SCHOOL OF PUBLIC HEALTH

TITLE: FACTORS ASSOCIATED WITH FAILURE TO PRODUCE SPUTUM FOR TUBERCULOSIS INVESTIGATION AMONG CLINIC ATTENDEES IN PRIMARY HEALTH CLINICS IN MANGAUNG, SOUTH AFRICA.

BY

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Declaration

I, Makeneuoe Fako 2194721, affirm that this research report is all my own work. It is being submitted to the University of the Witwatersrand in Johannesburg for the MSc Epidemiology degree in the field of Implementation Science. It has not been submitted for a degree or exam at this or another university before.

(Signature of candidate)

24 September 2024

Dedication

I wholeheartedly dedicate this report to God Almighty by his mercy and grace to keep me going even when I was at the edge of giving up. To my husband for always being there for me and looking out for me. Thank you for making it look possible while it looked impossible to me. Finally, to my aunt who took care of my son during the time of my study.

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Abbreviations

ART	Antiretroviral Therapy
CAD score	Computer Aided Detection score
CI	Confidence Interval
COVID-19	Coronavirus 2019
d-CXR	digital chest X-ray
HIV/AIDS	Human Immunodeficiency Virus/Acquired Immunodeficiency Syndrome
MTB	Mycobacterium tuberculosis
OR	Odds Ratio
PHC	Primary health clinic
PI	Principal Investigator
PLHIV	People living with HIV
SA	South Africa
SSA	Sub-Saharan Africa
SI	Sputum induction
TB	Tuberculosis
WHO	World Health Organisation

Definition of Keywords

Sputum: is phlegm produced and coughed up from the trachea and bronchi.

Sputum Production: is spitting out/ expectorating and or coughing up thick mucus produced in the lungs.

TB Investigation: includes a screening step where a symptom screen is done and if any of cough, fever, weight loss or night sweats is present then further investigations are done to test whether the person has TB disease.

Primary Health Clinic: is a public health facility that offers primary care services including all preventative and regular medical treatment, manages chronic diseases and minor ailments, and is linked to the district hospital for secondary management in cases of complicated cases/disease or decision.

Abstract

Background: Tuberculosis **(**TB) is an infectious disease that can be prevented or cured if diagnosed early. TB can be fatal if not treated. In an effort to diagnose TB early, the WHO recommends universal TB screening in health facilities among clinic attendees. Any clinic attendee who has at least one symptom requires further investigations to confirm if they have TB and they are asked to produce sputum for TB tests. However, there are clinic attendees who fail to produce sputum despite the fact that they might have the disease. Therefore, this study will contribute to the efforts for eliminating TB by investigating factors associated with failure to produce sputum.

Methods: This study is a secondary analysis of a cross sectional study that was carried out between November 2017 and June 2018 in three Primary Health Clinics (PHCs) in Mangaung South Africa (SA). If a clinic attendee had at least one symptom or abnormal chest x-ray, they were asked to produce sputum. All clinic attendees who were asked to produce sputum were included in this study, including those who failed to produce sputum. The outcome variable was a binary variable (failure to produce sputum). The main exposure variables were reason for being asked to produce sputum and HIV status. Univariable and multivariable logistic regression models were fitted to examine factors associated with failure to produce sputum. The analysis was done using STATA 17. Factors were considered significant at $P<0.05$.

Findings: A total of 1356 clinic attendees were asked to produce sputum, of whom 530 (39.1%) failed to produce sputum. A higher proportion of females (48.1%) failed to produce sputum compared to males (27.0%). A highest proportion of those aged 18-29 (46.2%) failed to produce sputum as compared to the lowest proportion of those aged 60+ (33.3%). A higher proportion of those who were asked to produce sputum based on the symptom screen only (45.9%) failed to produce sputum compared to those who were asked to produce sputum due

to both symptoms and the chest x-ray (11.0%) and those who were asked to produce sputum due to the chest x-ray only (33.7%). The proportion failing to produce sputum was higher among People living with HIV (PLHIV) on ART (43.9%) and lower among PLHIV not on ART (23.5%) compared to HIV negative (38.9%) or HIV unknown (31.9%). In univariable logistic regression analysis, one factor was positively associated with failure to produce sputum and four factors negatively associated with failure to produce sputum. Method of screening was positively associated with failure to produce sputum (P<0.001) while HIV/ART status (P=0.001), sex (P<0.001), having had TB previously (P=0.002) and being an ex-miner (P=0.004) were negatively associated with failure to produce sputum. In the multivariable logistic regression analysis, there were three factors associated with failure to produce sputum namely the method by which a clinic attendee was screened positive, sex and being a clinic attendee that was a TB contact.

Conclusion: TB prevalence remains high in SA and the crowded health facilities with inadequate resources foster the spread of TB. If a high proportion of the clinic attendees fail to produce sputum this could lead to the spread of TB as some of those who fail to produce sputum may be infected. In addition, if an individual fail to produce sputum this may result in a delay in TB diagnosis which increases the risk of an unfavourable TB outcome. Therefore, the fact that some people fail to produce sputum warrants more work on alternative methods of TB diagnosis.

CHAPTER ONE: INTRODUCTION

1. Introduction

TB is an infection caused by a bacterium known as Mycobacterium TB (MTB) (Sanyaolu *et al.*, 2019). TB is transmitted when TB patients release bacteria into the air (for instance by coughing or sneezing) (World Health Organization, 2020). Despite the efforts to eliminate TB, before the coronavirus (COVID-19) pandemic, TB had been the worldwide leading infectious cause of death and a significant contributor to poor health, surpassing HIV/AIDS (World Health Organization, 2022a). Furthermore, about 90% of those who contract TB annually are adults, with men experiencing the disease at a higher rate than women (Bagcchi, 2023). Annually, an estimated 3.6 million people with TB may remain undiagnosed, in part because in community and basic healthcare settings access to confirmatory TB diagnostics, such as molecular assays or culture, is limited (Nathavitharana *et al.*, 2019). The recently completed TB prevalence survey done in South Africa in 2018 showed that a high proportion (57%) of people who tested positive for TB did not report having the standard symptoms of TB which consists of cough, fever, night sweats and weight loss (Moyo *et al.*, 2022). Among 9066 participants who were eligible for sputum collection, 1490 (16.4%) participants failed to provide sputum (Van der Walt and Moyo, 2018).

In this chapter, there is a global overview of the TB problem and a more detailed consideration of the SA setting. A literature review is also provided to summarise previous research. The conceptual framework used to guide this study, the problem description and study importance are also shown in this chapter. Lastly, the objectives and the study question are presented at the end of the chapter.

1.1. Background

1.1.1. TB Globally

In 2021, 10.6 million people were estimated to have contracted TB in comparison to 10.1 million in 2020. There were also 100,000 more TB deaths than there were in 2020 (1.6 million vs 1.5 million) (WHO, 2022). As a result, TB remains a pandemic and a major concern globally. Moreover, COVID-19 globally caused a 20% to 30% drop in TB notification rates in 2020 (McQuaid *et al.*, 2021). The reduction in the reported number of people with TB in 2020 and 2021 which coincided with the "Covid-19 pandemic" could correspond to a rise in undiagnosed and untreated TB, which in turn would lead to an increase in TB related mortality and TB transmission (Scheunemann *et al.*, 2023).

1.1.2. TB Detection and Control

To reduce the number of TB patients missed, WHO recommends screening every clinic attendee for TB symptoms at every visit using a four- symptom tool of "a cough of 2 weeks' duration or of any duration if living with HIV, unexplained weight loss of more than 1.5 kg in the past month, a persistent fever of more than 2 weeks, and drenching night sweats," (WHO, 2013). Any clinic attendee who has at least one of these symptoms requires further tests to check for the presence of TB; such attendees are asked to produce sputum for testing. However, among those clinic attendees there are still some who do not produce sputum (Moodley *et al.*, 2022). Amongst PLHIV, who are unable to provide a sputum sample, a chest x-ray (CXR) is recommended to diagnose TB (WHO, 2016). The Global Strategy to Stop TB is built on the principle that all TB patients must be identified and properly treated but a significant barrier to this strategy is the difficulty in locating and treating every patient (Claassens *et al.*, 2013). HIV negative patients who are unable to produce sputum are allowed to leave without any further

TB tests even if they have at least one symptom of TB (Claassens *et al.*, 2013; Moodley *et al.*, 2022).

