RISK FACTORS FOR CARRIAGE OF DRUG-RESISTANT *STREPTOCOCCUS PNEUMONIAE* AMONG ADULTS WITH COUGH UNDERGOING EVALUATION FOR TUBERCULOSIS DISEASE IN BLANTYRE, MALAWI

UNIVERSITY OF THE
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JOHANNESBURG

BY

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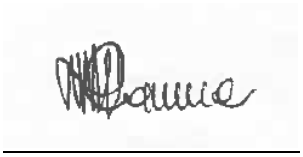
Declaration

I, Nawane Dorothy Sekgala, declare that this dissertation is my own. It is being submitted for the Degree of Master of Science in Epidemiology: Epidemiology and Biostatistics at the University of Witwatersrand, Parktown, Johannesburg, South Africa. This work has not been submitted before for any degree or examination at any university.



Student signature

I declare this in June 2025, at Johannesburg, South Africa.

Dedication

I dedicate this research work to my son in heaven, Puleng Vincent Sekgala. I wish you were here to celebrate this milestone with me. To my two children, Khumo Gilah Matemeke Sekgala and Mosa Raymond Dithomo Sekgala. You have made me stronger, better and more fulfilled than I could have ever imagined.

Ye, ke ya lena!!

Abstract

Background

Streptococcus pneumoniae is one of the major causes of infectious diseases across the world. It can cause life threatening diseases such as pneumonia to less severe illness such as sinusitis. Carriage is defined as Streptococcus pneumoniae that occurs in the upper airway. It is harmless, however; can cause severe illness and death if it subjugates the immune system. Carriage is common in children and decreases with an increasing age. However, older people are at higher risk of developing carriage due to their compromised immune system. This study investigated the factors associated with the development of carriage of drug resistant Streptococcus pneumoniae in adults and inform strategies for prevention and treatment.

Methods

This study was a secondary data analysis of a three-arm, individually randomised, open-label, control trial which investigated accuracy and consequences of using Trial-of-antibiotics for TB diagnosis. The primary study enrolled 1,583 adult's participants who presented with cough of at least 14 days at 2 primary health care centres in Blantyre, Malawi. Nasopharyngeal swabs were collected from the participants and were tested for antibiotics resistance against *S. pneumoniae.* Descriptive analysis was carried out and we performed univariable and multivariable logistic regressions to determine significant factors associated with the development of carriage of drug-resistant *S. pneumoniae.* We adjusted for possible confounding factors during multivariable analysis.

Results

The study had 1,576 participants for analysis, including 139 participants who tested positive for drug resistant *S. pneumoniae*. The participants comprised of 635 (40%) males and 941 (60%) females.There were 502 (32%) from Limbe Health Centre while 1073 (68%) were from Ndirande Health Centre. There were 1,309 (83%) participants who were HIV negative, while 221 (14%) were HIV positive and only 46 (2.9%) didn't know their HIV status. There were 98 (6.2%) participants who had confirmed TB status and 1478 (94%) were not confirmed for TB. In univariable analysis, the following factors were significantly associated with the development of carriage of drug-resistant *S. pneumoniae* in adults: underweight with OR 1.89 (95% CI: 1.13 – 3.03, p-value= 0.011) compared to not being underweight, having a history of previous TB with OR 2.12 (95% CI: 1.17 – 3.36, p-value= 0.009) compared to no history of previous TB, presence of sweat with OR 1.43 (95% CI: 1.01 – 2.03, p-value= 0.045) compared to absence of sweat, producing sputum with OR 1.69 (95% CI: 1.01 – 2.81, p-value= 0.031) compared to not producing sputum, positive HIV status with OR 1.71 (95% CI: 1.09 – 2.62, p-value= 0.016) compared to negative HIV status and unknown HIV status. In multivariable analysis, having a history of previous TB was the only factor significantly associated with the development of carriage of drug resistant *S. pneumoniae* in adults with an adjusted OR 1.86 (95% CI: 0.99 – 3.31, p-value 0.044). The study found that the other factors (treatment arm, site, education, fever, chest pain, blood in sputum, weight loss and TB microbiology) were not significantly associated with the development of carriage of drug-resistant *S. pneumoniae* in adults.

Conclusion

The study concluded that having a history of previous TB is a risk factor in the development of carriage of drug-resistant *S. pneumoniae* among older population. Therefore, stakeholders should explore context-specific strategies such as strengthened mycobacterial resistant monitoring mechanisms.

Keywords: Carriage, *Streptococcus pneumoniae*, Tuberculosis, Colonization, Pneumococcal Conjugate Vaccine

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List of Acronyms

AMR …… Antimicrobial Resistance

ART ……. Antiretroviral Therapy

AWaRe……. Access Watch Reserve

CAP…….. Community Acquired Pneumonia

CI…….. Confidence Interval

COMREC……. College of Medicine Research and Ethics Committee

EML…….. Essential Medicines List

IPD…….Invasive Pneumococcal Disease

LMIC……. Low-Middle Income Countries

MSTGs…….. Malawi Standard Treatment Guidelines

MTB…….. Mycobacterium Tuberculosis

OR……..Odds Ratio

PCV…….Pneumococcal Conjugate Vaccine

SARS- CoV2……Severe Acute Respiratory Syndrome – Coronavirus 2

SOC…….Standard of Care

S. Pneumoniae…….. Streptococcus Pneumoniae

TB……Tuberculosis

WHO……World Health Organization

Definition of terms

Adult: Any person older than 18 years

Carriage: The presence of meningococcal bacteria in the upper respiratory tract without any signs or symptoms of infection.

Colonization: Presence of a microorganism in a host with growth and multiplication of the organism; however without interaction between the host and the organism (no clinical expression, no immune response).

Cough: a voluntary or involuntary act that clear the throat and breathing passage of foreign particles, microbes, irritants, fluids and mucus; it is a rapid expulsion of air from the lungs.

Drug resistance: it is a reduction in effectiveness of a drug in curing a disease or condition. The ability of microbes such as bacteria, viruses, parasites, or fungi to grow in the presence of a drug that would normally kill it or limit its growth.

Streptococcus Pneumoniae: A Gram-positive encapsulated commensal bacterium that colonize the human upper respiratory tract .

Tuberculosis (TB): Is an infectious disease caused by a bacteria, Mycobacterium tuberculosis that mostly affects the lung.

CHAPTER 1: Introduction

1.1. Chapter Outline

This chapter provides a general summary of *Streptococcus pneumoniae* in sub-Saharan Africa and Malawi. It consists of background to the study, problem statement, study justification, the research question, aim and the objectives of the study. The background section of this chapter outlines the overall view and history of the *Streptococcus pneumoniae*.

1.2. Background

Streptococcus pneumoniae (S. pneumoniae) is one of the bacteria that naturally colonises the upper airways in human beings. *S. pneumoniae* existing in the upper airways (carriage) is harmless but can sometimes evade the immune system to cause severe illness and death.[1] *S. pneumoniae* can cause a wide spectrum of diseases ranging from life threatening to less severe infections. Life threatening diseases caused by *S. pneumoniae* include pneumonia, meningitis and septic shock. Less severe infections include non-bacteraemia pneumonia, otitis media or sinusitis.[2] Children younger than 5 years, elderly and people living with HIV are the most vulnerable population to develop *S. pneumoniae*. Factors such as high-density living conditions and poverty also exacerbate the risk of developing pneumococcal pneumonia and transmission of the infections. Nose and throat are the main reservoir for *S. pneumoniae* as commensal in 20% to 40% of healthy children. *S. pneumoniae* is normally found in public places such as creches, playgrounds and preschools, in gatherings or where people spend a lot of time.[3]

World Health Organization (WHO) estimated that an approximation of more than 300,000 deaths among children younger than 5 years old across the world every year results from *S. pneumoniae*, and most of these deaths are reported from developing countries.[4] The reduction in deaths was noted after the introduction of Pneumococcal Conjugate Vaccine (PCV) in immunization programmes for children in 2000.[5] Younger children have an immature immune system and are more vulnerable to infections. In 2015, pneumococcal pneumonia accounted for 55.4% of respiratory deaths in all ages. If the strain of *S. pneumoniae* causing disease is also drug resistant, meaning that the strain of *S. pneumoniae* bacteria has developed mechanisms to evade or withstand the effects of antibiotics that would normally kill or inhibit its growth. This can complicate treatment. Reducing the prevalence of carriage of *S. pneumoniae* in the population can contribute to the reduction of incidence of severe infections. The carriage of *S. pneumoniae* depends on many factors such as age, sex, HIV status and availability and coverage of vaccination in the population.[1]

1.3. Problem statement

Several studies have investigated pneumococcal carriage among children, proving colonization is more prevalent in children than in adults. There is only limited information available on colonization among adults. Children are regarded as population susceptible to carriage and carriers of pneumococcal diseases. Study by Ameida et al reported that in most cases colonization in children exceed 50% at a given time.[6] Currently the use of PCV in immunization programme for infants has reduced the burden of pneumococcal diseases, however few studies indicated that incidence of carriage are still reported among adults living with children.[4,6] Furthermore, literature revealed that people infected with HIV regardless of age are at

higher risk of developing carriage.[1] Despite the availability of PCV, pneumococcus remain a leading cause of invasive bacterial diseases and the main cause of community acquired pneumonia among older adults.[7] Many African countries still battle with the burden of pneumococcal diseases even after introduction of PCV. Given the current gap in knowledge about the prevalence of carriage among adults, this study aimed to investigate risk factors for carriage of *S. pneumoniae* development in adults.

