# Exploring drug-resistant tuberculosis profiles within the West Coast, South Africa

by

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# Declaration

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# Summary

Tuberculosis is still to this day the most widespread infectious disease globally. The spread of drug-resistant strains which has been reported to be attributed to primary transmission threatens TB control and prevention programmes. Previous molecular epidemiological studies have reported that the dynamics of tuberculosis transmission varies geographically.

The aim of the present study was to describe the drug-resistant tuberculosis epidemic and identify transmission hotspots and possible outbreaks of drug-resistant tuberculosis within the West Coast region of the Western Cape, South Africa. We used the internationally standardised spoligotyping method to classify *Mycobacterium tuberculosis* into different lineages and strain family and DNA sequencing of drug resistance conferring genes to genotypically characterise drug-resistant tuberculosis isolates collected from the West Coast region over a 5 year period (2008-2012).

Spoligotyping data revealed the X-family as the most dominant *M. tuberculosis* strain family, followed by the Beijing family. Our findings are contradicting to what has been reported in the rest of the Western Cape Province that identified the Beijing family to be the predominant family responsible for drug-resistant tuberculosis within the province.

We identified a cluster of multidrug-resistant isolates mainly located in the Northern parts of the region, harbouring similar spoligotyping patterns and identical mutations conferring resistance to the 4 first-line drugs used in tuberculosis treatment. This is indicative of transmission. Isolates belonging to this outbreak, but with different additional mutations conferring to resistance to second-line drugs were also identified, indicating that Pre-XDR and XDR-TB are primarily acquired on an existing MDR strain genotype.

Even though spoligotyping is considered to have a low discriminatory power and could overestimate the extent of transmission, this study described an epidemic spread of multi-drug resistant strains in the West Coast region of the Western Cape. Our study highlighted the regional variation of outbreaks and the need for molecular epidemiology studies in various regions to tailor interventions to curb TB and drug-resistant TB spread.

# Opsomming

Tuberkulose (TB) is steeds die mees wydverspreide aansteeklike siekte wêreldwyd. TB beheer en voorkomingsprogramme word bedreig deur middel-weerstandige TB wat ook wêreldwyd aangemeld word en meestal toegeskryf word aan primêre oordrag van die siekte tussen pasiënte. Vorige molekulêre epidemiologiese studies het gewys dat die dinamika van tuberkulose oordrag verskillend is in verskillende geografiese areas.

Die doel van hierdie studie was om die middel-weerstandige tuberkulose epidemie in die Weskus distrik van die Wes-Kaap, Suid-Afrika te beskryf, en om potensiële uitbrake van middelweerstandige tuberkulose te identifiseer. Ons het gebruik gemaak van die internasionaal gestandaardiseerde spoligotipering metode om *Mycobacterium tuberculosis* in verskillende stamme en families te klassifiseer en DNA volgorde bepaling om mutasies wat middelweerstand veroorsaak te indentifiseer. Die studie is gedoen op isolate van TB pasiënte van die Weskus streek oor 'n tydperk van 5 jaar (2008-2012).

Spoligotipering het gewys dat die X-familie as die mees dominante *M. tuberculosis* stamfamilie is, gevolg deur die Beijing stamfamilie. Ons bevindinge is teenstrydig teen wat voorheen giving is in the res van die Wes-Kaap Province, data die Beijing family oorheersend is en meestal verantwoordelik is vir middelweerstandige tuberkulose in die provinsie.

Ons het verder 'n groep van multi-weerstandige isolate geïdentifiseer in die noordelike dele van die streek, met soortgelyke spoligotipering patrone en identiese mutasies wat weerstandigheid teen die 4 eerste linie middels veroorsaak. Dit is 'n aanduiding van oordrag van 'n multi-weerstandige stam familie in die streek. Isolate wat deel is van hierdie uitbraak, maar met verskillende bykomende mutasies wat weerstandigheid teen tweede linie middels het is ook geïdentifiseer, en dui daarop dat Ekstreem Weerstandige TB ontwikkel het deur mutasies wat later ontwikkel.

Selfs al word spoligotipering beskou as 'n tegniek met minder diskriminerende waarde as ander ginotiperings tegnieke, en daarom die mate van tuberculose oordragf kan oorskat, bewys hierdie studie dat 'n multi-middelweerstandige epidemie versprei in die Weskus van die Wes-Kaap. Ons studie beklemtoon die noodsaaklikheid vir molekulêre epidemiologie

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# List of abbreviations

**Abbreviations**

| | |
|---|---|
| °C | Degree Celsius |
| μL | Microliter |
| AFB | Acid fast bacilli |
| AIDS | Acquired immune deficiency syndrome |
| AMK | amikacin |
| BCG | Bacilli Calmette-Guérin |
| bp | Base pair |
| CAF | Central Analytical Facility |
| CAP | capreomycin |
| CDC | Centres for Disease Control and Prevention |
| CIP | ciprofloxacin |
| CRISPs | Clustered regularly interspersed short palindromic |
| CS | cycloserine |
| DNA | Deoxyribonucleic acid |
| dNTP | Deoxyribonucleotide triphosphate |
| DOTs | Directly observed treatment short course |
| DR | Drug-resistance |
| DRs | Direct repeats |
| DST | Drug susceptibility testing |
| ECL | Enhanced Immunohistochemistry |
| EDTA | Ethylenediaminetetraacetic acid |

| | |
|---|---|
| EMB | ethambutol |
| GIS | Geographical information system |
| GTR | The general time-reversal |
| H$_2$0 | Water |
| HCW | Health care worker |
| HIV | Human immunodeficiency virus |
| INH | isoniazid |
| KAN | kanamycin |
| KZN | KwaZulu-Natal |
| LCC | Low Copy Clade |
| LT | local spoligotype |
| *M. tuberculosis* | *Mycobacterium tuberculosis* |
| MDR | Multidrug-resistant |
| MIRU-VNTR | Mycobacterial interspersed repetitive units-variable |
| ml | Millilitres |
| mM | milimolar |
| NHLS | National Health Laboratory Service |
| OFL | ofloxacin |
| PAS | para-aminosalicylic acid |
| PCR | Polymerase Chain Reaction |
| PGRS | Polymorphic guanine-cytosine rich repetitive sequence |
| PHRI | Public Health Research Institute |
| pmol | Picomol |
| PZA | pyrazinamide |

| | |
|---|---|
| RAPET | Rapid epidemiological typing |
| RaxML | Randomized Axelerated Maximum Likelihood |
| RFLP | Restriction Fragment Length Polymorphism |
| RIF | rifampicin |
| RRDR | Rifampicin resistant determining region |
| SA | South Africa |
| SDS | Sodium dodecyl sulfate |
| SIT | spoligo international type |
| SNA | Social network analysis |
| SNP | Single nucleotide polymorphism |
| STR | streptomycin |
| TB | Tuberculosis |
| TDR | Totally drug-resistant |
| WGS | Whole genome sequencing |
| WHO | World Health Organisation |
| XDR | Extensively drug-resistant |

# Chapter 1

# General introduction

## 1.1 Introduction

Tuberculosis (TB) caused by *Mycobacterium tuberculosis* is still to this day the most widespread infectious disease globally (1, 2). In addition, the World Health Organisation (WHO) reported an estimated 1.1 million people co-infected with human immunodeficiency virus (HIV) and TB worldwide (3). Furthermore, more than 50% of the TB cases co-infected with HIV were recorded in Sub-Saharan Africa (3). This global problem is further complicated by the emergence and rise of drug-resistant (DR) TB, most of which is believed to be attributed to primary transmission (4).

An estimated 480 000 cases of multidrug-resistant (MDR) TB, defined as (resistance to at least isoniazid (INH) and rifampicin (RIF), the two key first-line drugs) were recorded in 2014 worldwide, threatening TB control programs (5). Moreover, the widespread emergence of extensively drug-resistant (XDR) TB (defined as MDR-TB with additional bacillary resistance to any of the fluoroquinolones and at least one of the three second-line injectable drugs: kanamycin [KAN], amikacin [AMK] or capreomycin [CAP]) cases is alarming (6). Several studies have reported that most cases of MDR-TB and XDR-TB in South Africa and globally result from primary transmission (4, 7–11). Continued transmission is not only exhibited in inadequately treated patients with no known drug resistance, but also in undetected, undiagnosed patients which predisposes communities to outbreaks (12–14).

South Africa has been reported to have high and increasing rates of DR-TB. Studies have documented that the drug resistance burden in the Western Cape province is due to ongoing transmission (15, 16). Furthermore, previous studies suggests that the W/Beijing *M. tuberculosis* strain family drives the TB epidemic, in particular DR-TB in the Western Cape Province (17–19). Molecular epidemiological tools used in combination with traditional investigation techniques have improved and broadened our understanding of TB epidemiology by providing insight into the transmission dynamics of *M. tuberculosis* (20–22). Application of geospatial mapping of case clusters and whole genome sequencing (WGS) coupled with social network analysis (SNA) have provided a guideline to aid in public health responses (23–25).

Although genotypic and phenotypic characterisation of DR-TB strains has been done in the Western Cape Province (7, 16, 26), very little has been reported in the West Coast district of this Province (27). Furthermore, this region has an alarmingly high estimated TB incidence of 1224.9/100 000 population (28). Molecular epidemiology studies have demonstrated that the dynamics of TB transmission vary geographically (29). A study by Hanekom *et al*. suggested that some strains disseminate and cause disease more efficiently than others (30), it is therefore vital to understand the dynamics governing DR-TB transmission in this region.

We hypothesise that a sudden increase in DR-TB cases in an area is indicative of recent transmission and a possible outbreak of a DR strain of tuberculosis. Therefore, the aim of the current study was to describe the DR-TB epidemic in the West Coast district of the Western Cape Province and to identify possible transmission hotspots. To achieve this aim, we have 3 specific objectives: 1) to describe the DR-TB strain population by spoligotyping, and thereby identify possible clusters of drug resistance; 2) to identify mutations associated with drug resistance of the most prominent strain families by polymerase chain reaction (PCR) amplification and Sanger sequencing; and 3) to identify and map out transmission hotspots of DR-TB.

Chapter 2 is an overview of the literature relevant to the study; it describes the molecular epidemiology tools used in outbreak investigations and their limitations and also reports on studies of DR-TB outbreaks in different settings and the lessons learned. Chapter 3 is a brief description of material and methods used in the study, buffers and solutions are included in the appendices. The results from data generated in the study are in Chapter 4, this includes the distribution of strain families in the area, mutations conferring drug resistance in the prominent strain family and areas were transmission is evident. In chapter 5 we discuss the results obtained in this study. An additional data set of results has also been included in the appendices.

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# Chapter 2

# Literature Review

Global molecular epidemiology of drug-resistant tuberculosis

## 2.1 Background

Tuberculosis remains an enormous public health burden globally (1). The WHO reported an estimated 9.6 million new cases of TB and 1.5 million deaths from TB, including about 1.1 million deaths among individuals who were HIV negative and among 0.4 million people who were HIV positive in 2014 (2). These reports emerge despite available antituberculous therapy that could cure most cases of TB (3).

### 2.1.1 Drug resistance

Acquired drug resistance emerges as a result of spontaneous selection of mutations in certain chromosomal genes during suboptimal therapy (4–6) whereas primary resistance develops when an individual is infected with a resistant strain (7). The emergence and increase in MDR-TB seriously threaten TB prevention programmes. Moreover, global reports of XDR-TB in 105 countries have emerged stressing the need for current control measures to be augmented (2). These forms of TB do not respond to the standard six-month regimen with first-line anti-TB drugs and can take up to two or more years to treat with drugs that are less effective and much more expensive (8). In 2009, Velayati *et al*. identified a strain of TB that showed in-vitro resistance to all first- and second-line drugs tested. The term "totally drug-resistant tuberculosis (TDR-TB)" was proposed for this strain (9). These dangerous forms of TB bacilli which have not been clearly defined have subsequently been reported in other countries (Italy, India and South Africa) (10–12). Failure to reinforce control and prevention of TB may result in outbreaks that are difficult and expensive to control.

## 2.2 Drug-resistant TB outbreaks

2.2.1 Definition of an outbreak

The WHO defines an outbreak of any sort as the unusual increase of disease in a defined community or geographical area (13). An outbreak may be confined to a geographical area, or spread over several countries. It may be short-lived or long-lived (13). A study by Marais *et al.* identified a large number of MDR-TB strains and multiple clusters circulating within the greater Johannesburg area of South Africa which could be considered as disease in excess

and fits the description of an outbreak. However, transmission in this study was attributed to numerous source cases and described as an epidemic spread and not an isolated clonal outbreak (14). Owing to the alarming outbreak reports and absence of a tailored definition of an outbreak of DR-TB, the present review is undertaken to describe global outbreaks of DR-TB and how the application of molecular epidemiology tools coupled with traditional epidemiological investigations aided in the control of outbreaks.

