UNIVERSIY OF CALIFORNIA, SAN DIEGO

SAN DIEGO STATE UNIVERSITY

Prevalence and Risk Factors of Drug Resistant Mycobacterium Tuberculosis in a Multisite Cohort Study

A dissertation submitted in partial satisfaction of the requirements for the degree

Doctor of Philosophy

in

Public Health (Epidemiology)

by

Elisea Estela Avalos

Committee in charge:

University of California, San Diego
- Professor Theodore Ganiats, Co-chair
- Professor Timothy Rodwell, Co-chair
- Professor Antonio Catanzaro

San Diego State University
- Professor John Alcaraz
- Professor Stephanie Brodine

2015

The Dissertation of Elisea Estela Avalos is approved, and it is acceptable in quality and form for publication on microfilm and electronically:

Co-chair

Co-chair

University of California, San Diego

San Diego State University

2015

TABLE OF CONTENTS

LIST OF ABBREVIATIONS

AMK: Amikacin

AP: Attributable Proportion

CAP: Capreomycin

CI: Confidence Interval

CIPRO: Ciprofloxacin

DRTB: Drug-Resistant Tuberculosis

DST: Drug Susceptibility Testing

FQ: Fluoroquinolone

GAT: Gatifloxacin

GCDD: Global Consortium for Drug-Resistant Tuberculosis Diagnostics

HIV: Human Immunodeficiency Virus

IDU: Injection Drug User

INH: Isoniazid

IRB: Institutional Review Board

KAN: Kanamycin

LEVO: Levofloxacin

LPA: Line Probe Assay

LTBI: Latent Tuberculosis Infection

MDRTB: Multidrug-Resistant Tuberculosis

MGIT: Mycobacterial Growth Indicator Tube

MIC: Minimum Inhibitory Concentration

***Mtb*:** *Mycobacterium tuberculosis*

MOX: Moxifloxacin

OFX: Ofloxacin

OR: Odds Ratio

PCR: Polymerase Chain Reaction

PSQ: Pyrosequencing

RERI: Relative Excess Risk Due to Interaction

RIF: Rifampin

SAS: Statistical Analysis System

SI: Synergy Index

SITA: Sitafloxacin

SLD: Second-line Drug

SPX: Sparfloxacin

TAT: Turn Around Time

TB: Tuberculosis

UCSD: University of California, San Diego

WHO: World Health Organization

XDRTB: Extensively Drug-Resistant Tuberculosis

LIST OF TABLES

LIST OF FIGURES

ACKNOWLEDGEMENTS

This dissertation would not have been possible without the guidance and the help of several individuals who in one way or another contributed and extended their valuable assistance in the preparation and completion of this study.

I would like to express my sincere gratitude to my committee co-chairs Dr. Ted Ganiats and Dr. Timothy Rodwell for the continuous support of my PhD study and research, for their motivation, enthusiasm, and immense knowledge. Their guidance helped me in my research and writing of this thesis. I could not have imagined having a better pair of advisors and mentors for my PhD.

I offer my sincere appreciation to Dr. Stephanie Brodine for introducing me to Dr. Antonio Catanzaro and to the world of infectious disease epidemiology; to Dr. Antonio Catanzaro for sharing his vast tuberculosis expertise and enthusiasm and welcoming me into his research study; to Dr. John Alcaraz for his clear, valuable statistical suggestions and explanations; to Dr. Donald Catanzaro and Dr. Richard Garfein for their support and valuable input; and to the study site PIs whose insights, enthusiasm, and generous sharing of knowledge were much appreciated and vital to the success of the project.

My thanks to Hollie Ward, Kristin Deveraux, Ruby Lopez and Vickie Saddler for their administrative help over the past years.

Above all, I would like to thank my husband Mike for his personal support and great patience at all times. My parents, who have put their lives on hold the past year to help me out. My brother and sisters who have given me their unequivocal support throughout, as always. My greatest achievement yet, my son Michael, who reminds me every day to smile and enjoy life.

Chapters from this dissertation have been or will be submitted for publication. The dissertation author was the primary investigator and author of these papers.

Chapter 2, in full, is a reprint of the material as it appears in PLOS ONE, 2015, Avalos, E, Catanzaro, D, Catanzaro, A, Ganiats, T, Brodine, S, Alcaraz, J, Rodwell, T. *Frequency and Geographic Distribution of gyrA and gyrB Mutations Associated with Fluoroquinolone Resistance in Clinical Mycobacterium Tuberculosis Isolates: A Systematic Review.* The dissertation author was the primary investigator and author of this paper.

Chapter 3, is currently being prepared for submission for publication of the material in The International Journal of Tuberculosis and Lung Disease. Avalos, E**,** Garfein, R, Catanzaro, D, Ganiats, T, Brodine, S, Alcaraz, J, Eisenach, K, Ajbani, K, Udwadia, Z, Crudu, V, Victor, T, Rodwell, T. *Prevalence of drug resistant tuberculosis in Mumbai, India, Chisinau, Moldova and Port Elizabeth, South Africa, 2012-2013.* The dissertation author was the primary investigator and author of this paper.

Chapter 4 is currently being prepared for submission for publication of the material in The International Journal of Tuberculosis and Lung Disease. Avalos, E, Garfein, R, Catanzaro, D, Ganiats, T, Brodine, S, Alcaraz, J, Eisenach, K, Ajbani, K, Udwadia, Z, Crudu, V, Victor, T, Rodwell, T. *Characteristics of multi and extensively drug resistant tuberculosis in a multisite study.* The dissertation author was the primary investigator and author of this paper.

VITA

Elisea Estela Avalos received the degree of Bachelors of Science in Health Sciences, with an emphasis in public health, epidemiology from Truman State University. She also received a graduate certificate in Geographic Information Science & Technology from the University of Southern California, her Master in Public Health in Epidemiology from the University of California, Los Angeles and her Doctor of Philosophy in Public Health in Epidemiology from the Joint Doctoral Program at San Diego State University and the University of California, San Diego.

SELECTED PUBLICATIONS

Avalos, E, Catanzaro, D, Catanzaro, A, Ganiats, T, Brodine, S, Alcaraz, J, Rodwell, T. Frequency and Geographic Distribution of gyrA and gyrB Mutations Associated with Fluoroquinolone Resistance in Clinical Mycobacterium Tuberculosis Isolates: A Systematic Review. PLOS ONE.

Avalos, E, Garfein, R, Catanzaro, D, Ganiats, T, Brodine, S, Alcaraz, J, Eisenach, K, Ajbani, K, Udwadia, Z, Crudu, V, Victor, T, Rodwell, T (in preparation). Characteristics of multi and extensively drug resistant tuberculosis in a multisite study.

Avalos, E, Garfein, R, Catanzaro, D, Ganiats, T, Brodine, S, Alcaraz, J, Eisenach, K, Ajbani, K, Udwadia, Z, Crudu, V, Victor, T, Rodwell, T (in preparation). Prevalence of drug resistant tuberculosis in Mumbai, India, Chisinau, Moldova and Port Elizabeth, South Africa, 2012-2013.

Garfein, R, Catanzaro, D, Rodwell, T, **Avalos, E,** Jackson, L, Kaping, J, Evasco, H, Rodriques, C, Crudu, V, Lin,G, Grossel, E, Hillery, N, Trollip, A, Ganiats, T, Victor,T, Eisenach, K, Valafar, F, Channick, J, Qian, LCatanzaro, A. (2015) 'Phenotypic and Genotypic Diversity in a Multinational Sample of Drug-resistant *M. Tuberculosis* Isolates.' *International Journal of TB and Lung Disease*.

SELECTED POSTERS

Avalos, E, Garfein, R, Catanzaro, D, Ganiats, T, Brodine, S, Alcaraz, J, Eisenach, K, Rodrigues, C. Crudu, V, Victor, T, Rodwell, T. Characteristics of multi and extensively drug resistant tuberculosis in a multisite study. University of California San Diego Public Health Research Day, San Diego, California, April 9, 2015.

Avalos, E, Garfein, R, Catanzaro, D, Ganiats, T, Brodine, S, Alcaraz, J, Eisenach, K, Rodrigues, C. Crudu, V, Victor, T, Rodwell, T. Characteristics of multi and extensively drug resistant tuberculosis in a multisite study. San Diego Epidemiology Research Exchange, San Diego, California, May 2, 2015.

FIELDS OF STUDY

Major Fields: Epidemiology

Clinical Outcomes Research and Preventive Medicine: Dr. Theodore Ganiats

Molecular Epidemiology and Global Health: Dr. Timothy Rodwell

Clinical Tuberculosis and Diagnostics Research: Dr. Antonio Catanzaro

Infectious Disease Epidemiology: Dr. Stephanie Brodine

Biostatistics: Dr. John Alcaraz

ABSTRACT OF THE DISSERTATION

Prevalence and Risk Factors of Drug Resistant Mycobacterium Tuberculosis in a Multisite Cohort Study

by

Elisea Estela Avalos

Doctor of Philosophy in Public Health (Epidemiology)

University of California, San Diego, 2015
San Diego State University, 2015

Professor Theodore Ganiats, Co-chair

Professor Timothy Rodwell, Co-chair

Drug -resistant tuberculosis (DRTB) has emerged as a major challenge in the control and prevention of TB. In the present study we first systematically reviewed the literature to characterize the diversity and frequency of *gyrA* and *gyrB* mutations in fluoroquinolone resistant *Mycobacterium Tuberculosis* (*Mtb*) and describe the global distribution of these mutations to help determine their potential utility and reliability as diagnostic markers for detecting phenotypic fluoroquinolone resistance in *Mtb*. Secondly, we describe the prevalence of and characteristics of DRTB in Mumbai, India, Chisinau, Moldova and Port Elizabeth, South Africa. The results from our systematic review revealed the *gyrA* mutations occurring most frequently in fluoroquinolone-resistant isolates, were D94G(21-32%) and A90V(13-20%) and that 83% and 80% of moxifloxacin and ofloxacin resistant strains respectively, were observed to have

mutations in the *gyrA* codons interrogated by the existing MTBDR*sl* line probe assay. In China and Russia, 83% and 84% of fluoroquinolone resistant strains respectively, were observed to have *gyrA* mutations in the gene regions covered by the MTBDR*sl* assay. The results from our study found the overall prevalence of MDRTB was 79.7%, 51.1% and 15% in Mumbai, Chisinau and Port Elizabeth, respectively. Among the MDRTB patients, the prevalence of XDRTB in Mumbai, Chisinau and Port Elizabeth was 13.9%, 12.1% and 41.4%, respectively. A multiple logistic regression analysis showed that those less than 25 years of age (OR 1.8, 95%CI 1.0 to 3.1), study site (Mumbai (OR 33.1, 95% CI 18.8 to 58.3) and Chisinau (OR 13.0, 95%CI 6.8 to 24.6)), higher education (OR 2.4, 95%CI 1.4 to 4.0), ever been hospitalized (OR 1.9, 95%CI 1.2 to 2.9) and previously treated for TB (OR 1.7, 95% CI 1.1 to 2.8) were associated with developing M/XDRTB. An interaction was also observed between study site and prior TB treatment. The burden of DRTB was high in all three sites highlighting the importance of continuous surveillance to identify DRTB, especially among patients who have been previously treated for TB. It is important to improve early diagnosis of MDRTB and to provide effective treatment to all MDRTB patients in order to prevent the development of additional drug resistance.

CHAPTER 1 BACKGROUND AND SIGNIFICANCE

TB, MDRTB and XDRTB

Tuberculosis (TB), a communicable disease caused by *Mycobacterium tuberculosis* (*Mtb*), is responsible for nearly 9 million cases and 1.3 million deaths worldwide every year [1]. Although the disease can affect any part of the body, only active pulmonary disease can be spread person to person via droplets carrying the TB bacillus. This can occur when a person with the disease coughs, sneezes or laughs [2]. The inhaled bacillus makes its way to the lungs and spreads to other parts of the body [3]. TB infection is established in approximately one-third of individuals exposed to the bacillus; among those infected only 10% become symptomatic. A number of comorbidities may influence disease progression, such as diabetes mellitus, renal failure or malnutrition [2].

No sooner were the first anti-TB drugs introduced than the emergence of drug resistant isolates of *Mtb* was observed [2]. Drug resistance typically occurs when patients are treated inappropriately or are exposed to sub-therapeutic drug levels. Lengthy treatment time combined with drug toxicity results in reduced patient compliance and, consequently a higher likelihood of drug resistance [4]. A growing public health concern is the emergence of resistance to multiple drugs. The worldwide emergence of multi-drug resistance (MDRTB) and extensively drug resistant *Mtb* (XDRTB) is a major setback to TB control [5]. MDRTB strains, defined by the World Health Organization (WHO), are *Mtb* strains resistant to the most effective "first-line" TB drugs: isoniazid (INH) and rifampin (RIF). XDRTB strains are characterized by resistance to INH and RIF plus any fluoroquinolone (FQ), and at least one of the three "injectable" anti-TB drugs: amikacin

(AMK), kanamycin (KAN), and capreomycin (CAP) [1]. As of 2013, the WHO estimated the global prevalence of MDRTB to be 3.5% among new TB cases and 20.5% among recurrent TB cases; as of 2013, XDRTB has been reported in 100 countries [1]. While treatment for MDRTB and XDRTB has improved, drug resistant TB (DRTB) is more difficult to treat and has been associated with high morbidity and mortality, prolonged treatment to cure and increased risk of spreading drug resistant isolates [6].

FQ Use

FQs, including the older generation drug ofloxacin and the newer generation drugs gatifloxacin and moxifloxacin, are second-line anti-TB drugs [7]. These drugs have high *in vitro* activity against *Mtb* and are used as the backbone drugs for MDRTB and for persons intolerant of current first line therapy [8]. Recently, the newer generation FQs have been recommended as first-line drugs to reduce the duration of therapy [6]. FQs inactivate *Mtb* by binding to gyrase-DNA complexes and inhibiting DNA replication [7]. FQs are also used to treat many other infections including community acquired pneumonia, sexually transmitted diseases and gastrointestinal infections [6, 9, 10]. The abuse and overuse of FQs contribute to the increasing emergence of FQ-resistant *Mtb* [11].

Mtb acquires FQ resistance through mutations in the conserved region known as the quinolone resistance determining region (QRDR) of the *gyrA* and *gyrB* gene which encode DNA gyrase [7]. It is estimated that roughly 60 to 90% of *Mtb* clinical isolates with FQ resistance, have mutations in the QRDR of *gyrA.* The most frequent mutations in clinical isolates are found at codons 90, 91 and 94 of *gyrA* [7, 9]. Mutations in the *gyrB* gene are also associated with FQ resistance, but at a much lower rate. Mutations in *gyrB*

are typically in association with *gyrA* mutations [12, 13] and most often occur in codons 500, 538, 539 and 540 of *gyrB* [14]. Whereas most *Mtb* strains with *gyrA* mutations in the QRDR are FQ resistant, nearly all wild type strains in this region are FQ-susceptible. The exceptions are the polymorphisms of *gyrA* at codons 21, 95 and 668 [15]. Since every generation of FQs has the same drug targets, cross-resistance to FQs is common [7]. However, the resistance levels of each isolate against individual drugs vary [16]. In general, the minimum inhibitory concentrations (MICs) of newer generation FQs are lower than those of older generation FQs [9].

Drug susceptibility testing (DST)

Accurate drug susceptibility testing (DST) for *Mtb* is important for both therapy guidance and surveillance of drug resistance [17]. DST has been shown to have a major impact on the effectiveness of anti-TB treatment and is the standard of care in the US and most of the developed countries. In regions with high prevalence of TB drug resistance, TB treatment without DST may lead to treatment failure and may help contribute to the growing drug resistance problem [18]. However, DST is costly and requires highly-skilled laboratories. In addition to this, results may not be available for up to three months; in the case of XDRTB/HIV co-infection, the patient may have already died at this point. These diagnostic delays complicate the public health control of TB. The use of liquid systems has improved turn around time (TAT) to about 25–45 days, but liquid culture systems are in most cases not available where the need is greatest [19].

Rapid DST

The emergence of MDRTB and XDRTB has emphasized the need for rapid drug susceptibility testing. The detection of DRTB traditionally has been accomplished by

time-consuming culture-based methods. Mycobacterial growth on culture requires three to eight weeks, followed by an additional two to three weeks before anti-TB DST results are available. The rapid method on liquid medium such as BACTEC MGIT 960 also requires one to two weeks [20]. The molecular methods to detect FQ resistance in *Mtb* provide a more rapid alternative [20-22]. The GenoType MTBDR*sl* test can process results in 5 hours either directly from patient specimens or from cultured samples. Pyrosequencing can analyze 96 samples simultaneously in less than one hour. Previous studies have demonstrated that molecular tests on resistance genes (FQ resistance mutations in 320- and 375-bp hypervariable regions of *gyrA* and *gyrB* genes) can facilitate the rapid diagnosis of MDRTB [23-25] to allow adequate adjustments in treatment and to minimize transmission of drug-resistant strains [20]. Unfortunately many developing countries lack these diagnostic methods.

Risk Factors for TB

Several risk factors for TB development have been reported including clinical and epidemiological characteristics such as socio-demographics (e.g. age, gender, marital status), TB history (e.g., prior TB diagnosis, prior TB treatment), TB contact history, medical conditions associated with TB (e.g., HIV status, diabetes), TB risk factors (e.g., substance abuse, homelessness, incarceration) and TB-related medical conditions (e.g., CD4 cell counts, HIV viral load). As not all infections lead to disease, risk factors can increase the risk of TB by increasing risk of acquiring infection or increasing risk of developing clinical disease.

Age

Studies have consistently reported higher prevalence rates of DRTB among older age groups [26]. In a study examining risk factors associated with TB, Yu et al [27] reported a relative risk of 2.7 in persons older than 50 years of age, compared to persons less than 30 years of age, signifying a strong association between aging and TB. In contrast, Macedo et al found that MDRTB and XDRTB incidences were associated with young adult age [28]. Ageing is a major risk factor for any disease. The effects of ageing have been attributed to a decline in numerous macrophage functions which figure prominently in host defense in pulmonary TB [26]. DRTB associated with young adult age can create obstacles towards economic and social development in countries where TB is endemic [29].

Race

The TB literature recognizes that certain ethnic minorities are at higher risk for TB [30, 31]. A systematic review by Nava-Aguilera et al which included 14 countries concluded that the most prominent risk factor for recent TB transmission was being a minority (OR = 3.0; 95% CI 2.2-4.2). Compared with the general population, ethnic minorities are more likely to experience overcrowding, higher poverty rates, less access to medical services, unemployment and lower education rates, all of which may contribute to increased risk for *Mtb* [31]. Genetically, some ethnic minorities (e.g., Native American) are still relatively ‘naïve’ regarding TB (i.e., they are at higher risk of contracting TB) [32].

Gender

Women are more likely to face socio-cultural barriers to accessing health care [33], but compared to men are less likely to develop TB [31]. When diagnosed with TB,

women are more likely to adhere to treatment [34] and have better treatment outcomes [35]. With respect to TB, the immune response in men and women is different, indicating sexual dimorphism. Evidence suggests that at physiological levels, estrogen is beneficial to the immune system, whereas testosterone, is immunosuppressive [26]. Perhaps this sexual dimorphism in the immune response may explain the higher risk of developing TB in men. Further work is needed to determine whether the increased risk of developing TB in men is due to biological or socio-cultural determinants [33].

Marital Status

Previous studies have shown that marital status affects the risk of TB, with singles having a greater risk of TB than married individuals [36]. Gustafson et al found that people living without children, alone or with adults of their own sex only, had higher risks of developing TB than people living in households with children or/and adults of the opposite sex (OR =5.0; 95% CI 1.0-24.8) [37]. The increased risk of adults living without children or individuals of the opposite sex may have to do with differences in lifestyles, but could possibly also be a result of some protection from contact with children. Protection from contact with children, possibly through immune stimulation from exposure to childhood infections, could be one of the reasons for the high TB incidence among young adults and old people, neither of whom would have much contact with young children. Additionally, it has been hypothesized that being single implies an absence of family support, which may in turn increase vulnerability to TB at times of psychosocial stress [38].

Crowding

Although the literature is conflicting on the role of crowding, Gustafson et al [37] found that adult crowding is a risk factor for TB (OR = 1.7; 95% CI 1.2-2.4 for >2 adults in household). Crowding is a known marker of poverty; both crowding and poverty are independently associated with TB [39]. Crowding increases the likelihood of coming into contact with persons excreting the bacilli in crowded environments [36]. Crowding has been well studied in prisons [40] as well as in homeless shelters [41]. In both situations, crowding increases the risk of exposure to an infectious TB case and therefore the risk of infection.

Educational level/SES

Socioeconomic status (SES) of individuals has been shown to influence a person's susceptibility to TB infection, with the poorest individuals having the highest risk for disease [42]. People with low SES typically live in poor housing and environmental conditions, have greater food insecurity and have less access to quality health care relative to those from higher SES groups [43]. All of these social determinants are also related to TB, and often work together to put the poor at greater risk of disease by acting on different stages in the pathogenetic pathway [44]. Younis et al reported that people of higher SES are more likely to receive better treatment and undergo additional diagnostic procedures, while patients with low educational levels have a poorer understanding of TB, resulting in diagnostic delay and incomplete treatment [45].

Smoking

The association between TB and smoking has been examined in several systematic reviews [42, 46, 47]. Bates et al [46] included 24 studies in their meta-analysis

on the effects of smoking on TB and showed that the relative risk of TB (RR = 2.7; 95% CI 2.2-3.3) was higher among smokers compared to nonsmokers. Biological explanations for how smoking could increase one's risk for TB include mechanical disruption of cilia function in the airways, defects in macrophage immune responses, decreased immune response and decreased CD4 lymphopenia due to the nicotine in the cigarettes have been given as reasons for increased susceptibility to pulmonary TB [42, 48].

Drinking

It has long been evident that there is a strong association between alcohol use and risk of TB. A systematic review by Loennroth et al concluded that the risk of active TB is significantly higher (RR = 2.9; 95% CI 1.9-4.6) among people who drink more than 40g of alcohol per day and/or abuse alcohol [49]. Reasons for increased risk include the idea that alcohol may assert a direct toxic effect on the immune system rendering the host more susceptible to TB disease. The association between alcohol use and TB could also be explained by specific social mixing patterns, which may increase the risk of exposure to people with infectious TB disease in settings such as bars, shelters for homeless, prisons, and social institutions [49].

Incarceration

Prisoners are at a disproportionately high risk of developing TB [50]. Many factors contribute to the high prevalence of TB in prison populations, mostly related to the prisoners themselves, their living conditions and other factors associated with incarceration. These factors include the predominance of young males from disadvantaged communities with low education levels, the use of illicit drugs, high rates

of TB, overcrowding, poor ventilation, inadequate nutrition and limited access to health services [51, 52].

Injection Drug Use

The physiological effects of drug use, along with the environment and risk behaviors of drug users, may contribute to the high prevalence of TB among drug users. A possible biological explanation is that opioids affect the immune response directly; *in vitro* studies have found deleterious effects of opioids in infections [53]. Lower access to health care, poor treatment compliance and increased exposure to *Mtb* due to homelessness, crowding and incarceration, increases the risk of TB among drug users [54]. Together, these physiological and epidemiological factors may contribute to observed outcomes—namely, that drug users are more likely to be infectious and take longer to achieve negative culture [55].

Hospitalization

In a study by Zetola at al researchers found that one-year TB incidence rate was associated with the number of days that the patient remained hospitalized, the number of days spent in the cohorting bay (regardless of whether the patient was eventually diagnosed with TB or not) and the number and proximity to TB index cases within the following 12 months after discharge [56]. This finding points to the possibility of nosocomial transmission as a catalyst to the growing TB epidemic. Delays in the diagnosis of drug resistance and large, congregate TB wards, that are typical in many high burden settings, remain a dangerous combination for the transmission of DRTB [57].

Diabetes

Previous studies have documented a strong association between diabetes and TB [58, 59]. Jeon et al conducted a meta-analysis and found that diabetes was strongly associated with an increased risk of developing TB (RR = 3.1; 95% CI 2.3-4.3) [58]. Biologically, it is believed that diabetes directly impairs the innate and adaptive immune responses, thereby accelerating the proliferation of TB [58]. Animal studies have shown higher bacterial load among diabetic mice experimentally infected with *Mtb* [60]. Decreased production of IFN-γ and other cytokines diminished T-cell immunity and reduced chemotaxis in neutrophils of diabetic patients are thought to play a role in increasing the propensity of diabetic patients to developing active TB [60]. Additionally, a reverse association where TB can induce glucose intolerance and deteriorate glycemic control in subjects with diabetes has also been identified [61].

Malnutrition

Malnutrition is thought to both predispose individuals to respiratory infection through deficits in innate immunity and contribute to progression from TB to active disease through altered gene expression and impaired cell-mediated immunity. The resulting inflammatory response further worsens nutritional status [62]. TB disease itself leads to malnutrition because of decreasing appetite and changes in metabolic processes [42]. Moran-Mendoza et al found that malnutrition was the most important risk factor for developing TB in their study (HR = 37.5; 95% CI 12.7-111.4); the authors further concluded that improved nutrition might reduce the risk of developing active TB [63].

HIV

TB is the leading killer of HIV-infected individuals worldwide. Several biological mechanisms linking DRTB to HIV infection have been suggested [42]. It is believed that

drug malabsorption in HIV-infected patients can lead to drug resistance and has been shown to lead to treatment failure [64]. Drug resistant strains may be less virulent and preferentially lead to disease progression in immune compromised patients, as opposed to immune-competent individuals. Additionally, the association between HIV infection and TB may be confounded by shared risk factors such as injection drug use, imprisonment, socioeconomic status, alcohol use and hospitalization. HIV-infected patients and TB are more likely to be hospitalized compared to those who are HIV negative or suffer from drug sensitive TB. HIV-infected patients may thus be more likely to be exposed to patients with drug resistant isolates, and thus be infected or re-infected with a resistant isolate [65].

Close contact with a known case

Contact history, as well as closeness of contact, is well-defined as a risk factor for TB. Contacts exposed to patients with TB, in a variety of settings, are at substantial risk of latent TB infection (LTBI) and active TB. Household contacts and care givers/health care workers [66] are at a higher risk of becoming infected with *Mtb* and developing TB. In a systematic review by Fox et al the prevalence of active TB and LTBI among TB contacts was 3.1% and 51.5% respectively. Additionally, a higher prevalence of TB among child contacts compared with adults, and a higher prevalence of TB among contacts in low–middle-income countries compared with high-income countries was found [67]. Contact investigations around TB patients enable early detection of infection and disease, and prevention of secondary TB cases [68].

Previously treated for TB

Having previously been treated for TB has been widely recognized as a risk factor for DRTB. A systematic review concluded that the risk of MDRTB was up to ten times higher in previously treated patients compared to newly treated patients [69]. Prior episodes of anti-TB treatment can increase the risk of receiving non-standard regimens or interrupted treatment [70]. A sub-minimum inhibitory concentration effect may occur when TB patients receive non-standard regimens (sensitive strains are killed and mutant MDRTB strains take the place of the sensitive ones) resulting in the emergence of MDRTB [71]. It is important to ensure TB patients receive standard regimens the first time, that interrupted treatment is avoided, and that poor adherence to treatment is reduced.

Global Consortium for Drug-resistant TB Diagnostics (GCDD)

The Global Consortium for Drug-resistant TB Diagnostics (GCDD) was established in 2008 to characterize the genetic basis of drug resistance and evaluate molecular and microbiological methods to quickly and efficiently detect DRTB. This international collaboration to improve current DRTB diagnostics gathered data from Mumbai, India, Port Elizabeth, South Africa and Chisinau, Moldova in an effort to improve accuracy and precision of novel diagnostics and reduce DRTB detection time [72].

Study Sites

Participants were enrolled from three diverse regions with a high prevalence of XDR-TB. These sites were carefully considered in the planning of the study and were selected due to high documented risk for DR-TB and the ethnic diversity of these regions.

India

Mumbai, India has a population of approximately 13 million people. In 2013, the WHO estimated that India accounted for 20.4% of the total number of TB cases worldwide, with 2.2% (1.9-2.6%) and 15% (11-19%) of the new and retreatment cases respectively being caused by MDRTB strains [1]. Patients in the GCDD were enrolled at the P.D. Hinduja National Hospital (PD-HNH) and Medical Research Centre (MRC), a tertiary care center in central Mumbai. The PD-HNH is the referral center for MDR and XDR-TB cases of the city and the state of Maharashtra. Therefore, the TB patient population is more likely to contain those who have previously been treated and were either unresponsive or relapsed [72].

Moldova

Chisinau, Moldova, has a population of roughly 700,000 people. In 2013, the WHO estimated that Moldova accounted for 0.07% of the total number of TB cases worldwide, with 24% (21-26%) and 62% (59-65%) of the new and retreatment cases respectively being caused by MDRTB strains [1]. In Chisinau, patients in the GCDD were enrolled at the Phthisiopneumology Institute (PPI) in Chisinau, Moldova. The PPI is the central unit of the National TB Control Programme, which leads all TB and unspecific upper respiratory tract diseases services for patients across all of Moldova [72].

South Africa

Port Elizabeth, South Africa has a population of approximately 1.3 million people. In 2013, the WHO estimated that South Africa accounted for 5.1% of the total number of TB cases worldwide, with 1.8% (1.4-2.3%) and 6.7% (5.4-8.2%) of the new and retreatment cases respectively being caused by MDRTB strains [1]. In Port Elizabeth,

patients in the GCDD were enrolled at six Primary Health Care facilities and one regional hospital [72].

Goals of Dissertation

In recognition of the lack of information regarding the role of FQ resistance and the prevalence of MDRTB and XDRTB, it was the purpose of this dissertation to review the current TB literature to characterize *gyrA* and *gyrB* mutations, describe the prevalence of drug resistance among individuals residing in Mumbai, Port Elizabeth and Chisinau and analyze the clinical and epidemiologic characteristics of multi and extensively drug resistant TB (M/XDRTB) to identify factors that are linked to M/XDRTB.

Our research questions were:

- What is the diversity and frequency of *gyrA* and *gyrB* mutations in FQ resistant *Mtb*?
- What is the global distribution of *gyrA* and *gyrB* mutations?
- What is the prevalence of first and second-line drug resistance in Mumbai, India, Port Elizabeth, South Africa and Chisinau, Moldova?
- What clinical and epidemiology risk factors are associated with M/XDRTB?

Intervention

The GCDD was established to characterize the genetic basis of drug resistance and evaluate molecular and microbiological methods to detect DRTB quickly and efficiently. This international collaboration to improve current DRTB diagnostics gathered data from Mumbai, Port Elizabeth and Chisinau in an effort to improve accuracy and precision of novel diagnostics and reduce DRTB detection time. The GCDD study design was conducted in two phases. Data from Phase II consisted of a prospective cohort

study of patients with suspected, but not confirmed XDRTB. Data for these analyses was collected from men and women enrolled in the GCDD funded study.