1.4. Justification

This study aimed to identify factors for carriage of drug resistant *S. pneumoniae* among adults with cough undergoing evaluation for Tuberculosis (TB) disease in Blantyre, Malawi. Patients presenting with cough resort to antibiotics use and put themselves at risk of developing resistant strains of *S. pneumoniae* that they naturally carry; however, little has been done to establish the factors associated with this carriage of resistance strains particularly following increased antibiotic use among patients with presumed tuberculosis disease. Findings of this study will assist in finding strategies to reduce emergence of drug resistant strains of *S. pneumoniae* and also lead to improvement in treating and monitoring of patients who may suffer invasive disease. In addition, policy makers will be informed in order to find interventions that address the gaps that are present in order to increase the health programmes effectiveness such as reduction in inappropriate use of antibiotics. Assessment of risk factors for carriage of *S. pneumoniae* can also help inform groups of patients to be prioritised for PCV among adults.

1.5. Research Question

What are the risk factors for carriage of drug resistant *S. pneumoniae* among adults with cough undergoing evaluation for TB disease in Blantyre, Malawi?

1.6. Aim

To investigate the factors associated with the development of carriage of drug-resistant *S. pneumoniae* among adults presenting with cough undergoing for evaluation for TB disease in two primary healthcare facilities, Ndirande and Limbe in Blantyre, Malawi in 2019.

1.7. Objectives

1. To describe characteristics of patients with cough attending primary care clinics for evaluation for TB disease in Blantyre, Malawi in 2019.

2. To describe the proportion of carriage of drug resistant *S. pneumoniae* in adults with cough undergoing evaluation for TB disease in Blantyre stratified by different characteristics.

3. To investigate risk factors for carriage of drug resistant *S. pneumoniae* among adults with cough undergoing evaluation for TB disease in Blantyre, Malawi.

CHAPTER 2: Literature review

2.1. Chapter Outline

This chapter reviews various studies conducted to determine the prevalence of *Streptococcus pneumoniae* in Africa including Malawi. It also reviews studies that have established the contributing factors related to *Streptococcus pneumoniae*, and the association between *Streptococcus pneumoniae* and other illnesses. The conceptual framework of the factors associated with the development of carriage of drug-resistant *Streptococcus pneumoniae* is also discussed.

2.2. Burden of pneumococcal disease in Africa

Pneumococcal disease is preventable despite it being a major cause of hospitalization and death among children in Africa. Evidence continues to accrue that carriage of *S. pneumoniae* is high in Africa. According to Zar et al, Africa reports 1 to 4 episodes of pneumonia resulting from *S. pneumoniae* per person every year.[8] In a systematic literature review, carriage was found to be high in children less than 5 years (63.2%), and decreased with increasing age as it was found to be 42.6% in children aged 5–15 years old and 28.0% in adults older than 15 years.[9] Heinsbroek et al. established that carriage of *S. pneumoniae* in Malawi is significant, particularly among HIV infected adults irrespective of the implementation of antiretroviral therapy.[10] Uganda introduced PCV in 2014, however in 2017-2018 pneumonia was reported as the fourth leading cause of mortality among children under 5 years of age across the country. The prevalence of pneumonia in west Uganda was 25.6%, 53.7% in central Uganda and 34% across East African countries post vaccination period.[5] South Africa amongst other countries in Africa has been reporting childhood mortality due to pneumonia for decades irrespective of improved access to health care and better economic growth.

In 2003, South Africa reported an increase of 1.6% mortality rate among children under 5 years increasing from 1.3% in 1999.[8]

2.3. Prevalence of pneumococcal carriage in Africa

The prevalence of pneumococcal carriage has been described as generally high in Africa especially in young children. This was due to the fact that many countries in Africa are economically deprived and have difficulty to introduce treatment strategies such as early detection and antimicrobial therapy for suspected pneumonia cases.[11] Additionally, death among children aged 0-59 months due to pneumococcal diseases still occur despite introduction of PCV in April 2011.[12] The systematic review conducted in Sub Saharan Africa reported that Mozambique, Ethiopia and The Gambia has a high prevalence of more than 85% of pneumococcal carriage in children less than 5 years old.[13] A cluster-controlled trial contacted in Malawi from 2015 to 2017 established that prevalence of carriage of *S. pneumoniae* was greater than 80% and the proportion of strain resistant to macrolides was 28%.[14]

Furthermore, people living with HIV are amongst groups considered to be vulnerable to develop pneumococcal diseases. Pneumococcal disease is an opportunistic disease that affects people living with weakened immune system than people with healthy immune system. The report by WHO African Region states that Sub Saharan African countries have a high prevalence of people living with HIV, accounting for 25.7 million people living with HIV on a global scale in 2018. Kalata et al pointed that several researchers had revealed that the prevalence of pneumococcal carriage of pneumonia among adults living with HIV and taking antiretroviral therapy (ART) in Africa is 40%-80%.[11]

2.4. Age, Sex and pneumococcal carriage

S. pneumoniae is a major cause of infectious diseases across the globe. It is imperative to target colonization to curb the spread of infections in the community. Carriage is common in children and decreases with age. Epidemiological studies have established that children and the aged population are more susceptible to infectious diseases because of their compromised immune system and inability to eliminate the pathogens before they become pathogenic.[15] The results of a cross sectional study conducted in Ethiopia revealed that 63.2% of pneumococcal carriage was found in children younger than 5 years, 42.6% in children aged 5-15 years and 28.0% in adults older than 15 years.[16] *S. pneumoniae* is a bacteria that targets individual with immature, weakened or deteriorating immune system.[15] There is only limited information available on colonization among adults. However, an estimated 1% -10% prevalence of colonization among adults was reported in developed countries despite effective implementation of PCV.[6] Additionally, in low and middle-income countries with limited resources such as adequately trained staff, diagnostic facilities and equipments, and effective respiratory management guidelines in primary facilities, many infections including carriage can be missed. Vaccination of PCV in infants was considered to be indirect protection for adults against vaccination serotype.[17] However, adults with low socioeconomic status, living with children under 5 years, living in overcrowded areas, smoking cigarette and using antibiotics are prone to the development of pneumococcal colonization.[18] Almeida et al confirmed that adult smokers aged between 25 and 50 years are at higher risk of developing pneumococcal carriage.[6] Smoking is among the culprits that compromises the immune system's ability to defend itself from organisms that causes sickness.

Sex is an important factor when determining clinical disease susceptibility and outcome. In general men are more susceptible to infectious diseases and unlike women, men carry a high burden of bacterial, fungal, parasitic and viral diseases.[19] Studies have established that the rate of pneumococcal carriage is higher in children and the transmitter of the *S. pneumoniae* within the population is children. However, there is no supported evidence to indicate systematic age-specific difference in asymptomatic pneumococcal nasopharyngeal carriage rates between boys and girls.[19] Dias et al pointed that, sex chromosome can influence disease severity, immune response to treatment and vaccination, and susceptibility to disease and outcome.[20]

S. pneumoniae is the frequent coloniser of the nasopharynx and most common bacterial pathogens in both sexes. Pneumococcal pneumonia and invasive pneumococcal diseases are seen to be most common in men than in females.[19] Difference in exposure to common infection, immune response against pathogens, hormonal factors, social and behavioural factors are the most contributing factors in both sexes.[21] Several studies show that women mount more robust innate and adaptive immune response to infections than men.[22]

2.5. Relationship between respiratory symptoms and carriage

The main function of the respiratory system is gaseous exchange whereby oxygen will be inhaled and carbon dioxide be exhaled. However, introduction of microorganisms during inhalation of polluted air can colonize the respiratory tract. Respiratory diseases are considered as the risk factor that predispose an individual to develop pneumococcal illness.

S. pneumoniae is the common gram-positive bacterium found in the respiratory tract. Several epidemiological studies established that *S. pneumoniae* colonize the upper respiratory tract,[23,24,25] and remain the leading cause of community acquired pneumonia as it has the ability to spread to the lower respiratory tract.[23] Additionally S. *pneumoniae* was reported as one of the common cause of bacterial coinfection that increases morbidity and mortality of influenza infection.[26] This was supported by the study by Troeger et al., 2018. The study revealed that in 2016 *S. pneumoniae* was noted as the primary cause of morbidity and mortality from lower respiratory infections resulting in 1, 189, 931 deaths across the globe.[27]

The relationship between pneumococcal diseases and viral respiratory infections was confirmed by numerous epidemiological studies. Study by Miellet et al suggest that disturbances by viruses in the upper respiratory tract and immune response to virus may disrupt the balanced host commensal relationship.[7]

Study conducted in Malawi indicated that pneumococcal nasopharyngeal carriage is highly associated with upper respiratory tract viral infections such as rhinovirus, influenza virus, parainfluenza virus and respiratory syncytial virus.[28] In children pneumococcal carriage is commonly influenced by the microbial composition of the upper respiratory tract including viral infections.[28] Another study conducted in California demonstrated a strong association between *S. pneumoniae* and severe acute respiratory syndrome coronavirus 2 (SARS-CoV2). The study revealed that the prevalence of SARS-CoV2 among pneumococcal carriers was higher with an estimation of 27.4% compared to non-carriers which accounted 9.2%.[29]

2.6. Environmental and household factors associated with pneumococcal carriage

Household air pollution is common in low income and middle-income areas as most people rely on wood fire, gas stove and other solid fuels for cooking and heating. There are 2.8 million people around the globe who use open fire, biomass and stove fuelled by coal or kerosene for cooking everyday.[30] Poor household energy practices result in a very high level of household air pollution. Other factors such as indoor cigarette smoking, dust, pets, overcrowding, use of pesticides and chemicals used for cleaning among other factors contribute to household air pollution.

