NASOPHARYNGEAL CARRIAGE AND ANTIBIOTIC SUSCEPTIBILITY OF *STREPTOCOCCUS PNEUMONIAE* AMONG DIABETIC AND NON-DIABETIC PATIENTS AT MOI TEACHING AND REFERRAL HOSPITAL, ELDORET, KENYA

BY

CHARITY WAMBUI KANYORO

A THESIS SUBMITTED IN PARTIAL FULFILLMENT OF THE REQUIREMENT FOR THE AWARD OF THE DEGREE OF MASTER OF MEDICINE IN INTERNAL MEDICINE, MOI UNIVERSITY.

DECLARATION

This thesis is my original work and has not been presented for a degree in any other university. No part of this thesis may be reproduced without the prior written permission of the author and/or Moi University.

Dr. Charity Wambui Kanyoro

SM/PGM/11/15

Signature:..... Date.....

Declaration by Supervisors

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

Dr. Jemimah Kamano,

Department of Internal Medicine,

School of Medicine, **Moi University, Eldoret, kenya**

Signature:..... Date.....

Prof. Henry DN Nyamogoba,

Department of Medical Microbiology & Parasitology,

School of Medicine, **Moi University, Eldoret, kenya**

Signature:.....Date.....

DEDICATION

I dedicate this work to my late mother, Dorcus Muthoni Kanyoro. She sacrificed her all to get me here. May this work be a testimony of her achievements.

ABSTRACT

Background: Respiratory infections are among the common infections in diabetes patients with *Streptococcus pneumoniae* being one of the common causative organisms. This organism colonizes the nasopharynx with varying frequency which has been stipulated to be higher in patients with diabetes. Elimination of colonization is possible through immunization. This immunization strategy is yet to be applied in Kenya due to paucity of data on the burden of colonization with the organism.

Objective: To determine the nasopharyngeal carriage and antibiotic susceptibility of *S. pneumonia* among diabetic and non-diabetic patients at the Moi Teaching and Referral Hospital (MTRH) in Eldoret, Kenya.

Methods: A cross-sectional comparative study among diabetic and non-diabetic patients attending the MTRH diabetes and eye clinics respectively. Participants were selected using systematic random sampling from the clinic queue. An interviewer administered questionnaire was used to collect social demographic data and risk factors. Blood samples were taken for measurement of random blood sugar and HbA1c. Nasopharyngeal swabs were taken and immediately delivered into Amies transport media and transported to the laboratory for culture and antibiotic susceptibility testing within 24 hours. Data analysis was done using STATA version 13. Association between nasopharyngeal carriage of *S. pneumoniae* was assessed using Pearson's Chi Square test and Fisher's exact test for categorical variables and unpaired t test and two-sample Wilcoxon test for continuous variables.

Results: A total of 124 and 121 participants were enrolled into the diabetes and non-diabetes groups respectively. Of all the participants 149 (60.8%) were female. The mean age was 43.6 with a standard deviation of 16.15 years. Among all participants, carriage of *S. pneumoniae* was 7.4% (95%CI 4.4, 11.4). Carriage was higher in the diabetes group at 12.1% (95%CI 0.07, 0.19) than the non-diabetes group 2.48% (95%CI 0.01, 0.07) with this difference being statistically significant at p=0.004. Only diabetes was associated with higher odds of carriage (adjusted OR 6.2, p=0.012). There was no association with age, gender, type of cooking fuel used, presence of under 5-year-old or previous antibiotic use. Among participants with diabetes, 66.13% had diabetes for less than 10 years and majority (56.46%) were on insulin with only 17.74% having good glycemic control. Nasopharyngeal carriage of *S. pneumoniae* was associated with insulin use. There was no association with duration of diabetes or glycemic control. Among the antibiotics used, Amoxicillin was the most frequently used at 67.31% with macrolides only used by 9.61%. Antibiotic resistance was highest for Cotrimoxazole (94.44%) followed by Amoxicillin at 16.7% and Cefuroxime at 11.1%. No resistance was reported to macrolides.

Conclusion: Nasopharyngeal carriage of *Streptococcus pneumoniae* is higher among persons with diabetes and isolates are resistant to routinely used antibiotics with macrolides being spared

Recommendations: The ministry of health should adapt policy guidelines to reduce the burden of nasopharyngeal carriage of *Streptococcus pneumoniae* among patients with diabetes such as vaccination. *Streptococcus pneumoniae* resistance to Cotrimoxazole is high and its use should be supported by a positive culture and sensitivity result.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENT

I wish to thank my supervisors Dr. Jemima Kamano and Prof. Henry Nyamogoba for their support during the development of the research proposal, conduct of the research and writing of this thesis. I also wish to thank my biostatisticians for the statistical analysis, the Lancet Laboratories for their professionalism in carrying out the transportation and culture of all the specimens, the AMPATH CDM program and Home glucose monitoring team for all the support with glucostrips and HbA1c cartridges, my research assistant for maintaining good research conduct throughout the research period, the department of Internal Medicine for the knowledge and skills which were key in the development and conduct of this study. In a special way I wish to thank the patients in the diabetes and eye clinics at MTRH for volunteering to be part of my study. I owe the success of this project to the support of my family, my friend and spouse Allan Sudoi and my son Sankei Sudoi who persevered as I worked on it and encouraged me to see it through.

LIST OF ABBREVIATIONS AND ACRONYMS

ACIP	Advisory Committee on Immunization Practices
ADA	American Diabetes Associations
BMI	Body Mass Index
BP	Blood Pressure
CAMP	Christie–Atkins–Munch-Petersen
CAP	Community Acquired Pneumonia
CDC	Centers for Disease Control
CKD	Chronic Kidney Disease
CO_2	Carbon dioxide
DM	Diabetes Mellitus
DMI	Diabetes Management Institute
HbA1c	Glycated Hemoglobin A1C
HIV	Human Immunodeficiency Syndrome
IDF	International Diabetes Federation
IFN-γ	Interferon gamma
IgG	Immunoglobulin G
IL-10	Interleukin 10
IL-6	Interleukin 6
IQR	Inter quartile range
IREC	Institutional research and ethics committee
LMIC	Low and Middle Income Countries
MIC	Minimum inhibitory concentration
MTRH	Moi Teaching and Referral Hospital
MUSOM	Moi University School of Medicine
NP	Nasopharyngeal

OR	Odds Ratio
PAF	Platelet activating factor
PCV13	Pneumococcal conjugate vaccine 13
PMNL	Polymorphonuclear leukocytes
PPSV 23	Pneumococcal polysaccharide vaccine 23
PspA	*Streptococcus pneumoniae* surface protein A
RBS	Random Blood Sugar
SD	Standard deviation
SOM	School of medicine
SSA	Sub Saharan Africa
STGG	Skim milk tryptone- glucose-glycerol
TNF-α	Tumor necrosis factor-alpha and
URTI	Upper respiratory tract infection
USA	United States of America
WHO	World Health organization

DEFINITION OF TERMS

Good glycemic control - HbA1c level below 7%

Poor glycemic control - HbA1c level above 7%

Rhinorrhea - Free discharge of thin nasal mucus

Indoor air pollution - Cooking and heating using solid fuels (i.e. wood, charcoal, coal, dung, crop wastes) on open fires or traditional stoves.

CHAPTER ONE: INTRODUCTION

1.1 Background information

Respiratory tract infections remain among the leading cause of morbidity and mortality in most populations. In kenya it is second only to Human immunodeficiency Virus (HIV)/Acquired Immunodeficiency Syndrome (AIDS) (Institute for Health Metrics and Evaluation, 2017). *Streptococcus pneumoniae*, also known as pneumococcus, is the most common documented causative organism of lower respiratory tract infections in Kenya with high mortality attributed to drug resistant strains of the bacteria (Scott *et al.*, 2000). *S. pneumoniae* colonizes the nasopharynx of healthy individuals. Colonization of the upper respiratory tract with the organism is key to the development of invasive disease (Kadioglu *et al.*, 2008; Simell *et al.*, 2012). Most people with intact mucosal immunity will be asymptomatic despite colonization. In persons with impaired immune function the risk of progression from asymptomatic carriage to invasive disease is higher (Garcia-Rodriguez and Fresnadillo Martinez, 2002).

Certain populations have a higher risk of colonization and invasive disease than others. Colonization is higher in childhood and reduces with age (Simell *et al.*, 2012). It is also higher in adults older than 65 years old and those with poor immune response. Diabetes Mellitus (DM) is one of the conditions associated with poor immune response and predisposition to infections. Patients with DM have higher rates infection and poor outcomes compared to persons without DM (Bertoni *et al.*, 2001; Muller *et al.*, 2005) contributing significantly to their morbidity and mortality (Shah and Hux, 2003).

The higher incidence of infections in DM has been attributed to immune dysfunction associated with DM which occurs as a result of inherent defects in immune cells (Casqueiro *et al.*, 2012; Geerlings *et al.*, 2000) as well as in relation to the hyperglycemic environment in DM (Mathews *et al.*, 2012; Price *et al.*, 2010). Respiratory tract infections are among the commonest infections in persons with DM and tend to have worse outcomes than in persons without DM (Joshi *et al.*, 1999; Kornum *et al.*, 2007, 2008; Peleg *et al.*, 2007). Colonization and progression to invasive disease with *S. pneumoniae* is higher in DM than in the general population (Garcia-Rodriguez and Fresnadillo Martinez, 2002). Elimination of colonization with *S. pneumoniae* in this population and others predisposed to colonization becomes an important public health measure to reduce the burden of invasive disease and the morbidity and mortality associated with it.

Elimination of *S. pneumoniae* from the carriage sites can be achieved through immunization and carriage can be used as a marker for vaccine induced protection (Marchisio *et al.*, 2002; Simell *et al.*, 2012). Immunization also confers additional benefits of reduction in invasive disease, number of hospitalizations and better disease outcomes. Hospitalized patients with respiratory pneumococcal disease who have previously been vaccinated against the organism have reduced length of hospitalization, deaths and risk of respiratory failure (Casqueiro *et al.*, 2012; Fisman *et al.*, 2006; Marchisio *et al.*, 2002; Shapiro *et al.*, 1991). Immunization also reduces the horizontal transmission of the bacteria reducing rates of community acquired respiratory infections (Bogaert *et al.*, 2004). These benefits have led to the introduction of mandatory pneumococcal vaccination in all children in most countries including Kenya (Ministry of Health, 2013).

The current global initiatives on immunization against *S. pneumoniae* mostly focus on vaccinating children. While this has been shown to confer some herd immunity extending to adults, the benefit is much less in those in extremes of age and those with preexisting co-morbid conditions such as DM (Hennessy *et al.*, 2005; Lexau *et al.*, 2005). Among children and adolescents with DM who had received the vaccine as part of the childhood immunization schedules, the protection is not lifelong and a booster vaccination is required to fully provide coverage (Principi *et al.*, 2015). This has led to vaccination recommendations for persons with DM. Despite the demonstrated disorders in immune response associated with DM, antibody response to pneumococcal vaccine is not impaired in diabetes and is safe (Lederman *et al.*, 1981).

There are two available pneumococcal vaccines; pneumococcal conjugate vaccine 13 (PCV13) and pneumococcal polysaccharide vaccine 23 (PPSV 23) which protect against 13 and 23 serotypes of pneumococcal bacteria respectively. It is recommended that persons with diabetes receive one dose of PPSV 23 at ages 19-64 years, one dose of PCV 13 after 65 years at least one year after the PPSV 23 vaccine and a final PPSV 23 vaccine after 65 years at least one year after the PCV 13 and 5 years after the most recent PPSV 23 vaccine (Centres for Disease Control and Prevention, 2017). This guideline has been adopted by most diabetes associations (American Diabetes Association, 2016; Canadian Diabetes Association Clinical Practice Guidelines Expert *et al.*, 2013) but is yet to be implemented in most low and middle income countries (LMIC) despite the significant rise in burden of DM and higher incidence of infectious diseases in these countries. While one may argue that inclusion of the vaccine in the routine management of diabetes patients will financially overwhelm economies of LMICS, cost effectiveness studies done on this recommendation

showed that it's indeed a cost effective vaccine strategy (Chen *et al.*, 2014) (Tomczyk *et al.*, 2014).

An emerging problem in the management of pneumococcal invasive disease is the rise in antibiotic resistant strains. This has been fueled by lack of proper diagnosis to guide antibiotic use, unqualified personnel prescribing drugs with no evidence of benefit or indication and the over availability and over use by the general public (Kimang'a, 2012; Neu, 1992). Colonization with drug resistant strains of pneumococcus leads to risk of invasive disease with the same drug resistant strain. This poses the risk of longer treatment durations and poor outcomes (Scott *et al.*, 2000). Most of the antibiotic resistant serotypes are covered by the current pneumococcal vaccines (Dagan *et al.*, 1997). As a result, apart from reducing carriage of pneumococcus, the pneumococcal vaccine also prevents carriage of drug resistant serotypes of the bacteria (Dagan *et al.*, 1997; Mbelle *et al.*, 1999) a benefit which is transferred to the larger population through herd immunity (Hennessy *et al.*, 2005). Knowledge of the resistant patterns is useful to not only inform choice of antibiotics but also to guide and inform pharmacologic stewardship programs.