The study was approved by the Institutional Review Boards (IRB) at the University of California, San Diego (UCSD), P.D. Hinduja National Hospital and Medical Research Centre, IRB Project Number. 507-09-CR; Ministry of Health Care of the Republic of Moldova, Institution of Public Health Phthisiopneumology Institute, Ethics Committee of IMSP Phthisiopneumology Institute (no applicable reference number); and Universiteit-Stellenbosch University Health Research Ethics Committee Tygerberg, South Africa, Ethics Reference Number N10/08/261.

CHAPTER 2 FREQUENCY AND GEOGRAPHIC DISTRIBUTION OF *GYRA* AND *GYRB* MUTATIONS ASSOCIATED WITH FLUOROQUINOLONE RESISTANCE IN CLINICAL *MYCOBACTERIUM TUBERCULOSIS* ISOLATES: A SYSTEMATIC REVIEW

Elisea Avalos[1], Donald Catanzaro[2], Antonino Catanzaro[3], Theodore Ganiats[1], Stephanie Brodine[4], John Alcaraz[4], Timothy Rodwell[5]

[1]Department of Family and Preventive Medicine, UCSD, [2]Department of Biological Sciences, University of Arkansas, [3]UCSD Medical Center, [4]Graduate School of Public Health, SDSU, [5]Division of Global Public Health, UCSD

Abstract

Background: The detection of mutations in the *gyrA* and *gyrB* genes in the *Mycobacterium tuberculosis* genome that have been demonstrated to confer phenotypic resistance to fluoroquinolones is the most promising technology for rapid diagnosis of fluoroquinolone resistance. Methods: In order to characterize the diversity and frequency of *gyrA* and *gyrB* mutations and to describe the global distribution of these mutations, we conducted a systematic review, from May 1996 to April 2013, of all published studies evaluating *Mycobacterium tuberculosis* mutations associated with resistance to fluoroquinolones. The overall goal of the study was to determine the potential utility and reliability of these mutations as diagnostic markers to detect phenotypic fluoroquinolone resistance in *Mycobacterium tuberculosis* and to describe their geographic distribution. Results: Forty-six studies, covering four continents and 18 countries, provided mutation data for 3,846 unique clinical isolates with phenotypic resistance profiles to

fluoroquinolones. The *gyrA* mutations occurring most frequently in fluoroquinolone-resistant isolates, ranged from 21-32% for D94G and 13-20% for A90V, by drug. Eighty seven percent of all strains that were phenotypically resistant to moxifloxacin and 83% of ofloxacin resistant isolates contained mutations in *gyrA.* Additionally we found that 83% and 80% of moxifloxacin and ofloxacin resistant strains respectively, were observed to have mutations in the *gyrA* codons interrogated by the existing MTBDR*sl* line probe assay. In China and Russia, 83% and 84% of fluoroquinolone resistant strains respectively, were observed to have *gyrA* mutations in the gene regions covered by the MTBDR*sl* assay. Conclusions: Molecular diagnostics, specifically the Genotype MTBDR*sl* assay, focusing on codons 88-94 should have moderate to high sensitivity in most countries. While we did observe geographic differences in the frequencies of single *gyrA* mutations across countries, molecular diagnostics based on detection of all *gyrA* mutations demonstrated to confer resistance should have broad and global utility.

Keywords: Fluoroquinolone resistance, gyrA, gyrB, mutations, resistance

Introduction

Mycobacterium tuberculosis (*Mtb*) is a worldwide public health threat responsible for approximately 8.6 million incident cases of tuberculosis (TB) and an estimated 1.3 million deaths annually [1]. The emergence and increasing prevalence of *Mtb* strains resistant to first and second line antituberculous medications are exacerbating the global TB epidemic [5]. Multidrug resistant (MDR) strains are *Mtb* strains resistant to rifampicin (RIF) and isoniazid (INH), the most effective first-line drugs. Extensively drug resistant (XDR) *Mtb* strains, are defined as strains with MDR plus resistance to any fluoroquinolone (FQ) and one of the second-line injectable drugs used commonly for

treating TB [73]. As of 2012, the World Health Organization (WHO) estimated the global prevalence of MDR-TB to be 3.6% among new TB cases and 20% among recurrent TB cases [1].

As M/XDRTB rates continue to increase, the development and implementation of rapid diagnostic systems for the detection of microbial resistance to prevent further transmission and promptly implement appropriate drug regimens are needed [74]. Automated liquid culture systems have significantly shortened turnaround times for drug susceptibility tests (DSTs) compared to solid media, but bacteriological assays are technically demanding and still require a sophisticated biosafety environment and approximately 7 to 10 days to complete [74]. Detection of genetic mutations that confer resistance to certain antimicrobial agents represents a more rapid alternative [74]. Currently, the only broadly available commercial assay for the rapid detection of second-line-drug resistance, including FQ resistance, the MTBDR*sl* assay (Hain Lifescience, Nehren, Germany), detects only the most common mutations found in the quinolone resistance determining region (QRDR) of *gyrA* [22].

The main cellular target of FQs in *Mtb* is the DNA gyrase, a type II topoisomerase, which consists of two A and two B subunits encoded by *gyrA* and *gyrB* genes, respectively [5]. The genetic mechanism of resistance to FQs is a result of changes in the DNA gyrase, particularly, mutations in the QRDR of *gyrA* (codons 74 to 113) [75] and *gyrB* (codons 500 to 538) [14]. It has been estimated that roughly 60 to 90% of *Mtb* clinical isolates with FQ resistance have mutations in the QRDR of *gyrA*, particularly in codons 88, 90, 91, and 94 [12, 76, 77]. Mutations in *gyrB* have also been associated with FQ resistance, but with lower sensitivity and specificity, and they often co-occur with

canonical *gyrA* mutations [13, 15, 78-80] and most often occur in codons 500 and 538 [81]. While most *Mtb* strains with *gyrA* mutations in the QRDR are FQ resistant, nearly all isolates with a wild type QRDR are FQ susceptible. The exceptions are the polymorphisms of *gyrA* at codons 21, 95 and 668 [8, 15, 23], which do not appear to be related to resistance.

FQs have potent *in vitro* activities against *Mtb* [82]. However, FQs are widely used to treat bacterial infections of the respiratory, gastrointestinal, and urinary tract as well as sexually transmitted diseases, further contributing to the increasing levels of FQ resistance in *Mtb* [83, 84]. FQs have proven to be among the most effective second-line anti-mycobacterial drugs [15, 84] and are recommended for the treatment of drug-resistant TB and for persons intolerant of current first-line therapy [8, 85]. While resistance to some of the older generation of FQs has been shown to emerge during treatment of patients infected with FQ-susceptible strains [83], newer generation FQs have become vital in the successful treatment of drug resistant TB [5, 73, 86]. As a result of the promising clinical activity of these newer FQs, the WHO currently recommends levofloxacin or moxifloxacin for the treatment of XDRTB when ofloxacin resistance is present [87, 88].

In order to characterize the *gyrA* and *gyrB* mutations associated with global phenotypic resistance to the most commonly used FQs in *Mtb* we conducted a systematic review of English language studies from May 1996 to April 2013. The overall goals of the study were to: 1) characterize the diversity and frequency of *gyrA* and *gyrB* mutations in FQ resistant *Mtb* and 2) to describe the global distribution of these mutations to help

determine their potential utility and reliability as diagnostic markers for detecting phenotypic FQ resistance in *Mtb*.

Methods

Literature Search: A Medline search was conducted of all publications investigating *gyrA* and *gyrB* mutations associated with phenotypic FQ resistance in *Mtb*. The search was restricted to studies published from May 1996 through April 15, 2013, including those studies available online prior to publication. MEDLINE/PubMed key search terms used with the help of Boolean operators ('and', 'or') were: "tuberculosis," "fluoroquinolone," "resistance," "resistant" "*gyrA*," "*gyrB*," "mutation," "sequence."

Study Selection Criteria: Study selection criteria were similar to those described in Georghiou et al. [89]. Studies were included if they met the following predetermined criteria: i) published in English ii) presented original data and iii) assessed drug resistance mutations in clinical *Mtb* strains resistant to FQs (*in vitro* studies were excluded as laboratory generated mutations have been observed to be different from those found in clinical isolates) [90]. Studies were also excluded if they did not mention the specific FQ tested, did not perform or describe details of phenotypic drug susceptibility testing, did not perform sequencing as a method for determining mutations associated with drug resistance. Additionally, studies were excluded if they did not mention the country the clinical isolates originated from or if they listed multiple countries and did not distinguish clinical isolates by country.

Data Extraction and Entry: The following background variables were collected from the selected publications: author(s), year of publication, geographic origin of clinical strains, the reference strain used, methods for testing phenotypic drug

susceptibility and genotypic mutations, MIC levels for each drug, genes sequenced, and loci of genes sequenced. The following mutation information was also recorded: specific gene mutation(s) found, FQ drug utilized for selection, number of resistant and susceptible isolates tested, and number of resistant and susceptible isolates demonstrating a mutation. Data was recorded and compiled using Microsoft Excel (Microsoft, Redmond, WA).

Data Collation and Cumulative Mutation Frequency Calculations: Data concerning mutations associated with FQ resistance were grouped by gene and stratified by the drug resistance phenotype associated with the mutation. Studies that specifically reported multiple mutations within a gene were also analyzed separately in order to determine the frequency of multiple mutations in genes associated with FQ resistance. Each mutation reported in a resistant *Mtb* isolate was considered independent of all others within and between studies (except where otherwise noted for multiple mutations in the same gene) and recorded as one instance of the mutation in the numerator of the cumulative mutation frequency calculations. Cumulative mutation frequency in *resistant* isolates was calculated as the number of resistant isolates in which the mutation was found, divided by the total number of phenotypically resistant isolates tested across studies. Cumulative mutation frequency in *susceptible* isolates was calculated as the number of susceptible isolates in which the mutation was found, divided by the total number of susceptible isolates tested across studies. As not all studies examined all mutations or all genes associated with resistance, isolates from a study were only included in the denominator of a cumulative frequency mutation calculation for a particular mutation if that mutation could have been detected in that study (i.e. the study

sequenced the appropriate section of the gene). In order to accurately assess which gene fragments had been sequenced for each isolate, the exact start and end points of the gene fragments sequenced had to be determined. These endpoints were identified by entering the published primer sequences into the NCBI BLAST (Basic Local Alignment Search Tool) with *Mtb* H37Rv complete genome selected as the reference genome, Accession number NC_000962.3 and mapping the coordinates on *Mtb* H37Rv. Sequence fragments were inferred for articles that did not include primer sequences by using the outermost identified mutations as sequence endpoints. If several primers were included and sequenced fragments overlapped, the final dataset included only the outermost/inclusive primers.

The cumulative mutation frequency tables presented in the main body of the review represent the mutations that reached a frequency threshold, described as the following: 1) Isolates were included if a mutation was observed in at least two studies and reported resistance to at least two FQs with a frequency of at least 1% for any one of the FQs tested; 2) Mutations were excluded from the main tables when the frequencies of the mutation were equal in resistant and susceptible strains. Due to the large number of mutations reported (146 total), this frequency threshold was used to report only the most frequently reported mutations in the main tables. All mutations not meeting the above mentioned criteria are available in **Appendix A**.

Results

Description of Included Studies: **Figure 2.1** illustrates the study selection and exclusion process utilized for this review. Initial search parameters identified 193 studies published from May, 1996 through April 15, 2013. (PRISMA checklist **Appendix B**).

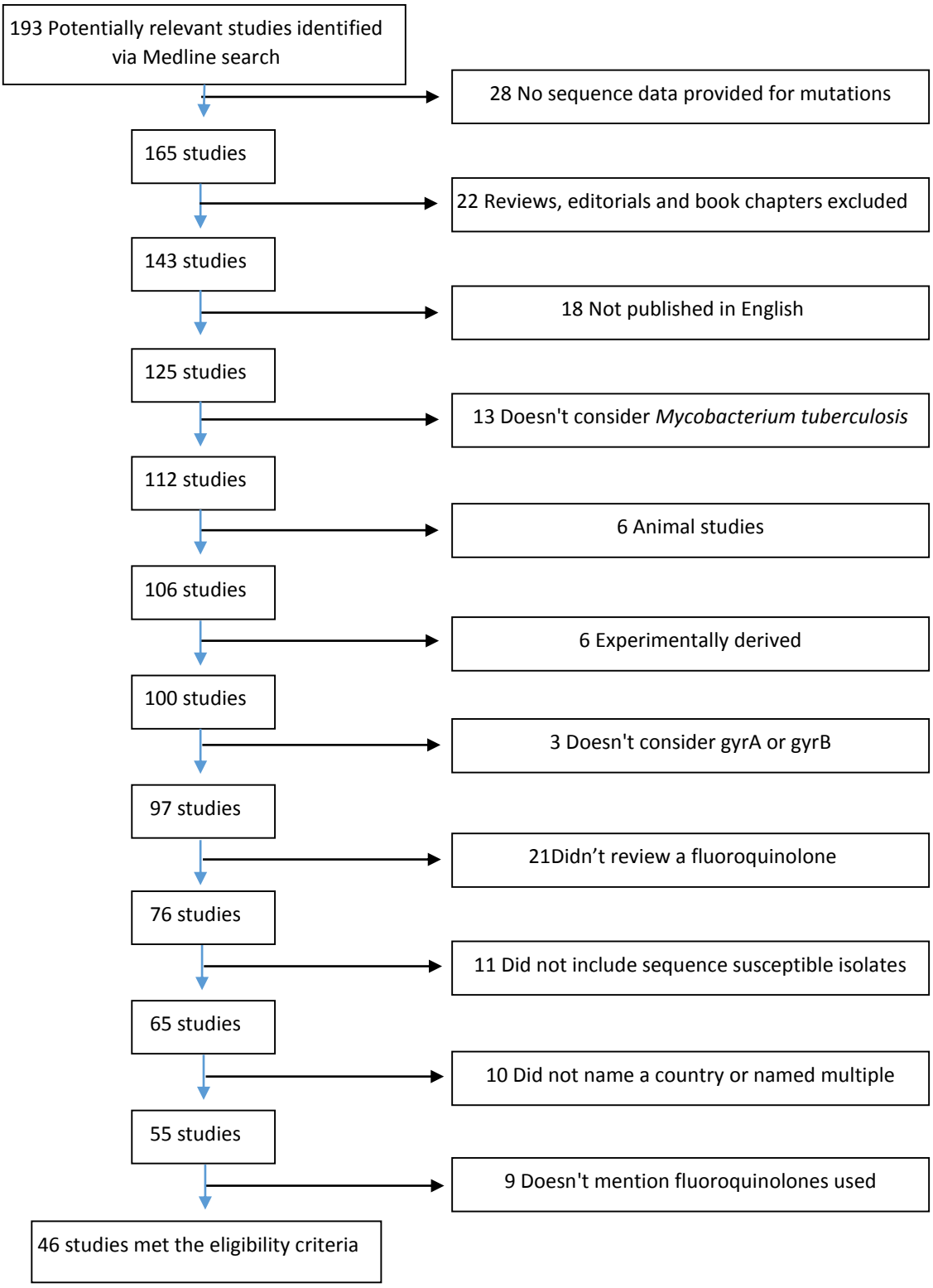


Figure 2.1: Study Selection Process and Reason for Exclusion of Studies

Forty-six publications met all eligibility criteria and were included in the review [12, 13, 16, 20, 21, 23, 73, 74, 76-78, 83, 85, 90-122].

Of the 46 studies included, the earliest was published in 1996, with 23 (50%) published in the last three years (**Table 2.1**). Altogether, mutation data was provided for 3,846 unique clinical *Mtb* isolates with various phenotypic resistance profiles to FQs. The reported geographic origins of these strains were diverse, covering four continents and 18 countries.

Table 2.1: Details of Studies Included in Review and Source of *Mycobacterium tuberculosis* Isolates

PubMed ID	Author (Year)	# of FLQ Isolates Examined [+]	Origin of Isolates	Molecular Technique	Clinical Institution(s) Providing Isolates	Year of Collection
23491718	Chernyaeva et al. (2013)	50	Russia	Sequencing	TB Dispensary	2011
23561273	Jnawali et al. (2013)	123	South Korea	PCR & Sequencing	Korea Mycobacterium Resource Center	2009-2010
23019190	Nosova et al. (2013)	68	Russia	Sequencing & TB-BIOCHIP-2	Not Stated	Not Stated
23146281	Poudel et al. (2013)	13	Nepal	PCR & Sequencing	German Nepal Tuberculosis Project	2007-2010
22552454	Chen et al. (2012)	93	China	PCR & Sequencing	Not Stated	2009-2010
22526012	Long et al. (2012)	177	China	PCR & Sequencing	National Tuberculosis Reference Laboratory	Not Stated
22357804	Sirgel et al. (2012)	177	South Africa	Sequencing	Not Stated	2007-2009
22330913	Streicher et al. (2012)	181	South Africa	Sequencing	National Health Laboratory Service	2006-2008
22421328	Suzuki et al. (2012)	59	Japan	PCR & Sequencing	11 Hospitals in Japan	Not Stated
23205246	Tahmasebi et al. (2012)	97	Iran	PCR-SSCP & Sequencing	Mycobacteriology Research Center, Masih Daneshvari Hospital	Not Stated
22553245	Yuan et al. (2012)	58	China	PCR & Sequencing	Jiangxi Chest Hospital	2010-2011
22560167	Zhu et al. (2012)	227	China	PCR & Sequencing	Not Stated	2007-2010
21911575	Ali et al. (2011)	39†	Pakistan	PCR & Sequencing	Aga Khan University Clinical Microbiology Laboratory	2004 - 2009
22152119	Anand et al. (2011)	39	India	Sequencing	Not Stated	Not Stated

Table 2.1: Details of Studies Included in Review and Source of *Mycobacterium tuberculosis* Isolates, Continued

PubMed ID	Author (Year)	# of FLQ Isolates Examined [+]	Origin of Isolates	Molecular Technique	Clinical Institution(s) Providing Isolates	Year of Collection
21051549	Ando et al. (2011)	33	Japan	PCR, Sequencing & LiPA	Nine Hospitals in Japan	2002
		17			National Center for Global Health and Medicine	2003-2008
21443804	Cui et al. (2011)	192	China	PCR & Sequencing	Not Stated	2009
21653760	El Sahly et al. (2011)	36	United States	Sequencing	Mycobacteriology Laboratory at Texas Department of State Health Services	2007-2008
21562102	Huang et al. (2011)	74‡	Taiwan	GenoType MTBDRsl & PCR	Various Hospitals	2008-2009
21450523	Hu et al. (2011)	31	China	Sequencing	Local TB Dispensaries	2004-2005
21632897	Kontsevaya et al. (2011)	51	Russia	Sequencing, Pyrosequencing & GenoType MTBDRsl	Various TB Clinics in Samara Region, Russian Federation	2008
21555766	Sekiguchi et al. (2011)	11	Japan	PCR	Not Stated	Not Stated
21623040	Singh et al. (2011)	8	India	PCR & Sequencing	Mycobacterial Repository Centre of the Institute	2004-2008
22115861	Zhao et al. (2011)	125	China	MAS-PCR, PCR-RFLP & Sequencing	Not Stated	Not Stated

Table 2.1: Details of Studies Included in Review and Source of *Mycobacterium tuberculosis* Isolates, Continued

PubMed ID	Author (Year)	# of FLQ Isolates Examined [+]	Origin of Isolates	Molecular Technique	Clinical Institution(s) Providing Isolates	Year of Collection
20335420	Brossier et al. (2010)	52	France	Sequencing & GenoType MTBDRsl	French Reference Center for Mycobacteria	2005-2009
20573868	Kiet et al. (2010)	62	Vietnam	Sequencing & GenoType MTBDRsl	Pham Ngoc Thach Hospital	2005-2006
20956608	Lau et al. (2010)	71	China	PCR & Sequencing	Queen Mary Hospital and Grantham Hospital	2003-2007
		99				2008-2009
20452372	Yin et al. (2010)	62	China	PCR & Sequencing	Guangdong Chest Hospital	2008-2009
19846642	Bravo et al. (2009)	102	Philippines	PCR & Pyrosequencing	University of the Philippines-Philippine General Hospital	Not Stated
19721073	Duong et al. (2009)	109	Vietnam	Sequencing	Pham Ngoc Thach Hospital	2005-2007

Table 2.1: Details of Studies Included in Review and Source of *Mycobacterium tuberculosis* Isolates, Continued

PubMed ID	Author (Year)	# of FLQ Isolates Examined [+]	Origin of Isolates	Molecular Technique	Clinical Institution(s) Providing Isolates	Year of Collection
19386845	Hillemann et al. (2009)	106	Germany	Sequencing & GenoType MTBDRsl	National Reference Laboratory	Not Stated
20028780	Perdigao et al. (2009)	26	Portugal	PCR & Sequencing	Hospitals and Laboratories in Lisbon's Health Region	2005
19024017	Antonova et al. (2008)	107	Russia	PCR, Biochip & Sequencing	Not Stated	Not Stated
18559646	Mokrousov et al. (2008)	71	Russia	PCR & Sequencing	St. Petersburg Research Institute of Phthisiopulmonology	2006
18164184	Sun et al. (2008)	110	China	PCR, DHPLC & Sequencing	Beijing Chest Hospital	2002-2004
18544197	van Doorn et al. (2008)	82	Vietnam	PCR, RT-PCR & Sequencing	Pham Ngoc Thach Hospital	2005-2006
17360809	Chan et al. (2007)	250	China	PCR-SSCP/ MPAC & Sequencing	Grantham Hospital and Public Health Laboratory	1994-2004
17934259	Escribino et al. (2007)	18	Spain	PCR & Sequencing	Not Stated	Not Stated
17434825	Sulochana et al. (2007)	118	India	PCR & Sequencing	Not Stated	Not Stated
17412727	Wang et al. (2007)	42	Taiwan	PCR & Sequencing	Tertiary Care Referral Centre	2004-2005
16584301	Kam at al. (2006)	143	China	Sequencing	TB Reference Laboratory, Department of Health	1999-2003

Table 2.1: Details of Studies Included in Review and Source of *Mycobacterium tuberculosis* Isolates, Continued

PubMed ID	Author (Year)	# of FLQ Isolates Examined [+]	Origin of Isolates	Molecular Technique	Clinical Institution(s) Providing Isolates	Year of Collection
16204341	Huang et al. (2005)	141	Taiwan	PCR & Sequencing	Kaohsiung Veterans General Hospital	1995-2003
15195248	Post et al. (2004)	13	South Africa	Sequencing	Not Stated	Not Stated
12044302	Lee et al. (2002)	100	Singapore	PCR & Sequencing	Central Tuberculosis Laboratory	Not Stated
11796356	Siddiqi et al. (2002)	68	India	PCR & Sequencing	Outpatient hospitals and National Mycobacterial Repository	1995-1998
8737156	Williams et al. (1996)	9	China	PCR & Sequencing	Not Stated	Not Stated
8896523	Xu et al. (1996)	19	United States	PCR & Sequencing	Public Health Research Institute Tuberculosis Center	Not Stated

[+]Does not include reference strain
[†]Included S95T; not reported here
‡Examined 234 isolates, reported 74

A total of 146 unique mutations were reported relative to the reference H37Rv genome: *gyrA* (76 unique mutations, 34 single mutations and 42 multiple mutations), *gyrB* (28 unique mutations, 25 single mutations and 3 multiple mutations) and *gyrA and gyrB* (42 multiple mutations). We evaluated the DST methods and critical drug concentrations used in each study to define whether a strain was phenotypically resistant or not. **Table 2.2** shows the DST methods and critical concentrations used in each of the included studies and whether or not they conformed to published reference standards. The drug concentrations used in 35 of the 46 (76%) studies conformed to at least one national or international published standard, 4 (9%) studies were conducted in national reference laboratories. The remaining 7 (15%) studies did not document a specific reference laboratory standard.

Table 2.2: Drug Susceptibility Testing (DST) Methods Employed in Publications

Author	DST Method	Second Generation		Third Generation		Fourth Generation		
		CIPRO	OFL	LEVO	SPAR	GAT	MOX	SITA
Tahmasebi et al.	LJ	2.0[+]	--	--	--	--	--	--
Wang et al.	LJ	2.0[+]	2.0[+]	1.0[‡‡]	--	--	0.5[‡‡]	--
Hu et al.	LJ	2.0[+]	2.0[+]	1.0[‡‡]	--	--	--	--
Chen et al.	LJ	1.0-16.0[††]	2.0[+]-16.0[†]	--		0.125-8.0[‡‡]	0.125-16.0[‡‡]	
Poudel et al.	LJ	--	2.0[+]	--	--	--	--	--
Yuan et al.	LJ	--	2.0[+]	--	--	--	-	--
Williams et al.	LJ	--	2.0[+]	--	--	--	--	--
Jnawali et al.	LJ	--	2.0[+]	--	--	--	--	--
Zhao et al.	LJ	--	2.0[+]	--	--	--	--	--
Brossier et al.	LJ	--	2.0[+]	--	--	--	--	--
Kiet et al.	LJ	--	2.0[+]	--	--	--	--	--
Duong et al.	LJ	--	2.0[+]	--	--	--	--	--
Mokrousov et al.	LJ	--	2.0[+]	--	--	--	--	--
van Doorn et al.	LJ	--	2.0[+]	--	--	--	--	--
Hillemann et al.	LJ/MGIT 960	--	2.0[+]	--	--	--	--	--

Table 2.2: Drug Susceptibility Testing (DST) Methods Employed in Publications, Continued

Author	DST Method	Second Generation		Third Generation		Fourth Generation		
		CIPRO	OFL	LEVO	SPAR	GAT	MOX	SITA
Nosova et al.	LJ	--	2.0[+]	2.0[++]	--	0.5[++]	0.5[++]	--
Anand et al.	LJ	--	2.0[+]-4.0[†]	--	--	2.0-5.0[‡‡]	2.0-5.0[‡‡]	--
Chernyaeva et al.	LJ	--	2.0[+]-10.0[++]	--	--	--	--	--
Antonova et al.	LJ	--	2.0[+], 10.0[†]	--	--	--	--	--
Long et al.	LJ	--	5.0-50.0[†]	2.0-20.0[‡‡]	--	--	--	--
Kam at al.	LJ/MGIT 960	--	0.5[‡], 1.0[‡], 2.0[+], 4.0[†], 8.0[†], 16.0[†]	--	--	--	0.5[+], 1.0[‡‡], 2.0[‡‡], 4.0[‡‡], 8.00[‡‡], 16.00[‡‡]	--
Sun et al.	LJ	--	0.5[‡], 1.0[‡], 2.0[+], 4.0[†], 8.0[†], 10.0[†], 16.0[†], 20.0[†]	--	--	--	--	--
Sulochana et al.	LJ	--	8.0[†]	--	--	--	--	--
Chan et al.	LJ	--	--	--	--	--	4.8[++]	--
Siddiqi et al.	LJ	--	--	--	--	--	2.0‡‡	--
Perdigao et al. 2007	BACTEC 460	--	2.0[+]	--	--	--	--	--

Table 2.2: Drug Susceptibility Testing (DST) Methods Employed in Publications, Continued

Author	DST Method	Second Generation		Third Generation		Fourth Generation		
		CIPRO	OFL	LEVO	SPAR	GAT	MOX	SITA
Zhu et al.	MGIT 960	--	2.0*	--	--	--	--	--
Kontsevaya et al.	MGIT 960	--	2.0*	--	--	--	2.0†	--
Streicher et al.	MGIT 960	--	2.0*	--	--	--	--	--
Cui et al.	MGIT 960	--	2.0*	--	--	--	--	--
Sirgel et al.	MGIT 960	--	0.5-10.0††	-	--	--	0.125-2.0††	--
Singh et al.	Middlebrook 7H9	--	8.0†, 16.0†, 32.0†	--	--	--	--	--
Sekiguchi et al.	Middlebrook 7H10	0.5‡	--	0.5‡	--	0.06‡	--	--
Xu et al.	Middlebrook 7H10	2.0*	--	--	--	--	--	--
Ali et al.	Middlebrook 7H11	2.0*	--	--	--	--	--	--
Huang et al.	Middlebrook 7H11	2.0*	2.0*	1.0‡‡	--	--	--	--
Suzuki et al.	Middlebrook 7H11	6.25-50.0†	--	3.13-25.0‡‡	1.56-12.5‡‡	0.78-6.25‡‡	0.78-12.5‡‡	0.39-12.5‡‡
Escribano et al.	Middlebrook 7H11	16.0†	16.0†	8.0‡‡	--	2.0‡‡	4.0‡‡	--
Bravo et al.	Middlebrook 7H10	--	2.0*	--	--	--	--	--

Table 2.2: Drug Susceptibility Testing (DST) Methods Employed in Publications, Continued

Author	DST Method	Second Generation		Third Generation		Fourth Generation		
		CIPRO	OFL	LEVO	SPAR	GAT	MOX	SITA
Lau et al.	Middlebrook 7H10	--	2.0⁺	--	--	--	1.0‡‡	--
Post et al.	Middlebrook 7H10	--	2.0⁺	--	--	--	--	--
Huang et al.	Middlebrook 7H11	--	2.0⁺	--	--	--	--	--
Yin et al.	Middlebrook 7H11	--	--	1.0, 10.0‡‡	--	--	--	--
El Sahly et al.	Agar proportion indirect susceptibility assay	--	--	--	--	--	0.5‡‡	--
Ando et al.	Broth MIC; Egg based Ogawa medium	2.0-16.0‡‡	--	2.0-16.0‡‡	1.0-8.0‡‡	--	--	--
Lee et al.	E-test	--	--	--	--	--	32.0‡‡°	--

CIPRO = Ciprofloxacin, GAT = Gatifloxacin, LEVO = Levofloxacin, MOX = Moxifloxacin, OFL = Ofloxacin, SITA=Sitafloxacin, SPX=Sparfloxacin, NM = MIC not mentioned, LJ= Lowenstein-Jensen

-- = Indicates fluoroquinolone not tested in this study

⁺DST conforms to published standard

†DST above published standard

‡DST below published standard

⁺⁺Absolute concentration, not yet validated

††DST range above and below published standard

‡‡No published standard

° In gyrB only

gyrA Mutations Associated with Fluoroquinolone Resistance: Of the 46 papers examined in this review, all 46 studied resistance-associated markers within *gyrA*. **Figure 2.2** shows the *gyrA* studies as a heat map of the number of isolates evaluated in all 46 studies as well as the locations of the mutations found in *gyrA*. Thirty-four studies sequenced the QRDR of the *gyrA* gene, 11 studies sequenced part of the QRDR of the *gyrA* gene; only one study sequenced the entire *gyrA* gene.