Outbreaks of DR-TB have been reported worldwide in communities and institutions (15). Prior to the era of molecular techniques, understanding the dissemination of TB relied on observational data (16). Detection of a cluster of *M. tuberculosis* resistant profiles was indicative of an outbreak; this was achieved through case history, contact investigation, tuberculin skin test and drug susceptibility tests (DST). Advances in molecular microbiology, that enable differentiation of strains of *M. tuberculosis* by use of deoxyribonucleic acid (DNA) fingerprinting have provided new insights into tuberculosis epidemiology (17). Isolates with identical fingerprints are termed a cluster and are indicative of recent transmission while isolates with unique fingerprints are considered unrelated (18).

## 2.3 Molecular epidemiological tools used to identify and confirm outbreaks

### 2.3.1   IS*6110*

Different techniques have been standardised and used in molecular epidemiology studies (19). IS*6110*-restriction fragment length polymorphism (RFLP) is the first standardised fingerprinting technique for TB epidemiology (20). This technique is based on the presence of the insertion sequence in different positions of the *M. tuberculosis* genome. *M. tuberculosis* complex has been reported to contain 0-25 copies of IS*6110*. Considered to be a gold standard until recently (21), IS*6110* typing is the most widely used method for molecular epidemiological studies because of the high degree of discriminatory power achieved with this method (22). However, this technique has its own flaws (23) as it is laborious and needs relatively large amounts of DNA. Furthermore, it requires prior culture and produces results that are not easy to compare across laboratories (24).

2.3.2 Polymorphic guanine-cytosine-rich sequence-RFLP

Polymorphic guanine-cytosine-rich sequence (PGRS) makes use of the, polymorphic guanine-cytosine (GC)-rich sequence contained in the recombinant plasmid pTBN12 as a probe (25, 26). The poor discriminatory power of IS*6110*-RFLP when applied to low copy number strains has been resolved by use of PGRS, which has high discriminatory power when applied to low copy strains. Although highly discriminatory, the process is lengthy and the results are difficult to interpret.

2.3.3 Polymerase Chain Reaction based typing

2.3.3.1 Spoligotyping

Spoligotyping is based on PCR amplification of the highly polymorphic direct repeat locus which is a member of the clustered regularly interspaced short palindromic repeats (CRISPRs) using membrane format or Luminex® technology (Luminex Corp., Austin, TX) (27). Spoligotyping is less discriminatory than IS*6110* typing when high copy number strains are being analysed but it is superior for the evaluation of low copy number strains (22). It is not complicated, rapid and robust, but low in discriminatory power (22).

2.3.3.2 Mycobacterial interspersed repetitive unit-variable number tandem repeats

Mycobacterial interspersed repetitive unit-variable number tandem repeats (MIRU-VNTR) typing is based on polymorphisms of the MIRU loci. MIRUs are short (40-100 base pairs) DNA elements, often found as tandem repeats and spread in intergenic regions in the genome of the *M. tuberculosis* complex (28). MIRU-VNTR is a portable, reproducible and discriminatory tool and has been adopted as an international standard (21, 28, 29). It produced more distinct patterns when compared with IS*6110*-RFLP and spoligotyping (30, 31). However, this technique is time consuming.

2.3.4 Drug resistance associated mutations

Drug resistance associated mutations have lately been used coupled with other molecular epidemiological techniques to define clustering of *M. tuberculosis* isolates (32, 33). Drug resistance mutations are determined by PCR amplification followed by sequencing of the target region or hybridization of the target amplicon with immobilized probes representative of a possible mutation causing drug resistance (34, 35). This approach is limited to isolates with drug resistance.

2.3.5 Whole genome sequencing

Whole genome sequencing (WGS) is based on analysis of whole genome sequence data generated by next-generation sequencing. The discriminatory power of analysing the whole genome makes it superior to all other genotyping methods, with the additional benefit that a complete drug resistance profile based on drug resistance causing mutations could be compiled. Unfortunately, this will not be feasible to identify outbreaks of TB or DR-TB in "real-time" in areas with a high burden of tuberculosis, since the cost of sequencing is still too high and the technique and analysis is time consuming (20).

2.3.6 The use of geographical information system in outbreak investigations

The use of geographical information system (GIS) coupled with molecular epidemiological tools can be a dual force in outbreak investigations, enabling identification of clusters and localising transmission. Although rarely used in resource limited settings, GIS has successfully been utilized in a study to localise an outbreak (36). However, the application of GIS is only effective in areas where streets and houses are properly numbered and mapped, which is not the case for low-income settings. In such instances, global position system (GPS) is utilized to gain access to geographical coordinates (36).

## 2.4 Global reports of outbreaks of DR-TB

### 2.4.1   Nosocomial outbreaks

Drug-resistant TB outbreaks within health care facilities have been reported in various geographical settings. This could possibly be resulting from an active spread of TB bacilli by undiagnosed and inadequately treated patients where poor prevention and infection control measures are in place. This was evident in an outbreak of a highly resistant strain that was reported in New York City (37), where more than two thirds of the patients were reported to have been infected with the strain designated as the "W-strain" in the nosocomial setting (37). A high HIV co-infection was also documented among patients. Genotyping was done using IS*6110*-RFLP which has a high discriminatory power in identifying identical or closely related cases. Poor infection control and inadequate treatment contributed to transmission. Ripples of nosocomial TB transmission were also documented in hospitals across the United States of America (USA), which stressed for effective prevention and control efforts to be augmented (38–40). The surge of nosocomial outbreaks was reported to be attributed to neglect and decline in public health efforts (41). Reinforcement of infection control, preventive measures and improvements in directly observed treatment short course (DOTs) resulted in a decline in transmission (42).

The lesson from the above mentioned outbreaks (37) has been poorly implemented by TB control programs, especially in resource limited settings such as sub-Saharan Africa, South Africa in particular, which has been reported to have a high prevalence in cases co-infected with HIV (43). The suboptimal treatment regimens of patients with susceptible TB and poor infection control has both allowed for selection of DR forms of TB and transmission. Lack of effective prevention and control measures cascaded into the highly publicised hospital transmission of XDR-TB in Tugela Ferry, a rural town in the South African province of KwaZulu-Natal (KZN). HIV co-infection was documented in all patients that were tested. Furthermore, all but one of the patients died within a fortnight of diagnosis. Genotypic analysis was enhanced by using both IS*6110*-RFLP and spoligotyping, which enabled identification of the patients that were in the same cluster and strain family which was subsequently named F15/LAM4/KZN strain (44). The high fatality among patients diagnosed

with XDR-TB and apparent transmissions in the hospital setting led to a major international rethink of treatment strategies. The finding in this study suggests that HIV positive patients are at high risk of acquiring resistant forms of TB. This is consistent with reports by other studies that documented rapid progression to TB infection in patients who were HIV positive (45, 46) and stresses for improved isolation of HIV infected patients from patients diagnosed with TB. In addition, prompt antiretroviral treatment should be administered for effective management of TB infection. These studies show that hospital settings can facilitate the transmission of tuberculosis if infection control measures are not in place. In addition, not only are patients at risk, health care workers (HCW) also contribute to the spread of TB in the health care facilities (47, 48). These findings have important implications for TB infection control. It has been reported that implementing effective infection control measures in health care facilities can reduce the risk of tuberculosis transmission (49). The WHO recommends that some set of guidelines at administrative, environmental and personal level be implemented. Administrative control measures include prompt identification of patients infected with TB, isolation of infected patients, administration of proper treatment, reduced hospital stays, improvement in lab testing and notification to prevent diagnostic delays and support of health care workers by implementing routine screening and HIV testing (49). This works effectively in conjunction with implementation of effective environmental control measures that involve reduction and control of infectious air by both mechanical and natural ventilation. However, implementation and maintenance of mechanical ventilation in low resource settings could be costly. Such settings rely on natural ventilation which has been reported to be effective provided that air is directed to the outside and not within the corridors (50). Use of ultraviolet germicidal irradiation (UVGI) has also been recommended as an infection control measure to limit transmission, especially in cold settings (51). However, the effectiveness of UVGI depends on the vertical air movement between the upper and the lower room and is only effective in areas with low relative humidity (52). Although it has been reported to reduce rates of transmission (51), further upgrades are necessary to improve safety and efficacy, more importantly in high risk regions. Furthermore, the WHO recommends particulate respirators for HCW who are attending to patients to prevent transmission of TB (49, 53). Nosocomial outbreaks have also been reported in Italy, England, USA, South Africa, Argentina and Spain (54–60), see **table 1**.

2.4.2 Community and household outbreaks

TB transmission is not only isolated to health care facilities but extends to households and community settings. Studies have reported that contacts exposed to patients with active TB are vulnerable to infection by primary transmission, especially in settings reported to have a high burden of HIV (61, 62).

2.4.2.1 Household outbreaks

Exposure of close contacts to index cases prior to diagnosis puts them at risk for TB transmission (61, 63). This was seen in a study that reported an outbreak of MDR-TB and tuberculous meningitis in a single family (64). All patients were reported to be immunocompetent. Genotyping by PCR based on amplification of IS*6110* revealed a closely related pattern. In addition, isolates from two of the patients harbored the *rpoB* mutation and had the same RFLP profile, suggesting an intra-familial transmission. Mortality of two of the patients was attributed to neurological complications. The study highlighted the importance of early detection, adequate treatment and adequate isolation measures. This outbreak could have been prevented if prompt and correct diagnosis was done. This report is consistent with a study that reported household settings as one of the transmission hotspots (65). A study by Vella *et al.* (66) stressed the importance of an early and strong contact tracing to limit transmission which could in turn reduce TB in high incidence areas (67, 68). It has been recommended that a contact investigation be initiated after detection of an infectious case. (69). However, this may be difficult to implement in resource limited settings (70).

2.4.2.2 Community outbreaks

Transmission is not only exhibited in households but frequently occurs in communities were social mixing is heightened. This could result from exposure of contacts to active cases in social areas. A community outbreak was reported among non-institutionalised and HIV seronegative patients in Cape Town, South Africa (71). Genotyping by IS*6110*-RFLP and use of dot blot hybridization of mutations conferring drug resistance to four first-line drugs enabled identification of a cluster of 16 MDR-TB patients. The strain was indiscriminately referred to as the "U strain" which is "strain W-like". Isolates from the initial MDR-TB episode exhibited mutations in the *katG*315, *katG*463, *rpoB*531, *rrs*513 and *embB*306 conferring resistance to INH, RIF, streptomycin (STR) and ethambutol (EMB) respectively. The study redefined what was known as a norm in a high incidence area. This study also

emphasised on the importance of prompt and correct diagnosis with administration of optimal treatment (71). Studies have further documented that the youth, progressing to adolescence and young adults are vulnerable to exposure of TB from infectious cases during social contact, particularly in an enclosed setting with minimal ventilation (65, 72). It has also been shown that contacts are at risk of transmission of highly resistant strains from active undetected cases who were infected in health care facilities (73). In addition, undetected miners who migrate to and from the mines in low resource and high prevalence regions such as sub-Saharan Africa serve as a potential threat to households and communities (74).

These studies gave insight into transmission dynamics in households and communities and stressed the need for intensive case detection and contact investigation, regardless of regional HIV and drug resistance prevalence (75). Adhering to the lessons learnt from these outbreaks will enable identification of undetected or undiagnosed cases and initiation of treatment to control the spread of TB (70, 76). Furthermore, incorporating recent genotyping tools such as WGS, could enhance findings that could not be achieved by classic epidemiological tools and genotyping alone, owing to the higher discriminatory power that WGS exhibits (77).

2.4.3 Outbreaks in prisons

Incarcerated persons are at risk of transmission of tuberculosis due to overcrowding and poor ventilation (78, 79). In addition, risk factors such as substance abuse and HIV infection contribute to high rates of tuberculosis in prisons. Furthermore, lack of screening programmes for early identification of TB allow for ongoing transmission from undetected infectious persons (79, 80). This was highlighted in a study by Valway *et al* (81). A contact investigation was initiated following the death of four inmates attributed to MDR-TB infection. Investigation identified nine cases with MDR-TB and 96 (30%) contacts with a positive skin test. All cases had the same drug resistance patterns. Identical patterns were obtained using IS*6110*-RFLP, confirming a true outbreak. Furthermore, all outbreak cases were immunocompromised, nine were HIV positive and one was diagnosed with cancer. This is consistent with a study by McLaughlin *et al*. that showed that transmission is elevated in settings where HIV infected persons are congregated (82). Transmission was attributed to an undiagnosed source case with active MDR-TB (81). The study highlights the importance of intensive screening programs and effective infection control measures. It has also been

documented that infected inmates also pose as a threat to community contacts after they are released (79, 83).