1.1.3. Challenges of Sputum Collection

The approved diagnostic algorithms, which use tests based on sputum to investigate TB disease, have significant drawbacks, especially in environments with limited resources (WHO, 2017). Some people may find it challenging to produce sputum, particularly those who belong to high risk categories (Kendall, 2017). This is a challenge, because if such individuals remain undiagnosed or are diagnosed late, they may be major drivers of TB transmission or have poor outcomes (Kendall, 2017). Sample collection and testing are further restricted in locations with limited resources due to a lack of skilled healthcare personnel to show or convince patients with presumptive TB to expectorate sputum (Wobudeya *et al.*, 2022). While TB investigation in the majority of settings with a lack of resources still relies on sputum collection, it remains challenging for some people with symptoms suggestive of TB to produce sputum for microbiological investigation of TB.

Furthermore, screening for TB is advised for everyone who enters a clinic regardless of their risk profile or the reason they are there (Claassens *et al.*, 2013). However, there is a knowledge gap regarding an appropriate screening method between targeted and general clinic attendees (Kweza *et al.*, 2018). Additionally, patients who are assessed for TB frequently suffer lengthy delays (Nathavitharana *et al.*, 2022) and by the time they are asked to produce sputum they are already in a hurry. At times patients must travel elsewhere because some primary care facilities lack access to diagnostics and provide low quality TB symptom screening (Hanson *et al.*, 2017). Finally, there may not be enough instruction or support during sputum sample collection (Sibbald, 2013) which can demotivate patients from producing sputum and limit the sample's appropriateness for TB investigations since submitted samples differ in volume and quality.

1.1.4. Production of sputum

Expectorated sputum is the biological sample used to diagnose pulmonary TB (Orina *et al.*, 2019). It is formed when someone coughs up mucus from their lungs and, given that it can be collected without causing any harm, it is most frequently used for investigations (Orina *et al.*, 2019). The best sputum samples are those with the least amount of saliva (Stop, 2013). Purulent sputum, which has consistently been the best sputum for culture is distinguished by higher quantities of lipid, DNA, and non-mucin proteins (Orina *et al.*, 2019).

1.1.5. Sputum Induction

It remains a problem diagnosing patients with suspected TB who have smear negative disease or who are unable to produce sputum. Consequently, it is critical to deal with the sputum specimen acquisition diagnostic bottleneck as soon as possible (Peter *et al.*, 2014). Characterising these patients will help to inform the choice of patients in whom sputum could be induced.

Sputum induction is a secure and efficient way in which samples for microbiologic testing can be collected. Patients who are unable to produce sputum or those with a "negative sputum smear" frequently undergo sputum induction and / or bronchoscopy (Saglam, Akgun and Aktas, 2005). For this reason, recognized procedures include obtaining specimens from gastric washings, inducing sputum with nebulized hypertonic saline, and fibreoptic bronchoscopy with bronchoalveolar lavage (Brown *et al.*, 2007).

In decentralized settings with minimal resources, sputum induction through ultrasonic nebulization of hypertonic saline is a relatively straightforward and secure process (Hepple, Ford and McNerney, 2012). Moreover, it has demonstrated particular value in TB screening of asymptomatic patients prior to the start of Anti-Retroviral Therapy (ART) (Lawn *et al.*, 2012).

As a result, there is growing support for the use and extensive application of sputum induction in primary care settings with limited resources and an HIV epidemic (Bell *et al.*, 2009).

1.2. Literature Review

1.2.1. TB in South Africa

According to the WHO Global TB Report for 2023, 23% of new cases of TB in 2021 occurred in the African region. South Africa (SA) has a high prevalence of TB and a widespread HIV epidemic (Churchyard *et al.*, 2014). According to Maja and Maposa (2022), out of the 22 countries with a high burden of TB, SA is said to have one of the worst epidemics worldwide. In 2020, 25% of TB deaths took place in the African region that accounts for 16% of the world population (WHO, 2021). Despite improved TB prevention and increasing access to ART, TB remains the top cause of mortality in SA (Tomita *et al.*, 2019).

Furthermore, even with the massive expansion of HIV diagnosis and treatment, with an estimated prevalence of 1734 cases (95% CI 1219-2249) per 100,000 persons, approximately twice that of HIV-negative people, SA continues to have one of the highest rates of TB among people living with HIV in the world (Ayles, Mureithi and Simwinga, 2022). SA guidelines advise routinely screening all adult clinic attendants for symptoms and testing anyone who presents with at least one symptom of TB (namely persistent cough, night sweats, weight loss or fever). However, there are challenges to facility based case finding, such as some clinic attendees with symptoms not producing sputum, which cause delays and missed opportunities for case detection (Chihota *et al.*, 2015).

SA still has a major public health issue with TB. According to recent surveys, TB is still widespread, with an estimated 852 prevalent cases (95% CI 679-1026) per 100,000 persons. Men and those over 65 are more likely to contract the disease (Ayles, Mureithi and Simwinga,

2022). Prioritizing the identification and treatment of those with active TB is crucial for TB control.

1.2.2. Challenges in implementing sputum induction in health facilities.

Sputum induction increases risk of infection from patient to healthcare worker. Hence, it is recommended that professionals induce sputum in a short period to minimize exposure as exposure to TB patients increases their risks (Rao *et al.*, 2016). Additionally, induction must be done in a well-ventilated laboratory or room at a health facility, and this may be challenging to have in countries with inadequate resources like SA. In the absence of required facilities this operation must be carried out outside (Peter *et al.*, 2014). Sputum induction can be laborious, needs fasting beforehand, and can be upsetting to both patients and medical personnel (Zar *et al.*, 2005). Furthermore, induced individuals could develop side effects such as nausea and vomiting (Peter *et al.*, 2014).

1.2.3. Non-Sputum Based TB Tests

The Alere Determine AlereLAM Ag test (AlereLAM; Abbott, Waltham, MA, USA) is a commercially accessible urine point of care test that is approved by the World Health Organization (Sossen and Meintjes, 2023a). AlereLAM is simple and quick to use, and it has been demonstrated to lower mortality when used in clinical investigations of critically ill PLHIV who are admitted to hospitals (Peter *et al.*, 2016; Gupta-Wright *et al.*, 2018). Inadequate diagnostic sensitivity, estimated at 62% in hospitalized patients and 31% in outpatients, limits the test's usefulness (Bjerrum *et al.*, 2019). A significant challenge in assessing non-sputum-based testing is an imprecise reference standard, especially for individuals with paucibacillary diseases such as HIV and extra pulmonary TB (Bulterys *et al.*, 2019). Sossen and Meintjes (2023b) indicated that Urine lipoarabinomannan (LAM) test, has been carried out in SA, Uganda, Mozambique and Kenya in people with high risk of TB and has shown that