Pneumococcal carriage studies are key in informing vaccination guidelines in persons with diabetes. Literature search revealed no such studies carried out in the African diabetes population thus lacking data to support such a guideline. As a result, the existing recommendations on vaccination and treatment have largely been ignored by care providers and health ministries denying the patient the immense benefit of this albeit simple intervention. This study was conducted to compare the burden of nasopharyngeal carriage of *S. pneumoniae* and antibiotic susceptibility patterns among persons with and those without DM in western Kenya with the goal of informing vaccination guidelines in this population.

1.2 Problem statement

Infections among patients with DM are of concern because they cause significant mortality and morbidity and increasing cost of hospitalization (Joshi *et al.*, 1999; Schuetz *et al.*, 2014). Respiratory infections are the most common infections among persons with DM and are caused by organisms that can most often be isolated from the nasopharynx of healthy individuals (Mackenzie *et al.*, 2010). *S. pneumoniae* is the most common causative organism of bacterial respiratory infections in the general population as well as among patients with diabetes (Casqueiro *et al.*, 2012; Ruiz-Gonzalez *et al.*, 1999). In patients with DM, there is a higher risk of asymptomatic carriage developing into invasive disease. This higher risk is due to immune dysfunction in diabetes that impairs mucosal immune response (Casqueiro *et al.*, 2012). Colonization with *S. pneumoniae* is key to the development of invasive disease and elimination from colonization sites has been proven to reduce the risk of invasive disease. Elimination of carriage of *S. pneumoniae* is possible through immunization and this has been recommended among patients in DM (Centres for Disease Control and Prevention, 2017; Shapiro *et al.*, 1991). This recommendation has been adopted and implemented in most developed countries but is yet to be replicated in SSA despite the higher burden of infections in this region. Lack of data on the burden and factors associated with nasopharyngeal carriage of *S. pneumoniae* among persons with DM in SSA could partly be blamed for failure to implement this guideline (Auranen *et al.*, 2013). Management of invasive *S. pneumoniae* disease faces the challenge of emerging drug resistance (Kimang'a, 2012) which is preceded by nasopharyngeal colonization with the drug resistant strain. There is limited data on drug resistance patterns of these organisms in this same population that would be useful in informing treatment guidelines.

1.3 Justification of the study

Studies on the carriage of *S. pneumoniae* are key to informing vaccination recommendations for at risk populations (Auranen *et al.*, 2013). Many studies have been conducted on the nasopharyngeal carriage of *S. pneumoniae* among children and adolescents (Bogaert *et al.*, 2001; Lehmann *et al.*, 1997; Marchisio *et al.*, 2002; Principi *et al.*, 2015). However, few studies have been done on the same among adult patients with DM particularly in developing countries (Kesavadev *et al.*, 2012). This has led to a knowledge gap in the burden and factors associated with nasopharyngeal carriage of *S. pneumoniae* in this population. As a result, there is no data to inform vaccination recommendation guidelines. Antibiotic susceptibility of colonizing species approximates that of the invasive species as these are likely to be the cause of invasive disease (Lehmann *et al.*, 1997). Vaccination in addition to reducing invasive disease, has also been associated with reduced carriage of drug resistant serotypes (Dagan *et al.*, 1997; Hennessy *et al.*, 2005).Most studies on nasopharyngeal carriage of *S. pneumoniae* also assessed the drug susceptibility of isolates (Blossom *et al.*, 2006; Gebre *et al.*, 2017; Hill *et al.*, 2006; Marchisio *et al.*, 2002).

This study sought to describe and compare the nasopharyngeal carriage of *S. pneumoniae* among DM and non-DM patients, and the drug susceptibility patterns of isolates. By demonstrating this, we hope to provide data on nasopharyngeal carriage of *S. pneumoniae* that will inform the vaccination policy among DM patients in Kenya as well as antibiotic use in the treatment of respiratory infections caused by this organism in this population.

1.4 Research questions

1. Is nasopharyngeal carriage of *S. pneumonia* higher among patients with DM than those without DM at MTRH?
2. What are the factors associated with nasopharyngeal carriage of *S. pneumonia* among patients at MTRH?
3. Are nasopharyngeal isolates of *S. pneumoniae* at MTRH susceptible to routinely used antibiotics?

1.5 Research Objectives

1.5.1 Broad objective

To determine nasopharyngeal carriage and antibiotic susceptibility patterns of *S. pneumonia* among DM and non-DM patients at MTRH.

1.5.2 Specific objectives

1. Compare the prevalence of nasopharyngeal carriage of *S. pneumonia* among DM and non-DM patients at MTRH.
2. Determine the socio-demographic and clinical factors associated with nasopharyngeal carriage of *S. pneumonia* among patients at MTRH.
3. Determine the antibiotic susceptibility of nasopharyngeal isolates of *S. pneumoniae* to routinely used antibiotics.

CHAPTER TWO: LITERATURE REVIEW

2.1 Infections and diabetes

DM is a recognized risk factor for infections, both the common ones affecting the general population as well as those that exclusively affect persons with DM. A retrospective study in a Canadian population compared infection and their outcomes in persons with DM and matched persons without DM. This study demonstrated that persons with DM had higher risk of infection, hospitalization and mortality attributable to infections. They also found that serious bacterial infections were more common in persons with DM(Shah and Hux, 2003). Similar findings were demonstrated in a Dutch cohort where DM was associated with increased risk of lower respiratory infections, urinary tract infections, skin and mucous membrane infections (Muller *et al.*, 2005). A review article on DM and the risk of infection concluded that DM is a risk factor for skin, nail and mucous membrane infections and also a risk factor for serious respiratory tract infections with an atypical presentation. The risk of infection in the DM population is also associated with increased risk of adverse outcomes (Peleg *et al.*, 2007). In a retrospective cohort study in a US population, higher infection related mortality was observed among patients with DM irrespective of age, sex, poverty status, smoking, body mass index (BMI) and hypertension was observed (Bertoni *et al.*, 2001).

This increased susceptibility to infection in DM is the result of interplay of various factors. These mechanisms of immune deficiency have been summarized in one review article as due to the hyperglycemic environment, reduced production of interleukins in response to infections, reduced chemotactic and phagocytic activity, immobilization of polymorphonuclear leukocytes (PMNL), glycosuria,

gastrointestinal and urinary dysmotility (Casqueiro *et al.*, 2012). One experimental study in Netherlands compared cytokine production in women with and without DM and the women with DM had reduced levels of tumor necrosis factor-alpha (TNF-α) and Interleukin 6 (IL-6). There was also reduced production of pro-inflammatory cytokines from monocytes of women with DM after stimulation with bacterial lipopolysaccharide (Geerlings *et al.*, 2000). This reduction in cytokine production appears to be due to an intrinsic defect in the cells of individuals with DM (Geerlings and Hoepelman, 1999). Antibody production and function also appears to be deranged in patients with DM. In a study to determine the baseline protective titers of antibodies to *S. pneumoniae* surface protein A (PspA), the authors demonstrated that PspA antibody titers were lower in persons with DM and had reduced ability for complement deposition and this was associated with hyperglycemia (Mathews *et al.*, 2012). Immune glycation contributes to both the cellular and innate immune dysfunction observed in DM. It reduces production on interleukin 10 (IL-10) from myeloid cells, TNF-α and interferon gamma (IFN-γ) from T cells (Price *et al.*, 2010) and interferes with antigen antibody recognition by modification of the Fab fragment of IgG (Lapolla *et al.*, 2002). These disorders in immune function have rendered DM an immunosuppressive state with patients having a higher than normal predisposition to infection.

2.2 Respiratory infections in DM

Respiratory infections are among the most common infections in persons with DM. in population based case control studies in Denmark to assess DM as a risk factor for pneumonia hospitalization, persons with DM had four times higher risk of hospitalization for pneumonia and higher odds of bacteremia than persons without

DM. Longer duration of DM, more than forty years of age and prolonged poor glycemic control were associated with higher risk of hospitalization (Kornum *et al.*, 2008; Thomsen *et al.*, 2004).

Respiratory infections in DM tend to have atypical presentation, higher risks complications and adverse outcomes (Casqueiro *et al.*, 2012; Peleg *et al.*, 2007). In Bangladesh, an analytic study comparing the differences in clinical presentation of respiratory infections in persons with and without DM demonstrated that persons with DM had more atypical presentation, more severe disease, higher rates of complications and were more likely to have multilobar infiltration (Saibal *et al.*, 2012). In Denmark, adults with DM hospitalized for pneumonia had a higher ninety-day mortality non-DM patients. A prospective study of pneumonia cases in Spain demonstrated that DM was associated with increased complications specifically pleural effusions and fatality (Falguera *et al.*, 2005).

The causative organisms of respiratory infections in DM include those affecting the general population as well as organisms that are more likely to affect persons with DM. *S. pneumoniae* is the most common causative agent of bacterial respiratory infections in the general population (Ruiz-Gonzalez *et al.*, 1999; Scott *et al.*, 2000) as well those with DM as demonstrated in several review articles on infections in persons with DM (Casqueiro *et al.*, 2012; Joshi *et al.*, 1999; Klekotka *et al.*, 2015). Other organisms causing respiratory infections with increasing frequency in persons with DM include *Staphylococcus aureus, Klebsiella pneumoniae* and *Mycobacterium tuberculosis (Klekotka et al., 2015; Peleg et al., 2007). S. pneumoniae* has generated interest not only because colonization with the organism is very common but also because prevention of its invasive disease is possible through vaccination.

2.3 Bacteriology of *S. pneumoniae*

S. pneumoniae is a gram positive lancet shaped cocci that is alpha hemolytic, catalase negative, non-spore forming, and non-motile bacteria. It is fastidious and grows best in a carbon dioxide (CO_2) rich environment and requires a source of catalase to grow on agar plates (Kenneth Todar, 2012). Over 90 serotypes of the bacteria exist but only a few are pathogenic. The structure of the bacteria aids in its ability to colonize, evade the host's immune protective mechanisms and to cause invasive disease. The outer surface of the bacteria is covered by a polysaccharide capsule that overlies the cell wall. The capsule forms the basis of serotyping and has antiphagocytic properties making it protective to the bacteria (Henriques-Normark and Tuomanen, 2013). Pilli are present on the bacteria which serve to facilitate adherence to epithelial cells of the nasopharynx during colonization (Elaine I Tuomanen, Aug 10, 2017.; Henriques-Normark and Tuomanen, 2013).

The bacteria possesses several surface proteins that are essential in its adherence, cell division and evasion of host immune responses. The surface protein LytA autolysin is the main pneumococcal autolysin and is a major virulence factor which aids in cell division. Pneumococcal surface protein A (PspA) inhibits complement activation and deposition and impairs phagocytosis. The Choline binding proteins A, D, E and G play a role in bacterial adherence (Henriques-Normark and Tuomanen, 2013). The bacteria is a leading cause of mortality and morbidity globally with the highest impact seen children under 5 years and persons older than 65 years of age (World health Organization, 2018). The spectrum of disease it causes ranges from asymptomatic colonization to severe invasive disease.

2.4 Carriage of *S. pneumoniae*, transmission and progression to invasive disease

S. pneumoniae colonizes the nasopharyngeal mucosa and upper airways of healthy individuals. Non-symptomatic colonization has been demonstrated in both adults and children. Human beings are its only host and are necessary for its survival. Transmission of the *S. pneumoniae* occurs between humans and is from infected droplets of persons with disease or from healthy nasopharyngeal carriers (Bogaert *et al.*, 2004; Sleeman *et al.*, 2005).

Nasopharyngeal colonization is higher in children. It starts from birth, peaks at 1-2 years and reduces with increase in age (Hussain *et al.*, 2005; Lloyd-Evans *et al.*, 1996). The initial asymptomatic colonization involves the binding of pneumococcal cell surface proteins to non-inflamed epithelium a process mediated by bacterial cell surface proteins (Bogaert *et al.*, 2004). Choline binding proteins present on the surface of *S. pneumoniae* contribute to the hydrophobic and electrostatic characteristics of the bacteria and aid in its adherence to respiratory epithelium (Swiatlo *et al.*, 2002).

Colonization with the bacteria is not always followed by invasive disease. In order for this to happen, a local host inflammatory response is required to facilitate the process. The inflammatory response leads to release of inflammatory mediators and increased the affinity of the pneumococcus for the platelet activating factor (PAF) receptor. Binding to this receptor results in internalization of the pneumococcus, migration through the respiratory epithelium and vascular endothelium resulting in invasive disease (Cundell *et al.*, 1995; McCullers and Rehg, 2002).

The prevalence of nasopharyngeal carriage of *S. pneumoniae* differs in different populations and age groups. In Kilifi Kenya in a rural and semi urban population the prevalence was found to be 6.4% in children older than 10 years and adults

(Abdullahi *et al.*, 2008). In an aboriginal Australian population a prevalence of 28.4% was found in the wet season and 22.8% in the dry season among adults (Mackenzie *et al.*, 2010). The prevalence appears to be higher in patients with DM with a prevalence as high as 49.8% among children and adolescents in Italy (Principi *et al.*, 2015). This is the only study identified that was done in an adult population. The prevalence in this group was much higher than the 8.6% what was found in a similar study in a general population in Italy (Marchisio *et al.*, 2002) demonstrating the much higher prevalence of carriage in persons with DM compared to the general population.