Early identification of cases infected with TB and initiation of appropriate treatment is mandatory in preventing transmission. This should be done in conjunction with improvement of infection control practices. The WHO recommends that managerial activities involving policy makers be implemented to ensure effective planning and implementation of infection control (49). Furthermore, administrative controls that include screening of newly infected and long term inmates, educating and encouraging staff to seek medical attention when they present with symptoms as well as screening for HIV and counselling must be instituted (49, 79). In addition, all case reports of suspected and confirmed TB cases must be reported to health departments. This will ensure that adequate resources are available for case management and contact investigations in and out of the correctional facility and enable follow up of released inmates who are infected with TB (79, 84). The Centres for Disease Control and Prevention (CDC) (Atlanta, Georgia, USA) also recommends that environmental control measures be instituted such as local exhaust ventilation and UVGI which has been reported to have limitations as mentioned above in health care infection control measures (52). Moreover, adequate personal respiratory protection is recommended for use when entering isolated rooms with infected patients and during transportation of infectious inmates (79). Further reports of community and household outbreaks have been reported in Benin, Taiwan, Argentina, Norway, Austria, Singapore and Virginia (36, 85–90), see **table 1**.

2.4.4 Outbreaks in schools

The long hours of contact among students and educators in enclosed spaces favour transmission, which makes schools potential outbreak settings. One such outbreak was reported in California and occurred from 1991-1993. A retrospective cohort study was initiated following  diagnosis of four high school students with TB resistant to three first-line drugs (91). A total of 18 active cases were identified. Eight culture positive isolates that were available for IS*6110*-RFLP typing exhibited identical patterns. The index case was documented to have been infectious for a prolonged period of time resulting in transmission. Furthermore, risk of infection was found to be high among contacts of an infectious student. Subsequent screening detected additional cases of positive reactors. The study demonstrated the discriminatory power of IS*6110*-RFLP in comparison to conventional phenotypic

techniques and the importance of continuous screening in schools to prevent transmission. In addition, patients receiving treatment should be monitored using effective DOTs to ensure treatment adherence. Prevention measures such as educating students about TB transmission and when to seek medical attention should also be instituted. Furthermore, infection control measures should be implemented; this may include adequate ventilation in classrooms and sitting areas. School outbreaks of DR-TB have also been reported in Ireland, Spain and South Africa, which is a high incidence area (91–93), see **table 1**.

2.4.5 Outbreaks among foreign born persons

Transmission among foreign born, homeless persons and illicit drug users continue to challenge TB control efforts in developed countries. Immigrants who migrate from areas with high prevalence to areas with low incidence of TB pose a threat to communities leading to an increase in rates of transmission (94). From 2005-2006 a cluster of four MDR-TB cases were identified in Austria with matching genotypes confirmed by MIRU-VNTR typing (24 loci). All four cases were HIV seronegative and refugees. Non-adherence, ineffective follow up and inappropriate treatment of the highly infectious index case led to transmission. Combination of MIRU-VNTR typing with high discriminatory power and spoligotyping as an initial screening tool was conclusive in confirming this study as an outbreak. The findings were consistent with reports from a meta-analysis that documented that refugees were four times more likely to be diagnosed with active pulmonary TB than other immigrants (95). Another study reported an outbreak of MDR-TB among US-bound Hmong refugees in Thailand (96). Of the screened refugees, a total of 272 patients were diagnosed with TB. Genotyping was done by MIRU, Spoligotyping and IS*6110*-RFLP. Twenty of the 23 MDR-TB patients had matching genotypes by the 3 molecular techniques. The other 3 exhibited unique patterns. The study defined an outbreak based on three criteria: social links, matching genotypes by three molecular techniques and increase in tuberculin reactivity among smear positive household contacts. The study underscored the importance of prompt diagnosis of active cases and initiation of treatment to prevent transmission and importation of virulent strains to different geographical locations. Moreover, the study also highlighted the effectiveness of utilizing genotypic techniques in conjunction with classic epidemiology to get to the root of transmission. Screening on entry into developed countries has been a main countermeasure, especially for migrants coming from countries with high prevalence of TB (94). Moreover,

subsequent screening to oversee the trends in specific populations is highly recommended (94). However, a study stressed that although screening of refugees is easy since they are under surveillance, it may be difficult for migrants owing to the various entry points that they may use (97). Furthermore, Menzies *et al*. stressed that screening upon entry and administration of treatment alone is not effective in reducing the TB incidence from the country of origin, since immigrants reactivate as soon as they enter the developed countries with low TB incidence. Hence a global coordinated effort is required to avert TB transmission (98). Another outbreak has also been reported in Norway (87), see **table 2.1.**

**Table 2.1 Summary of global reports of DR-TB outbreaks**

| Reference | Geographical area | Year(s) of study | Type of study | Setting | Cases | Screening and surveillance | Drug resistance | Genotyping/ mutation analysis | Strain family | Lessons learned |
|---|---|---|---|---|---|---|---|---|---|---|
| Nosocomial outbreaks | | | | | | | | | | |
| (39) | New York, USA | 1989-1990 | Case control study | Teaching Hospital | Patients co-infected with AIDS | | MDR | IS*6110*-RFLP | | Inadequate isolation among immunocompromised and infectious TB patients favours transmission |
| (37) | New York City, USA | 1990-1993 | Epidemiological study | Multi-institutional | Every tuberculosis patient reported in New York City from 1 January 1990 to 1 August 1993 | | MDR | IS*6110*-RFLP | Beijing/ W-like strain | Implementation of DOTs can improve patient outcome and there is a need for improved diagnosis, treatment and infection control |
| (46) | Milan, Italy | 1991-1995 | Molecular epidemiological investigation | Hospital | HIV infected patients | | MDR | IS*6110*-RFLP | | Infection control measures are mandatory in settings with immunocompromised patients |
| (55) | North London, | 1995-2000 | Molecular | Hospital and | Isoniazid | | INH | IS*6110*-RFLP | | prompt diagnosis |

| | England | | retrospective epidemiological study | prison | resistant patients | | monoresistant | and rapid epidemiological typing (RAPET) | | of infected patients and inmates aids in preventing transmission |
|---|---|---|---|---|---|---|---|---|---|---|
| (56) | South Carolina, USA | 1995 | Outbreak investigation | Hospital and community | Patients diagnosed with MDR-TB having an identical genotype | | MDR | IS*6110*-RFLP | Strain W1 | Adherence to published infection control guidelines is important in preventing the spread of DR-TB |
| (40) | New York City, USA | 1989-1991 | Retrospective case control study | Teaching hospital | Patients diagnosed with DR-TB and susceptible patients | TST | MDR | IS*6110*-RFLP | | The study highlighted the need for effective isolation facilities and infection control measures |
| (57) | Johannesburg, South Africa | 1996-1998 | Retrospective epidemiological study | Hospital | HIV infected patients | | MDR | IS*6110*-RFLP | | The study emphasised the importance of isolation of infected patients and effective infection control in hospitals |
| (58) | Buenos Aires, Argentina | 1991-1994 | Outbreak investigation | Hospital | HIV infected patients, co-infected with MDR-TB | | MDR | IS*6110*-RFLP | | The study demonstrated the effectiveness of IS*6110*-RFLP as a powerful tool to analyse |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| | | | | | | | | | | transmission |
| (99) | Buenos Aires , Argentina | 1994-1995 | Epidemiological investigation | Hospital | Patients with HIV associated with MDR | | MDR | IS*6110*-RFLP | | Transmission occurs where infection control procedures are minimal. Stressing for reinforcement |
| (38) | New York City, USA | 1993-1994 | Retrospective epidemiological study | Hospital nursery | All patients diagnosed with TB or resistant to INH, RIF and STR or had an initial diagnosis or exposure to hospital A | TST | MDR | IS*6110*-RFLP | | Study highlighted the importance of implementation of preventive and control measures in hospitals |
| (100) | Madrid, Spain | 1991-1995 | Outbreak investigation | Hospital | Patients with HIV infection | none | MDR | IS*6110*-RFLP | | The study underscored the importance of routine screening among health care workers and ensuring that effective infection control measures are in place |
| Outbreaks among households, communities and foreign born cases | | | | | | | | | | |
| | | | | | | | | | | The study |

| | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|
| (85) | Taiwan | 2006 | Molecular epidemiological investigation | Familial | HIV negative patients | TST and chest radiography | MDR | Spoligotyping and MIRU-VNTR | | highlighted the importance of an effective and tailored treatment program and isolation of active cases |
| (86) | Buenos Aires , Argentina | 2001-2004 | Epidemiological investigation | Low category hotel | Transvestite sex workers | TST | MDR | IS*6110*-RFLP | | Overcrowding fuels transmission and that imparting knowledge of how TB spreads is important in congregated settings |
| (87) | Norway | 1994-2000 | Retrospective epidemiological study | Community | Culture positive cases (foreign born and natives) | none | INH and MDR | Sequencing, reverse hybridisation, IS*6110*-RFLP and Spoligotyping | | The study stressed the importance of continuous screening of immigrants |
| (101) | Austria | 2005-2006 | Epidemiological investigation | Community | HIV seronegative patients | Chest X ray | MDR | Spoligotyping and MIRU-VNTR | Beijing | The study demonstrated the utility of Molecular tools and that non-adherence and inappropriate treatment can lead to transmission |

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|---|---|---|---|---|---|---|---|---|---|
| (89) | Singapore | 2012 | Outbreak investigation | Community | Immunocompet ent patients | QuantiFERON-TB Gold In-Tube test | MDR | Spoligotyping, MIRU-VNTR and WGS | Beijing | The study highlighted on the importance of prompt diagnosis and appropriate treatment and gave insight on the resolution that WGS offers |
| (102) | Virginia, USA | 2004-2010 | Retrospective outbreak investigation | Community | PZA mono-resistance cases with matching genotypes and clinically diagnosed cases who are epidemiological ly linked to cluster cases | TST and chest radiography | PZA mono-resistance | IS6110-RFLP, Spoligotyping, MIRU-VNTR | | The young and adolescent are vulnerable to transmission of resistant forms of TB and should be routinely screened |
| (36) | Conotou, Benin | 2005-2006 | Cohort study | Community | Patients with pulmonary tuberculosis | GIS | STR mono-resistance | MIRU-VNTR typing and RFLP | Beijing strain | The study highlighted that clustered areas enable transmission and coupling of molecular tools with GIS enhances confirmation an outbreak |
| | | | | | | | | | | |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Outbreaks in Prisons | | | | | | | | | |
| (103) | New York State, USA | 1990-1991 | Retrospective epidemiological investigation | State prison | Inmates diagnosed with TB | TST | MDR | IS*6110*-RFLP | | The study emphasised on the importance of isolation of active cases and continuous screening among prison inmates to prevent outbreaks |
| Outbreaks in schools | | | | | | | | | |
| (92) | Castellon, Spain | 2008-2014 | Prospective observational study | Schools | High school personnel | | MDR | IS*6110*-RFLP | | Prompt diagnosis is necessary to prevent treatment delays and transmission |
| (91) | California, USA | 1991-1993 | Retrospective cohort study with case investigation | School | High school students | TST | Poly-resistant | IS*6110*-RFLP and DNA Sequencing | | The study highlighted the importance of continuous screening in schools to prevent spread of infection |

**Conclusion**

An understanding of how outbreaks emerge and the molecular epidemiological tools used to confirm transmission can help in controlling the spread of DR-TB strains. We reviewed outbreak reports of DR-TB in different settings.

Reports suggested that the most vulnerable cases were the immunocompromised and those that were in congregated environments, such as schools, prisons and hospitals. Findings from the above studies clearly indicated that transmission was the driving force for the DR-TB outbreaks and was fuelled by inadequately treated and undetected cases. Furthermore, poor infection control contributed to the dissemination of the DR strains.

Lack of knowledge also contributed to the spread of DR-TB as the general public are in most cases ill-informed about the symptoms of the disease and thereby, patients with TB did not seek medical attention when they presented with symptoms and only did when the disease had progressed, infecting a number of contacts before they were diagnosed. This could have also resulted from fear of the stigma associated with diseases such as HIV, especially in resource limited settings. Follow up of cases was highlighted, which stressed for routine follow up of patients undergoing treatment and those that were found to be latently infected with TB. The studies also emphasised on promptly reporting to TB control.

There is a deficit in prevention and control measures; clearly what is in place is not effective enough to combat this scourge. An explanation could be poor management of the recommended programs. The resurgence of TB in New York City bears as evidence of what neglect or poor TB management could lead to. Hence, there is a need for control efforts to be instituted in various institutions and settings to halt transmission (37). A rapid and effective approach is mandatory to tackle this situation. There is a need for prompt diagnosis of infected cases using rapid diagnostic tests such as The Genotype® MTBDR*plus* and MTBDR*sl* (Hain Life Science, Nehren, Germany) and GeneXpert™ MTB/RIF (Cepheid, Germany). In future, routine use of WGS could be implemented to aid in identification of drug resistance and identifying possible transmission chains.

