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Spatial and temporal analysis of xpert confimed MDR-TB in the North-West province of South Africa from 2011-2015

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SPATIAL AND TEMPORAL ANALYSIS OF XPERT CONFIRMED MDR-TB IN THE NORTH-WEST PROVINCE OF SOUTH AFRICA FROM 2011-2015

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A Research Report submitted to the Faculty of Health Sciences, University of the Witwatersrand, Johannesburg, in fulfilment of the requirements for the degree of Master of Science in the Field of Epidemiology and Biostatistics

Johannesburg, November 2018

DECLARATION

I Oladimeji Matthew declare that this research report is my own unaided work. It is being submitted for the degree of Masters of Science in Epidemiology in the field of Epidemiology and Biostatistics, Faculty of Health Sciences at the University of the Witwatersrand, Johannesburg. It has not been submitted before for any degree or examination at any other University.

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Oladimeji Matthew

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ABSTRACT

The burden of multi-drug resistant Tuberculosis (MDR-TB) cases is a reflection of the continuous and recent transmission of infection within the population. This study therefore aimed to describe the spatiotemporal distribution of Xpert tested Rifampicin (RIF) resistant cases using the Gene Xpert CT probe dropout/delay (a test to diagnose the presence and the type of RIF resistance from specific *Mycobacterium tuberculosis* strain), and also evaluate the demographic and socio-economic factors that are associated with the distribution of MDR-TB in the North West province from 2011-2015. Spatial and spatiotemporal autocorrelation were explored using the Global Moran's I, local Anselin Moran's I and Kulldorff's scan statistics. A multivariate zero-truncated Poisson model was used to generate estimates of parameters for demographic and socioeconomic factors.

A total of 2949 RIF resistant cases, reported by the National Health Laboratory Services (NHLS) testing sites from 2011-2015 was added to the analysis. The cumulative incidence rate of MDR-TB was 139.4 cases/100,000 inhabitants. The highest incidence rate of 223.7 cases/100,000 inhabitants was seen in the City of Matlosana municipality. There was no evidence of temporal clustering but spatial and spatiotemporal clustering of MDR-TB cases were mostly observed in the South Western to the South Eastern region of the province where potential overcrowding from seasonal migration and economic activities like mining are prevalent. *Mycobacterium tuberculosis* (MTB) strains with genotypes causing the probe dropout/delay of 17 (RR=5.00, P-value<0.001), 20 (RR=5.22, P-value<0.001) and 24 (RR=5.3, P-value<0.001) were mostly transmitted within these MDR-TB hotspots in 2012. The Municipal risk for more MDR-TB cases, was positively associated with, MTB bacterial load (IRR=1.15, 95% CI: 1.05-1.26), youth male to female ratio (IRR=1.59, 95% CI: 1.19-2.14) and male proportion (IRR=1.09, 95% CI: 1.01-1.16) and negatively associated with the number of households (IRR=0.87, 95% CI: 0.79-0.94, P-value: 0.001). There is evidence of hotspots in the South Western and South Eastern regions of North West Province resulting from direct transmission of specific MTB strains between clustered facilities during the study period. Also, beyond the direct transmission, other ecological factors such as increasing median bacterial load and increased male to female ratio can increase the municipality risk of MDR-TB. These hotspots should be prioritized in the implementation of strategic MDR-TB control programs

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CONTENTS

DECLARATION ..... **ii**

ABSTRACT ..... **iii**

ACKNOWLEDGEMENTS ..... **iv**

CONTENTS ..... **v**

ABBREVIATION AND ACRONYMS ..... **viii**

LIST OF FIGURES ..... **ix**

LIST OF TABLES ..... **x**

CHAPTER ONE – INTRODUCTION **1**

1.1 General Introduction 1

1.2 Background 1

1.3 Problem Statement 2

1.4 Justification 3

1.5 Literature Review 4

1.5.1 Pathology of Tuberculosis and Multi-Drug Resistance-TB 4

1.5.2 Mechanism of RIF resistance 5

1.5.3 Acquisition/Transmission of MDR resistance TB 5

1.5.4 Predictors of Rifampicin resistant Tuberculosis 6

1.5.5 Geographical Distribution of MDR-TB 9

1.5.6 Spatial Analysis Methodology 10

1.5.6.1 Spatial Scan Statistics 10

1.5.6.2 Global Moran's I 11

1.5.6.3 Anselin's Local Moran's I 12

1.5.6.4 Getis-Ords Gi* statistic 12

1.5.7 The Assay test for TB and MDR-TB 12

1.5.8 The Sensitivity and Specificity of the Xpert MTB/RIF assay 14

1.6 Research Question 15

1.7 Research Hypothesis 15
1.8 Aim 16
1.8.1 Specific Objectives 16
CHAPTER TWO – METHODS AND MATERIALS 17
2.1 Chapter Overview 17
2.2 Study Settings 17
2.3 Study Design 19
2.4 National Health Laboratory Services Gene Xpert roll out in South AFrica 19
2.5 Target population 20
2.5.1 Study Population and Sampling 20
2.5.2 Eligibility criteria ..... 21~~20~~
2.5.3 Definition of terms 21
2.6 Data Description and Management 21
2.7 Data Analysis 25
2.7.1 MDR-TB hotspots in the North West province between 2011-2015 25
2.7.2 Hotspots of median facility Bacterial load 27
2.7.3 Spatial and spatiotemporal distribution of probe dropout/delay 28
2.7.4 Objective 4: Ecological factors associated with the distribution of MDR-TB 29
CHAPTER THREE – RESULTS 31
3.1 Introduction 31
3.2 MDR-TB hotspots in the North West province between 2011-2015 31
3.2.1 MDR-TB distribution, incidence rates and Interpolation 31
3.2.2 Spatial Cluster Analysis of annual MDR-TB Incidence rates ..... 35~~34~~
3.2.3 Spatiotemporal analysis of annual MDR-TB Incidence rates ..... 36~~35~~
3.3 Hotspots of Median Facility Bacterial load ..... 38~~37~~
3.4 Spatial and spatiotemporal distribution of probe dropout/delay ..... 38~~37~~
3.4.1 Spatial analysis of Probe dropout/delay using Moran's I statistic ..... 38~~37~~
3.4.2 Spatiotemporal analysis of Probe dropout/delay clusters using scan statistic ..... 39~~37~~

3.4.3 Most likely probe dropout/delay ..... 41~~39~~
3.5 Ecological factors associated with the distribution of MDR-TB ..... 42~~41~~
3.5.1 Univariable Analysis ..... 42~~41~~
3.5.2 Multivariable Analysis ..... 43~~41~~
CHAPTER FOUR – DISCUSSION ..... 47~~44~~
4.1 Introduction ..... 47~~44~~
4.2 MDR-TB hotspots in the North West province between 2011-2015 ..... 47~~44~~
4.3 Spatial and spatiotemporal distribution of probe delay/dropout ..... 48~~46~~
4.4 Hotspots of Median Facility Bacterial load ..... 49~~46~~
4.5 Ecological factors associated with the distribution of MDR-TB ..... 49~~46~~
4.6 Strength and Limitation ..... 50~~47~~
CHAPTER FIVE – CONCLUSIONS AND RECOMMENDATIONS ..... 52~~49~~
5.1 Conclusion ..... **52~~49~~**
REFERENCES ..... 54~~51~~
APPENDICES ..... 67~~62~~

ABBREVIATION AND ACRONYMS

AIC	Akaike Information Criterion
BIC	Bayesian Information Criterion
CFU	Colony Forming Unit
CT	Cycle Threshold value
DOTs	Directly Observed Therapies
DS TB	Drug Susceptible Tuberculosis
GIS	Geographic Information System
HIV	Human Immunodeficiency Virus
INH	Isoniazid
ISA	Incremental Spatial Autocorrelation
LLR	Log Likelihood Ratio
MDR-TB	Multi Drug Resistance TB
MTB	Mycobacterium Tuberculosis
NHLS	National Health Laboratory Services
PCR	Polymerase Chain Reaction test
RIF	Rifampicin
rtPCR	reverse transcriptase PCR
EMB	Ethambuthol
SEM	Structural Equation Modeling
SPC	Sample Processing Control
TB	*Mycobacterium Tuberculosis*
UID	Unique Identifier
WHO	World Health Organization
Xpert	Gene Xpert
Xpert MTB/RIF	Xpert *Mycobacterium Tuberculosis*/Rifampicin assay
Z	Pyrazinamide

LIST OF FIGURES

CHAPTER TWO

Figure 1: A hybrid physical/political map of the North West Province in the North West Province, indicating the major cities, towns, game, nature reserves and national roads Extracted from https://www.sa-venues.com/maps/north-west-regional.htm..... 1918

CHAPTER THREE

Figure 2: Spatial distribution of annual MDR-TB crude infection rate by district, North West, 2011-2015 31

Figure 3: The distribution of annual MDR-TB crude infection rate by municipality, North West, 2011-2015 32

Figure 4: Integrated map of the facilities annual crude infection rate using IDW and facilities number of years with greater than expected rate of MDR-TB..... 33

Figure 5: Six spatial (point layer) and eight visible spatiotemporal clusters (circle layers) identified by local Moran's (Anselin) and the SaTScan statistic respectively..... 3836

Figure 6: left: spatiotemporal clusters resulting from all similar probe dropout/delay; Right: spatiotemporal cluster of the most likely probe dropout/delay within MDR-TB hotspots. 4240

LIST OF TABLES

CHAPTER TWO

Table 1: NHLS, DHIS and STATSSA variables definition. 2333

CHAPTER THREE

Table 2: Temporal Distribution of the annual rates of MDR-TB cases..... 3544
Table 3: List of clustered facilities by the Moran's I statistics (Anselin)..... 3544
Table 4: Clusters of annual MDR-TB by SaTScan 3746
Table 5: Moran's results for pure spatial analysis of median facility probe dropout/delay 3947
Table 6: Characteristic of the spatiotemporal clusters of probe dropout/delay by SaTScan 3948
Table 7: Characteristic of the most likely spatiotemporal clusters of probe dropout/delay 4150
Table 8: Crude and adjusted risk ratio of ecological factors associated with the annual distribution MDR-TB crude rates from 2011-2015 4351
Table 9: Crude and adjusted risk ratio of ecological factors associated with the annual municipal MDR-TB crude rates for 2015 only. 4553

CHAPTER ONE – INTRODUCTION

1.1 General Introduction

This chapter describes the global and national burden of multi-drug resistant Tuberculosis (MDR-TB) and states the relevance of the study for strategic infection control to policy makers in South Africa. Relevant literature was reviewed on the transmission pattern and known risk factors of MDR-TB, especially in South Africa(1).

1.2 Background

Globally, Tuberculosis (TB) is of public health concern with about 6.3 million new cases and 1,674,000 estimated deaths in 2016 (2). In the same year, 10.4 million people fell ill from the disease, of which 90% were adults, 65% were males (2). The same report ranked TB as the ninth leading cause of death globally above Human Immunodeficiency virus infection (HIV) in 2016 (2).

In 2007, World Health Organization (WHO), ranked South Africa as the seventh country with the largest global incidence of TB (3), while in 2014, the country was ranked as the second in the world only after her neighbouring country Lesotho (4). Presently, TB is the leading cause of death among South Africans between ages of 15 and 64 years. In addition, about 61% of the TB cases are co-infected with HIV(5–7).

North West specifically was reported to have the second largest prevalence of TB (8). Seven percent (7%) of deaths in the province are attributable to the disease (8). The department of health of South Africa has identified Bojanala and Dr Kenneth Kaunda district, to have the highest TB prevalence in the province of North West. According to the report, this high prevalence is because there are many mines in the region (8).

Specific features have been identified to complicate the control of TB. These include complex biology and pathogenesis of the disease (most of which are still poorly understood); potentially long latency period allowing for transmission a number of months before patient seek clinical care; unrecognized primary and re-infections. Thus, a huge unrecognized reservoir of latent TB infection coupled with unavailability of effective and affordable TB drugs also contribute to the

poor TB control. Furthermore, poor adherence on account of lengthy treatment and multiple drug therapy can consequently increase the development of MDR-TB (9).

The emergence of MDR-TB, has majorly contributed to poor TB control (10). In 2016, 490,000 MDR-TB cases occurred worldwide, of which 350,000 were detected and notified, and more than half of these cases were found in Brazil, India, China and South Africa (2). Similarly, about 240,000 people died of MDR-TB in the same year (2). Sub-Saharan African countries like Nigeria, Ethiopia, DR Congo, South Africa among others, were ranked among countries with the highest burden of MDR-TB globally (2).

In 2016, South Africa was ranked among the 20 countries with the highest number of incident cases of MDR-TB in the world (2). Its MDR-TB prevalence, was about 3.4% among new TB cases and about 7.1% among TB retreated cases (2). The number of MDR-TB cases diagnosed and laboratory confirmed in the country were estimated to be 218,231 and 18,734 respectively in 2014 (11). A report released by the National Health Laboratory Service (NHLS), estimated that the prevalence of MDR-TB in the North West province among the new TB cases in 2011, 2012, 2013 and 2014 was 8.0%, 7.1%, 5.4% and 5.2% respectively (12). This temporal trend is suggestive of a slow decline but still very high rate of MDR-TB in the province.

1.3 Problem Statement

Efforts have been geared towards stemming the tide of MDR-TB in the country. One of those is the deployment of Gene Xpert Mycobacterium tuberculosis/Rifampicin assay (Xpert MTB/RIF assay), to replace smear microscopy, as the initial test for the diagnosis of TB in all government laboratories in 2010 (13). This was based on the evidence that Xpert MTB/RIF assay has a better sensitivity and will also provide an immediate Rifampicin (RIF) resistance result. However, poor infection control still remains a major challenge confronting the control of MDR-TB in the country (14–16). Over the years, the country has been ranked among the countries with the highest number of incident cases of MDR-TB in the world (2,11). The 2012-2014 TB drug resistance survey in the country, indicated that the prevalence of RIF resistant-TB was almost doubled between 2001-02 and 2012-14 most likely from the direct transmission of the infection (17). Another report from the country, stated that half of the drug resistant cases were never exposed to TB treatment (15,16). Also in the North West province, the prevalence of RIF resistant cases among the new TB cases in 2011, 2012, 2013,2014 and 2015 was 8.9%, 7.5%,

5.5% 5.4% and 5.6 % respectively (18). All these findings are an indication that there are areas with active ongoing MDR-TB transmission in the country including the North West province.

Many studies have identified the geographic hotspots (places with greater than expected risk) of MDR-TB in different settings (19–21). These studies also indicated that hotspots were as a result of direct transmission of the infection, and identified the genotypes of specific MTB strains driving this localized transmission within hotspots (19,22). In addition, MDR-TB hotspots can also be influenced by the social and biological risk factors in different settings (22). Hence, other studies have examined the socio economic factors (23–28), climatic factors (21), health factors (1,21), demographic and environmental factors (29) that are associated with MDR-TB hotspots. If there are regions with greater than expected risk of MDR-TB and unique transmission dynamics, the design of region specific interventions and strategic allocation of the interventions to these regions, may be more effective than blanket screening and treatment (22). In South Africa, no provincial specific studies have been conducted to evaluate the hot spots of MDR-TB and other socio-economic and demographic factors that might be driving transmission within hotspots. This will address the problem of poor infection control through the design and strategic allocation of region specific MDR-TB interventions in the study area.

1.4 Justification

The Xpert TB/RIF assay has been proven not only to be highly sensitive to the presence of MTB, but also very specific to the detection of different RIF resistance conferred by specific mutations on the probe gene (30–32). This study describes the distribution of Xpert confirmed Rif resistant (MDR-TB) cases and identified hot spots of the infection in the North West province in space and time. It also explored the technology of Xpert probe dropout/delay, to examine the transmission pattern and distribution of region-specific genetically identical MTB isolates. This will set a foundation for public health interventions of region-specific infection control for MDR-TB in the North-West province.