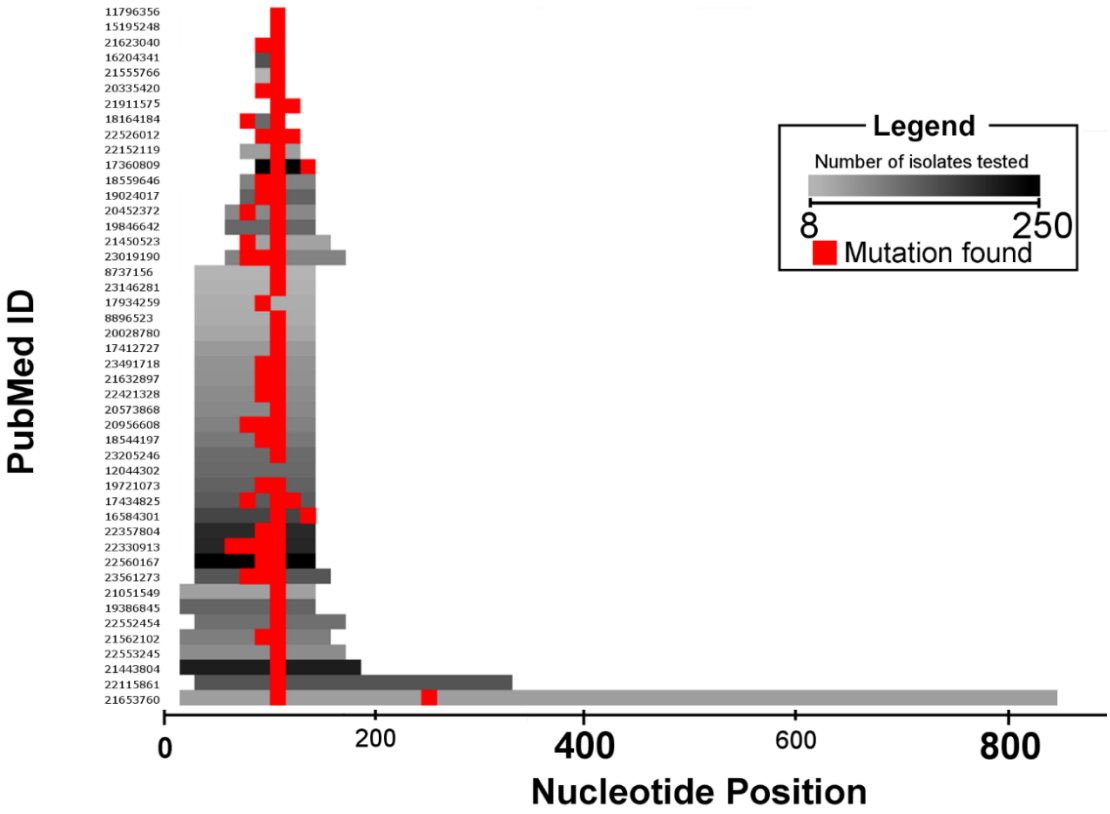


Figure 2.2: Heat map of Reviewed Studies that Evaluated *gyrA* Gene Mutations in *Mtb*, Heat map of individual papers indicating the number of isolates and the region of the *gyrA* gene studied. The number of isolates testes ranges from 8 (light grey) to 227 (black). Red indicates that a mutation has been found.

Table 2.3 shows the cumulative frequencies of the most commonly reported mutations in the *gyrA* gene associated with resistance to the primary FQs across

publications. Resistance to ofloxacin, levofloxacin and moxifloxacin was studied in the largest number of isolates, but it is important to note that the primary canonical mutations listed in **Table 2.3** appeared to be associated with resistance to all of the clinically relevant FQs, suggesting some level of cross-resistance is likely. Additionally, a subset of studies specifically examined and found evidence of cross resistance associated with these mutations, for example, most moxifloxacin resistant isolates with the A90V mutation (18%) were also resistant to ofloxacin (17%).

Table 2.3: Cumulative Frequencies of the Most Frequently Occurring Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates Resistant to Fluoroquinolones. Mutations are listed in order of descending frequency

Codon	Substitution	FLQ Tested	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates with Mutation	# Susceptible Isolates with Mutation	Frequency of Mutation among Resistant Isolates	Frequency of Mutation among Susceptible Isolates
94	Asp→Gly	OFL	1995	1572	566	0	0.28	0.00
		MOX	357	540	114	0	0.32	0.00
		LEVO	412	248	105	0	0.25	0.00
		CIPRO	334	287	81	0	0.24	0.00
		GAT	198	91	56	0	0.28	0.00
		SPX	109	0	23	0	0.21	NA
		SITA	59	0	15	0	0.25	NA
	Asp→Ala	OFL	1995	1572	177	1	0.09	0.00
		MOX	357	540	43	0	0.12	0.00
		LEVO	412	248	46	0	0.11	0.00
		CIPRO	334	287	36	0	0.11	0.00
		GAT	198	91	26	0	0.13	0.00
		SPX	109	0	19	0	0.17	NA
		SITA	59	0	10	0	0.17	NA
	Asp→Asn	OFL	1995	1572	122	1	0.06	0.00
		MOX	357	540	22	1	0.06	0.00
		LEVO	412	248	22	0	0.05	0.00
		CIPRO	334	287	28	1	0.08	0.00
		GAT	198	91	13	1	0.07	0.01
		SPX	109	0	5	0	0.05	NA
		SITA	59	0	5	0	0.08	NA

Table 2.3: Cumulative Frequencies of the Most Frequently Occurring Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates Resistant to Fluoroquinolones. Mutations are listed in order of descending frequency, Continued

Codon	Substitution	FLQ Tested	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates with Mutation	# Susceptible Isolates with Mutation	Frequency of Mutation among Resistant Isolates	Frequency of Mutation among Susceptible Isolates
94	Asp→Tyr	OFL	1995	1572	79	0	0.04	0.00
		MOX	357	540	14	0	0.04	0.00
		LEVO	412	248	11	0	0.03	0.00
		CIPRO	334	287	19	0	0.06	0.00
		GAT	198	91	11	0	0.06	0.00
		SPX	109	0	6	0	0.06	NA
		SITA	59	0	5	0	0.08	NA
	Asp→His	OFL	1995	1572	21	0	0.01	0.00
		MOX	357	540	4	0	0.01	0.00
		LEVO	412	248	3	0	0.01	0.00
		CIPRO	334	287	1	0	0.00	0.00
		GAT	198	91	1	0	0.01	0.00
	Asp→Val	OFL	1995	1572	4	0	0.00	0.00
		MOX	357	540	1	0	0.00	0.00
		LEVO	412	248	2	0	0.00	0.00
		CIPRO	334	287	2	0	0.01	0.00
		GAT	198	91	2	0	0.01	0.00
		SPX	109	0	1	0	0.01	NA
		SITA	59	0	1	0	0.02	NA

Table 2.3. Cumulative Frequencies of the Most Frequently Occurring Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates Resistant to Fluoroquinolones. Mutations are listed in order of descending frequency, Continued

Codon	Substitution	FLQ Tested	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates with Mutation	# Susceptible Isolates with Mutation	Frequency of Mutation among Resistant Isolates	Frequency of Mutation among Susceptible Isolates
90	Ala→Val	OFL	1995	1572	330	4	0.17	0.00
		MOX	357	540	65	0	0.18	0.00
		LEVO	412	248	82	0	0.20	0.00
		CIPRO	334	287	45	0	0.13	0.00
		GAT	198	91	36	0	0.18	0.00
		SPX	109	0	16	0	0.15	NA
		SITA	59	0	12	0	0.20	NA
91	Ser→Pro	OFL	1995	1572	84	0	0.04	0.00
		MOX	357	540	14	0	0.04	0.00
		LEVO	412	248	9	0	0.02	0.00
		CIPRO	334	287	18	0	0.05	0.00
		GAT	198	91	7	0	0.04	0.00
		SPX	109	0	4	0	0.04	NA
		SITA	59	0	4	0	0.07	NA
88	Gly→Cys	OFL	1982	1504	17	0	0.01	0.00
		MOX	357	540	5	0	0.01	0.00
		LEVO	412	248	2	0	0.00	0.00
		CIPRO	295	287	1	0	0.00	0.00
		GAT	198	91	2	0	0.01	0.00
		SPX	109	0	1	0	0.01	NA
		SITA	59	0	1	0	0.02	NA
126	Ala→Arg	OFL	1676	1283	4	0	0.00	0.00
		MOX	335	523	2	0	0.01	0.00

CIPRO = Ciprofloxacin, GAT = Gatifloxacin, LEVO = Levofloxacin, MOX = Moxifloxacin, OFL = Ofloxacin, SITA=Sitafloxacin, SPX=Sparfloxacin, NA=Not Applicable

Eighty seven percent of the moxifloxacin resistant isolates and 83% of the ofloxacin resistant isolates had mutations in their *gyrA* genes, with most mutations occurring in codons 88-94 (**Table 2.3**, **Appendix A** and **Appendix C**). The cumulative frequency of individual mutations associated with FQ resistance was highest for the *gyrA* mutation D94G, ranging from 21-32% in FQ-resistant isolates depending on the specific FQ tested. The *gyrA* A90V mutation was the second most frequent mutation observed in FQ resistant isolates, and was found in 13-20% of FQ-resistant isolates depending on the FQ tested. Across all drugs tested, the *gyrA* mutations G88C and D94V were least frequent (1-2%).

Most importantly, none of the mutations listed in **Table 2.3** occurred in more than a few of the many thousands of FQ susceptible isolates evaluated. Of the 41 studies reporting single A90V mutations, only two studies (n=4) reported the A90V mutation in FQ susceptible isolates. Two other mutations were reported in susceptible isolates: D94A and D94N, but less than 1% of susceptible isolates contained these mutations, leaving open the possibility these were likely phenotypic DST errors.

gyrB Mutations Associated with Fluoroquinolone Resistance: Eighteen of the 46 (39%) publications included sequence data for *gyrB*. However, overall the *gyrB* mutations have only been evaluated in a few hundred FQ-resistant strains. Mutations of the *gyrB* gene occurred most frequently within ofloxacin resistant isolates (**Table 2.4**). The *gyrB* N538D mutation (also reported as N510D in some publications depending on the numbering system used), as well as D500H, T539N and A543V were reported to be rare among ofloxacin-resistant isolates, at frequencies of less than 1%. While the number

of susceptible isolates examined for *gyrB* mutations was low, it is important to note than none of them contained mutations listed in **Table 2.4**.

Table 2.4: Cumulative Frequencies of the Most Frequently Occurring Mutations within gyrB Gene among *Mycobacterium tuberculosis* Isolates Resistant to Fluoroquinolones. Mutations are listed in order of descending frequency.

Codon	Substitution	FLQ Tested	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates with Mutation	# Susceptible Isolates with Mutation	Frequency of Mutation among Resistant Isolates	Frequency of Mutation among Susceptible Isolates
538	Asn→Asp	OFL	838	393	3	0	0.00	0.00
		MOX	118	70	2	0	0.02	0.00
		LEVO	315	112	2	0	0.01	0.00
		CIPRO	119	40	1	0	0.01	0.00
		GAT	104	42	1	0	0.01	0.00
500	Asp→His	OFL	838	393	3	0	0.00	0.00
		MOX	118	70	1	0	0.01	0.00
		LEVO	315	112	2	0	0.01	0.00
		GAT	104	42	1	0	0.01	0.00
543	Ala→Val	OFL	536	191	4	0	0.01	0.00
		LEVO	137	40	2	0	0.01	0.00
539	Thr→Asn	OFL	708	239	2	0	0.00	0.00
		LEVO	256	42	2	0	0.01	0.00

CIPRO = Ciprofloxacin, GAT = Gatifloxacin, LEVO = Levofloxacin, MOX = Moxifloxacin, OFL = Ofloxacin, NA=Not Applicable

Double Mutations in gyrA Associated with Fluoroquinolone Resistance: Several studies reported double mutations in *gyrA*, *gyrB* or both *gyrA* and *gyrB*; **Appendix C** includes double mutations reported within the *gyrA* gene. The most commonly reported double mutations largely included the previously examined A90V mutation. While the cumulative frequencies of *gyrA* double mutations ranged from 1-3% among resistant isolates, no susceptible isolates were reported to contain any of the double mutations, suggesting that although rare, double *gyrA* mutations are highly specific predictors of FQ-resistance.

Mutations in gyrA Associated with Fluoroquinolone Resistance by Country: **Table 2.5** shows the cumulative frequencies of *gyrA* point mutations in FQ resistant isolates by country. The greatest number of studies came from China (n=13), followed by Russia (n=5), with all other countries contributing less than four studies each. Both China and Russia reported the *gyrB* mutation D500H in FQ resistant isolates. In China, 85% of mutations reported were found in codons 88-94, whereas 89% of mutations in Russia were in these codons (the remainder of the mutations occurred outside of these codons and in *gyrB*). Of the 18 country-specific studies included in our review, 14 reported mutations in codon 90 (all in A90V) with frequencies ranging from 6% of FQ resistant strains in Iran to 30% of FQ resistant strains in the Philippines. Sixteen countries reported mutations in codon 94. For *gyrA* D94G, the cumulative frequency of the mutation in all FQ resistant strains ranged from 6% in Iran to 56% in South Korea. While A90V and D94G were the most frequently reported mutations overall, four countries reported mutations other than these mutations with higher frequency. In India, the most commonly reported mutation was D94A (20%); in Iran the most commonly reported mutation was

D94N (11%); in Portugal the most commonly reported mutation was S91P (42%) and in Spain the most commonly reported mutation was D84G (17%).

Table 2.5: Cumulative Frequencies of Selected Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates by Country

Country	Mutation	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates with Mutation	# Susceptible Isolates with Mutation	Frequency of Mutation among Resistant Isolates	Frequency of Mutation among Susceptible Isolates
China (n=13)	A90V	1391	1088	253	0	0.18	0.00
	D94G	1391	1088	394	0	0.28	0.00
	D94A	1391	1088	111	1	0.08	0.00
	D94N	1391	1088	117	4	0.08	0.00
	S91P	1391	1088	51	0	0.04	0.00
	D94Y	1391	1088	63	0	0.05	0.00
	D94H	1391	1088	18	0	0.01	0.00
	G88C	1391	1088	3	0	0.00	0.00
	D500H	674	220	3	0	0.00	0.00
France (n=1)	A90V	24	28	4	0	0.17	0.00
	D94G	24	28	6	0	0.25	0.00
	D94A	24	28	2	0	0.08	0.00
	D94N	24	28	2	0	0.08	0.00
	D94H	24	28	1	0	0.04	0.00
	G88C	24	28	1	0	0.04	0.00
	N538D	24	28	1	0	0.04	0.00
Germany (n=1)	A90V	32	74	4	0	0.13	0.00
	D94G	32	74	13	0	0.41	0.00
	D94A	32	74	5	0	0.16	0.00
	D94N	32	74	1	0	0.03	0.00
	S91P	32	74	1	0	0.03	0.00
India (n=4)	A90V	153	158	15	0	0.10	0.00
	D94G	153	158	14	0	0.09	0.00
	D94A	153	158	31	0	0.20	0.00
	D94N	153	158	4	0	0.03	0.00
	S91P	153	158	2	0	0.01	0.00
	D94Y	153	158	2	0	0.01	0.00

Table 2.5: Cumulative Frequencies of Selected Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates by Country, Continued

Country	Mutation	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates with Mutation	# Susceptible Isolates with Mutation	Frequency of Mutation among Resistant Isolates	Frequency of Mutation among Susceptible Isolates
Iran (n=1)	A90V	18	79	1	0	0.06	0.00
	D94G	18	79	1	0	0.06	0.00
	D94N	18	79	2	0	0.11	0.00
Japan (n=3)	A90V	537	0	93	0	0.17	NA
	D94G	537	0	120	0	0.22	NA
	D94A	537	0	90	0	0.17	NA
	D94N	537	0	33	0	0.06	NA
	S91P	537	0	24	0	0.04	NA
	D94Y	537	0	36	0	0.07	NA
	G88C	537	0	6	0	0.01	NA
Nepal (n=1)	D94G	13	0	7	0	0.54	NA
	D94A	13	0	2	0	0.15	NA
	D94N	13	0	1	0	0.08	NA
	S91P	13	0	1	0	0.08	NA
	D94Y	13	0	1	0	0.08	NA
	D94H	13	0	1	0	0.08	NA
Pakistan (n=1)	A90V	39	0	9	0	0.23	NA
	D94G	39	0	14	0	0.36	NA
	D94A	39	0	2	0	0.05	NA
	D94N	39	0	2	0	0.05	NA
	S91P	39	0	1	0	0.03	NA
	D94Y	39	0	5	0	0.13	NA
Philippines (n=1)	A90V	10	92	3	0	0.30	0.00
	D94G	10	92	3	0	0.30	0.00
Portugal (n=1)	D94G	52	0	12	0	0.23	NA
	D94A	52	0	16	0	0.31	NA
	S91P	52	0	22	0	0.42	NA

Table 2.5: Cumulative Frequencies of Selected Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates by Country, Continued

Country	Mutation	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates with Mutation	# Susceptible Isolates with Mutation	Frequency of Mutation among Resistant Isolates	Frequency of Mutation among Susceptible Isolates
Russia (n=5)	A90V	364	238	67	3	0.18	0.01
	D94G	364	238	122	0	0.34	0.00
	D94A	364	238	42	0	0.12	0.00
	D94N	364	238	14	0	0.04	0.00
	S91P	364	238	10	0	0.03	0.00
	D94Y	364	238	18	0	0.05	0.00
	D94H	364	238	5	9	0.01	0.04
	G88C	364	238	10	0	0.03	0.00
	D500H	250	143	4	0	0.02	0.00
	N538D	250	143	4	0	0.02	0.00
Singapore (n=1)	D533A	48	24	1	0	0.02	0.00
South Africa (n=3)	A90V	280	258	65	0	0.23	0.00
	D94G	280	258	92	0	0.33	0.00
	D94A	280	258	30	0	0.11	0.00
	D94N	280	258	27	0	0.10	0.00
	S91P	280	258	15	0	0.05	0.00
	D94Y	280	258	2	0	0.01	0.00
	G88C	275	250	3	0	0.01	0.00
South Korea (n=1)	A90V	108	15	16	0	0.15	0.00
	D94G	108	15	60	0	0.56	0.00
	D94A	108	15	2	0	0.02	0.00
	D94N	108	15	3	0	0.03	0.00
	S91P	108	15	9	0	0.08	0.00
	D94Y	108	15	2	0	0.02	0.00
	D94H	108	15	2	0	0.02	0.00

Table 2.5: Cumulative Frequencies of Selected Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates by Country, Continued

Country	Mutation	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates with Mutation	# Susceptible Isolates with Mutation	Frequency of Mutation among Resistant Isolates	Frequency of Mutation among Susceptible Isolates
Spain (n=1)	D84G	35	60	5	0	0.14	0.00
Taiwan (n=3)	A90V	145	520	15	0	0.10	0.00
	D94G	145	520	51	0	0.35	0.00
	D94A	145	520	3	0	0.02	0.00
	D94N	145	520	5	0	0.03	0.00
	S91P	145	520	2	0	0.01	0.00
	D94Y	145	520	6	0	0.04	0.00
	G88C	145	520	6	0	0.04	0.00
	N538D	56	112	4	9	0.07	0.08
United States (n=2)	A90V	23	26	4	0	0.17	0.00
	D94G	23	26	3	0	0.13	0.00
	D94A	23	26	1	0	0.04	0.00
	D94N	23	26	3	0	0.13	0.00
	D94Y	23	26	3	0	0.13	0.00
	D94H	23	26	2	0	0.09	0.00
Vietnam (n=3)	A90V	192	40	37	0	0.19	0.00
	D94G	192	40	48	0	0.25	0.00
	D94A	192	40	20	0	0.10	0.00
	D94N	192	40	3	0	0.02	0.00
	S91P	192	40	2	0	0.01	0.00
	D94Y	192	40	7	0	0.04	0.00
	D94H	192	40	1	0	0.01	0.00

NA=Not applicable, division by zero

Discussion

From the literature reviewed, it is evident that the QRDR of *gyrA* has been widely studied in FQ resistant *Mtb* isolates; while the remainder of the *gyrA* gene and the *gyrB* gene have been only rarely evaluated. In this review, we found that mutations occurring in the QRDR, specifically in codons 88-94, were found in 85% and 82% of phenotypic moxifloxacin and ofloxacin resistant strains, respectively. These results suggest that *gyrA* mutations in codons 88-94 are likely to be very sensitive markers of phenotypic resistance to FQ drugs in *Mtb* isolates, with high likelihood of cross-resistance to all the major FQs. Only one study included in the review sequenced the entire *gyrA* gene, explaining why very few mutations were reported outside of the QRDR region. The understudied *gyrA* regions may contain mutations that help explain the 15-18% of reported FQ resistant strains that did not appear to have mutations in codons 88-94 of the QRDR of *gyrA*. Additionally the 15-18% of FQ resistant *Mtb* strains with no identified mutation may possess an alternate mechanism of resistance [97, 123, 124]. Low cell wall permeability, efflux-related mechanisms, and drug sequestration or inactivation have been proposed to account for FQ resistance in these isolates [90, 124]. Equally important to the high frequency of the *gyrA* mutations in FQ resistant isolates, is the fact that these mutations occurred in only a few (<1%) FQ susceptible isolates, suggesting that these mutations will have close to 100% specificity as markers of phenotypic FQ resistance. The very few susceptible isolates with QRDR mutations may also have been DST errors as most QRDR mutations (the canonical mutations) have been shown to confer resistance at WHO approved critical concentrations [84].

Mutations in the *Mtb gyrB* gene were also associated with FQ resistance but at a much lower frequency. In this study, these mutations were only evaluated in a few hundred FQ resistant strains and were rare (1-2% of FQ isolates observed). Mutations in *gyrB* typically occur in association with *gyrA* mutations [13, 15, 78, 80] and most often occur in codons 500 and 538 [81], making it difficult to assess their individual contributions to phenotypic resistance. In a recent study by Malik et al. [84] functional genetic analysis of *gyrB* indicated that certain mutations in *gyrB* confer FQ resistance, however the level and pattern of resistance varied among the different mutations. Nonetheless, the results from their study provide support for the inclusion of mutations in the QRDR of *gyrB* in next generation molecular assays used to detect FQ resistance in *Mtb*. In this review, some *gyrB* mutations did occur independently of *gyrA* mutations which could help explain the phenotypic resistance in isolates that don't have mutations in the QRDR region of *gyrA*. In our study, the most common *gyrB* mutations occurred in codons 500, 538, 539 and 543. No susceptible isolates were reported to contain *gyrB* mutations, suggesting these rare mutations are highly specific markers of FQ-resistance.

Although rare, *gyrA* double mutations were found to occur in codons 90 and 94. Double mutations suggest *Mtb* may be undergoing adaptive evolution to improve the fitness of the bacteria in response to global FQ treatment [125]. Although the data from this review were limited by the lack of geographical diversity of strains with double mutations, double *gyrA* mutations were never reported in FQ susceptible *Mtb* strains and are likely highly specific markers of FQ resistance in *Mtb*.

In this study, we noted that ofloxacin-resistant clinical isolates were consistently cross-resistant to the newer FQs (eg. moxifloxacin). While there is building evidence to

suggest that certain *gyrA* mutations are associated with differential cross resistance to the different FQs, it would appear from our study that many of the canonical *gyrA* mutations should probably be considered broadly cross resistant while evidence of mutation-specific differential resistance is being verified.

The WHO has listed 27 "high burden" TB countries; data from seven of these countries (China, India, Pakistan, Philippines, Russia, South Africa and Vietnam) were included in this review. While several studies have commented on potential geographic differences [5, 6, 11, 21, 78, 95, 96, 100, 116, 118, 126] in frequencies of resistance conferring *gyrA* and *gyrB* mutations within and between countries, few attempts have been made to characterize these differences. In our study, we demonstrated that single *gyrA* mutations and resistance to FQs varies geographically. One possible reason for the diversity of mutations between countries may be attributed to different social and geographic transmission environments giving rise to different pressures of natural selection. A second possible reason for this diversity may be attributed to differences in treatment regimens containing FQs, which can result in geographically diverse drug-based selection pressures. Identifying geographical areas with high frequencies of unique mutations may help improve molecular surveillance methods and identify areas of concern for molecular diagnostic assay scale up. However, as long as next generation molecular diagnostics or whole gene/genome approaches are able to detect all of the canonical *gyrA* mutations known to confer resistance, and geographically diverse mutations show the same specificity, the observed spatial diversity of mutations will not decrease sensitivity or specificity of next generation assays.

The WHO Stop TB Program has emphasized the need to strengthen diagnostic testing and the need to develop rapid diagnostics [127]. The only commercial assay for rapid detection of FQ resistance in clinical samples currently is the MTBDR*sl* line probe assay (Hain Lifescience, Nehren, Germany). The MTBDR*sl* assay can detect *Mtb* mutations A90V, S91P, D94A, D94N/Y, D94G, and D94H, with a recently reported pooled sensitivity and specificity of 87% and 97% respectively on direct clinical samples [128]. While we did observe mutations in *gyrA* outside of the codons interrogated by the MTBDR*sl* assay, and in *gyrB (*1-2% of FQ-resistant strains showed single mutations in *gyrB*), our findings indicate that at least 85% and 82% of moxifloxacin and ofloxacin resistant strains, respectively, were observed to contain mutations in the codons interrogated by the MTBDR*sl* assay. This data is consistent with the pooled sensitivity of the MTBDR*sl* assay recently reported in a Cochrane review [129] and suggests that the MTBDR*sl* assay is likely to have good sensitivity for detection of moxifloxacin and ofloxacin resistance globally depending on its ability to detect these mutations in clinical samples. Based on the frequency of QRDR mutations observed in FQ resistant strains in China and Russia (83% and 84% respectively), the MTBDR*sl* assay may have a similar sensitivity in those countries. However, it is important to understand that biases in the collection of strains in the studies from those countries may have contributed to the frequencies observed. This emphasizes the need for representative national and global surveillance of resistance mutations to obtain more reliable estimates of global frequencies of these mutations in order to design next generation molecular diagnostics and optimize global performance.

Recently the WHO Expert Group concluded that based on available evidence, the GenoType MTBDR*sl* assay had a pooled sensitivity and specificity of 84% and 97% respectively. The expert panel determined that while the specificity was sufficient for a "rule-in" test of FQ resistance, it should not be used as a replacement test for conventional phenotypic testing yet [130] due to a high proportion of phenotypic FQ resistant isolates that it appears to be unable to detect. Our review of the global frequencies of *gyrA* mutations in FQ resistant isolates suggests that next generation assays able to detect all of the *gyrA* mutations presented in this review should have sensitivities of at least 87% and 83% for detection of moxifloxacin and ofloxacin resistance respectively, depending on their ability to detect these mutations in clinical samples. Based on our review and previously published work on *gyrA* frequencies by others [81, 131, 132], it seems unlikely that molecular diagnostics based on *gyrA* mutations alone will have global sensitivities exceeding 95%, and may suffer from geographic variability. But it is important to view this limitation in the context of the fact that less than 30-45% of MDR-TB, and likely less FQ resistant TB, is currently being detected by standard phenotypic methods [133]. Existing molecular diagnostics based on detection of QRDR mutations could significantly improve the number of FQ resistant TB cases being detected and treated appropriately.

Limitations: This study has several limitations. The cumulative frequencies calculated were based on two main assumptions. First, it was assumed that all the mutations reported were independent of each other. If some isolates were misclassified as independent when they were, in fact, not, this could have caused an overestimation in our cumulative frequencies of that specific mutation. Every effort was made to ensure that the

isolates and the mutations presented in one study were not also reported in another study. Every manuscript was scrutinized for evidence of the same isolates being reported on and to the best of our knowledge all isolates reported were unique. A second potential source of misclassification error was in our use of the DST results as reported. For example, if an isolate was misclassified as resistant based on faulty DST data, when it was, in fact, susceptible, and it did not have the expected mutation then we would have underestimated the cumulative frequency of that mutation among resistant isolates. To minimize the chances of such misclassification, we excluded manuscripts with no explicit descriptions of their DST methods and clear definitions of what constituted a resistant or susceptible isolate using accepted DST drug concentrations and methodologies. For those studies that did not state which section of a gene was sequenced, this was assumed based on the mutations reported, possibly introducing misclassification bias. Identified "hot spots" were grouped by country (as not all studies reported the city the isolates were collected in) regardless of the year the isolates were collected. Additionally it was assumed that these mutations would remain in the same locations between the time the data were collected and the time of this publication. Moreover, studies reporting from only one country were generalized to the entire country, possibly introducing misclassification bias. Lastly, the exclusion of laboratory generated mutations may have led to the under-reporting of *gyrA* mutations. While laboratory generated mutations and clinical isolates have common features, they also have some key differences. Sun et al. [90]observed mutations occurring in clinical isolates most often did not occur in the laboratory generated mutations. Furthermore, clinical isolates and laboratory generated mutations differed in frequency for various mutation patterns. Thus, while laboratory

generated mutations are critical to the understanding of the mechanism of mutations, these mutations do not always accurately reflect the mutations and frequencies of mutations observed in clinical isolates and were therefore excluded from this review of mutations for the purposes of understanding molecular diagnostics for clinical isolates.

Conclusion

To maximize the sensitivity and specificity of molecular diagnostics based on detection of mutations conferring FQ resistance in *Mtb*, we need an understanding of the frequency and geographic distribution of these mutations. In this review, *gyrA* mutations reported in codons 88-94 appeared to account for at least 82% of phenotypic ofloxacin resistance and 85% of moxifloxacin resistance globally, while g*yrB* mutations and *gyrA* double mutations occurred only rarely. While we did observe geographic differences in the frequencies of specific *gyrA* mutations between countries, it is likely that next generation molecular assays that can detect all of the *gyrA* and *gyrB* mutations documented to confer resistance, will have good sensitivity and specificity globally. Using existing molecular diagnostics to rapidly detect FQ resistance in clinical *Mtb* strains could substantially enhance drug resistance control efforts, with the goal of interruption of disease transmission and ultimately incidence reduction, especially in countries with cross-resistance. While it appears the line probe assay, Genotype MTBDR*sl* should have good sensitivity and specificity for detecting phenotypic FQ resistance globally, future national and international surveillance studies focusing on prevalence of mutations across all of *gyrA* and *gyrB*, could improve design and optimization of next generation molecular diagnostics for detecting FQ resistance.