Household air pollution is a major risk factor for acute lower respiratory infections amongst the population. It was estimated that household air pollution contributes to 3.2 million deaths and 21% of these deaths resulted from lower respiratory infections.[30] Furthermore, children exposed to household air pollution have higher risk of developing lower respiratory infection. Household air pollution is highly associated with the risk of developing respiratory infections mainly pneumonia and TB. WHO indicated that 44% of deaths in children less than 5 years old are due to pneumonia. The WHO report of 2014 has revealed that approximately 120 million children have pneumonia and 1.1 million succumb to pneumonia, 50% of these pneumonia deaths are attributed to household air pollution.[30] The study conducted on Malawian infants confirmed that the association between household air pollution and nasopharyngeal pneumococcal carriage exist. The results reported that the prevalence of *S. pneumoniae* was 86% in children aged 6 months old who are exposed to household air pollution.[31]

2.7. Antibiotic use and pneumococcal carriage

Antibiotics are commonly used by individuals suffering from various respiratory diseases including influenza and TB. However inappropriate use of antibiotics prompt the development of drug resistance.[32] Inappropriate use of antibiotics is commonly attributed by over prescription, taking inadequate dosage or taking antibiotics for nonbacterial infections. In case of influenza antibiotics use can be high in case of individuals suffering from repeated bouts of infections.[33] For TB people delay to seek care in health facility and they tend to self-medicate with antibiotics with the hope that the symptoms they are experiencing will resolve.[34] Even when they attend health facilities because of inefficiencies in diagnosis of TB disease, health workers also tend to prescribe more antibiotics to individuals undergoing evaluation for TB disease in what is termed clinically as “trial of antibiotics”.[34] People undergoing evaluation for TB disease are a group of interest for emergence of drug resistance bacterial strains such as *S. pneumoniae*.

2.8. Pneumococcal carriage and co-morbidities

Anyone can get pneumococcal disease, however; some people are at an increased risk. Living with a certain medical condition can increase a person’s risk for pneumococcal disease. Several studies have demonstrated that HIV infections exacerbate the risk of developing pneumococcal carriage irrespective of antiretroviral therapy (ART). Pneumonia is an opportunistic disease that affects people living with weakened immune system than people with healthy immune system. HIV compromises the immune system and exposes a host to the risk of multiple infections including pneumonia. Carriage occurs in the nasopharynx and it remains a major transmitter of pneumococcal diseases especially in people living with HIV.

Study by Thindwa et al pointed that the risk of invasive pneumococcal disease in HIV infected person is high compared to uninfected person because HIV affects the T and B cell function resulting in impaired response to control pneumococcal carriage at mucosal level.[35] Another study conducted in South Africa between 2016 and 2018 revealed that HIV infected people were more likely to be colonized and had higher risk of pneumococcal diseases than any other groups. More evidence was revealed by another study conducted in Mozambique looking at the nasopharyngeal carriage of *S. pneumoniae* among HIV infected and uninfected children. The study reported that prevalence of carriage of *S. pneumoniae* was found to be high (81.5%) among HIV infected children as compared to 79.1% in uninfected children.[36] *S. pneumoniae* can cause a range of illnesses depending on the part of the body that is infected, this may include lung diseases such as Community acquired pneumonia (CAP) and IPD.[37]

2.9. Pneumococcal vaccine in Africa

Pneumococcal vaccine (PCV 10 and PCV 13) was introduced in 2000 to target the serotypes resulting from severe pneumococcal diseases. In 2007, WHO recommended PCV in routine immunization programmes for children in developing countries.[5] The introduction of PCV played an important role in reducing the burden of pneumococcal infections caused by vaccine serotype and a reduction in resistant strains of pneumococcus globally. Most countries in Africa adopted the recommendation made by WHO to introduce PCV in their immunization programme for children. The study conducted in Morocco between 2015 and 2018 showed a significant reduction in pneumococcal diseases among children younger than 2 years, the incidence declined from 34.6 per 100,000 before the vaccination to 13.5 per 100,000 after the introduction of PCV.[2] Another study conducted in South Africa reported a significant reduction of hospitalization and deaths due to pneumococcal

diseases among children aged 0-59 months after the implementation of PCV programme in the country. In South Africa an approximation of 107,600 cases of pneumococcal diseases with an approximation of 5000 deaths were reported annually pre-introduction of PCV in 2009.[12] However, Uganda reported different results whereby the incidence of pneumococcal diseases among children younger than 2 years remained high at 40.6% post-vaccination rollout in 2014 slightly declining from 42.3% pre-vaccination period.[2]

PCV was regarded as the main intervention to curb the development of pneumococcus diseases. However, vaccine serotype carriage can still occur even after a person received PCV. Neal et al reported that, in Low-Middle Income Countries (LMIC) such as Malawi where PCV was introduced in 2011 and vaccine coverage still persist, the rate of vaccine serotype carriage among children aged 3-5 years remained high compared to high income countries.[9] This revelation opposes the evidence that PCV reduces the carriage prevalence in vaccinated populations, especially in LMIC where the burden and transmission of pneumococcal diseases is high.[9]

CHAPTER 3: Methodology

3.1. Chapter Outline

This chapter provides an outline of the methods and design of the study. It also details the primary study description and the secondary study description, the outcome variable's description and the selected exposure variable description. It provides details of the study design, the primary study population and study sample. The chapter further elaborates on how sample size and power calculations were done, the discussion of the data source, data management, data analysis plan, study limitations and ethical considerations.

3.2. Study setting

The study was conducted in two health centres (Ndirande Health Centre and Limbe Health Centre) in Blantyre, Malawi, a landlocked country in southeastern Africa. It is defined by its topography of highlands split by the Great Rift Valley and enormous Lake Malawi. Blantyre is Malawi's commercial city with a population of 800,264. Ndirande Health Centre and Limbe Health Centre are both government owned primary health care facilities overseen by Blantyre District Health Office.

3.3. Description of the primary study

The primary study was titled "Accuracy and consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB study)". This was a three-arm, individually randomised, open-label, controlled trial among adults (defined as 18 years and older) who presented with cough for at least 14 days at 2 primary health care centres in Blantyre, Malawi (Limbe and Ndirande Health Centres).[34] 1,583 participants were enrolled for the study. Out of 1,583 participants 139 (9%) had positive *S. pneumoniae* swab with resistance to the tested antibiotics. *S. pneumoniae* resistance was defined as resistance to any of the following commonly used antibiotics selected from all three WHO AWaRe classes in 2019: ceftriaxone, amoxicillin, cefoxitin, azithromycin, and erythromycin.[38] The study was reviewed and approved by the Kamuzu University of Health Sciences Research and Ethics Committee, London School of Hygiene & Tropical Medicine Research Ethics Committee, Regional Committee for Health and Research Ethics- Norway, and Malawi Pharmacy Medicines, and Poisons Board.

3.4. Conceptual framework

Figure 1, shows the conceptual framework that informed the selection and extraction of variables of interest from the primary study to answer the research question for this study. The conceptual framework outlines the possible exposure variables theoretically and empirically associated with the development of carriage of drug-resistant *S. pneumoniae*. The exposure variables include presenting symptoms, socio-demographic factors, previous TB, HIV status, and antibiotics given.

Asymptomatic carriage of *S. pneumoniae* is considered to be a significant source of transmission of pathogens and usually precedes invasive pneumococcal disease. *S. pneumoniae* and Mycobacterium tuberculosis (MTB) are the most common pathogens of respiratory tract infections particularly in people living with HIV and elderly due to their compromised immune system. The presenting symptoms for *S. pneumoniae* and MTB are similar since they both attack the lungs. The chest X-ray results for positive *S. pneumoniae* will indicate abnormal shadow due to the fibrinous oedema fluid and phagocytic cells in the air sacs of the lungs.[39] The rate of carriage in a given population perpetuates transmission of *S. pneumoniae*. Carriage is higher in children and decreases as the age increases. However, it was stated that 28.0% of adults older than 15 years are found to have developed carriage.[9,16] Pneumococcal disease is common in people living with HIV due to the alteration of defence mechanisms in the respiratory tract that increases the risk for pulmonary infections.

Smoking is associated with an increased risk of respiratory infections and it was proven by several studies that there is a strong link between pneumococcal disease and viral respiratory infections. The use of antibiotics prior to proper diagnoses among individual suffering from respiratory diseases such as influenza and TB is common.[9] While antibiotics can treat the symptoms of the disease, suboptimal use, prolonged

use of antibiotics and use of broad-spectrum antibiotics can lead to high level of antimicrobial resistance (AMR).