Other Studies have shown that there is a constant log linear relationship between Cytochrome threshold (CT) values and MTB Colony Forming Unit (cfu), valid for sputum with 10^2 -10^7 cfu/ml (33,34). Thus, this study also explored this semi-quantitative capacity of Xpert CT values and Geographic Information System (GIS), in determining places with hotspots and clusters of highly infectious MDR-TB active cases with very high MTB bacterial load in the province. This

is to know where the risk of disease is highest for strategic allocation of interventions. Factors associated with MDR-TB distribution in the municipalities of the province were also investigated to provide evidence for strategic planning for TB infection control and management in the North-West Province of South Africa.

1.5 Literature Review

1.5.1 Pathology of Tuberculosis and Multi-Drug Resistance-TB

Tuberculosis (TB) is an infectious disease caused by mycobacterium tuberculosis (MTB) (35). It usually affects the lungs (pulmonary TB), but can also affect other parts of body including the kidney, spine and the brain (extra-pulmonary TB) (35). It is transmitted when a person with an active pulmonary or laryngeal disease coughs, sneezes, sings or shouts (36). This allows for the suspension of MTB in the air in form of nuclei droplets that is about 1-5 microns diameter and which last for about 3 hours (36). The chances of infectivity depend on factors such as recipient's susceptibility (immune status), the infectiousness (the number of bacilli expelled into the air), and the environment that affects the concentration of the bacilli in the air (space, ventilation, air circulation, air pressure, physical proximity to the infectious person, frequency of exposure and duration of exposure among others) (36).

MDR-TB is defined as TB that is resistant to at least two of the first line drugs for TB (37). Rifampicin (RIF) is one of the four drugs that are generally utilized as first line combination therapy for TB treatment. Other drugs in the combination include Isoniazid (INH), Ethambuthol (EMB) and Pyrazinamide (Z) (14). Resistance to RIF is due to mutations in the 81-bp core region of the rpoB gene (38). The World Health Organisation (WHO) in 2004 recommended that RIF resistance should be designated as a "surrogate marker" for MDR-TB detection (39). This is because, about 90% of all RIF resistant clinical isolates are also resistant to INH, especially in regions where RIF mono-resistance is rare (39). Conventionally, drug resistant TB patients are categorized as having acquired resistance or primary resistance based on previous exposure to TB treatment (40). TB patients that have had prior TB treatment, are categorized as patients with 'acquired resistance', while TB patient without prior treatment who developed MDR-TB are designated as patients with 'primary resistance'(40).

1.5.2 Mechanism of RIF resistance

Rifampicin is a derivative of Rifamycin, and it was introduced in 1972 as an anti-tuberculosis agent (41). It is one of the most effective drugs against TB and is used together with INH as a multi-drug treatment regimen for TB (41). It acts by binding to the ß-subunit of the RNA polymerase, thus inhibiting the elongation of the messenger RNA of the bacteria during transcription (42). Most RIF- resistant MTB harbors mutation in the rpoB gene that code for the ß-subunit of the RNA polymerase (41). This conformational change in the ß-subunit, prevents RIF from binding (41,43). When this happens, the bacteria is said to develop resistance to RIF (44). About 96% of all the MTB clinical isolates mutate in the codons spanning from 507-533 of the 81bp of the rpoB gene (45). This region is called the hot spots or RIF resistance determining region for most molecular diagnostic tests like the Gene Xpert (45). The most common mutations are those in the codon 516, 526 and 531 (46,47). Mutations in some codons like the 518 or 529 are less common and said to be associated with low level resistance to RIF but still susceptible to other Rifamycins such as Rifabutin and Rifalzil (48,49). RIF mono-resistance is rare, therefore, MTB that are resistant to RIF, are also resistant to other TB drugs, especially INH (50). Such suppositions led to RIF resistance being suggested as a surrogate marker for MDR-TB (50).

More than 95% of the mutations that confers RIF resistance, are localized within the 81 bp core region of the RNA polymerase rpoB gene. The PCR assay amplifies this 192bp segment of the MTB rpoB gene in a hemi-nested real-time reaction, hence, it is called rt-PCR assay (32,51). This core region is in specific DNA sequence of the MTB. Also, the wild type nucleotide sequence of the susceptible isolates of MTB, are almost always the same and flanked in specific DNA-sequence. This makes the simultaneous detection of MTB and RIF resistance using the Polymerase Chain Reaction (PCR) technology possible (30).

1.5.3 Acquisition/Transmission of MDR resistance TB

Multi-drug resistant-TB (MDR-TB) in patients occurs through two pathways. The first is the primary drug resistance in patients without prior TB treatment. The second is the acquired (secondary) resistance in patients with prior TB treatment (8). Acquired resistance results when mutant resistant strains of MTB are selected as a result of inadequate, incomplete or poor treatment quality (52). A typical example is when a patient is exclusively treated with a single

ineffective TB regimen which allows the selection and multiplication of the bacteria with drug resistant mutations (52). Another scenario occurs when a patient who already has resistance to a regimen is treated with that regimen and another new regimen, allowing the bacteria to develop resistance to the new regimen (52). Thus, subsequent addition of a new regimen to another regimen allows for more severe patterns of drug resistance in MDR-TB (52). Natural mutations of MTB to more than one TB drug are very rare, thus the problem of MDR-TB is regarded as man-made (52).

Primary or initial resistance occurs when a patient develops TB after being infected with a strain of MTB that was transmitted from a patient with MDR-TB (52). People in regions with high prevalence of MDR-TB are at risk of direct transmission of drug resistant TB (52). Environments with overcrowding, poor ventilation and poor infection control also allow for the direct transmission of MDR-TB (52). Just like drug susceptible TB (DS-TB), MDR-TB can be latent for a very long time before it progresses into active disease (52). Immunosuppression is a major factor that hastens this progression (52). Thus, factors like HIV, under nutrition, diabetes, silicosis, smoking, alcohol abuse and other diseases that impair the immune system are risk factors for MDR-TB (52).

Globally, the proportion of patients with primary resistance has remained 3.5% from 2008 to 2013, and these patients were disproportionately distributed (53). The highest of these primary MDR-TB cases were reported in central Asia and Eastern Europe (53). However, this rate has increased from 3.3% to 4.1% for primary resistance and decreased from 20% to 19% in previously treated cases between 2014 and 2016 (2). A population based study that was conducted in South Africa and other settings with high MDR-TB prevalence, showed that transmission is a major contributor to the rising global prevalence of MDR-TB (54). This is a reflection of direct transmission and poor infection control of MDR-TB.

1.5.4 Ecological predictors of Rifampicin resistant Tuberculosis

Various patient level factors like age, HIV, genetic susceptibility, urbanisation and occupation, were found to be associated with MDR-TB (6,55–59). Previous treatment was consistently identified as a strong predictor for MDR-TB (60–62). Inadequate treatment (in the form of non-adherence to first line anti-TB treatment, drug side effects during first line treatment, treatment not directly observed by the health worker and interruption of treatment for at least one day) has

been identified as the strongest predictor of MDR-TB (63,64). It causes the increased death and growth cycle of MTB, allowing for accumulation of individual mutations of different independent genes (65).

Many studies have established the association between the prevalence of MDR-TB and patient level risk factors. Most of these factors have been validated to be a risk for MDR-TB in almost all settings (60–62). A typical example of this kind of risk is treatment default and exposure to previous TB treatment (61,62,66). However, the distribution of MDR-TB in different regions and settings varies (21).

Further studies have therefore looked into the association between ecological factors and the risk of MDR-TB. Different ecological factors have been observed in different settings. The ecological factors that have been implicated include socioeconomic factors (23–28), climatic factors (21), health factors (1,21), demographic and environmental factors (29).

HIV is another major ecological factor associated with MDR-TB. Studies in France and Ukraine, found an association between the primary MDR-TB and HIV (56,57). This is because HIV suppresses the immune system allowing drug susceptible TB to develop (56,57). While researchers in Ethiopia did not find any significant association between HIV and MDR-TB (55). A South African study, found that MDR-TB clusters were associated with HIV infected patients in institutional and community settings (67).

Other population level factors have also been considered to be risk factors for TB and MDR-TB. People who work in the mines have been seen to be at very high risk of TB because of prolonged exposure to Silicosis from mines shaft (68,69). The South Africa mining industry has reported the highest incidence of TB in the world of about 7000 cases per 100,000 inhabitants. Apart from the high prevalence of HIV amongst miners, which increases their chance of having TB by 30 times, their circular migration between South Africa and neighboring countries leads to interrupted TB/HIV treatment and poor access to care, which further predisposes them to the development of MDR-TB (70). A 2010 report revealed that 4% of the national MDR-TB burden is from those who reside in mining areas (70). The South African government has regarded people in the mining area as vulnerable groups to be targeted with TB screening and testing interventions (71).

Migration and Tourism are other factors which are associated directly or indirectly with MDR-TB. Migration is a risk factor for MDR-TB (27,28). Refugees and asylum seekers are likely to be at high risk of MDR-TB and develop poor MDR-TB treatment outcomes (27,28). Migrants have higher chances of being infected with MDR-TB resulting from reactivation of latent TB and from proximity to other active cases migrating for MDR-TB treatment (health tourist) (28). Seasonal migrants have increased tendencies of poor compliance and TB treatment default (27), which are risk factors for acquired drug resistance (52,63,64). Another report in South Africa, also revealed travelling as a risk factor for the infection (72).

Overcrowding is a known predictor for MDR-TB because it increases the concentration of the MTB bacilli in the air from reduced space, poor ventilation, increased air pressure, close physical proximity to the infectious person, and frequency of exposure and duration of exposure (36,52). Periodic overcrowding and population hikes have been identified with tourism especially during tourism peak seasons. A study in Malindi town of Kenya, describes how commercial workers who arrive in search of employment and other trading activities during tourism season, were congested in small houses and experienced poor living conditions (73). In addition, a WHO report stated that there is poor access to quality health services and poor living conditions among refugees and other migrants during periodic large influx of immigration (74). This potential overcrowding during tourism season could increase the risk of MDR-TB infection (36,52).

Urbanization has also been found to be associated with MDR-TB cases (27). According to the study, urban settings most times are characterized by overcrowding and higher population density (27). This was consistent with another study in Georgia on the geographical heterogeneity of MDR-TB cases, where living in urban areas and capital cities were significantly associated with high rate of primary MDR-TB from direct transmission (75). In contrast, a study in Dhaka in Bangladesh, found people living in the rural area to be 14 times more likely to develop the disease compared to their urban counterparts (76). This disparity is not unexpected, because other studies have confirmed that the MDR-TB dynamics vary in different settings (11,20,21,77–79).

Agricultural activities have been associated with MDR-TB. Farming most of the time is done in rural areas where primary health care is unavailable. Hence, most farmer populations are out of reach for the MDR-TB screening programs (27). Another study has also established that people

involved in agricultural production and agricultural products transportation were more likely to develop MDR-TB (76). This is because the laborious nature of the profession demotivates farmers from completing their TB treatment especially when not under DOTs, hence increasing their chance of MDR-TB (76).

Ecological factors have been identified to be responsible for the significant variations in the MDR-TB distribution that occurred in different regional settings (21). No study has been done in the North West province of South Africa on the spatial distribution of MDR-TB and their association with different ecological factors.

1.5.5 Geographical Distribution of MDR-TB

Many studies have shown the geographic distribution of TB and MDR-TB clustering. For example, a study on the spatial epidemiology of DR-TB worldwide showed variation across study regions (21). Also, spatiotemporal patterns of MDR-TB and drug sensitive TB examined in South American setting, confirmed that MDR-TB cases were clustered (19). The same study revealed that the clustering was driven by MDR-TB direct transmission (19). Another study in Portugal and Africa showed spatiotemporal clustering of TB and MDR-TB clusters in hyper endemic hotspots, as a result of crowding, HIV infection and other social determinants (23–26). Reports from various studies, indicated a variation in the space and time (spatial and temporal) distribution and clustering of MDR-TB in different settings (20).

In the mid-1980s, MDR-TB was first discovered in South Africa and subsequent surveillance data showed that its prevalence was low with considerable geographical variation (77,78). However, a report on a drug resistance survey revealed that the percentage of MDR-TB among TB cases in the country including the North West province has increased in the last 15 years with considerable geographical variations (79).

Geographical information systems (GIS) have therefore been increasingly used to map the clustering, spatial and temporal distribution of region specific TB and MDR-TB in different settings around the world (20). This information has given a clue to the disease distribution, transmission pattern, risk factors and evaluation of the interventions (20). It can also guide in the development of regional specific control measures for MDR-TB (20).

1.5.6 Spatial Analysis Methodology

The knowledge of where and when the transmission risk of a disease is high, and also where the cases are clustered, can give a clue to the underlying causes, and where the intervention can be targeted to prevent further spread and ultimately implement effective disease control (80).Various statistical techniques like hotspot analysis, cluster detection and regression have been explored to reveal the spatiotemporal distribution of different diseases. Some of these techniques are built into Geographic Information Systems (GIS) software packages and others are stand-alone programmes. Examples include SaTScan, GeoDa, Crime Stat, Clusterer and extensions of R. Anselin gave a recommendation after comparing the methodologies behind 4 spatial statistical techniques incorporated in four software packages (GeoDa, SaTScan, Crime stat and the open source statistical program in R), that Kulldorf spatial scan statistic and local Global Moran's I specifically, should be used jointly for disease clusters analysis (80). This study, has therefore explored the use of at least two analytical approaches, mainly spatial scan statistic and Global Moran's I statistic, to evaluate the spatiotemporal distribution of MDR-TB in North West province of South Africa.

1.5.6.1 Spatial Scan Statistic

The Kulldorf Spatial scan is a widely used spatial statistical technique for cluster analysis and the result is influenced by the methods and parameters applied. It has been used to study a variety of infectious diseases such as TB and Malaria, non-infectious diseases such as cancer and vector borne pathogens such as the West Nile Virus (80). It can be used to detect disease clusters in purely temporal, purely spatial or spatiotemporal settings. It generates a two-dimensional cylindrical window, with height representing the time and a circular geographical base for the space (81). The windows vary in sizes, from the smallest unit of analysis to the largest (user defined % of a population usually 50%) (81).

The cylindrical window scans for the disease clusters within it in space and time, comparing the observed and expected prevalence/incidence inside the window with that outside it (82). A p-value is assigned to windows with the maximum likelihood of being a cluster (primary clusters) (82). SaTScan also always describes other clusters that are similar to the primary clusters but with relatively low Log Likelihood Ratio (LLR) (secondary clusters). Secondary clusters give additional information about the primary clusters (81). The evaluation of the secondary clusters

is done by running the analysis without the data of the primary clusters to produce a geographically distinct cluster with a more homogenous cluster risk (83). This usually produces a ring of windows around a vacuum (83). The cluster shape can be specified as circular or elliptic. Circular windows are best used for small compact clusters while elliptic windows provide the greatest power with long narrow clusters (83).

The LLR for each cluster is formed from the comparison and a p value is assigned to it (82). Clusters with p values <0.05 and relative risk (RR) greater than one were regarded as clusters with more than expected risk of MDR-TB (82). In addition, the higher the LLR, the less likely the clusters formed were due to chance (82). The RR for each significant cluster is calculated from the ratio of the risk within and outside it for that area at that time (82).

The null hypothesis assumes the RR within the window is the same as that outside it, while the alternative hypothesis assumes they are different (81). Different probability models are used by the scan statistic depending on the data available. For example, count data on the number of Asthma cases with controls will use the Bernoulli model, without controls will use discrete Poisson model, without a population file will use the space time Permutation model. Multinomial data on cancer histology will use the Multinomial model; Ordinal model will be used for ordered categorical data; Exponential model will be used for survival time data and similarly the Normal model will be used for continuous data such as birth weight and blood sugar level (81). For this study, where the counts of MDR-TB cases are aggregated by facility, the Poisson discrete model will be used to evaluate the spatiotemporal distribution of MDR-TB cases by facility.