Acknowledgements

The specimens, data, and funding for this project were provided by the Global Consortium for Drug-Resistant TB Diagnostics (GCDD; http://gcdd.ucsd.edu), funded by a grant (U01-AI082229) from the National Institute of Allergy and Infectious Diseases (NIAID), US National Institutes of Health (NIH), Bethesda, MD, USA. TCR was also supported by NIAID, grant # R01AI111435 and grant #R01AI105185. The authors thank the clinical and laboratory staff at each of the study sites for their assistance in acquiring and preparing *Mtb* isolates.

Chapter 2, in full, has been accepted for publication of the material as it may appear in PLOS ONE, 2015, Avalos, E, Catanzaro, D, Catanzaro, A, Ganiats, T, Brodine, S, Alcaraz, J, Rodwell, T. *Frequency and Geographic Distribution of gyrA and gyrB Mutations Associated with Fluoroquinolone Resistance in Clinical Mycobacterium Tuberculosis Isolates: A Systematic Review.* The dissertation author was the primary investigator and author of this paper.

CHAPTER 3 PREVALENCE OF DRUG RESISTANT TUBERCULOSIS IN MUMBAI, INDIA, CHISINAU, MOLDOVA AND PORT ELIZABETH, SOUTH AFRICA, 2012-2013

Elisea Avalos[1], Richard Garfein[2], Donald Catanzaro[3], Theodore G. Ganiats[1], Stephanie Brodine[4], John Alcaraz[4], Kathleen Eisenach[5], Camilla Rodrigues[6], Valeriu Crudu[7], Thomas C. Victor[8] and Timothy Rodwell[2]

[1]Department of Family and Preventive Medicine, UCSD, [2]Division of Global Public Health, UCSD, [3]Department of Biological Sciences, University of Arkansas, [4]Graduate School of Public Health, SDSU, [5]Department of Pathology, University of Arkansas, [6]P. D. Hinduja Hospital & Medical Research Centre, Department of Microbiology, [7]Microbiology and Morphology Laboratory, Institute of Phthisiopneumology, [8]Department of Biomedical Sciences, Stellenbosch University

Abstract

Background: Drug -resistant tuberculosis (DRTB) has emerged as a major challenge in the control and prevention of TB. While the World Health Organization (WHO) has emphasized the importance of collecting surveillance data, true rates of DRTB remain unknown throughout the world, especially in regions where the burden of TB is high. The purpose of this study was to describe the pattern of drug resistance in new and previously treated TB patients at risk for DRTB. Methods: We performed drug susceptibility testing (DST) on Mycobacterium tuberculosis (*Mtb*) isolates, with first and second-line anti-TB drugs in 908 culture-positive TB patients using the MGIT 960. Patients enrolled from May 2012 to August 2013 in Mumbai, India, Chisinau, Moldova

and Port Elizabeth, South Africa were included in the analysis. Results: Among the 908 patients, 603 (66.4%) had isolates that exhibited resistance to at least one drug. The overall prevalence of multi-drug resistant TB (MDRTB) was 79.7% (72.6% of newly diagnosed patients and 81.7% of previously treated patients), 51.1% (44.1% of newly diagnosed patients and 74.0% of previously treated patients) and 15% (62.5% of newly diagnosed patients and 13.0% of previously treated patients) in Mumbai, Chisinau and Port Elizabeth, respectively. Among the MDRTB patients, the prevalence of XDRTB in Mumbai, Chisinau and Port Elizabeth was 13.9%, 12.1% and 41.4%, respectively. Conclusions: The burden of DRTB was high in all three sites highlighting the importance of continuous surveillance to identify DRTB, especially among patients previously treated for TB. It is important to improve early diagnosis of MDRTB and to provide effective treatment to all MDRTB patients in order to prevent the development of additional drug resistance in these high-risk populations.

Keywords: MDRTB, XDRTB, Drug resistance

Clinical Trials Registration Number: ClinicalTrials.gov under number NCT02170441.

Introduction

Although the global prevalence of tuberculosis (TB) has been on a slow decline [133], drug-resistant TB (DRTB) remains a serious public health concern. The increasing incidence of DRTB, specifically multi-drug resistant tuberculosis (MDRTB) and extensively drug resistant tuberculosis (XDRTB) presents tremendous challenges to global TB control [134]. MDRTB, defined as resistance to both isoniazid (INH) and rifampicin (RIF), is difficult to cure and requires prolonged treatment with expensive and

often toxic multidrug regimens [134]. XDRTB is defined as MDRTB with additional resistance to any fluoroquinolone (FQ) (ie. ofloxacin (OFX) or moxifloxacin (MOX)) and at least one of three injectable drugs (amikacin (AMK), capreomycin (CAP) or kanamycin (KAN)) [133]. According to the World Health Organization (WHO) 2014 Global Tuberculosis Report, 5% of global TB cases were estimated to have had MDRTB in 2013 (3.5% of new and 20.5% of previously treated TB cases). One hundred countries have reported XDRTB; an estimated 9% of people with MDRTB are estimated to have XDRTB [133]. Former States of the Soviet Union, India and China have the greatest burden of XDRTB [135].

The WHO has listed 27 "high-burden" TB countries, with four of these countries (India, China, the Russian Federation and South Africa) responsible for roughly 60% of the world's cases of MDRTB [133]. Several studies [136-141] have described the prevalence of MDRTB in a number of different countries and the WHO has emphasized the importance of collecting surveillance data on the proportion of TB cases that are MDRTB or XDRTB. However, true rates of DRTB remain unknown throughout the world, especially in regions where the burden of TB is high. This is primarily a result of the lack of long-term cohort studies to detect trends due to the lack of human and financial resources, selection bias of some studies (ie. studies conducted among hospitalized or incarcerated patients) and the absence of high quality laboratory culture facilities [142]. As TB is one of the leading causes of morbidity and mortality, knowledge of true drug resistance rates in high TB burden regions are essential for developing appropriate treatment strategies [142].

The emergence of DRTB is of great concern as few treatment options remain against such highly resistant strains [143]. Thus, prevention of DRTB is paramount to curb this epidemic. The Global Consortium for Drug-resistant TB Diagnostics (GCDD) was established in 2008 to characterize the genetic basis of drug resistance and evaluate molecular and microbiological methods to detect DRTB quickly and efficiently. In an effort to improve accuracy and precision of novel diagnostics and reduce DRTB detection time, this international collaboration enrolled patients from Mumbai, India; Chisinau, Moldova; and Port Elizabeth, South Africa [72]. These sites were carefully considered in the planning of the study and were selected due to the high documented risk for DRTB and the ethnic diversity of these regions. The purpose of this study was to describe the patterns of first and second-line drug resistance in new and previously treated TB patients enrolled in the GCDD. Specifically we assessed the resistance to first-line drugs (INH and RIF) and second-line drugs (MOX, OFX, AMK, CAP and KAN) among all TB patients.

Methods

Study setting: The study was a prospective, observational study using collected laboratory data. The details of the study method have been described previously [72]. Patients were prospectively enrolled at the P.D. Hinduja National Hospital (PD-HNH) and Medical Research Centre (MRC) a tertiary care center in central Mumbai, India. In Chisinau, Moldova patients were enrolled at the Phthisiopneumology Institute (PPI), a scientific research and medical consultation and training center and two hospitals (Municipal TB Hospital in Chisinau and Municipal TB Hospital in Balti). In Port Elizabeth, South Africa patients were enrolled at one of six Primary Health Care facilities

and one regional hospital (Chatty Primary Health Care Clinic, Kwazakhele Primary Health Care Clinic, Motherwell NU2 Primary Health Care Clinic, New Brighton Clinic, Soweto Primary Health Care Clinic, Zwide Primary Health Care Clinic and Empilweni TB Hospital).

Patient enrollment: Patients at least 5 years of age, who were acid-fast bacilli sputum smear-positive, 1+ or greater (within previous 14 days), positive on GeneXpert, or with high suspicion of active TB **and**: previously received treatment for a prior TB episode **or** were failing TB treatment **or** had close contact with a known DRTB case **or** were newly diagnosed with MDRTB **or** were previously diagnosed with MDRTB and failed TB treatment, were recruited from each of the study clinics, between April 2012 and August 2013. The eligibility criteria were designed to identify patients at increased risk for DRTB. Following screening and informed consent, eligible patients were asked to provide sputum specimens and complete a baseline interview. Based on the interview and review of medical records, each patient was classified as new or previously treated. Patient treatment history was assigned according to WHO standards. A new patient was defined as a patient who had never had treatment for TB or who had taken anti-TB drugs for less than one month. A previously treated patient was defined as a patient who had ever received treatment for TB for more than one month [144].

Drug Susceptibility Testing (DST): Phenotypic drug susceptibility of the *Mycobacterium tuberculosis* (*Mtb)* isolates collected from enrolled patients was determined using the Mycobacterial Growth Indicator Tube (MGIT) 960 platform (BD Diagnostic Systems, Franklin Lakes, NJ, USA) following the manufacturer's recommendations as described in Hillery et al [72]. The following critical concentrations

were used: 0.1 μg/ml for INH and 1.0 μg/ml for RIF [145]. DST for second-line drugs (SLDs) was performed by using validated critical concentrations of in-house (locally prepared by each site) drug solutions consistent with WHO recommendations. Critical concentrations were as follows: 2.0 μg/ml for OFX, 0.25 μg/ml for MOX, 1.0 μg/ml for AMK, and 2.0 μg/ml for CAP [146]. As there were no published WHO recommended critical concentrations for KAN DST by MGIT 960 at the time of the study, we used 2.5 μg/ml based on the literature [72, 147, 148].

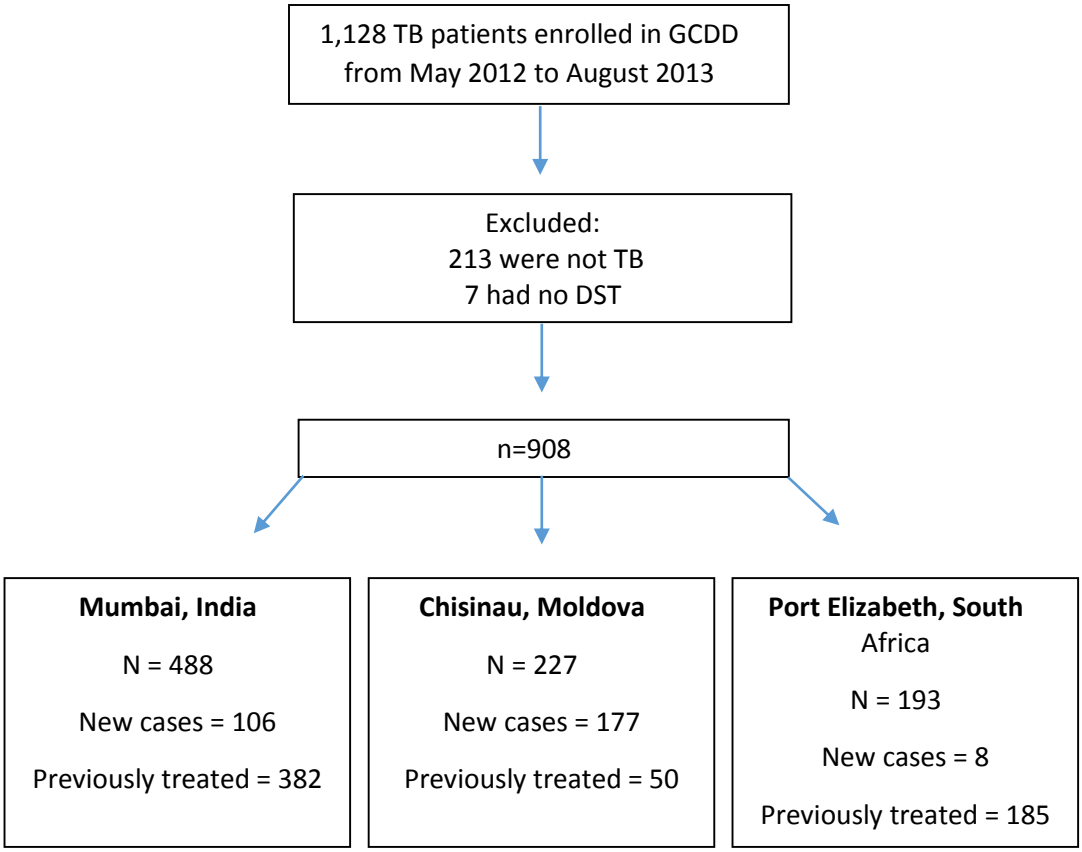
Ethical considerations: This study was approved by the Institutional Review Board (IRB) at the University of California, San Diego (UCSD) (IRB Project No. 110383) and at each enrolling site: P.D. Hinduja National Hospital and Medical Research Centre, IRB Project Number. 507-09-CR; Ministry of Health Care of the Republic of Moldova, Institution of Public Health Phthisiopneumology Institute, Ethics Committee of IMSP Phthisiopneumology Institute (no applicable reference number); and Universiteit-Stellenbosch University Health Research Ethics Committee Tygerberg, South Africa, Ethics Reference Number N10/08/261. Written informed consent was obtained from each patient prior to collection of sputum sample. The trial is registered on ClinicalTrials.gov under number NCT02170441.

MDRTB, pre-XDRTB and XDRTB: MDRTB was defined as an isolate in which the culture was positive for *Mtb* and found to have phenotypic resistance to INH and RIF with or without resistance to other anti-TB drugs. pre-XDRTB was defined as an MDRTB case with additional resistance to either a FQ (OFX or MOX) *or* a second-line injectable anti-TB drug (AMK, CAP or KAN), but not both. XDRTB was defined as having an MDRTB strain that was resistant to any FQ *and* at least one second-line injectable anti-TB drug.

Statistical analysis: The overall prevalence of each drug by site was calculated. Prevalence rates and their 95% confidence intervals (CI) for those who were newly diagnosed with TB versus those who were previously treated for TB were also calculated for each site. A two-sample z-test was used to compare differences in treatment category by site. A p-value of < 0.05 was considered as statistically significant. All statistical analyses were carried out using SAS version 9.3 (SAS Institute Inc, Cary, NC).

Results

Patient characteristics: A total of 1128 patients were recruited from May 2012 and August 2013; 213 (18.9%) patients were excluded as they did not have TB and 7 (0.6%) patients were excluded as the MGIT DST failed to provide a valid result. DST results were available for analysis for 908 patients. Of these, 488 (53.7%) were from Mumbai; 227 (25.0%) were from Chisinau and 193 (21.3%) were from Port Elizabeth (**Figure 3.1**). The majority of patients were male (63.9%); the mean age was 35.1 (±13.6) (the median was 33.0 years; IQR = 24-45). A total of 592 (65.2%) patients were resistant to isoniazid, followed by rifampicin 540 (59.5%), ofloxacin 314 (34.6%), moxifloxacin 310 (34.1%), kanamycin 145 (16.0%), amikacin 82 (9.0%), and capreomycin 79 (8.7%).



TB = tuberculosis; GCDD = Global Consortium for Drug-resistant TB Diagnostics; DST=drug susceptibility testing

Figure 3.1: Flow chart for selection of patients included in the study

Resistance to first-line drugs: A high prevalence of resistance to first-line drugs was reported in Mumbai where 84.4% of *Mtb* isolates were resistant to at least one first-line drug with 84.0% resistant to isoniazid and 80.1% resistant to rifampicin. In Chisinau, the prevalence of resistance was reported in at least one first-line drug in 60.4% of isolates with 60.4% resistant to isoniazid and 51.1% resistant to rifampicin. In Port Elizabeth, 25.4% of isolates were resistant to at least one first-line drug, 23.3% were resistant to isoniazid and 17.1% were resistant to rifampicin. The prevalence of MDRTB in Mumbai, Chisinau and Port Elizabeth was 79.7%, 51.1% and 15%, respectively.

Resistance to second-line drugs: In Mumbai, isolates from 58.4% of patients were resistant to at least one second-line drug, ofloxacin had the highest prevalence of resistance (57.8%). In Chisinau, 34.4% of patients were resistant to at least one second-line drug, with 31.3% reporting resistance to kanamycin. In Port Elizabeth, 10.4% of patients were resistant to at least one second-line drug with the highest prevalence of resistance in capreomycin (9.3%).

Seventy-three percent of MDRTB isolates in Mumbai demonstrated resistance to second-line drugs with 57.8% identified as pre-XDRTB with FQ resistance. Among the 116 MDRTB isolates in Chisinau, 59.5%, showed resistance to second-line drugs with 44.0% classified as pre-XDRTB with resistance to an injectable. In Port Elizabeth, 62.1% of MDRTB isolates demonstrated resistance to second-line drugs with 17.2% identified as pre-XDRTB with resistance to an injectable. Among the MDRTB patients, the prevalence of XDRTB in Mumbai, Chisinau and Port Elizabeth was 13.9%, 12.1% and 41.4%, respectively (**Table 3.1**).

Table 3.1: Drug susceptibility profile of the M. tuberculosis isolates in the present study

	India		Moldova		South Africa		Total	
Drug resistance profile	**No.**	**%**	**No.**	**%**	**No.**	**%**	**No.**	**%**
Total strains tested	**488**		**227**		**193**		**908**	
Susceptible to all drugs	75	15.4%	87	38.3%	143	74.1%	305	33.6%
Susceptible to first-line drugs	76	15.6%	90	39.6%	144	74.6%	310	34.1%
Susceptible to second-line drugs	203	41.6%	149	65.6%	173	89.6%	525	57.8%
Resistant to any drug	413	84.6%	140	61.7%	50	25.9%	603	66.4%
Overall first line drug resistance	412	84.4%	137	60.4%	49	25.4%	598	65.9%
Overall second line drug resistance	285	58.4%	78	34.4%	20	10.4%	383	42.2%
Any resistance[a]								
INH	410	84.0%	137	60.4%	45	23.3%	592	65.2%
RIF	391	80.1%	116	51.1%	33	17.1%	540	59.5%
MOX	276	56.6%	20	8.8%	14	7.3%	310	34.1%
OFX	282	57.8%	19	8.4%	13	6.7%	314	34.6%
AMK	52	10.7%	13	5.7%	17	8.8%	82	9.0%
CAP	50	10.2%	11	4.8%	18	9.3%	79	8.7%
KAN	57	11.7%	71	31.3%	17	8.8%	145	16.0%
Monodrug resistance[b]								
INH	19	3.9%	16	7.0%	16	8.3%	51	5.6%
RIF	2	0.4%	0	0.0%	3	1.6%	5	0.6%
OFX	1	0.2%	0	0.0%	0	0.0%	1	0.1%
AMK	0	0.0%	1	0.4%	0	0.0%	1	0.1%
CAP	0	0.0%	0	0.0%	1	0.5%	1	0.1%
Multidrug resistance[c]								
MDRTB	107	21.9%	47	20.7%	11	5.7%	165	18.2%
pre-XDRTB (FQ)	225	46.1%	4	1.8%	1	0.5%	230	25.3%
pre-XDRTB (INJ)	3	0.6%	51	22.5%	5	2.6%	59	6.5%
Polydrug resistance[d]								
INH, KAN, MOX, OFX	0	0.0%	1	0.4%	0	0.0%	1	0.1%
CAP, KAN, MOX, OFX	0	0.0%	1	0.4%	0	0.0%	1	0.1%
INH, MOX, OFX	2	0.4%	0	0.0%	0	0.0%	2	0.2%
AMK, CAP, MOX	0	0.0%	1	0.4%	0	0.0%	1	0.1%
INH, KAN	0	0.0%	4	1.8%	0	0.0%	4	0.4%
RIF, MOX	0	0.0%	0	0.0%	1	0.5%	1	0.1%
XDRTB[e]	54	11.1%	14	6.2%	12	6.2%	80	8.8%

INH = isoniazid; RIF = rifampin; MOX = moxifloxacin; OFX = ofloxacin; AMK = amikacin, CAP = capreomycin, KAN = kanamycin

[a] Any drug resistance: resistance to any of the anti-TB drugs

[b] Mono-resistance: resistance to only one drug

[c] MDRTB: resistance to at least INH and RIF

[d] Polydrug-resistance: resistance to at least two or more drugs, but excluding the INH and RIF combination

[e] XDRTB: resistance to at least INH, RIF, a FQ, and a second-line injectable

Resistance in newly diagnosed patients and previously treated TB patients:

Eighty-six percent of previously treated patients and 79.3% of newly diagnosed patients had TB resistant to any of the two first-line drugs and five second-line drugs in Mumbai. Thus previously treated patients were more likely to harbor drug resistance compared to newly treated patients (chi-square = 3.0, p= 0.0822). Statistically significant differences were observed between new and previously treated TB patients regarding rifampicin resistance (72.6% vs. 82.2%; p=0.0293) and having at least MDRTB (72.6% vs. 81.7%; p=0.0408) (**Table 3.2**).

Table 3.2: Prevalence of any drug resistance in new and previously treated cases (n=488) Mumbai, India

Any resistance	New Cases (N=106)		Previously Treated Cases (N=382)		p-value
	Resistant (N)	Resistant (% and 95% CI)	Resistant (N)	Resistant (% and 95% CI)	
INH	84	79.2 (71.5 to 87.0)	326	85.3 (81.8 to 88.9)	0.1298
RIF	77	72.6 (64.2 to 81.1)	314	82.2 (78.4 to 86.0)	0.0293
MOX	58	54.7 (45.2 to 64.2)	218	57.1 (52.1 to 62.0)	0.6592
OFX	60	56.7 (47.2 to 66.0)	222	58.1 (53.2 to 63.1)	0.7821
AMK	4	3.8 (0.1 to 7.4)	48	12.6 (9.2 to 15.9)	0.0095
CAP	4	3.8 (0.1 to 7.4)	46	12.0 (8.8 to 15.3)	0.0137
KAN	5	4.7 (0.7 to 8.8)	52	13.6 (10.2 to 17.1)	0.0116
MDRTB	18	17 (9.8 to 24.1)	89	23.3 (19.1 to 27.5)	0.1655
pre-XDRTB(FQ)	54	50.9 (41.4 to 60.5)	171	44.8 (39.8 to 49.8)	0.2650
pre-XDRTB(INJ)	0	--	3	0.8 (0.0 to 1.7)	0.3556
XDRTB	5	4.7 (0.7 to 8.8)	49	12.8 (9.5 to 16.2)	0.0186

CI = confidence interval, INH = isoniazid, RIF = rifampicin, MOX = moxifloxacin, OFX = ofloxacin, AMK = amikacin, CAP = capreomycin, KAN = kanamycin, MDRTB = multi drug resistant TB, pre-XDRTB (FQ) = pre- extensively drug resistant TB with FQ resistance, pre-XDRTB (INJ) = pre- extensively drug resistant TB with injectable resistance, XDRTB = extensively drug resistant TB

In Chisinau, previously treated patients (82%) were more likely to harbor drug resistance compared to newly diagnosed patients (56.0%) (chi-square = 11.2, p= 0.0008). Statistically significant differences were observed between new and previously treated TB patients regarding isoniazid resistance (54.2% vs. 82.0%; p=0.0004), rifampicin resistance (44.6% vs. 74.0%; p=0.0002) and having at least MDRTB (44.1% vs. 74.0%; p=0.0002) (**Table 3.3**).

Table 3.3: Prevalence of any drug resistance in new and previously treated cases (n=227) Chisinau, Moldova

Any resistance	New Cases (N=177)		Previously Treated Cases (N=50)		p-value
	Resistant (N)	Resistant (% and 95% CI)	Resistant (N)	Resistant (% and 95% CI)	
INH	96	54.2 (46.9 to 61.6)	41	82.0 (71.4 to 92.6)	0.0004
RIF	79	44.6 (37.3 to 52.0)	37	74.0 (61.8 to 86.2)	0.0002
MOX	8	4.5 (1.5 to 7.6)	12	24.0 (12.2 to 35.8)	<0.0001
OFX	8	4.5 (1.5 to 7.6)	11	22.0 (10.5 to 33.5)	<0.0001
AMK	8	4.5 (1.5 to 7.6)	5	10.0 (1.7 to 18.3)	0.1389
CAP	8	4.5 (1.5 to 7.6)	3	6.0 (0.0 to 12.6)	0.6622
KAN	47	26.6 (20.0 to 33.1)	24	48.0 (34.2 to 61.8)	0.0040
MDRTB	34	19.2 (13.4 to 25.0)	12	24.0 (12.2 to 35.8)	0.4558
pre-XDRTB(FQ)	1	0.6 (0.0 to 1.7)	3	6.0 (0.0 to 12.6)	0.0110
pre-XDRTB(INJ)	37	20.9 (14.9 to 26.9)	14	28.0 (15.6 to 40.4)	0.2881
XDRTB	6	3.4 (0.7 to 6.1)	8	16.0 (5.8 to 26.2)	0.0011

CI = confidence interval, INH = isoniazid, RIF = rifampicin, MOX = moxifloxacin, OFX = ofloxacin, AMK = amikacin, CAP = capreomycin, KAN = kanamycin, MDRTB = multi drug resistant TB, pre-XDRTB (FQ) = pre- extensively drug resistant TB with FQ resistance, pre-XDRTB (INJ) = pre- extensively drug resistant TB with injectable resistance, XDRTB = extensively drug resistant TB

Newly diagnosed patients (87.5%) were more likely to harbor drug resistance compared to previously treated patients (23.0%) (chi-square = 16.5, p <0.0001) in Port Elizabeth. Statistically significant differences were observed between new and previously treated TB patients regarding isoniazid resistance (75.0% vs. 21.2%; p= 0.0004), rifampicin resistance (75.0% vs. 14.6%; p <0.0001) and having at least MDRTB (62.5% vs. 13.0%; p= 0.0001) (**Table 3.4**).

To determine if the eight new cases of DRTB in Port Elizabeth, South Africa were clustered, we conducted a sub-analysis of these patients. As you can see from **Table 3.5**, the only two cases that appear to be related are patients 3 and 4.

Table 3.4: Prevalence of any drug resistance in new and previously treated cases (n=193) Port Elizabeth, South Africa

Any resistance	New Cases (N=8)		Previously Treated Cases (N=185)		p-value
	Resistant (N)	Resistant (% and 95% CI)	Resistant (N)	Resistant (% and 95% CI)	
INH	6	75.0 (45.0 to 100.0)	39	21.2 (15.2 to 27.0)	0.0004
RIF	6	75.0 (45.0 to 100.0)	27	14.6 (9.5 to 19.7)	<0.0001
MOX	3	37.5 (4.0 to 71.0)	11	5.9 (2.5 to 9.4)	0.0007
OFX	3	37.5 (4.0 to 71.0)	10	5.4 (2.1 to 8.7)	0.0004
AMK	5	62.5 (29.0 to 96.1)	12	6.5 (2.9 to 10.0)	<0.0001
CAP	5	62.5 (29.0 to 96.1)	13	7.0 (3.3 to 10.7)	<0.0001
KAN	5	62.5 (29.0 to 96.1)	12	6.5 (2.9 to 10.0)	<0.0001
MDRTB	0	--	11	5.9 (2.5 to 9.4)	0.4794
pre-XDRTB(FQ)	0	--	1	0.5 (0.0 to 1.6)	0.8411
pre-XDRTB(INJ)	2	25.0 (0.0 to 55.0)	3	1.6 (0.0 to 3.4)	<0.0001
XDRTB	3	37.5 (4.0 to 71.0)	9	4.9 (1.8 to 8.0)	0.0002

CI = confidence interval, INH = isoniazid, RIF = rifampicin, MOX = moxifloxacin, OFX = ofloxacin, AMK = amikacin, CAP = capreomycin, KAN = kanamycin, MDRTB = multi drug resistant TB, pre-XDRTB (FQ) = pre- extensively drug resistant TB with FQ resistance, pre-XDRTB (INJ) = pre- extensively drug resistant TB with injectable resistance, XDRTB = extensively drug resistant TB

Table 3.5 Drug Resistance Profile of the 8 New Cases of Tuberculosis in Port Elizabeth, South Africa

Patient	Drug Resistance	Age	Gender	Educational Level	Previously Hospitalized	Previously Jailed	Marital Status	# of People Currently Living With	Close Contact With a Known TB Case	# of rooms used for sleeping
1	RIF	17	Female	Primary/Secondary	Yes	No	Single	10	Yes	2
2	INH	47	Male	Primary/Secondary	No	No	Living w/partner	2	Unknown	2
3	pre-XDRTB (INJ)	51	Male	Primary/Secondary	No	Yes	Single	2	Unknown	3
4	pre-XDRTB (INJ)	51	Male	Primary/Secondary	Yes	Yes	Single	2	Yes	3
5	PS	44	Male	Primary/Secondary	Yes	Yes	Single	0	Yes	2
6	XDRTB	23	Female	Primary/Secondary	No	No	Single	4	No	1
7	XDRTB	21	Male	Primary/Secondary	No	No	Single	10	Unknown	6
8	XDRTB	27	Male	Primary/Secondary	Yes	Yes	Single	4	Unknown	3

INH = isoniazid, RIF = rifampicin, pre-XDRTB (INJ) = pre- extensively drug resistant TB with injectable resistance, PS = pan susceptible, XDRTB = extensively drug resistant TB

In Mumbai, the prevalence of drug resistance in the second-line injectable drugs was statistically different between new and previously treated TB patients for all drugs: amikacin (3.8% vs. 12.6%; p=0.0095), capreomycin (3.8% vs. 12.0%; p=0.0137) and kanamycin (4.7% vs. 13.6%; p=0.0116). In Chisinau, drug resistance to the FQs was statistically different between new and previously treated TB patients: moxifloxacin (4.5% vs. 24.0%; p= <0.0001) and ofloxacin (4.5% vs. 22.0%; p= <0.0001). Kanamycin was the only injectable with statistically significant differences in the prevalence of resistance between new and previously treated TB patients (26.6% vs. 48.0%; p=0.004). In Port Elizabeth, statistically significant differences were observed between new and previously treated TB patients for moxifloxacin (37.5% vs. 5.9%; p=0.0007) and ofloxacin (37.5% vs. 5.4%; p=0.0004) as well as all injectables: amikacin (62.5% vs. 6.5%; p<0.0001), capreomycin (62.5% vs. 7.0%; p<0.0001) and kanamycin (62.5% vs. 6.5%; p <0.0001). Among the XDRTB isolates, statistically significant differences were observed between new patients and previously treated TB patients for all sites; Mumbai (p= 0.0186), Chisinau, (p= 0.0011) and Port Elizabeth (p= 0.0002).

A high prevalence of cross-resistance among all SLDs was observed in this study. The cross-resistance to moxifloxacin was found among 97.9% (276/282), 94.7% (18/19) and 100.0% (13/13) of ofloxacin resistant isolates in Mumbai, Chisinau and Port Elizabeth, respectively (**Table 3.6**). As for the injectables, a high proportion of amikacin resistant isolates were also resistant to capreomycin and kanamycin 96.2% (50/52) in Mumbai and 100.0% (17/17) in Port Elizabeth (**Table 3.7**).