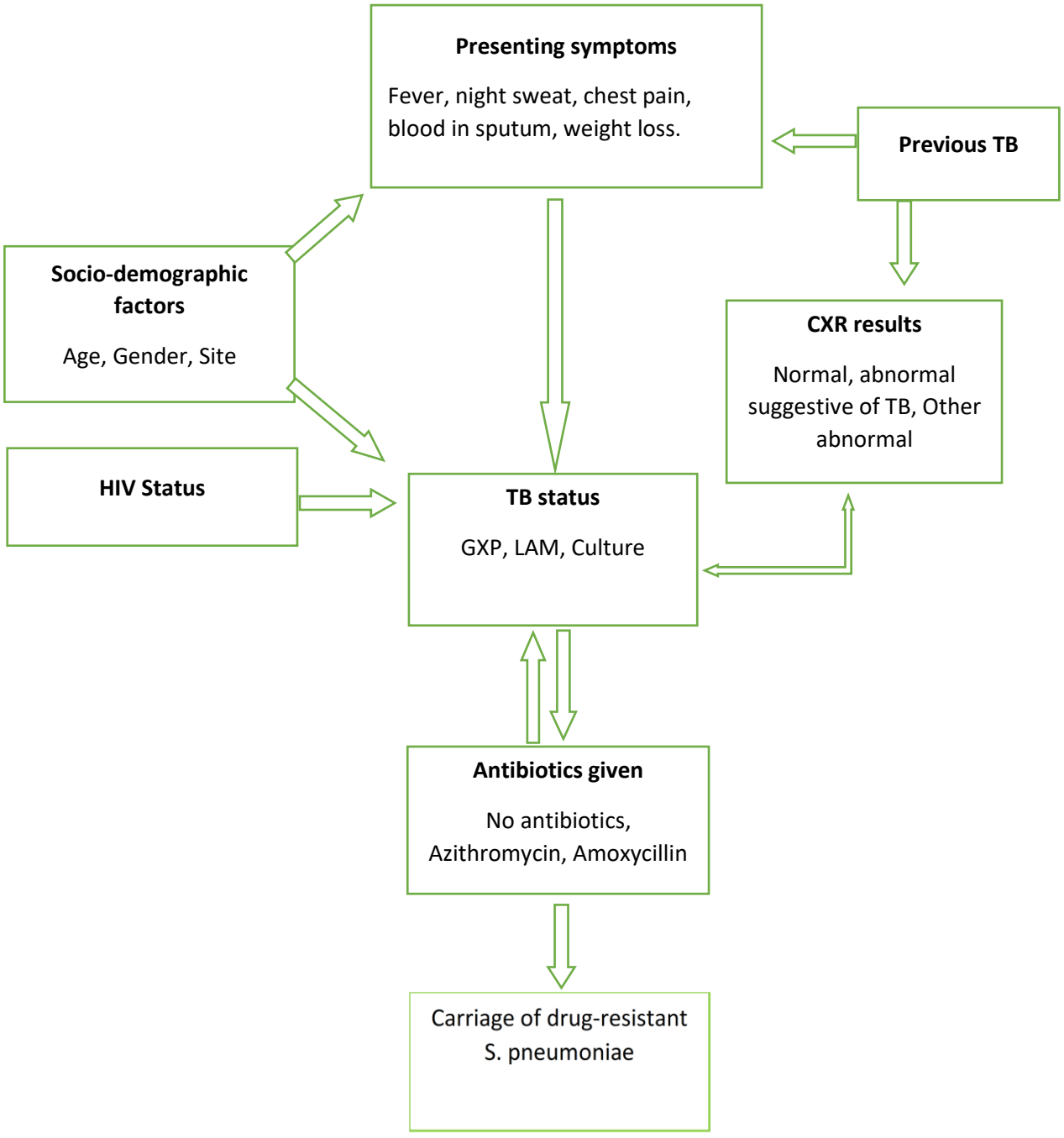

Figure 1: Conceptual framework for risk factors of carriage of drug resistant S. pneumoniae

3.5. Description of the secondary study

This study addressed the research question as a secondary data analysis using the data obtained from the primary study, a randomised, open-label, control trial design.

3.5.1. Study population

The study population were all individuals aged ≥18 years with cough of more than 14 days and attended primary care clinics in Blantyre, Malawi. According to primary study 1,583 participants were identified from Limbe Health Centre and Ndirande Health Centre for eligibility. All participants aged ≥ 18 years with cough of more than 14 days were enrolled in the study.

3.6. Definition of variables

3.6.1. Study outcomes

The main outcome in the primary study was swab culture results if it was either negative or positive for *S. pneumoniae* and assessed for resistance. Nasopharyngeal aspirate swab was collected at day 1 and 29 from the participants who reported being unwell and coughing for 14 days without immediate indication for hospitalisation. In the context of this study, the outcome of interest was a drug resistant *S. pneumoniae* carriage at baseline (day 1).

3.6.2. Study exposure

There were several exposures being assessed in this study and these included: TB status (confirmed or not), previous history of TB, antibiotic use and assignment (Azithromycin, Amoxicillin, and no antibiotics); Other exposure variables were age, gender, presenting symptoms, previous TB, body mass index (BMI), HIV status, symptoms improvement at day 8 (aided by audio computer-assisted self-interview), vital signs, CXR results and TB treatment.

3.7. Sample size and power calculations

The outcome of interest was carriage of drug resistant *S. pneumoniae* at day 1. For sample size calculation, the main exposure variable of interest was a TB status i.e., TB negative or TB positive (consistent x-ray findings, current diagnosis plus past positive history). The primary study had 1,583 participants and we estimated that 15% of participants would have a positive TB status.[40] We assumed that 15% of TB positive participants would have resistant *S. pneumoniae* carriage compared to 7.5% of TB negative participants. Using the formula **sampsi 0.15 0.075, alpha (0.05) n1(238) n2(1345)** to detect the difference in outcomes between TB positive and TB negative patients, 91% power was found.

3.8. Data management

After obtaining permission to use the dataset, a new dataset was created using the variables appropriate to address the research question for ease analysis. The dataset was password protected and stored safely in google drive to avoid access by unauthorised personnel. Patient demographic data including age, sex, TB and HIV test results matched against each patient and sample identification number, no patient names were used. STATA version 18 was used for data management and analysis. Data screening was performed to check for the following: missing data, duplicates, outliers, unique identifiers and errors. Any identified data errors were handled accordingly. Missing data was handled according to extent and nature of occurrence. Where data were missing completely at random, a complete case analysis was used; where data were missing at random, a multiple imputation was used to predict the missing values and where data were missing not at random, a sensitivity analyses was used to measure bias. The risk factors for carriage of drug resistant *S. pneumoniae* were recorded into categories *(see Table 1 in Appendix).*

3.9. Data analysis

Statistical analysis

For statistical analysis, to describe participant characteristics and the distribution of outcomes by characteristics [Objective 1 and 2] we used proportions and chi-square test comparisons for categorical variables *(See Table 1 and 2 in Appendix*), and we stratified by site, i.e. Ndirande Health Centre and Limbe Health Centre. For numerical variables we used means/medians and standard deviation/interquartile range for summarization and t-tests and Wilcoxon rank-sum test for comparison.

For objective 3 we used univariable and multivariable binary logistic regression to estimate odds ratios and 95% confidence intervals (CI) of the association of each variable and the outcome of interest *(See Table 3 in Appendix).* Firstly we fitted a univariable logistic regression model to estimate the association between outcome variable and each selected exposure variable *(See Table 2 in Appendix).* The outcome variable was regressed on each exposure variable separately to estimate unadjusted odds ratio, p-value and corresponding 95% CI.

All exposure variables with a p-value of < 0.05 in the univariable logistic regression model were selected as candidates for inclusion in the multivariable logistic regression model to assess their main effects.

Stepwise elimination was used to eliminate those variables which are not statistically significant i.e. p- values > 0.05 from our multivariable logistic regression model.

The final model fit to evaluate how well the predicted probabilities from multivariable logistic regression model match the observed outcome was assessed using Hosmer-Lemeshow goodness-of-fit test. The final model's output i.e. the adjusted odds ratios, p-values and 95% CI were reported.

3.10. Ethical considerations

An application for ethical clearance was made to the Human Research Ethics Committee (Medical) of the University of the Witwatersrand and the approval was obtained. Appendix A is an ethics clearance certificate for the secondary study **M231112**. Furthermore, an authorization to use data from the gatekeeper of the primary study **"Accuracy and consequences of using Trial-of-antibiotics for tuberculosis diagnosis**" (ACT-TB study: P.04/18/2381) was obtained and utilized with strict confidentiality for only academic purposes. Appendix B is a proof of authorization by the primary study gatekeeper. The primary study was approved by the College of Medicine Research and Ethics Committee (COMREC) in Malawi. Appendix C is an ethics clearance certificate for the primary study.

CHAPTER 4: Results

4.1. Chapter Outline

This chapter presents the results of the analysis conducted in relation to the study objectives. The results are presented in tables and the interpretation of the tables have been discussed. The results in the tables are presented in proportion or percentage, odds ratios (crude odds ratios and adjusted odds ratios), 95% confidence intervals and p-values. Figures were used to present some of the results.