1.5.6.2 Global Moran's I

Global Moran's I statistic with Inverse Distance Weight (IDW) matrices is another spatial technique used to assess whether there is an aggregation of similarly high/low values in space of the entire study region. A significant positive spatial autocorrelation suggests that there is a clustering/aggregation in the spatial distribution pattern of the MDR-TB patient and that this distribution is not due to chance/random underlying spatial process (29). The IDW interpolation divides the study region into grid cells of MDR-TB density surfaces and identifies the neighbor count values around the point of interest. The interpolation is done by averaging the values of the nearest points in space and inversely weighting their distance to the point of interest (36,84). Thus, the influence of the neighboring point is inversely proportional to the its distance from the

point of interest (84). In most spatial studies, the Global Moran's statistic is used as an initial test for determining any kind of clustering in the study region. If the result indicates lack of clustering (Z value<1.96 and p-value>0.05), then it is often not necessary to carry on with the local statistics (Anselin's Moran's I) (36,84).

1.5.6.3 Anselin's Local Moran's I

This is a spatial statistical technique used to identify clusters of features with similarly high or low values in space in a specific location of the study region (84). It also identifies outliers by comparing a given feature to neighboring features and the mean of the entire population (84). The major advantage of this technique over the SaTScan statistic is its ability to explicitly identify outliers and also evaluate feature near edges that have fewer neighbors. This technique is also incorporated in the Arc tool box in the ArcGIS (84).

1.5.6.4 Getis-Ords Gi* statistic

The Getis-Ords Gi* statistic was used to examine the aggregation of the MDR-TB patient (local autocorrelation) at the facility level of the study region. This statistical technique is incorporated as a tool in the optimized hotspot analysis tool function of the ArcGIS. The Gi* statistic measures the ratio of the scale of analysis to the sum of all values (the ratio of the MDR-TB proportion of the facility to that of the whole study region) (85). Thus, when there is a large difference between the local sum and the expected local sum and that difference is unlikely to be due to chance, a significant Z score results (85). Also, the resulting clusters indicate a high/low values surrounded by other similarly high/low values (Hotspots/cold spots).

1.5.7 The Assay test for TB and MDR-TB

Xpert MTB/RIF assay is a test for the simultaneous detection of MTB DNA and RIF resistance mutations of the rpoB gene in a sample (sputum/sediments) (30,86,87). It uses five molecular beacon probes to detect DNA sequence that is amplified by a reverse transcriptase Polymerase Chain Reaction (rtPCR) (88). Probes are oligonucleotides labeled with differently colored fluorophore. Each of the five probes is complementary to a different target sequence of the rpoB gene of the wild type (RIF susceptible) MTB, such that hybridization (binding) only occurs with the DNA sequence of a wild type (RIF sensitive) MTB. Any mutation in the rpoB gene inhibits the hybridization (binding) of one or more rpoB gene specific probes which in turn leads to the reduction (delay) or elimination (dropout) of the signal (fluorescing) from that probe (88). Drug

resistant MTB strains have mutations in the rpoB gene which inhibits the hybridization of one or more probes. The Gene Xpert technology also has an internal control *B.globigii* assay hemi-nested with the MTB/RIF assay. This control assay must be positive (CT≤38) for a MTB negative result or positive/negative for MTB positive result for the result to be considered valid (88). The result of the assay test is interpreted using the cycle threshold value (CT), which is defined as the number of PCR cycles required for each probe to signal (fluoresce) (34,87). MTB is detected when at least two of the five probes signal with CT values that are ≤38 and differ by no more than two cycles from each other (32). RIF resistance is detected based on the difference between the CT value of the first probe (early CT) and the CT value of the last probe (late CT) (ΔCTmax) (32,34). RIF resistance is present when ΔCTmax is >3.5 cycles and absent if ΔCTmax is ≤3.5 cycles (32). The result is considered indeterminate if the first probe gives a CT of >34.5 and the last gives a CT >38 (the assay's terminating CT) because ΔCTmax of >3.5 cannot be measured (32).

Studies have shown that there is an inverse relationship between MTB DNA concentration (bacterial load) and the number of cycles needed for one probe to be positive (signal). If the concentration of MTB DNA (bacterial load) is very high, the number of cycles is very low and vice versa (34). A previous study found there is a 50% decrease in the MTB DNA amount for one cycle increase in the CT value (31,34). Previous studies have used CT as a semi-quantitative method for marking bacteria load (proxy for MTB burden) in a sputum sample (87). This is because it has demonstrated a strong correlation with microscopy and culture based methods for quantifying MTB DNA concentration (bacterial load) in sputum with a detection limit of 100-131 bacilli cfu/ml (31,34,89). Thus, the log linear relationship between CT values and MTB cfu is only valid for cfu between10^2 -10^7 cfu/ml of sputum. Just as the community viral load can be used to identify HIV high risk areas, community MTB load which is calculated as the median CT value for MDR-TB testing center over a period of time can be used to identify high risk areas for MDR-TB (90).

For TB diagnosis using Gene Xpert, suspected TB patients who have never received anti TB therapy before, nor have been on any kind of therapy in the last seven days, give two sputum specimens (at least with one early morning's specimen for greater yield) (86). The specimen sample is processed with the MTB/RIF sample reagent and then loaded into the Gene Xpert

machine for testing. There is an in-built sample processing control (SPC), to verify the bacteria processing and monitor any form of PCR reaction inhibitor. Hence a test is considered valid only if the SPC control is positive for a negative sample (no MTB) or positive or negative for a positive sample (MTB present) (30,86). There is also a Probe check control to verify reagent rehydration, probe integrity, PCR tube filling and dye stability. It passes the check if the probe signal meets a set criterion (30,86).

1.5.8 The Sensitivity and Specificity of the Xpert MTB/RIF assay

Studies confirmed the high sensitivity of Xpert to the presence of MTB and its high specificity to detect specific mutation conferring RIF resistance in sputum samples (31,32). An example is a study done in the United States, where Xpert sensitivity was assessed with 79 known phylogenetically and geographically diverse strains of MTB. Out of the 79 strains, 42 were drug susceptible isolates and 37 were RIF resistant with 13 different mutations on the rpoB gene (32). The same study also tested the specificity of the assay using 89 non-tuberculosis fungi, bacteria, and viruses. Their result showed that Xpert could correctly identify all 79 MTB isolates and excluded the 89 non-tuberculosis isolates (32). Also, RIF resistance was correctly identified in all 37 RIF resistant strain and undetected in all 42 RIF susceptible strains (32). The detection of specific mutations was found to be dependent on the mutation, and often requires between 65%-100% of the mutant DNA to be present for 95% confidence of RIF resistance (32). The same study found a constant linear relationship between the Xpert CT and the MTB cfu over different known concentration ranges of 10^2 to 10^7 cfu (32).

Another study was done to confirm the level of agreement between the two WHO endorsed probe based molecular susceptibility testing methods (Xpert and Genotype MTBDR*plus (*DR*plus)*) for the rapid detection of drug resistance TB. These two methods were compared against the gold standard LJ proportion method (LJ-DST). The result revealed that Xpert and DR*plus* have RIF resistance detection rate of 93.5% and 92.4% respectively (89) . Also, both methods rightly detect the molecular locus of the RIF Resistance Determining Region (RRDR) in 84 of the 92 tested samples. However, Xpert showed more accuracy (7 out of 8) over DR*plus* (none) in the detection of discrepant cases (89).

1.6 Research Question

What are the patterns and factors associated with spatiotemporal distribution of Xpert confirmed MDR-TB cases and Xpert probe dropout/delay from specific resistant mutations in the North-West province of South Africa (2011-2015)?

1.7 Research Hypothesis

Four research hypotheses have been proposed as stated below

Null Hypothesis 1: There is no clustering of MDR-TB cases in space and time in the North-West Province of South Africa from 2011-2015.

Alternative Hypothesis 1: There is clustering of MDR-TB cases in space and time in the North-West province of South Africa between 2011-2015.

Null Hypothesis 2: There is no active transmission between regions with high MTB bacterial load and regions with high crude rates of MDR-TB cases in the North-West Province of South Africa from 2011-2015.

Alternative Hypothesis 2: There is active transmission between regions with high MTB bacterial load and regions with high crude rates of MDR-TB cases in the North-West Province of South Africa from 2011-2015.

Null Hypothesis 3: There is no significant clustering of Xpert probe dropout/delay from specific resistant mutations in space and time in the North-West Province of South Africa from 2011-2015.

Alternative Hypothesis 3: There is a significant clustering of Xpert probe dropout/delay from specific resistant mutations in space and time in the North-West Province of South Africa from 2011-2015.

Null Hypothesis 4: There is no association between ecological factors and the distribution of MDR-TB cases in the North-West province of South Africa from 2011-2015.

Alternative Hypothesis 4: There is an association between ecological factors and the distribution of MDR-TB cases in the North-West province of South Africa from 2011-2015.

1.8 Aim

The aim of this study is to identify the MDR-TB hotspots, examine the spatiotemporal distribution of MDR-TB cases; Xpert probe drop out/delay and evaluate the ecological factors associated with the distribution of MDR-TB cases in the North West province of South Africa, 2011-2015.

1.8.1 Specific Objectives

1. To identify MDR-TB hotspots in the North West province of South Africa between 2011-2015.
2. To identify the hotspots of MTB bacterial load in the North-West province of South Africa between 2011 and 2015.
3. To map the spatial and temporal distribution of probe dropout/delay from MTB strains in MDR-TB hotspots in the province between 2011 and 2015.
4. To evaluate the ecological factors associated with the spatiotemporal pattern of MDR-TB burden in North-West province of South Africa between 2011 and 2015.

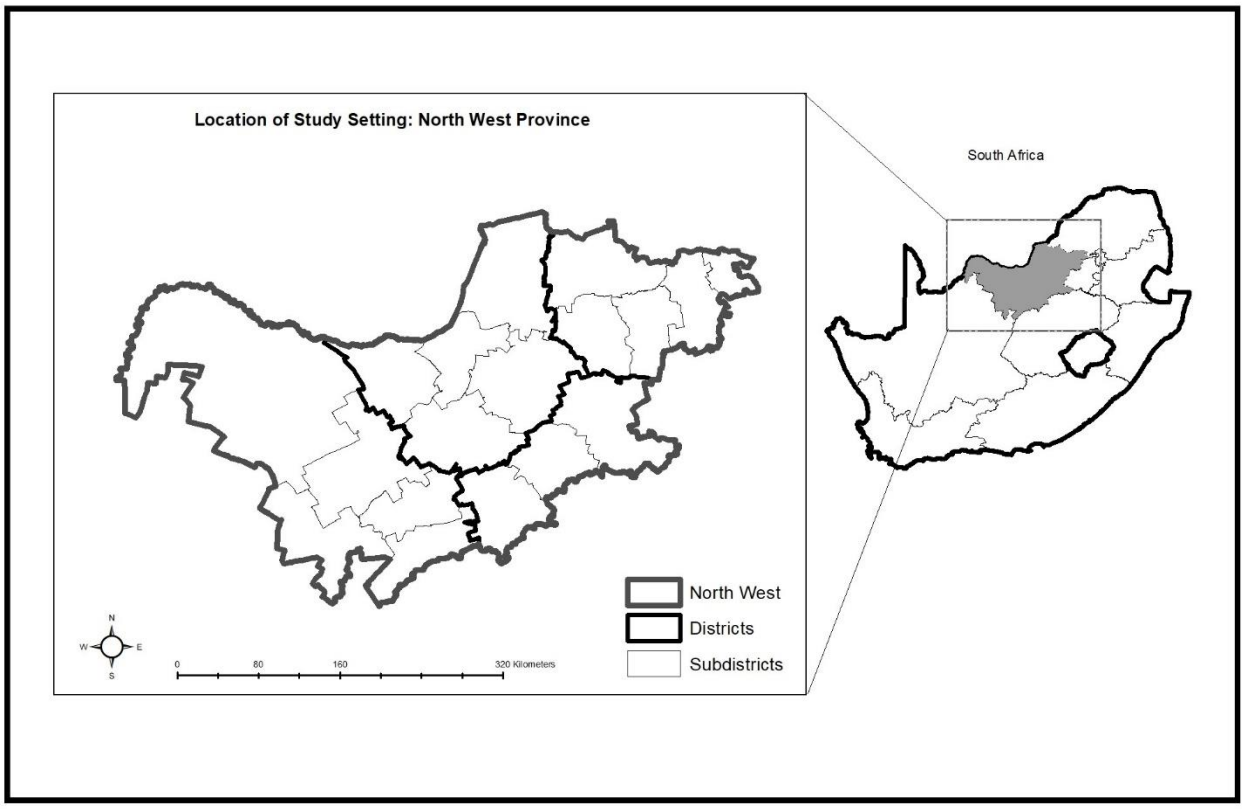
CHAPTER TWO – METHODS AND MATERIALS

2.1 Chapter Overview

This chapter describes the demography of North West province of South Africa, the study settings and design, the study population (MDR-TB cases) and their distribution at the facility and municipal level. It also explains how the data management and analysis was conducted for each objective.

2.2 Study Settings

The North West lies in the North of South Africa on the Botswana border. On the west of the province is the Kalahari Desert, Gauteng on the East and Free State to the South. For the most part the province consists of flat areas of scattered trees and grass land with the Vaal river flowing along the Southern part (91). It is the fifth largest province in South Africa, with a total land mass of 116,320km^2 which is about 9.5% of the total land mass of the country (92). The population increased slightly from 3.5 to 3.7 million between 2011 and 2015 (93), which accounted for about 6.7% of the total South African population according to the 2015 mid-year population estimates (94). Administratively, the North West province has four major districts which are Bojanala platinum district, Dr Kenneth Kaunda district, Dr Ruth Segomotsi Mompati district and Ngaka Modiri Molema district (95). The Bojanala district has the highest population of 1.7 million followed by Ngaka Modiri Molema district (889108) and Dr Kenneth Kaunda district (742 821). Dr Ruth Segomotsi Mompati district has the smallest population size in the province (459 358) (93). Mahikeng (formally Mafikeng) municipality in Ngaka Modiri Molema district, is the capital city of the province while Potchefstroom and Klerksdorp are the biggest cities of the province (91).


Location of Study Setting: North West Province
South Africa
North West
Districts
Subdistricts
0
80
160
320 Kilometers


FRANCISTOWN
THABAZIMBI
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Gaborone
LIMPOPO
BOTSWANA
MADIKWE GAME RESERVE
Northam
PILANESBERG GAME RESERVE
Mogwase
N1
Sun City
N4
Boshoek
Brits
Phokeng
Zeerust
Marikana
Terra Firma
Swartruggens
Rustenburg
Hartbeespoort
Mafikeng
Koster
Kroondal
PRETORIA
Lichtenburg
NORTH WEST
JOHANNESBURG
GAUTENG
Stella
N14
N12
Potchefstroom
Klerksdorp
Vryburg
Stilfontein
Schweizer-Reneke
Orkney
Wolmaransstad
Taung
Bloemhof
FREE STATE
N14
N12
N1
NORTHERN CAPE
BLOEMHOF DAM
Christiana
VAAL
VAAL DAM
0 20 40 80 KM
0 25 40 MLS 80
UPINGTON
KIMBERLEY
BLOEMFONTEIN
POLOKWANE
DURBAN

Figure 1: A hybrid physical/political map of the North West Province in the North West Province indicating the major cities, towns, game, nature reserves and national roads Extracted from https://www.sa-venues.com/maps/north-west-regional.htm

The number of households increased from 1.1 million in 2011 to 1.2 million in 2016 (93). The province form forms the southern part of the Kalahari Desert hence having almost a year-round sunshine. The temperature can range from 31 degrees Celsius during summer to 3 degrees Celsius in winter (93). Mafikeng, the capital city for the province has weather that is indicative for the whole of the province with the average rainfall of 300mm-700mm annually and an average humidity of 72% (96). The economic activities are mainly in the Southern region (Potchefstroom and Klerksdorp) and the Eastern Region in Rustenburg. The province's economy is centered round mining as it represents about one-quarter of the South African mining industry with Brits and the Rustenburg District producing more platinum than any other single area in the world. The province as a whole also produces one-quarter of the country's gold, marble, granite, fluorspar and diamonds. Other economic activities include tourism with international tourist attraction centres such as Sun City, Pilanesberg National Park, Madikwe Game Reserve and Rustenburg Natural Reserve. The province is also known for cattle farming. Rustenburg and Brits are fertile for mixed cropping and the area is a major producer of white maize in the country (91).