Table 3.6: Cross-resistance to fluoroquinolones among clinical isolates

Mumbai, India			Chisinau, Moldova			Port Elizabeth, South Africa		
MOX	**OFX**	**Total**	**MOX**	**OFX**	**Total**	**MOX**	**OFX**	**Total**
R	R	276	R	R	18	R	R	13
S	R	6	S	R	1	S	R	0
R	S	0	R	S	2	R	S	1
S	S	206	S	S	205	S	S	179

MOX = moxifloxacin, OFX = ofloxacin, R = resistant, S = susceptible

Table 3.7: Cross-resistance to injectables among clinical isolates

Mumbai, India				Chisinau, Moldova				Port Elizabeth, South Africa			
AMK	**CAP**	**KAN**	**Total**	**AMK**	**CAP**	**KAN**	**Total**	**AMK**	**CAP**	**KAN**	**Total**
R	R	R	50	R	R	R	7	R	R	R	17
R	R	S	0	R	R	S	1	R	R	S	0
S	R	R	0	S	R	R	3	S	R	R	0
R	S	R	2	R	S	R	3	R	S	R	0
R	S	S	0	R	S	S	0	R	S	S	0
S	S	R	5	S	S	R	58	S	S	R	0
S	R	S	0	S	R	S	0	S	R	S	1
S	S	S	431	S	S	S	153	S	S	S	175

AMK = amikacin, CAP = capreomycin, KAN = kanamycin, R = resistant, S = susceptible

Resistance patterns in patients diagnosed between 2012 and 2013: As our sample was collected over the course of two years, it was possible for us to perform a sub-analysis of the difference in DRTB prevalence by year to determine how the prevalence of DRTB changed during this time. The differences in the prevalence of resistance to the two first-line and five second-line drugs are shown in **Figures 3.2a-3.2d**. Overall, the prevalence of resistance to first-line drugs and the FQs decreased from 2012 to 2013. During this same time, the prevalence of resistance of second-line injectables increased, although these changes were not statistically significant. In Mumbai, the prevalence of resistance to first-line drugs decreased from 2012 to 2013; however the prevalence of resistance increased in all second-line drugs. These differences were not statistically significant ($p > 0.05$). During this same time, the prevalence of MDRTB decreased from 25.6% to 16.7% (p= 0.0191). In Chisinau the prevalence of resistance to all first and second-line drugs, except for amikacin decreased from 2012 to 2013; however, these differences were not statistically significant. In Port Elizabeth, the prevalence of resistance for all first and second-line drugs increased from 2012 to 2013, with statistically significant differences in the prevalence of resistance in isoniazid (17.3% to 29.5%; p=0.0450) and rifampicin (10.2% to 24.2%; p = 0.0098). Additionally the prevalence of MDRTB increased (from 2.0% to 9.5%) during this same time period (p= 0.0246).

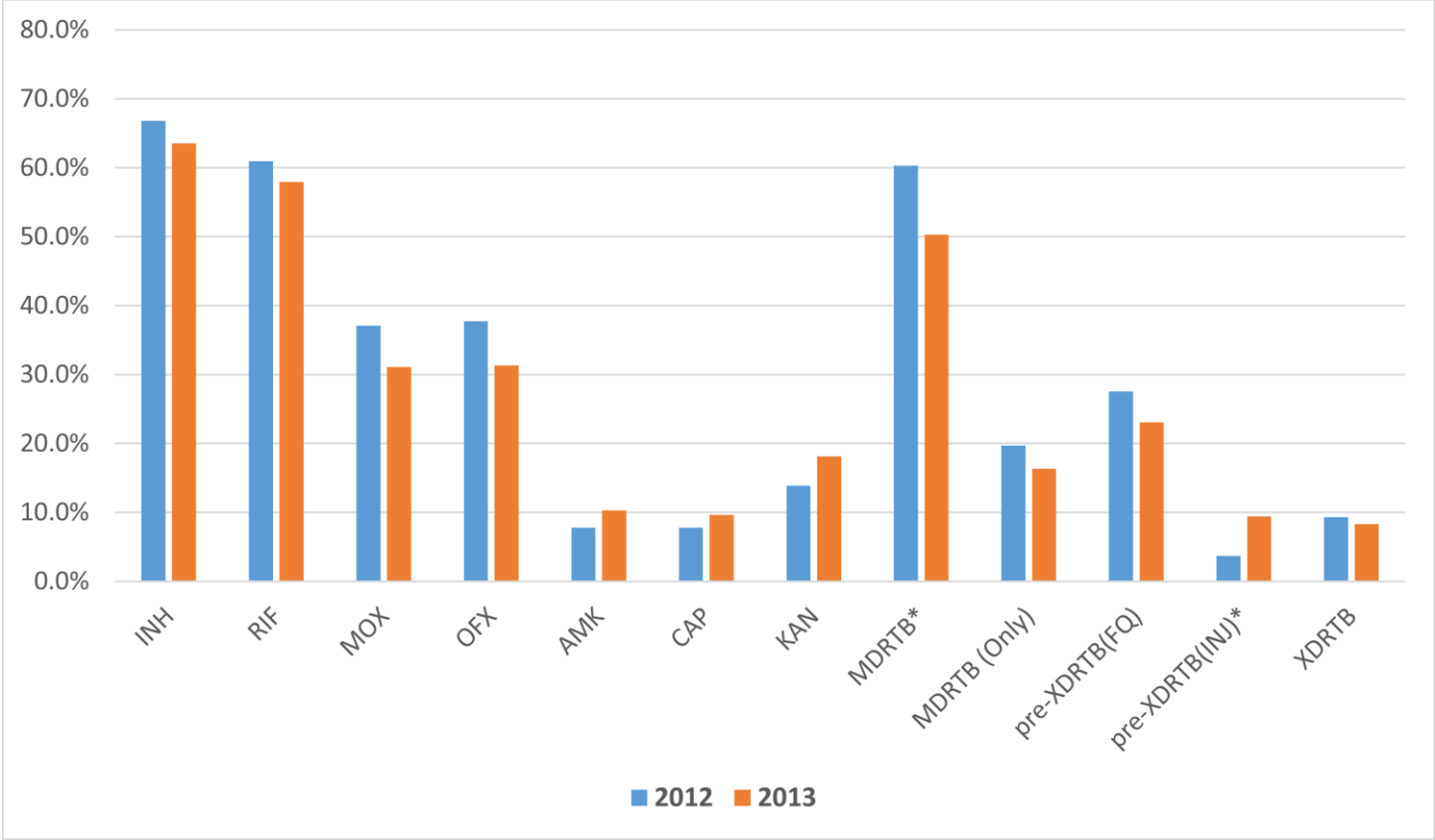


Figure 3.2a: Drug resistance rates, all sites 2012-2013

INH = isoniazid, RIF = rifampicin, MOX = moxifloxacin, OFX = ofloxacin, AMK = amikacin, CAP = capreomycin, KAN = kanamycin, MDRTB = multi drug resistant TB, pre-XDRTB (FQ) = pre- extensively drug resistant TB with FQ resistance, pre-XDRTB (INJ) = pre- extensively drug resistant TB with injectable resistance, XDRTB = extensively drug resistant TB
*Indicates significant differences between years

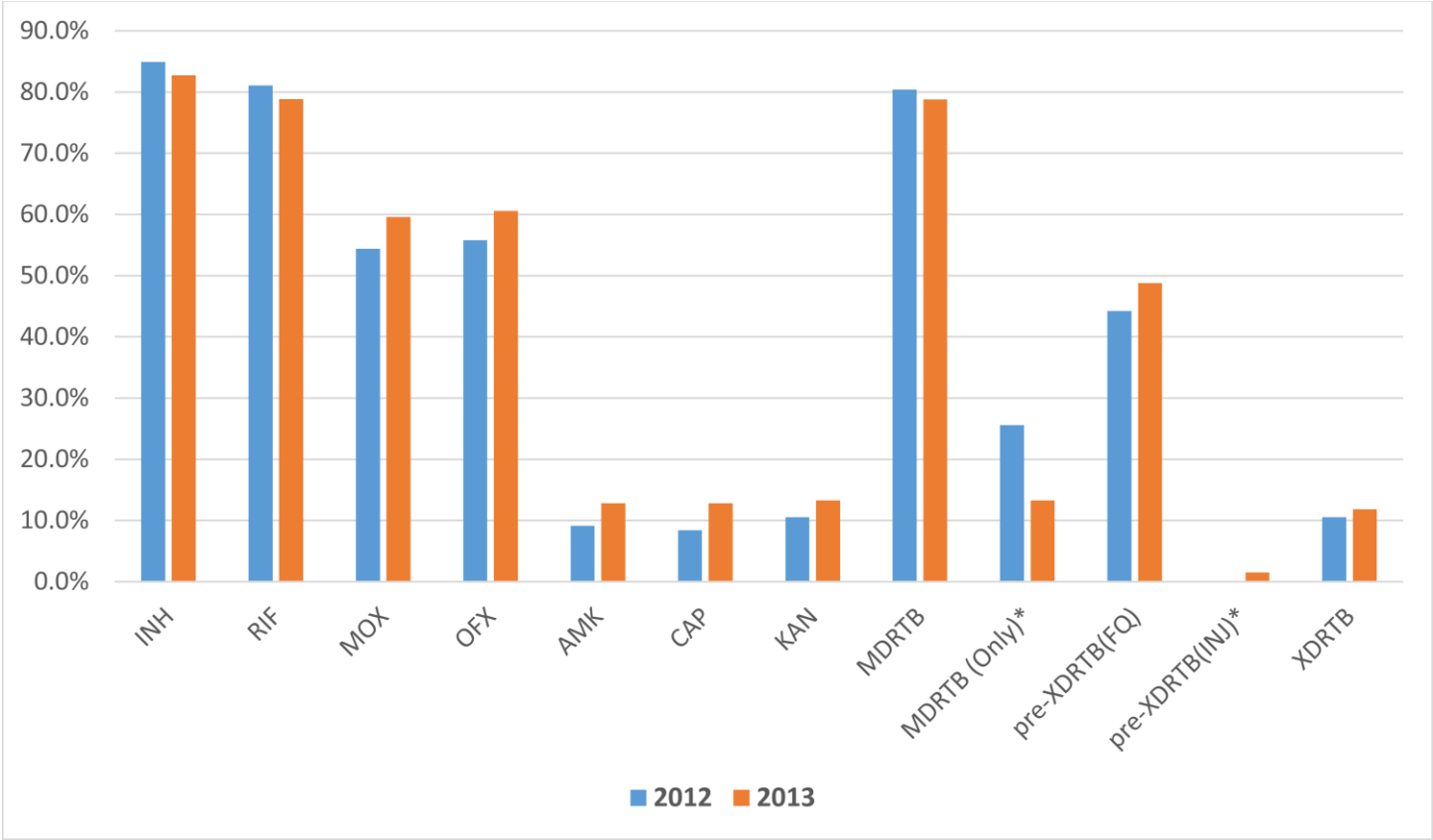


Figure 3.2b: Drug resistance rates in Mumbai, India 2012-2013

INH = isoniazid, RIF = rifampicin, MOX = moxifloxacin, OFX = ofloxacin, AMK = amikacin, CAP = capreomycin, KAN = kanamycin, MDRTB = multi drug resistant TB, pre-XDRTB (FQ) = pre- extensively drug resistant TB with FQ resistance, pre-XDRTB (INJ) = pre- extensively drug resistant TB with injectable resistance, XDRTB = extensively drug resistant TB
*Indicates significant differences between years

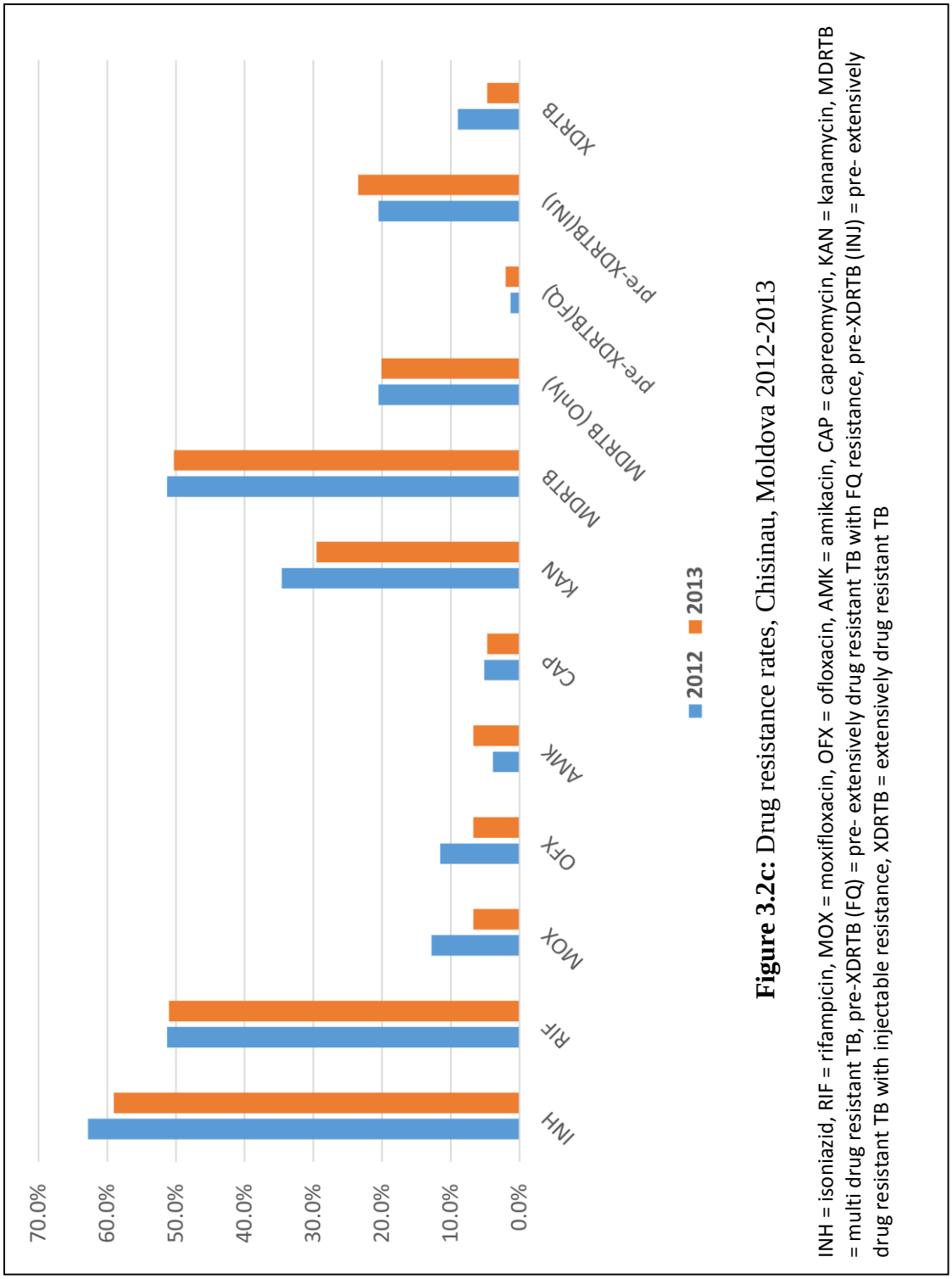


Figure 3.2c: Drug resistance rates, Chisinau, Moldova 2012-2013

INH = isoniazid, RIF = rifampicin, MOX = moxifloxacin, OFX = ofloxacin, AMK = amikacin, CAP = capreomycin, KAN = kanamycin, MDRTB = multi drug resistant TB, pre-XDRTB (FQ) = pre- extensively drug resistant TB with FQ resistance, pre-XDRTB (INJ) = pre- extensively drug resistant TB with injectable resistance, XDRTB = extensively drug resistant TB

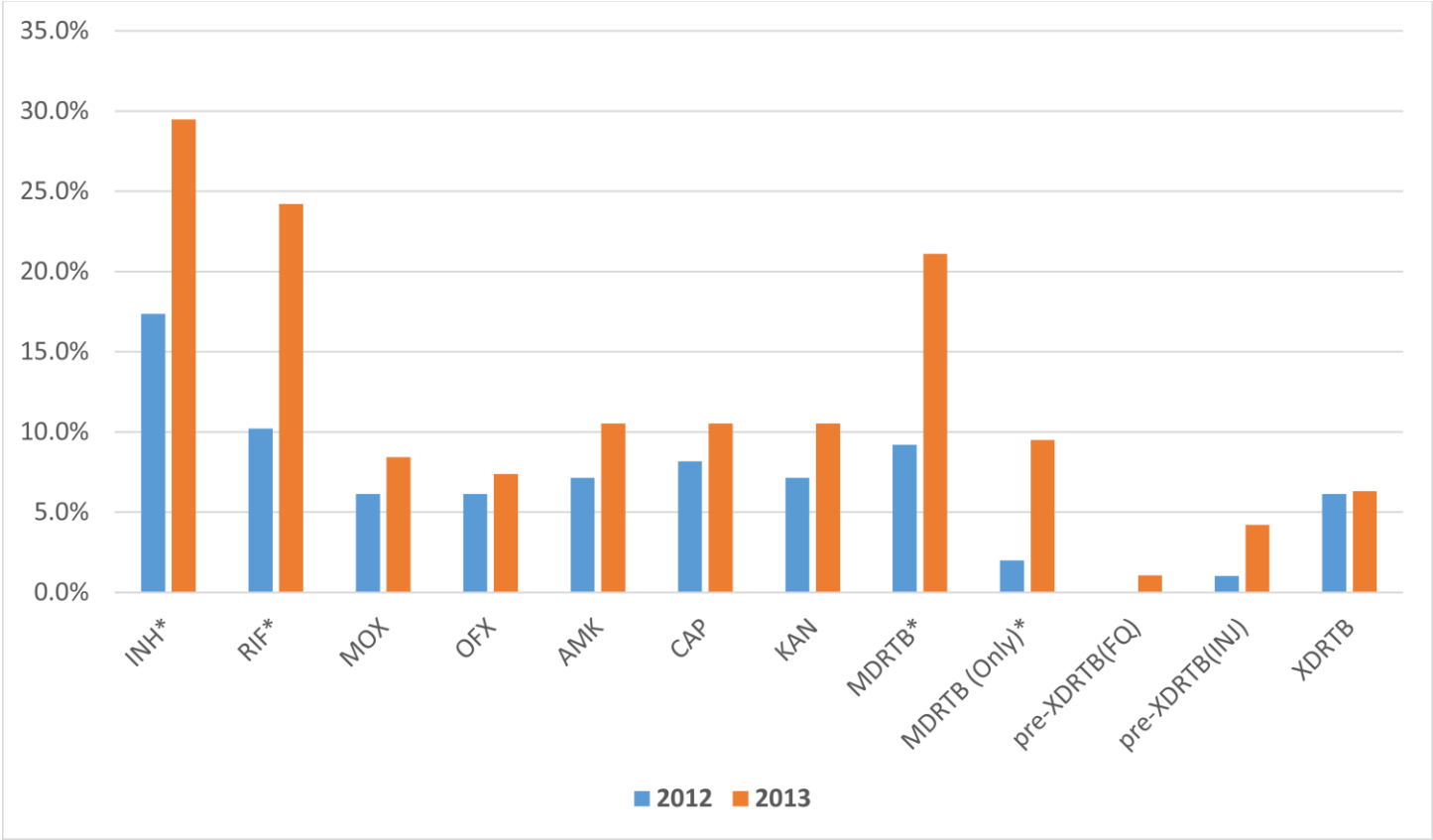


Figure 3.2d: Drug resistance rates, Port Elizabeth, South Africa 2012-2013

INH = isoniazid, RIF = rifampicin, MOX = moxifloxacin, OFX = ofloxacin, AMK = amikacin, CAP = capreomycin, KAN = kanamycin, MDRTB = multi drug resistant TB, pre-XDRTB (FQ) = pre- extensively drug resistant TB with FQ resistance, pre-XDRTB (INJ) = pre- extensively drug resistant TB with injectable resistance, XDRTB = extensively drug resistant TB
*Indicates significant differences between years

Discussion

The emergence of DRTB is a major global health issue as high rates of DRTB can impede TB control activities. India, Moldova and South Africa have been described as hotspots for DRTB. In this study, we systematically investigated the prevalence of drug resistant *Mtb* isolates from Mumbai, India; Chisinau, Moldova; and Port Elizabeth, South Africa in patients considered at risk for drug resistant TB. In analyzing the first and second-line drug resistance patterns, high rates of drug resistance to all seven drugs in all three sites was revealed. A large proportion (65.9%) of isolates showed resistance to the first-line drugs. Poor patient adherence and interrupted treatment have been shown to contribute to the emergence on MDRTB [135]. These individuals pose a challenge for the management and treatment of TB. Having a high proportion of MDRTB patients resistant to all first-line drugs places a financial burden on a nation as second-line drugs are more expensive and more toxic [149]. Overall, the first-line drugs exhibited the highest prevalence of resistance, followed by the FQs and the injectables. However, differences by site revealed a higher prevalence of injectable resistance, compared to FQ resistance, in Moldova (KAN resistance 31.3% vs MOX resistance 8.8%) and South Africa (KAN resistance 8.8% vs MOX resistance 7.3%).

High rates of MDRTB were reported from all sites. In Mumbai we found the rate of MDRTB to be 79.7%, the rate of pre-XDRTB was 46.7% and the rate of XDRTB was 11.1%. In Chisinau, we found that 51.1% of TB patients met the definition of MDRTB, 24.2% were pre-XDRTB and 6.2% were XDRTB. In Port Elizabeth, we found that 58.8% of TB patients met the definition of MDRTB, 31.8% were pre-XDRTB and 8.8% were XDRTB. The prevalence of MDRTB in our study is higher than that reported by the

WHO. The high prevalence of MDRTB in our study is also higher than that of previously published rates in Mumbai [136, 137] and Chisinau [138, 139], but not Port Elizabeth [140, 141].

We identified a large subset of patients with pre-XDRTB. Globally, the number of pre-XDRTB strains has increased. In Mumbai, the majority of pre-XDRTB cases were resistant to a FQ whereas in Chisinau the majority of pre-XDRTB cases were resistant to an injectable. The emergence of pre-XDRTB is a major concern for TB control programs and highlights the use of FQs and the injectables in the treatment of non-tubercular infections. These findings highlight the importance of implementing country-specific strategies to identify and cure patients with pre-XDRTB before they develop XDRTB [149]. Blower and Supervie conducted a modeling study and concluded that if the evolution of MDRTB to XDRTB is not slowed, a tipping point could be reached, after which the number of XDRTB cases could increase exponentially [150]. XDRTB is associated with high morbidity and mortality and requires individualized treatment to address first and second-line drug resistance accurately.

In 2013, the WHO estimated that India accounted for 20.4% of the total number of TB cases worldwide, with 2.2% (1.9-2.6%) and 15% (11-19%) of the new and retreatment cases respectively being caused by MDRTB strains; Moldova accounted for 0.07% of the total number of TB cases worldwide, with 24% (21-26%) and 62% (59-65%) of the new and retreatment cases respectively being caused by MDRTB strains and South Africa accounted for 5.1% of the total number of TB cases worldwide, with 1.8% (1.4-2.3%) and 6.7% (5.4-8.2%) of the new and retreatment cases respectively being caused by MDRTB strains. The results of our study demonstrate that MDRTB is common

in both new and previously treated TB patients enrolled in this study. The prevalence of MDRTB is alarmingly high among new TB cases: 72.6% in Mumbai; 44.1% in Chisinau; and 62.5% in Port Elizabeth and among previously treated TB patients: 81.7% in Moldova; 74.0% in Chisinau; and 13.0% in Port Elizabeth.

The prevalence of FQ resistance in Mumbai was high among MDRTB cases with 50.9% among new patients and 44.8% among previously treated patients. In Chisinau, a high prevalence of second-line injectable drugs was observed with 20.9% among new patients and 28.0% among previously treated patients. A meta-analysis of 26 studies by Falzon et al [151] reported a prevalence of FQ and SLD injectable resistance in MDRTB of 12% and 34.5% respectively. Based on the results of our study, there is concern about the increasing resistance of SLDs in MDRTB and the possible reduced efficacy of drug combinations used to treat MDRTB.

If an effective TB control program is in place, the proportion of previously treated patients with MDRTB should be low. In our study, the proportion of previously treated patients with MDRTB was 81.7% in Mumbai, 74.0% in Chisinau and 13.0% in Port Elizabeth. These results indicate that previously treated patients were more likely to harbor MDRTB than new patients. High rates of MDRTB in previously treated patients is an indicator of current treatment practices (inadequate treatment regimens or poor treatment adherence) whereas drug resistance in new patients is an indicator of disease transmission with resistant bacilli [152, 153].

Several programmatic and patient factors are responsible or contribute to the development of high drug resistance detected in these populations. First off, some of these drugs are readily available on the open market [154]. A second factor is poor

adherence; some patients may stop treatment due to the inability to pay for the costly, lengthy treatment [151]. Prescribing errors such as prescribing a FQ for a respiratory, gastrointestinal, or sexually transmitted diseases, inappropriate treatment regimen, inadequate dosage and insufficient treatment duration further contribute to multiple drug resistance [83, 84]. The inappropriate use of second-line anti-TB drugs in MDRTB patients will lead to amplification of resistance and the development of XDRTB [133, 153].

Knowledge of true drug resistance rates is essential for developing appropriate treatment strategies [142]. DRTB, especially XDRTB, is more expensive and difficult to treat. The increased information on SLD resistance reported in this study could be valuable for the development of rapid diagnostics for the timely detection of pre-XDRTB and XDRTB. Additionally this information can be used to determine effective drug combination of SLDs for the treatment of MDRTB.

In our study, similar resistance rates were reported by site, across all three sites, in the FQs and the injectables, suggesting cross-resistance. Cross-resistance among these SLDs is concerning as they have a mode of action different from that of the first-line anti-TB drugs. FQs are widely used for other infectious diseases and are even available without prescription in several countries, increasing the burden of selective pressure and compromising their efficacy in the treatment of TB [155]. Patients on SLDs often experience serious adverse events that require a change in therapy [156]. This change may further contribute to the growing problem of multiple drug resistance.

This multisite study reported varying rates of DRTB between all three sites even though the same eligibility criteria were applied to all patients during the screening

process. These differences may be due to the differences in the underlying prevalence of DRTB in these areas or due to the fact that patients from Mumbai, India and Chisinau, Moldova were recruited from hospitals whereas in Port Elizabeth, South Africa patients were recruited from one hospital and six primary health care facilities. Hospitals tend to have more serious TB cases compared to primary health care facilities. Thus the higher rates of DRTB in Mumbai and Chisinau observed in this study may be due to differences in the patient populations in which these sites recruited from.

Limitations: Our study does have a few limitations. First off, due to logistics, only the previously mentioned hospitals/clinics were included in our analysis. While a substantial number of TB patients present to these hospital/clinics, the results of this study can only be generalized to these specific hospitals/clinics; thus the results might not reflect the overall situation in each respective city and country. Additionally, these hospitals may have higher inclusions rates of serious TB patients than other hospitals in the region which may lead to the overestimation of DRTB. For example the Mumbai site is not representative of the city but rather the data is from one tertiary care center with a referral bias towards non-responders. A potential source of misclassification bias among new and previously treated patients might have occurred if some patients registered as new patients when in fact they may have actually have had TB treatment in the past. Lastly, as the inclusion criteria for our study included suspected DRTB patients who were sputum smear-positive, the results of our study reflect the prevalence of DRTB in a population suspected of having DRTB and not the general public.

Conclusions

This study reported the prevalence of resistance to seven major anti-TB drugs in Mumbai, India, Chisinau, Moldova and Port Elizabeth, South Africa. Our study showed that the high prevalence of drug resistance continues to be a major challenge for TB control as the transmission of DRTB is extensive and widespread. The prevalence of MDRTB remains high and the presence of pre-XDRTB and XDRTB will impose new challenges in the global effort to control TB. Continuous surveillance is needed to identify DRTB, especially among patients previously treated for TB. It is important to improve early diagnosis of MDRTB and to provide effective treatment to all MDRTB patients in order to prevent the development of additional drug resistance in these high-risk populations.

Acknowledgements

The specimens, data, and funding for this project were provided by the Global Consortium for Drug-Resistant TB Diagnostics (GCDD; http://gcdd.ucsd.edu), funded by a grant (U01-AI082229) from the National Institute of Allergy and Infectious Diseases (NIAID), US National Institutes of Health (NIH), Bethesda, MD, USA. TCR was also supported by NIAID, grant # R01AI111435 and grant #R01AI105185. The authors thank the clinical and laboratory staff at each of the study sites for their assistance in acquiring and preparing *Mtb* isolates.

Chapter 3, is currently being prepared for submission for publication of the material in The International Journal of Tuberculosis and Lung Disease. Avalos, E**,** Garfein, R, Catanzaro, D, Ganiats, T, Brodine, S, Alcaraz, J, Eisenach, K, Ajbani, K, Udwadia, Z, Crudu, V, Victor, T, Rodwell, T. *Prevalence of drug resistant tuberculosis*

in Mumbai, India, Chisinau, Moldova and Port Elizabeth, South Africa, 2012-2013. The dissertation author was the primary investigator and author of this paper.