2.5 Factors associated with carriage of *S. pneumoniae*

Immune status of the individual determines *S. pneumoniae* colonization, decolonization as well as progression to invasive disease (Garcia-Rodriguez and Fresnadillo Martinez, 2002) .Persons with impaired immunity as is the case with DM are at higher risk with carriage rate of 49.8% among adolescents with DM (Principi *et al.*, 2015) which was significantly higher than the 8.6% observed in healthy children in the same population (Principi *et al.*, 2015). Another study in Georgia US demonstrated an incidence of invasive pneumococcal infection to be 8.8/100000 among healthy adults and 51.4/100000 among persons with DM (Kyaw *et al.*, 2005). Higher carriage has also been demonstrated in persons living with HIV (Conklin *et al.*, 2016; Heinsbroek *et al.*, 2015). Other factors that have been demonstrated to be associated with carriage of *S. pneumoniae* include younger age, male gender, recent rhinorrhea, exposure to indoor air pollution (Abdullahi *et al.*, 2008; Cardozo *et al.*, 2008; Mackenzie *et al.*, 2010; Principi *et al.*, 2015). Factors associated with reduced carriage include antibiotic use and previous pneumococcal vaccination (Garcia-Rodriguez and Fresnadillo Martinez, 2002).Living with a vaccinated child is associated with reduced carriage among adults (Hennessy *et al.*, 2005). Vaccination of

children under 5 years of age against pneumococcus confers protection to adults, a benefit referred to as herd immunity.

2.6 *S. pneumoniae* antibiotic susceptibility

Microbial drug resistance is an emerging threat in the management of infectious diseases. This problem has not spared Africa. A review article on antimicrobial resistance in the continent highlighted its increase in the region. This rise has been attributed to indiscriminate use of antibiotics, wrong prescriptions by unqualified personnel and wrong diagnoses (Kimang'a, 2012).

Drug susceptibility testing is key in determining the choice of antibiotics. In the assessment of drug susceptibility of *S. pneumoniae* serotypes causing invasive disease, the use of drug susceptibility testing on isolates from carriage sites has been shown to provide an estimate of the drug susceptibility of invasive strains (Lehmann *et al.*, 1997). Studies of drug susceptibility patterns of *S. pneumoniae* have highlighted differences in resistant patterns between the developed and the developing nations. In Gambia 14.3%, 39%, 0.3%, 39%, 32.3% and 6.3% of the isolates were resistant to penicillin, cotrimoxazole, erythromycin, tetracycline, chloramphenicol and cefotaxime respectively (Hill *et al.*, 2006). A similar study in Ethiopia demonstrated 53.2%, 43.7%, 36.1%, 13.3%, and 8.9% of *S. Pneumoniae* isolates being resistance to tetracycline, cotrimoxazole, penicillin, chloramphenicol and erythromycin respectively (Gebre *et al.*, 2017). In Uganda 99% of the isolates were resistant to cotrimoxazole and only one isolate was resistant to macrolides (Blossom *et al.*, 2006). In Italy, the resistance to macrolides was higher at 52.1%, that to penicillin was lower at 9.1% and resistance to cotrimoxazole was 23.9% (Marchisio *et al.*, 2002). The

same was observed in China with macrolide resistance at 74%, tetracycline at 87.6% and only 1.2% to penicillin (Wang *et al.,* 1998).

2.7 Clinical and laboratory diagnosis of *S. pneumoniae*

To demonstrate colonization with *S. pneumoniae*, the organism can be isolated from the nasopharynx or the oropharynx. Nasopharyngeal specimens have a higher yield than oropharyngeal specimens. A study on nasopharyngeal carriage among adults, demonstrated 11.1% yield of nasopharyngeal specimens compared to 5.8% from oropharyngeal specimens. (Watt *et al.*, 2004). A study to compare the sensitivities of sampling the nasopharynx and the oropharynx for identification of potential respiratory pathogens colonization found higher sensitivity of nasopharyngeal swab at 89% than oropharyngeal swab was 30% (Lieberman *et al.*, 2006).

In Kenya, nasopharyngeal sampling was validated in the Kilifi study. In this study the sensitivity of nasopharyngeal swabs was high at 85%. Sampling from both nostrils by nasopharyngeal swabbing and nasopharyngeal wash were found to increase sensitivity but the discomfort associated with use of the two methods concurrently was not justifiable with the marginal increase in sensitivity (Abdullahi *et al.,* 2007). The WHO pneumococcal working group recommends the use of nasopharyngeal swabs for collection of samples as opposed to nasopharyngeal washes or aspirates. One swab is recommended as multiple swabs increase the discomfort to the patient without increasing the yield (Satzke *et al.*, 2013).

Culture of the specimen to demonstrate growth of *S. pneumoniae* remains the gold standard for detection of the bacteria from nasopharyngeal samples. For culture based studies, the swab material used to collect the sample should be made from calcium alginate, rayon, Dacron or nylon materials. Skim milk tryptone- glucose-glycerol

(STGG) medium or Amies transport media can be used as transport and storage medium with no evidence of one being more superior to the other (Satzke *et al.*, 2013). The samples should be transported in ice and stored at ultralow temperatures. Blood agar, either Columbia or trypticase soy agar base with sheep, horse, or goat blood, supplemented with 5 µg/ml gentamicin is considered the core primary isolation media (Satzke *et al.*, 2013). Confirmation of *S. pneumoniae* is based on alpha hemolytic colony morphology, bile solubility, catalase negativity, and optochin sensitivity (Paul G. Engelkirk, 2008; Satzke *et al.*, 2013).

2.8 Vaccination against *S. pneumoniae*

Due to the high burden of infections caused by *S. pneumoniae*, a vaccine was developed to help protect against colonization and invasive disease especially in children under 5 years of age and adults at high risk. There are two available pneumococcal vaccines; pneumococcal conjugate vaccine 13 (PCV13) and pneumococcal polysaccharide vaccine 23 (PPSV 23) which protect against 13 and 23 serotypes of pneumococcal bacteria respectively. PCV13 contains 13 serotypes and is recommended for use in children aged 6 weeks-71 months (Nuorti *et al.*, 2010). It is widely used globally including in Kenya where it was introduced in 2010 but as PCV10 into routine vaccination (Ministry of Health, 2013). PPSV 23 contains 12 of the serotypes in PCV13 and 11 additional serotypes. It is recommended for use in adults older than 65 years of age and persons aged 19-64 at high risk of colonization and invasive disease (Centres for Disease Control and Prevention, 2017).

It is recommended that persons with DM receive one dose of PPSV 23 at ages 19-64 years, one dose of PCV 13 after 65 years at least one year after the PPSV 23 vaccine and a final PPSV 23 vaccine after 65 years at least one year after the PCV 13 and 5

years after the most recent PPSV 23 vaccine (Centres for Disease Control and Prevention, 2017). The antibody response among patients with DM receiving the vaccine is not impaired and is not related to age, glycemic control, insulin dose or duration of DM and therefore they should receive the vaccine without any reservations (Lederman *et al.*, 1981).

Vaccination reduces the carriage of serotypes contained in the vaccines and leads to a reduction in the incidence of invasive disease caused by these serotypes (Dagan *et al.*, 1997; Marchisio *et al.*, 2002). The impact of the vaccine on progression to invasive disease has been assessed after vaccination in children under 5 years old and the vaccine type invasive disease decreased by 91% in children and 40% in adults (Hennessy *et al.*, 2005). This benefit of herd immunity conferred to unvaccinated adults by vaccinating children was demonstrated in most studies looking into the effects of the vaccine (Dagan *et al.*, 1997; Hennessy *et al.*, 2005). According to one such study, this benefit does not apply to all adults. Adults over 65 years and those with a comorbid condition for which the vaccine is indicated did not benefit as much from herd immunity (Lexau *et al.*, 2005). Even children and adolescents with conditions predisposing to infection with *S. Pneumoniae* who receive the PCV 13 do not appear to be protected for life and require a booster vaccine to maintain the protection offered (Principi *et al.*, 2015). Administration of the polyvalent pneumococcal vaccine to adults at high risk of invasive disease has been demonstrated to be effective in the prevention on invasive pneumococcal disease and should be used more widely (Shapiro *et al.*, 1991).

Another benefit of vaccination is the reduction of carriage of drug resistant *S. pneumoniae* strains. Mbelle, N., et al demonstrated a reduction in carriage of penicillin and cotrimoxazole resistant pneumococci after pneumococcal vaccination

(Mbelle *et al.*, 1999). Similar findings were reported in a study in Alaska where there was noted a reduction in invasive disease due to isolates resistant to penicillin, erythromycin and cotrimoxazole after introduction of the pneumococcal conjugate vaccine (Hennessy *et al.*, 2005). Other benefits of the vaccine include a reduction in mortality from pneumococcal invasive disease, reduced likelihood of complications and reduced length of hospital stay (Casqueiro *et al.*, 2012; Fisman *et al.*, 2006). Despite these benefits, one downside observed with the use of the vaccine is the increase in colonization by non-vaccine serotypes (Mbelle *et al.*, 1999; Usuf *et al.*, 2014). While these serotypes may not be as pathogenic as those covered by the vaccine, there is still a need for continued monitoring of colonization and invasive disease strains to inform future vaccination strategies.

2.9 Knowledge gap on nasopharyngeal carriage of *S. pneumoniae*

Studies on the colonization of the nasopharynx with organisms are key to informing vaccine practices and for the backbone of vaccine research and recommendations (Kesavadev *et al.*, 2012; Satzke *et al.*, 2013). Vaccination against *S. pneumoniae* has been recommended in most developed countries where the bulk of colonization studies have been carried out. There are currently few studies highlighting the carriage of *S. pneumoniae* among healthy as well as persons with DM in most developing countries. Where these have been conducted, they have been in mostly in children and adolescents (Adegbola *et al.*, 2014; Usuf *et al.*, 2014). This has resulted in a lack of epidemiologic data to guide the vaccine guidelines in adults with DM despite its availability globally and demonstration of its benefits and cost effectiveness (Chen *et al.*, 2014).

CHAPTER THREE: METHODOLOGY

3.1 Study site

The study was conducted at the Moi Teaching and Referral Hospital (MTRH) which is the second largest referral hospital in the country located in Eldoret town, Western Kenya (310 kilometres Northwest of Nairobi, the capital city of Kenya). The hospital serves a catchment population of 24 million people (Moi Teaching and Referral Hospital (MTRH), 2014). The hospital runs DM and eye outpatient clinics with approximately 150 and 250 patients respectively seen in clinic per week.

3.2 Study design

This study was a cross sectional comparative study comparing nasopharyngeal colonization with *S. pneumoniae* between DM patients attending the DM clinic and a non-DM patients attending the eye clinic at MTRH. The eye clinic was selected as it provided a population of participants who were likely to be healthy clinic walk in.

3.3 Study population

3.3.1 Target population

DM group- DM patients attending the DM clinic at MTRH who met the inclusion criteria.

Comparison group - Non DM patients attending the eye clinic at MTRH who met the inclusion criteria.

3.3.2 Sample Size calculation

A formula for the comparison of two proportions was used (Kirkwood R Betty, 2003.). To detect a 23.8% difference in carriage of *S. pneumoniae* between persons with and without DM with 90% power at 0.05 level of significance and with a 20%

adjustment for non-responders and missing data, a sample size of 123 was required in each study group. The estimates used in this calculation were derived from the study done by Principi, N., et al in a DM population (Principi *et al.*, 2015) and that by Mackenzie, G. A., et al done in a general adult population (Mackenzie *et al.*, 2010)

$$\mathbf{n= \{((Z_{\alpha/2}+Z_{\beta})^2 * (p_1 (1-p_1) + p_2 (1-p_2)))/ (p_1-p_2)^2 * 100\%}$$

$Z_{\alpha/2}$ = the critical value of the normal distribution at $\alpha/2= 0.05$, critical value=1.96

Z_{β} = the critical value of the normal distribution at $\beta = 0.1$, critical value = 1.64

p_1 = Burden of NP carriage among DM populations =0.498

p_2 =Burden of NP carriage among non DM patients = 0.26

3.3.3 Sampling technique

Participants were selected using systematic random sampling from the clinic queues. Given the 8 weeks allocated for data collection and the target of 123 participants, we needed to enroll at least 15 participants per week in each study group. At the time of conducting the study, an average of 150 and 250 adult patients were seen each week in the DM and eye clinics respectively. To meet our target of we enrolled every 10^{th} patient (150/15) in the clinic queue at the DM clinic and every 16th patient (250/15) in the eye clinic. This process was continued until the desired sample size was achieved.

3.3.4 Inclusion and exclusion criteria

Inclusion criteria

Study arm

1. Known DM patient
2. Above 18 years of age
3. Attending DM clinic at MTRH

Comparison arm

1. Non-DM patient
2. Above 18 years of age
3. Attending eye clinic at MTRH clinic

Exclusion criteria

Study arm

1. Presence of craniofacial abnormalities limiting access to nasopharynx.

Comparison arm

1. Known DM patient or new diagnosis of dysglycemia.
2. Presence of craniofacial abnormalities limiting access to nasopharynx

3.4 Study methods

3.4.1 Recruitment of participants

Participants were recruited from MTRH DM and eye clinics by the principal investigator (PI) and/or the research assistant. The study was explained to all recruited participants and informed consent sought. Those participants who did not meet the inclusion criteria or declined participation were omitted from the study. The recruitment process is summarized in Figure 1 below.