2.3 Study Design

This is an ecological study design using routinely collected National Health Laboratory Services (NHLS) secondary laboratory data of Xpert tested TB patients in North West province. The NHLS conducted the initial TB diagnostic test using the Xpert assay on sputum samples from suspected TB cases in the province. The test results were sent to a centralized data warehouse, in real time.

2.4 National Health Laboratory Services Gene Xpert roll out in South Africa

In 2011, South Africa inaugurated a health policy, to implement the use of Xpert TB/RIF assay to replace smear microscopy, as the initial test in the diagnosis of TB in all government laboratories (13). This policy was based on the evidence that Xpert TB/RIF assay, has a better sensitivity and will also provide an immediate RIF resistance result (97). The department of health of South Africa (National) collaborated with the NHLS to roll out the newly implemented

XpertMTB/RIF assay (98). This was done because of the high TB incidence rate, TB/HIV co-infection and the increasing burden of MDR-TB in the country. By the end of September 2013, 287 Gene Xpert instruments have been placed in 207 Health facilities both in the rural and the urban settings (98). Between March 2011 and April 2014, 3,300,544 tests were performed, and this accounted for over 60% of global cartridges procured (98). The NHLS conducted Xpert TB and RIF resistance tests on all sputum samples from suspected TB cases in the province. The test results were sent to a centralized data warehouse, in real time. So far, approximately seven per cent of all the Xpert positive tests for TB were also positive for RIF resistance (98).

2.5 Target population

The target population for the spatiotemporal distribution of both Xpert confirmed MDR-TB cases and Xpert probe dropout/delay are all the population of the North-West province of South Africa. This is because the dataset used for this study is at the North-West provincial level. In addition, the results from this study will inform decisions on the development of provincial specific intervention strategies for the North-West province.

2.5.1 Study Population and Sampling

All Xpert confirmed RIF resistant TB patients were included in the study as cases. These patients were tested for TB in the NHLS-collaborative TB testing sites in all the districts of the province, within the study period of 2011-2015. No sampling was done. Also, RIF resistant patients were considered as MDR-TB patients in agreement with the WHO recommendation (39).

2.5.1.1 Outcome Variable for Objective 1: Annual facility MDR-TB cases as indicated by the RIF resistance case count per year for each facility**.**

2.5.1.2 Outcome Variable for Objective 2: Annual facility median MTB bacterial load (NHLS_NW_2011-2015_v12, code facility burden minimum/mean).

2.5.1.3 Outcome Variable for Objective 3: Annual median facility probe dropout/delay from specific mutations (calculated as the difference between the first (early CT) and the last (late CT) Code ΔCTmax).

2.5.1.4 Outcome Variable for Objective 4: Annual municipality MDR-TB crude rates.

2.5.2 Eligibility criteria

2.5.2.1 Inclusion criteria for objectives 1 2 3 & 4: All patients that are positive to Xpert RIF resistance assay test, from any of the NHLS testing facilities in the North West province between the year of 2011-2015.

2.5.2.2 Exclusion criteria for objectives 2: All patients who have all probe CT values (A-E) missing in the assay test result were excluded for the bacterial load analysis.

2.5.2.3 Exclusion criteria for objective 3: Patients with indeterminate Xpert result of minimum CT value of >34.5 and the maximum CT value of >38 (assay's terminating CT), were excluded because ΔCTmax of >3.5 could not be measured (88). Also, patients with internal control assay result (*B.globigii*) CT value of >38 were excluded and also patients with all CT values missing were also excluded from the analysis (32).

2.5.3 Definition of terms

Probes: They are oligonucleotides with DNA sequence complementary to different target sequence of the rpoB gene in a wild type (RIF susceptible) MTB strain. Each of them is labeled with differently colored fluorophore which fluoresces (signals) when it hybridizes (binds) with its complementary target sequence of the repoB gene. The five probes overlap to cover the entire length of the 81bp of the repoB gene (30,88,99).

Cycle Threshold (CT): Is the number of PCR cycles required for each probe to signal (fluoresce) (34,87).

Probe dropout/delay: Is the complete elimination (dropout) or delay in the signal of a probe during assay. It is measured as the difference in the CT values between the first (early) probe and the last (late) probe (ΔCTmax) (32).

Polymerase Chain Reaction: It is a technique used for the amplification of small segments of DNA and RNA. It is very cost effective as it can make million copies in few hours.

***Mycobacterium tuberculosis* (MTB)**: Is the causative agent for Tuberculosis (TB) (35).

Bacterial Load: Is the number of the MTB DNA molecules present in the sputum sample of a patient (33).

2.6 Data Description and Management

This analysis was done using data from 3 sources. The NHLS data has variables on the patient test result for RIF resistance, the assay CT values (A-E) for each patient who tested, facility

names where patients are registered, laboratory where they tested, district and municipalities of facilities among others. Based on the result of the assay, there were 531,600 observations in 412 facilities with 14 unique values for RIF resistant test results (E, ERR, INV, MTBCN, NORES, NULL, PR, R, RIFR, RIFS, S, S+, U and Z). Only records of RIF resistant patients, (R and RIFR) were retained in the study, and this amounted to 2949 observations in 292 facilities. Other records were RIF susceptible or indeterminate results.

The District Health Information System (DHIS) had three datasets. The DHIS population headcount data has variables on the facility name, district names and the monthly population allocated to this facility from 2011-2015 in a wide format. The DHIS coordinate file has variables on the unique identifier (UID) and the coordinates (longitude and latitude) of all the facilities in North West province in a long format. The DHIS UID file has variables on the facility UID and facility name. To generate a complete population dataset with all the variables from all DHIS datasets, the UID dataset, was first merged with the coordinate dataset on the key variable "facility UID", to join the facilities names to their coordinates. The population file, was reshaped to long format and merged with the coordinate file using m:1 merging on the key variable facility name. This added in the facility monthly headcount variable. The "testing year" variable, was generated from the year of the test date and the annual headcount per facility, was generated from the average of all monthly population estimates. The final population dataset has variables on the facilities names, coordinates, UID, the annual and monthly headcounts per facility. For all objective specific analysis, the NHLS dataset was merged with the population file using the facility name and year as key variables. Also, for all the maps (rates, spatial and spatiotemporal distribution), merged data was exported as an excel csv file format from STATA, and imported into ArcGIS using the facility geocodes.

Statistics South Africa (STATSSA) 2016 community survey data has data on the demography of the municipality including proportions of males, females, youth and socio economic variables such as the number of households with access to electricity, pipe borne water as shown in Table 1.

Table 1: NHLS, DHIS and STATSSA variables definition.

Data set	Variable	Variable definition
NHLS Assay Data Variables	Year	The year when cases tested
	Municipal Mean age	The annual mean age of all cases in a facility
	Municipal Probe dropout/delay	The annual median difference between the CT values of the first and the last probe for each facility
	Median District bacterial load	Minimum bacterial load $=10^{(ct_min-39.09)/(-2.9865)}$
	CT values of probe set A-E	Cytochrome threshold of Xpert CT values of the probes
	RIF resistant	All RIF resistant test results of patients
DHIS Variables	Monthly Head count	The total population of people living in the vicinity of a facility in a month
	Facility coordinates	The longitude and latitude of a facility for location on earth surface
	Facility UID	System generated unique identifier for all the health facilities in the province
2016 Community survey population estimates	Male youth	Weighted proportions of Males between age 15-35
	Female youth	Weighted proportions of Females between age 15-35
	Youth proportion	Weighted proportions of persons between age 15-35
	Total youth	Weighted sum of all persons between age 15-35
	Male total	Weighted proportions of males by biological status
	Female total	Weighted proportions of females by biological status
	Total population	Weighted sum of all Males and Females
	People who completed grade 12 at age 20	Weighted proportion of persons who completed grade 12 at age 20

Table 1 continued

Data set	Variable	Variable definition
2016 Community survey socio economic variables	Total Household	Total number of surveyed houses
	Household Size	Weighted average of the number of persons living in a house of all the surveyed houses
	Proportion of household in formal house	If the main dwelling that a household currently live in is a structure built according to government approved plan.
	Proportion of household in traditional dwelling	If the main dwelling that a household currently live in is a house made of clay reeds and other locally made materials.
	Proportion of household in Informal dwelling	If the main dwelling that a household currently live in is a structure erected not according to government architectural plan.
	Proportion of household in other kind of dwelling Others	If the main dwelling that a household currently live in does not fall in any of the above dwelling unit
	Access to pipe born water	Weighted proportions of household which have access to pipe borne water
	No Access to pipe born water	Weighted proportions of household which do not have access to pipe borne water
	Flush toilet	Weighted proportions of household with access to Flush toilet in their main dwelling
	Other source of toilet sanitation	Weighted proportions of household which do not have access to Flush toilet in their main dwelling
	Connection to Electricity	Weighted proportions of households which have access to electricity in their main dwelling
	Connection to other sources of Electricity	Weighted proportions of households which do not have access to electricity in their main dwelling

2.7 Data Analysis

Data was analyzed using STATA version 12, SaTScan version 9.4.and ArcGIS version 10.2.1 (ESRI, Redlands, CA, USA).

2.7.1 MDR-TB hotspots in the North West province between 2011-2015

2.7.1.1 MDR-TB incidence rates and Interpolation

To calculate the cumulative incidence rate of MDR-TB, the annual district and municipal MDR-TB number of cases was generated by aggregating cases in each district and municipality per year respectively. The aggregated MDR-TB count at each level was divided by the annual population head count at that level and multiplied by 100,000 to obtain rate per 100,000 inhabitants. The district crude rate was calculated as shown in the formula below.

$$\text{District crude rate per 100,000 inhabitants} = \frac{\text{District annual MDR-TB cases}}{\text{District annual population}} \times 100{,}000 \quad (29{,}100)$$

The crude rates of MDR-TB at the district and municipal level was plotted on map to identify district and municipalities with the highest rates of MDR-TB and likely to be identified as hotspots by the cluster analysis.

The annual facility MDR-TB crude rates map was observed using IDW interpolation technique. The number of years that a facility had greater than expected rate of MDR-TB cases was also drawn on the same map. The integrated map of both inputs (annual MDR-TB incidence and number of years with greater than expected rate of MDR-TB) describes the distribution of facilities that are likely to be hotspots of infections in the province (80).

2.7.1.2 Pure Spatial Cluster Analysis of annual MDR-TB Incidence rates

The NHLS MDR-TB data was geo-referenced using ArcGIS (ESRI, Redlands, CA, USA). The annual facility MDR-TB cumulative incidence rate/100,000 inhabitants, was displayed on the South African shape file using the longitude (X-coordinates) and latitude (Y-coordinates). All features were defined and projected in the Global Coordinate System (GCS_WGS_1984).

Global Moran's I with row standardized inverse distance weight matrices, was used to assess if there are any MDR-TB hotspots in the study region without necessarily indicating where the hotspots occur (29). Then Anslin local Moran's I, was used to indicate where in the region significant hotspots of MDR-TB cases were located. Getis Ord General G was also used to

identify significant hot and cold spots at different significance levels (99%, 95% and 90% CI). Higher CI indicate a more presumable aggregation of hotspots/cold spots (29). The results were compared with those of the spatiotemporal analysis using SaTScan methodology for the MDR-TB cases. Facilities identified by both statistical methods as hotspots were defined as "Strong evidenced high risk facilities" (101). Those identified by one of the methods are defined "high risk facilities" (101).

2.7.1.3 Spatiotemporal analysis of Annual MDR-TB Incidence rates

To evaluate the temporal trend in the spatial distribution of cases, SaTScan was used to examine the spatiotemporal distribution of annual rates of facility MDR-TB cases. The probability model was specified as the discrete Poisson model because the study was based on a retrospective space-time analysis of a four-year period MDR-TB cases count data without controls, or any other patient level risk factors (81). Maximum cluster size is a major parameter that affects the output of spatiotemporal analysis. Different studies used different cluster sizes (50%/25% of the population at risk), to optimize the detected clusters in order to produce easily interpreted clusters/hotspots that are distinct (81,101). Some other studies used a range of scales for the cluster size and visualize the output to know which cluster size produced the best outputs on the map (80,83). This method of determining maximum cluster size is subjected to pre-selection error (81). However, a study proposed a methodology to determine the optimum cluster size (80). It suggested an optimum cluster size as those producing clusters covering most regions with greater than expected risk of the disease under study. In that light, the maximum cluster size for this objective was imputed as 50% of the population, but the maximum reported cluster size, reported cluster size that are 20% of the population at risk. The 20% cluster size provided the best coverage of facilities with greater than expected rate of MDR-TB. Facilities with greater than expected rate of MDR-TB were determined from the observed rate and expected rate calculated as below:

$$\text{Observed rate} = \frac{\text{Annual facility MDR-TB cases}}{\text{Annual facility head count}}$$

$$\text{Expected rate} = \frac{\text{Annual MDR-TB cases for all facilities}}{\text{Annual head count of all facility}}$$ (This is based on the assumption of random distribution of cases in the study area)

A facility has greater than expected rate of MDR-TB when observed rate is greater than expected rate. A binary variable of zero and one was generated, allocating one to any facility with greater than the expected rate and a zero to a facility less than the expected rate. This method was found from literature (80).

For the number of Monte Carlo replications parameters, 999 was selected for increased statistical power. The higher the value, the greater the statistical power of the analysis. The limitation is that beyond the 999 values, the statistical power only marginally increases and also delays the run time of the analysis especially for large dataset (81).

2.7.2 Hotspots of median facility Bacterial load

The Cycle threshold of the Gene Xpert, has demonstrated a strong correlation with microscopy and culture based method for quantifying MTB in sputum with a detection limit of 100-131 bacilli cfu/ml (31,34,89). How CT is used to quantify MTB burden is fully discussed in the literature (31,32,34). In 2015, Scott Lesley and Van Rie proposed that, as the community viral load can be used to identify HIV high risk areas, community MTB load taken as the median CT value for MDR-TB testing center over a period of time can be used to identify high risk areas for MDR-TB (90).