4.2. Study flowchart

1583 individuals were recruited to participate in the study. Out of 1583 in the dataset, 0.4% (7/1583) had no data on sex and were excluded from the analysis. 99.5% (1576/1583) had data and were used for analysis. The first analysis conducted was to determine whether *S. pneumoniae* was isolated or not in a participant. 99.5%

(1576/1583) were used for analysis in this regard and 82.6% (1303/1576) of patient samples had no *S. pneumoniae* isolated while 17.3% (273/1576) had *S. pneumoniae* isolated. In participants who had *S. pneumoniae* isolated, 49% (134/273) did not have drug resistant *S. pneumoniae* and 49.8% (139/273) had drug resistant *S. pneumoniae*. Figure 2, below is a flow chart showing the number of individuals who developed drug resistant *S. pneumoniae.*

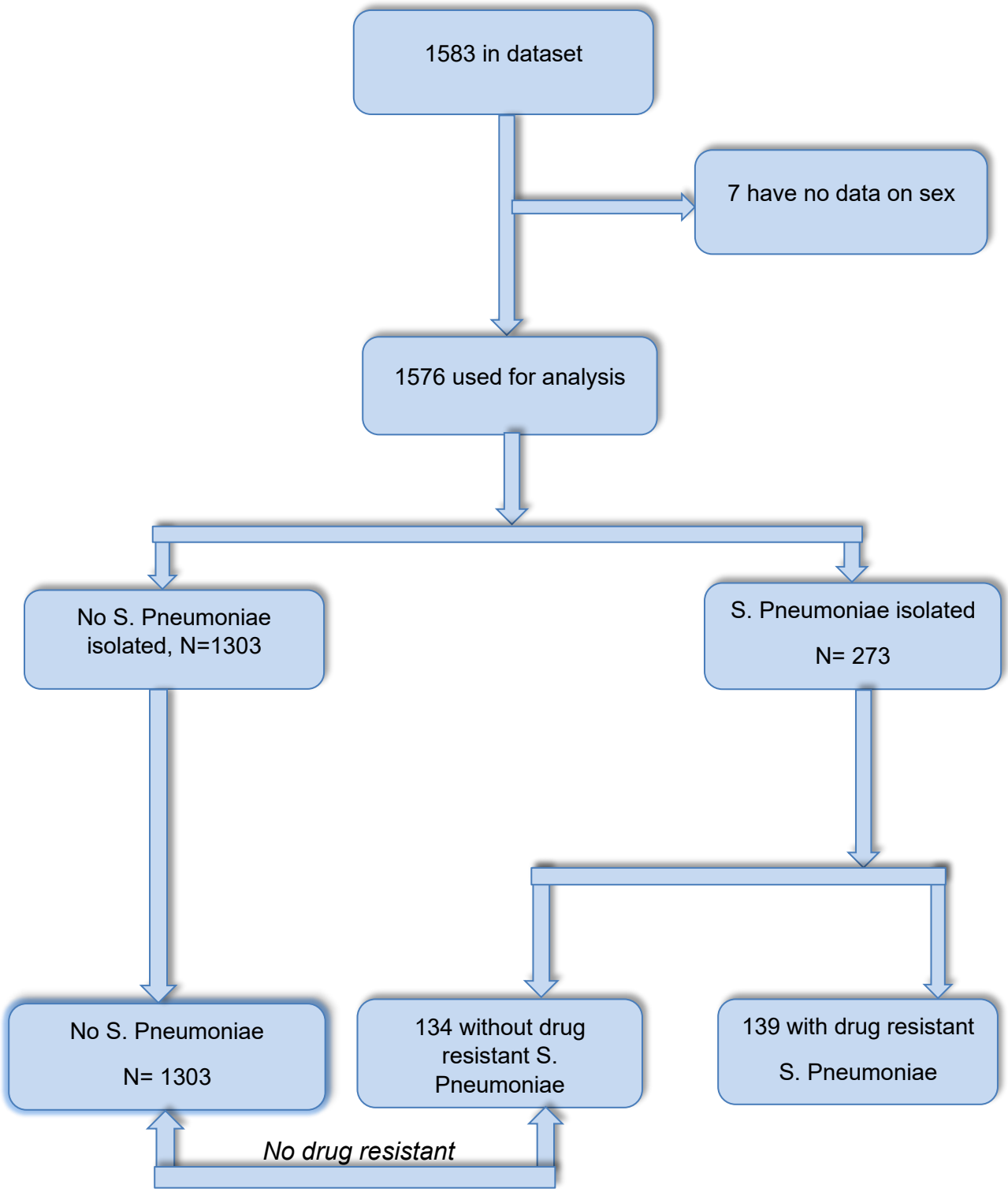

Figure 2: Flowchart showing the number of individuals who had drug resistance S. pneumoniae

4.3. Description of study characteristics

Table 3.1 below shows the baseline characteristics of the study participants and the proportions of either resistant or non-resistant to *S. pneumoniae*. The current study included 1,576 participants of which 941 were women and 635 were men. The median age was 32 (IQR: 24;44).

For the distribution of site, most participants were found in Ndirande 68% (1,073/1,576) and out of 1,073, 65% were resistant to *S. pneumoniae*. Limbe had fewer participants 32% (503/1,576) and only 35% were confirmed to have resistant *S. pneumoniae.* Table 3.1 also presents the distribution in education level whereby secondary is leading with higher number of cases 48% (757/1,576) as compared to other three levels. However, the level of resistance for secondary education was slightly lower 46% than in primary education (44% vs 47%).

Among the presenting symptoms, a lot of people 78% (1,222/1,576) were presenting with sputum, 73% (1,149/1,576) had chest pains and only a few 6.2% (76/1,576) had blood in their sputum.

Table 1: Baseline characteristics of individuals in the study

Characteristics	Overall N= 1,576[1]	Non-resistant, N= 1,437[1]	Resistant, N = 139[1]	P-Value[2]
Sex				0.205
Female	941 (60%)	865 (60%)	76 (55%)	
Male	635 (40%)	572 (40%)	63 (45%)	
Age	32 (24,44)	32 (24,44)	32 (26,44)	0.510
Arm				0.520
Amoxicillin	523 (33%)	471 (33%)	52 (37%)	
Azithromycin	524 (33%)	482 (34%)	42 (30%)	
SOC	529 (34%)	484 (34%)	45 (32%)	
Site				0.377
Limbe	503 (32%)	454 (32%)	49 (35%)	
Ndirande	1,073 (68%)	983 (68%)	90 (65%)	
BMI category				0.010
Not underweight	1,424 (90.4%)	1,307 (91%)	117 (84%)	
Underweight	152 (9.6%)	130 (9.0%)	22 (16%)	
Education				0.283
None	67 (4.3%)	60 (4.2%)	7 (5.0%)	
Primary	669 (42%)	604 (42%)	65 (47%)	
Secondary	757 (48%)	693 (48%)	64 (46%)	
Tertiary	83 (5.3%)	80 (5.6%)	3 (2.2%)	
Previous TB	99 (6.3%)	83 (5.8%)	16 (12%)	0.008
Fever	988 (63%)	895 (62%)	93 (67%)	0.282
Sweat	711 (45%)	637 (44%)	74 (53%)	0.044
Chestp	1,149 (73%)	1,045 (73%)	104(75%)	0.595
Sputum	1,222 (78%)	1,104 (77%)	118(85%)	0.030
Blood in sputum	76 (6.2%)	65 (5.9%)	11(9.3%)	0.142
Unknown previous TB	354	333	21	
Weight loss	554 (35%)	503 (35%)	51(37%)	0.691
HIV status				0.060
Negative	1,309 (83%)	1,203 (84%)	106(76%)	
Unknown	46 (2.9%)	42 (2.9%)	4 (2.9%)	
Positive	221 (14%)	192 (13%)	29 (21%)	
PTB status				0.813
Confirmed PTB	98 (6.2%)	90 (6.3%)	8 (5.8%)	
No PTB confirmed	1,478 (94%)	1,347 (94%)	131(94%)	

Abbreviations: SOC= Standard of care, BMI=Body mass index

[1] n (%); Median (IQR)

2. Pearson's Chi-squared test; Wilcoxon rank sum; Fisher's exact test

4.4. Drug resistance outcome

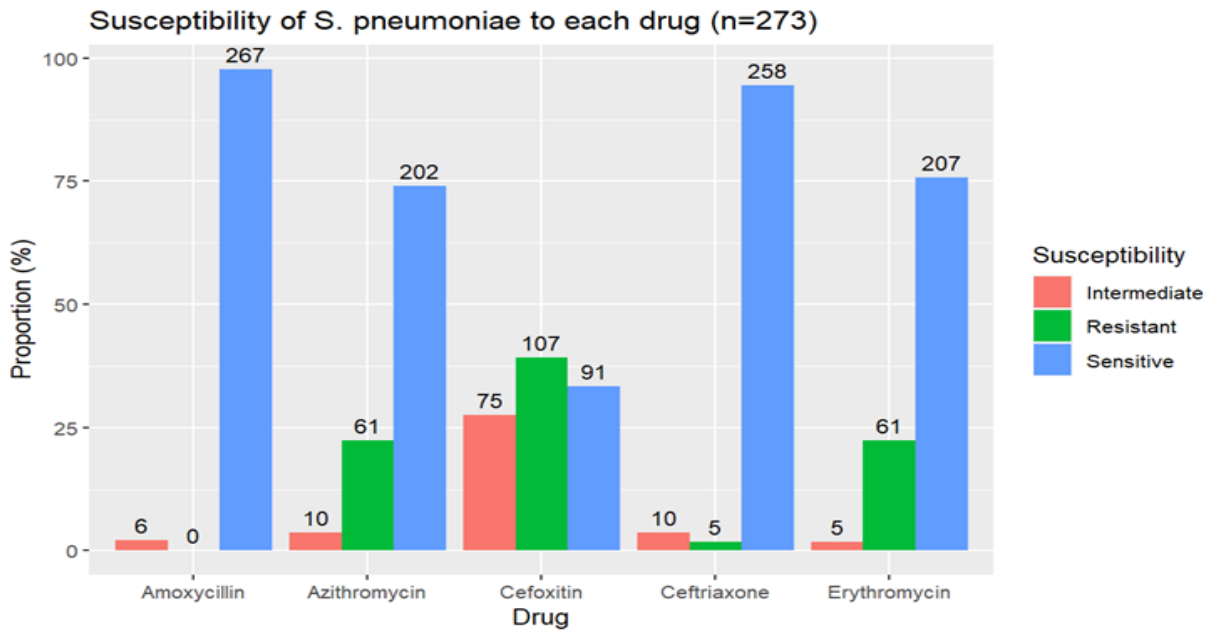

Figure 3: susceptibility of S. pneumoniae to individual drug

The susceptibility of *S. pneumoniae* to Amoxycillin, Azithromycin, Cefoxitin, Ceftriaxone and Erythromycin was presented in the bar graph above. A total of 273 individuals were tested for each drug respectively. The results showed that the drug with a high number of resistance was Cefoxitin 39.1% (107/273). Azithromycin and Erythromycin indicated an equal amount of resistance 22.3% (61/273). Ceftriaxone had a lowest 1.8% (5/273) resistance while Amoxicillin indicated no resistance. Overall, the bar graph provided a clear visual representation of the susceptibility patterns of *S. pneumoniae* to the tested drugs.