CHAPTER 4 CHARACTERISTICS OF MULTI AND EXTENSIVELY DRUG RESISTANT TUBERCULOSIS IN A MULTISITE STUDY

Elisea Avalos[1], Richard Garfein[2], Donald Catanzaro[3], Theodore G. Ganiats[1], Stephanie Brodine[4], John Alcaraz[4], Kathleen Eisenach[5], Kanchan Ajbani[6], Zarir Udwadia[6], Valeriu Crudu[7], Thomas C. Victor[8] and Timothy Rodwell[2]

[1]Department of Family and Preventive Medicine, UCSD, [2]Division of Global Public Health, UCSD, [3]Department of Biological Sciences, University of Arkansas, [4]Graduate School of Public Health, SDSU, [5]Department of Pathology, University of Arkansas, [6]P. D. Hinduja Hospital & Medical Research Centre, Department of Microbiology, [7]Microbiology and Morphology Laboratory, Institute of Phthisiopneumology, [8]Department of Biomedical Sciences, Stellenbosch University

Abstract

Objectives: To analyze the clinical and epidemiologic characteristics of multi and extensively drug resistant tuberculosis (M/XDRTB). *Mycobacterium tuberculosis* (*Mtb)* isolates from Mumbai, India; Chisinau, Moldova; and Port Elizabeth, South Africa were selected due to the high documented risk for drug resistant tuberculosis (DRTB) and the ethnic diversity of these regions. Methods: A cross-sectional study was conducted from April 2012 to August 2013. *Mtb* strains isolated from patients were subjected to drug susceptibility testing (DST). Cases were defined as patients with M/XDRTB. Controls were patients selected from the cohort during the same period who were non-MDRTB. Results: Of the 1,128 patients enrolled in the study, 838 patients met the inclusion criteria, the overall prevalence of multidrug-resistant tuberculosis (MDRTB) and

extensively drug resistant tuberculosis (XDRTB) were 63.6% (n = 533) (61.3% of newly diagnosed patients and 64.6% of previously treated cases) and 9.5% (n = 80) (5.4% of newly diagnosed patients and 11.4% of previously treated cases), respectively. Multivariable logistic regression analysis showed that those less than 25 years of age (OR 1.8, 95%CI 1.0 to 3.1), study site (Mumbai [OR 33.1, 95% CI 18.8 to 58.3] and Chisinau [OR 13.0, 95%CI 6.8 to 24.6]), higher education (OR 2.4, 95%CI 1.4 to 4.0), ever been hospitalized (OR 1.9, 95%CI 1.2 to 2.9) and previously treated for TB (OR 1.7, 95% CI 1.1 to 2.8) were associated with having M/XDRTB. An interaction was also observed between study site and treatment for a prior episode of TB; however, the multiplicativity and additivity between these factors were not significant. Conclusions: The results of this study reflect the growing drug resistance situation in Mumbai, Chisinau and Port Elizabeth. Thus, the timely detection of drug resistance is of great importance to optimize treatment and to direct infection control measures to prevent M/XDRTB transmission.

Keywords: Multidrug-resistant TB (MDRTB), Extensively drug resistant TB (XDRTB), epidemiology

Clinical Trials Registration Number: ClinicalTrials.gov under number NCT02170441.

Introduction

Drug-resistant tuberculosis (DRTB) has emerged as a serious threat to global tuberculosis (TB) control [157]. According to the World Health Organization (WHO) Global Tuberculosis Report, in 2013 roughly 9 million people developed TB and 1.5 million died from the disease [133]. Worldwide, the proportion of new cases with multidrug-resistant TB (MDRTB defined as *Mycobacterium tuberculosis* (*Mtb*) resistant

to isoniazid (INH) and rifampin (RIF)) was 3.5%; 20.5% of previously treated TB cases were estimated to have had MDRTB. Combined, India, China, the Russian Federation and South Africa have almost 60% of the world's cases of MDRTB [133]. It is estimated that 9.0% of patients with MDRTB have extensively drug resistant TB (XDRTB) [133]. XDRTB is defined as TB with resistance to at least INH and RIF plus one fluoroquinolone (FQ) (e.g. moxifloxacin (MOX), ofloxacin (OFX)) and one of three injectable second-line drugs (SLDs) (capreomycin (CAP), kanamycin (KAN), and amikacin (AMK)). Pre- XDRTB is defined as resistance to INH and RIF and either a FQ or an injectable, but not both [157, 158].

The increase in the incidence of DRTB, specifically M/XDRTB presents challenges to the global efforts to eradicate TB [134]. XDRTB is more expensive and difficult to treat than MDRTB. Compared to first-line drugs, SLDs are more expensive, less effective, more toxic, must be taken for longer duration and have higher rates of treatment failure and death [149]. Given the lack of accurate, rapid drug susceptibility testing (DST) for MDRTB and XDRTB [134], the epidemiology of DRTB in high-burden settings has been limited.

Several studies [136-141] have described the prevalence of and risk factors associated with DRTB in a number of different countries. Additionally, the WHO has emphasized the importance of collecting surveillance data on the proportion of TB cases that are MDRTB or XDRTB. However, true rates of DRTB remain unknown throughout the world, especially in regions where the burden of TB is high.

The Global Consortium for Drug-resistant TB Diagnostics (GCDD) was established in 2008 to characterize the genetic basis of drug resistance and evaluate

molecular and microbiological methods to detect DRTB quickly and efficiently. The objectives of this study were to analyze the clinical and epidemiologic characteristics of multi and extensively drug resistant tuberculosis (M/XDRTB) to estimate the prevalence of M/XDRTB and to identify factors that are linked to M/XDRTB. Patients from Mumbai, India; Chisinau, Moldova; and Port Elizabeth, South Africa were selected due to the high documented risk for DRTB and the ethnic diversity of these regions.

Methods

Study Population: To evaluate the performance of rapid drug susceptibility tests among patients with suspected, but not confirmed DRTB, we enrolled previously diagnosed TB cases into a longitudinal cohort study conducted by the GCDD. The study methods have been described elsewhere [72] and the methods for collecting baseline data that were used for the current study are briefly described here. To ensure generalizability of study findings, TB patients were prospectively enrolled in three countries selected for their high prevalence of drug resistant TB and proven laboratory capacity. They included: (i) The P.D. Hinduja National Hospital (PD-HNH) and Medical Research Centre (MRC) a tertiary care center in central Mumbai, (ii) the Phthisiopneumology Institute (PPI) in Chisinau, Moldova a scientific research and medical consultation and training center, and (iii) in Port Elizabeth, South Africa patients were enrolled at one of six primary health care facilities and one regional hospital.

Patients at least 5 years of age, who were acid-fast bacilli sputum smear-positive, 1+ or greater (within previous 14 days), positive on GeneXpert, or with high suspicion of active TB **and**: previously received treatment for a prior TB episode **or** were failing TB treatment **or** had close contact with a known DRTB case **or** were newly diagnosed with

MDRTB **or** were previously diagnosed with MDRTB and failed TB treatment, were recruited from each of the study clinics, between April 2012 and August 2013. The eligibility criteria were designed to identify patients at increased risk for DRTB. Following screening and informed consent, eligible patients were asked to provide sputum specimens and complete a baseline interview.

Data Collection: During the study period, a total of 1,128 patients were enrolled and sputum samples collected from the three different sites mentioned above. Clinical and epidemiological characteristics were analyzed to identify possible associations with M/XDRTB. Patient information was collected via patient interviews and chart reviews (case report forms can be found in **Appendix D**). Clinical and epidemiological characteristics collected included information on socio-demographics (e.g. age, gender, marital status), TB history (e.g., prior TB diagnosis, treatment for a prior TB episode), TB contact history, medical conditions associated with TB (e.g., HIV status, diabetes) and TB risk factors (e.g., substance abuse, homelessness, incarceration). The patient's medical record was reviewed to obtain data on TB signs and symptoms, chest x-ray results, HIV test results, CD4 cell counts, HIV viral load, antiretroviral therapy and TB treatment history. Height and weight were also measured to compute body mass index. Patient treatment history was assigned according to WHO standards. A new patient was defined as a patient who had never had treatment for TB or who had taken anti-TB drugs for less than 1 month. A previously treated patient was defined as a patient who had ever received treatment for TB for more than 1 month [144].

Determination of Drug Resistance: The standard protocol for DST of INH and RIF on the Mycobacterial Growth Indicator Tube (MGIT) 960 (BD Diagnostic Systems,

Franklin Lakes, NJ, USA) was followed according to the manufacturer's instructions [145]. The following critical concentrations were used: 0.1 μg/ml for INH and 1.0 μg/ml for RIF [145]. DST for SLDs was performed by using validated critical concentrations of in-house (locally prepared by each site) drug solutions compatible with the WHO recommendations: 2.0 μg/ml for OFX, 0.25 μg/ml for MOX, 1.0 μg/ml for AMK, and 2.0 μg/ml for CAP [146]. As there were no published WHO recommended critical concentrations for KAN DST by MGIT 960 at the time of the study, we used 2.5 μg/ml based on the literature [147, 148]. The results of the MGIT 960 were used to categorize participants by type of drug resistance. An M/XDRTB case was defined as having either MDRTB, pre-XDRTB or XDRTB. MDRTB was defined as having resistance to INH and RIF. Pre- XDRTB was defined as MDRTB with resistance to either a FQ or a second-line injectable, but not both. XDRTB was defined as MDRTB with resistance to a FQ and an injectable. As MOX and OFX resistance was similar, we combined these two drugs and created a single variable for FQ resistance.

Statistical analysis: As the objective of this study was to analyze the clinical and epidemiologic characteristics associated with M/XDRTB, we used the χ^2 test or Fisher's exact test to determine if the characteristics were associated with M/XDRTB. We investigated the association of each covariate with the outcome of interest by using regression. We first performed univariate analysis to determine factors related to M/XDRTB. Associations between selected factors were estimated by computing odds ratios (ORs) and their 95% confidence interval (CI). A multiple logistic regression model was used to estimate the effect of each covariate on the odds of M/XDRTB versus non-MDRTB, while simultaneously adjusting for all other variables in the model. A p-value

of < 0.05 was considered as statistically significant. All statistical analyses were carried out using SAS version 9.3 (SAS Institute Inc, Cary, NC).

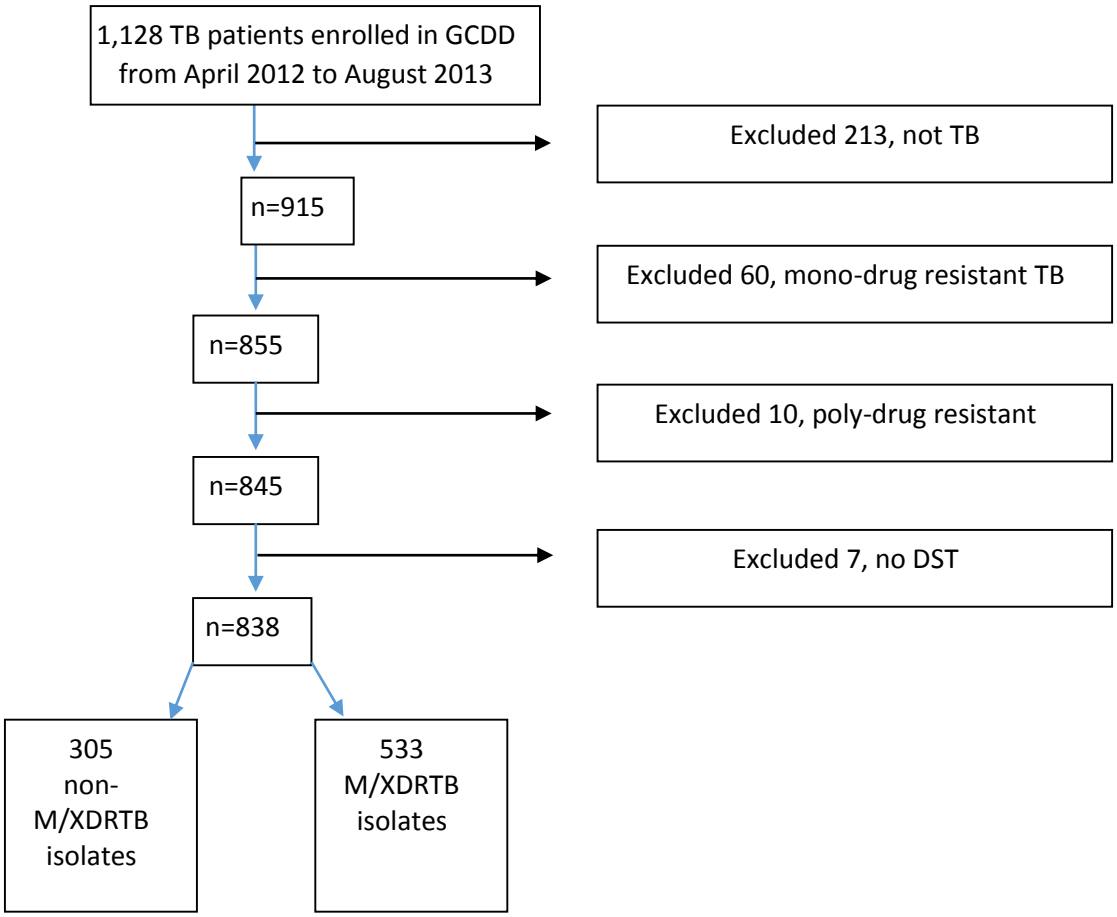
Since characteristics of M/XDRTB vary by location, we examined the interaction between study site and each significant correlate of M/XDRTB. Multiplicative and additive models of interaction were used. To test for multiplicative interactions, ORs were first examined across different strata and then an interaction term was included in a logistic regression model. A p-value below 0.05 was used to indicate a significant interaction. In addition to this, a multiple logistic regression model was used to evaluate departure from additivity. To assess the deviation from the additive model of no interaction between variables, three measures of additive interaction were used: relative excess risk due to interaction (RERI), attributable proportion (AP) and synergy index (SI). We considered RERI and AP to be equal to 0 and SI equal to 1 to indicate the absence of additive interactions [159]. Conversely, additive interaction is considered present if RERI and AP do not equal 0 and SI exceeds unity. Furthermore, if RERI is greater than 0, this denotes a synergetic interaction, which implies that the combined action between two exposures in an additive model is greater than the sum of the individual effects. The SI and its 95% CI, as proposed by Rothman, was calculated [160]; $SI = (OR_{11} - 1)/(OR_{01} + OR_{10} - 2)$. OR_{10} and OR_{01} mean the OR for the presence of each factor in the absence of the other, whereas OR_{11} means the OR of the joint effect of two factors.

Ethical considerations: This study was approved by the Institutional Review Board (IRB) at the University of California, San Diego (UCSD) and at each enrolling site: P.D. Hinduja National Hospital and Medical Research Centre, IRB Project Number.

507-09-CR; Ministry of Health Care of the Republic of Moldova, Institution of Public Health Phthisiopneumology Institute, Ethics Committee of IMSP Phthisiopneumology Institute (no applicable reference number); and Universiteit-Stellenbosch University Health Research Ethics Committee Tygerberg, South Africa, Ethics Reference Number N10/08/261. Written informed consent was obtained from each patient prior to study enrollment.

Results

Between April 2012 and August 2013, a total of 1,128 patients with clinical suspicion of DRTB were enrolled in the study. A total of 838 patients were included in the present analyses (**Figure 4.1**).



TB = tuberculosis; GCDD = Global Consortium for Drug-resistant TB Diagnostics; DST=drug susceptibility testing; X/MDRTB = multi and extensively drug resistant TB

Figure 4.1: Flow chart for selection of patients included in the study

Among these patients, 305 (36.4%) were non-M/XDRTB and 533 (63.6%) had M/XDRTB. Among those with M/XDRTB, 164 (30.8%) had MDRTB only, 230 (43.2%) had pre-XDRTB with FQ resistance, 59 (11.1%) were pre-XDRTB with injectable resistance and 80 (15.0%) were XDRTB. The average age of participants was 34.7 years with 529 (63%) of the cohort being male. **Table 4.1** shows the distribution of the clinical and epidemiological data by drug resistance.

Table 4.1: Clinical and demographic characteristics of patients (N=838)

Characteristic	Total (N=838) (%)	Non-MDRTB (N=305) (%)	M/XDRTB (N=533) (%)	OR (95%CI)
Age (yrs)				
<25	225 (26.8)	43 (14.1)	182 (34.1)	3.5 (2.3 to 5.3)
25-34	231 (27.6)	94 (30.8)	137 (25.7)	1.2 (0.8 to 1.8)
35-44	165 (19.7)	70 (23.0)	95 (17.8)	1.1 (0.7 to 1.7)
45+	217 (25.9)	98 (32.1)	119 (22.3)	ref
BMI				
<18	398 (47.5)	131 (43.0)	267 (50.1)	1.3 (1.0 to 1.8)
18+	440 (52.5)	174 (57.0)	266 (49.9)	ref
Study site				
Mumbai, India	464 (55.4)	75 (24.6)	389 (73.0)	25.6 (16.0 to 40.9)
Chisinau, Moldova	202 (24.1)	87 (28.5)	115 (21.6)	6.5 (4.0 to 10.6)
Port Elizabeth, South Africa	172 (20.5)	143 (46.9)	29 (5.4)	ref
Gender				
Male	529 (63.1)	218 (71.5)	311 (58.3)	ref
Female	309 (36.9)	87 (28.5)	222 (41.7)	1.8 (1.3 to 2.4)
Marital status				
Single	401 (47.9)	160 (52.5)	241 (45.2)	ref
Married/living with partner	381 (45.5)	120 (39.3)	261 (49.0)	1.4 (1.1 to 1.9)
Divorced/widowed	56 (6.7)	25 (8.2)	31 (5.8)	0.8 (0.5 to 1.5)
# of people currently living with				
Less than or equal to 4	437 (52.1)	175 (57.4)	262 (49.2)	ref
Greater than 5	401 (47.9)	130 (42.6)	271 (50.8)	1.4 (1.1 to 1.9)
# of rooms for sleeping				
Less than or equal to 2	639 (76.3)	191 (62.6)	448 (84.1)	3.2 (2.3 to 4.4)
Greater than 2	199 (23.7)	114 (37.4)	85 (15.9)	ref
Own home				
No	337 (40.2)	160 (52.5)	177 (33.2)	ref
Yes	498 (59.4)	143 (46.9)	355 (66.6)	2.2 (1.7 to 3.0)
Refuse to answer	3 (0.4)	2 (0.7)	1 (0.2)	
Education level				
Primary-secondary	690 (82.3)	279 (91.5)	411 (77.1)	ref
University/Higher	148 (17.7)	26 (8.5)	122 (22.9)	3.2 (2.0 to 5.0)
Income source				
Full-time	284 (33.9)	69 (22.6)	215 (40.3)	1.7 (1.2 to 2.5)
Part-time	189 (22.6)	107 (35.1)	82 (15.4)	0.4 (0.3 to 0.6)
Other	365 (43.6)	129 (42.3)	236 (44.3)	ref
Income (US$)				
<$100	432 (51.6)	193 (63.3)	239 (44.8)	ref
>$100	406 (48.4)	112 (36.7)	294 (55.2)	2.2 (1.7 to 3.0)

Table 4.1: Clinical and demographic characteristics of patients (N=838), Continued

Characteristic	Total (N=838) (%)	Non-MDRTB (N=305) (%)	M/XDRTB (N=533) (%)	OR (95%CI)
Smoking				
Cigarettes/Bidis				
No	543 (64.8)	134 (43.9)	409 (76.7)	4.3 (3.2 to 5.8)
Yes	293 (35.0)	171 (56.1)	122 (22.9)	ref
Refuse to answer	2 (0.2)	0 (0.0)	2 (0.4)	
Marijuana				
No	758 (90.5)	243 (79.7)	515 (96.6)	8.6 (4.8 to 15.5)
Yes	76 (9.1)	61 (20.0)	15 (2.8)	ref
Refuse to answer	4 (0.5)	1 (0.3)	3 (0.6)	
Drank alcohol in the past 3 months				
No	687 (82.0)	215 (70.5)	472 (88.6)	3.2 (2.3 to 4.7)
Yes	151 (18.0)	90 (29.5)	61 (11.4)	ref
Ever jailed				
No	738 (88.1)	228 (74.8)	510 (95.7)	7.3 (4.5 to 11.9)
Yes	98 (11.7)	75 (24.6)	23 (4.3)	ref
Refuse to answer	2 (0.2)	2 (0.7)	0 (0.0)	
Ever hospitalized				
No	522 (62.3)	166 (54.4)	356 (66.8)	1.7 (1.2 to 2.2)
Yes	314 (37.5)	137 (44.9)	177 (33.2)	ref
Refuse to answer	2 (0.2)	2 (0.7)	0 (0.0)	
Seen a doctor in past 2yrs other than TB				
No	699 (83.4)	234 (76.7)	465 (87.2)	2.0 (1.4 to 2.9)
Yes	137 (16.3)	69 (22.6)	68 (12.8)	ref
Refuse to answer	2 (0.2)	2 (0.7)	0 (0.0)	
Close contact with known TB case				
No	280 (33.4)	66 (21.6)	214 (40.2)	3.2 (2.3 to 4.5)
Yes	351 (41.9)	174 (57.0)	177 (33.2)	ref
Unknown	207 (24.7)	65 (21.3)	142 (26.6)	
Previously treated for TB				
Yes	577 (68.9)	204 (66.9)	373 (70.0)	1.2 (0.9 to 1.6)
No	261 (31.1)	101 (33.1)	160 (30.0)	ref
Smear positive				
No	45 (5.4)	25 (8.2)	20 (3.8)	ref
Yes	769 (91.8)	273 (89.5)	496 (93.1)	2.3 (1.2 to 4.2)
Unknown	24 (2.9)	7 (2.3)	17 (3.2)	
Cough				
No	110 (13.1)	40 (13.1)	70 (13.1)	ref
Yes	728 (86.9)	265 (86.9)	463 (86.9)	1.0 (0.7 to 1.5)
Fever				
No	290 (34.6)	139 (45.6)	151 (28.3)	ref
Yes	544 (64.9)	165 (54.1)	379 (71.1)	2.1 (1.6 to 2.8)
Unknown	4 (0.5)	1 (0.3)	3 (0.6)	

Table 4.1: Clinical and demographic characteristics of patients (N=838), Continued

Characteristic	Total (N=838) (%)	Non-MDRTB (N=305) (%)	M/XDRTB (N=533) (%)	OR (95%CI)
Night sweats				
No	332 (39.6)	116 (38.0)	216 (40.5)	ref
Yes	503 (60.0)	188 (61.6)	315 (59.1)	0.9 (0.7 to 1.2)
Unknown	3 (0.4)	1 (0.3)	2 (0.4)	
Unintentional weight loss				
No	246 (29.4)	104 (34.1)	142 (26.6)	ref
Yes	584 (69.7)	194 (63.6)	390 (73.2)	1.5 (1.1 to 2.0)
Unknown	8 (1.0)	7 (2.3)	1 (0.2)	
Hemoptysis				
No	762 (90.9)	278 (91.1)	484 (90.8)	ref
Yes	74 (8.8)	25 (8.2)	49 (9.2)	1.1 (0.7 to 1.9)
Unknown	2 (0.2)	2 (0.7)	0 (0.0)	
Diabetes				
No	776 (92.6)	289 (94.8)	487 (91.4)	ref
Yes	46 (5.5)	9 (3.0)	37 (6.9)	2.4 (1.2 to 5.1)
Unknown	16 (1.9)	7 (2.3)	9 (1.7)	
HIV status				
Negative	491 (58.6)	169 (55.4)	322 (60.4)	5.3 (3.3 to 8.6)
Positive	98 (11.7)	72 (23.6)	26 (4.9)	ref
Pending/Not Tested	249 (29.7)	64 (21.0)	185 (34.7)	

***Due to rounding percents may not add up to 100%**

BMI = Body mass index

HIV = Human Immunodeficiency Virus

M/XDRTB = Multi and extensively drug resistant tuberculosis

TB = Tuberculosis

CI = Confidence interval

OR = Odds ratio

Ref=reference category

[a]p-value of chi-square comparing those with M/XDRTB vs. non-MDRTB

Second-line drug resistance: Of the 838 people included in our analysis, 577 (68.9%) had been previously treated for TB. Statistically significant differences were observed between new and previously treated TB cases regarding drug resistance to all SLDs except KAN. Statistically significant differences were not observed between new and previously treated TB cases regarding MDRTB (P=0.352); however statistically significant differences were observed in those with XDRTB (P=0.006) (**Figure 4.2**). As there were differences between study sites regarding drug resistance, we categorized resistance to SLDs by site (**Table 4.2**). In Mumbai, previously treated patients had a higher prevalence of SLD resistance compared to those newly diagnosed with TB across all drugs: AMK (P = 0.0108), CAP (P= 0.0148), KAN (P=0.0134) and FQ (P = 0.9039). In Chisinau, previously treated patients had a higher prevalence of SLD resistance than those previously treated for TB across all drugs tested: AMK (P = 0.0653), CAP (P= 0.4406), KAN (P <0.0074) and FQ (P <0.0001). In Port Elizabeth, *newly* diagnosed patients had a higher prevalence of SLD resistance compared to those previously treated for TB across all drugs tested: AMK (P <0.0001), CAP (P <0.0001), KAN (P <0.0001) and FQ (P <0.0001).

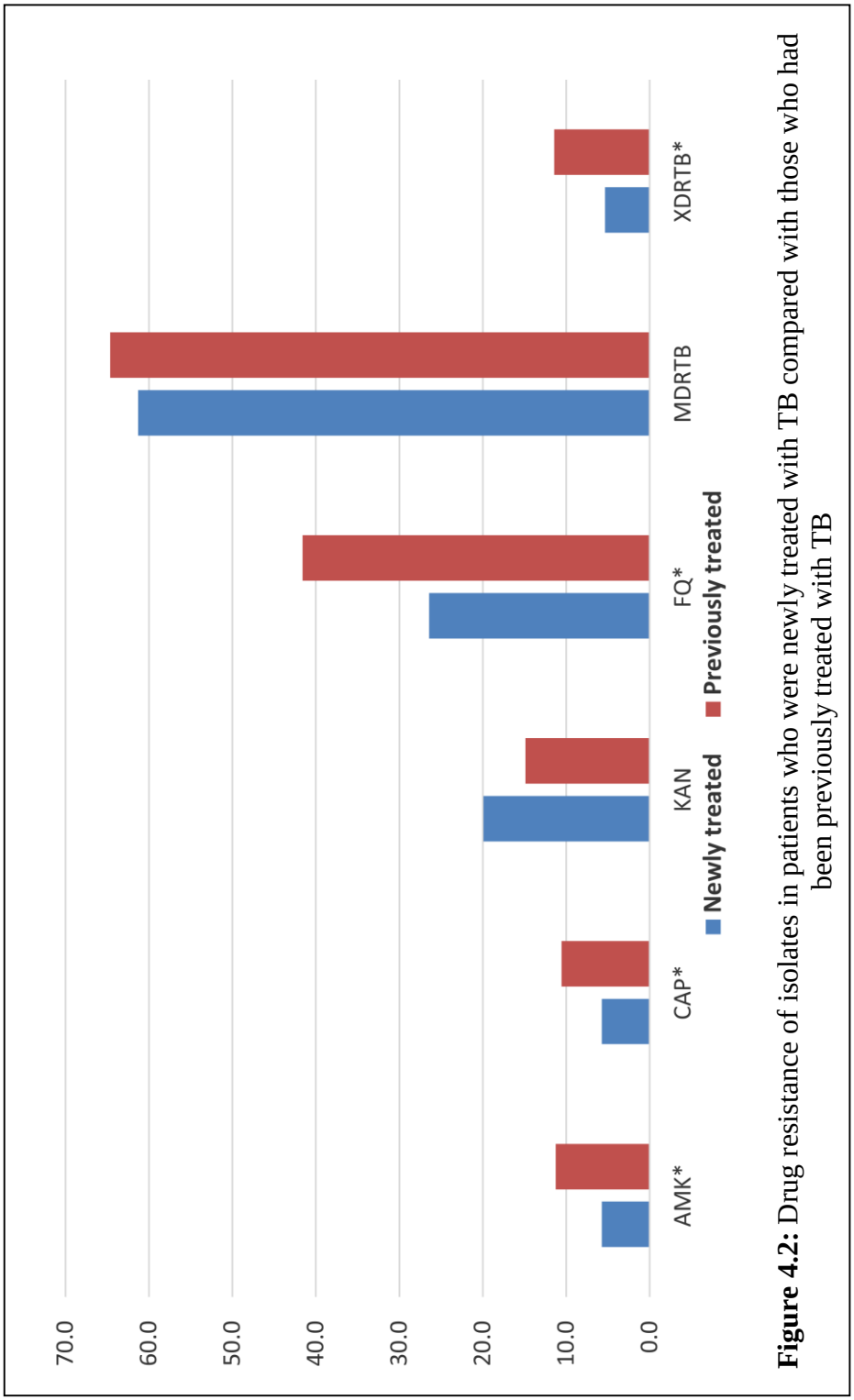


Figure 4.2: Drug resistance of isolates in patients who were newly treated with TB compared with those who had been previously treated with TB

Table 4.2: Drug resistance profile of second-line drugs in new and previously treated patients by study site

Drug	Previously treated AMK			Newly treated AMK			
Study Site	R	S	% Resistant	R	S	% Resistant	p-value*
Mumbai, India	48	317	13.2%	4	95	4.0%	0.0108
Chisinau, Moldova	5	41	10.9%	6	150	3.9%	0.0653
Port Elizabeth, South Africa	12	154	7.2%	5	1	83.3%	<0.0001
Total	**65**	**512**		**15**	**246**		

Drug	Previously treated CAP			Newly treated CAP			
Study Site	R	S	% Resistant	R	S	% Resistant	p-value*
Mumbai, India	46	319	12.6%	4	95	4.0%	0.0148
Chisinau, Moldova	3	43	6.5%	6	150	3.9%	0.4406
Port Elizabeth, South Africa	12	154	7.2%	5	1	83.3%	<0.0001
Total	**61**	**516**		**15**	**246**		

Drug	Previously treated KAN			Newly treated KAN			
Study Site	R	S	% Resistant	R	S	% Resistant	p-value*
Mumbai, India	52	313	14.3%	5	94	5.1%	0.0134
Chisinau, Moldova	22	24	47.8%	42	114	26.9%	0.0074
Port Elizabeth, South Africa	12	154	7.2%	5	1	83.3%	<0.0001
Total	**86**	**491**		**52**	**209**		

Drug	Previously treated FQ			Newly treated FQ			
Study Site	R	S	% Resistant	R	S	% Resistant	p-value*
Mumbai, India	220	145	60.3%	59	40	59.6%	0.9039
Chisinau, Moldova	11	35	23.9%	7	149	4.5%	<0.0001
Port Elizabeth, South Africa	10	156	6.0%	3	3	50.0%	<0.0001
Total	**241**	**336**		**69**	**192**		

R = resistant; S = susceptible; AMK = amikacin, CAP = capreomycin, KAN = kanamycin, FQ= fluoroquinolones

*p-value represents difference in prevalence in previously treated TB patients compared to newly treated TB patients

Patients previously treated for TB: Of the 838 people included in our analysis, 577 (68.9%) had been previously treated for TB. The median age of these subjects was 27 years. This patient population had significant prior TB treatment history. The median number of previous treatment episodes was 2 (range, 1–10) and the median number of drugs resistant to at baseline was 2 (range, 0-7). Overall, 309 (53.6%) of the previously treated patients were currently failing TB treatment, with 166 (28.8%) failing MDRTB treatment. Of the 577 previously treated patients, 169 (29.3%) reported having previously received a FQ and 123 (21.3%) had previously received an injectable. On the basis of previous treatment category, 290 subjects (50.3%) were classified as "relapse", 67 (11.6%) were classified as "treatment after default" and 209 (36.2%) were classified as "treatment after failure" (**Table 4.3**).