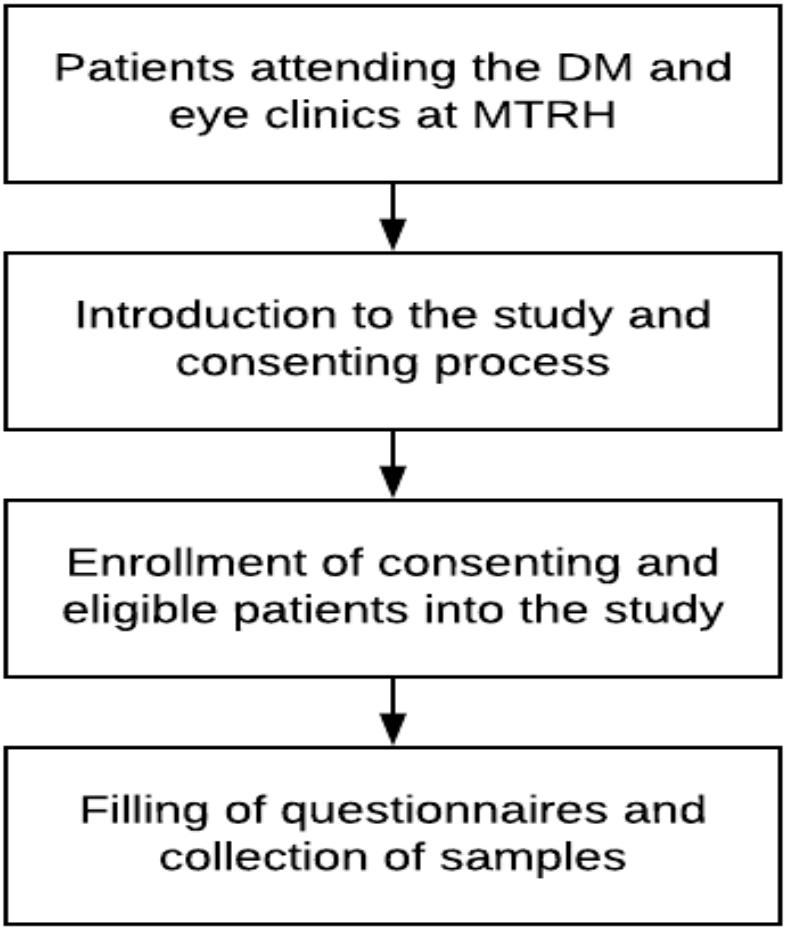

Figure 1: Participants Recruitment Schema

3.4.2 Consenting process

Each participant was taken through the informed consent process by the PI or the research assistant. An English version (Appendix Ia) and Swahili version (Appendix Ib) of the consent form were available an administered based on the participants preference. For participants who were illiterate, the consent was read to them in the presence of a witness/translator where necessary.

3.4.3 Study procedure

After consent was obtained, an interviewer administered questionnaire was used to record clinical and demographic characteristics. Procedures to be done were explained to the participants. Blood pressure, height and weight measurements were then taken as outlined in Appendix III. Blood samples were drawn using finger prick for measurement of random blood sugar an HbA1c (Appendix III).

3.4.4 Nasopharyngeal swab collection

The procedure was explained to the participant and consent obtained. A trained research assistant (clinical officer) and/or the PI then performed the procedure in keeping with WHO standards (Satzke *et al.*, 2013). The participant's head was tilted slightly backwards and the swab made of flexible polystyrene on nylon introduced into the nasal cavity and passed directly backwards parallel to the base of the nasopharyngeal passage. The swab was pushed in to a distance equivalent to two thirds of the distance between the tip of the nose and the earlobe until the nasopharynx was reached. The swab was left in place for 5 seconds and rotated 180^0 to saturate the tip. If resistance was met before the swab reached the nasopharynx, the swab was withdrawn and the procedure repeated in the opposite nostril. Once the specimen was collected, the swab was gently withdrawn and immediately placed into

Amies transport media. The samples were then transported to the Lancet Laboratory offices in Eldoret where cold chain was initiated for transportation to the Nairobi main laboratory within 24 hours.

3.4.5 Nasopharyngeal swab culture procedure

As soon as the swabs were received in Lancet laboratory Nairobi, they were inoculated into culture media. Where delay was anticipated between arrival and inoculation, the specimens were stored in the refrigerator which was maintained at 2-8degrees Celsius. Inoculation was done in sheep blood agar with Bactrim, Erythromycin, and penicillin discs and also into Saboraud dextrose agar with chloramphenicol (Dex/Chlor). The blood agar culture was incubated a CO_2 atmosphere while the Dex/Chlor culture was incubated in an oxygen rich atmosphere.

3.4.6 Identification of *S. pneumoniae*

S. pneumoniae was identified by colony morphology, alpha hemolysis and optochin sensitivity. Confirmation was by gram staining in which they appeared as lancet shaped cocci existing in pairs, single colonies and positive on gram staining. They were catalase negative, negative on bile esculin test, Christie–Atkins–Munch-Petersen (CAMP) test negative and 6.5 percent salt negative.

3.4.7 Drug susceptibility testing

Drug susceptibility was carried out using disk diffusion (modified Kirby -Bauer) and E test. A bacterial suspension of 0.5 density check was inoculated into sheep blood agar in a CO_2 atmosphere. For the disk diffusion test, the disks containing the test antibiotics were placed aseptically on the plate using sterile forceps then plates were incubated in a 5–10% CO_2 atmosphere at 37^0C for 24 hours. The zone of inhibition around each disk was then measured and compared to a standard interpretation chart used to categorize the isolate as susceptible, intermediately susceptible or resistant. This method was used to test sensitivities to cotrimoxazole, Erythromycin, Clindamycin, Moxifloxacin, Tetracycline and Vancomycin. For the E test, the E test strips containing Penicillin and Ceftriaxone were applied onto the agar plate and incubated in a 5–10% CO_2 atmosphere at 37^0C for 24 hours. The colonies were assessed for an elliptical zone of growth inhibition around the test strip. Where the lower part of the ellipse intersected the test strip, this was interpreted as the minimum inhibitory concentration (MIC). E test was done for Penicillin and Ceftriaxone. MIC for parenteral penicillin was considered sensitive when equal or less than 2, intermediate when 4, resistant when equal 8 or more. Oral Penicillin was considered sensitive when the MIC was equal 0.6 or less, intermediate 0.12-1 and resistant when equal or greater than 1. Ceftriaxone was considered sensitive when the MIC was equal or less than 1, intermediate when equal to 2 and resistant when equal or greater than 4. Based on the E test results, sensitivities were reported for Augmentin, Cefuroxime and Ampicillin.

3.5 Data management

3.5.1 Data collection

An interviewer administered questionnaire (Appendix II) was used to collect data on demographic and clinical characteristics as well as risk factors for nasopharyngeal carriage of *S. pneumoniae*. These included age, gender, area of residence, history and duration of hypertension, history and duration of DM, history of chronic kidney disease, HIV status, indoor air pollution, presence of under 5 year olds in the household, prior pneumococcal vaccine, type of DM medication used and results of nasopharyngeal culture. Each questionnaire was assigned a unique identifier that was unique to each participant. The questionnaire was a simplified checklist thus did not need to be validated prior to administration.

3.5.2 Data entry and validation

The questionnaires were checked for completeness before data entry was done. Data from the questionnaire was then entered, into EpiData (version 3.1, EpiData Association, Odense, Denmark). Validation was ensured by use of data entry checks, use of unique identifiers and double data entry.

3.5.3 Data protection and security

All the data was de-identified and stored in a password protected computer that only the PI had access to. The computer was protected using an antivirus and all the data was backed up in password protected cloud storage. The data will be kept for a minimum of three years after completion of the study.

3.5.4 Data cleaning

All data was assessed for missing data and consistency. Assessment for outliers was done using scatter plots and box plots. Distribution of continuous variables was assessed using histograms and normal probability plots. Missing data was excluded from the univariate and multivariate analysis, this did not affect the sample as this had been factored into the sample size calculation.

3.5.5 Data analysis

Data analysis was done using STATA version 13. Categorical variables were summarized as frequencies with corresponding percentages. Continuous variables were assessed for Gaussian assumptions using graphical methods such as normal probability plots and histograms. They were then summarized using the mean and corresponding standard deviation (SD) where the distribution was normal and using median and the corresponding inter quartile range (IQR) where the assumptions were violated. Association between nasopharyngeal carriage of *S. pneumoniae* and categorical variables was assessed using Pearson's Chi Square test. Fisher's exact test was used whenever the Chi Square assumptions were violated. Association between continuous variables and nasopharyngeal carriage of *S. pneumoniae* was assessed using the unpaired t test where the distribution was normal and using Wilcoxon rank sum test where the Gaussian assumptions were violated. Factors associated with nasopharyngeal carriage of *S. pneumoniae* were assessed using univariate logistic regression with results presented as odds ratios (OR) with corresponding 95% confidence intervals. Multivariate logistic regression was carried out on all factors associated with nasopharyngeal carriage of *S. pneumoniae* from the univariate analysis and those that were determined as a priori including age, sex, area of

residence, type of cooking fuel used, presence of a child under 5 years old in the household, tobacco use, presence of URTI and use of antibiotics in the previous three months. Results were presented as adjusted ORs with corresponding 95% confidence intervals. Drug sensitivity was analyzed and presented as frequencies.

3.6 Study limitations

This study did have some limitations. We collected data on HIV status and chronic kidney disease from self-report which could have underestimated the persons with HIV and chronic kidney disease in the study. Data on vaccination status of children under 5 years of age living with the participants was not confirmed with the vaccination cards.

3.7 Ethical considerations

This study was approved by the Institutional research and ethics committee (IREC) at MTRH.

Informed consent was sought from all participants prior to performing any study procedures. The study was explained to the participants in detail in a language they were comfortable with. A translator was sought where required. All study procedures were explained to the patients prior to performing them and consent obtained. Participants were free to decline participation in the study and were not denied care.

3.8 Dissemination of results

The results of the tests and procedures were communicated to all the participants and their clinicians. The findings will also be shared with DM patient support groups, stakeholders such as the Ministry of Health, DM management Institute (DMI) and the

International DM Federation (IDF). The results will also be disseminated through a written thesis and an oral defense in a forum that shall be convened by the School of Medicine (SOM). The results will also be shared with MTRH and published in peer-reviewed journal. The printed thesis will be available at the Moi University School of Medicine (MUSOM) library. This study will be published in a peer reviewed journal and presented in professional conferences and seminars.

CHAPTER FOUR: RESULTS

4.1 Participants recruited

The study was conducted between the months of April and June 2017. A total of 135 participants were approached for enrollment into the study from the DM clinic. Of these, 124 completed the study and were included in the final analysis.

A total of 132 participants were approached for enrollment into the study from the eye clinic. Of these 121 completed the study and were included in the final analysis. (Figure 2)

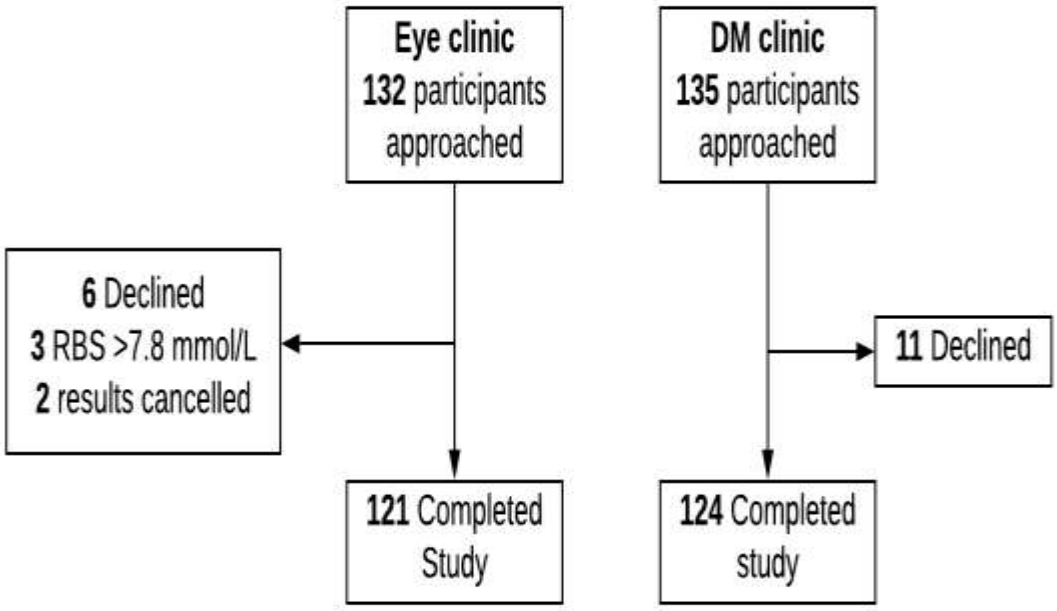

Figure 2: Participant Recruitment flow chart

4.2 Demographic and clinical characteristics of participants

Table 1: Demographic characteristics of participants screened for *S. pneumoniae* carriage at MTRH, Eldoret, Kenya