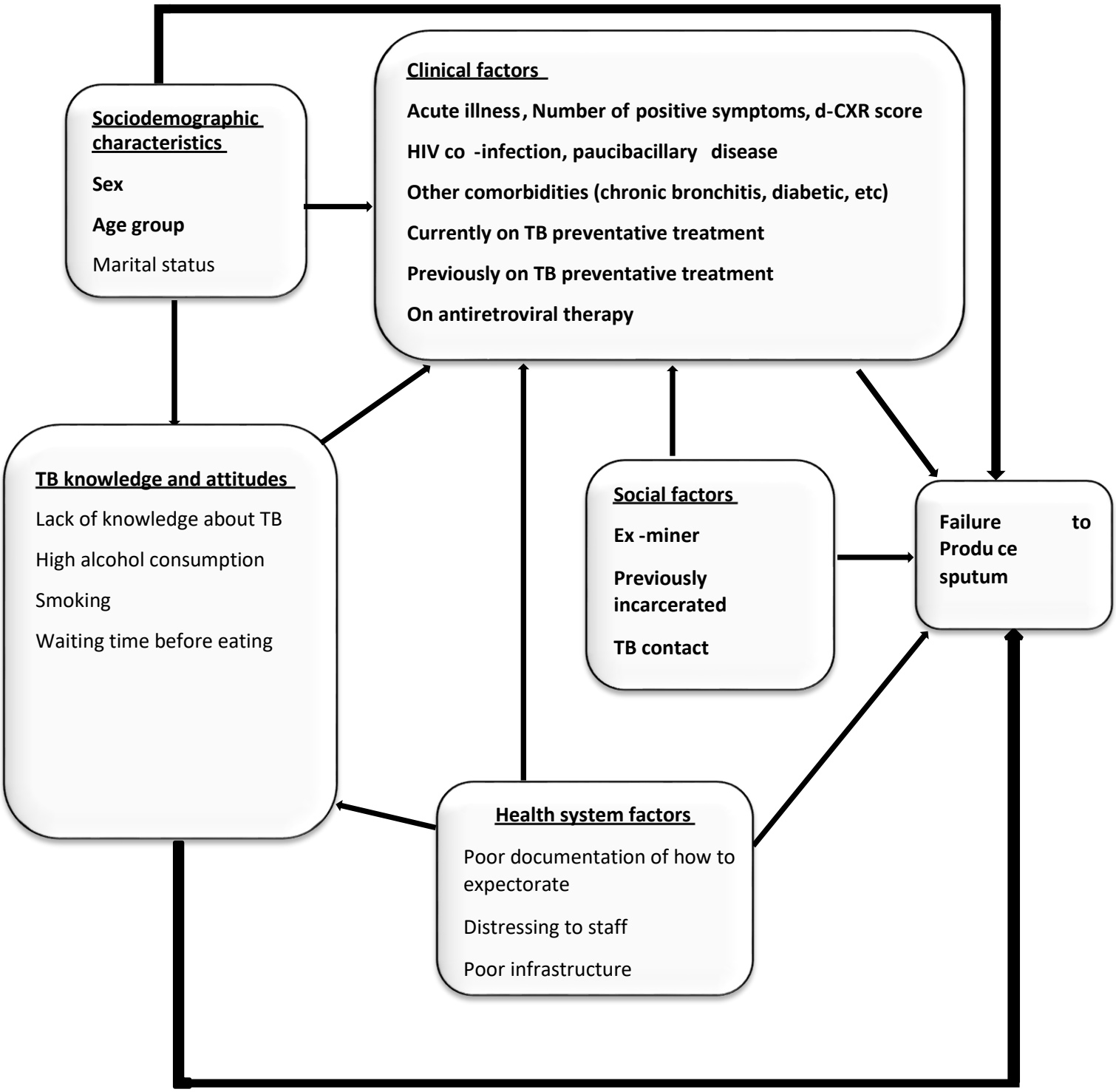
continuously excellent diagnostic accuracy, giving comfort that further advanced urine LAM diagnoses are yet achievable

1.2.4. Factors associated with sputum production.

Children (Drain *et al.*, 2019) and PLHIV (Peter *et al.*, 2014) frequently are unable to spontaneously expectorate sputum and are also at a greater likelihood of developing extra-pulmonary TB. Furthermore, the place where the patient is getting services and the reason for referral for sputum collection could be other factors that influence patients to produce sputum (Peter *et al.*, 2014). Patients with minimal symptoms and possibly with early TB disease may find it unnecessary to provide sputum for further TB investigations and confirmatory sputum tests will not be possible (Nathavitharana *et al.*, 2022). Jeon *et al.* (2009) added that old age, severity of symptoms and presence of co-morbidities can make it difficult for people with symptoms suggestive of TB to expectorate sputum. The clinic attendees' medical history, whether they have been exposed to the likelihood of contracting TB and their current illness or problem could influence the production of sputum (Kendall, 2017). Lastly, some people, such as little children, those with dry coughs, and those who are very sick or seriously disabled, are unable to produce sputum (Byanyima *et al.*, 2022).

1.2.5. Conceptual framework: Factors associated with production of sputum.

This study's conceptual framework (Fig.1) is based on the fusion of two conceptual models. The 1950s-era Health Belief Model, that was later modified by Rosenstock *et al*. and offers potential theories to explain health behaviour (Rosenstock, Strecher and Becker, 1988) and the Conceptual framework for TB treatment initiation (Mwansa-Kambafwile *et al.*, 2019). The framework acknowledges that sputum production can be influenced by patient and health system factors. Moreover, clinical factors can be factors that inhibit production of sputum.



[1]

Figure 1. A framework mapping factors that influence the production of sputum.

[1] The variables in bold were included in the analysis.

1.3. Problem statement

WHO has included SA in all three different categories of priority nations (TB, TB/HIV, and RR-MDR-TB) because of the high burden of TB in the country, per capita (National Institute for Communicable Diseases, 2020). In 2022, an estimated 54,200 persons in SA died of TB, while around 280,000 became ill with the disease (WHO, 2023).

In 2019, 360,000 TB event cases were estimated to exist in SA; of these, 58% were people living with HIV, and 17% of people living with HIV died as a result of TB (Osman, 2021). High death rates exist, especially in those who are hospitalized, co-infected with HIV, and who have undiagnosed TB. (Osman *et al.,* 2021). In 2018, 10% of TB patients in the Free State Province died, with the Mangaung District accounting for 60% of all people with TB (Moodley *et al.*, 2022). To diagnose TB, individuals with presumptive TB are typically asked to produce sputum for further investigations. Currently testing of a sputum specimen using Xpert Ultra is the Gold Standard however alternative methods of diagnosing TB are being investigated (Waters *et al.*, 2024). However, some participants find providing a sputum sample for TB diagnosis a challenge, despite the fact that some of those not providing a sputum specimen might be infected. Moreover, WHO (2016) indicated that although PLHIV are considered to be at high risk of TB, some of them are unable to produce sputum. For high-risk populations like PLHIV on ART, newly diagnosed PLHIV, pregnant PLHIV clinic attendees, people who received TB treatment in the previous two years and the people who were close contacts with a person with TB within the previous three months, the National Department of Health of SA has introduced a universal targeted testing strategy whereby sputum is collected for testing for TB using Xpert regardless of symptoms (Western Cape Government Circular 64. 2023). If a large proportion of people fail to produce sputum, then the implementation of the strategy might be challenging. In the study carried out by Moodley *et al.* (2022) in Mangaung District, Free

State Province, SA, 39.4% (534/1356) participants who were suspected of having TB were unable to produce sputum.

1.4. Justification

Healthcare professionals must first screen for the presence of symptoms, initiate and complete TB diagnostic investigations before beginning the diagnosis and care pathway for TB (Subbaraman *et al.*, 2016). TB diagnosis is the important step in the TB care continuum since without a diagnosis treatment cannot be initiated. Due to its non-invasive nature, sputum specimen testing continues to be recommended for detection of Mtb (Orina *et al.*, 2019). The need to screen for TB in high-burden countries is accepted by most such countries. However, among presumptive people with TB, sputum collection remains a problem with sputum collected being of low volume or poor quality (World Health Organization, 2013a). Those individuals who are unable to produce sputum may be diagnosed using an alternative method such as a urine LAM test or a chest x-ray, although currently these alternative methods of diagnosis have their own limitations. Characterizing these individuals and assessing factors associated with the non-production of sputum will help understand some of the demographic characteristics and clinical profile of clinic attendees unlikely to provide a sputum specimen and potentially requiring alternative sampling methods or investigations. Understanding the characteristics of those who are likely to fail to produce sputum will also inform implementation of targeted universal testing where a sputum sample for TB investigations is requested regardless of symptoms among groups at high risk of TB. (Berhanu *et al.*, 2023).

1.5. Research question, Aim and Objectives

1.5.1. Research Question

What are the factors associated with failure to produce sputum for TB investigations among clinic attendees in primary health clinics (PHCs) in Mangaung, South Africa between November 2017 and June 2018?

1.5.2. Aim

To investigate factors associated with failure to produce sputum for TB investigations among individuals attending for care at PHCs in Mangaung, South Africa.

1.5.3. Objectives

1. To describe the proportion of participants who were able to produce sputum by socio-demographic and clinical characteristics.