In order to prevent future DR-TB outbreaks globally, there is a need for continuous roll out of treatment, decentralised treatment should be tightened by having trained personnel and

adequate resources. This will enable patients to achieve high cure rates. Failure to reinforce prevention and control programs could result in outbreaks of strains that do not respond to available treatment which could be disastrous.

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# Chapter 3

# Materials and methods

**3.1 Geographical location**

The West Coast is among the district municipalities of the Western Cape Province, it is bordered by the Atlantic Ocean on the west and the Northern Cape on the north and east. This area is characterised by a high TB prevalence of 1224.9/100 000 population (28). The five local municipalities within the West Coast are: Matsikama, Swartland, Bergrivier, Cederberg and Saldanha Bay.

**3.2 Sample selection**

A longitudinal reference database and corresponding sample bank house in the Division of Molecular Biology and Human Genetics were available for this study. All viable DR-TB isolates diagnosed at the reference laboratory of the National Health Laboratory Service (NHLS) from the Western Cape Province were collected and stored since 2007, with phenotypic drug susceptibility testing (DST) results and basic demographic data of the patients. Ethical clearance was obtained from the Ethics Committee (reference number N09/11/296). All isolates from patients who originated from the West Coast district, collected from 2008-2012 were extracted from the database (n=786). One isolate from each patient was selected for analysis. If a patient had more than 1 isolate in the sample bank, the first isolate with resistance to the most drugs was selected (n=611).

**3.3 Genotyping**

**3.3.1 Spoligotyping**

To describe the DR-TB strain population in the West Coast region we made use of spoligotyping. This genotyping technique makes use of the DR region present in the genome of members of the *M. tuberculosis* complex (31). The DR region is made up of direct repeats (DRs), interspersed with unique DNA sequences, termed spacers (see **figure 3.1**). Spoligotyping is a reverse line probe assay, where the primers in the PCR step are based on the repeat sequences, thereby amplifying the unique sequences in between the DRs, labelled

with Biotin for downstream visualization with Streptavidin and Enhanced Immunohistochemistry (ECL). The PCR products are then hybridised to a membrane containing 43 probes compliment to spacer sequences derived from the laboratory strain H37Rv and the vaccine strain *M. bovis* bacilli Calmette-Guérin (BCG) (32). Evolution of the DR region can be via homologous recombination, single nucleotide polymorphisms, or disruptions by the insertion element, IS*6110* (33). After hybridization of the DNA on the membrane, the presence or absence of the spacer DNA is visualized by enhanced ECL on an X-Ray. The presence of the spacer DNA is indicated by a black block, and the absence of a block indicates the absence of the spacer DNA. Thereby generating a characteristic pattern for each strain analysed.
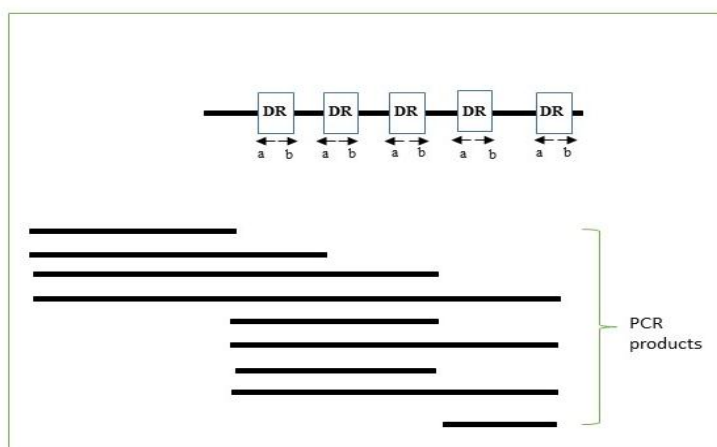


**Figure 3.1** The principle of PCR amplification of the DR region using primer "a" and primer "b" based on the DR region of the *M. tuberculosis* complex

### 3.3.1.1 Spoligotyping Protocol

Spoligotyping was performed using the internationally standardised method to identify strain families (34). For PCR amplification: the PCR master mix was made up as set out in **table 3.1**

**Table 3.1** PCR master mix

| H20 | 6.5µl |
|---|---|
| Kapa Taq ready mix | 12.5µl |

| DRb (supplied with manufacturer) | 2µl |
|---|---|
| DRa (biotinylated 5' end) (supplied with manufacturer) | 2µl |
| Template DNA | 2µl |

*M. bovis* BCG and H37Rv DNA were included as positive controls. The PCR mixture was placed in the thermocycler and subjected to the following conditions: 3 minutes at 95°C followed by 30 cycles at 94°C for 1 minute, 55°C for 1 minute, 72°C for 30 seconds and 72°C for 10 minutes. For hybridization, 20µl of PCR product was diluted by adding 160µl of 2XSSPE/0.1%SDS and heat denatured for 10 minutes at 99°C and immediately cooled on ice. The membrane (Mapmygenome™, India) with covalently bound oligonucleotides was washed at 60°C in 2XSSPE for 10 minutes and placed on a support cushion in a miniblotter (Mapmygenome™, India) with slots perpendicular to 43 immobilised oligonucleotides. The residual fluid from the slots was removed by aspiration and denatured PCR products were pipetted into parallel slots with caution to avoid bubbles. Hybridization was done for 60 minutes at 60°C on a horizontal surface. PCR products were removed from the miniblotter by aspiration. The membrane was washed twice for 5-10 minutes each time in 2XSSPE/0.5%SDS at 60°C and then incubated in 40ml of 2XSSPE/0.5%SDS with 12.5µl streptavidin-peroxidase conjugate (500U/ml) (Roche, Germany) for 60 minutes at 42°C. Following incubation, the membrane was washed twice for 5-10 minutes each time in 2XSSPE/0.5%SDS at 42°C and rinsed with 2xSSPE for 5 minutes at room temperature. The membrane was incubated in 20ml of (10 ml solution A and 10 ml solution B) of enhanced chemiluminescence ECL (Amersham ECL Direct™ nucleic acid labelling and detection system, GE Healthcare Limited, Little Chalfont, UK) detection liquid for 1½ minutes. The X-ray film was exposed to the membrane for 5-20 minutes in a dark room and developed using an automated developer. The membrane was stripped by washing twice in 1%SDS for 30 minutes each time at 80°C and stored at 4°C in 20Mm EDTA (Sigma Aldrich) at pH 7.0 in a sealed plastic container for repeated use.

**3.3.2 Targeted gene sequencing**

Genes or gene regions, associated with drug resistance were PCR amplified and sequenced using Sanger sequencing to detect mutations conferring drug resistance.

3.3.2.1 PCR amplification

*M. tuberculosis* target genes (*inhA* promoter, *katG, rpoB, embB*, *pncA, gyrA* and *rrs*) were PCR amplified using designed primers, **table 3.2**.

**Table 3.2** Primers used for amplification of genes conferring resistance

| Drug resistance phenotype | primer | Sequence (5'to 3') | Fragment length | Tm |
|---|---|---|---|---|
| **INH** | *katG* gene RTB 59 RTB 35 | TGGCCGCGGCGGTCGACATT GGTCAGTGGCCAGCATCGTC | 419 | 62°C |
| **INH** | *inhA* prom inhA P5 inhA P3 | CGCAGCCAGGGCCTCGCTG CTCCGGTAACCAGGACTGA | 246 | 55°C |
| **RIF** | *rpoB* gene rpoB For rpoB Rev | TGGTCCGCTTGCACGAGGGTCAGA CTCAGGGGTTTCGATCGGGCACAT | 437 | 69°C |
| **EMB** | *embB* gene emb 151 emb 131 | CGGCATGCGCCGGCTGATTC TCCACAGACTGGCGTCGCTG | 260 | 64°C |
| **PZA** | *JpncA* JpncA_For JpncA_Rev | GGCGTCATGGACCCTATA GTGAACAACCCGACCCAG | 700 | 62°C |
| **Fluoroquinolones** | *gyrA* gyrA For gyrA Rev | TGACATCGAGCAGGAGATGC GGGCTTCGGTGTACCTCATC | 344 | 62°C |
| **Aminoglycosides KAN, AMK and CAP)** | *rrs* (16 RNA) rrs290_For rrs290_Rev | TGCTACAATGGCCGGTACAA CTTCCGGTACGGCTACCTTG | 290 | 62°C |

PCR reactions were made up to 25µl total volume, containing 6.375µl of $H_20$, 5µl of Q solution, 2.5µl 10x buffer, 4µl $MgCl_2$ (25 Mm), 4µl dNTPs (10mM), 1µl each of forward and reverse primer (50pmol/uL), 1µl Syto9 florescent dye (1:00 dilution) (Life technologies, Oregon, USA), 0.125 µl HotStar Taq polymerase (Qiagen, Hilden, Germany) and 2µl DNA that was grown on liquid culture and boiled for half an hour. Amplification conditions were as follows: 15 minutes at 95°C, followed by 40 cycles at 94°C for 1 min, Tm for 1 minute, 72 °C for 1 minute and 72°C for 10 minutes (see **table 3.2** for each specific Tm). The *rpoB* target gene was the exception, because of the high Tm of the primers, the reaction mixture was subjected to a touchdown PCR as follows: 15 minutes at 95°C, followed by 2 cycles at 94°C for 1 minute, 72°C for 1 minute, 72°C for 1 minute, 2 cycles at 94°C for 1 minute, 71°C for 1 minute, 72°C for 1 minute, 2 cycles at 94°C for 1 minute, 70°C for 1 minute, 72°C for 1 minute, 40 cycles at 94°C for 1 minute, 69°C for 1 minute, 72°C for 1 minute and 72°C for 10 minutes. H37Rv laboratory strain was used as a positive control and water was included as a negative control. Successful PCR Amplification was confirmed using the Rotor gene V1.787 high resolution melt (HRM) analysis. The H37Rv laboratory strain DNA was used as a positive control and water as a negative control. The PCR products were subjected to a high resolution melt as follows: ramp from 80°C to 95°C rising by 0.1 °C each step, waiting for 90 seconds of pre-melt conditions on the first step and for 2 seconds of each step afterwards.  As the temperature increases, the double stranded DNA becomes single stranded and releases the intercalated Syto9 fluorescence dye. The decrease in fluorescence is measured in real time as represented in an example plotted with Fluorescence (df) vs. temperature (dt) which generates a melting curve, see **figure 3.2.** The peaks differ in different genes owing to the DNA fragments.
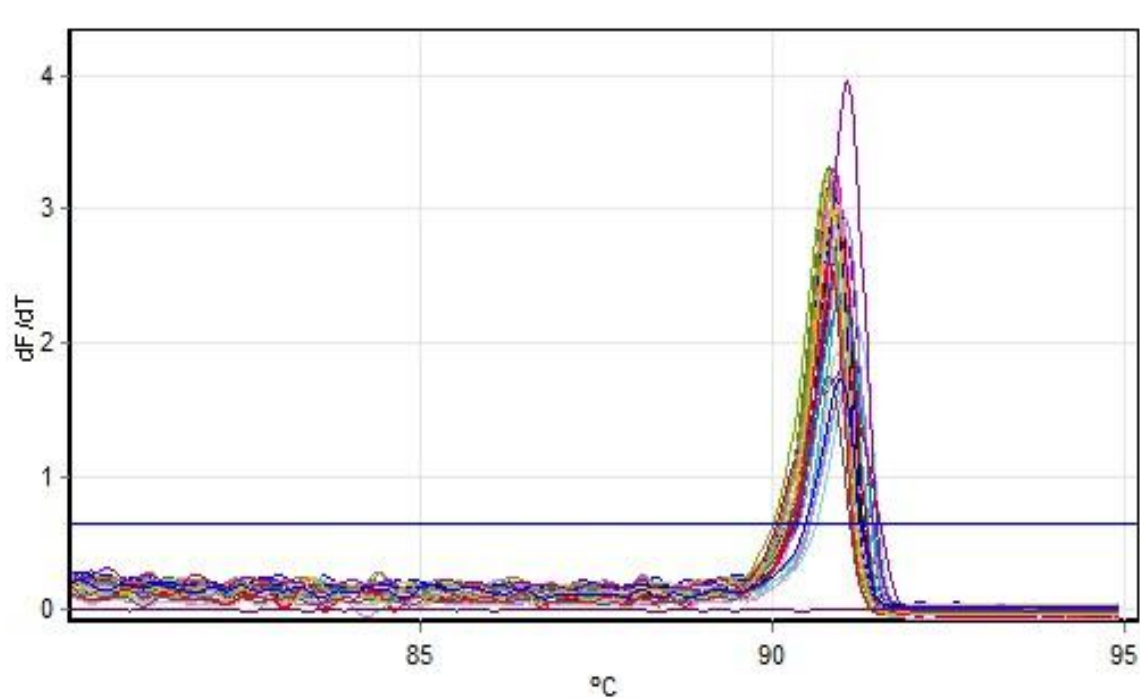
**Figure 3.2 Example of a Melt curve of High resolution Melt to confirm successful PCR amplification of *rpoB* gene.**

### 3.3.2.2 DNA Sequencing

PCR products were sequenced by Sanger sequencing at the Central Analytical Facility (CAF) at Stellenbosch University. Briefly, Post PCR clean-up of the PCR products was done using the Agencourt AMPure XP PCR Purification system (Agencourt Bioscience Corporation, Beckman Coulter, Beverly, Massachusetts) according to the manufacturer's instructions. PCR products were sequenced using the forward amplification primer. The DNA sequencing reaction was done using a GeneAmp 9700 thermal cycler (Applied Biosystems, Foster City, CA 94404 USA). The following sequence program was selected; 5 minutes at 94°C, followed by 25 cycles at 94°C for 10 seconds, 55°C for 10 seconds and 60°C for 4 minutes. The sequencing reaction was cleaned using Centri-Sep™ columns (Princeton Separations Inc, Adelphia, NJ 07710 USA) and then electrophoresed on an ABI3730xl genetic analyser (Applied Biosystems, Foster City, CA 94404 USA) using the standard run module for the 50cm capillary. Base calling was performed using Sequencing analysis V5.3.1 with the

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"mixed base setting" set at 10% allowing for the detection of minority single nucleotide polymorphisms.