Variable	Total N=245	DM	Non-DM	P value
	n(%),mean (SD	n(%),mean (SD)	n(%),mean (SD)	
Age	43.69 (16.15)	50.50 (16.44)	36.71(12.50)	<0.001
Sex				
Male	96 (39.18)	45 (36.29)	51 (42.15)	0.348
Female	149 (60.82)	79 (63.71)	70 (57.85)	
Residence				
Rural	128 (52.24)	68 (55.28)	60 (49.59)	0.373
Urban	117 (47.76)	55 (44.72)	61 (50.41)	
Fuel type				
Gas	46 (18.78)	13 (10.48)	33 (27.27)	0.001
Firewood/charcoal/ Kerosene	199 (81.22)	111 (89.52)	88 (72.73)	
Presence of under 5 year old				
Present	64 (26.12)	41 (33.06)	23 (19.01)	0.012
None	181 (73.88)	83 (66.94)	98 (80.99)	
Smoking				
Never smoked	220 (89.80)	105 (84.68)	115 (95.04)	0.007
Past/current smoker	25 (10.20)	19 (15.32)	6 (4.96)	

Table 2: Clinical characteristics of participants screened for *S. pneumoniae* carriage at MTRH, Eldoret, Kenya

Variable	Total N=245	DM	Non-DM	P value
	n (%), Mean (SD)	n(%),Mean (SD)	n(%), Mean (SD)	
URTI in last 3 months				
Present	41 (16.73)	32 (25.81)	9 (7.44)	<0.001
None	204 (83.27)	92 (74.19)	112 (2.56)	
Antibiotic use last 3 months				
Used	52 (21.22)	32 (25.81)	20 (16.53)	0.076
None	193 (78.78)	92 (74.19)	101 (83.47)	
HIV status				
Positive	7 (2.86)	5 (4.03)	2(1.65)	0.447
Negative	238 (97.14)	119 (95.97	119 (98.35)	
SBP	128.69 (17.05)	131.61 (20.33)	125.69 (12.24)	0.003
DBP*	86 (17)	83 (16.50)	87 (14.00)	0.390
BMI	25.52 (5.21)	25.76 (5.68)	25.28 (4.69)	0.240
Duration of DM N=124				
<10 years		82 (66.13)		
>10 years		42 (33.87)		
HbA1c N=124		9.9 (2.52)		
<7.0%		22 (17.74)		
>7.0%		102 (82.26)		
Type of DM medication N=124				
Insulin based		85 (68.55)		
OGLA		36 (29.03)		
Lifestyle only		3 (2.42)		

*Median (IQR)

There were more women (60.82%) than men enrolled into the study. There was an almost equal distribution between rural (52.46%) and urban (47.54%) dwellers. The mean age among all participants was 43.69 with only 10% being older than 65 years of age. Participants with DM were significantly older than those without DM

(p<0.001), had more history of smoking (15.32%, p=0.007) and upper respiratory tract infection in the previous 3 months (25.81%, p<0.001) than those without DM. More DM participants had used antibiotics in the preceding 3 months (25.81%) but this difference was not statistically significant (p=0.076). The mean systolic blood pressure among DM participants was 139.33 and was significantly higher than that in non-DM participants 127.84 (p=0.005). There were less participants with DM using safer fuel options than those without DM P<0.001. More DM than non DM participants had children under 5 years living with them (33.06%, p= 0.012). There was no difference in BMI between the two groups. Among the participants with DM, 68.55% were on an insulin based regimen and of these 87.06% had poor glycemic control p=0.02 with HbA1c above 7%. Majority (66.13%) had a diagnosis of DM for less than 10 years. Of those who had had DM for more than 10 years, 85.71% were on insulin based regimen and were more likely to have poor glycemic control (97.62%, p=0.001) than those on other treatment modalities.

4.3 Objective 1: Compare the prevalence of nasopharyngeal carriage of *S. pneumoniae* among DM and non-DM patients at MTRH

Nasopharyngeal carriage of *S. pneumoniae* was 7.4% (95% CI 4.4, 11.4) among all the participants. It was 12.1% among participants with DM and 2.48% among non-DM participants. This difference was statistically significant with a p value of 0.004.

4.4 Objective 2: Determine the socio-demographic and clinical factors associated with nasopharyngeal carriage of *S. pneumoniae* among patients at MTRH

Table 3: Factors Associated with nasopharyngeal carriage of *S. pneumoniae* among DM and non-DM patients in MTRH, Eldoret, Kenya

Variable	Total N= 245	Culture Positive	Culture Negative	P value
	n (%), Mean (SD)	n(%),Mean (SD)	n(%), Mean (SD)	
Study Group				
DM	124 (50.61)	15 (12.10)	109 (87.90)	0.004
Non-DM	121 (49.39)	3 (2.48)	118 (97.52)	
Sex				
Male	96 (39.18)	10 (10.42)	86 (89.58)	0.139
Female	149 (60.82)	8 (5.37)	141 (94.62)	
Age	43.69 (16.15)	48.83 (18.34)	43.28 (15.94)	0.161
Residence				
Rural	128 (52.24)	10 (7.81)	118 (92.19)	0.770
Urban	117 (47.76)	8 (6.84)	109 (93.16)	
Smoking				
Never smoked	220 (89.80)	16 (7.27)	204 (92.73)	0.895
Past/current smoker	25 (10.20)	2 (8.00)	23 (92.00)	
HIV status				
HIV positive	7 (2.86)	0 (0.00)	7 (100)	0.582
HIV negative	238 (97.14)	18 (7.56)	220(92.44)	
Fuel type				
Gas	46 (18.78)	2 (4.35)	44 (95.65)	0.378
Firewood/ Kerosene/ Charcoal	199 (81.22)	16 (8.04)	183 (91.96)	
Presence of under 5 year old				
Present	64 (26.12)	7 (10.94)	57 (89.06)	0.200
None	181 (73.88)	11 (6.08)	170 (93.92)	

URTI in last 3 months				
Positive	41 (16.73)	3 (7.32)	38 (92.68)	0.646
Negative	204 (83.27)	15 (7.35)	189 (92.65)	
Antibiotic use last 3 months				
Used	52 (21.22)	3 (5.77)	49 (94.23)	0.444
None	193 (78.78)	15 (7.77)	178 (92.23)	
HbA1c N=124				
<7%	22(17.74)	2 (9.09)	20 (90.91)	0.479
>7%	102 (82.26)	13 (12.75)	89 (87.25)	
Duration DM N=124				
< 10 years	82 (66.13)	8 (9.76)	74 (90.24)	0.264
>10 years	42 (33.87)	7 (16.67)	35 (83.33)	
DM medication type N=124				
Insulin based	85 (68.55)	15 (17.65)	70 (82.35)	0.012
OGLAs	36 (29.03)	0 (0.00)	3 (100.00)	
Lifestyle modification only	3 (2.42)	0(0.00)	0 (100.00)	

*Median (IQR)

Table 4: Results of multivariate analysis of factors associated with nasopharyngeal carriage of *S. pneumoniae* among DM and non- DM patients at MTRH, Eldoret, kenya

Participant Factor	Unadjusted OR (95% CI)	P value	Adjusted OR (95% CI)	P value
DM	5.4 (1.53, 11.92)	0.009	6.2 (1.50, 12.57)	0.012
Female gender	0.49 (0.19, 1.28)	0.146	0.39 (0.13, 1.11)	0.057
Age	1.02 (0.99, 1.05)	0.785	0.98 (0.94, 1.02)	0.750
Urban residence	0.87 (0.33, 2.30)	0.164	1.08 (0.37, 3.20)	0.993
Fuel type	0.77 (0.5, 1.21)	0.395	0.82 (0.51, 1.31)	0.808
Presence of under 5 year old	1.89 (0.70, 5.13)	0.206	1.72 (0.55, 5.39)	0.395
Smoking	1.10 (0.24, 5.13)	0.895	0.83 (0.16, 4.22)	0.390
URTI last 3 months	0.99 (0.27, 3.61)	0.994	0.98 (0.12, 7.85)	0.729
Use of antibiotics last 3 months	0.73 (0.20, 2.61)	0.625	0.74 (0.18,2.93)	0.660
Hba1c >7%*	1.46 (0.31, 6.99)	0.670	1.32 (0.20, 8.56)	0.713
Insulin use*	3.52 (0.94, 13.17)	<0.001	5.19 (1.13, 23.64)	<0.001
Duration of DM>10 years*	1.85 (0.62,5.51)	0.269	1.11 (0.28,4.32)	0.716

*Factors assessed among DM participants only

Having DM was associated with 5.4 higher odds of carriage of *S. pneumoniae* and this association was statistically significant (p=0.009). This association was observed even in the multivariate analysis where the odds rose to 6.2 times higher among DM participants. In the multivariate analysis, female gender was protective of colonization and this had borderline statistical significance (OR=0.36, p=0.057).There were more participants using unsafe fuel options who had a positive culture result for *S. pneumoniae* , this difference was however not statistically significant (p=0.378).There was no association between nasopharyngeal carriage of *S. pneumoniae* and presence

of under 5 year old child in the household, HIV infection status, age, area of residence, or smoking. Among participants with DM, those on an insulin based regimen were more likely to have nasopharyngeal colonization by *S. pneumoniae* (P=0.012). Participants on insulin based regimens also had longer duration of DM and worse glycemic control. There was, however, no association between nasopharyngeal carriage of *S. pneumoniae* with glycemic control or duration of DM.

4.5.Objective 3: Determine the antibiotic susceptibility of nasopharyngeal isolates of *S. pneumoniae* to routinely used antibiotics.

Table 5: Antibiotic susceptibility patterns of nasopharyngeal *S. pneumoniae* isolates in DM and non- DM patients at MTRH, Eldoret, Kenya

Antibiotic Type	***Streptococcus pneumoniae* Resistant N (%)**	***Streptococcus pneumoniae* susceptible N (%)**
Amoxicillin	3 (16.67)	15 (83.33)
Azithromycin	0	18 (100%)
Erythromycin	0	18 (100%)
Cotrimoxazole	17 (94.44)	1(5.56)
Cefuroxime	2 (11.1)	16 (88.89)
Amoxicillin/Clavunate	1 (5.56)	17 (94.44

Amoxicillin was the most routinely used at 67.31%. Macrolides were used by 9.61% with cotrimoxazole only used by 3.85%. cephalosporins were not routinely used. Of the *S. pneumoniae* isolates, 94.44% were resistant to cotrimoxazole and 16.67 % to amoxicillin. Despite their limited use oral cephalosporins (Cefuroxime) showed resistance in 11.1 % of the isolates while there was no documented resistance to macrolides. There was one cases each (5.56%) of resistance to intravenous cephalosporins and Carbapenems.

CHAPTER FIVE: DISCUSSION

5.1 Prevalence of nasopharyngeal carriage of *S. pneumoniae* among all participants

The prevalence of nasopharyngeal carriage of *S. pneumoniae* was 7.5% among all the participants. This is comparable to the 6.4% prevalence found in Kilifi Kenya among persons aged between 10 and 85 years (Abdullahi *et al.*, 2008). Studies done in other populations have demonstrated different prevalence. In native Australian communities, the prevalence was as high as 22.8% (Mackenzie *et al.*, 2010). This study differs with our studies in that their participants were exclusively a rural population, they used a modified WHO procedure to collect samples where the swab was left in the nasopharynx for 10 rather than 5 seconds and rotated through 360^0 rather than 180^0. This would have led to the higher prevalence observed.

5.2 Factors Associated with nasopharyngeal carriage of *S. pneumoniae* among all participants.

In this study only DM was associated with nasopharyngeal carriage of *S. pneumoniae* among all participants. Female gender was protective of colonization with borderline statistical significance. Other studies have found conflicting association between sex and carriage of *S. pneumoniae*. In some studies female gender was protective (Cardozo *et al.*, 2008; Mackenzie *et al.*, 2010; Principi *et al.*, 2015) while in others there was no association between carriage and sex (Abdullahi *et al.*, 2008; Garcia-Rodriguez and Fresnadillo Martinez, 2002; Usuf *et al.*, 2014).

There was no association of pneumococcal carriage with age. This is similar to findings in the Kilifi study (Abdullahi *et al.*, 2008) which was conducted in a population comparable with ours. It differs with the Australian and American studies

which found reduction in pneumococcal carriage with increasing age. These studies had included children and adults while ours included only adults. The mean age of our participants 43.69years with only 10 percent being older than 65 years the age at which carriage is expected to peak. This could explain the observed lack of association with age.

While indoor air pollution is expected to predispose to colonization by *Streptococcus pneumoniae*, there was no association with fuel type used in cooking in our study. This was despite most of the participants with a positive culture result using indoor polluting fuel sources. This finding differs from findings in the Australian study (Mackenzie *et al.*, 2010) and Brazil study (Cardozo *et al.*, 2008) where exposure to indoor smoke was associated with higher risk of colonization. It is however consistent with findings in a study conducted in Andean population which compared nasopharyngeal colonization among participants enrolled in a trial with improved stoves to reduce smoke and participants living in homes with open fires. There was no difference in nasopharyngeal colonization by *S. pneumoniae* among these groups (Howard *et al.*, 2017).

A history of cigarette smoking was not associated with colonization in our study. This is contrary to findings by Raman et al who demonstrated higher pneumococcal mucosal adherence among smokers than non-smokers (Raman *et al.*, 1983). The difference in our study could be due to the small numbers of self-reported cigarette users.

There were very few participants with rhinorrhea at the time of the study and we found no association with nasopharyngeal colonization with *S. pneumoniae*. This is contrary to findings in other studies where presence of rhinorrhea was associated with

higher *S. pneumoniae* carriage (Abdullahi *et al.*, 2008; Cardozo *et al.*, 2008; Mackenzie *et al.*, 2010).The small number of participants with rhinorrhea in our study may explain this observed discrepancy with other studies.