2. To determine factors associated with failure to produce sputum for TB investigation among clinic attendees.

CHAPTER TWO: METHODS

2. Methods

2.1. Introduction

This chapter discusses the methods that were utilized in this secondary data analysis to address the study question. It starts with a succinct explanation of the primary study from which the data used in the current analysis were collected, including the study setting of the primary study. This chapter also describes study design, study population, how sampling was done, and the data acquisition procedure carried out to get the data set used for analysis in the study. A thorough description of data management done in the study, variables included in the data analysis, and how data analysis was done is also provided in this chapter. Lastly there is a discussion of the ethical issues involved in the study.

2.2. Description of the primary study

The primary study was a cross-sectional study carried out between November 2017 and June 2018 in three Primary Health Clinics (PHCs) in the District of Mangaung SA. Clinic attendees for acute and chronic services were systematically identified and screened using the WHO four symptom-screen questions and a digital chest x-ray (d-CXR). Clinic attendees who were eligible and gave consent completed a questionnaire recording socio- demographic and clinical information. Clinic attendees were considered positive at screening if they had at least one symptom of the four symptoms or if they had an abnormal d-CXR. Those who screened positive were asked to produce sputum and if they failed to produce sputum, sputum induction was not done. In the study 3041 participants were screened of whom 1356 participants had at least one TB symptom or had a score of ≥ 60 on the d-CXR and were asked to produce sputum: 247 tested positive on d-CXR alone, 325 tested positive on both the symptom screen and d-CXR

and 784 were positive on the symptom screen alone. The Free State Department of Health funded the primary study.

2.3. Study Setting

The primary study was conducted in three PHCs in Mangaung, Free State, SA where there is a higher population density than the average for the Free State Province with high burdens of unemployment and poverty (Massyn, Pillay and Paradath, 2018). In 2017, Free State Province reported an HIV prevalence of 13% and a TB death rate of 10%. In Mangaung, the incidence of TB was 616 per 100,000 (Kigozi, Heunis and Engelbrecht, 2019). However, only 60% of projected people with TB were diagnosed in Mangaung District in 2018 (The Aurum Institute, 2018).

2.4. Study Design

The present study was a secondary data analysis of a cross-sectional study.

2.5. Study Population

Between November 2017 and June 2018, clinic attendees aged 18 years or older and who provided written informed consent for participation in the primary study, were asked to provide sputum if they screened positive using either the WHO 4-symptom TB screen or d-CXR or both were included in the study. Participants were excluded from the study if they were pregnant, currently on TB treatment or had been diagnosed with TB in the previous two years.

2.6. Sampling

All clinic attendees who were asked to produce sputum and who provided data for the primary study were included in the analysis, giving a total sample size of 1356. A power calculation was carried out using results published in Moodley *et al.* (2022). If we assume that there are in

total 1350 participants of whom about 1100 tested positive on the symptom screen and 250 tested negative on the symptom screen (but positive on the d-CXR) and that overall about 40% of participants failed to produce sputum, then the study will have over 80% power to detect as significant (at the 5% significance level) an absolute difference of 10% in the percentage of patients who failed to produce sputum between those who tested positive on the symptom screen and those who tested negative on the symptom screen (but positive on the d-CXR).

2.7. Data Acquisition

An application requesting the use of and access to the data was sent to the primary study team at The Aurum Institute. A consent letter to use the data set was received from the Principal Investigator of the primary study and is included in appendix B.

2.8. Description of Variables

2.8.1. Outcome variable

The outcome variable in this study is a binary variable (failure to produce sputum). Participants were asked to provide two spot sputum samples for TB testing. There was no induction of sputum, since this is not routine practice. Participants who did not provide sputum samples for further investigations when requested to provide two spot sputum samples in the primary study were deemed to have failed to produce sputum.

2.8.2. Exposure variables

The main exposure variables studied were how participants were screened positive (symptom screen alone, d-CXR alone or both symptom screen and d-CXR) and HIV status. These variables were considered to be the main exposure variables because symptomatic people and people living with HIV (PLHIV) are considered to be at high risk of TB. Other exposure variables were sex, whether or not the participant was on antiretroviral therapy, whether or not

he/she had previously had TB or diabetes, whether or not he/she was an ex-miner or previously incarcerated and whether or not he/she was suffering from an acute illness. Age was considered to be a possible confounder.

Table 1. Definition of variables

Name of the variable	Description
Outcome Variable	
Produced Sputum	0 = Yes defined as being able to produce sputum 1 = No defined as unable to produce to produce sputum
Exposure variables	
Sex	Binary 1 = Female 2 = Male
Age groups	5-points ordinal scale 1 = 18-29 (respondents aged 18 to 29) 2 = 30-39 (respondents aged 30 to 39) 3 = 40-49 (respondents aged 40 to 49) 4 = 50-59 (respondents aged 50 to 59) 5 = 60+ (respondents aged 60 or more)
Screened positive	3-point nominal scale 1 = Symptoms (respondents had at least one symptom of TB when screened) 2 = d-CXR (respondents who had CAD score >=60 when screened) 3 = Both (respondents who had at least one symptom and CAD score >=60.
HIV Status	4-point nominal scale 1 = Negative (respondents who self-reported HIV negative or from clinic records) 2 = Positive on ART (respondents who self-reported that they are living with HIV on ART or for whom the clinic records show that they are living with HIV on ART) 3 = Unknown (respondents whose HIV status was not documented or who refused to disclose their status in the primary study) 4=Positive not on ART (respondents who self-reported positive but not on ART
Previously had TB	binary nominal 1 = No (respondents who self-reported that did not have TB previously) 2 = Yes (respondents who self-reported that they had TB previously but not currently on TB treatment)
Diabetic	binary nominal 1 = No (respondents who self- reported not diabetic) 2 = Yes (respondents who self- reported diabetic)
Ex-miner	binary nominal 1 = No (respondents who were not ex-miners) 2 = Yes (respondents who were ex-miners)
Previously incarcerated	binary nominal 1 = No (respondents who were not former inmate at correctional centre) 2 = Yes (respondents who were former inmate at correctional centre)
Acutely ill	binary nominal 1 = No (respondents who self-reported not being acutely ill) 2 = Yes (respondents who self-reported being acutely ill)
TB Contact	binary nominal 1 = No (respondents who were not exposed to a TB case) 2 = Yes (respondents who were exposed to a TB case)

2.9. Data Management

After receiving the data, as a password protected Excel spreadsheet, the data were filtered to include only those who were asked to produce sputum i.e. those who had at least one symptom or a CAD score ≥ 60 or both. The outcome variable was recoded as unable to produce sputum = 1, able to produce sputum= 0. Age was calculated from date of birth and categorized into the following groups (18-29,30-39,40-49,50-59,60+). HIV status was recoded as a composite variable that took the values HIV negative, PLHIV on ART, PLHIV not on ART and unknown HIV status.

2.10. Data Analysis

A table of frequencies, broken down by whether the participant was able to produce sputum or failed to produce sputum, together with row percentages was used to summarize the data. The main exposure variables were how the participant was selected to produce sputum (screened positive on symptom screen, screened positive with the d-CXR or screened positive with both) and the composite variable defined above for HIV and ART status. The association between the exposure variables and the outcome was further investigated by fitting univariable logistic regression models which summarised the effects as odds ratios with 95% confidence intervals. An association was deemed to be statistically significant if the P-value was less than 0.05.

The final model contained the variables pre-selected as specified above together with all variables that were significant at the nominal 20% level (i.e. for which $P<0.20$) together with variables such as being a TB contact, that have been shown in the literature to be predictors of production of sputum. Age group (as potential confounder) was adjusted for by being included in the final model irrespective of statistical significance. Note that Likelihood ratio tests were used to test the significance of individual terms in the model, while the goodness of fit of the

model was tested using the Hosmer-Lemeshow goodness of fit test. STATA software version 17 was used to perform data analysis.

2.11. Ethical Consideration

The University of the Witwatersrand Human Research Ethics Committee as well as the Free State Provincial Research Ethics Committee provided ethical approval for the primary study. The data sharing agreement was signed with The Aurum Institute. For this study ethics approval was acquired from University of Witwatersrand Human Research Ethics Committee Medical (HREC-M) (on appendix D).