Table 4.3: Treatment histories among 577 previously treated subjects

	Previously treated (n=577)	
Age at first diagnosis	N = 438	median 27 years (range 1-70)
Number of previous treatments	N = 438	median 2 treatments (range 1-10)
Number of drugs resistant to at baseline	N = 577	median 2 drugs (range 0-7)
Failing TB treatment	N = 577	309 (53.6%)
Failing MDRTB treatment	N = 577	166 (28.8%)
Exposure to fluoroquinolones	N = 577	169 (29.3%)
Exposure to injectable	N = 577	123 (21.3%)
MDRTB	N = 577	373 (64.6%)
XDRTB	N = 577	66 (11.4%)
Category	N = 577	
Relapse		290 (50.3%)
Treatment after default		67 (11.6%)
Treatment after failure		209 (36.2%)
Other		9 (1.6%)
Unknown		2 (0.4%)

MDRTB = Multidrug resistant tuberculosis
TB = Tuberculosis
XDRTB = extensively drug resistant tuberculosis

Factors associated with M/XDRTB: Results of univariate analysis of correlates of drug resistance are shown in **Table 4.1**. Drug resistance was associated with young age, low BMI, study site, female gender, being married/living with partner, currently living with five people or more, using two rooms or less for sleeping, owning a home, higher education, having a full-time job, averaging over $100 per month (US$) in income, being a non-cigarette smoker, not having consumed alcohol in the past three months, no prior jail history, no prior hospitalizations, not having seen a doctor in the past two years other than for TB, no close contact with a known TB case, smear positive status, having a fever, unintentional weight loss, having diabetes and HIV negative status (all p-values <0.05).

Multivariable logistic regression analysis was performed to identify factors independently associated with M/XDRTB. The following covariates were included in the final model: age, study site, gender, education level, ever hospitalized, previously treated for TB and the interaction between study site and previous TB treatment (**Table 4.4**). Those less than 25 years of age, compared to those 45 years of age and older were twice (OR 1.8, 95%CI 1.0 to 3.1) as likely to have M/XDRTB. Individuals in Mumbai, India, compared to those in Port Elizabeth, South Africa, were 33 (OR 33.1, 95% CI 18.8 to 58.3) times more likely to have M/XDRTB. Individuals in Chisinau, Moldova, compared to those in Port Elizabeth, South Africa, were 13 (OR 13.0, 95%CI 6.8 to 24.6) times more likely to have M/XDRTB. Compared to those with primary/secondary education, those with higher education were 2.4 (OR 2.4, 95%CI 1.4 to 4.0) times more likely to have M/XDRTB. Individuals who had ever been hospitalized, compared to those who had never been hospitalized were twice (OR 1.9, 95%CI 1.2 to 2.9) as likely to have

M/XDRTB. Patients previously treated with TB, compared to those newly diagnosed with TB, were twice as likely to have M/XDRTB (OR 1.7, 95% CI 1.1 to 2.8).

Table 4.4: Multivariable analysis of factors associated with M/XDRTB (N=838).

Characteristic	Adjusted Odds Ratio (95%CI)
Age (yrs)	
<25	**1.8 (1.0 to 3.1)**
25-34	1.0 (0.6 to 1.6)
35-44	1.3 (0.8 to 2.2)
45+	ref
Study site	
Mumbai, India	**33.1 (18.8 to 58.3)**
Chisinau, Moldova	**13.0 (6.8 to 24.6)**
Port Elizabeth, South Africa	ref
Gender	
Male	ref
Female	1.2 (0.8 to 1.8)
Education level	
Primary-secondary	ref
University/Higher	**2.4 (1.4 to 4.0)**
Hospitalized	
No	ref
Yes	**1.9 (1.2 to 2.9)**
Previously treated for tuberculosis	
Yes	**1.7 (1.1 to 2.8)**
No	ref

CI = Confidence interval
M/XDRTB = Multi and extensively drug resistant tuberculosis
Ref=reference category

Interaction between factors and M/XDRTB: After evaluating the independent effects of each significant factor on M/XDRTB, the interactions of these factors were investigated. When examining the interaction between having been previously treated for TB and study site on the odds of having M/XDRTB, the relative excess risk of M/XDRTB in patients by study site and prior TB treatment together exceeded the sum of the relative excess risks for each factor alone. Assuming a multiplicative scale, the OR for interaction was 2.3 (95% CI 0.02, 246.2) and 17.2 (95% CI 0.2, 1816.0) for Mumbai, India and Chisinau, Moldova, respectively. Under an additive scale, the SI was 2.7 (95% CI 0.03, 287.0) and 20.2 (95% CI 0.1, 3812.8), respectively, indicating the synergistic effect of study site and having previously been treated for TB; however the multiplicative ORs and additive SIs were not statistically significant (**Table 4.5, Figure 4.3**).

Table 4.5: Interaction between study site and previous TB treatment

Interaction variables						
Study site	**Previously treated for TB**	**AOR (95%CI)**	**Multiplicative (95%CI)**	**SI (95%CI)**	**RERI (95%CI)**	**AP 95%CI)**
Mumbai, India	Yes	66.6 (6.8 to 655.5)	2.3	2.7	40.9	0.6
Mumbai, India	No	1.8 (1.0 to 3.2)	(0.02 to 246.6)	(0.03 to 287.0)	(-169.4 to 251.2)	(-1.2 to 2.4)
Chisinau, Moldova	Yes	118.9 (11.3 to 1254.3)	17.2	20.2	112.1	0.9
Chisinau, Moldova	No	3.2 (1.4 to 7.4)	(0.2 to 1816.0)	(0.1 to 3812.8)	(-176.6 to 400.8)	(0.7 to 1.2)

*Adjusted for age, gender, educational level and having been hospitalized
AOR = Adjusted odds ratio
AP = Attributable proportion due to interaction
CI = Confidence interval
RERI = Relative excess risk due to interaction
SI = Synergy index
TB = Tuberculosis

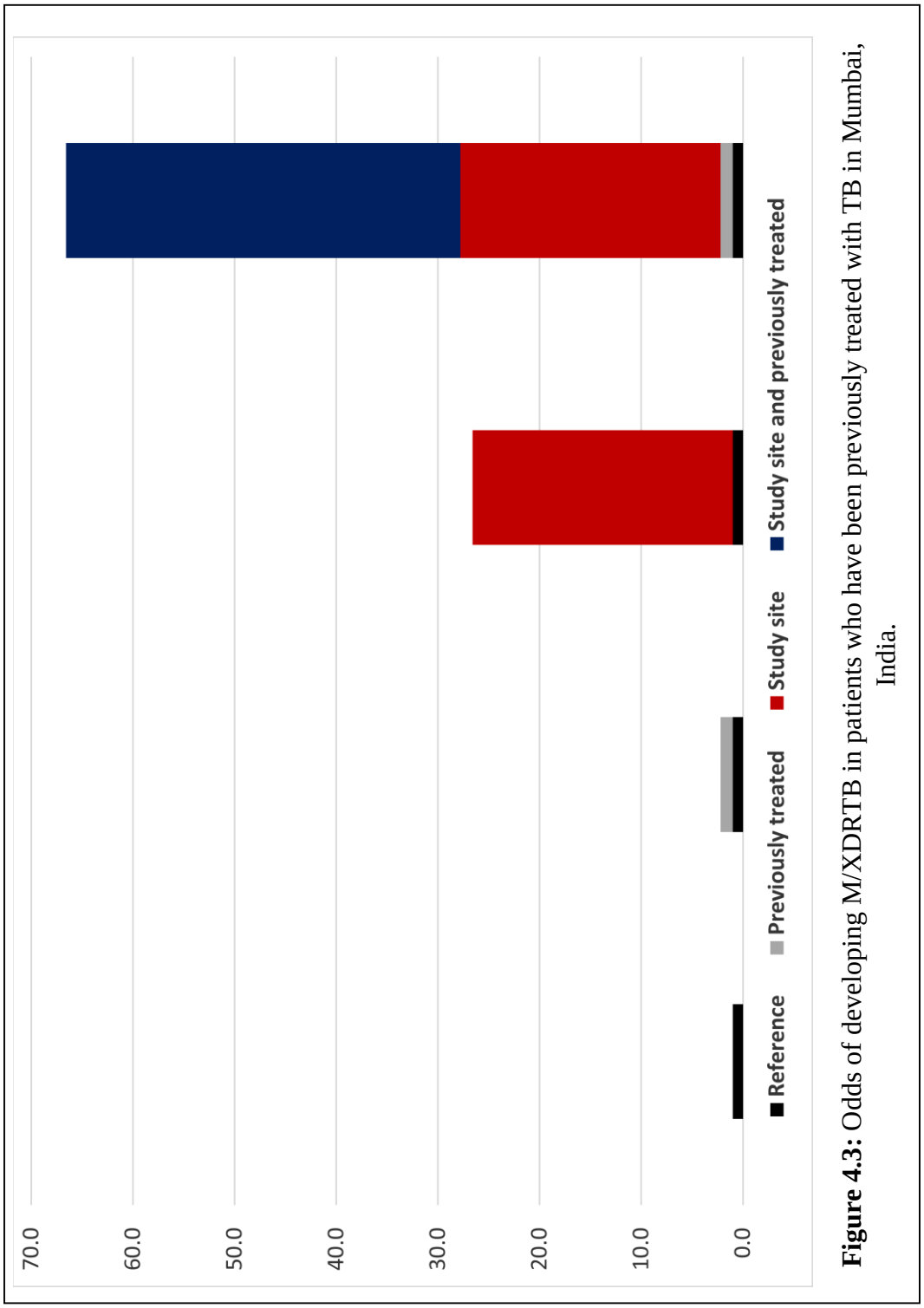


Figure 4.3: Odds of developing M/XDRTB in patients who have been previously treated with TB in Mumbai, India.

Discussion

We found the most important factors associated with developing M/XDRTB were young age (<25 yrs), study site, higher educational level, having ever been previously hospitalized and having previously been treated for TB. Additionally there was an interaction between study site and having previously been treated for TB. To our knowledge, this is the first study to explore the characteristics of developing M/XDRTB and report an interaction between study site and having been previously treated for TB.

SLD resistance is associated with poor treatment outcomes and delayed diagnosis [161]. Resistance in previously treated cases is an indicator of current treatment practices whereas drug resistance in new cases is an indicator of disease transmission with resistant bacilli [142]. In our study, the prevalence of SLD resistance in previously treated patients was higher than new cases for each drug, except for KAN. When examining the drug resistance profile by study site, Port Elizabeth, South Africa had a higher prevalence of KAN resistance in newly diagnosed patients compared to those previously treated for TB (83.3% vs. 7.23%). In fact, Port Elizabeth had higher rates of resistance in newly diagnosed patients compared to those previously treated for TB for all SLDs indicating higher rates of primary resistance in this site. Resistance to SLDs at the start of treatment is a critical risk factor for the subsequent development of acquired resistance to other drugs in the regimen [162]. In our study, the prevalence of XDRTB was 5.4% in new cases and 11.4% in previously treated cases. In a study designed to identify DRTB in new and previously treated patients in South Korea, resistance to at least one first-line drug was found in 11.7% of new cases and in 41.6% of previously treated cases. The proportion of XDRTB among MDRTB patients in their study was 16.7% [29].

In this study, individuals less than 25 years of age were more likely to have M/XDRTB, than those older than 45 years of age. In a study examining risk factors associated with TB, Yu et al. [27] reported a relative risk of 2.7 in persons older than 50 years of age, compared to persons less than 30 years old, signifying a strong association between aging and DRTB. In contrast, Macedo et al. found that MDRTB and XDRTB incidences were associated with young adult age [28] which is consistent with our findings. Buu et al. also observed an association between MDRTB and young age and concluded that the strong association with young age suggests recent transmission [163]. MDRTB associated with young adult age can create obstacles towards economic and social development in countries where TB is endemic [3].

The WHO has listed 27 "high-burden" MDRTB countries, three of these countries are India, Moldova and South Africa. India is a high-burden country for both TB and MDRTB. In 2013, the WHO estimated that India accounted for 20.4% of the total number of TB cases worldwide, with 2.2% and 15% of the new and retreatment cases respectively being caused by multidrug-resistant strains [133]. In a recent study by Isaakidis et al. [136] assessing the burden of drug-susceptible and DRTB in Mumbai, researchers found that almost one in four new TB cases and one in two of those previously treated for TB had DRTB. Moldova has one of the highest reported nationwide proportions of TB patients with MDRTB in the world. Almost one fourth of people newly diagnosed with TB in Moldova and two-thirds of those returning for treatment, have MDRTB [133]. In a study by Jenkins et al., researchers found that between 7.2% and 9.2% of non-MDRTB cases in Moldova were subsequently diagnosed

with MDRTB during treatment [138]. These findings suggest that there is a growing DRTB epidemic in both India and Moldova.

Socioeconomic status (SES) of individuals has been shown to influence a person's susceptibility to TB infection. People with low SES are exposed to several risk factors (including malnutrition, indoor air pollution, overcrowding, alcohol, etc.) which increases their risk for TB [42]. SES is difficult to measure, and there are no uniform criteria to assess it. In the present study, we collected information on a range of variables expected to reflect SES. Several of them showed some degree of association with having M/XDRTB in univariate analysis. Only educational level had an independent effect on the odds of having M/XDRTB in multivariate analysis, when adjusting for main demographic variables. Our study showed that a higher educational level was associated with M/XDRTB, which is in contrast to other reports of low educational level and TB, in general. Individuals with higher education tend to work and are more likely to come in contact with and spread M/XDRTB as they are mobile and active.

Our study also identified having ever been hospitalized (for any reason) as an important factor for M/XDRTB, with having previously been hospitalized associated with a two-fold increase. This finding points to the possibility of nosocomial transmission as a catalyst to the growing TB epidemic. In a study by Zetola at al. researchers found that the number of days spent in medical wards was strongly associated with the development of TB within the following 12 months after discharge. In addition to this, TB-related mortality was also higher among these previously hospitalized patients [56]. Delays in the diagnosis of drug resistance and large, congregate TB wards which are

typical in many high-burden settings remain a dangerous combination for the transmission of MDRTB [57].

Prior TB treatment is a well-established risk factor for DRTB [158, 161, 164, 165]. In our study, having previously been treated for TB was associated with a two-fold increase for having M/XDRTB. A systematic review concluded that the risk of MDRTB was up to ten times higher in previously treated patients compared to newly treated patients [69]. The process of resistance in TB is particularly dangerous for patients who have received prior treatment without success. In many of these individuals, lesions advance by repeated reactivations and inadequate treatments, which can be a risk factor for mutant bacilli resistant to one or more drugs [166].

The most noteworthy finding of this study is the interaction between study site and having previously been treated for TB on the odds of M/XDRTB development. In our study, the combined effects of these two variables dramatically increased one's odds of developing M/XDRTB. Patients previously treated with TB, in Mumbai, were sixty-six times more likely to have M/XDRTB whereas patients previously treated with TB in Chisinau, were one hundred nineteen times more likely to have M/XDRTB (compared to patients newly diagnosed with TB in Port Elizabeth, South Africa). Prior episodes of anti-TB treatment can increase the risk of receiving non-standard regimens or interrupted treatment [70]. A sub-minimum inhibitory concentration effect may occur when TB patients receive non-standard regimens (sensitive strains are killed and mutant MDRTB strains take the place of the sensitive ones) resulting in the emergence of MDRTB [71]. As the risk of contracting TB is higher for people who live in areas with high rates of TB; it is important to ensure TB patients in these "high-burden" areas receive standard

regimens the first time, that interrupted treatment is avoided, and that poor adherence to treatment is reduced.

Our study showed no effect on several clinical and epidemiological factors and M/XDRTB in multivariate analysis. However, univariate analysis demonstrated several key associations with M/XDRTB and BMI, gender, marital status, number of people currently living with, number of rooms used for sleeping, home ownership, source of income, income (in US dollars), smoking cigarettes, smoking marijuana, alcohol consumption in the past three months, ever been jailed, having seen a doctor in the past two years other than for TB, close contact with a known TB case, smear positive status, having a fever, unintentional weight loss, hemoptysis, diabetes and HIV. It is possible that there was not enough power to demonstrate these effects in multivariate analysis; further studies are needed to clarify these associations.

Limitations: While this study made use of a comprehensive clinical and laboratory database, there are several limitations to this study. First, of the 1,128 consecutive patients enrolled 19% did not have TB (Figure 1). Secondly, data on some factors (close contact with a known case (24.7%), HIV status (29.7%) and abnormal chest x-ray (20.9%)) were unknown at the time the study was performed. Our analysis was also limited by missing and misclassified data, problems frequently encountered when analyzing surveillance data sets. However, it seems unlikely that there should be systematic differences in the quality of data collected at baseline for individuals with and without M/XDRTB, and, therefore, our central conclusions should be unaffected. Despite the study limitations, our approach in evaluating factors associated with M/XDRTB provides information that may help identify vulnerable patients.

Conclusions

This study identified young age (<25 yrs), study site, educational level, having ever been previously hospitalized and having previously been treated for TB as factors associated with M/XDRTB. In order to control TB, it is essential to understand the complex risk factors and socio-demographic dimensions of the disease. Knowledge of the main risk factors for M/XDRTB permits the identification of populations at highest risk of MDRTB, pre-XDRTB and XDRTB. Additionally, these findings can provide useful information for controlling the transmission of DRTB.

Acknowledgements

The specimens, data, and funding for this project were provided by the Global Consortium for Drug-Resistant TB Diagnostics (GCDD; http://gcdd.ucsd.edu), funded by a grant (U01-AI082229) from the National Institute of Allergy and Infectious Diseases (NIAID), US National Institutes of Health (NIH), Bethesda, MD, USA. TCR was also supported by NIAID, grant # R01AI111435 and grant #R01AI105185. The authors thank the clinical and laboratory staff at each of the study sites for their assistance in acquiring and preparing *Mtb* isolates.

Chapter 4 is currently being prepared for submission for publication of the material in The International Journal of Tuberculosis and Lung Disease. Avalos, E, Garfein, R, Catanzaro, D, Ganiats, T, Brodine, S, Alcaraz, J, Eisenach, K, Ajbani, K, Udwadia, Z, Crudu, V, Victor, T, Rodwell, T. *Characteristics of multi and extensively drug resistant tuberculosis in a multisite study.* The dissertation author was the primary investigator and author of this paper.

CHAPTER 5 OVERALL CONCLUSIONS AND DISCUSSION

To maximize the sensitivity and specificity of molecular diagnostics based on detection of mutations conferring FQ resistance in *Mtb*, we need an understanding of the frequency and geographic distribution of these mutations. In this review, *gyrA* mutations reported in codons 88-94 appeared to account for at least 82% of phenotypic ofloxacin resistance and 85% of moxifloxacin resistance globally. While we did observe geographic differences in the frequencies of specific *gyrA* mutations between countries, it is likely that next generation molecular assays that can detect all of the *gyrA* and *gyrB* mutations documented to confer resistance, will have good sensitivity and specificity globally. Using existing molecular diagnostics to rapidly detect FQ resistance in clinical *Mtb* strains could substantially enhance drug resistance control efforts, with the goal of interruption of disease transmission and ultimately incidence reduction, especially in countries with cross-resistance.

Our study showed that the high prevalence of drug resistance continues to be a major challenge for TB control as the transmission of DRTB is extensive and widespread. The prevalence of MDRTB remains high and the presence of pre-XDRTB and XDRTB will impose new challenges in the global effort to control TB. Young age (<25 yrs), study site, educational level, having ever been previously hospitalized and having previously been treated for TB were characteristics associated with having M/XDRTB. Continuous surveillance is needed to identify DRTB, especially among patients previously treated for TB. It is important to improve early diagnosis of MDRTB and to provide effective treatment to all MDRTB patients in order to prevent the development of additional drug resistance in these high-risk populations.

The three papers included in this dissertation all reveal that resistance to commonly used anti-TB drugs is emerging worldwide. Early detection of DRTB is crucial both for patient management and infection control. The diagnosis of MDRTB and XDRTB is based on mycobacterial culture and DST on liquid or solid media, with results available in weeks to months. As the rate of DRTB continues to rise, rapid tests to promptly identify resistance to first- and second-line anti-TB drugs are urgently needed. The increased prevalence of MDRTB and XDRTB in Mumbai, India, Chisinau, Moldova and Port Elizabeth, South Africa is a growing threat to TB control. The high prevalence of DRTB, observed in our study, highlights the importance of developing more rapid and effective DRTB detection methods for the initiation of early and proper treatment of patients and for the effective management of TB in these respective countries.

Strengths and Limitations

In the course of conducting this study, a few issues came to light. A potential limitation of this study is its cross-sectional nature. Cross-sectional studies are carried out at one time point or over a short period, which makes it difficult to determine whether the outcome followed exposure in time or exposure resulted from the outcome (ie. Did a patient develop FQ-resistant TB as a result of prior FQ exposure or did FQ resistance develop during the current treatment?). Cross-sectional studies are typically conducted to estimate the prevalence of the outcome of interest for a given population, for the purposes of public health planning. Data can also be collected on individual characteristics, including exposure to risk factors, alongside information about the outcome. Thus, cross-sectional studies provide a “snapshot” of the outcome and the characteristics associated with it, at a specific point in time.

One weakness of cross-sectional studies is the bias associated with the way the data are collected. As cross-sectional studies measure prevalent, rather than incident cases, the data reflect determinants of survival (survival bias). Additionally our study was susceptible to misclassification due to recall bias. A potential for recall bias exists whenever historical self-reported information is elicited from respondents (have you ever been treated with a fluoroquinolone?).

While there are limitations to this study design, there are also strengths. In this study, we were able to study multiple outcomes and exposures. Additionally, the information we collected on the prevalence of DRTB, by site, is important in assessing the burden of DRTB and in planning and allocating health resources to each respective site. A second strength of this study was the training provided to the study personnel. All study personnel were trained prior to the beginning of the study and completed training on how to protect human subjects. Additionally the clinical and laboratory personnel from each site were trained in the use of laboratory techniques and on how to properly use case report forms for data collection. A third strength of the study was the data quality control measures. These quality assurance measures were used to prevent incorrect data from being entered. As this method could not prevent every error, some data were verified via internal quality checks and corrected in a timely manner. A fourth strength of the study was the multi-site study design. While the sample is not globally representative and does not generalize to all DRTB patients; the sites from which patients were sampled do encompass a broad geographical distribution. Multisite studies provide large, diverse samples with sufficient statistical power to detect significant associations between exposures and outcomes. The findings are more generalizable than studies in a

single institution and, therefore, more likely to influence practices and policies. This multisite study was successful as it established communication, trust and collaboration among participating sites and assured data integrity ***before*** study initiation and continued to maintain an open line of communication as the study progressed.

Public Health Implications

Mtb is a major public health concern as the bacterium spreads from person to person. Effective and rapid diagnosis is a key objective of worldwide TB control strategies. Currently, the only commercial assay available for the rapid detection of FQ resistance in clinical samples is the MTBDR*sl* line probe assay (Hain Lifescience, Nehren, Germany). The reported pooled sensitivity of this assay is 87% indicating that there is room for improvement. Before this assay can be improved, we must have an understanding of the frequency of mutations in FQ resistant *Mtb* isolates (aim of paper 1).

Before a successful TB treatment strategy can be implemented in a country, we must have an idea of the prevalence of DRTB of that country (aim of paper 2). The prevalence of DRTB describes the severity of the problem; an effective TB treatment program should be familiar with the prevalence of DRTB in new and previously treated patients. Once an effective TB treatment strategy is implemented, continuous surveillance of DRTB prevalence rates can be used to evaluate the success of the TB treatment strategy.

This study identified young age (<25 yrs), study site, educational level, having ever been previously hospitalized and having previously been treated for TB as factors associated with M/XDRTB. Knowledge of the main characteristics of M/XDR-TB permits the identification of populations at highest risk of DRTB, specifically MDR-TB,

pre-XDR-TB and XDR-TB (aim of paper 3). Recognizing factors conferring risk permits the prompt identification of patients at risk for developing DRTB, thus allowing effective treatment regimens targeted to these high-risk populations to be implemented.

Currently, the WHO has a standardized treatment regimen for the treatment of DRTB. Treating DRTB is complex and no single strategy will apply to all patients. Based on the differences in the prevalence of DRTB reported, by country, in this study, the WHO treatment regimen is not working. Treatment for DRTB should be individualized and 1) based on prior medications taken by the patient, 2) consider commonly used drugs and 3) consider the prevalence of drug resistance to the first and second-line drugs on a per country basis.

Future Directions

Our study identified characteristics associated with *having* M/XDRTB, in Mumbai, India, Chisinau, Moldova and Port Elizabeth, South Africa. To determine if these characteristics are associated with *developing* M/XDRTB, a prospective cohort study should be designed. In an ideal prospective cohort study, investigators would enroll subjects and collect baseline exposure information from individuals who are at risk for DRTB, but do not have DRTB. These individuals would be prospectively followed until they developed and were treated for DRTB. In a prospective cohort study, baseline information is collected from all subjects in the same way using exactly the same questions and data collection methods for all subjects. The investigators design the questions and data collection procedures carefully in order to obtain accurate information about exposures before DRTB develops in any of the subjects. After baseline information is collected, subjects in a prospective cohort study are then followed over a period of time

to determine if and when they develop DRTB and whether their exposure status changes. In this way, investigators can eventually use the data to answer many questions about the associations between “risk factors” and developing DRTB. As an example, one could identify patients previously hospitalized and not previously hospitalized in the past year at baseline and compare their subsequent incidence of developing DRTB.