4.5. Overview of the overlap of drug resistance on other drugs

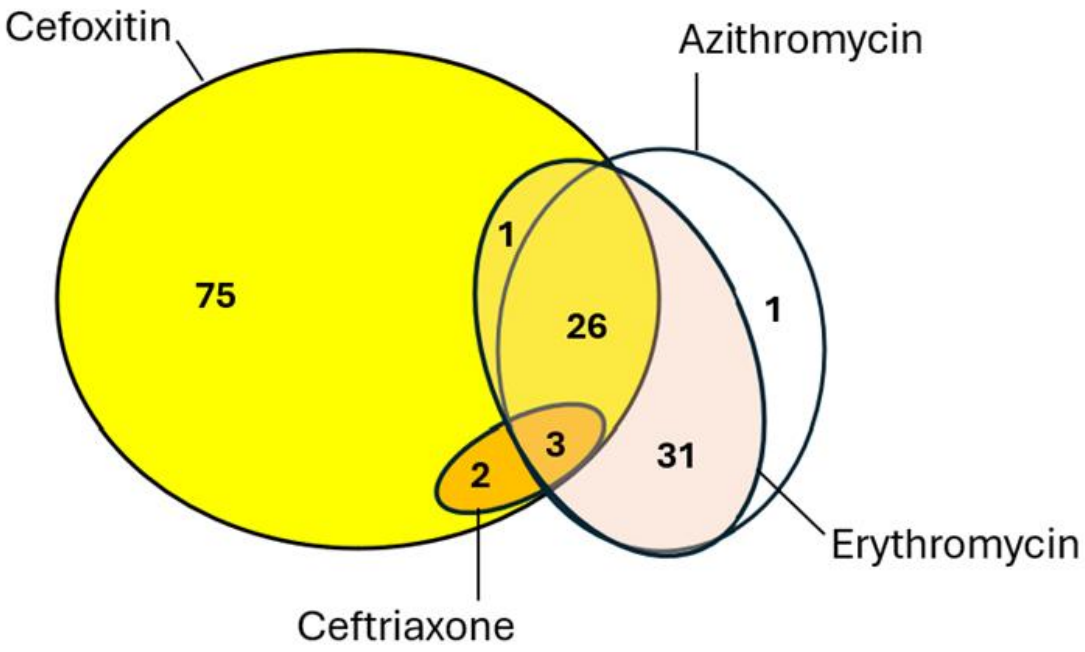

Figure 4: Overlap of drug resistance

The overlap of drug resistance on other drugs was presented in the diagram above. The diagram represents all individuals (139) who showed resistant *S. pneumoniae.* Out of 139, only 2% (3 cases) were resistant to all tested drugs. Those who were resistant to Cefoxitin alone were 53% (75/139), and 1% (2/139 cases) was resistant to both Cefoxitin and Ceftriaxone, only 0.7% (1/139 case) was resistant to both Cefoxitin and Erythromycin, those who were resistant to Cefoxitin, Erythromycin and Azithromycin were 19% (26/139), 22% (31/139 cases) were resistant to Erythromycin alone and 0.7% (1/139 case) was resistant to Azithromycin alone.

4.6. Univariate regression of the association between each variable and the development of carriage of drug-resistant *S. Pneumoniae*

Table 2, shows the univariate analysis of the association between each variable and development of carriage of drug resistant *S. pneumoniae.* Several factors were tested

and the following variables were significantly associated with the development of carriage of drug resistant *S. pneumoniae*: BMI category (being either underweight or not underweight), previous TB, sweat, sputum and positive HIV status.

For BMI category, the univariate analysis suggested that the underweight individuals had 89% higher likelihood of developing drug resistant *S. pneumoniae* as compared to those who were not underweight, i.e. OR 1.89 (95%CI: 1.13 to 3.03, p= 0.011). The 95% CI:1.13 to 3.03 reinforced the significance of the association, and the p-value (0.011) further supported that this association was unlikely due to chance.

For previous TB, the odds of developing carriage of drug resistant *S. pneumoniae* was 112% higher among individuals with a history of previous TB as compared to those without a history of previous TB, i.e. OR 2.12 (95% CI: 1.17 – 3.65, p = 0.009). This association was statistically significant as it was supported by the 95% CI (1.17 to 3.65) and the p-value (0.009).

For sweat, individuals who sweat had significantly 43% higher odds of developing carriage of drug resistant *S. pneumoniae* compared to those who did not sweat, i.e. OR 1.43, (95% CI: 1.01 – 2.03, p = 0.045). The 95% CI (1.01 to 2.03) and the p-value (0.045) confirmed that the association was statistically significant.

For sputum, the odds of developing carriage of drug resistant *S. pneumoniae* among individuals with sputum production was 69% compared to those without sputum production i.e. OR 1.69, (95% CI 1.07 to 2.81, p-value = 0.031).

For positive HIV status, there was 71% probability of developing carriage of drug resistant *S. pneumoniae* among participants with HIV positive status, i.e. OR 1.71, (95% CI:1.09 to 2.62, p-value=0.016) which indicated that HIV positive individuals had

significantly higher odds of developing *S. pneumoniae* compared to those who were HIV negative.

For sex, males had 25% higher odds of developing carriage of drug resistant *S. pneumoniae* compared to females i.e. OR 1.25 (95% CI:0.88-1.78). However this difference was not statistically significant (p = 0.206).

For age, for each year increase in age the likelihood of developing carriage of drug resistant *S. pneumoniae* decreases by 1%, i.e. OR 1.00 (95% CI:0.99 to 1.01, p = 0.524). With a non-significant p-value (0.524) and a 95% CI:0.99 to 1.01, there was no significant association observed between age and the odds of developing carriage of drug resistant *S. pneumoniae*.

For treatment arms, individuals who were in Azithromycin treatment arm had 21% reduction in the odds of developing carriage of drug resistant *S. pneumoniae* compared to individuals who were in Amoxicillin treatment arm. There was no strong evidence to suggest a significant effect of Azithromycin treatment arm on the development of carriage of drug resistant *S. pneumoniae* in the studied population. This was evident by the non-significant p-value of 0.276 and the wide confidence interval i.e. OR 0.79, (95% CI: 0.51- 1.21, p value= 0.276). Furthermore; individuals who were in standard of care (SOC) treatment arm had 16% reduction in the odds of developing carriage of drug resistant *S. pneumoniae* compared to individuals who were in Amoxicillin treatment arm, OR 0.84, (95% CI: 0.55 – 1.28, p-value = 0.421).

For site, the univariable analysis indicated that the odds of developing carriage of drug resistant *S. pneumoniae* among individual who lived in Ndirande was 15% lower as compared to individuals who lived in Limbe, i.e. OR 0.85, (95% CI: 0.59 - 1.23, p-value = 0.377). Therefore, there was no statistically significant difference between the

odds of developing carriage of drug resistant *S. pneumoniae* among individuals who lived in Ndirande and individuals who lived in Limbe.

For education level: The univariable analysis depicted that individuals with primary education level had 90.8% lower odds of developing carriage of drug resistant *S. pneumoniae* as compared to those without formal education i.e. OR 0.092, (95% CI:043 - 2.29, p-value = 0.848).

For fever, the odds of developing carriage of drug resistant *S. pneumoniae* among individuals with fever was 22% higher than the odds of developing carriage of drug resistant *S. pneumoniae* among individuals without fever i.e. OR 1.22, (95% CI 0.85-1.78, p value= 0.282). However, the p-value suggested that this likelihood was not statistically significant since.

For chest pain, the univariable analysis indicated that the was no statistically significant association between the presence of chest pain in an individual and the development of carriage of drug resistant *S. pneumoniae*. The OR 1.11, (95% CI: 0.75 – 1.68, p-value = 0.595) indicated non- significant increase in the odds of developing carriage of drug resistant *S. pneumoniae* among individuals with chest pain as compared to those without chest pain.