CHAPTER THREE: RESULTS

3. Results

3.1. Introduction

The main objective of this research was to investigate factors associated with failure to produce sputum while describing the study population by whether they produced sputum or failed to produce sputum during their clinic attendance. This chapter gives the findings of this study, and it starts by showing the characteristics of clinic attendees by sputum production. Thereafter factors associated with failure to produce sputum are investigated.

3.2. Descriptive statistics

Table 2 gives the characteristics of the clinic attendees broken down by whether they produced sputum or failed to produce sputum. A total of 1356 clinic attendees were asked to provide sputum samples based on the symptoms and/or the d-CXR score and of these 530 (39.1%) failed to produce sputum. Failure to produce sputum was highest amongst participants who screened positive by symptoms only, with 45.9% failing to produce sputum compared to 33.7% among those who screened positive by d-CXR only and 11.0% for those who screened positive by both symptoms and d-CXR. Furthermore, the proportion failing to produce sputum differed by the level of HIV/ART status, being higher among PLHIV on ART (43.9%) and lower among PLHIV not on ART (23.5%). A higher proportion of females failed to produce sputum compared to males (48.1% women vs 27.0% men). Additionally, failure to produce sputum was higher among clinic attendees who were aged 18-29 (46.2%), who self-reported not having had TB previously (41.6% vs 32.3%), who were not ex-miners (39.9% vs 22.7%), and who were TB contacts (42.2% vs 35.1%). Moreover, a higher proportion of participants who were

not acutely ill (41.5%) failed to produce sputum. The proportion who failed to produce sputum was similar among diabetic clinic attendees (39.3%) and those who were not diabetic (39.1%).

Table 2. Characteristics of clinic attendees by sputum production

Factor	**Level**	**Produced sputum (n=826)**	**Failed to produce sputum(n=530)**	**Total (N=1356)**
Sex	Male Female	424(73.0%) 402(51.9%)	157(27.0%) 373(48.1%)	581 775
Age (years)	18-29 30-39 40-49 50-59 60+	119(53.8%) 144(59.5%) 171(58.6%) 202(63.9%) 190(66.7%)	102(46.2%) 98(40.5%) 121(41.4%) 114(36.1%) 95(33.3%)	221 242 292 316 285
How screened positive	Symptoms only d-CXR (CAD4TB>=60) Both	424(54.1%) 313(66.3%) 89(89.0%)	360(45.9%) 159(33.7%) 11(11.0%)	784 472 100
HIV Status	Negative Positive not on ART Positive on ART Unknown	432(61.0%) 62(76.5%) 253(56.1%) 79(68.1%)	276(38.9%) 19(23.5%) 198(43.9%) 37(31.9%)	708 81 451 116
Previously had TB	Yes No	249(67.7%) 577(58.4%)	119(32.3%) 411(41.6%)	368 988
Diabetic	Yes No	74(60.7%) 752(60.9%)	48(39.3%) 482((39.1%)	122 1234
Ex-miner	Yes No	51(77.3%) 775(60.1%)	15(22.7%) 515(39.9%)	66 1290
Previously incarcerated	Yes No	55(65.5%) 771(60.6%)	29(34.5%) 501(39.4%)	84 1272
Acutely ill	Yes No	462(62.9%) 364(58.5%)	272(37.1%) 258(41.5%)	734 622
TB Contact	Yes No	441(57.8%) 385(64.9%)	322(42.2%) 208(35.1%)	763 593

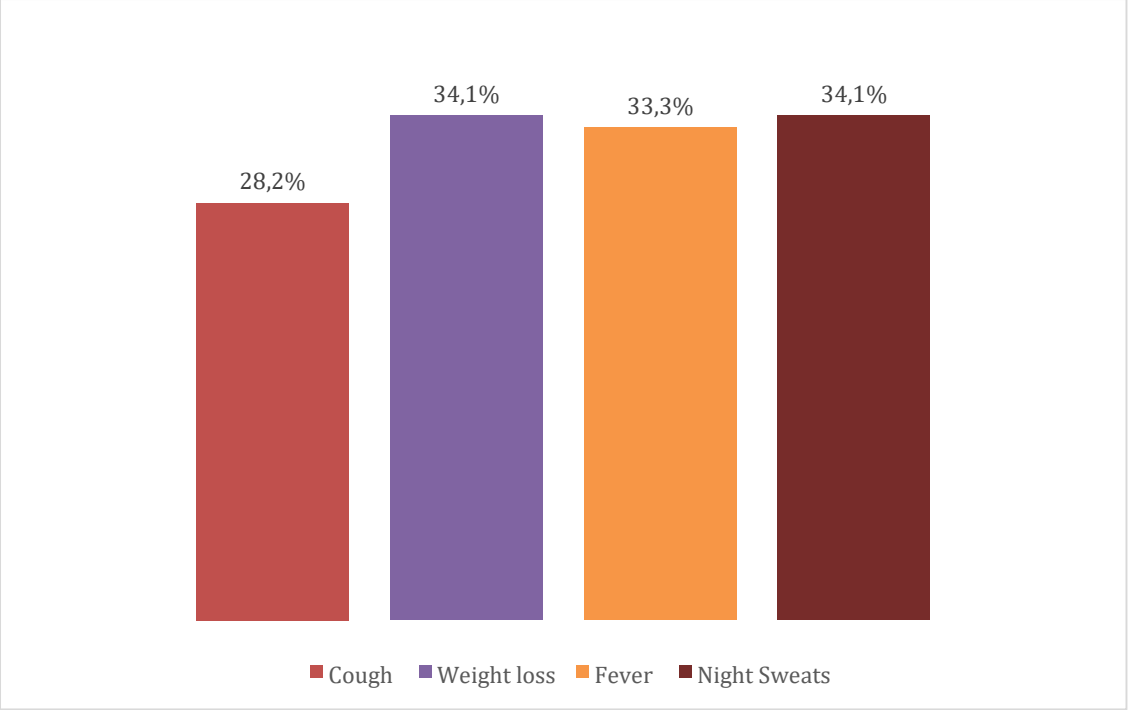


Figure 2. The proportion of clinic attendees who failed to produce sputum by screened positive symptom

In figure 2, it was found that those who reported to have cough for more than 2 weeks were least likely to fail to produce sputum (28.2%). Moreover, failure to produce sputum, was similar among clinic attendees who self-reported that they had night sweats, or weight loss, or fever.