### 3.3.3 Sequencing analysis

Gene sequences were aligned using Bio Edit version 7.1.3 and the respective gene sequence of *M. tuberculosis* H37Rv reference strain obtained from tuberculist. (http://genolist.pasteur.fr/TubercuList/)

### 3.4 Phylogenetic analysis

In order to identify possible outbreak clones of the X-family circulating in the West Coast region, a phylogeny was constructed with the isolates belonging to this family and all sequencing results from the 7 conferring genes were available. The spoligotype pattern, and each variable region sequenced were concatenated to produce a FASTA file for each isolate. The general time-reversal (GTR) model of substitution was applied to construct a maximum likelihood phylogeny of the isolates included in this analysis with Randomized Axelerated Maximum Likelihood (RaxML) with 1000 bootstrap pseudo-replicates (35, 36).

# Chapter 4

# Results

## 4.1 Demographic data

We conducted a retrospective study of DR-TB isolates that were collected in the West Coast district of the Western Cape Province, South Africa, from 2008-2012. Patient drug-susceptibility tests and demographic data were routinely collected from the five local municipalities within the West Coast district (see **table 4.1**). A total of 611 DR-TB patients were included in the study. Of these, 349 (57%) were male and 251 (40%) were female. Data was not available for 11 (1.8%) of the patients and were designated as unknown. The average age among the patients with confirmed DR-TB was 35 years. The HIV status and other clinical data were not available for this study.

**Table 4.1** The Distribution of patients in the West Coast District within the local municipalities and respective towns.

| Local Municipality | | Town | |
|---|---|---|---|
| **Matsikama** | 212 | Vredendal | 100 |
| | | Lutzville | 65 |
| | | Klawer | 33 |
| | | van Rhynsdorp | 6 |
| | | Bitterfontein | 5 |
| | | Rietpoort | 3 |
| **Swartland** | 127 | Malmesbury | 77 |
| | | Moorreesburg | 33 |
| | | Riebeeck Kasteel | 8 |
| | | Riebeeck West | 5 |
| | | Darling | 2 |
| | | Melkbosstrand | 2 |
| **Cederberg** | 112 | Clanwilliam | 55 |
| | | Citrusdal | 30 |
| | | Graafwater | 15 |
| | | Lamberts Bay | 7 |
| | | Elands Bay | 5 |
| **Saldanha Bay** | 100 | Vredenburg | 93 |
| | | Hopefield | 3 |
| | | Langebaan | 3 |
| | | Saldanha | 1 |
| **Berg River** | 60 | Porterville | 32 |
| | | Piketberg | 28 |

**4.2 Phenotypic drug resistance**

Drug-susceptibility data for the isolates was routinely collected from the National Health Laboratory Service (NHLS) at Greenpoint, Western Cape Province. These tests were routinely done as requested by the clinicians. The INH/RIF mono-resistance was defined as resistance to either one of the first-line antituberculous drugs. Polyresistance was defined as resistance to more than one antituberculous drug but not MDR. MDR-TB was defined as resistance to at least INH and RIF. Pre-XDR-TB was defined as MDR-TB with added resistance to either the fluoroquinolone, ofloxacin (OFL) or injectable drug, (AMK). Extensively drug-resistant TB was defined as MDR-TB with resistance to both OFL and AMK.

We identified a high proportion of patients diagnosed with MDR-TB (n=297; 49%). We also identified more INH monoresistant isolates (n=133; 22%) as compared to the isolates that were RIF monoresistant (n=81; 13%). Furthermore 60 (10%) patients had pre-XDR-TB and 19 (3%) had XDR-TB. Five patients (1%) were classified as poly-resistant, while for 16 (3%) patients, we did not have adequate DST results and could not classify them, hence designated as unclassified.

**4.3 Genotyping**

To determine the distribution of DR-TB strain families in the West Coast district, the isolates from each case with the highest number of resistance phenotype were genotyped using spoligotyping. The strain families were classified according to the international SITVIT database (37). The spoligotypes that have not been assigned a spoligo international type (SIT) were referred to as orphans and numbered according to the local spoligotype (LT) and classified to belong to a specific spoligotype family and lineage according to distinct signatures. Spoligotype patterns that were not previously identified in SITVIT, were also assigned a LT, but not classified into an international family/clade. The spoligotyping signatures of *M. tuberculosis* that were identified in this region are shown in **table 4.2**.

### 4.3.1 Spoligotyping analysis

We identified 9 spoligotype lineages of *M. tuberculosis* within the region, these included: the Beijing family (n=154; 25%), X-family (n=246; 40%), T-family (n=63; 10%), S-family (n=45; 8%), Latin American Mediterranean (LAM) (n=44; 7%), Central Asia (CAS) (n=11; 2%), Manu (n=8 ; 1%), Haarlem (n=8; 1%) and East African Indian (EAI) family (n=5; 1%), see **figure 4.1**. The predominant family identified was the X-family, followed by the Beijing family. Patients infected with *M. tuberculosis* X-family were male (54%) the average ages were 35 years. This is not statistically different to the total population or patients infected with the Beijing strain family of *M. tuberculosis* (61% male, average age 34). The breakdown of *M. tuberculosis* strain families within the lineages are shown in **table 4.2.**
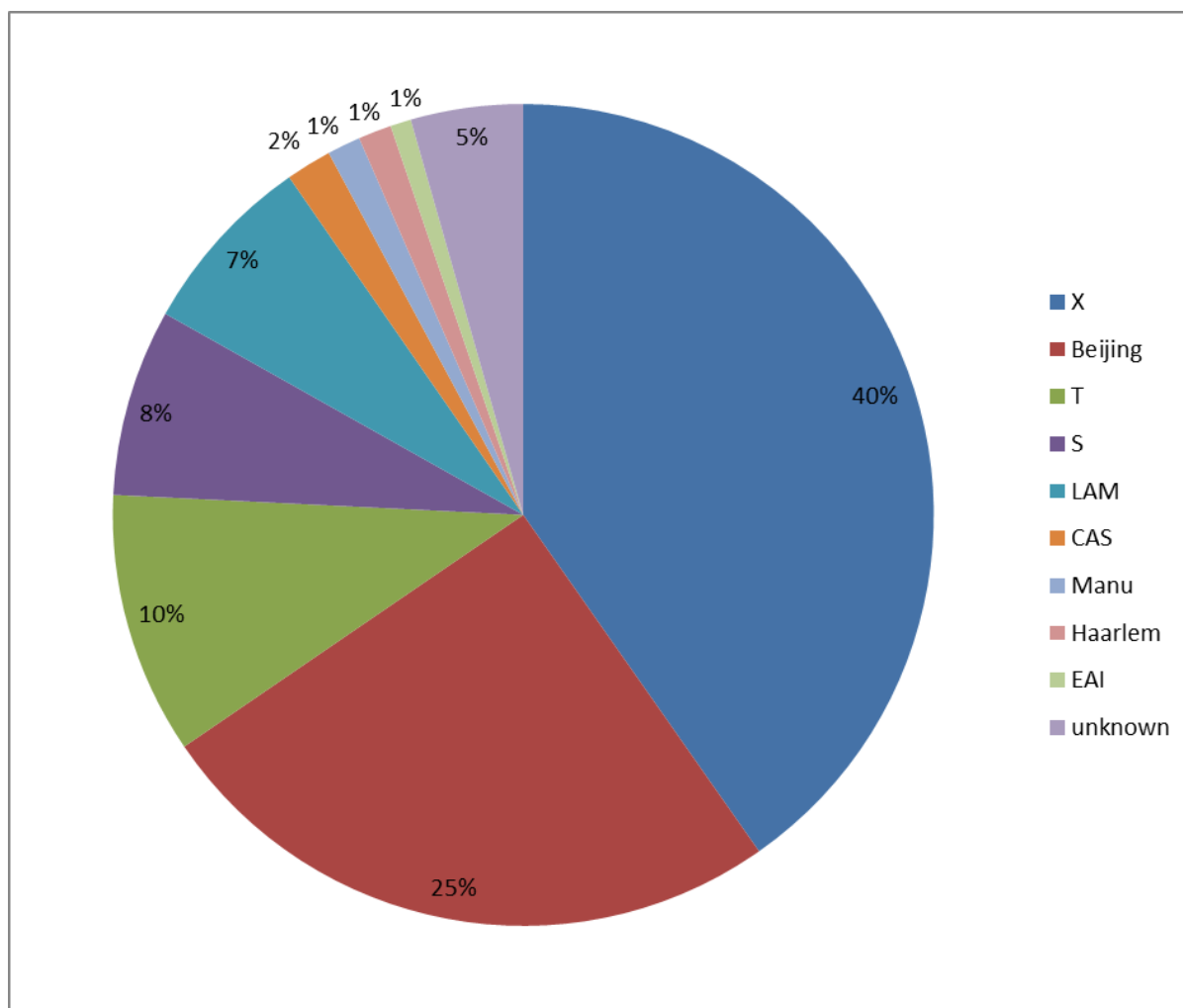


**Figure 4.1.**  Distribution of spoligotype lineages that were identified from the West Coast district.

**Table 4.2.** Spoligotyping signatures of *M. tuberculosis* identified in the West Coast

| Lineage | nr of isolates | Int family[c] | nr of isolates | SIT[#] | Spoligotype pattern | nr of isolates |
|---|---|---|---|---|---|---|
| X | 246 | X1 | 192 | 119 | | 186 |
| | | | | Orphan[^] (LT[*] -837) | | 1 |
| | | | | 336 | | 3 |
| | | | | 302 | | 2 |
| | | X3 | 36 | 92 | | 35 |
| | | | | 2286 | | 1 |
| | | X2 | 18 | 137 | | 17 |
| | | | | 347 | | 1 |
| Beijing | 154 | BEIJING | 154 | 1 | | 153 |
| | | | | 190 | | 1 |
| T | 63 | T1 | 48 | 53 | | 17 |
| | | | | 244 | | 11 |
| | | | | 1067 | | 8 |
| | | | | 136 | | 4 |
| | | | | 462 | | 3 |
| | | | | 628 | | 1 |
| | | | | 732 | | 1 |
| | | | | 913 | | 1 |
| | | | | 1202 | | 1 |
| | | | | 1737 | | 1 |
| | | T1_RUS2 | 6 | 280 | | 6 |
| | | T4_CEU1 | 3 | 39 | | 3 |
| | | T5 | 4 | Orphan[^] (LT[*] -838) | | 1 |
| | | | | 44 | | 3 |
| | | T5_RUS1 | 2 | 254 | | 2 |
| S | 45 | S | 44 | 34 | | 32 |
| | | | | 789 | | 9 |
| | | | | Orphan[^] (LT[*] -939) | | 1 |
| | | | | 494 | | 1 |
| | | | | 1333 | | 1 |
| | | U | 1 | 790 | | 1 |
| LAM | 44 | LAM3 | 29 | 33 | | 17 |
| | | | | 719 | | 4 |
| | | | | Orphan[^] (LT[*] -43) | | 1 |
| | | | | 2345 | | 2 |
| | | | | 1331 | | 1 |
| | | | | 1335 | | 1 |
| | | | | 2302 | | 1 |