For the analysis the NHLS data was merged with the population headcount data and the 577 patients in 153 facilities with missing values for all probes were deleted. The CT minimum variable and the CT mean variable was generated from the individual minimum CT values of probe set A-E and the average of all the CT values of probe set A-E for each case respectively. The rationale is to calculate the median facility bacterial load from both the minimum and the average CT values for comparison. The result from a studies, indicated that the first CT value (the minimum CT) is unlikely to be due to resistance of any kind, thus more appropriate to quantify the number of MTB cells than the average of the CT values (30,87). The individual bacterial load was generated from the minimum/mean CT value of probe set A-E as given by the formula below in the literature (32).

$$\text{Minimum Bacterial load} = 10^{(\text{ct_min}-39.09)/(-2.9865)}$$

$$\text{Average Bacterial load} = 10^{(\text{ct_mean}-39.09)/-2.9865)}$$

'ct_min' and 'ct_mean' represent the minimum and average CT values for each case respectively. To compute the annual facility bacterial load, the median of all the individual bacterial load in that facility for each year was first computed (bacterial load is not normally distributed), and then multiplied by the annual aggregated absolute number of cases in the facility in the same year as shown in the formula:

$$\text{Facility bacterial load} = \frac{(\text{Number of RIF resistant cases per facility per year})}{\text{head count of the total number of people the facility is servicing}} \times \text{Median Bugload}$$

The Global Moran's I was used to check for hotspots using the facility bacterial load as the input parameter. The incremental spatial autocorrelation (ISA) using ArcGIS was then used to determine the scale (increasing distance) at which spatial autocorrelation was maximized which is the distance with peaks in Z scores. This tool performs many Global Moran's I statistic to check for the intensities of clusters at each increasing distance (29,85). The rationale for this objective is to compare the spatial distribution of the facility bacterial load to that of the MDR-TB hotspots. If facilities with very high bug load are clustered and share the same spatial pattern with places of high MDR-TB hotspots, then it is reflective of direct active transmission, from highly infectious individual within and between facilities (90).

2.7.3 Spatial and spatiotemporal distribution of probe dropout/delay

2.7.3.1 Spatial analysis of Probe dropout/delay

Median facility Xpert probe dropout/delay (ΔCTmax) caused by genotypes of specific MTB strains, was used as a proxy for transmission of specific resistant mutations at the facility level. Xpert probe dropout/delay was calculated from the difference between the early and the late CTs for individual patients (31,32) The calculation and interpretation of Xpert probe drop out/delay as a proxy of specific mutation was fully discussed by literature (31,32).

The CT maximum variable was generated as the highest CT values for probes set A-E for each case and ΔCTmax was then computed as the difference between the maximum and the minimum CT per case. This annual facility median probe dropout/delay was calculated as the median of the all individual ΔCTmax in each facility for each year. Clusters of specific mutations were taken as any two facilities with similar ΔCTmax (29). Global Moran's I was used to identify hotspots of probe dropout/delay using the ΔCTmax calculated as the input parameter. The rationale behind

this objective is to identify all possible MTB with specific resistant mutations that are locally transmitted within municipalities and also clusters of facilities that are potentially linked by direct transmission of specific genotypes/mutations.

2.7.3.2 Spatiotemporal analysis of Probe drop out/delay clusters

The spatiotemporal analysis with SaTScan statistic was done using ΔCTmax as the input parameter. For this objective, only the hotspot (places of greater than expected risk) of similar probes dropout/delay was computed, and the space time probability model was used. This is because the outcome of interest is any similar probe dropout/delay resulting from specific mutations which is independent of the underlying population at risk. The spatiotemporal analysis of probes was done to identify all possible clusters of probe within the study setting and also to identify possible MTB with specific resistant mutations that are driving the localized transmission between facilities in MDR-TB hotspots.

2.7.3.3 Most likely Probe dropout/delay

To identify the specific probe most likely driving the transmission within MDR-TB hotspots, a spatiotemporal hotspot analysis for each probe dropout/delay that appeared in more than ten facilities and present in each of the clusters for similar probe dropout/delay was done (22). Any probe that has its most likely cluster located within the MDR-TB hotspots has the highest probability of being directly transmitted between hotspot facilities (22). This was based on the assumption that, given the random effect of that particular probe across the study region, it is most likely to occur within that cluster (81). An integrated map of similar probe dropout/delay and that of the most likely probe was plotted only within regions identified as MDR-TB hotspots.

2.7.4 Objective 4: Ecological factors associated with the distribution of MDR-TB

The NHLS data was merged with the 2011/2016 STATSSA community survey data on the key variables, municipality name and year. The survey data has weighted individual and household variables aggregated to the provincial, district and municipality level, so that the sample estimates are representative of the general population (102).

It is of note that the 2011/2016 community survey data only has population estimates for 2010 and 2015. For 2011 2012, 2013 and 2014, the formula for calculating inter-survey population increase which assumed a constant annual population growth rate was used. There South African

community surveys were done in 2010 and 2015 which was a period of five years. Thus the annual inter-survey increase between 2010 and 2015 was calculated using the formula below.

$$\frac{\text{2015 population estimates} - \text{2010 population estimates}}{5}$$

The annual change was added arithmetically to 2010 population estimates to get that of the unavailable years (103–105). This principle is not applicable to the non-population estimate socio-economic variables (Table 1) hence the second model was restricted to the municipality level variables (NHLS, socio economic and population estimates) for 2015 only.

2.7.4.1 Regression analysis

Univariable analysis was used to assess the independent association between municipality level variables and municipalities MDR-TB incidence rate. Stepwise regression at probability <0.2, was used with the negative binomial regression (as an alternative to Poisson regression) to identify variables that will be included in the multivariable analysis. A Negative binomial regression was used because the outcome variable across municipalities violates the assumption of constant mean and variance (annual municipalities MDR-TB rates was over dispersed)

Forward stepwise model building techniques were used with the zero-truncated Poisson regression to evaluate the association of a variable while adjusting for other ecological variables (Table 1) . The best fitted model was selected based on Akaike Information Criterion (AIC). The model with the lowest AIC statistic was taken as the best fitted model. Two models were fitted; the first was fitted with all the municipal level variables except for the survey socio economic variables (Table 1) from 2011-2015. The second model was fitted for all municipality level variables (NHLS, population estimates DHIS and socioeconomic variables) for 2015 only.

CHAPTER THREE – RESULTS

3.1 Introduction

This chapter describes the spatial and spatiotemporal distribution of MDR-TB crude rates in the districts and municipalities of North West province from 2011-2015. It also evaluates the ecological factors that are associated with municipalities risk for more MDR-TB cases.

3.2 MDR-TB hotspots in the North West province between 2011-2015

3.2.1 MDR-TB distribution, incidence rates and Interpolation

A total of 2949 RIF resistant cases were reported by the NHLS testing sites from 2011-2015 in the North West province. Dr Kenneth Kaunda district had the highest number of 1288 (44%) MDR-TB cases, which was followed by the Bojanala platinum and the Ngaka Modiri district with 761 (26%) and 900 (18%) cases from 2011-2015. Dr Ruth Segomotsi district had the lowest number of 368 (12%) cases from 2011-2015. The expected annual incidence rate of MDR-TB in the entire province from 2011-2015 was 139.4/100,000 inhabitants. Dr Kenneth Kaunda district had the highest rate of 193.2/100,000 inhabitants (Figure 2), while Dr Ruth Segomotsi district had the second highest rate across the study period. The lowest rate of 112.6/100,000 inhabitants was from Bojanala Platinum district.

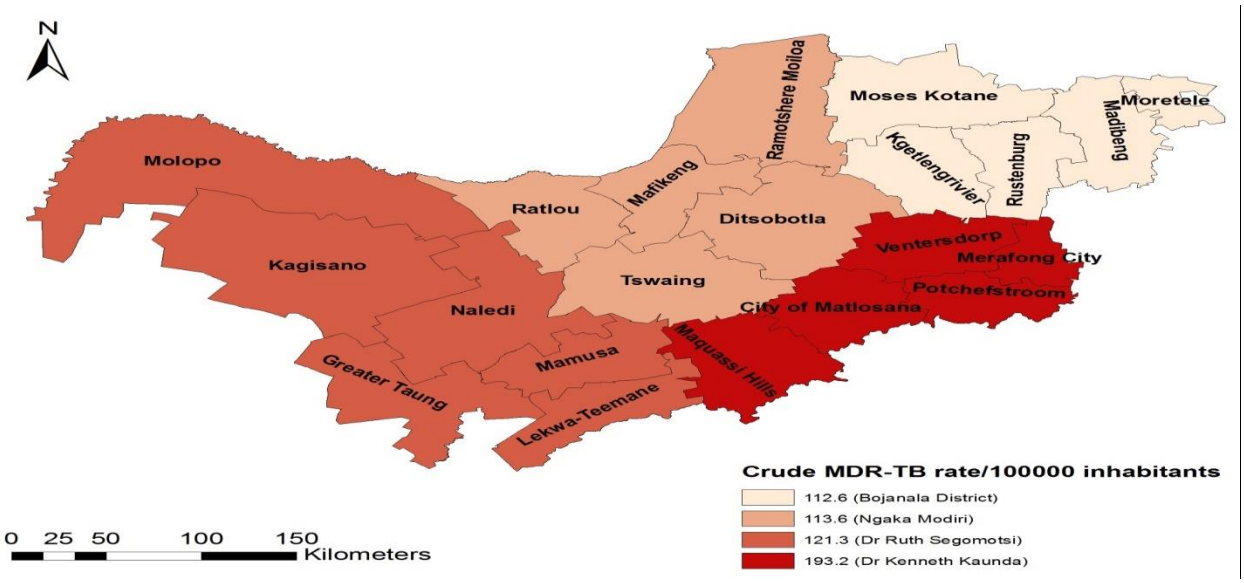


Figure 2: Spatial distribution of annual MDR-TB crude infection rate by district, North West, 2011-2015

At the municipality level, the City of Matlosana municipality alone had the highest MDR-TB rate of 223.7/100,000 inhabitants across the study period. Figure 3 indicates that the very high rate seen in the Dr Kenneth Kaunda district was majorly contributed by the City of Matlosana and Maquassi hills municipality, because they are surrounded by other municipalities with relatively low rates of the infection. The same was seen for Dr Ruth Segomotsi district where Greater Taung municipality in the South Western region contributed majorly to the second highest rate seen in the district. Other municipalities (figure 3) had relatively low rates of MDR-TB across the study period.

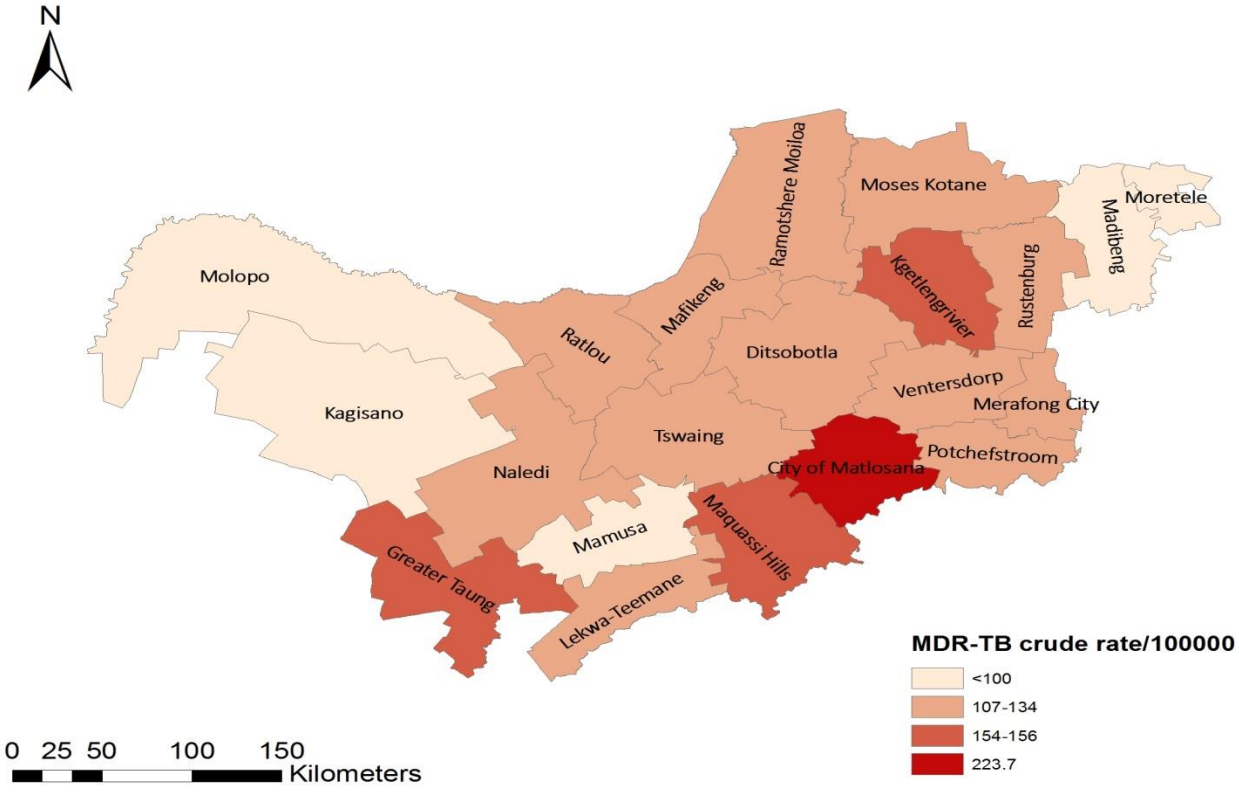


Figure 3: The distribution of annual MDR-TB crude infection rate by municipality, North West, 2011-2015

Also from the IDW interpolation results of the annual facilities incidence rates, the highest rates (240.43-1702.61/100,000 inhabitants) were from facilities in the City of Matlosana municipality, the central of Kgetlengrivier municipalities, part of Moses Kotane and Maquassi hills municipalities (Figure 2). In contrast, Tswaing, Mamusa and Molopo municipality had the

lowest MDR-TB rates (23.08-124.46/100000 inhabitants). Most of the municipalities in the high rate regions, had facilities with greater than expected rate of the infection for more than one year (Figure 2). A typical example is the City of Matlosana where most of the facilities had greater than expected rate of MDR-TB across the study period.

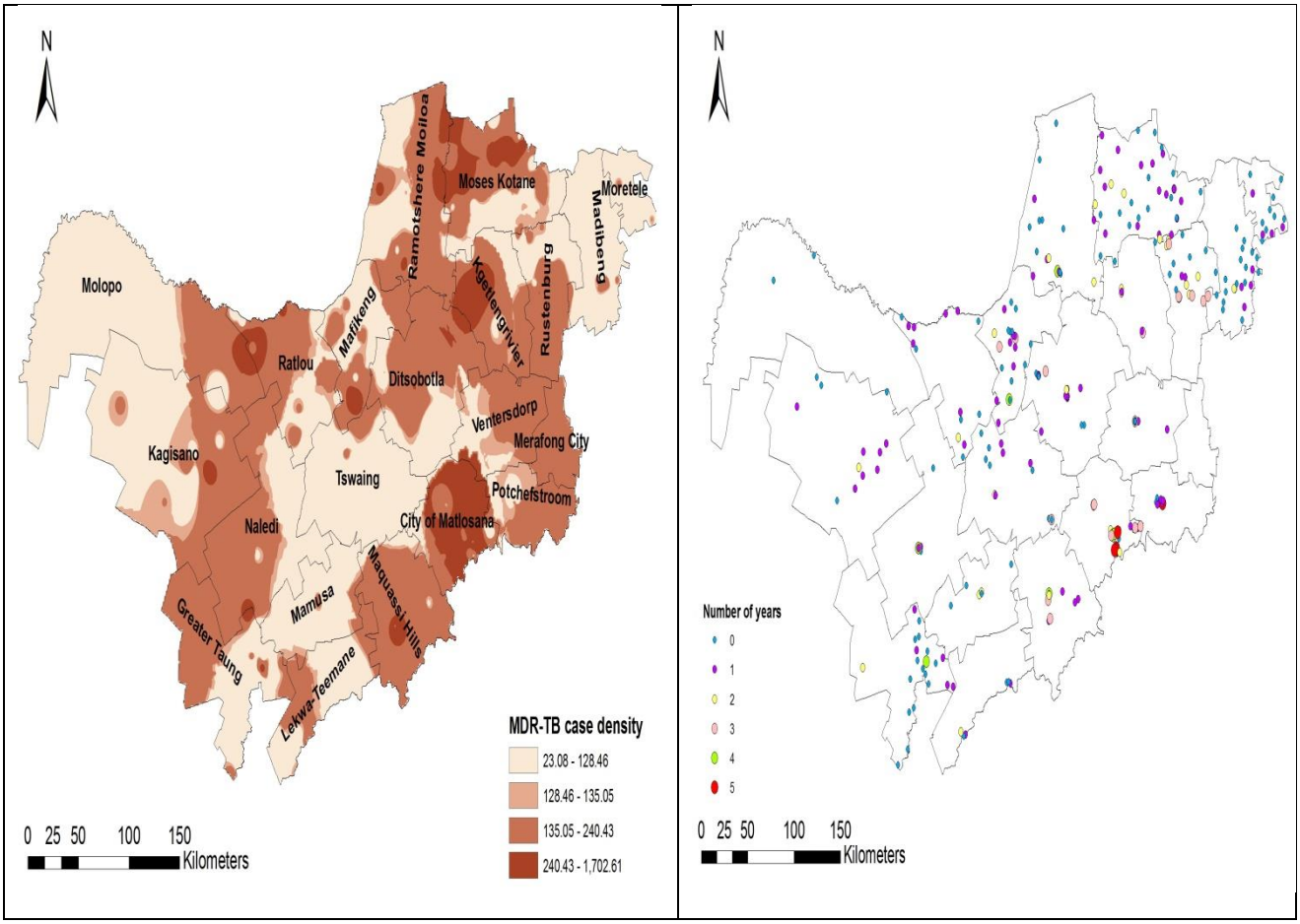


Figure 4: Integrated map of the facilities annual crude infection rate using IDW and facilities number of years with greater than expected rate of MDR-TB

The number of MDR-TB cases increased from between 2011 and 2014, with a slight decline from 2014 to 2015 (Table 2). The crude infection rates showed an increase year after year from 3893/100,000 inhabitants in 2011 to 5,455/100,000 inhabitants in 2015 which accounted for

about 98.438% increment. In contrast, temporal analysis of the SaTScan did not indicate any temporal clusters considering the underlying population at risk at one year increment.