There were only six participants who reported being positive for the Human Immunodeficiency Virus and none was found to have a positive culture for *S. pneumoniae*. These small numbers may also have led to an underestimation of the association as most studies have demonstrated higher colonization among persons living with HIV (Conklin *et al.*, 2016; Heinsbroek *et al.*, 2015) .

5.3 Prevalence of nasopharyngeal carriage of *S. pneumoniae* among participants with DM

The prevalence of nasopharyngeal carriage of *S. pneumonia* was significantly higher among participants with DM at 12.1%. This is lower than the prevalence reported by the only other study done in a DM population in Italy which demonstrated a prevalence of 49.8% (Principi *et al.*, 2015). This study, however, differs from our study as it included children and adolescents with DM and carriage of the pneumococcus has been demonstrated to be higher in younger persons and reduces with age (Abdullahi *et al.*, 2008; Adegbola *et al.*, 2014; Hill *et al.*, 2006; Mackenzie *et al.*, 2010). Although the participants with DM were older than the those with no DM in our study, this difference observed cannot purely be attributed to this as the mean age of the DM population was 50.5 years which is still below 65 years, the age at which susceptibility to colonization is anticipated to increase (Garcia-Rodriguez and Fresnadillo Martinez, 2002). The association with DM also remained after controlling for age in the multivariate analysis.

The higher prevalence of nasopharyngeal carriage of *S. pneumoniae* among participants with DM was observed despite a significantly higher proportion of participants with DM having a household member less than 5 years old. Presence of children under 5 years of age is expected to confer herd immunity to adults since this age group receives the pneumococcal vaccine as part of routine vaccination (Lexau *et al.*, 2005; Ministry of Health, 2013). The observation in our study could indicate that the predisposition to colonization by *S. pneumoniae* in DM, as in other comorbid conditions for which vaccination indicated, is greater than would be sufficiently covered under herd immunity (Lexau *et al.*, 2005). It could also signify an increase in colonization by non vaccinatable serovars of *S. pneumoniae* (Lexau *et al.*, 2005; Mbelle *et al.*, 1999). Vaccination status of these children was not confirmed by examination of vaccination cards and if a majority had not actually received the vaccine, then there was no benefit of herd immunity to be conferred to the adults in our study.

In addition to higher prevalence of pneumococcal carriage, there were more cases of recent upper respiratory tract infection (URTI) and antibiotic use among participants with DM. This could be attributed to the higher rates of URTI expected in DM due to the immune dysfunction. There was however no association between nasopharyngeal colonization with *S. pneumoniae* and glycemic control suggesting some aspects of the immune dysfunction observed in DM is independent of glycemic control. This finding has been described in a study among women with DM that demonstrated low levels of interleukins independent of glycemic control and attributable to intrinsic defects in cells of persons with DM (Geerlings *et al.*, 2000).

Participants on insulin alone had higher prevalence of carriage than those on other modes of treatment. They were also more likely to have DM longer and to have poor

glycemic control suggesting long standing dysglycemia may be a contributing factor to susceptibility to colonization with the bacteria. Long standing dysglycemia leads to suppression of myeloid and T cell function making it harder for patients with DM to mount an immune response to the initial colonization by *S. pneumonia* (Price *et al.*, 2010)

5.4 Antibiotic susceptibility patterns of *S. pneumoniae* isolates

Among the antibiotics investigated in this study, Amoxicillin was the most frequently used, this could be due to its affordability and over the counter availability. The pneumococcal resistance to amoxicillin was 16.7% which is comparable to that observed in the Gambia (Hill *et al.*, 2006) and china (Wang *et al.*, 1998) studies. It is much lower than that the 72% observed in the Uganda study (Blossom *et al.*, 2006) .There was high resistance (94.4%) demonstrated to trimethoprim-sulphamethoxazole which can be attributed to its overuse and availability in previous years which could also explain its current unpopularity among clinicians with preference for use of amoxicillin. This finding is consistent with findings from the Uganda study which demonstrated a 99.9% rate of resistance. The Gambia and china studies had less resistance at 39% and 43.7% respectively. There was no resistance to macrolides in our study which is similar to findings in the Gambia and study. The Uganda study had only one resistance case with the Chinese study having 74% resistance. This difference could be attributed to the less availability and affordability of macrolides in developing countries as opposed to more developed nations like China. Our study demonstrated resistance to oral and intravenous Cephalosporins. With the increases in their use, this could signal rising resistance to these agents with the need to regulate their use to when absolutely indicated.

CHAPTER SIX: CONCLUSION AND RECOMMENDATIONS

6.1 Conclusions

1. Patients with DM have higher rates of nasopharyngeal colonization with *S. pneumoniae* than patients without DM.

2. There is demonstrated resistance of *S. pneumoniae* isolates to amoxicillin and cotrimoxazole with macrolides being relatively spared.

6.2 Recommendations

1. The ministry of health to adapt policy guidelines on pneumococcal vaccination aimed at reduction of the nasopharyngeal carriage of *S. pneumoniae* among patients with DM.

2. Given the *S. pneumoniae* isolates resistance to cotrimoxazole, it should not be used for treating invasive RTIs unless supported by a positive culture and sensitivity result.

3. Future research- longitudinal study to determine progression of colonization to invasive disease and outcomes among patients with DM in Kenya.

REFERENCES

Abdullahi, Osman, et al. "The descriptive epidemiology of Streptococcus pneumoniae and Haemophilus influenzae nasopharyngeal carriage in children and adults in Kilifi district, Kenya." *The Pediatric infectious disease journal* 27.1 (2008): 59.

Abdullahi, O., Wanjiru, E., Musyimi, R., Glass, N., & Scott, J. A. G. (2007). Validation of nasopharyngeal sampling and culture techniques for detection of Streptococcus pneumoniae in children in Kenya. *Journal of clinical microbiology*, *45*(10), 3408-3410.

Adegbola, R. A., DeAntonio, R., Hill, P. C., Roca, A., Usuf, E., Hoet, B., & Greenwood, B. M. (2014). Carriage of Streptococcus pneumoniae and other respiratory bacterial pathogens in low and lower-middle income countries: a systematic review and meta-analysis. *PloS one*, *9*(8), e103293.

American Diabetes Association. (2016). Standards of medical care in diabetes—2016 abridged for primary care providers. *Clinical diabetes: a publication of the American Diabetes Association*, *34*(1), 3.

Auranen, K., Rinta-Kokko, H., Goldblatt, D., Nohynek, H., O'Brien, K. L., Satzke, C., ... & Käyhty, H. (2013). Design questions for Streptococcus pneumoniae vaccine trials with a colonisation endpoint. *Vaccine*, *32*(1), 159-164.

Bertoni, A. G., Saydah, S., & Brancati, F. L. (2001). Diabetes and the risk of infection-related mortality in the U.S. *Diabetes Care*, *24*(6), 1044-1049.

Blossom, D. B., Namayanja-Kaye, G., Nankya-Mutyoba, J., Mukasa, J. B., Bakka, H., Rwambuya, S., ... & Kityo, C. (2006). Oropharyngeal colonization by Streptococcus pneumoniae among HIV-infected adults in Uganda: assessing prevalence and antimicrobial susceptibility. *International journal of infectious diseases*, *10*(6), 458-464.

Bogaert, D., de Groot, R., & Hermans, P. W. M. (2004). Streptococcus pneumoniae colonisation: the key to pneumococcal disease. *The Lancet infectious diseases*, *4*(3), 144-154.

Bogaert, D., Engelen, M. N., Timmers-Reker, A. J., Elzenaar, K. P., Peerbooms, P. G., Coutinho, R. A., ... & Hermans, P. W. (2001). Pneumococcal carriage in children in The Netherlands: a molecular epidemiological study. *Journal of clinical microbiology*, *39*(9), 3316-3320.

Cardozo, D. M., Nascimento-Carvalho, C. M., Andrade, A. L. S., Silvany-Neto, A. M., Daltro, C. H., Brandao, M. A. S., ... & Brandileone, M. C. C. (2008). Prevalence and risk factors for nasopharyngeal carriage of Streptococcus pneumoniae among adolescents. *Journal of Medical Microbiology*, *57*(2), 185-189.

Casqueiro, J., Casqueiro, J., & Alves, C. (2012). Infections in patients with diabetes mellitus: A review of pathogenesis. *Indian journal of endocrinology and metabolism*, *16*(Suppl1), S27.

Centres for Disease Control and Prevention. (2017). Pneumococcal Vaccination. from https://www.cdc.gov/pneumococcal/vaccination.html

Chen, J., O'Brien, M. A., Yang, H. K., Grabenstein, J. D., & Dasbach, E. J. (2014). Cost-effectiveness of pneumococcal vaccines for adults in the United States. *Advances in therapy*, *31*(4), 392-409.

Conklin, L. M., Bigogo, G., Jagero, G., Hampton, L., Junghae, M., da Gloria Carvalho, M., ... & Laserson, K. F. (2015). High Streptococcus pneumoniae colonization prevalence among HIV-infected Kenyan parents in the year before pneumococcal conjugate vaccine introduction. *BMC infectious diseases*, *16*(1), 18.

Cundell, D. R., Gerard, N. P., Gerard, C., Idanpaan-Heikkila, I., & Tuomanen, E. I. (1995). Streptococcus pneumoniae anchor to activated human cells by the receptor for platelet-activating factor. *Nature*, *377*(6548), 435.

Dagan, R., Muallem, M., Melamed, R., Leroy, O., & Yagupsky, P. (1997). Reduction of pneumococcal nasopharyngeal carriage in early infancy after immunization with tetravalent pneumococcal vaccines conjugated to either tetanus toxoid or diphtheria toxoid. *The Pediatric infectious disease journal*, *16*(11), 1060-1064.

Elaine I Tuomanen, M. (Aug 10, 2017.). *UpToDate*. Waltham, MA: UpToDate.

Falguera, M., Pifarre, R., Martin, A., Sheikh, A., & Moreno, A. (2005). Etiology and outcome of community-acquired pneumonia in patients with diabetes mellitus. *Chest*, *128*(5), 3233-3239.

Fisman, D. N., Abrutyn, E., Spaude, K. A., Kim, A., Kirchner, C., & Daley, J. (2006). Prior pneumococcal vaccination is associated with reduced death, complications, and length of stay among hospitalized adults with community-acquired pneumonia. *Clin Infect Dis*, *42*(8), 1093-1101. doi: 10.1086/501354

García-Rodríguez, J. Á., & Fresnadillo Martínez, M. J. (2002). Dynamics of nasopharyngeal colonization by potential respiratory pathogens. *Journal of Antimicrobial Chemotherapy*, *50*(suppl_3), 59-74.

Gebre, T., Tadesse, M., Aragaw, D., Feye, D., Beyene, H., Seyoum, D., & Mekonnen, M. (2017). Nasopharyngeal carriage and antimicrobial susceptibility patterns of Streptococcus pneumoniae among children under five in Southwest Ethiopia. *Children*, *4*(4), 27.

Geerlings, S. E., & Hoepelman, A. I. (1999). Immune dysfunction in patients with diabetes mellitus (DM). *FEMS Immunology & Medical Microbiology*, *26*(3-4), 259-265.

Geerlings, S. E., Brouwer, E. C., Van Kessel, K. C., Gaastra, W., Stolk, R. P., & Hoepelman, A. I. (2000). Cytokine secretion is impaired in women with diabetes mellitus. *European journal of clinical investigation*, *30*(11), 995-1001.

Heinsbroek, E., Tafatatha, T., Phiri, A., Ngwira, B., Crampin, A. C., Read, J. M., & French, N. (2015). Persisting high prevalence of pneumococcal carriage among HIV-infected adults receiving antiretroviral therapy in Malawi: a cohort study. *AIDS (London, England)*, *29*(14), 1837.

Hennessy, T. W., Singleton, R. J., Bulkow, L. R., Bruden, D. L., Hurlburt, D. A., Parks, D., ... & Butler, J. C. (2005). Impact of heptavalent pneumococcal conjugate vaccine on invasive disease, antimicrobial resistance and colonization in Alaska Natives: progress towards elimination of a health disparity. *Vaccine*, *23*(48-49), 5464-5473.

Henriques-Normark, B., & Tuomanen, E. I. (2013). The pneumococcus: epidemiology, microbiology, and pathogenesis. *Cold Spring Harbor perspectives in medicine*, *3*(7), a010215.

Hill, P. C., Akisanya, A., Sankareh, K., Cheung, Y. B., Saaka, M., Lahai, G., ... & Adegbola, R. A. (2006). Nasopharyngeal carriage of Streptococcus pneumoniae in Gambian villagers. *Clinical infectious diseases*, *43*(6), 673-679.

Howard, L. M., Fan, R., Zhu, Y., Griffin, M. R., Edwards, K. M., Hartinger, S., ... & Lanata, C. F. (2017, July). Nasopharyngeal pneumococcal density is associated with viral activity but not with use of improved stoves among young Andean children. In *Open forum infectious diseases* (Vol. 4, No. 3). Oxford University Press.