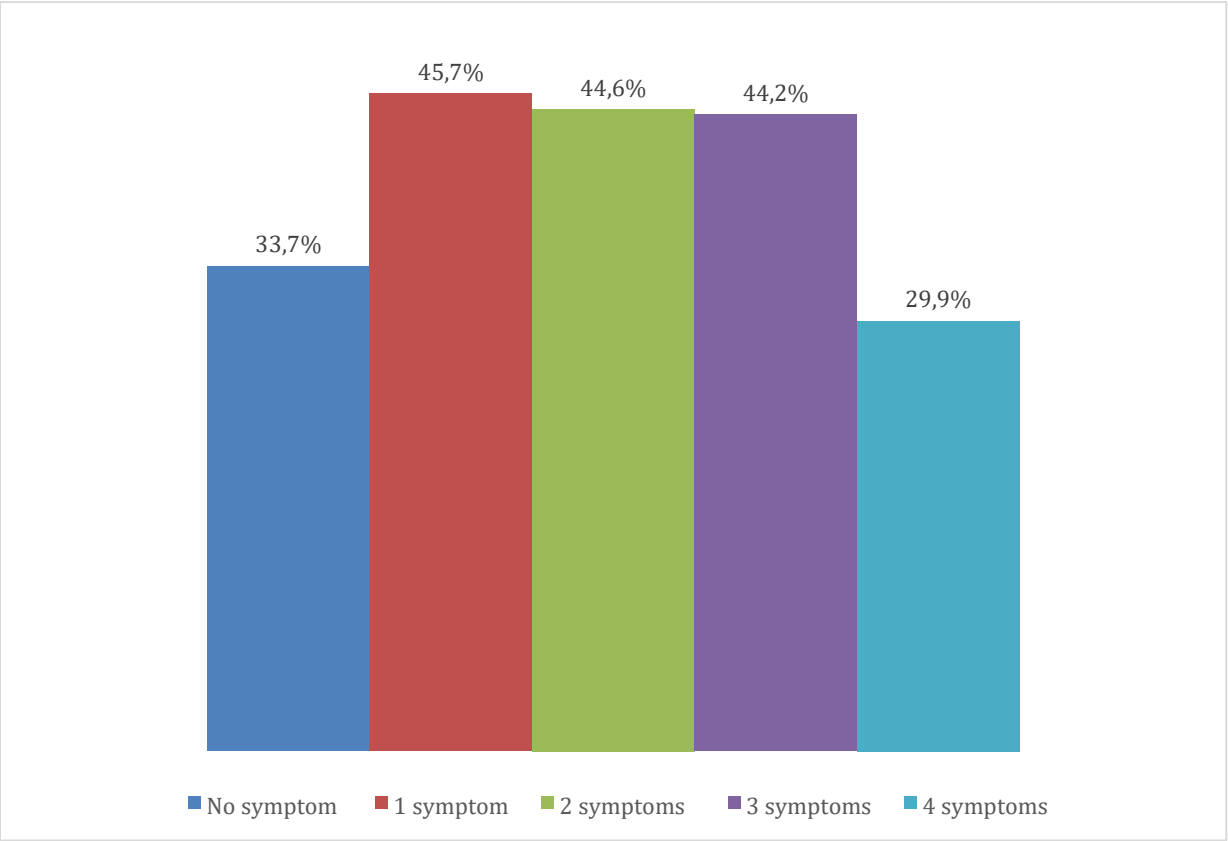


Figure 3. The proportion of clinic attendees who failed to produce sputum by the number of symptoms reported.

Among clinic attendees who were screened positive with one, two, or three symptoms, the proportion who failed to produce sputum was similar. However, the lowest proportion of those who failed to produce sputum was among clinic attendees reporting four symptoms.

3.3. Factors associated with failure to produce sputum

From Table 3, in univariable logistic regression analysis, two factors were positively associated with failure to produces sputum method of screening and being a TB contact. Method of screening was positively associated with failure to produce sputum with 67% increase in predicted failure to produce among clinic attendees who screened positive by symptoms only and being TB contact was also positive associated with failure to produce sputum with 35% increase in predicted failure to produce among clinic attendees who were TB contacts. Furthermore, four factors were negatively associated with failure to produce sputum. Particularly HIV/ART status, sex, having had TB previously and being an ex-miner was

negatively associated with failure to produce sputum. Those who reported living with HIV and not on ART were 52% less likely to fail to produce sputum as compared to who self-reported HIV negative. However, those who self-reported living with HIV and on ART were 22% more likely to fail to produce sputum as compared to who self-reported HIV negative. Males were 60% less likely to fail to produce sputum as compared to females. In addition, those who have reported having had TB previously were 33% less likely to fail to produce sputum. Finally, ex-miners were 56% less likely to fail to produce sputum compared to those who did not work in the mines.

The multivariable model included terms for sex, age, how screened positive, HIV status, TB contact, prior TB. In the multivariable logistic regression analysis in table 4, adjusting for all other terms in the model, there were three factors associated with failure to produce sputum. In particular, the method by which the clinic attendee was screened positive was strongly associated with failure to produce sputum. Compared to the reference level of participants who were screened positive using the d-CXR score only, those who screened positive with symptoms only were 45% more likely to fail to produce sputum (aOR 1.45; 95% CI 1.12 - 1.89; P<0.001) while those who screened positive based on both symptoms and d-CXR were 72% less likely to fail to produce sputum (aOR 0.28; 95% CI 0.15 - 0.55; P<0.001). Furthermore, sex was associated with failure to produce sputum. Males were less likely to fail to produce sputum (aOR 0.46; 95% CI 0.36-0.59; p-value <0.001).

Additionally, being a clinic attendee that was a TB contact was associated with failure to produce sputum. Clinic attendees who were TB contacts were 30% more likely to fail to produce sputum than those who were not TB contacts (aOR 1.30; 95% CI 1.00-1.69; p-value=0.047). Age group, prior TB, ex-miner, being acutely ill were found not to be associated with failure to produce sputum but were included in the final model because they have been shown to be important predictors of failure to produce sputum in the literature.

Table 3. Association of baseline characteristics with failure to produce sputum.

Factor	**Level**	**Univariable Logistic Regression**		
		OR	**95% CI**	**P-Value**
Sex	Female Male	1 0.40	(0.32-0.50)	<0.001
Age (years)	18-29 30-39 40-49 50-59 60+	1 0.79 0.83 0.66 0.58	 (0.55-1.15) (0.58-1.17) (0.46-0.93) (0.41-0.84)	 0.030
How screened positive	Symptoms d-CXR (CAD4TB>=60) Both	1.67 1 0.24	(1.32-2.12) (0.13-0.47)	<0.001
HIV/ART status	Negative Positive not on ART Positive on ART Unknown	1 0.48 1.22 0.73	 (0.28-0.82) (0.96-1.56) (0.48-1.11)	0.001
Previously had TB	No Yes	1 0.67	 (0.52-0.86)	 0.002
Diabetic	No Yes	1 1.01	 (0.69-1.48)	 0.951
Ex-miner	No Yes	1 0.44	 (0.25-0.80)	 0.004
Previously incarcerated	No Yes	1 0.81	 (0.51-1.29)	 0.373
Acutely ill	No Yes	1 0.83	 (0.67-1.03)	 0.097
TB Contact	No Yes	1 1.35	 (1.08-1.67)	 0.008

Table 4. Final model for factors associated with failure to produce sputum.

Factor	Level	OR	95% CI	P-Value
Sex	Female Male	1 0.46	Reference (0.36-0.59)	<0.001
Age (years)	18-29 30-39 40-49 50-59 60+	1 0.80 0.80 0.70 0.64	Reference (0.54-1.17) (0.55-1.17) (0.48-1.02) (0.43-0.95)	0.2279
How screened positive	Symptoms only d-CXR (CAD4TB>=60) Both	1.45 1 0.28	(1.12-1.89) Reference (0.15-0.55)	<0.0001
HIV/ART status	Negative Positive not on ART Positive on ART Unknown	1 0.58 1.14 0.85	Reference (0.33-1.01) (0.86-1.52) (0.55-1.32)	0.1074
TB contact	No Yes	1 1.24	Reference (0.97-1.58)	0.087
Previously had TB	No Yes	1 0.80	Reference (0.60-1.07)	0.133

CHAPTER FOUR: DISCUSSION AND CONCLUSION

4. Discussions and Conclusion

4.1. Introduction

The spread of pulmonary TB should be stopped and prevented by prioritizing early detection, timely treatment initiation, and efficient infection control measures (Ko *et al.*, 2017). Sputum collection is an important but understudied component of case finding in high workload, resource limited settings (Armstrong-Hough *et al.*, 2018). This chapter aims to present a summary of the main results and draw conclusions from the study.

4.2. Discussion

In this study, we assessed factors associated with failure to produce sputum among clinic attendees who were asked to produce sputum for TB investigations due to being symptomatic. We determined three factors associated with failure to produce sputum which are the method by which participants screened positive, sex, and being TB contact. As a result, we found that participants who screened positive on the four symptom screen alone or had an abnormal chest x-ray only were more likely to fail to produce sputum compared to participants who were positive on both the four-symptom screen and the chest x-ray. This is different from another study that was conducted in Cape Town that found that those who could produce sputum were those with at least one of the five symptoms that were screened which were cough, haemoptysis, fever, night sweats and weight loss (Den Boon *et al.*, 2006). Furthermore, a study that was carried out in Uganda showed that contacts with a cough were more likely to provide sputum than those with other symptoms or risk factors (Elgstrand *et al.*, 2017).

This study further found that a higher proportion of females failed to produce sputum compared to males (48.1% women vs 27.0% men, P<0.001). This is similar to another study that found that those who could produce sputum were more likely to be males than females (Den Boon *et al.*, 2006). In this study model, HIV status was adjusted for, so the HIV status could not be said to be the reason for the difference between females and males in the proportion who failed to produce sputum. Additionally, in the study that was conducted at Uganda in 2018, it was mentioned that during sputum collection interviews, women mentioned stigma more frequently than males so women may be less likely than men to produce sputum due to variations in their experience or anticipation of TB stigma.