For blood in sputum, the odds of developing carriage of drug resistant *S. pneumoniae* among individuals with blood in their sputum was 81% higher as compared to individuals without blood in their sputum, i.e. OR 1.81, (95% CI: 0.89 – 3.39, p-vale = 0.079). However, this was not supported by the wide 95%CI and the p-value which concluded that the association was not statistically significant.

For weight loss, the odds of developing carriage of drug resistant *S. pneumoniae* among individuals with weight loss was 8% higher odds compared to individuals

without weight loss, i.e. OR 1.08, (95% CI: 0.75 – 1.54, p-value = 0.691). However this difference was not supported by the 95%CI and the p-value which concluded that the association was not statistically significant.

For TB microbiology, the odds of developing carriage of drug resistant *S. pneumoniae* among individuals with confirmed TB was 9% higher odds compared to individuals without confirmed TB i.e. OR 1.09, (95% CI: 0.55 – 2.49, p-value = 0.813). However the univariate analysis suggested that the observed association was not statistically significant 95% CI (0.55 to 2.49) and p-value (0.813).

Table 2 : Univariate logistic regression of the association between each variable and the development of carriage of *S pneumoniae*.

Characteristics	N	OR	95% CI	p-value
Sex	1,576			
Female		1	-	
Male		1.25	0.88, 1.78	0.206
Age	1,576	1.00	0.99, 1.01	0.524
Arm	1,576			
Amoxicillin		1	-	
Azithromycin		0.79	0.51, 1.21	0.276
SOC		0.84	0.55, 1.28	0.421
Site	1,576			
Limbe		1	-	
Ndirande		0.85	0.59, 1.23	0.377
BMI category	1,576			
Not underweight		1	-	
underweight	1,576	1.89	1.13, 3.03	0.011*
Education	1,576			
None		1	-	
Primary		0.092	0.43, 2.29	0.848
Secondary		0.79	0.37, 1.97	0.578
Tertiary		0.32	0.07, 1.21	0.110
Previous TB	1,576			
No		1	-	
Yes		2.12	1.17, 3.65	0.009*
Fever	1,576			
No		1	-	
Yes		1.22	0.85, 1.78	0.282
Sweat	1,576			
No		1	-	

Yes		1.43	1.01, 2.03	0.045*
Chest pain	1,576			
No		1	-	
Yes		1.11	0.75, 1.68	0.595
Sputum	1,576			
No		1	-	
Yes		1.69	1.07, 2.81	0.031*
Blood sputum	1,576			
No		1	-	
Yes		1.81	0.89, 3.39	0.079
Weight loss	1,576			
No		1	-	
Yes		1.08	0.75, 1.54	0.691
HIV status	1,576			
No		1	-	
Unknown		1.08	0.31, 2.74	0.884
Yes		1.71	1.09, 2.62	0.016*
TB microbiology	1,576			
Confirmed		1	-	
No TB confirmed		1.09	0.55, 2.49	0.813

*OR = Odds Ratio, CI = Confidence Interval, * Statistically significant p-value < 0.05, 1= reference category*

4.7. Multivariable logistic regression analysis of the association between each variable and the development of carriage of drug resistant *S. Pneumoniae.*

Table 3 below shows the results of multivariable analysis of factors which were statistically significant to develop carriage of drug resistant *S. pneumoniae*. Stepwise elimination was used to eliminate those variables which are not statistically significant i.e. p- values > 0.05 from our multivariable logistic regression model.

The multivariable logistic regression analysis indicated that previous TB was significantly associated with higher odds of developing carriage of drug resistant *S. pneumoniae* as compared to other factors, adjusted OR 1.86. Although 95%CI: 0.99 to 3.31 is on the borderline of statistical significance, the p-value 0.044 suggest that the observed association is statistically significant. Individuals who reported positive HIV status adjusted OR 1.51, 95%CI: 0.94 to 2.37, p-value 0.079 and individuals with

presence of sputum adjusted OR 1.51, 95%CI: 0.94 to 2.52, p-value 0.101 were associated with equal odds of developing carriage of drug resistant *S. pneumoniae.* However, these associations were not statistically significant as the p-values were >0.05. Additionally, Sex, age, BMI category (whether the participant was underweight or not underweight) and sweat (whether the participant had a presence of sweat or not) were not associated with the development of carriage of drug resistant *S. pneumoniae.* In conclusion, previous TB was the only factor associated with the development of carriage of drug resistant *S. pneumoniae.*

Table 3: Multivariable logistic regression of the association between each variable and the development of carriage of drug resistant *S. pneumoniae*.

Characteristics	**AOR**	**95% CI**	**p-value**
Sex			
Female	1	-	
Male	1.18	0.81, 1.71	0.373
age	1.00	0.99, 1.01	0.752
BMI category			
Not underweight	1	-	
Underweight	1.57	0.90, 2.62	0.094
Previous TB			
No	1	-	
Yes	1.86	0.99, 3.31	0.044*
Sweat			
No	1	-	
Yes	1.31	0.92, 1.89	0.139
Sputum			
No	1	-	
Yes	1.51	0.94, 2.52	0.101
HIV status			
No	1	-	
Yes	1.51	0.94, 2.37	0.079

*AOR = Adjusted Odds Ratio, CI = Confidence Interval, *Statistically significant p-value < 0.05*

CHAPTER 5: Discussion

5.1. Summary of main findings

This study investigated the factors associated with carriage of drug resistant *S. pneumoniae* among adults with cough undergoing evaluation for TB disease at Ndirande and Limbe health care centres in Blantyre, Malawi. A majority of studies on carriage have been done on children. This study aimed to investigate carriage development in adults as a results of self-medicating with antibiotics when coughing. The main finding of this study is that patients who had TB before are significantly associated with the development of carriage of drug resistant *S. pneumoniae,* adjusted OR 1.86, 95% CI: 0.99 - 3.31, p-value 0.044. This study finding was similar to the results of the study reported from Dilla University Referral Hospital, Dilla in Ethiopia. The study reported that the prevalence of *S. pneumoniae* in presumptive tuberculosis adults cases was 21.4%, 95% CI: 17.7 - 25.5.[41] Although our findings are lower compared to the results of this study, we assume that the reason could be due to geographical distribution, sample size and other socio- economic factors.

Several epidemiological studies had proven that most people suffering from various respiratory sicknesses such as cough resort to self-medicate with antibiotics before seeking help or get a proper diagnosis.[32,34] Chikovore et al, revealed that most people affected by TB delay to seek care in health facility and they tend to self-medicate with antibiotics with the hope that the symptoms they are experiencing will resolve.[42] This has caused a significant challenge of antimicrobial-resistant on public health globally. In Malawi, the ministry of health has emphasized the need to optimize antimicrobial use through stewardship approaches while preserving this crucial group of medicines.[43]. This approach was implemented to avoid antimicrobacterial resistance

and good health outcome among Malawian population. To ensure standardised prescribing patterns and use of antimicrobacterial drugs, the Malawi Standard Treatment Guidelines (MSTGs) has incorporated the Access Watch and Reserve (AWaRe) categorization of antibiotics into their Essential Medicines List (EML).[43]

5.2. Associated factors of development of carriage of drug resistant *S. pneumoniae*

In the univariable analysis of the association between each factor and the development of carriage of *S pneumoniae*. The following factors: BMI category (underweight/overweight), sweat, sputum and HIV status had a p-value <0.05. These factors were found potentially associated with development of carriage of drug-resistant *S. pneumoniae* among adults with cough. All these factors became candidates for multivariable analysis. Underweight leads to impaired immune function, thereby elevates the vulnerability of an individual especially older adults to the risk of TB reactivation.[41] In this study, the probability of developing carriage of drug-resistant *S. pneumoniae* was proven to be possible among individuals with previous TB. Our findings are consistent with the study by Halala et al., which proved that individuals with a history of TB are more likely to be colonized with drug-resistant strains due to various factors including increased exposure to antibiotics, immunosuppression and healthcare exposure.[41] Therefore underweight in adults is considered as the risk factor for developing carriage of S. pneumoniae within these age group.

Furthermore, the univariable analysis of this study proved that HIV infected adults are highly associated with the development of carriage of drug resistant *S. pneumoniae* risk of pneumococcal colonisation, OR 1.71, 95% CI: 1.07; 2.62, p-value = 0.016. This study finding was similar to the results of the study published from South Africa in

2016. The study results indicated that the incidence of invasive pneumococcal disease in HIV infected population was 43 times higher than in HIV uninfected population (52 per 100 000 vs 1.2 per 100 000), peaking at 237 per 100 000 in the HIV infected elderly group.[44] Additionally, most HIV infected individual presented with bacteraemia (74% [3091/4 190]) and S. pneumoniae is the most frequent cause of bacteraemia pneumonia in individuals infected with HIV.[44] TB is an opportunistic disease amongst people living with HIV. The chance of TB reinfection amongst HIV infected group is very high. TB was proven to be the highest risk factor to develop carriage of drug resistant *S. pneumoniae.*

5.3. Limitations

This study was a secondary analysis, therefore some important variables such as environmental exposures (indoor pollution), viral or bacterial co-infections and number of immunised under-five children in the household were not available. These variables were not evaluated in the current study.

CHAPTER 6: Conclusions and Recommendations

6.1. Conclusions

In conclusion this study showed that development of carriage of drug resistant *S. pneumoniae* in adults with a history of previous TB is frequent. This suggests that adults who had TB before may act as a reservoir of carriage of drug resistant *S. Pneumoniae*. Taking into considerations their body weight, HIV statuses, production of sputum during cough and sweating. TB and *S. pneumoniae* are opportunistic pathogens which causes lower respiratory tract infections in an individual with weakened immune system.