Hussain, M., Melegaro, A., Pebody, R. G., George, R., Edmunds, W. J., Talukdar, R., ... & Miller, E. (2005). A longitudinal household study of Streptococcus pneumoniae nasopharyngeal carriage in a UK setting. *Epidemiology & Infection*, *133*(5), 891-898.

Institute for Health Metrics and Evaluation. (2017). Health Data Kenya. from http://www.healthdata.org/kenya

Joshi, N., Caputo, G. M., Weitekamp, M. R., & Karchmer, A. W. (1999). Infections in patients with diabetes mellitus. *New England Journal of Medicine*, *341*(25), 1906-1912.

Kadioglu, A., Weiser, J. N., Paton, J. C., & Andrew, P. W. (2008). The role of Streptococcus pneumoniae virulence factors in host respiratory colonization and disease. *Nature Reviews Microbiology*, *6*(4), 288.

Kenneth Todar, P. D. (2012). *Todar's Online Textbook of Bacteriology* Retrieved from http://textbookofbacteriology.net/S.pneumoniae.html

Kesavadev, J., Misra, A., Das, A. K., Saboo, B., Basu, D., Thomas, N., ... & Unnikrishnan, R. (2012). Suggested use of vaccines in diabetes. *Indian journal of endocrinology and metabolism*, *16*(6), 886.

Kimang'a, A. N. (2012). A situational analysis of antimicrobial drug resistance in Africa: are we losing the battle? *Ethiop journal of laboratory medicine22*(2), 135-143.

Kirkwood R Betty, S. A. J. (2003.). *Essential Medical Statistics*, (Vol. Vol 2): Oxford: Blackwell publishing company.

Klekotka, R. B., Mizgała, E., & Król, W. (2015). The etiology of lower respiratory tract infections in people with diabetes. *Advances in Respiratory Medicine*, *83*(5), 401-408.

Kornum, J. B., Thomsen, R. W., Riis, A., Lervang, H. H., Schønheyder, H. C., & Sørensen, H. T. (2007). Type 2 diabetes and pneumonia outcomes: a population-based cohort study. *Diabetes Care*, *30*(9), 2251-2257.

Kornum, J. B., Thomsen, R. W., Riis, A., Lervang, H. H., Schønheyder, H. C., & Sørensen, H. T. (2008). Diabetes, glycemic control, and risk of hospitalization with pneumonia: a population-based case-control study. *Diabetes care*, *31*(8), 1541-1545.

Kyaw, M. H., Rose Jr, C. E., Fry, A. M., Singleton, J. A., Moore, Z., Zell, E. R., ... & Active Bacterial Core Surveillance Program of the Emerging Infections Program Network. (2005). The influence of chronic illnesses on the incidence of invasive pneumococcal disease in adults. *The Journal of infectious diseases*, *192*(3), 377-386.

Lapolla, A., Tonani, R., Fedele, D., Garbeglio, M., Senesi, A., Seraglia, R., ... & Traldi, P. (2002). Non-enzymatic glycation of IgG: an in vivo study. *Hormone and metabolic research*, *34*(05), 260-264.

Lederman, M. M., Schiffman, G., & Rodman, H. M. (1981). Pneumococcal immunization in adult diabetics. *Diabetes*, *30*(2), 119-121.

Lehmann, D., Gratten, M., & Montgomery, J. (1997). Susceptibility of pneumococcal carriage isolates to penicillin provides a conservative estimate of susceptibility of invasive pneumococci. *The Pediatric infectious disease journal*, *16*(3), 297-305.

Lexau, C. A., Lynfield, R., Danila, R., Pilishvili, T., Facklam, R., Farley, M. M., ... & Hadler, J. (2005). Changing epidemiology of invasive pneumococcal disease among older adults in the era of pediatric pneumococcal conjugate vaccine. *Jama*, *294*(16), 2043-2051.

Lieberman, D., Shleyfer, E., Castel, H., Terry, A., Harman-Boehm, I., Delgado, J., ... & Lieberman, D. (2006). Nasopharyngeal versus oropharyngeal sampling for isolation of potential respiratory pathogens in adults. *Journal of clinical microbiology*, *44*(2), 525-528.

Lloyd-Evans, N., O'Dempsey, T. J., Baldeh, I., Secka, O., Demba, E., Todd, J. E., . . . Greenwood, B. M. (1996). Nasopharyngeal carriage of pneumococci in Gambian children and in their families. *Pediatr Infect Dis J*, *15*(10), 866-871.

Mackenzie, G. A., Leach, A. J., Carapetis, J. R., Fisher, J., & Morris, P. S. (2010). Epidemiology of nasopharyngeal carriage of respiratory bacterial pathogens in children and adults: cross-sectional surveys in a population with high rates of pneumococcal disease. *BMC infectious diseases*, *10*(1), 304.

Marchisio, P., Esposito, S., Schito, G. C., Marchese, A., Cavagna, R., & Principi, N. (2002). Nasopharyngeal carriage of Streptococcus pneumoniae in healthy children: implications for the use of heptavalent pnemococcal conjugate vaccine. *Emerging infectious diseases*, *8*(5), 479.

Mathews, C. E., Brown, E. L., Martinez, P. J., Bagaria, U., Nahm, M. H., Burton, R. L., ... & Mirza, S. (2012). Impaired function of antibodies to pneumococcal surface protein A but not to capsular polysaccharide in Mexican American adults with type 2 diabetes mellitus. *Clin. Vaccine Immunol.*, *19*(9), 1360-1369.

Mbelle, N., Huebner, R. E., Wasas, A. D., Kimura, A., Chang, I., & Klugman, K. P. (1999). Immunogenicity and impact on nasopharyngeal carriage of a nonavalent pneumococcal conjugate vaccine. *The Journal of infectious diseases*, *180*(4), 1171-1176.

McCullers, J. A., & Rehg, J. E. (2002). Lethal synergism between influenza virus and Streptococcus pneumoniae: characterization of a mouse model and the role of platelet-activating factor receptor. *The Journal of infectious diseases*, *186*(3), 341-350.

Ministry of Health. (2013). *National Policy Guidelines on Immnunization* Kenya: Ministry of Health.

Moi Teaching and Referral Hospital (MTRH). (2014). About Moi teaching and Referral Hospital. Retrieved 26th January 2015, 2014, from http://www.mtrh.or.ke/index.php/about-us

Muller, L. M. A. J., Gorter, K. J., Hak, E., Goudzwaard, W. L., Schellevis, F. G., Hoepelman, A. I. M., & Rutten, G. E. H. M. (2005). Increased risk of common infections in patients with type 1 and type 2 diabetes mellitus. *Clinical infectious diseases*, *41*(3), 281-288.

Neu, H. C. (1992). The crisis in antibiotic resistance. *Science*, *257*(5073), 1064-1073.

Nuorti, J. P., Whitney, C. G., Centers for Disease, C., & Prevention. (2010). Prevention of pneumococcal disease among infants and children - use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine - recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR Recomm Rep, 59*(RR-11), 1-18.

Paul G. Engelkirk, J. D.-E. (2008). *laboratory Diagnosis of Infectious Diseases; Eseentials of diagnostic microbiology*. Philadelphia: Lippincot Williams & Wilkins.

Peleg, A. Y., Weerarathna, T., McCarthy, J. S., & Davis, T. M. (2007). Common infections in diabetes: pathogenesis, management and relationship to glycaemic control. *Diabetes/metabolism research and reviews*, *23*(1), 3-13.

Price, C. L., Hassi, H. O. A., English, N. R., Blakemore, A. I., Stagg, A. J., & Knight, S. C. (2010). Methylglyoxal modulates immune responses: relevance to diabetes. *Journal of cellular and molecular medicine*, *14*(6b), 1806-1815.

Principi, N., Iughetti, L., Cappa, M., Maffeis, C., Chiarelli, F., Bona, G., ... & Marigliano, M. (2016). Streptococcus pneumoniae oropharyngeal colonization in school-age children and adolescents with type 1 diabetes mellitus: Impact of the heptavalent pneumococcal conjugate vaccine. *Human vaccines & immunotherapeutics*, *12*(2), 293-300.

Raman, A. S., Swinburne, A. J., & Fedullo, A. J. (1983). Pneumococcal adherence to the buccal epithelial cells of cigarette smokers. *Chest*, *83*(1), 23-27.

Ruiz-González, A., Falguera, M., Nogués, A., & Rubio-Caballero, M. (1999). Is Streptococcus pneumoniae the leading cause of pneumonia of unknown etiology? A microbiologic study of lung aspirates in consecutive patients with community-acquired pneumonia. *The American journal of medicine*, *106*(4), 385-390.

Saibal, M. A. A., Rahman, S. H. Z., Nishat, L., Sikder, N. H., Begum, S. A., Islam, M. J., & Uddin, K. N. (2012). Community acquired pneumonia in diabetic and non-diabetic hospitalized patients: presentation, causative pathogens and outcome. *Bangladesh Medical Research Council Bulletin*, *38*(3), 98-103.

Satzke, C., Turner, P., Virolainen-Julkunen, A., Adrian, P. V., Antonio, M., Hare, K. M., ... & Sá-Leão, R. (2013). Standard method for detecting upper respiratory carriage of Streptococcus pneumoniae: updated recommendations from the World Health Organization Pneumococcal Carriage Working Group. *Vaccine*, *32*(1), 165-179.

Schuetz, P., Friedli, N., Grolimund, E., Kutz, A., Haubitz, S., Christ-Crain, M., . . . Pro, H. S. G. (2014). Effect of hyperglycaemia on inflammatory and stress responses and clinical outcome of pneumonia in non-critical-care inpatients: results from an observational cohort study. *Diabetologia*, *57*(2), 275-284. doi: 10.1007/s00125-013-3112-9

Scott, J. A. G., Hall, A. J., Muyodi, C., Lowe, B., Ross, M., Chohan, B., ... & Marsh, K. (2000). Aetiology, outcome, and risk factors for mortality among adults with acute pneumonia in Kenya. *The Lancet*, *355*(9211), 1225-1230.

Shah, B. R., & Hux, J. E. (2003). Quantifying the risk of infectious diseases for people with diabetes. *Diabetes care*, *26*(2), 510-513.

Shapiro, E. D., Berg, A. T., Austrian, R., Schroeder, D., Parcells, V., Margolis, A., ... & Clemens, J. D. (1991). The protective efficacy of polyvalent pneumococcal polysaccharide vaccine. *New England Journal of Medicine*, *325*(21), 1453-1460.

Sharma, A. M., & Lau, D. C. (2013). Weight management in diabetes. *Macrovascular and Microvascular Complications*, *37*, 82.

Simell, B., Auranen, K., Käyhty, H., Goldblatt, D., Dagan, R., O'Brien, K. L., & Pneumococcal Carriage Group (PneumoCarr). (2012). The fundamental link between pneumococcal carriage and disease. *Expert review of vaccines*, *11*(7), 841-855.

Sleeman, K. L., Daniels, L., Gardiner, M., Griffiths, D., Deeks, J. J., Dagan, R., ... & Crook, D. W. (2005). Acquisition of Streptococcus pneumoniae and nonspecific morbidity in infants and their families: a cohort study. *The Pediatric infectious disease journal*, *24*(2), 121-127.

Swiatlo, E., Champlin, F. R., Holman, S. C., Wilson, W. W., & Watt, J. M. (2002). Contribution of choline-binding proteins to cell surface properties of Streptococcus pneumoniae. *Infection and immunity*, *70*(1), 412-415.

Thomsen, R. W., Hundborg, H. H., Lervang, H. H., Johnsen, S. P., Schonheyder, H. C., & Sorensen, H. T. (2004). Risk of community-acquired pneumococcal bacteremia in patients with diabetes: a population-based case-control study. *Diabetes Care*, *27*(5), 1143-1147.

Tomczyk, S., Bennett, N. M., Stoecker, C., Gierke, R., Moore, M. R., Whitney, C. G., ... & Pilishvili, T. (2014). Use of 13-valent pneumococcal conjugate vaccine and 23-valent pneumococcal polysaccharide vaccine among adults aged≥ 65 years: recommendations of the Advisory Committee on Immunization Practices (ACIP). *MMWR. Morbidity and mortality weekly report*, *63*(37), 822.

Usuf, E., Bottomley, C., Adegbola, R. A., & Hall, A. (2014). Pneumococcal carriage in sub-Saharan Africa—a systematic review. *PloS one*, *9*(1), e85001.

Wang, H., Huebner, R., Chen, M., & Klugman, K. (1998). Antibiotic Susceptibility Patterns ofStreptococcus pneumoniae in China and Comparison of MICs by Agar Dilution and E-Test Methods. *Antimicrobial agents and chemotherapy*, *42*(10), 2633-2636.

Watt, J. P., O'brien, K. L., Katz, S., Bronsdon, M. A., Elliott, J., Dallas, J., ... & Santosham, M. (2004). Nasopharyngeal versus oropharyngeal sampling for detection of pneumococcal carriage in adults. *Journal of clinical microbiology*, *42*(11), 4974-4976.

World health Organization, W. (2018). Pneumococcal disease. from http://www.who.int/ith/diseases/pneumococcal/en/

APPENDICES

Appendix Ia: Consent for Participation (English)

You are being invited to participate in a research study on the burden and risk factors of nasopharyngeal carriage of *Streptococcus pneumoniae* among DM and non-DM patient attending clinics in Moi Teaching and Referral Hospital. This study is being conducted by Dr. Wambui Charity who is a resident in Moi University School of Medicine Department of internal medicine.

