika kwenye ripoti inayotokana na utafiti huu. Fomu hii ya idhini itawekwa salama mahali ambapo jopo linaloshughulikia utafiti huu pekee linawesa kupata ujumbe unaotakikana.

MATOKEO YA UTAFITI
UTARATIBU

FAIDA
Deta itakayotokana na stadi hiitatumika kutoa misingi ya jinsi chanjo hii inaweza kuimarishwa na ujumbe kuhusu umuhimu wa chanjo na uzuri unaotokana na kuendelea kuitimia. Ujumbe utatumika na wasayansi kuibuka na chanjo faafu kwa watoto inchini Keny.

WATAFITI
Ukiwa na maswali yoyote kuhusu kuhusika katika utafiti huu unaweza ukawarejelea watafiti.

Sahihi ya Mchunguzi_____________________________ Tarehe_____________________________

ARIFA NA SAHIHI YA MADA
Nimeelezwa kwa tafsili kuhusu stadi hii na nimekubali kumwachilia mtoto wangu ahusika katika utafiti huu. Pia, nimeshapa muda wa kuuliza maswali, hata hivyo, nikiwa na maswali zaidi nitamwuliza mmojawepo wa watafiti husika.

Jina la Mzazi/Mlezi_____________________________

Sahihi au alama za vidole za mzazi/mlezi

Asante kwa kukubali kwa hiari kuhusika katika stadi/utafiti huu.
Appendix III

Hojaji

UBAINISHI WA KUKOLEA KWA SERUM IgG KAMA DHIHIRISHO LA IMMUNOGENECITY KWA WATOTO WACHANGA WALIOCHANJWA NA PCV-10 KATIKA HOSPITALI YA WILAYA YA MBAGATHI


MTAFITI
Walekhwa Michael Nyongesa
Mwanafunzi wa Shahada ya uzamili kitika kitivo cha Sayansi, Chuo Kikuu cha Kenyatta
S.L.P. 43844,
Nairobi, Kenya

1. UMRI
i. Mtoto wako ana miezi mingapi?
ii. Ulikuwa na umri gani ulipompata mtoto huyu?
iii. Je, ulikuwa na mtoto mwingine kabla ya kumpata huyu?
   a. Kama Ndio, mtoto huyo alikuwa na umri gani wakati ulipojifungua huyu?
   b.

2. MATUMIZI YA MVINYO/MAJADAWA
i. Je, wewe hutumia mvinyo au dawa zozote usizelekezwa na daktari?
   a. Kama ndio, kwa mazoea yapi?
   b. Je, kuna mmoja wa aila yako ambaye hutumia mvinyo au dawa zozote zisizelekezwa?

3. LISHE
Andika kwa ufupi aina za vyakula ulivyokuwa ukitumia ulipokuwa mja mzito
Je, una mazoea yapi katika kumnyonyesha mtoto wako?__________________

Andika kwa ufupi kuhusu lishe lako wakati unaponyonyesha.________________________________________________________

4. HUDUMA ZA HOSPITALI
Mtoto wako amepata huduma za hospitali hivi karibuni? __________________________
   a. Kama ndio, kwa muda gani?____________________________
   b. Katika hospitali gani?____________________________

Je, ulipata huduma za hospitali wakati ulikuwa mja mzito? _________kama ndio, kwa muda gani?____________________________

5. MAKAZI
Unaishi katika mtaa upi?________________________________________
Nyumba yako ni kubwa kiasi gani?________________________________________
Ni watu wangapi unaoishi nao katika nyumba yako?____________________________

6. HISTORIA YA MATIBABU
Je, mtoto wako amewahi kuugua ugonjwa hatari/mkali?____________________________
   a. Kama ndio, ni ugonjwa upi?________________________________________
   b. Mtoto amekuwa akiugua kwa muda gani?____________________________

7. MATIBABU
Je, mtoto wako anatumia dawa zozote za antibiotic?____________________________
   a. Kama Ndio, amekuwa akizitumia kwa muda gani?____________________________