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| Family | | Sub-family | | Type/SIT | Spoligotype pattern | Count |
|---|---|---|---|---|---|---|
| | | | | 4 | | 2 |
| | | LAM1 | 6 | 20 | | 6 |
| | | LAM11_ZWE | 4 | 59 | | 2 |
| | | | | 2017 | | 1 |
| | | | | 2196 | | 1 |
| | | LAM4 | 4 | 60 | | 4 |
| | | LAM9 | 1 | 42 | | 1 |
| CAS | 11 | CAS1_DELHI | 10 | 26 | | 9 |
| | | | | 25 | | 1 |
| | | CAS | 1 | 357 | | 1 |
| Manu | 8 | MANU2 | 8 | 54 | | 2 |
| | | | | 226 | | 2 |
| | | | | 2229 | | 2 |
| | | | | 1634 | | 1 |
| | | | | 2117 | | 1 |
| Haarlem | 8 | H1 | 3 | 47 | | 2 |
| | | | | 62 | | 1 |
| | | H3 | 3 | 50 | | 3 |
| | | H4 | 1 | 921 | | 1 |
| | | U (likely H) | 1 | 46 | | 1 |
| EAI | 5 | EAI1_SOM | 4 | 48 | | 3 |
| | | | | 806 | | 1 |
| | | EAI5 | 1 | 236 | | 1 |
| unknown | 27 | | NOT IN SITVIT | LT747 | | 5 |
| | | | | LT974 | | 1 |
| | | | | LT1017 | | 1 |
| | | | | LT1013 | | 1 |
| | | | | LT1028 | | 1 |
| | | | | LT1030 | | 1 |
| | | | | LT1160 | | 1 |
| | | | | LT1139 | | 1 |
| | | | | LT1157 | | 1 |
| | | | | LT994 | | 1 |
| | | | | LT1161 | | 1 |
| | | | | LT1092 | | 1 |
| | | | | 2306 | | 2 |
| | | | | 519 | | 1 |
| | | | | 602 | | 1 |
| | | | | 1241 | | 1 |
| | | | | no result | | 6 |

**Legend to Table 4.2** [*]LT-local type, 'Int-international family, [#]SIT- spoligo international type, ^Orphan- Present in the international database only once.

**4.3.2 Drug resistance and sequencing of drug resistance conferring genes of the X-family**

Since the X-family was the predominant cause for DR-TB of the West Coast region, further analysis was focused on isolates from this lineage. Genes or gene regions associated with drug resistance were analysed by PCR amplification followed by DNA sequencing.

**4.3.3 Sequencing analysis (n=246)**

Of the isolates that were identified to belong to the X-family, we identified mutations at codon 315(AAC, ACA, ACC and AGA) of the *katG* gene and mutations in the *inhA* promoter region (-15C-T) conferring INH resistance. The rifampicin resistance determining region (RRDR) harboured mutations at codon 511(CCG), 516(TAC), 526(GAC and CTC), 531 (TTG) and 533(CCG) of the *rpoB* gene conferring RIF resistance. Mutations conferring PZA resistance were identified throughout the *pncA* gene at position 105(GAC), 132(AGT), 139 (ATG), 143(GTC), 154(GGG), 164(CCG), 180(GCC), 35(CGG), 62(CTG), 68(GGG) and 85(CCG). We identified codons harbouring the *embB* mutation, codon 306(ATA, ATC, ATT, GTG) and codon 328(TAT) conferring EMB resistance. The *gyrA* gene conferring OFL resistance harboured mutations at codons, 89(GGC), 90(GTG), 91(CCG) and 94(AAC, GCC, GGC). Mutations conferring kanamycin resistance were identified at position 1401A-G, see **table 4.3.**

**Table 4.3** Different mutations identified in 7 drug resistance conferring genes

| Gene | Mutation | Nr of isolates |
|------|----------|----------------|
| *rpoB* | CTG511CCG | 17 |
| | GAC516GTC | 2 |
| | GAC516TAC | 1 |
| | CAC526CTC | 28 |
| | CAC526GAC | 1 |
| | TCG531TTG | 184 |
| | CTG533CCG | 1 |

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|  |  |  |
|---|---|---|
|  | Wild type | 10 |
|  | no result | 2 |
|  |  |  |
| *katG* | AGC315AAC | 1 |
|  | AGC315ACA | 196 |
|  | AGC315ACC | 21 |
|  | AGC315AGA | 1 |
|  | Wild type | 22 |
|  | no result | 5 |
|  |  |  |
| *inhA* prom | -15 C-T | 7 |
|  | Wild type | 237 |
|  | no result | 2 |
|  |  |  |
| *pncA* | GGC105GAC | 1 |
|  | GGT132AGT | 3 |
|  | GTG139ATG | 134 |
|  | GCC143GTC | 3 |
|  | AGG154GGG | 1 |
|  | TCG164CCG | 1 |
|  | GTC180GCC | 4 |
|  | CTG35CGG | 1 |
|  | CCG62CTG | 1 |
|  | TGG68GGG | 1 |
|  | CTG85CCG | 1 |
|  | Wild type | 82 |
|  | no result | 13 |
|  |  |  |
| *embB* | ATG306ATA | 6 |
|  | ATG306ATC | 1 |
|  | ATG306ATT | 1 |
|  | ATG306GTG | 137 |
|  | GAT328TAT | 4 |
|  | Wild type | 83 |
|  | no result | 14 |
|  |  |  |
| *gyrA* | GAC89GGC | 1 |
|  | GCG90GTG | 14 |
|  | TCG91CCG | 7 |
|  | GAC94AAC | 1 |

| | | |
|---|---|---|
| | GAC94GCC | 7 |
| | GAC94GGC | 29 |
| | Wild type | 185 |
| | no result | 2 |
| | | |
| *rrs* | 1401 A-G | 12 |
| | 1402 C-A | 1 |
| | Wild type | 224 |
| | no results | 9 |

DNA sequencing of drug resistance conferring genes and DST correlates as has been previously reported (38–41) with the best correlation between RIF and *rpoB* (38) and the lowest EMB and *embB* (39), Ethambutol susceptibility testing been known to be inaccurate and has been suspended by the routine lab during the course of the study (as indicate by the limited numbers in **table 4.4**.)

**Table 4.4** Correlation of drug resistance conferring genes and DST

| Antituberculous drugs | Associated gene | Sensitivity | Specificity | correlation | Nr of isolates |
|---|---|---|---|---|---|
| RIF | *rpoB* | 99.1 | 66.7 | 97.5 | 242 |
| INH | *katG* and *inhA* promoter | 96.4 | 68.8 | 94.6 | 239 |
| EMB | *embB* | 100.0 | 32.3 | 35.0 | 103 |
| OFL | *gyrA* | 67.4 | 86.7 | 82.7 | 226 |
| AMI | *rrs* 1400 region | 69.2 | 99.0 | 97.2 | 217 |

## 4.4 Phylogeny of the X-family

A combination of spoligotype patterns and the identified gene mutations associated with drug resistance were used to construct a maximum likelihood phylogenetic tree, in order to identify possible outbreak strains, see **figure 4.2**. For this, all isolates that had complete sequencing data of all 7 drug resistance conferring mutations were included (n=221). A large MDR cluster of 50 isolates were identified harbouring identical *katG*315ACA, *rpoB*531TTG, *pncA*139ATG and *embB*306GTG mutations. Furthermore, 53 isolates with additional

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mutations, leading to pre-XDR-and XDR-TB that form part of this outbreak were present on this branch.
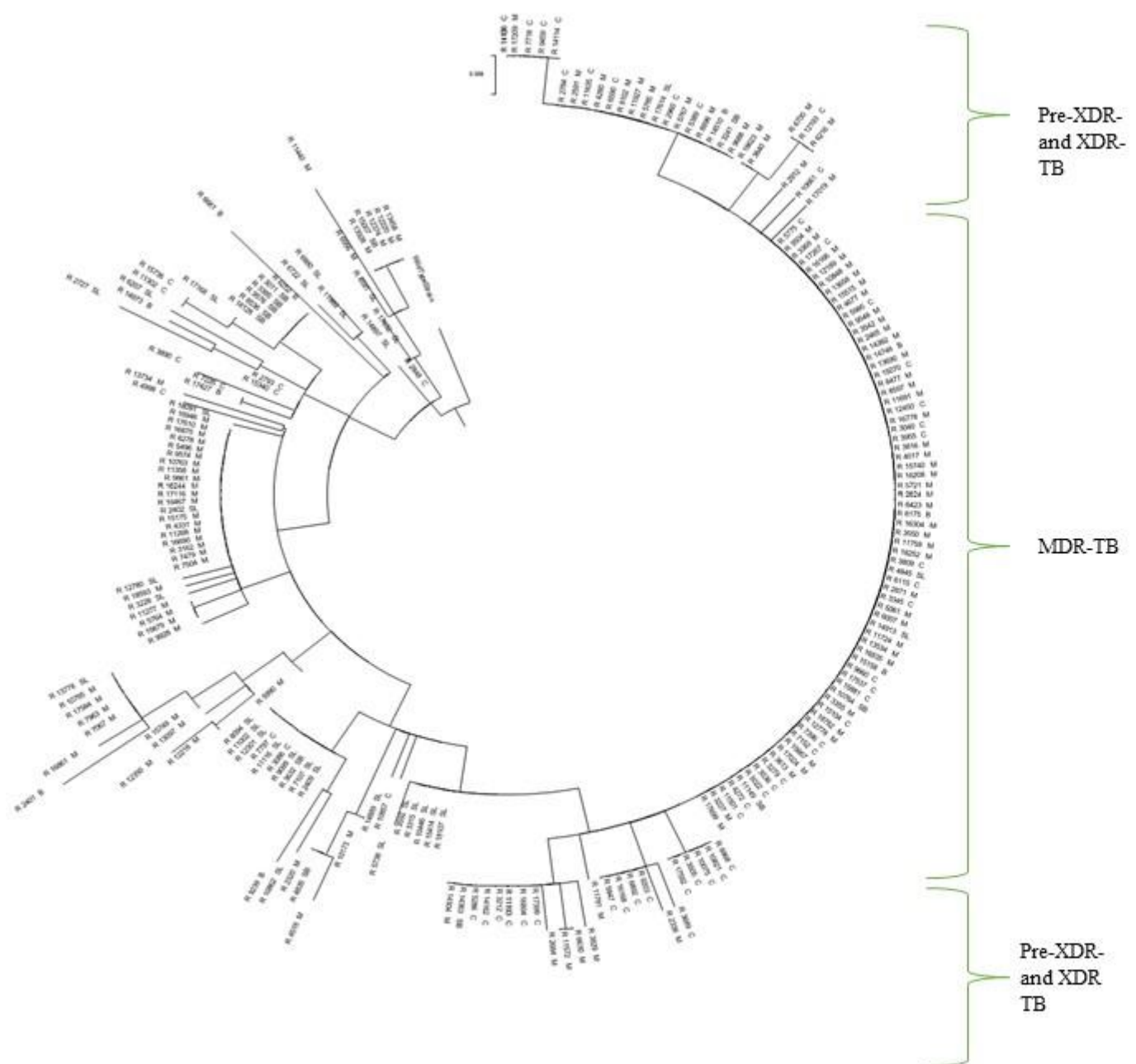
**Figure 4.2 Maximum likelihood phylogenetic tree selected clinical isolates of *M. tuberculosis*.** A maximum likelihood phylogeny was constructed using model parameters GTR+G and 1000 bootstraps with the RAxML phylogenetic software based on selected drug resistance associated regions of *M. tuberculosis*. The municipalities are symbolised by letters, Matsikama (M), Cederberg (C), Swartland (SL), Bergrivier (B) and Saldanha bay (SB).

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## 4.5 Geographical distribution of an outbreak.