6.2. Recommendations

Early diagnose of TB is essential step in the prevention process of drug-resistant *S. pneumoniae.* Clinicians must utilize practical strategies for history taking and physical examination to identify risk factors and exposures presenting with unresolved cough. Selection of appropriate diagnostic tests is very crucial, considering their sensitivity and specificity to enhance diagnostic accuracy. Health care workers must implement evidence-based antimicrobial management and guide effective therapeutic interventions during management of patients with suspected TB. Furthermore, healthcare workers must enforce health education for people at risk of developing TB. People must be encouraged to seek help when experiencing unresolved cough, avoid using over the counter antibiotics and resort to health care facilities for proper diagnose. Awareness on the use of antibiotics and prompt initiation of proper treatment will halt the development of drug resistance in infected person. There is a need to continue to monitor the sensitivity pattern of *S. pneumoniae* isolates to the available antibiotics so as to ensure that there are little or no cases of drug resistance. There's a need to develop a guidelines to assist clinicians in their drug prescription as well. These findings are important when designing strategies to prevent pneumococcal diseases in adults.

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Appendices

Appendix A: Ethical clearance Certificate of the study

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Nawane Sekgala

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M231112 M240205-A-0001

NAME: (Principal Investigator)	Miss Nawane Sekgala
DEPARTMENT:	Wits School of Public Health
DEGREE:	MSc Epidemiology - Epidemiology and Biostatistics
PROJECT TITLE:	Risk factors for carriage of drug-resistant Streptococcus Pneumoniae among adults with cough undergoing evaluation for Tuberculosis disease in Blantyre, Malawi
DATE CONSIDERED:	24-Nov-2023
DECISION:	Approved unconditionally
CONDITIONS:	N/A
NOTE:	Application Number: MED23-09-304
SUPERVISOR:	Dr Marriott Nliwasa and Prof Kennedy Otwombe

APPROVED BY: Mrs M. Van Niekerk, Co-Chairperson, HREC (Medical)

DATE OF APPROVAL: 05-Feb-2024 **EXPIRY DATE:** 04-Feb-2029

This clearance certificate is valid for 5 years from data of approval. Extension may be applied for.

DECLARATION OF INVESTIGATOR(S)

I/we fully understand the conditions under which I am/we are authorized to carry out the abovementioned research and I/we undertake to ensure compliance with these conditions. Should any departure be contemplated, from the research protocol as approved, I/we undertake to resubmit the application to the Committee. **I agree to submit a yearly progress report** in the month date of convened meeting each year until study is closed. Failure to submit annual report will result in ethics clearance certificate suspension. The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. Unreported changes to the application may invalidate the clearance given by the HREC (Medical). Email a signed copy of ethics clearance certificate to HREC-Medical.ResearchOffice@wits.ac.za

Signature of Principal Investigator _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix B: Confirmation of permission to use the dataset

KAMUZU UNIVERSITY
OF HEALTH SCIENCES

Vice Chancellor
Prof. M. Mallewa (MBBS, MRCP, PhD)

Our Ref:
Your Ref:

Kamuzu University of Health Sciences
Private Bag 360
Blantyre 3
Malawi
Tel: 01 871 911
Fax: 01 8747 00

15 May 2023

Confirmation of permission to use data for MSc Dissertation

I would like to confirm that Nawane Sekgala has been given permission to use a dataset from the study titled **"Accuracy and consequences of using trial-of-antibiotics for tuberculosis diagnosis"** clinical trial registration number: ClinicalTrials.gov, NCT03545373. I have been made aware that the data will be used for submission of dissertation for Master of Science Degree at University of the Witwatersrandin Johannesburg, South Africa.

The dataset will be sent to the candidate in a protected electronic format. Please do take note that the dataset is anonymised hence all identifiers in the dataset have been de-identified.

Yours faithfully

Titus Divala MD MPH MS PhD
Epidemiologist

Appendix C: Ethics Clearance Certificate for the primary study

CERTIFICATE OF ETHICS APPROVAL

This is to certify that the College of Medicine Research and Ethics Committee (COMREC) has reviewed and approved a study entitled:

P.04/18/2381 - Accurancy and Consequences of using Trial-of-antibiotics for TB diagnosis (ACT-TB Study) by Titus H Divala

On 03-Jul-18

As you proceed with the implementation of your study, we would like you to adhere to international ethical guidelines, national guidelines and all requirements by COMREC as indicated on the next page

Dr. YB. Mlombe - Chairperson (COMREC)

03-Jul-18

Date

Appendix D: Plagiarism Declaration

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

FACULTY OF HEALTH SCIENCES

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I Nawane Dorothy Sekgala (Student number: 2177905) am a student

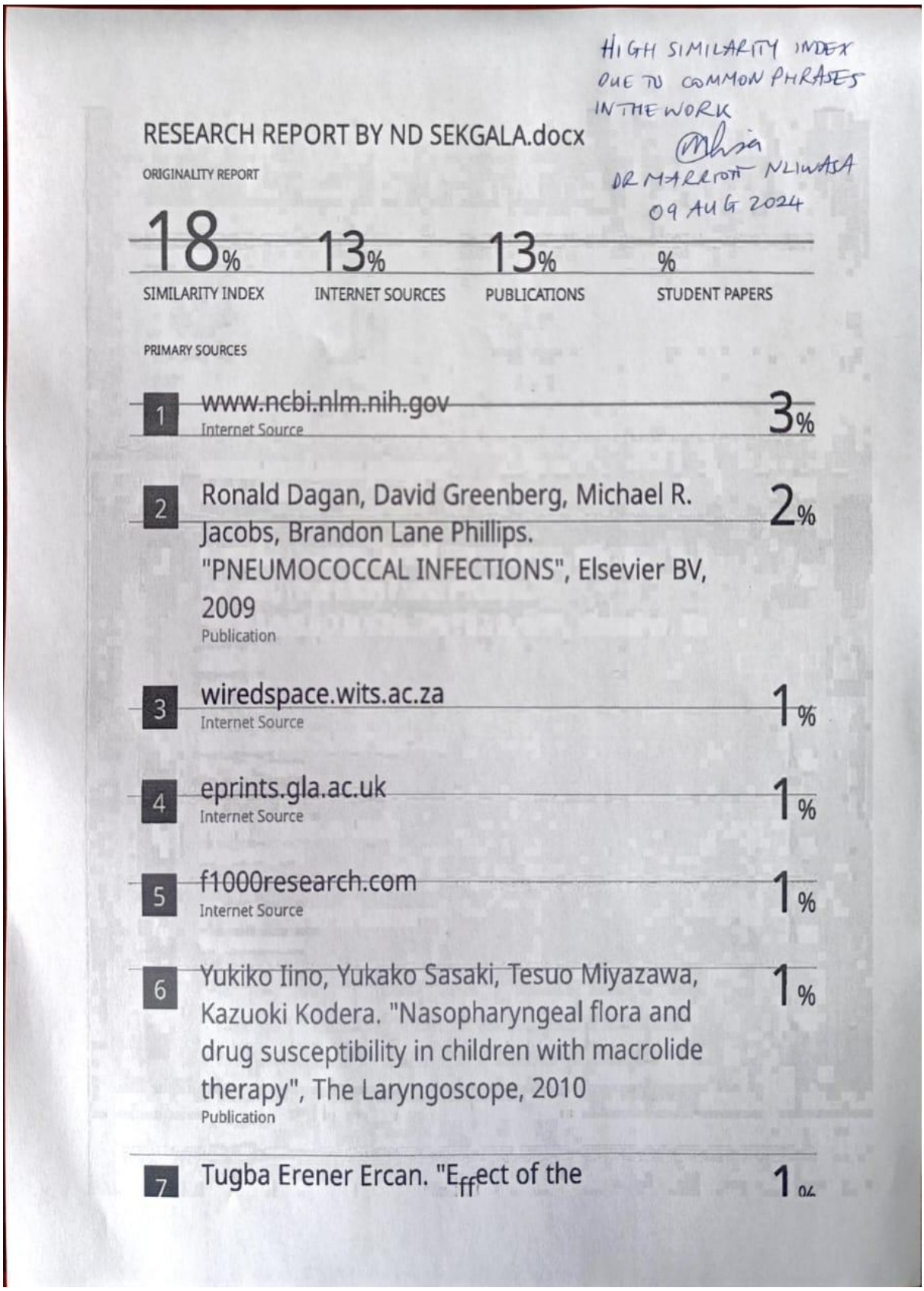
registered for the degree of MSc: Epidemiology and Biostatistics in the academic year 2021

I hereby declare the following:

- I am aware that plagiarism (the use of someone else's work without their permission and/or without acknowledging the original source) is wrong.
- I confirm that the work submitted for assessment for the above degree is my own unaided work except where I have explicitly indicated otherwise.
- I have followed the required conventions in referencing the thoughts and ideas of others.
- I understand that the University of the Witwatersrand may take disciplinary action against me if there is a belief that this is not my own unaided work or that I have failed to acknowledge the source of the ideas or words in my writing.
- I have included as an appendix a report from "Turnitin" (or other approved plagiarism detection) software indicating the level of plagiarism in my research document.

Signature:  Date: 02/06/2025

Appendix E: Turnitin Report



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DR MARRIOTT NLIWASA
09 AUG 2024

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