Table 2: Temporal Distribution of the annual rates of MDR-TB cases

Years	Number of NHLS testing facilities	Population at risk	Number of MDR-TB cases	MDR-TB crude rates per 100,000 inhabitants
2011	35	142071	199	3893
2012	79	275469	393	4435
2013	184	530321	690	5038
2014	226	588002	911	6175
2015	208	568283	756	5455

3.2.2 Spatial Cluster Analysis of annual MDR-TB Incidence rates

The result of the Global Moran's I indicated significant MDR-TB hotspots with Moran's index of 1.19, Z-score of 37.69 and p-value of <0.001. Thus, the distribution of MDR-TB cases in the province was significantly more spatially clustered than to be due to chance or a random effect. From 2011 to 2015, the local Moran's I identified six high risk facilities as significant hotspots. Four of which were also identified by Kulldorff's spatial scan statistic.

Table 3: List of clustered facilities by the Moran's I statistic (Anselin)

Facility Name	District	Sub-District	Year	Annual Burden	Strong Evidence High risk facility
Taung Gateway Clinic	Dr Ruth Segomotsi Mopati	Greater Taung	2014	27	Yes
Nic Bodenstein Hospital	Dr Kenneth Kaunda	Maquassi Hills	2015	187	Yes
Tshepong Hospital	Dr Kenneth Kaunda	City of Matlosana	2015	1694	Yes
Thusong Community Hospital	Ngaka Modiri Molema	Ditsobotla	2013	233	No
Zeerust Hospital	Ngaka Modiri Molema	Ramotshere Moiloa	2012	154	No
Montsana Clinic	Bojanala Platinum	Moses Kotane	2014	407	No

Table 3 shows the six facilities identified by the local Moran's I spatial statistic with the first three being the "strong evidenced high risk facilities" because they were also identified as hotspot by the SaTScan statistic (101). The other facilities were randomly distributed in the study area within the study period.

3.2.3 Spatiotemporal analysis of annual MDR-TB Incidence rates

Sixteen clusters were detected in the province across the study period (Table 5). Six of these were significant hotspots and 3 of the hotspots (1 5 & 8) span from the South western region to the South Eastern region of the province. These were strong evidenced hotspot because they were also identified by the spatial analysis as hotspots within the same time and space. The other three (4 7 & 3) were just high risk clusters. Eight of all the clusters detected had 0km radius and were not visible on the map (Figure 3). Cluster 1 which was the most likely cluster had the highest log-likelihood ratio (LLR) of 869.46 ($p< 0.001$) in the City of Matlosana municipality from 2011-2015. It only revolved round one facility (Tshepong clinic). The risk of MDR-TB in the region covered by the cluster 1 was 19.06 as compared to the other regions of the province. In other words, people in this cluster are 19.06 times as likely to have MDR-TB compared to people in other parts of the province.

Other clusters are secondary clusters arranged in the order of LLR and statistical significance. Cluster 4 and 5 had elevated risk ratio (RR) of 1.92 and 1.63. They revolved round 4 and 17 facilities from 2011-2015 and 2013-2015 respectively. Cluster 5, revolved round part of Maquassi Hills and City of Matlosana municipalities, while cluster 4 revolved round the middle region of Rustenburg municipality. Cluster 7 in the Tlokwe City Council revolved round the Potchefstroom hospital from 2012-2013 with elevated risk of 3.07. Cluster 8 in the Greater Taung municipality revolved round the Taung Gateway clinic and the Taung Hospital from 2014 to 2015 respectively. This cluster had an elevated risk of 2.68. Cluster 10 was across the Moses Kotane Hospital with an elevated risk of 3.39 from 2014 to 2015. The significant cold spot clusters of <1 RR spread across the remaining part of the study region.

Table 4: Clusters of annual MDR-TB by SaTScan

	Cluster	Number of facilities clustered	Time frame	Radius (KM)	Observed cases	Expected cases	RR	LLR	P-value
The most likely cluster	1	1	2011-2015	0.00	452	27.75	19.06	869.45	<0.001
	2	30	2011-2015	64.94	131	291.91	0.42	60.72	<0.001
	3	6	2011-2015	14.22	110	238.32	0.44	46.26	<0.001
	4	4	2011-2015	10.04	125	65.02	1.96	22.35	<0.001
	5	17	2013-2014	49.69	201	123.12	1.68	21.72	<0.001
	6	24	2011-2015	52.69	155	244.68	0.61	20.39	<0.001
	7	1	2012-2013	0.00	44	14.46	3.07	19.58	<0.001
Secondary clusters	8	2	2014-2015	0.00	51	19.25	2.68	18.12	<0.001
	9	22	2011-2015	53.30	156	240.04	0.63	18.10	<0.001
	10	1	2014-2015	0.00	33	9.83	3.39	16.89	<0.001
	11	19	2011-2012	63.77	8	35.30	0.22	15.55	<0.001
	12	27	2012-2013	93.68	21	47.71	0.44	9.59	0.14
	13	1	2011-2015	0.00	7	24.23	0.29	8.59	0.32
	14	1	2014-2015	0.00	14	3.80	3.7	8.07	0.44
	15	1	2014-2015	0.00	14	3.93	3.58	7.73	0.55
	16	All	2014		911	814.82	1.17	7.67	0.58

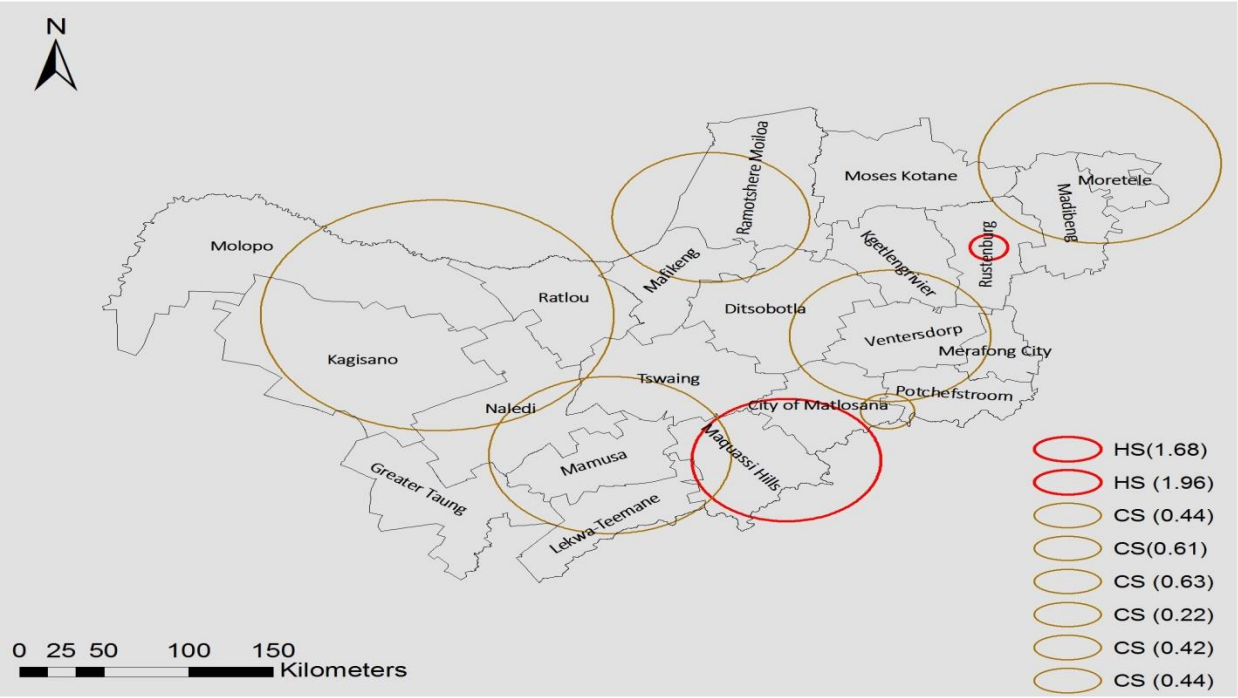


Figure 5: Six spatial (point layer) and eight visible spatiotemporal clusters (circle layers) identified by local Moran's (Anselin) and the SaTScan statistic respectively.

3.3 Hotspots of Median Facility Bacterial load

As shown by the result output (Appendix III), there was no evidence of significant clustering for both the average and the minimum facility bacterial load as indicated by the Moran's I index of 0.022060 given the Z score of 0.56 (P-value 0.58) and Moran's index of 0.0029 given the Z score of 1.29 (p value 0.89) respectively. This is an indication that facilities with similarly high or low MTB bacterial load were not clustered. The incremental spatial autocorrelation results (Appendix III) were consistent with the Global Moran's I results for the average and minimum facility bacterial load. There was also no distance with peak Z scores.

3.4 Spatial and spatiotemporal distribution of probe dropout/delay

3.4.1 Spatial analysis of Probe dropout/delay using Moran's I statistic

The spatial analysis of the probe dropout/delay using the Global Moran's I, did not indicate any significant clustering across the study period as shown in the table below. In contrast, 2015 result showed significant cold spot of probe dropout/delay. This was interpreted as there was no cluster of similar probes in the province that is significantly different from the others in space even

though there were possibilities of pockets of similar probes in different places driving transmission (85).

Table 5: Moran's results for pure spatial analysis of median facility probe dropout/delay

Year	Moran's Index	Z-scores	P-Values
2012	-0.061796	-0.220799	0.825
2013	-0.24074	-0.38113	0.70
2014	-0.3964	-0.942581	0.35
2015	-1.266781	-2.969757	0.00
All	-0.252596	-0.720686	0.47

3.4.2 Spatiotemporal analysis of Probe dropout/delay clusters using scan statistic

Four significant hotspots of similar probe dropout/delay were detected and they all together revolved around 39 facilities as shown in Figure 5 (left). All except one (cluster 8), were spread from the South to the South Eastern region of the province (Figure 5). Cluster 8 covered the North Western region of the Mafikeng municipality as shown (Figure 5). Twenty nine (74%) of the cluster period, were in 2012, while 5% (2 facilities in cluster 5) occured in 2011. Thirteen (33%), of all the facilities included in the clusters, were from City of Matlosana municipality, ten (26%) from Potchefstroom municipality, five (12%) from Maquassi hills municipality, 4 (10%) from Mafikeng municipality and the remaining seven (18%) revolved round Ventersdorp and Tswaing municipalities. Table 6 shows facilities and the time period of each cluster. The similar probe dropout/delay clusters identified all possible potential similar probe dropout/delay from MTB strains that were directly transmitted between the facilities within the clusters. Out of the seven municipalities consisting of facilities with significant MDR-TB hotspots only three (City of Matlosana, Potchefstroom and Maquassi Hills), showed evidence of probe dropout/delay clusters, others did not. The probe dropout/delay cluster was likely due to transmission of the same MTB strain between hotspot facilities.

Table 6: Characteristic of the spatiotemporal clusters of probe dropout/delay by SaTScan

Cluster number	Municipality name	Facility name	Facility median	Year

			probe dropout/delay	
	City Of Matlosana	Empilisweni Clinic [Jouberton]	13	2012
	City Of Matlosana	Park Street Clinic	16	2012
	City Of Matlosana	Duff Scott Memorial Hospital	17	2012
	City Of Matlosana	Tshepong Hospital	20	2012
	City Of Matlosana	Grace Mohomomo Clinic	20	2012
	City Of Matlosana	Tsholofelo	21	2012
1	City Of Matlosana	Stilfontein Manucipal Clinic	23	2012
	City Of Matlosana	Jouberton Clinic	24	2012
	City Of Matlosana	Nm Pretorius Gateway Clinic	27	2012
	City Of Matlosana	Orkney Municipal Clinic	27	2012
	City Of Matlosana	Majara Sephapha Dist Clinic	27	2012
	City Of Matlosana	Alabama	28	2012
	City Of Matlosana	Kanana Clinic [Orkney]	28	2012

Table 6 continued

Cluster number	Municipality name	Facility name	Facility median probe dropout/delay	Year
	Tlokwe City Council	Potchefstroom	7	2011
	Tlokwe City Council	Lesego Clinic	6	2013
	Ventersdorp	Jb Marks Community Centre	11	2011
	Tlokwe City Council	Potchefstroom Prison	16	2012
	Ventersdorp	Ventersdorp Gateway Hospital	4	2013
	Ventersdorp	Ventersdorp Hospital	14	2014
	Ventersdorp	Vernersdorp Hospital	17	2012
5	Ventersdorp	Jb Marks Community Centre	20	2012
	Tlokwe City Council	Potchefstroom Mun Clinic	13	2013
	Tlokwe City Council	Potchefstroom Mun Clinic	14	2014
	Tlokwe City Council	Potchefstroom Gateway Clinic	20	2012
	Tlokwe City Council	Mohadin Clinic	24	2012
	Tlokwe City Council	Rysmierbult Mobile Clinic	26	2012
	Tlokwe City Council	Rysmierbult	26	2012