Based on the results obtained from the phylogenetic tree, we selected all isolates that harboured the same *katG*315ACA, *rpoB*531TTG, *pncA*139ATG and *embB*306GTG mutations and plotted them on a map of where they originated and classification of drug resistance based on mutations identified. The patients were clustered mainly in and around 3 major towns: Vredendal, Lutzville and Clanwilliam. These towns are located in the Northern most District Municipalities of Matsikama and Cederberg, see **figure 4.3.**
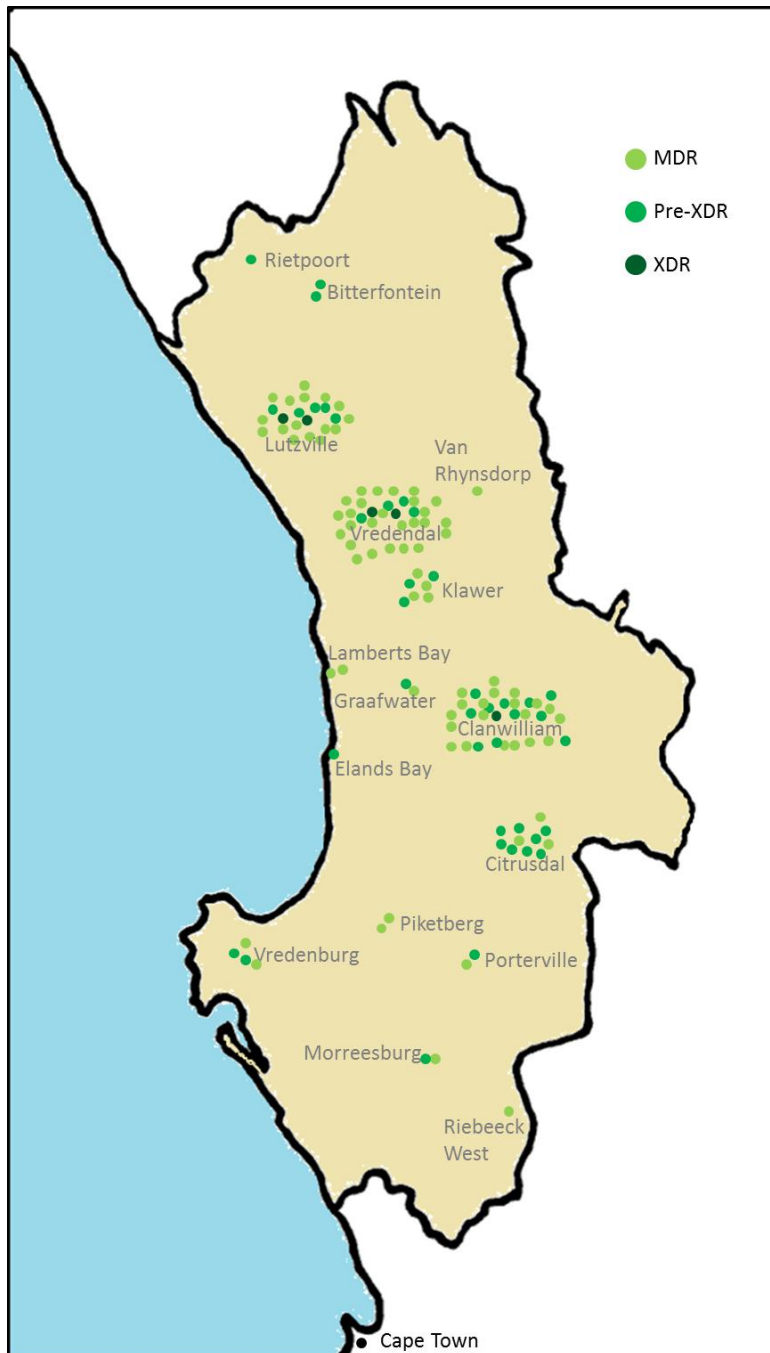
**Figure 4.3** Geographical distribution of patients infected with an outbreak strain of *M. tuberculosis* which have similar spoligotype patterns and at least 4 identical drug resistance conferring mutations.

# Chapter 5

# Discussion and Conclusion

DR-TB continues to be a major problem globally, even though prevention and control measures have been recommended (3). The emergence and spread of DR-TB has been attributed to inadequate treatment, delay in diagnosis of drug resistance and infection control measures. In the current study, we aimed to describe the DR-TB epidemic in the West Coast region of the Western Cape Province. Very limited data has been reported on molecular epidemiology in this region as compared to the rest of the Western Cape. Furthermore, a very high prevalence (1224.9/100 000 population) of TB has been reported which is alarming.

Analysis of DST by the routine laboratory showed that INH mono-resistant strains were more represented than RIF mono-resistant strains. This could be because RIF was not yet selected or that phenotypic DST could not pick up low level RIF resistance (42). Another possible explanation could be because INH resistance occurs more frequently than RIF resistance, since multiple genes and gene regions could be the reason to confer INH resistance compared to RIF, which only the RRDR are involved in drug resistance (43). We observed a low specificity for detecting RIF resistance by genotyping. This is skewed by the limited number of RIF sensitive strains that was present in the cohort tested. Furthermore, 3 of the 4 isolates that was resistant to RIF, had a *rpoB*526CTC mutation that is associated with low level RIF resistance (1ug/ml) (M. Whitfield, unpublished data), that could give false positive DST results.

Using spoligotyping as a molecular tool coupled with sequencing of *M. tuberculosis* target genes which confer resistance, we showed that there was an epidemic spread of DR-TB strains. The dominant strain families identified were the X-family, Beijing family, T-family, S-family and LAM. This is similar to other previously reported studies in the Western Cape Province that reported primary transmission as the major contributor of the circulating DR strains (16, 26). The X-family was identified as the predominant family in this region which is contrary to other studies that have reported the Beijing strain as the dominating strain in the Western Cape (7, 18). Furthermore, large outbreaks have been attributed to the Beijing lineage (44), suggesting that the Beijing strain is able to spread more efficiently as compared to other genotypes (45, 46).

The X-family has also been identified as one of the predominant *M. tuberculosis* strain families in the Western Cape (47). Other strains that were identified in this study included the Beijing, LAM and T families (47). Our group also previously reported a clone of the X-family which is characterized by deletion of spacer 18 and spacers 33-36 with the same *katG*315ACA mutation (26). The X-family strain has also been reported to be highly prevalent in the United States and the United Kingdom (48).

Phylogeny based on spoligotyping and drug resistance conferring mutations suggested high similarities of MDR strains, and more variations on a pre-XDR and XDR-TB level. This is indicative that MDR-TB is mainly due to transmission, and subsequently pre-XDR and XDR-TB are due to acquisition of additional mutations. These strains either have not had time to spread or could have an additional fitness cost, making them less able to spread as successfully as the susceptible or MDR counterparts.

We also identified various clusters of identical genotypes in the X-family, including a large cluster of the X-family with 4 identical first-line drug resistance markers in (*rpoB*, *katG, pncA* and *embB*). Sub-branches of this cluster indicate subsequent mutations leading to pre-XDR- and XDR-TB. These strains were mainly found in 2 of the Northern most municipalities of the region. This provides evidence of an outbreak of DR-TB in this region that contributes to the TB overall epidemic in the region.

To overcome the delay of diagnosing drug resistance in tuberculosis, a rapid, point-of-care DR diagnostics, GeneXpert has been introduced on a large scale in South Africa, including in this region. This will hopefully aid in identifying DR-TB more rapidly, in order to slow down transmission and possible outbreaks of DR-TB.

Possible interesting future studies would be to determine if rapid diagnostics of DR-TB would have slowed down this epidemic, by doing this kind of study over a different time period. It would also be interesting to incorporate WGS of the identified outbreak, to establish possible transmission chains of this outbreak, thereby aiding in contact tracing and

implementation of correct control measures. Another future study could focus on presenting the chronology of the primary X-family outbreak to identify if the outbreak is continuing despite the introduction of GeneXpert as the primary screening tool.

We acknowledge the fact that, although spoligotyping is rapid and less expensive than other genotyping methods including IS*6110*-RFLP, it has less discriminatory power (49). In this study however, the predominant strain family identified was the X-family, a strain family that has less than 6 copies of IS*6110* (50), rendering IS*6110*-RFLP also less discriminatory than when applied to other strain families with more copies of the insertion element. We also acknowledge that drug resistance conferring mutations are not ideal to use in generating phylogeny, as these mutations could have happened in parallel, and thereby indicating false transmission chains. However, the outbreak identified had a rare *katG* mutation with a 2 base pair change in the 315 codon which was also previously reported by our group (26) and also a specific *pncA* mutation. Mutations conferring PZA resistance in *pncA* is found throughout the gene, with no hotspot or preferential areas, suggesting that convergence will be minimal for this gene.

In conclusion, we applied molecular epidemiological techniques in order a describe the DR-TB population of the West Coast region of the Western Cape Province, South Africa – a region with high levels of tuberculosis disease including DR-TB, but with limited knowledge on the epidemiology of this region. We identified a predominant lineage responsible for the DR-TB epidemic – the X-family and showed the DR-TB epidemic is driven by transmission of MDR-TB strains, and acquisition of mutations leading to XDR-TB.

This study emphasised the importance of using genotypic techniques in different regions to effectively implement surveillance studies in neglected areas. This will ultimately enable prevention and control programmes to be implemented.

# Chapter 6

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# Appendices

**Appendix A**

Preparations of reagents

**Spoligotyping**

**Stock solutions**

- **10XSSPE**:

  13.7g Sodium Hydrogen Phopshate – 100mM

  105.19g Sodium Chloride-1.8M

  3.36g EDTA- 10mM

  Add distilled $H_2O$ to a volume of 1000ml

  pH = 7.40

  Store at room temperature

- **10% SDS**:

  50g SDS

  Add dH2O to a volume of 500ml

- **0.5 EDTA**:

  93g EDTA

  Add distilled $H_2O$ to a volume of 500ml

**Working stocks**

- **500ml 2xSSPE/0.1%SDS**:

  100ml 10x SSPE

  5ml 10% SDS

  Add $dH_2O$ to a final volume of 500ml

  Store at room temperature

- **1000ml 2xSSPE/0.5%SDS**:

  200ml 10x SSPE

  50ml 10% SDS

  Add distilled $H_2O$ to make a volume of 1000ml

  Store at room temperature

- **1000ml 1% SDS:**

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100ml 10% SDS

Add distilled $H_2O$ to make up a final volume of 1000ml

- **500ml 20mM EDTA:**

  25ml 0.5M EDTA

  Add $dH_2O$ to make up a final volume of 500ml

  Store at 4°C

## PCR

- **Syto9 fluorescent dye (sensitive to light)**

  5µl of Syto9

  Add 495µl of $H_2O$ and cover tube in foil

  Store at -20°C

- **dNTPs**

  20µl of 100mM dATP

  20µl of 100mM dCTP

  20µl of 100mM dGTP

  20µl of 100mM dTTP

  Add 720µl of $H_2O$

  Store at -20°C

## Primers

- **Primer stock:**

  Add xµl per nmole of IDT primer

  Store at -20°C

- **Primer mix for Forward and reverse**

  5µl of 1000µM of each primer stock

  Add 95µl of $H_2O$ to make a final volume of 100µl

  Store at -20°

# Appendix

Data set of isolates harbouring the X-family strain

| DB | number | Clade | ETHIO | ETHAM | Amikacin | Ofloxacin | Inh | Rif | Drug resistance | *rpoB* | *katG* | *embB* | *rrs* | *inhAPROM* | *gyrA* | *pncA* |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 2269 | X | | S | | | R | R | MDR | 531TTG | 315ACC | 306ATA | WT | -15 C-T | WT | no result |
| R | 2320 | X | S | R | S | S | R | R | MDR | 531TTG | 315ACA | 328TAT | WT | WT | 94GGC | 180GCC |
| R | 2338 | X | | S | | | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 91CCG | 139ATG |
| R | 2401 | X | S | S | S | S | R | R | MDR | 511CCG | 315AAC | WT | WT | WT | WT | WT |
| R | 2402 | X | S | S | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 2409 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 2465 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 2591 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 2624 | X | | S | | | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 2684 | X | | S | | | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | -15 C-T | 90GTG | 139ATG |
| R | 2727 | X | | S | | | R | R | MDR | 531TTG | 315ACC | 306ATA | WT | -15 C-T | WT | 154GGG |
| R | 2784 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 2793 | X | | S | | | R | S | Inh Mono | WT | 315ACC | WT | WT | WT | WT | WT |
| R | 2848 | X | | S | | | R | S | Inh Mono | WT | WT | WT | WT | WT | WT | WT |
| R | 2871 | X | | | | | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 2912 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 89GGC | 139ATG |
| R | 2960 | X | | S | | | R | S | inh Mono | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 3011 | X | | S | | | R | R | MDR | 531TTG | 315ACC | WT | WT | WT | WT | WT |
| R | 3040 | X | S | S | LV | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3086 | X | | S | | | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 3162 | X | | S | | | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |

| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 3212 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | 139ATG |
| R | 3227 | X |  | S |  |  | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3228 | X |  | S |  |  | R | S | Inh Mono | 526CTC | 315ACA | 306ATA | WT | WT | WT | WT |
| R | 3241 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 3279 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3315 | X | S | S | R | S | R | R | Pre XDR | 531TTG | 315ACA | WT | WT | WT | WT | 139ATG |
| R | 3345 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3355 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3369 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3385 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACC | WT | WT | WT | WT | WT |
| R | 3504 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3505 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | WT |
| R | 3536 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3542 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3550 | X |  | LV |  |  | R | R | MDR | 531ttg | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3592 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | 139ATG |
| R | 3613 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3632 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 3640 | X | S | S | R | S | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | 1401A-G | WT | WT | 139ATG |
| R | 3689 | X | S | R | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 91CCG | 139ATG |
| R | 3809 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3816 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 3890 | X | S | S | S | R | R | R | Pre XDR | 526GAC | 315ACC | WT | WT | WT | WT | 85CCG |
| R | 3929 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | WT |
| R | 3955 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 4017 | X | S | S | S |  | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |

| R | 4272 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 4280 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 4331 | X | R | S | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 4518 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 328TAT | WT | WT | 94GGC | 180GCC |
| R | 4582 | X | S | S | S | S | R | R | MDR | 531TTG | no result | No result | WT | WT | WT | WT |
| R | 4591 | X | S | S | S | S | S | R | Rif Mono | 531TTG | WT | WT | WT | WT | WT | WT |
| R | 4677 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 4835 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 328TAT | WT | WT | 94GGC | 180GCC |
| R | 4845 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 4998 | X | R | S | S | R | R | R | Pre XDR | WT | 315ACA | 306GTG | WT | WT | 94GGC | WT |
| R | 5022 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 5061 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 5286 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | 139ATG |
| R | 5389 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 5496 | X | R | S | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 5721 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 5738 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306ATA | WT | WT | WT | 139ATG |
| R | 5764 | X | S | S | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | 143GTC |
| R | 5767 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 5775 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 5785 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 5847 | X | S | S | R | R | R | R | XDR | 531TTG | 315ACA | 306GTG | WT | WT | 91CCG | 139ATG |
| R | 5985 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 5990 | X | S | S | S | S | S | R | Rif Mono | 511CCG | 315ACA | WT | WT | WT | WT | WT |
| R | 6007 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 6115 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |

| R | 6175 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 6207 | X | S | S | S | S | R | S | Inh Mono | WT | WT | WT | WT | -15 C-T | WT | WT |
| R | 6216 | X | S | R | R | R | R | R | XDR | 531TTG | 315ACA | 306GTG | 1401A-G | WT | 94GGC | 139ATG |
| R | 6252 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACC | WT | WT | WT | WT | WT |
| R | 6278 | X | S | S | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 6423 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 6477 | X | S | R | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 6590 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 6630 | X | S | S | R | R | R | R | XDR | 531TTG | 315ACA | 306GTG | 1401A-G | WT | 90GTG | 139ATG |
| R | 6661 | X | S | S | S | S | R | R | MDR | 516TAC | 315ACC | 306ATA | WT | WT | WT | 68GGG |
| R | 6700 | X | S | S | R | R | R | R | XDR | 531TTG | 315ACA | 306GTG | 1401A-G | WT | 94GGC | 139ATG |
| R | 6722 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACC | WT | WT | WT | WT | WT |
| R | 6880 | X | S | S | S | S | R | S | Inh Mono | WT | 315ACC | WT | WT | WT | WT | WT |
| R | 6892 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 91CCG | 139ATG |
| R | 6956 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACC | 306GTG | WT | WT | WT | WT |
| R | 7067 | X | S | S | S | S | S | R | Rif Mono | 511CCG | WT | WT | WT | WT | WT | WT |
| R | 7107 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 7152 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 7226 | X | S | S | S | R | R | R | Pre XDR | 516GTC | 315ACC | WT | WT | WT | WT | WT |
| R | 7396 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 7479 | X | S | S | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 7504 | X | S | S | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 7718 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GCC | 139ATG |
| R | 7797 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 7963 | X | LV | LV | LV | LV | S | R | Rif Mono | 511CCG | WT | WT | WT | WT | WT | WT |
| R | 8094 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |

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| | | | | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 8102 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 8536 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACC | WT | WT | WT | WT | WT |
| R | 8597 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 8868 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | WT |
| R | 8896 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 9099 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 9203 | X | S | S | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 91CCG | 139ATG |
| R | 9239 | X | S | S | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306ATT | WT | WT | 94GGC | WT |
| R | 9459 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GCC | 139ATG |
| R | 9548 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 9574 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 9576 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACC | WT | WT | WT | WT | WT |
| R | 9660 | X | S | ND | S | S | ND | ND | Unclassified | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 9688 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 9861 | X | S | ND | S | S | R | S | Inh Mono | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 9928 | X | S | ND | R | S | R | R | Pre XDR | 526CTC | 315ACA | WT | WT | WT | 90GTG | WT |
| R | 10075 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | WT |
| R | 10173 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 328TAT | WT | WT | 94GGC | 180GCC |
| R | 10446 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | 139ATG |
| R | 10661 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94AAC | 139ATG |
| R | 10763 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 10764 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 10785 | X | S | ND | S | S | ND | ND | Unclassified | 511CCG | WT | WT | WT | WT | WT | WT |
| R | 10821 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | WT |
| R | 10848 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 10857 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |

| R | 10862 | X | S | ND | R | S | R | R | Pre XDR | 531TTG | 315ACA | 306ATC | 1401A-G | WT | WT | WT |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 11002 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 11116 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 11149 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 11193 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | 139ATG |
| R | 11268 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 11277 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | 143GTC |
| R | 11302 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACC | WT | WT | WT | 94GGC | 132AGT |
| R | 11358 | X | R | ND | S | S | R | S | Poly | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 11440 | X | S | ND | S | S | R | R | MDR | 531TTG | WT | 306GTG | WT | -15 C-T | 94GGC | 164CCG |
| R | 11501 | X | S | ND | R | S | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 11572 | X | S | ND | R | R | R | R | XDR | 531TTG | 315ACA | 306GTG | 1401A-G | WT | 90GTG | 139ATG |
| R | 11610 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | no result | 306GTG | WT | WT | WT | WT |
| R | 11635 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 11691 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 11724 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 11759 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 11781 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 11927 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 11989 | X | ND | ND | S | S | R | R | MDR | WT | 315ACC | WT | WT | WT | WT | WT |
| R | 12007 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | No result | 306GTG | WT | WT | WT | WT |
| R | 12144 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | No result | WT | WT | 139ATG |
| R | 12189 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 12193 | X | S | ND | R | R | R | R | XDR | 531TTG | 315ACA | 306GTG | 1401A-G | WT | 94GGC | 139ATG |
| R | 12218 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | 315ACA | WT | WT | WT | 94GGC | WT |
| R | 12220 | X | S | ND | S | S | R | R | MDR | 531TTG | WT | 306GTG | WT | WT | WT | 139ATG |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 12301 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | WT | WT | WT | WT | WT |
| R | 12350 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | 315ACA | WT | WT | WT | 94GCC | WT |
| R | 12374 | X | S | ND | S | S | R | R | MDR | 531TTG | WT | 306GTG | WT | WT | WT | 139ATG |
| R | 12450 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 12533 | X | S | ND | R | R | R | R | XDR | no result | no result | no result | 1401A-G | no result | WT | 139ATG |
| R | 12778 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 12780 | X | S | ND | S | R | R | R | Pre XDR | 526CTC | 315ACA | WT | WT | WT | WT | 105GAC |
| R | 13097 | X | Cont | ND | Cont | Cont | S | R | Rif Mono | 511CCG | 315ACA | WT | WT | WT | 91CCG | WT |
| R | 13458 | X | S | ND | S | S | R | R | MDR | 531TTG | WT | 306GTG | WT | WT | WT | 139ATG |
| R | 13534 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 13658 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 13690 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 13734 | X | S | ND | S | S | R | S | Inh Mono | WT | 315ACA | WT | WT | -15 C-T | 94GCC | 35CGG |
| R | 13778 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | WT | WT | WT | WT | WT | WT |
| R | 13928 | X | S | ND | S | S | R | R | MDR | 531TTG | WT | 306GTG | WT | WT | WT | 139ATG |
| R | 14106 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GCC | 139ATG |
| R | 14114 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GCC | 139ATG |
| R | 14162 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | 139ATG |
| R | 14304 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | 139ATG |
| R | 14363 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | 139ATG |
| R | 14382 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 14510 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 14748 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 14889 | X | S | ND | R | S | S | R | Poly | 531TTG | 315ACA | WT | 1401A-G | WT | WT | WT |
| R | 14897 | X | S | ND | S | S | R | R | MDR | 526CTC | WT | WT | WT | WT | WT | WT |
| R | 14913 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 14973 | X | S | ND | S | S | R | S | Inh Mono | WT | 315ACA | 306ATA | WT | -15 C-T | WT | WT |
| R | 15007 | X | S | ND | S | S | R | R | MDR | 531TTG | WT | 306GTG | WT | WT | WT | 139ATG |
| R | 15070 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 15104 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 15158 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 15175 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 15340 | X | S | ND | S | S | R | S | Inh Mono | WT | 315ACC | WT | WT | WT | WT | WT |
| R | 15414 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | WT | WT | WT | WT | 139ATG |
| R | 15515 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 15667 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 15679 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | 143GTC |
| R | 15736 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACC | WT | WT | WT | 94GGC | 132AGT |
| R | 15740 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 15749 | X | S | ND | S | S | R | R | MDR | 511CCG | 315AGA | WT | WT | WT | WT | WT |
| R | 15881 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 15952 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | no result | WT | WT | 139ATG |
| R | 15982 | X | R | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | no result | WT | WT | 139ATG |
| R | 16010 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | WT | WT | no result | WT | no result | WT |
| R | 16166 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 16168 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 91CCG | 139ATG |
| R | 16208 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 16244 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 16304 | X | R | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 16467 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 16690 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 16778 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |

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|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| R | 16804 | X | R | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | 139ATG |
| R | 16835 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 16861 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | WT | WT | 1402A | WT | WT | WT |
| R | 16875 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 16946 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 17019 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 17024 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 17116 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 17168 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACC | WT | WT | WT | WT | 132AGT |
| R | 17209 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GCC | 139ATG |
| R | 17267 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 17399 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 90GTG | 139ATG |
| R | 17427 | X | S | ND | S | S | R | R | MDR | 516GTC | 315ACC | WT | WT | WT | WT | WT |
| R | 17537 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 17584 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | WT | WT | WT | WT | WT | WT |
| R | 17592 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | WT |
| R | 17610 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | WT |
| R | 17614 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | WT | WT | 94GGC | 139ATG |
| R | 17632 | X | S | ND | S | S | S | R | Rif Mono | 531TTG | WT | WT | WT | WT | WT | WT |
| R | 17699 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 18091 | X | S | ND | S | R | R | R | Pre XDR | 533CCG | 315ACA | WT | WT | WT | WT | WT |
| R | 18107 | X | S | ND | S | R | R | R | Pre XDR | 531TTG | 315ACA | WT | WT | WT | WT | 139ATG |
| R | 18128 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACC | WT | WT | WT | WT | WT |
| R | 18252 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 18593 | X | S | ND | S | S | R | R | MDR | 526CTC | 315ACA | WT | WT | WT | WT | 62CTG |
| R | 18623 | X | S | ND | R | S | R | R | Pre XDR | 531TTG | 315ACA | 306GTG | 1401A-G | WT | WT | 139ATG |

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| R | 18782 | X | R | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | WT | WT | WT | 139ATG |
| R | 18824 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | no result | WT | WT | WT | no result |
| R | 18875 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | no result | WT | WT | WT | no result |
| R | 18949 | X | S | ND | S | S | R | R | MDR | no result | no result | no result | no result | no result | no result | no result |
| R | 18972 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | no result | WT | WT | WT | no result |
| R | 18999 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | no result | WT | WT | 90GTG | no result |
| R | 19106 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | no result | WT | WT | WT | no result |
| R | 19118 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | WT | no result | WT | WT | WT | no result |
| R | 19242 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | no result | WT | WT | WT | no result |
| R | 19248 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | no result | WT | WT | WT | no result |
| R | 19269 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | WT | no result | WT | WT | WT | no result |
| R | 19409 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | no result | 1401A-G | WT | WT | no result |
| R | 19529 | X | S | ND | R | R | R | R | XDR | 531TTG | 315ACA | no result | 1401A-G | WT | WT | no result |
| R | 19881 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | 306GTG | no result | WT | WT | 139ATG |
| R | 20008 | X | S | ND | S | S | R | R | MDR | 531TTG | 315ACA | WT | no result | WT | WT | 139ATG |
| R | 20113 | X | S | ND | S | S | S | R | Rif Mono | 511CCG | WT | WT | no result | WT | WT | WT |
| R | 20119 | X | S | ND | S | S | R | S | Inh Mono | WT | WT | WT | no result | WT | WT | WT |

- DB-data base, R-resistant, S-susceptible, WT- wild type, ND- not done, LV-lost viability