Furthermore, this may imply that because women are more likely than men to be HIV-positive and frequently produce sputum specimens of lower quality, relying solely on microscopy for TB diagnosis could significantly underestimate the prevalence of TB in these situations (Boum *et al.*, 2014). These results echo those of another study that was done in Uganda that found that males provided sputum at a higher rate than females and stigma was cited by women as a barrier in their study (Armstrong-Hough *et al.*, 2018). This can be considered good as men are more likely than women to develop TB (Bagcchi, 2023). However, there is a need for further studies to explore this stark difference between males' and females' failure to produce sputum. Moreover, this will inform gender-specific strategies to help encourage females to produce sputum.

We further found that a higher proportion of TB contacts failed to produce sputum and clinic attendees who were TB contacts were 30% more likely to fail to produce sputum (OR 1.30; 95% CI 1.00-1.69; p= 0.047). In addition, 28.2% of those who failed to produce sputum were clinic attendees who self-reported having a cough for more than 2 weeks. This is similar to other studies that have found that patients who are ill with early symptoms may not expectorate sputum (Nathavitharana *et al.*, 2022). Other human samples that have been investigated as

potential substitutes for sputum in order to assess the diagnostic efficacy of TB include exhaled breath condensate, saliva, urine, blood, and stool. Unfortunately, compared to sputum, these substitute samples have shown reduced sensitivity or specificity (Zhang *et al.*, 2024). TUTT approach can help find "missing" TB patients in SA since more people were diagnosed by sputum testing than by standard symptom-directed TB testing (Martinson *et al.*, 2023). Therefore, failing to produce sputum may increase missed cases or poor TB outcomes.

4.3. Limitations of the study

The sample size was small as the data were collected from one Province so the study may not be generalizable. The data used were collected for a short period of time and collected by the research staff not the daily programmatic staff which may have impacted the time and space the clinic attendees were given to produce sputum, and this might have impacted the production of sputum. Participants were asked to provide sputum at the facility not at home which may have influenced failure to produce sputum as they may be in hurry for other services they came for in the clinic. There is lack of previous studies on the topic however, having searched the literature on TB diagnosis.

Not all variables that were included in the final model were found to be associated with failure to produce sputum and this may have been due to small sample size as discussed above. Despite the limitations this study adds to the still scanty literature on factors associated with clinic attendees being unable to produce sputum.

4.4. Conclusion

Having tested positive on the basis of the four-symptom screen alone was found to be associated with failure to produce sputum. Additionally, this study discovered that a larger percentage of women than men failed to produce sputum (48.1% vs. 27.0%, P<0.001). This research also found that clinic attendees with a TB contact were more likely to fail to produce sputum (OR

1.30; 95% CI 1.00 - 1.69; p=0.047). Therefore, factors positively correlated with non-production of sputum were the method of screening positive, female sex, and being a TB contact.

4.5. Recommendations

TB prevalence remains high in SA and the crowded health clinics with inadequate resources foster the spread of TB. Though screening of all patients has been started as recommended by the WHO, further investigations of TB should be carried out among clinic attendees who screen positive on the four symptom screen. Though this study has found that being screened positive through questions only, sex, and being TB contact is associated with failure to produce sputum, larger and home-based controlled studies are needed to find factors associated with failure to produce sputum. Furthermore, new techniques to investigate TB that do not depend on sputum production may be introduced among high risk populations if sputum production remains a problem to scale up early TB diagnosis and treatment. This is supported by the suggestions of Drain *et al.*, (2019) that testing non-sputum samples such as urine, breath, or blood may provide a more thorough and quick diagnosis in primary healthcare environments where sputum production is a challenge.

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Appendices

Appendix A: Plagiarism Declaration Report

PLAGIARISM DECLARATION TO BE SIGNED BY ALL HIGHER DEGREE STUDENTS

SENATE PLAGIARISM POLICY: APPENDIX ONE

I __Makeneuoe Fako___ (Student number: 2194721_____) am a student registered for the degree of

__MSc Epidemiology (Implementation Science) _____ in the academic year __2024__.

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: 10 January 2024_____

Appendix B: Data Use Authorization Letter

THE AURUM INSTITUTE
South Africa
Registration No. 2021/785844/08

Aurum House
The Ridge, 29 Queens Road
Parktown 2193
South Africa

PostNet Suite300
Private Bag X30500
Houghton 2041
South Africa

Tel: +27 (0) 10 590 1300
Fax: +27 (0) 866 180 268
Website: www.auruminstitute.org

16 January 2023

Ethics Committee
University of the Witwatersrand
Human Research Ethics Committee

RE: APPROVAL FOR ACCESS TO A DE-IDENTIFIED DATABASE EXTRACT –FOR SECONDARY DATA ANALYSIS

Dear Members of the Ethics committee,

We have considered the request by Ms Makeneuoe Fako (student no 2194721) for secondary data analyses using a de-identified database extract from the Free State TB Screening Study. The full title of the parent study is: **Optimising tuberculosis screening practices in the Free State Province (Wits HREC Ethics Reference Number-170605).**
This data will be used for the purposes of Ms. Fako's Master's report entitled: *Factors associated with the production of sputum for TB investigations among primary health clinic attendees in Mangaung, South Africa.*

The data points requested have been attached to this application.

Sincerely,

Professor Salome Charalambous
Principal Investigator

Appendix C: Primary Study Ethics Clearance Certificate

University
of the Witwatersrand,
Johannesburg

Human Research Ethics Committee: (Medical)
FWA Registered No IRB 00001223

SECRETARIAT: Suite 189, Private Bag x2600, Houghton 2041, South Africa Tel: +27-11-274 9200 Fax: +27-11-274 9281

04 July 2017

FAXED & COURIERED

Dr N Moodley,
The Aurum Institute
The Ridge, Aurum House
29 Queens Road
Parktown
2193
Fax: 086 759 2728

Dear Dr Moodley,

PROTOCOL: AUR 2-8-211 - OPTIMISING TUBERCULOSIS SCREENING PRACTICES IN THE FREE STATE PROVINCE

ETHICS REFERENCE NO: 170605

RE : FINAL ETHICS APPROVAL

This is to certify that the above-mentioned trial has been approved by the University of the Witwatersrand, Human Research Ethics Committee (HREC), and the Protocol/Expert Reviewer. Date of Meeting where trial was reviewed: 30 June 2017.

The University of the Witwatersrand, Human Research Ethics Committee Approval Granted for the above mentioned study is valid for five years. Where required by Sponsor to have approval on a more frequent basis it remains the responsibility of the Sponsor and Investigator to apply for continuing review and approval, or for the duration of the Trial.

1. It is the responsibility of the Sponsor and Principal Investigator to ensure, where required, that relevant approvals are in place and compliance with the following is adhered to before a trial may begin:

- If trial is being conducted in Provincial Health facilities: Approval from the Hospital CEO / Clinic Manager / District Research Committee (whichever is applicable) be obtained.
- The study is submitted onto The National Health Research Database (NHRD).
- The relevant approvals are uploaded onto the NHRD system: Ethics Approval, MCC Approval, Hospital CEO / Clinic Manager / District Research Committee Approval.

* A copy of the MCC Approval and/or MCC Notification letter must be submitted to the Ethics Secretariat Office for record purposes (IF MCC APPROVAL / NOTIFICATION IS APPLICABLE).

* The study is conducted according to the protocol submitted to the University of the Witwatersrand, Human Research Ethics Committee. Any amendments to the protocol must first be submitted to the Human Research Ethics Committee for approval.

* During the study, the University of the Witwatersrand, Human Research Ethics Committee is informed immediately of :

- Any Unexpected Serious Adverse Events or Unexpected Adverse Drug Reactions, which, in the Investigator and/or the Sponsor's opinion are suspected to be related to the study drug. (Refer to POL-IEC-001 and SOP-IEC-005, Item 3.4).
- Any data received during the trial which, may cast doubt on the validity of the continuation of the study.

* The University of the Witwatersrand, Human Research Ethics Committee is notified of any decision to discontinue the study and the reason stated.

* The Investigators authorised by this approval participate in this study. Additional Investigators shall be submitted to the University of the Witwatersrand, Human Research Ethics Committee for approval prior to their participation in the study.