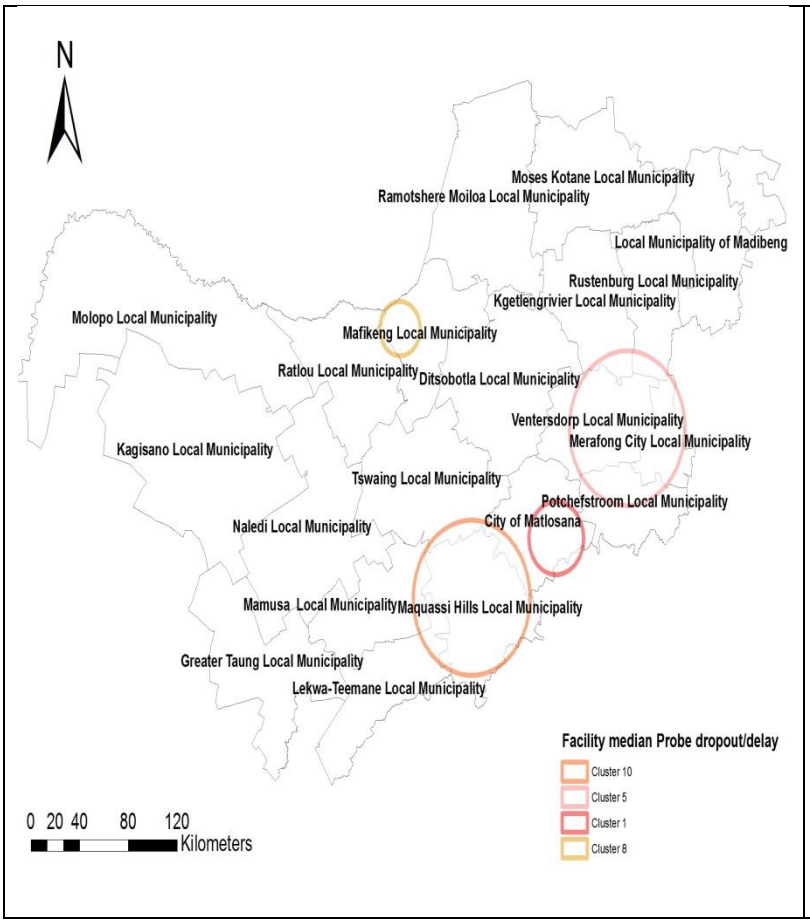
	Tlokwe City Council	Boiki Thlapi	27	2012
8	Mafikeng	Unit 9 Clinic	17	2012
	Mafikeng	Lesoko Clinic	28	2015
	Mafikeng	Motlhabeng Clinic	29	2013
	Mafikeng	Modimola Clinic	35	2013
10	Maquassi Hills	Leeudoringstad Phc Clinic	6	2012
	Tswaing	Letsopa Clinic	14	2012
	Maquassi Hills	Tshwelelang Clinic	17	2012
	Maquassi Hills	Kgakala	17	2012
	Maquassi Hills	Lebaleng Clinic	19	2012
	Tswaing	Ottosdal	27	2012
	Maquassi Hills	Wolmaranstad	32	2012

3.4.3 Most likely probe dropout/delay

Figure 6 (right) shows the map of the most likely cluster of the specific probe dropout/delay integrated on the map of all possible probe dropout/delay around MDR-TB hotspots. Probe dropout/delay of 24 was 5.3 times as likely to occur in the facilities within the City of Matlosana municipality cluster compared to facilities outside the cluster in 2012 (RR=5.3, P-value<0.001). Probe dropout/delay of 17 was 5 times as likely to occur in the facilities within the clusters across the Maquassi Hills and the City of Matlosana as compared to other facilities outside this cluster also in 2012 (RR=5.00, P-value<0.001). The probe dropout/delay of 20 was 5.22 times as likely in facilities within the central Potchefstroom cluster compared to other facilities outside the cluster in 2012 (RR=5.22, P-value<0.001). Table 7 shows the characteristic of the most likely clusters from the spatiotemporal analysis of specific probes.

Table 7: Characteristic of the most likely spatiotemporal clusters of probe dropout/delay

Most likely probe cluster	Number of facilities clustered	Time frame	Radius (KM)	RR	P-value
Probe 24	4	2012	17.61	5.30	<0.001*
Probe 17	2	2012	28.77	5.00	<0.001*
Probe 20	3	2012	24.80	5.22	<0.001*



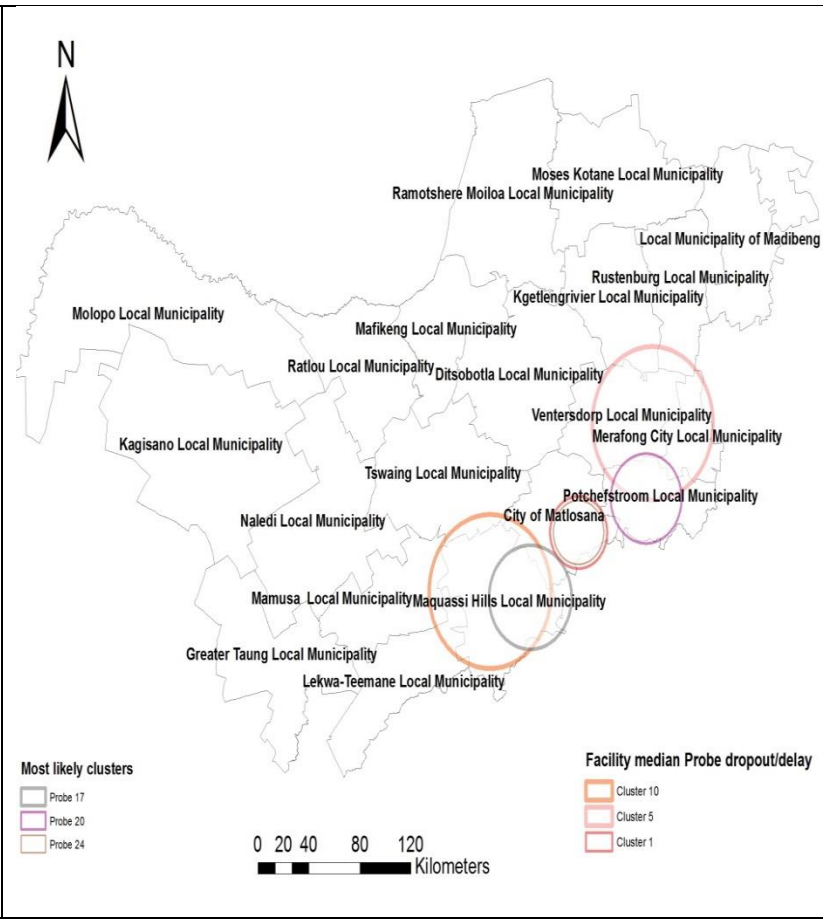


Figure 6: left: spatiotemporal clusters resulting from all similar probe dropout/delay; Right: spatiotemporal cluster of the most likely probe dropout/delay within MDR-TB hotspot

3.5 Ecological factors associated with the distribution of MDR-TB

3.5.1 Univariable Analysis

Proportion of male, female, total population, male youth, female youth, persons who completed grade 12 at age 20, total population, household size, total household, all kind of households dwelling (except for traditional dwelling), proportion of households with access to pipe borne water, electricity, other source of electricity, and flush toilet sanitation system were independently associated with annual MDR-TB crude rates (Table 8). Out of all the study period, 2014 had 2.85 times the risk of more MDR-TB in 2011 (IRR=2.85, 95% CI: 1.09 -7.41, P-value: 0.03) and 2015 had 2.34 times the risk of more MDR-TB in 2011 (IRR= 2.34, 95% CI: 0.90 - 6.09, P-value: 0.08) although marginally significant. The year 2012 and 2013 did not show any significant association with risk of more MDR-TB.

3.5.2 Multivariable Analysis

After adjusting for all significant ecological variables from the univariable analysis, the risk of more MDR-TB cases in a municipality increased by 1.15 times for every 100,000 unit increase in the MTB bacterial load (IRR=1.15, 95% CI: 1.05-1.26, P-value: 0.003) from 2011-2015. Municipalities with more males than females between the ages of 15-35 were 59% more likely to have more MDR-TB cases across the study period (IRR=1.59, 95% CI: 1.19-2.14, P-value: 0.002).

The results from the second model on 2015 municipality level variables indicated that municipalities with more households were 13% less likely to develop more MDR-TB cases (IRR=0.87, 95% CI: 0.79-0.94, P-value: 0.001). In contrast, municipalities with more males of all age groups, were 9% more likely to develop more MDR-TB cases (IRR=1.09, 95% CI: 1.01-1.16, P-value: 0.012). Just like in the first model, the municipality risk for more MDR-TB cases increased by 3.06 times for every 100,000 unit increase in municipal MTB bacterial load (IRR=3.06, 95% CI: 1.44-6.35, P-value: 0.003).

Table 8: Crude and adjusted risk ratio of ecological factors associated with the annual municipality MDR-TB crude rates from 2011-2015

	Univariable analysis			Multivariable analysis		
2011-2015	**CIRR**	**95%CI**	**P-value**	**AdjIRR**	**95%CI**	**P-value**
Year						
2011	Ref	_	_			
2012	1.32	0.49-3.49	0.58			
2013	2.15	0.83-5.57	0.12			
2014	2.85	1.09-7.41	0.03			
2015	2.34	0.90-6.09	0.08			
Median district bacterial load	0.72	0.53-0.97	0.03	1.15	1.05-1.26	0.003
Mean age	1.01	0.99-1.03	0.29			
Median probe dropout/delay	1.08	0.97-1.02	0.59			
Youth proportion	1.01	0.96-1.06	0.77			
Sex ratio	1.02	0.98-1.06	0.18			
Youth sex ratio	1.00	0.91-1.02	0.46	1.59	1.19-2.14	0.002
Male proportion	1.10	1.07-1.13	<0.001*			
Female proportion	1.11	1.08 -1.14	<0.001*			
Total population	1.05	1.04-1.07	<0.001*			
Male youth proportion	1.29	1.18-1.41	<0.001*			
Female youth proportion	1.30	1.20-1.41	<0.001*			
Total youth proportion	1.05	1.03-1.07	<0.001*			

Table 9: Crude and adjusted risk ratio of ecological factors associated with the annual municipality MDR-TB crude rates for 2015 only.

	Univariate analysis			Multivariate analysis		
2015 District level variables	**CIRR**	**95%CI**	**P-value**	**AdjIRR**	**95%CI**	**P-value**
Year						
2011	Ref	_	_			
2012	1.32	0.49 - 3.49	0.58			
2013	2.15	0.83 - 5.57	0.12			
2014	2.85	1.09 -7.41	0.03			
2015	2.34	0.90 - 6.09	0.08			
Sub-district Mean age	1.01	0.99 -1.03	0.29			
District median bug load	0.72	0.53-0 .97	0.03	3.06	1.4-6.35	0.003
Median Prob dropout/delay	1.08	0.97 - 1.02	0.59			
Youth Proportion	1.01	0.96-1.06	0.77			
Household Size	0.13	0.06-0.25	<0.001*			
Sex ratio	1.02	0.98-1.06	0.18			
Youth Sex Ratio	1.00	0.91-1.02	0.46			
Male proportion	1.06	1.04-1.09	<0.001*	1.09	1.0-1.16	0.012
Female proportion	1.11	1.08 - 1.14	<0.001*			
Total population	1.05	1.04-1.07	<0.001*			
Male youth proportion	1.29	1.18-1.41	<0.001*			
Female youth proportion	1.30	1.20-1.41	<0.001*			
Total youth proportion	1.14	1.09 - 1.19	<0.001*			
Proportion completed grade 12 at age 20	1.22	1.15-1.29	<0.001*			
Total Household	1.09	1.05-1.12	<0.001*	0.87	0.79-0.9	0.001
Formal dwelling	1.19	1.13 - 1.25	<0.001*			
Traditional Dwelling	10.47	0.24-1.53	0.22			
Informal Dwelling	1.52	1.20-1.92	<0.001*			
Other dwelling	2.43	1.54-3.85	<0.001*			
Access to pipe born water	1.16	1.10-1.22	<0.001*			
No access to pipe born water	1.15	0.89-1.47	0.29			
Flush toilet	1.20	1.14-1.27	<0.001*			
Other source of toilet sanitation	1.03	0.95 -1.11	0.47			
Connection to electricity	1.15	1.09 -1.20	<0.001*			
Connection to other sources of Electricity	1.52	1.04 - 2.22	0.03			

CHAPTER FOUR – DISCUSSION

4.1 Introduction

In this chapter, we summarize and discuss the spatiotemporal distribution and hotspots of MDR-TB in the North West Province of South Africa within the context of available literature and the overall socio-economic and public health environment of South Africa. Implications of this study for MDR-TB control in South Africa are also highlighted.

4.2 MDR-TB hotspots in the North West province between 2011-2015

There is clustering of MDR-TB cases in space and time in the North-West province of South Africa between 2011-2015

We tested the alternative hypothesis of whether there was clustering of MDR-TB cases in space and time in the North-West province of South Africa between 2011-2015 using two analytical methodologies (Moran's I statistic and Kulldorff's spatial scan statistic). The result at the significance level of 0.05 indicated that there was no evidence of pure temporal clusters of MDR-TB. The hotspots detected from the pure spatial analysis were homogenously distributed across the study period. Also, there were no pure temporal clusters detected by the spatiotemporal analysis. These foregoing results were consistent with the results obtained from the regression analysis, where the variable 'year', did not show any significant association with MDR-TB distribution. In contrast, other studies found significant temporal trends in MDR-TB distribution (19,106).

However, pure spatial and spatiotemporal hotspots were mostly detected in municipalities located in the South Western to South Eastern region of the province, specifically the City of Matlosana, Maquassi hills and Greater Taung municipalities. Hence we rejected the null hypothesis of no clustering of MDR-TB cases in space and time in the North-West Province of South Africa from 2011-2015.These municipalities had "Strong Evidenced High Risk facilities" according to Xia C et al. (101). Furthermore, we observed that Rustenburg, Potchefstroom, Moses Kotane and Ramotshere municipalities, had either pure spatial or spatiotemporal hotspots.

The Klerksdorp City in the City of Matlosana municipality which had the highest likelihood of the clustering of MDR-TB cases, is one of the two largest cities in the province (91). The economic activities of the inhabitants of Klerksdorp City include mining, tourism and agriculture (107,108). Living in mining areas, agricultural regions with inhabitants that are not easily

reached with primary health care, tourist and urban areas with high level of seasonal migration and potential overcrowding, increase the risk of MDR-TB infection (27,52,68,69,109,110). The City of Matlosana municipality has mining villages as part of the urban areas where 92% of the inhabitants reside (111). This disproportionate distribution around urban areas where mining and tourism activities are prevalent, led to overcrowding with higher risk MDR-TB transmission (36,52). Our study further confirms that initiative of the South African government which identified Dr Kenneth Kaunda district (where the City of Matlosana is located) as one of the six priority district for the 2015 comprehensive TB screening and testing campaign (71). We also detected MDR-TB hotspots at the major towns (Rustenburg, Marikana and Kroondal) of the Rustenburg municipality. Mining and urbanization are possible drivers of the hotspots in the municipality because the region is the largest producer of Platinum (27,68,69,75,91).

In addition, there are possibilities of cross border transmission between the City of Matlosana and its neighbouring municipalities (Potchefstroom and Maquassi Hills) resulting from seasonal migration for mining and other economic activities (27,72). This inference is supported by the cluster of a specific MTB strain causing the probe dropout/delay of 20, which spreads across Maquassi Hills and the City of Matlosana. There was also some evidence of cross border transmission in the Greater Taung municipality, as its hotspots were detected in the same period (2014-2015) the Northern Cape province (which shares the same border with it), was reported to have very high MDR-TB incidence rate of 764 and 645 per 100,000 inhabitants (14). Moses Kotane and Ramotshere municipalities that had only spatial clusters share borders with Rustenburg and Mafikeng which are economic centres and capital city respectively (75).