The study shall involve an initial interview of about 10 minutes. Subsequently you shall have your weight, height and blood pressure taken. An HbA1c test shall be done or a recent one obtained from the clinic file if you have DM. You will then have a sterile swab inserted into nasal cavity to perform the nasopharyngeal swab. If you are in the comparison arm, your blood glucose shall also be measured by finger prick.

The benefit of participating in this study to you as an individual, is to get to know your health status as regards carriage of *Streptococcus pneumoniae* in your nasopharynx and your glycemic control by Hba1c. If you are in the control arm you will also benefit from knowing your blood sugar level. If any of these is found to be abnormal, it shall be communicated to your primary physician to facilitate further management.

This research has minimal risk to you and will cause no harm. You however will experience some mild discomfort and pain during finger prick and collection of the nasopharyngeal swab and will on average spend an additional 30 minutes in the hospital. There are no costs to you for participating in the study.

The results of the study will be stored in a database that is password protected and only accessible by those conducting the study. No one will be able to identify you or your results. Should the data be published, no individual information will be disclosed. Your participation in this study is voluntary. If you decide to participate, you can change your mind later and quit the study before the end of the study. If you

decide not to participate, or if you quit the study, it will not affect the health care services you receive at the clinic. By signing this document, you are voluntarily agreeing to participate. You are free to decline to answer any particular question you do not wish to answer for any reason.

If you have any questions about the study, please contact Dr. Wambui Charity on cell phone number 0721270365. The Institutional Review and Ethics Committee of Moi University and Moi teaching & Referral Hospital has reviewed our request to conduct this project.

I have read this informed consent and authorization form. ALL OF MY QUESTIONS HAVE BEEN SATISFACTORILY ANSWERED, AND I WANT TO TAKE PART IN THIS RESEARCH STUDY. By signing below, I give my permission to participate in this research study and for the described uses and releases of information.

Signature of the participant _____ Date_____

Witness (if appropriate)_____ Date_____

Signature of person giving consent_____ Date_____

Appendix Ib: Consent for Participation (Swahili)

IDHINI YA KUHUSISHWA

Unaalikwa kushiriki kwenye utafiti juu ya ukoloni wa pua na vimea vya ugonjwa wa "pneumonia"kati ya wagonjwa wa kisukari kwenye Kliniki ya MTRH Kenya. Utafiti huu unafanywa na Wambui Charity daktari mwanafunzi katika Chuo Kikuu Cha Moi.

Uchunguzi huu utahusisha majadiliano ya awali ya dakika 10. Kisha, utapimwa kilo, urefu na shinikizo la damu. Kiwango cha sukari kwa damu na kipimo maalum cha miezi tatu(HbA1c). kisha usufi utatiwa kwenye pua kutafuta vimea. Utafiti huu hautakugharimu malipo yoyote.

Matokeo ya utafiti huu yatawekwa siri kwenye orodha iliyo na ulinzi kamilifu na ambao unaweza kufikiwa na watafiti peke yao. Hamna yeyote mwingine atakayeweza kufikia matokeo ya vipimo yako. Ikiwa matokeo yatachapishwa, habari zozote za kibinafsi hazitachapishwa. Uhusishwaji wako kwenye utafiti huuni ni wa hiari. Ukipenda kushirikishwa, unaweza kubadili nia baadaye na kujiondoa kabla ya utafiti kumalizika. Ikiwa hungependa kushirikishwa ama ukijiondoa, hio haitaadhiri matibabu utakayopewa. Kwa kutia sahihi hati hii unatoa idhini ya kushirikishwa kwa hiari yako. Pia una uhuru wa kutojibu swali lolote utakalopenda kutojibu kwa sababu yoyote ile.

Kwa maswali yoyote juu ya utafiti huu, tafadhali wasiliana na daktari Wambui Charity kwa nambari 0721270365 au Kamati ya Maadiliya Tathmini na Utafitiya Chuo Kikuu cha Moi na Hospitali ya Rufaa na Mafunzo ya Moi imetathmini ombi letu la kuendeleza utafitihuu.

Nimesoma nakuelewahatihiiyaidhiniyahiarinakutoaridhaa. MASWALI YANGU YOTE YAMEJIBIWA KWA NJIA INAYORIDHISHA. Kwakutiasahihihapa, naidhinishakuhusishwakwangukwenyeutafitihuupamojanakuchapishwanakutumiwakwama tokeoyake.

Sahihi ya mshiriki _____ Tarehe_____

Shahidi (kama inafaa)_____Tarehe_____

Sahihi ya mtu anayepana idhini_____Tarehe_____

Appendix II: Questionnaire

Study number ……

Age…….

Gender …….

Weight……..

Height…….

BMI……..

Blood Pressure…SBP……../DBP…….

Known hypertensive?

Yes □ No □ Unknown □

Duration of known hypertension diagnosis……

Random Blood Sugar…….. (For non DM only)

Area of residence …….

Location □Rural □urban □unknown

Known DM

Yes □ No □ Unknown □

Duration of known DM diagnosis……

Recent HbA1c ----------% test date……

Known CKD

Yes □ No □ Unknown □

HIV status

Positive□ Negative □ Unknown □

Source of energy for cooking

Firewood□ charcoal □ Biogas□ kerosene□ other □

Any under 5 year old in the homestead

Yes □ No □

Previous pneumococcal vaccination

Yes □ No □

Current medication use

Insulin □ oral glucose lowering agent's□ lifestyle only □ all □

Use of antibiotics in the last 3 months

Yes □ No □

If yes, which one …….

Does the patient have active rhinorrhoea?

Yes □ No □

Has the patient had an upper respiratory infection in last 3 months?

Yes □ No □

NP culture results

Streptococcus Pneumoniae culture Positive □ Negative□

If positive; Antibiotic susceptibility

Amoxicillin	Susceptible□	Not Susceptible □
Erythromycin	Susceptible□	Not Susceptible □
Azithromycin	Susceptible□	Not Susceptible □
Cotrimoxazole	Susceptible□	Not Susceptible □
Cefuroxime	Susceptible□	Not Susceptible □

Other organisms cultured:

Appendix III: Procedures Performed

Measurement of Blood Pressure

Blood pressure was measured using an Omron M2 compact upper arm blood pressure (BP) monitor (Omron Healthcare, Inc., 1200 Lakeside Drive, Bannockburn, Illinois 60015). The patients were allowed at least 15 minutes rest in a relaxed sitting position with no tight fitting clothing on the upper arm, or any thick clothing such as a sweater. Measurement of blood pressure was done before drawing of any samples.

Steps followed:

1. The participant was positioned seated upright with the back straight and right arm placed on the table so that the cuff was on the same level as the heart.
2. The blood pressure machine cuff was wrapped on the arm so that the bottom of the cuff was at least 1cm above the elbow. The cuff was then fastened snugly.
3. The start button on the blood pressure machine was then be pressed and the cuff allowed to automatically inflate to take the blood pressure reading.
4. Blood pressure and pulse rate results were the displayed on the on the screen of the blood pressure machine.
5. The results of the blood pressure readings were then recorded on the data collection pro-forma.
6. Where an error occurs during the process, the cuff was deflated and the process repeated.
7. High blood pressure readings (Systolic BP >140mmHg and Diastolic BP >90mmHg) were confirmed manually using a mercury sphygmomanometer.

Measurement of Height

The participants' height was measured using a Mechanical roll-up measuring tape (Seca 260) with wall attachment at the nursing station.

Below are the steps that were followed in measuring height:

1. The participant was asked to remove their footwear (shoes, slippers, sandals, etc.) and head gear (hat, cap, hair bows, comb, ribbons, etc.). However, those with a scarf or veil were not be asked to remove them as measurement may be taken over light fabric.
2. Participants were asked stand next to the wall facing the research assistant or the principal investigator with feet together, heels against the wall and knees straight.
3. Participants were then asked to look straight ahead and not tilt their head up and the research assistant or principal investigator made sure eyes were the same level as the ears to ensure no tilting.
4. The measuring arm was then moved gently down onto the head of the participant and the participant asked to breathe in and stand tall.
5. The height was read in centimeters at the exact point and the participant asked to step away from the wall.
6. The height measurements in centimeters were recorded in the participant's data collection pro-forma.

Weight Measurement

The weight was measured with a 762 Dial Bathroom Floor Scale positioned at the nursing station.

Below are the steps that were followed in measuring weight:

1. The scale was placed on a firm, flat surface.
2. Participants were asked to remove their footwear (shoes, slippers, sandals, etc.).
3. The participants were then asked to step onto scale with one foot on each side of the scale.
4. Participants were asked to: stand still, face forward, place arms on the side and wait until they were asked to step off after their weight had been measured.
5. The weight in kilograms was recorded on the participant's data collection pro-forma.

BMI calculation:

BMI was calculated using the measured weight and height using the formula

$$\frac{\text{Weight (kg)}}{\text{Height (m)}^2}$$

Expressed as Kg/M^2

Procedure for Drawing Finger Stick Blood

Finger stick blood was drawn for HbA1c and RBS measurement. The procedure was explained to the participant and verbal consent obtained. Universal safety procedures were observed. Finger stick blood was drawn from the second or third finger of the less dominant hand.

Steps followed;

1. The finger and site were selected.
2. Finger was cleaned with alcohol/methylated spirit and allowed to air dry to decrease hemolysis and not alter glucose results.
3. Spring activated lancet was applied to the selected finger and a little puncture is made.
4. The first drop of blood wiped away.
5. Approximately 4 drops of blood (35µL) were collected using a capillary tube.
6. An adhesive bandage was placed on the puncture site once the procedure was done.

Procedure for Measuring Hba1c

Finger stick blood was drawn following the procedure outlined above.

1. Using the capillary tube, the whole blood was transferred to a DCA HbA1c immunoassay test cartridge for use with the DCA vantage analyzer.
2. The DCA vantage analyzer was pre calibrated using a provided calibrator.
3. The Cartridge containing the sample was then loaded into the analyzer.
4. The analyzer automatically run the test and results given within 6 minutes.
5. The results were displayed on the screen as a percentage with ranges from 2.5% to 14%
6. The results were recorded in the participants' data collection pro forma.

Procedure for Measuring RBS

Finger stick blood was drawn as outlined above

1. Using the capillary tube, the whole blood was transferred onto an Optium H test strip which was read on an Abbott Optium Exceed Glucometer
2. Results were displayed on the screen in the range of LO (<1 mmol/L) to HI(>30mmol/L)
3. Results were recorded in the participants' data collection pro forma.

Appendix IV: Approval letters from MTRH and IREC

MOI TEACHING AND REFERRAL HOSPITAL

Telephone: 2033471/2/3/4
Fax: 61749
Email: director@mtrh.or.ke
P. O. Box 3
ELDORET

Ref: ELD/MTRH/R.6/VOL.II/2008

30th September, 2016

Dr. Wambui Charity,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

RE: APPROVAL TO CONDUCT RESEARCH AT MTRH

Upon obtaining approval from the Institutional Research and Ethics Committee (IREC) to conduct your research proposal titled:-

"A Comparison of Nasopharyngeal Carriage of Streptococcus Pneumoniae among Diabetes and Non –Diabetes Patients at Moi Teaching and Referral Hospital, Eldoret, Kenya".

You are hereby permitted to commence your investigation at Moi Teaching and Referral Hospital.

30/09/2016

DR. WILSON ARUASA
CHIEF EXECUTIVE OFFICER
MOI TEACHING AND REFERRAL HOSPITAL

CC - Deputy Director (CS)
- Chief Nurse
- HOD, HRISM

INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE (IREC)

MOI TEACHING AND REFERRAL HOSPITAL
P.O. BOX 3
ELDORET
Tel: 33471/2/3

MOI UNIVERSITY
SCHOOL OF MEDICINE
P.O. BOX 4606
ELDORET

Reference: IREC/2016/75
Approval Number: 0001755

27th September, 2016

INSTITUTIONAL RESEARCH & ETHICS COMMITTEE
27 SEP 2016
APPROVED
P. O. Box 4606-30100 ELDORET

Dr. Wambui Charity,
Moi University,
School of Medicine,
P.O. Box 4606-30100,
ELDORET-KENYA.

Dear Dr. Wambui,

RE: FORMAL APPROVAL

The Institutional Research and Ethics Committee has reviewed your research proposal titled:-

"A Comparison of Nasopharyngeal Carriage of Streptococcus Pneumoniae among Diabetes and Non-Diabetes Patients at Moi Teaching and Referral Hospital, Eldoret, Kenya".

Your proposal has been granted a Formal Approval Number: ***FAN: IREC 1755*** on 27th September, 2016. You are therefore permitted to begin your investigations.

Note that this approval is for 1 year; it will thus expire on 26th September, 2017. If it is necessary to continue with this research beyond the expiry date, a request for continuation should be made in writing to IREC Secretariat two months prior to the expiry date.

You are required to submit progress report(s) regularly as dictated by your proposal. Furthermore, you must notify the Committee of any proposal change (s) or amendment (s), serious or unexpected outcomes related to the conduct of the study, or study termination for any reason. The Committee expects to receive a final report at the end of the study.

Sincerely,

For

PROF. E. WERE
CHAIRMAN
INSTITUTIONAL RESEARCH AND ETHICS COMMITTEE

cc CEO - MTRH Dean - SOP Dean - SOM
Principal - CHS Dean - SON Dean - SOD