* In the event of an authorised Investigator ceasing to participate in the study, the University of the Witwatersrand, Human Research Ethics Committee must be informed and the reason for such cessation given.

2. PRINCIPLES OF INFORMED CONSENT:

* The University of the Witwatersrand, Human Research Ethics Committee requires that in all studies, the Principles of Informed Consent are adhered to. This applies to volunteers as well as patients.

3. PROGRESS REPORTS:

* The University of the Witwatersrand, Human Research Ethics Committee requests that the MCC Progress Reports be submitted twice a year either in March and September or six monthly from start of study to the HREC Secretariat Office - 011 274 9281 and a report of the final results, at the conclusion of the study. (IF APPLICABLE)

4. REIMBURSEMENT TO PATIENTS FOR TRANSPORT:

* The Human Research Ethics Committee: (Medical) is in agreement that reimbursement per visit is according to the Medicines Control Council of SA and that reimbursement should be appropriate according to the situation.

5. TRANSPORT AND STORAGE OF BLOOD AND TISSUE SAMPLES IN SOUTH AFRICA:

* If blood specimens are to be stored for future analysis and is planned that such analysis will be done outside Wits, then the blood must be stored at a facility in South Africa agreed with the relevant IRB, with release of sub-samples only once projects have been approved by the local Research Ethics Committee applicable to where the analysis will be done as well as by the Wits Human Research Ethics Committee: (Medical).

6. GENETIC TESTING:

* The Human Research Ethics Committee: Medical; will not approve open-ended genetic testing as this does not fit the Human Research Ethics Committee criteria.

7. GOOD CLINICAL PRACTICE:

* The South African Department of Health, Medicines Control Council requires Good Clinical Practice (GCP) Training for all Investigators in Clinical Trials, and that GCP training be renewed every three (3) years.

As yet, there are no National Guidelines for the content of GCP courses. Until these are available the Wits Human Research Ethics Committee (Medical) will note courses completed by Investigators without approval of the content of the individual courses.

8. THE SUPPORTING APPROVAL DOCUMENTS ARE ATTACHED:

8.1 Ethics Approval Form signed by the Chairperson of the HREC - Kindly return the copy of the Approval Form signed by the Principal Investigator(s) per fax: 011 274 9281 for our records (this is applicable with the initial Approval).

8.2 List of members present at the HREC meeting held as per INDEPENDENT ETHICS COMMITTEE APPROVAL FORM

Appendix D: Ethics Clearance Certificate

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Miss Makeneuoe Fako

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M230210 MED22-11-035

NAME: (Principal Investigator)	Miss Makeneuoe Fako
DEPARTMENT:	School of Public Health
PROJECT TITLE:	*Factors associated with failure to produce sputum for TB investigations among primary health clinic attendees in Mangaung, South Africa*
DATE CONSIDERED:	24/02/2023
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Prof J. Levin
APPROVED BY:	Dr M Vorster, Co-Chairperson, HREC (Medical)
DATE OF APPROVAL:	08/05/2023 (Initial approval); 28/07/2023 (Title change)
EXPIRY DATE:	08/05/2028

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

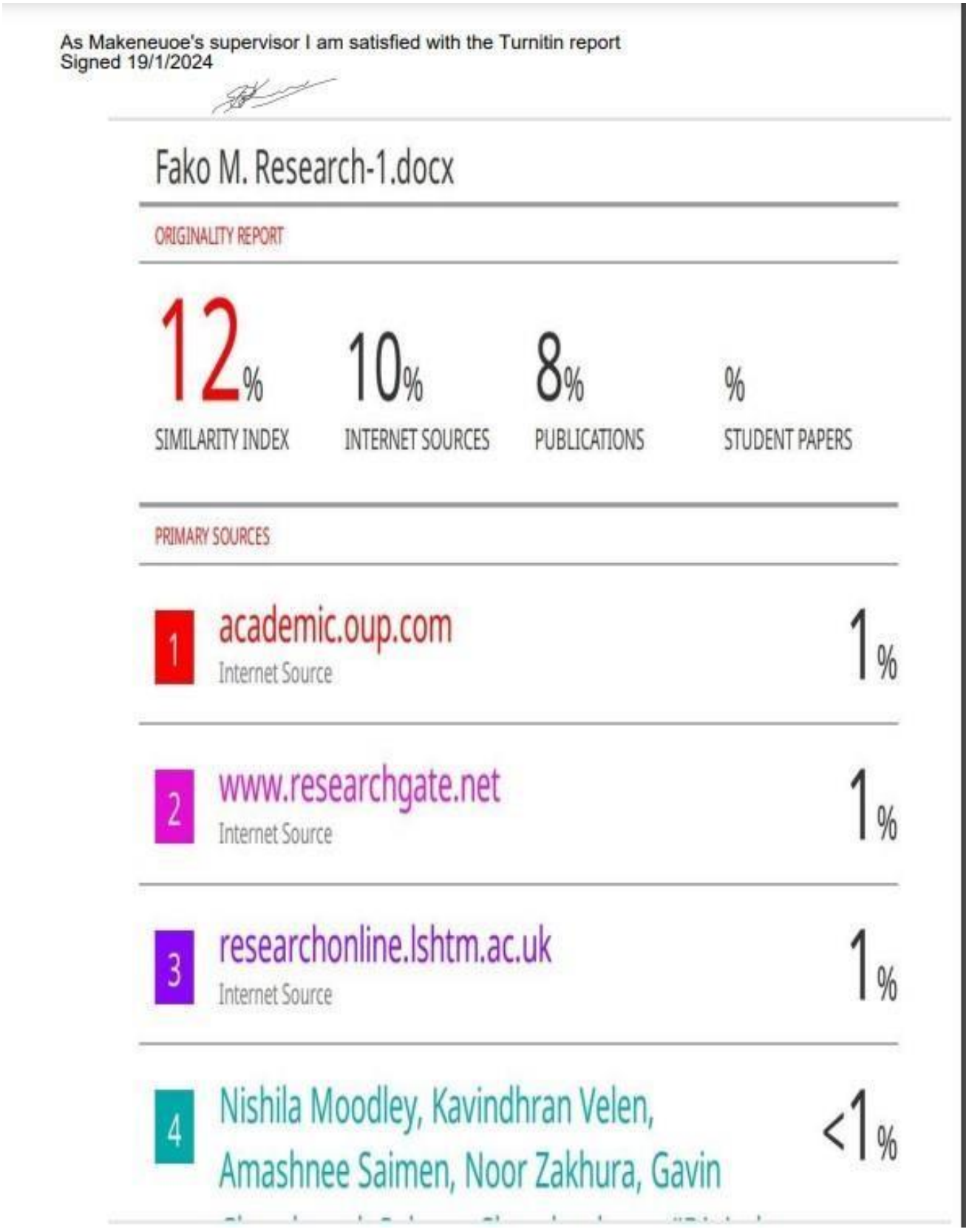
DECLARATION OF INVESTIGATORS

To be completed in duplicate and **ONE COPY** returned to the Research Office Secretary on the Third Floor, Faculty of Health Sciences, Phillip Tobias Building, 29 Princess of Wales Terrace, Parktown, 2193, University of the Witwatersrand. I/we fully understand the conditions under which I am/we are authorized to carry out the above-mentioned 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.** The date for annual re-certification will be one year after the date of convened meeting where the study was initially reviewed. In this case, the study was initially reviewed in **February** and will therefore be due in the month of **February** each year. Unreported changes to the application may invalidate the clearance given by the HREC (Medical).

Principal Investigator Signature _____ Date _____

PLEASE QUOTE THE PROTOCOL NUMBER IN ALL ENQUIRIES

Appendix E: Turnitin Report



As Makeneuoe's supervisor I am satisfied with the Turnitin report
Signed 19/1/2024

Fako M. Research-1.docx

ORIGINALITY REPORT

12%	10%	8%	%
SIMILARITY INDEX	INTERNET SOURCES	PUBLICATIONS	STUDENT PAPERS

PRIMARY SOURCES

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4	Nishila Moodley, Kavindhran Velen, Amashnee Saimen, Noor Zakhura, Gavin	<1%