4.3 Spatial and spatiotemporal distribution of probe delay/dropout

Also, in agreement with other studies which found periodic clustering of specific MTB strains (19,22), we observed that the most likely transmitted MTB strains causing the probe dropout/delay of 17, 20 and 24 in the Maquassi hills, City of Matlosana and Potchefstroom hotspots, were clustered in 2012, hence we rejected the null of no significant clustering of Xpert probe dropout/delay from specific resistant mutations in space and time in the North-West Province of South Africa from 2011-2015. Although, further genetic epidemiological studies need to be conducted to examine the genetic composition of these MTB strains and the environmental factors that enhanced their high infectivity in 2012

Clustering of MDR-TB in space and time has been attributed to various underlying factors in different settings (23–26). Furthermore, clustering resulting from direct transmission of the MDR-TB infection has been increasingly reported globally including South Africa (19,22,29,54). The geographic variation in the aggregation of MDR-TB genotypes could be as a result of the differences in infectivity between the different MTB strains (22). The findings of this study is not unexpected, because a report from Western Cape Province of South Africa, had earlier indicated that half of the new drug resistant cases were never exposed to TB treatment. Thus, direct transmission of the disease is a major driver of its increasing prevalence in the country (15,16).

4.4 Hotspots of Median Facility Bacterial load

We also tested the hypothesis of active transmission between places with high MTB bacterial load and places with high prevalence of MDR-TB cases in the North-West Province of South Africa from 2011-2011. The analysis on the median facility bacterial load using the Moran's I and Kulldorff's spatial scan statistic did not indicate significant hotspots of MTB bacterial load (in other words places with highly infectious active cases), hence we failed to reject the null hypothesis. However, adjusting for bacterial load in the multivariable analysis indicated that municipalities with higher bacterial load were more at risk of the infection. This agrees with the findings of other studies which indicated that the probability of transmission increases with the number and the size (Infectiousness) of droplet nuclei released by the TB/MDR-TB active patient (36,112). The report of the WHO in 2014 also stated that people in MDR-TB prevalent regions (more active cases) had higher risk of direct transmission of infection (52). Other studies have also shown that patients with more bacterial burden are more infectious and they are major drivers of transmission (33).

4.5 Ecological factors associated with the distribution of MDR-TB

We also rejected the null hypothesis of no association between ecological factors and the distribution of MDR-TB cases in the North-West province of South Africa from 2011-2015 because some socioeconomic factors were associated with the municipalities' risk of more MDR-TB cases. The youth male to female ratio and the proportion of all males were found to be associated with increased risk of MDR-TB. This is consistent with the results of other studies (27,113,114). The strong association between the higher population of the male gender and increased risk of MDR-TB at the municipal level may not be far-fetched. This is because

multiple risk factors of MDR-TB which include smoking, alcohol abuse, treatment default and sessional migration are commoner among the males (6,27,28,55–62,70). Also within the context of the study setting, more males engaged in mining and agricultural activities that were predominant in regions with peak transmission risk (27). Although most prior studies did not observe a relationship between total number of households and MDR-TB infection in other regions (29,84), this study found that increasing number of household was protective against MDR-TB infection. As opposed to previous findings, a positive association is possible because the distribution of MDR-TB in various settings tends to be unique (20). Homelessness has been identified as a primary predictor for MDR-TB transmission (29,115). Hence our result can possibly be an indirect effect of increased number of household in the province which possibly reduced number of homeless people. Further studies are needed to investigate this association in different settings.

4.6 Strength and Limitation

This is the first study in the North West province that explored the semi-quantitative ability of Xpert CT values to describe: MDR-TB hotspots, mutations from specific MTB genotypes transmitted within hotspots and quantified the bacterial load at the facility and municipal level. However, our result and conclusion should be interpreted within the context of its limitations. Since our study was an ecological study that utilised routinely collected National Health Laboratory Services (NHLS) secondary laboratory data of Xpert tested TB patients in North West province and population data that were routinely collected by STATSSA, the ecological fallacy may not be discounted. However, our results are still comparable to studies that were conducted based on other study designs (22,27). Nevertheless, findings from our study have produced some hypotheses that can then be further explored to improve TB control in North West Province and South Africa generally. Furthermore, this study did not take into account the patients' treatment history and the HIV status, since such variables were not available in the data set. Prior treatment can reduce the ability of Gene Xpert test to reliably quantify MTB bacterial load (34,116). Also, the level of immunosuppression (as indicated by CD4 count) is an independent predictor of microbial burden (34,116). Other patient level factors (such as like silicosis, smoking and alcohol abuse) that may impair the immune system were also not available in the datasets that were utilized for the study (52). We also acknowledge that MDR-TB transmission is multifactorial and can be influenced by other health, climatic and socio-economic

factors such as occupation, urbanization, health services, temperature and humidity that were not considered in this study (1,6,21,55–59).

CHAPTER FIVE – CONCLUSIONS AND RECOMMENDATIONS

This section summarizes the study results and gives recommendation to policy makers.

5.1 Conclusion

We identified MDR-TB hotspots in the North West province of South Africa. We have also evaluated probe dropout/delay from MTB strains that were more likely to be transmitted within these hotspots. Furthermore, other ecological factors that are associated with MDR-TB distribution in the province were identified. We also identified hotspots of MDR-TB in the South Western and South Eastern regions of North West Province (Greater Taung, City of Matlosana and Maquassi hills municipalities) during the study period. We also found that facilities in City of Matlosana, Maquassi Hills and Greater Taung as hotspots most likely resulting from direct transmission of specific strains while clustered facilities in Rustenburg were unlikely to be due to direct transmission of infection between facilities. Apart from direct transmission between hotspots facilities, other ecological factors such as increasing median bacterial load, increased male to female ratio can increase the municipality risk of MDR-TB However an increased number of households per municipality can reduce the risk**.** Hence of the 4 proposed hypotheses for this study using different analytical methodologies, we had enough evidence to reject the null for three of them (Hypothesis 1 3 and 4) and failed to reject the null for the hypothesis 2.

5.2 Recommendation

The following are some recommendation based on the results of the study

1. Intervention should target regions with high risk facilities

Municipalities identified by this study as hotspot for MDR-TB such as City of Matlosana, Maquassi Hills and Rustenburg, Greater Taung, Potchefstroom, Mafikeng and Moses Kotane should be given priority in the implementation of MDR-TB control programs. Further genotypic epidemiological studies should be conducted to further explore the transmission link between facilities in the hotspot clusters and also examine the ecological or environmental factors that increase the rate of reproduction of highly transmissible MTB strains.`

2. Maximization of Gene Xpert MTB/RIF for MDR-TB monitoring and Surveillance tool

We recommend that beyond using the Xpert MTB/RIF assay as an initial diagnostic test of TB and MDR-TB in South Africa, the semi-quantitative capacity of the assay can be explored and combined with GIS techniques for real time identification of the MDR-TB hotspots. We further recommend that the spatiotemporal distribution of hybrid probe drop outs/delay can be a good approach in the molecular epidemiology of MDR-TB. Exploring the spatiotemporal changes of probe distribution could be a cost effective method for real time monitoring of MDR-TB outbreaks in high burden regions.

3. Spatiotemporal studies should be conducted regularly to guide the design and implementation of MDR-TB Management and Infection control

In agreement with other studies, we have shown probable aggregation and clustering of MDR-TB cases in time and space. There was also an increasing trend of MDR-TB aggregation in the region, which may be related to direct transmission of the infection. Studies have also proven that there is regional variation in the transmission dynamics of MDR-TB cases (11,20,21,77–79). Therefore for effective disease management and infection control, the spatiotemporal pattern of MDR-TB should be evaluated as a guide in the implementation of any TB control intervention. This will inform policy makers to know where and when to target region specific interventions as opposed to blanket screening and treatment intervention programs that do not take into account the spatial and temporal variation of the infection

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1.

APPENDICES

APPENDIX 1

Ethics Clearance certificate for the research

UNIVERSITY OF THE WITWATERSRAND, JOHANNESBURG

R14/49 Mr Matthew Tunde Oladimeji

HUMAN RESEARCH ETHICS COMMITTEE (MEDICAL)

CLEARANCE CERTIFICATE NO. M160844

NAME: (Principal Investigator)	Mr Matthew Tunde Oladimeji
DEPARTMENT:	School of Public Health Division of Epidemiology and Biostatistics National Health Laboratory Service
PROJECT TITLE:	Spatial and Temporal Analysis of Xpert Confirmed MDR-TB in the North-West Province of South Africa from 2011-2014
DATE CONSIDERED:	26/08/2016
DECISION:	Approved unconditionally
CONDITIONS:	
SUPERVISOR:	Jabulani Ncayiyana
APPROVED BY:	Professor CB Penny, Chairperson, HREC (Medical)
DATE OF APPROVAL:	07/02/2018

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 in Room 301, 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 August and will therefore be due in the month of August 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 II

Plagiarism Report

APPENDIX III

UNIVERSITY OF THE WITWATERSRAND JOHANNESBURG
School of Pathology
Department of Molecular Medicine & Haematology
7 York Road Parktown 2193 SA, Telephone +2711 7172519/2000

NATIONAL HEALTH LABORATORY SERVICES
PO Box 1038 Johannesburg 2000 SA, Telephone +2711 489 8505 /9000

Busi Ngoyi
Research Administrator
School of Public Health
WITS

12th September, 2016

Dear Busi

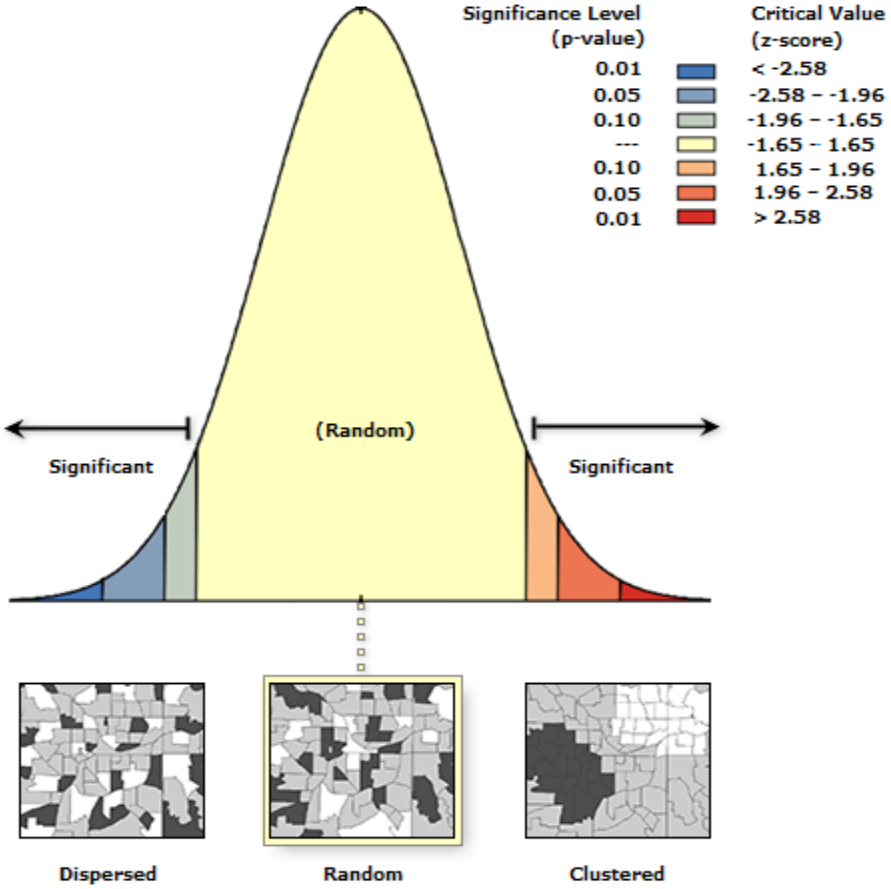
RE: corrections to MSc protocol for Mathew Oladimeji (#1161715)

This is to confirm that I am happy with the corrections made by Mathew to his MSc protocol as suggested by the assessors. All comments have been adequately addressed and necessary changes made in the final protocol document. I support Mathew to now proceed with his analyses.

Yours sincerely

Prof Lesley Scott, PhD
Senior Medical Scientist
Department of Molecular Medicine and Haematology
University of the Witwatersrand, Johannesburg,
South Africa lesley.scott@nhls.ac.za,
011-489-8567

APPENDIX IV



Given the z-score of 0.12937090509, the pattern does not appear to be significantly different than random.

Fig 5: Spatial autocorrelation report for the facility bugload

Moran's index: 0.002933

Z-score: 0.068061

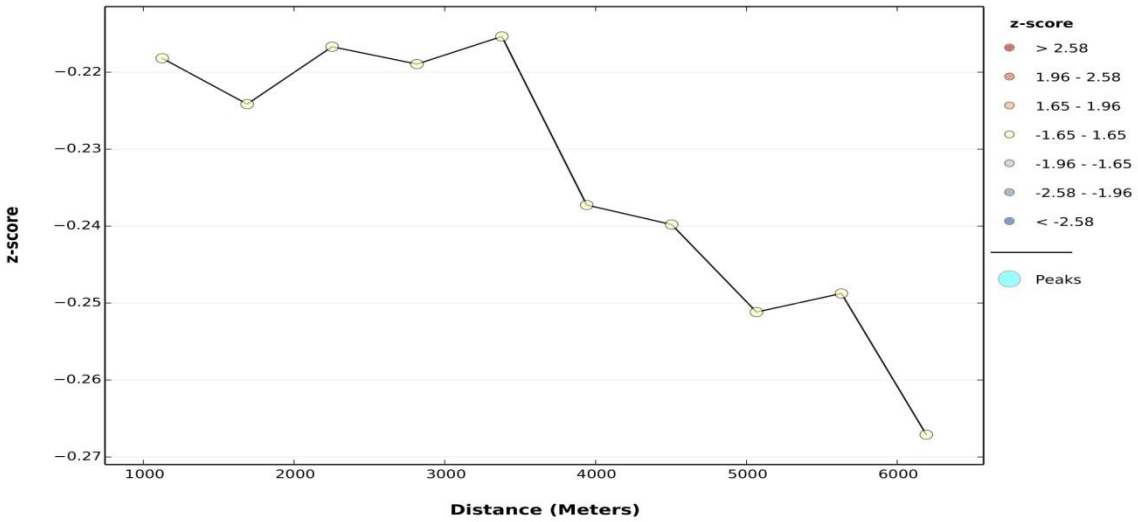
P-value: 0.897064

Moran's Index: 0.002933

z-score: 0.129371

p-value: 0.897064

Spatial Autocorrelation by Distance



Global Moran's I Summary by Distance

Distance	Moran's Index	Expected Index	Variance	z-score	p-value
1126.00*	-0.004375	-0.001866	0.000132	-0.218191	0.827280
1689.00*	-0.004337	-0.001859	0.000122	-0.224146	0.822644
2252.00*	-0.004174	-0.001852	0.000115	-0.216690	0.828450
2815.00*	-0.004116	-0.001848	0.000107	-0.218943	0.826694
3378.00*	-0.004014	-0.001838	0.000102	-0.215356	0.829490
3941.00*	-0.004175	-0.001828	0.000098	-0.237259	0.812456
4504.00*	-0.004112	-0.001815	0.000092	-0.239794	0.810490
5067.00*	-0.004171	-0.001795	0.000089	-0.251172	0.801681
5630.00*	-0.004103	-0.001779	0.000087	-0.248754	0.803551
6193.00*	-0.004180	-0.001767	0.000082	-0.267104	0.789389

First Peak (Distance, Value): None, None

Max Peak (Distance, Value): None, None

Distance measured in Meters

* At least one distance increment resulted in features with no neighbors which may invalidate the significance of the corresponding results.

APPENDIX V

	District MDR-TB cases				lnalpha_cons
	Median district bacterial	Total population	Youth sex ratio	_cons	
District MDR-TB cases					
Median district bacterial	1				
Total population	0.0938	1			
Youth sex ratio	0.0282	-0.3402	1		
_cons	-0.1296	-0.0785	-0.872	1	
lnalpha_cons	-0.0728	0.0418	-0.0389	0.0809	1

. vif

not appropriate after regress, nocons;

use option uncentered to get uncentered VIFs

r(301);