APPENDICES

Appendix A: List of all mutations not meeting criterion for inclusion

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
A74S	*gyrA*	MOX	357	540	1	0	0.28%
		OFL	1737	1121	1	0	0.06%
A74S & D94G	*gyrA*	OFL	1737	1121	11	0	0.63%
A74S & D94N	*gyrA*	OFL	1737	1121	1	0	0.06%
		CIPRO	279	151	1	0	0.36%
		LEVO	259	72	1	0	0.39%
A90A	*gyrA*	OFL	1995	1572	1	0	0.05%
A90E & T80A	*gyrA*	OFL	1761	1149	1	0	0.06%
A90G & D94G & T80A	*gyrA*	OFL	1761	1149	1	0	0.06%
A90G & T80A	*gyrA*	OFL	1761	1149	0	1	0.00%
A90P & D94G	*gyrA*	OFL	1995	1572	1	0	0.05%
A90V & D94C & D94G & D94Y	*gyrA*	OFL	1995	1572	1	0	0.05%
A90V & D94G & S91P	*gyrA*	OFL	1995	1572	1	0	0.05%
A90V & D94H	*gyrA*	OFL	1995	1572	1	0	0.05%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
A90V & D94V	*gyrA*	CIPRO	334	287	1	0	0.30%
		GAT	198	91	1	0	0.51%
		LEVO	412	248	1	0	0.24%
		MOX	357	540	1	0	0.28%
		SITA	59	0	1	0	1.69%
		SPX	109	0	1	0	0.92%
A90V & G88A	*gyrA*	OFL	1982	1504	2	0	0.10%
A90V & L96P	*gyrA*	CIPRO	334	287	1	0	0.30%
A90V & S91A	*gyrA*	OFL	1995	1572	1	0	0.05%
D84G	*gyrA*	CIPRO	279	151	1	0	0.36%
		GAT	187	91	1	0	0.53%
		LEVO	259	72	1	0	0.39%
		MOX	357	540	1	0	0.28%
		OFL	1737	1121	1	0	0.06%
D89G	*gyrA*	MOX	357	540	1	0	0.28%
		OFL	1982	1504	2	0	0.10%
D89N	*gyrA*	OFL	1982	1504	4	0	0.20%
D94A & D94G	*gyrA*	OFL	1995	1572	6	0	0.30%
D94A & D94G & S91P	*gyrA*	OFL	1995	1572	1	0	0.05%
D94A & S69T	*gyrA*	OFL	1627	1121	1	0	0.06%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
D94A & S91P	*gyrA*	CIPRO	334	287	1	0	0.30%
		GAT	198	91	1	0	0.51%
		MOX	357	540	1	0	0.28%
		OFL	1995	1572	1	0	0.05%
D94A/Y	*gyrA*	OFL	1995	1572	1	0	0.05%
D94C	*gyrA*	OFL	1995	1572	2	0	0.10%
D94C & D94G & D94N & D94S & D94Y	*gyrA*	OFL	1995	1572	1	0	0.05%
D94C & D94G & D94Y	*gyrA*	OFL	1995	1572	1	0	0.05%
D94F	*gyrA*	OFL	1995	1572	1	0	0.05%
D94G & D111N	*gyrA*	CIPRO	318	151	2	0	0.63%
D94G & D94N & D94S	*gyrA*	OFL	1995	1572	4	0	0.20%
D94G & D94N & D94Y	*gyrA*	OFL	1995	1572	1	0	0.05%
D94G & D94Y	*gyrA*	OFL	1995	1572	2	0	0.10%
D94G & S91P	*gyrA*	OFL	1995	1572	5	0	0.25%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
D94H & S91P	*gyrA*	CIPRO	334	287	1	0	0.30%
		GAT	198	91	1	0	0.51%
		MOX	357	540	1	0	0.28%
		OFL	1995	1572	1	0	0.05%
D94N & D94Y	*gyrA*	OFL	1995	1572	1	0	0.05%
D94N & G88C	*gyrA*	OFL	1982	1504	1	0	0.05%
D94N & G112H	*gyrA*	LEVO	396	112	1	0	0.25%
		OFL	1813	1323	1	0	0.06%
D94N & S91P	*gyrA*	CIPRO	334	287	1	0	0.30%
D94N/G	*gyrA*	OFL	1995	1572	2	0	0.10%
D94S	*gyrA*	LEVO	412	248	1	0	0.24%
		OFL	1995	1572	1	0	0.05%
D94V & G88R	*gyrA*	OFL	1982	1504	1	0	0.05%
D94Y & R98L	*gyrA*	OFL	1843	1340	1	0	0.05%
D94Y & S91P	*gyrA*	CIPRO	334	287	1	0	0.30%
G247S	*gyrA*	MOX	10	26	0	1	0.00%
G668D	*gyrA*	OFL	38	20	6	0	15.79%
G88A	*gyrA*	LEVO	412	248	1	0	0.24%
		OFL	1982	1504	4	0	0.20%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
G88A & B94Y	*gyrA*	CIPRO	295	287	1	0	0.34%
		LEVO	412	248	1	0	0.24%
		OFL	1982	1504	1	0	0.05%
G88A & H70R	*gyrA*	GAT	187	91	1	0	0.53%
		LEVO	259	72	1	0	0.39%
		MOX	357	540	1	0	0.28%
		OFL	1737	1121	1	0	0.06%
H52Q	*gyrA*	OFL	1474	1026	1	0	0.07%
H70R	*gyrA*	LEVO	259	72	2	0	0.77%
		OFL	1737	1121	1	0	0.06%
L109V	*gyrA*	OFL	1835	1340	0	1	0.00%
P102H	*gyrA*	MOX	357	540	0	1	0.00%
		OFL	1835	1340	0	1	0.00%
Q60R	*gyrA*	OFL	1605	1104	1	0	0.06%
R68G	*gyrA*	OFL	1627	1121	2	1	0.12%
S90P	*gyrA*	OFL	1995	1572	1	0	0.05%
S91A	*gyrA*	LEVO	412	248	4	0	0.97%
S91L	*gyrA*	OFL	1995	1572	1	0	0.05%
S91T	*gyrA*	CIPRO	334	287	1	0	0.30%
		GAT	198	91	1	0	0.51%
		MOX	357	540	1	0	0.28%
		OFL	1995	1572	1	0	0.05%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
T80S	*gyrA*	MOX	357	540	1	0	0.28%
		OFL	1761	1149	1	0	0.06%
A471V	*gyrB*	CIPRO	39	0	1	0	2.56%
A543T	*gyrB*	OFL	536	191	1	0	0.19%
D500A	*gyrB*	OFL	838	393	2	0	0.24%
D500H & G509A	*gyrB*	OFL	838	393	1	0	0.12%
D500N	*gyrB*	OFL	838	393	2	0	0.24%
D533A	*gyrB*	OFL	838	393	1	0	0.12%
E419K & T539P	*gyrB*	OFL	206	21	1	0	0.49%
E424K	*gyrB*	OFL	206	21	4	0	1.94%
E498K	*gyrB*	LEVO	234	70	1	0	0.43%
		OFL	609	236	1	0	0.16%
E540A	*gyrB*	OFL	684	211	1	0	0.15%
E540D	*gyrB*	OFL	684	211	1	0	0.15%
E540V	*gyrB*	OFL	684	211	1	0	0.15%
G425E	*gyrB*	OFL	206	21	1	0	0.49%
G551R	*gyrB*	LEVO	137	40	1	0	0.73%
		OFL	486	191	1	0	0.21%
G551R & T539N	*gyrB*	LEVO	137	40	1	0	0.73%
		OFL	486	191	1	0	0.21%
G570R	*gyrB*	MOX	10	26	0	1	0.00%
K679R	*gyrB*	MOX	10	26	0	1	0.00%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
Q577H	*gyrB*	OFL	254	44	1	0	0.39%
R485H	*gyrB*	GAT	38	30	0	1	0.00%
		LEVO	38	30	0	1	0.00%
		MOX	38	30	0	1	0.00%
		OFL	339	158	0	1	0.00%
R485L	*gyrB*	OFL	339	158	1	0	0.29%
S434A	*gyrB*	OFL	206	21	1	0	0.49%
S486F	*gyrB*	OFL	472	186	1	1	0.21%
S540L	*gyrB*	OFL	684	211	2	0	0.29%
T539P	*gyrB*	OFL	708	239	1	0	0.14%
A90V & D500A	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
A90V & D500N	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
A90V & D94A & N538T	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
A90V & D94A & D94G & S91P & N538T	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
A90V & E498K	*gyrA/gyrB*	LEVO	234	70	1	0	0.43%
		OFL	609	236	1	0	0.16%
A90V & G551R	*gyrA/gyrB*	LEVO	137	40	3	0	2.19%
		OFL	486	191	5	0	1.03%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
A90V & N538T	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
A90V & R485C	*gyrA/gyrB*	OFL	339	158	1	0	0.29%
A90V & T539A	*gyrA/gyrB*	LEVO	256	42	1	0	0.39%
		OFL	708	239	1	0	0.14%
A90V & T539N	*gyrA/gyrB*	CIPRO	98	0	2	0	2.04%
		GAT	59	0	2	0	3.39%
		LEVO	256	42	2	0	0.78%
		MOX	59	0	2	0	3.39%
		OFL	708	239	2	0	0.28%
		SITA	59	0	2	0	3.39%
		SPX	59	0	2	0	3.39%
A90V & T539P	*gyrA/gyrB*	OFL	708	239	1	0	0.14%
D94A & A543T	*gyrA/gyrB*	LEVO	137	40	6	0	4.38%
		OFL	536	191	6	0	1.12%
D94A & D500N	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
D94A & E424K	*gyrA/gyrB*	OFL	206	21	1	0	0.49%
D94A & E481Q & D483H	*gyrA/gyrB*	OFL	206	21	1	0	0.49%
D94A & E540D	*gyrA/gyrB*	OFL	684	211	1	0	0.15%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
D94A & I458M	*gyrA/gyrB*	OFL	206	21	1	0	0.49%
D94A & N538K	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
D94A & N538T	*gyrA/gyrB*	OFL	838	393	2	0	0.24%
D94A & N538I	*gyrA/gyrB*	LEVO	314	112	2	0	0.64%
		OFL	838	393	2	0	0.24%
D94A & T539P	*gyrA/gyrB*	OFL	708	239	2	0	0.28%
D94G & A543V	*gyrA/gyrB*	LEVO	137	40	2	0	1.46%
		OFL	536	191	6	0	1.12%
D94G & D414K	*gyrA/gyrB*	OFL	206	21	1	0	0.49%
D94G & D414P	*gyrA/gyrB*	OFL	206	21	1	0	0.49%
D94G & E424K	*gyrA/gyrB*	OFL	206	21	4	0	1.94%
D94G & E522Q	*gyrA/gyrB*	OFL	838	393	3	0	0.36%
D94G & G551R	*gyrA/gyrB*	OFL	486	191	1	0	0.21%
D94G & N538T	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
D94G & R485G	*gyrA/gyrB*	OFL	339	158	1	0	0.29%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
D94G & S486F	*gyrA/gyrB*	CIPRO	98	0	1	0	1.02%
		GAT	97	30	1	0	1.03%
		LEVO	97	30	1	0	1.03%
		MOX	97	30	1	0	1.03%
		SITA	59	0	1	0	1.69%
		SPX	59	0	1	0	1.69%
D94N & A543V	*gyrA/gyrB*	LEVO	137	40	2	0	1.46%
		OFL	536	191	2	0	0.37%
D94N & D500N	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
D94N & E419K & E424K & R460K	*gyrA/gyrB*	OFL	206	21	1	0	0.49%
D94N & G551R	*gyrA/gyrB*	LEVO	137	40	1	0	0.73%
		OFL	486	191	1	0	0.21%
D94N & N538K	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
D94N & N538S	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
D94N & V461A	*gyrA/gyrB*	OFL	206	21	1	0	0.49%
D94V & N538T	*gyrA/gyrB*	OFL	838	393	1	0	0.12%
D94Y & E419K	*gyrA/gyrB*	OFL	206	21	1	0	0.49%

Appendix A: List of all mutations not meeting criterion for inclusion, Continued

Mutation	Gene	Drug	# Resistant Isolates Examined	# Susceptible Isolates Examined	# Resistant Isolates w/Mutation	# Susceptible Isolates w/Mutation	Frequency of Mutation Among Resistant Isolates
G88A & G509C	*gyrA/gyrB*	LEVO	315	112	2	0	0.63%
		OFL	830	333	2	0	0.24%
S91P & N464S	*gyrA/gyrB*	OFL	206	21	1	0	0.49%
S91P & D500N	*gyrA/gyrB*	OFL	838	393	1	0	0.12%

Appendix B: PRISMA Checklist

Section/topic	#	Checklist item	Reported on page #
TITLE			
Title	1	Identify the report as a systematic review, meta-analysis, or both.	15
ABSTRACT			
Structured summary	2	Provide a structured summary including, as applicable: background; objectives; data sources; study eligibility criteria, participants, and interventions; study appraisal and synthesis methods; results; limitations; conclusions and implications of key findings; systematic review registration number.	15-16
INTRODUCTION			
Rationale	3	Describe the rationale for the review in the context of what is already known.	16-18
Objectives	4	Provide an explicit statement of questions being addressed with reference to participants, interventions, comparisons, outcomes, and study design (PICOS).	18-19
METHODS			
Protocol and registration	5	Indicate if a review protocol exists, if and where it can be accessed (e.g., Web address), and, if available, provide registration information including registration number.	NA
Eligibility criteria	6	Specify study characteristics (e.g., PICOS, length of follow-up) and report characteristics (e.g., years considered, language, publication status) used as criteria for eligibility, giving rationale.	19
Information sources	7	Describe all information sources (e.g., databases with dates of coverage, contact with study authors to identify additional studies) in the search and date last searched.	19
Search	8	Present full electronic search strategy for at least one database, including any limits used, such that it could be repeated.	19
Study selection	9	State the process for selecting studies (i.e., screening, eligibility, included in systematic review, and, if applicable, included in the meta-analysis).	19
Data collection process	10	Describe method of data extraction from reports (e.g., piloted forms, independently, in duplicate) and any processes for obtaining and confirming data from investigators.	19-20
Data items	11	List and define all variables for which data were sought (e.g., PICOS, funding sources) and any assumptions and simplifications made.	19-20

Appendix B: PRISMA Checklist, Continued

Section/topic	#	Checklist item	Reported on page #
METHODS			
Risk of bias in individual studies	12	Describe methods used for assessing risk of bias of individual studies (including specification of whether this was done at the study or outcome level), and how this information is to be used in any data synthesis.	20-21
Summary measures	13	State the principal summary measures (e.g., risk ratio, difference in means).	20-21
Synthesis of results	14	Describe the methods of handling data and combining results of studies, if done, including measures of consistency (e.g., I2) for each meta-analysis.	20-21
Risk of bias across studies	15	Specify any assessment of risk of bias that may affect the cumulative evidence (e.g., publication bias, selective reporting within studies).	20-21
Additional analyses	16	Describe methods of additional analyses (e.g., sensitivity or subgroup analyses, meta-regression), if done, indicating which were pre-specified.	20-21
RESULTS			
Study selection	17	Give numbers of studies screened, assessed for eligibility, and included in the review, with reasons for exclusions at each stage, ideally with a flow diagram.	21-22
Study characteristics	18	For each study, present characteristics for which data were extracted (e.g., study size, PICOS, follow-up period) and provide the citations.	23-28
Risk of bias within studies	19	Present data on risk of bias of each study and, if available, any outcome level assessment (see item 12).	20-21
Results of individual studies	20	For all outcomes considered (benefits or harms), present, for each study: (a) simple summary data for each intervention group (b) effect estimates and confidence intervals, ideally with a forest plot.	NA
Synthesis of results	21	Present results of each meta-analysis done, including confidence intervals and measures of consistency.	21-46
Risk of bias across studies	22	Present results of any assessment of risk of bias across studies (see Item 15).	21-46
Additional analysis	23	Give results of additional analyses, if done (e.g., sensitivity or subgroup analyses, meta-regression [see Item 16]).	21-46

Appendix B: PRISMA Checklist, Continued

Section/topic	#	Checklist item	Reported on page #
DISCUSSION			
Summary of evidence	24	Summarize the main findings including the strength of evidence for each main outcome; consider their relevance to key groups (e.g., healthcare providers, users, and policy makers).	47-51
Limitations	25	Discuss limitations at study and outcome level (e.g., risk of bias), and at review-level (e.g., incomplete retrieval of identified research, reporting bias).	51-52
Conclusions	26	Provide a general interpretation of the results in the context of other evidence, and implications for future research.	53
FUNDING			
Funding	27	Describe sources of funding for the systematic review and other support (e.g., supply of data); role of funders for the systematic review.	56

Appendix C: Cumulative Frequencies of the Most Frequently Occurring Double Mutations within *gyrA* Gene among *Mycobacterium tuberculosis* Isolates Resistant to Fluoroquinolones

Mutation		FLQ Tested	# Resistant (R) Isolates Examined	# Susceptible (S) Isolates Examined	# R Isolates with Mutation	# S Isolates with Mutation	Freq. of Mutation among R Isolates	Freq. of Mutation among S Isolates
A90V	D94G	OFL	1995	1572	53	0	0.03	0.00
		MOX	357	540	4	0	0.01	0.00
		LEVO	412	248	1	0	0.00	0.00
		GAT	198	91	1	0	0.01	0.00
A90V	S91P	OFL	1995	1572	17	0	0.01	0.00
		MOX	357	540	1	0	0.00	0.00
		LEVO	412	248	2	0	0.00	0.00
		CIPRO	334	287	3	0	0.01	0.00
		GAT	198	91	2	0	0.01	0.00
		SPX	109	0	1	0	0.01	NA
		SITA	59	0	1	0	0.02	NA
A90V	D94A	OFL	1995	1572	8	0	0.00	0.00
		MOX	357	540	1	0	0.00	0.00
		LEVO	412	248	5	0	0.01	0.00
		CIPRO	334	287	3	0	0.01	0.00
		GAT	198	91	2	0	0.01	0.00
		SPX	109	0	1	0	0.01	NA
		SITA	59	0	1	0	0.02	NA
A90V	D94N	OFL	1995	1572	10	0	0.01	0.00
		LEVO	412	540	1	0	0.00	0.00
		CIPRO	334	287	1	0	0.00	0.00
D94G	D94N	OFL	1995	1572	5	0	0.00	0.00
		MOX	357	540	3	0	0.01	0.00
		LEVO	412	248	1	0	0.00	0.00
		GAT	198	91	1	0	0.01	0.00
A90V	P102H	OFL	1835	1340	3	0	0.00	0.00
		MOX	357	540	2	0	0.01	0.00
D94A	D94N	OFL	1995	1572	3	0	0.00	0.00
		MOX	357	540	2	0	0.01	0.00
A90V	D94Y	OFL	1995	1572	2	0	0.00	0.00
		MOX	357	540	1	0	0.00	0.00
		LEVO	412	248	1	0	0.00	0.00
		GAT	198	91	1	0	0.01	0.00

Appendix D: Case Report Forms

Screening CRF

Screening ID Number: _____
Study ID Number: _____
Today's Date: _____ (DD-MM-YYYY)
Initials: _____ (3 letters)
☐ Direct subject interview ☐ Transcribed
Check the box if subject gives verbal consent (or assent) to be screened for the GCDD XDR-TB Study
☐ Verbal consent/assent given

A. DEMOGRAPHIC CHARACTERISTICS

1. Study Site:

☐ India
- ☐ P.D. Hinduja National Hospital

☐ Moldova
- ☐ Phthisiopneumology Institute,Chisinau
- ☐ Municipal TB Hospital, Chisinau
- ☐ Municipal TB Hospital, Balti
- ☐ Vorniceni Clinic

☐ South Africa
- ☐ Jose Pearson
- ☐ Empilweni TB Hospital
- ☐ New Brighton Clinic
- ☐ Zwide Primary Health Care Clinic
- ☐ Chatty Primary Health Care Clinic
- ☐ Motherwell NU11 Primary Health Care Clinic
- ☐ Motherwell NU2 Primary Health Care Clinic
- ☐ Soweto Primary Health Care Clinic
- ☐ Kwazakhele Primary Health Care Clinic
- ☐ Mabandla Primary Health Care Clinic

2. Subject Year of birth: _____ (e.g.,1950)

3. Subject Gender: ☐ Male ☐ Female ☐ Transgender

4. Subject Ethnicity: ☐ Hispanic ☐ Non-Hispanic

5. Race (specify one **or** more)**:**

I. ☐ Black (specify one **or** more below if applicable):
- ☐ *Xhosa* ☐ *Zulu*
- ☐ *Other Black:* _____

II. ☐ Colored (specify one **or** more below if applicable):
- ☐ *Xhosa* ☐ *Zulu*
- ☐ *Other Black:* _____

III. ☐ Asian (specify one **or** more below if applicable):
- ☐ *Maharashtrian* ☐ *Gujrathi* ☐ *South Indian*
- ☐ *Bikol*
- ☐ *Punjabi* ☐ *Bihari* ☐ *Bengali*
- ☐ *Indo-Aryan*
- ☐ *North East* ☐ *Chinese* ☐ *Dravidian*
- ☐ *Filipino*
- ☐ *Other Asian:* _____

IV. ☐ White (specify one **or** more below if applicable):
- ☐ *Russian* ☐ *Bulgarian* ☐ *Gagauz*
- ☐ *Ukrainian*
- ☐ *Moldovan/Romanian*
- ☐ *Other White:* _____

V. ☐ Native Hawaiian or other Pacific Islander

VI. ☐ American Indian / Alaskan Native
VII. ☐ Other:_____
VIII. ☐ Unknown

B. ELIGIBILITY AND PARTICIPATION

1. Is the subject 5 years of age or older?

☐ Yes [Go to #2] ☐ No/Unknown [Not eligible]

2a. Is the subject AFB smear-positive from a sputum specimen obtained within the past 14 days (1+ or greater)?

☐ Yes ☐ No ☐ Unknown

2b. Is the subject positive for M. tuberculosis by GeneXpert within the past 14 days?

☐ Yes [Go to #3] ☐ No ☐ Unknown

If Study Site=India, Go to #3
If 2a=Yes, Go to #3
If 2a=No/Unknown *AND* Study Site=Moldova or South Africa, Not Eligible. Stop Here.

3. Has the subject previously received >1 month of treatment for a prior TB episode (not including the current TB illness)?

☐ Yes [Go to #4] ☐ No [Go to #4] ☐ Unknown [Go to #4]

4. Has the subject had close contact with a known drug-resistant TB case? (ever lived, worked, or gone to school with a person known to have M/XDR-TB)

☐ Yes [Go to #5] ☐ No [Go to #5] ☐ Unknown [Go to #5]

5. Is the subject failing standard TB treatment? (Defined as persistently positive sputum smear or culture after ≥ 3 months of a standard TB treatment regimen)

☐ Yes [Go to #6] ☐ No [Go to #6] ☐ Unknown [Go to #6]

6. Has the subject been diagnosed with MDR-TB within the last 30 days?

☐ Yes [Go to #7] ☐ No [Go to #7] ☐ Unknown [Go to #7]

7. Is the subject failing MDR-TB treatment for presumed or confirmed MDR-TB? (Defined as persistently positive sputum smear or culture after ≥ 3 months of a standard MDR-TB treatment regimen).

☐ Yes [Go to#8] ☐ No [Go to #8] ☐ Unknown [Go to #8]

[If Yes for at least one of #3-7; subject may be eligible, Go to #8.]
[If No/unknown for #3-7, Subject is not eligible. Stop Here.]

8. Has anti-tuberculosis drug susceptibility testing for the fluoroquinolone and injectables (second line drug testing) been performed and reported within the previous 3 months?

☐ Yes [Not eligible] ☐ No [Go to #9] ☐ Unknown [Not eligible]

(Conduct all applicable procedures for obtaining informed consent before answering this question)

9. Did the subject provide informed consent to participate in the study?

☐ Yes
☐ No [Record reasons for refusal to participate]:_____

Appendix E
Enrollment Interview CRF

Screening ID Number: _____
Study ID Number: _____
Today's Date: _____ (DD-MM-YYYY)
Initials: _____ (3 letters)
☐ Direct Subject Interview ☒ Transcribed

A. SOCIODEMOGRAPHICS

1. What is your age? _____ Years
2. Subject Ethnicity: ☐ Hispanic ☐ Non-Hispanic
3. Race (specify one **or** more)**:**

I. ☐ Black (specify one **or** more below if applicable):
- ☐ *Xhosa* ☐ *Zulu*
- ☐ *Other Black:* _____

II. ☐ Colored (specify one **or** more below if applicable):
- ☐ *Xhosa* ☐ *Zulu*
- ☐ *Other Black:* _____

III. ☐ Asian (specify one **or** more below if applicable):
- ☐ *Maharashtrian* ☐ *Gujrathi* ☐ *South Indian* ☐ *Bikol*
- ☐ *Punjabi* ☐ *Bihari* ☐ *Bengali* ☐ *Indo-Aryan*
- ☐ *North East* ☐ *Chinese* ☐ *Dravidian* ☐ *Filipino*
- ☐ *Other Asian:* _____

IV. ☐ White (specify one **or** more below if applicable):
- ☐ *Russian* ☐ *Bulgarian* ☐ *Gagauz* ☐ *Ukrainian*
- ☐ *Moldovan/Romanian*
- ☐ *Other White:* _____

V. ☐ Native Hawaiian or other Pacific Islander
VI. ☐ American Indian / Alaskan Native
VII. ☐ Other*:* _____

4. What is your gender? ☐ Male ☐ Female ☐ Transgender
5. What is your marital status?
   ☐ Single/Never Married ☐ Married ☐ Widowed
   ☐ Living with partner but not married ☐ Divorced
6. Where were you born?
   City:_____ State/Province:_____ Country:_____
   6a. What Map Grid Code was this address in? Row: _____ Column: _____
   ☐ Refused Map Grid Code ☐ Not On Map
   6b. How old were you when you first moved away from that address? _____ Years-Old
   ☐ Still living at address where born [Skip to Question 10]
7. Where were you living when you were FIRST diagnosed with tuberculosis?
   City:_____ State/Province:_____ Country:_____
   7a. What Map Grid Code was this address in? Row:_____ Column:_____
   ☐ Refused Map Grid Code ☐ Not On Map ☐ Don't Remember
   7b. Please estimate how long you lived there before you were FIRST diagnosed with tuberculosis?
   Years: _____ Months: _____ ☐ Don't Remember
   7c. Please estimate in the two years prior to FIRST diagnosis of tuberculosis approximately how many times did you change your place of residence?
   ☐ None ☐ 1-2 times ☐ 3-5 times ☐ 6-10 times ☐ More than 10 times ☐ Don't Remember
8. Where do you currently live?
   ☐ Same as Question 7 (address where FIRST diagnosed with tuberculosis)

[If checked, Skip to Question 9]

City:_____ State/Province:_____ Country:_____

8a. What Map Grid Code is your current address in? Row: _____ Column: _____

☐ Refused Map Grid Code ☐ Not On Map

8b. Please estimate how long you have lived at your current address?

Years: _____ Months: _____ ☐ Don't Know

9. Did you move to your current address specifically to get TB treatment? ☐ No ☐ Yes

10. In what year were you diagnosed with tuberculosis? _____ ☐ Don't Remember

11. How many people do you currently live with (besides yourself)? _____ People

(If subject lives in a homeless shelter, on the street, in an informal dwelling, or other similar situation, ask them to estimate the total number of people who live there)

11a. How many rooms are used for sleeping? _____ Rooms

12. Do you own your own home/apartment?

☐ No ☐ Yes ☐ Refuse to Answer

12a. If No, where do you currently live?

☐ Rented house/Apartment
☐ Friend's or Family's home
☐ Homeless shelter
☐ Informal dwelling
☐ On the street
☐ Other, specify: _____

13. In the past 3 months, what has been your primary source of income? (Check all that apply)

☐ Regular full-time job
☐ Part-time work
☐ Friends/family members
☐ Bartering/trading
☐ Panhandling/begging
☐ Farming
☐ Sex work
☐ Welfare/government aid
☐ None (no income)
☐ Other, specify: _____

14. In the past 3 months, how much money did you earn on average per month? _____

14a. ☐ Rupee ☐ Leu ☐ Rand ☐ Other (specify) _____

15. What is the highest level of education you have attended?

☐ None
☐ 1-6th Grade
☐ 7-12th Grade
☐ Any trade school
☐ Any college graduate
☐ Any graduate program

16. Have you ever smoked cigarettes or bidis? ☐ No ☐ Yes ☐ Refuse to Answer

16a. If **Yes**, have you smoked in the last 3 months? ☐ No ☐ Yes ☐ Refuse to Answer

16b. If **Yes**, how many cigarettes/bidis did you typically smoke per day in the past 3 months? _____

☐ Don't know ☐ Refuse to Answer

17. Have you ever smoked marijuana, cannabis or hashish? ☐ No ☐ Yes ☐ Refuse to Answer

17a. If **Yes**, have you smoked in the last 3 months? ☐ No ☐ Yes ☐ Refuse to Answer

18. Have you ever smoked hookah? ☐ No ☐ Yes ☐ Refuse to Answer

18a. If **Yes**, have you smoked in the last 3 months? ☐ No ☐ Yes ☐ Refuse to Answer

19. During a typical week in the last 3 months, how many days did you drink alcohol (beer, wine, spirits)? _____ Days

20. On a typical day when you drank in the last 3 months, how many drinks did you have? (One drink is 12 oz of beer, 4 oz of wine or 2 oz of spirits) _____ Drinks

21. Have you ever taken street drugs (e.g., heroin, cocaine, methamphetamine, opium, mushrooms, LSD)? ☐ No ☐ Yes ☐ Refuse to Answer
    21a. If **Yes**, have you done so in the past 2 years? ☐ No ☐ Yes ☐ Refuse to Answer
22. Have you ever injected any drug not prescribed by a doctor? ☐ No ☐ Yes ☐ Refuse to Answer
    22a. If **Yes**, have you done so in the past 2 years? ☐ No ☐ Yes ☐ Refuse to Answer
23. Have you ever been in jail or correctional facility? ☐ No ☐ Yes ☐ Refuse to Answer
    23a. If **Yes**, was it within the past 2 years? ☐ No ☐ Yes ☐ Refuse to Answer
24. Have you ever been hospitalized? ☐ No ☐ Yes ☐ Refuse to Answer
    24a. If **Yes**, was it within the past 2 years? ☐ No ☐ Yes ☐ Refuse to Answer
25. Have you seen a doctor or health care worker in the past 2 years for anything other than TB? ☐ No ☐ Yes ☐ Refuse to Answer
26. Have you ever traded sex for money, goods, or shelter? ☐ No ☐ Yes ☐ Refuse to Answer
    26a. If **Yes**, have you done so in the past 2 years? ☐ No ☐ Yes ☐ Refuse to Answer
27. Have you ever lived in group housing? (shelter, old age home, etc.) ☐ No ☐ Yes ☐ Refuse to Answer
    27a. If **Yes**, have you done so in the past 2 years? ☐ No ☐ Yes ☐ Refuse to Answer

B. TB AND DRUG-RESISTANCE RISK INFORMATION

1. Have you ever lived, worked, or gone to school with a person who was diagnosed with TB?
   ☐ No ☐ Yes ☐ Unknown
   1a. If **Yes,** is this person known to have M/XDR-TB? ☐ No ☐ Yes ☐ Unknown

2. Have you previously been treated for TB (prior to the current TB illness)?
   ☐ No ☐ Yes ☐ Unknown
   2a. If **Yes**, how old were you the first time you were treated for TB? _____ Years
   2b. How many prior TB episodes have you had? _____ Number of episodes

C. CLINICAL HISTORY

Do you currently have any of the following:

1. Cough for more than 2 weeks ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

2. Fever? ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

3. Night sweats? (wet sheets ≥3 times/week): ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

4. Unintentional weight loss? ☐ No. ☐ Yes ☐ Unknown ☐ Refuse to Answer

5. Hemoptysis? ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6. Please select all the conditions that <u>currently</u> apply to the subject:
(Responses should be based on the subject's reported history <u>and</u> the physician's examination.)

6a. Does the subject have diabetes mellitus? ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer
   6a1. If **Yes**, does the subject take oral hypoglycemic agents? ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer
   6a2. If **Yes**, does the subject take insulin? ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer
6b. Does the subject have silicosis? ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer
6c. Does the subject have chronic liver disease (hepatitis B/C, cirrhosis)? ☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6d. Does the subject have lung cancer or neck cancer?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6e. Does the subject have leukemia or lymphoma?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6f. Does the subject have chronic kidney disease (e.g., end stage renal disease) requiring hemodialysis?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6g. Has the subject ever had a transplant requiring immunosuppressant therapy?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6g1. If **Yes**, what organ?
☐ Heart ☐ Kidney ☐ Liver ☐ Cornea ☐ Pancreas ☐ Intestine ☐ Bone

☐ Vasculature ☐ Lungs ☐ Refuse to Answer ☐ Other: _____

6g2. If **Yes**, was it:
☐ Within the past 2 years ☐ 2 years ago or longer ☐ Unknown

6g3. If Yes, 15 mg prednisone/day ≥ 1 month?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6g4. If Yes, TNF-alpha inhibitors [e.g., Infliximab/Etanercept]?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6g5. Other?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6g5a. If Yes, specify: _____

6h. Does the subject currently use an immunosuppressant drug for something other than a transplant?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6h1. If yes 15 mg prednisone/day ≥1 month?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6h2. If yes, TNF-alpha inhibitors [e.g., Infliximab/Etanercept]?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6h3. Other?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6h3a. If yes, specify: _____

6i. Does the subject have any other serious illness/condition (hypertension, heart disease, etc.)?
☐ No ☐ Yes ☐ Unknown ☐ Refuse to Answer

6i1. If yes, specify: _____

D. HIV/AIDS STATUS

1. Have you ever been tested for HIV? ☐ No ☐ Yes ☐ Refuse to Answer

↓

*If **Yes**, most recent test (DD-MM-YYYY):* _____ ☐ Unknown

*If **Yes**, what was the result of your last HIV test:* ☐ Positive ☐ Negative ☐ Pending
☐ Not Tested ☐ Refuse to Answer

E. PHYSICAL EXAMINATION

Users should use a period (.) as a decimal mark

1. Weight: _____ ☐ Kg ☐ Lbs
2. Height:_____ ☐ Cm ☐ Inches

☐ Measured ☐ Estimated

F. SPECIMEN COLLECTION

1. Was at least 5ml sputum collected (Spot 1) at this visit? ☐ No ☐ Yes

   1a. If **No**, did subject attempt to provide sputum, but could not produce at least 5ml? ☐ No ☐ Yes

2. Was blood collected at this visit? ☐ No ☐ Yes

Appendix F
Enrollment Chart Review CRF

Study ID Number: _____
Today's Date: _____ (DD-MM-YYYY)
Initials: _____ (3 letters)
☐ Direct Chart Review ☒ Transcribed

A. TB History

1. Has the subject previously received treatment for TB (>1month of treatment for a prior TB episode)?

☐ No ☐ Yes ☐ Unknown

If Yes,

1a. Was the subject treated with Fluoroquinolones? ☐ No ☐ Yes ☐ Unknown
1b. Was the subject treated with 2nd line Injectables? ☐ No ☐ Yes ☐ Unknown

2. Subject TB category is (check one):

☐ **New**. (A subject who has never had treatment for TB or who has taken antituberculosis drugs for less than 1 month).

☐ **Previously Treated**. (A subject who has ever received treatment for TB for ≥ 1 month)

If previously treated, specify if possible:

☐ **Relapse**. A subject previously treated for TB who has been declared cured or treatment completed, and is diagnosed with bacteriologically positive (smear or culture) tuberculosis.

☐ **Treatment after failure.** A subject who is started on a re-treatment regimen after having failed previous treatment.

☐ **Treatment after default.** A subject who returns to treatment, positive bacteriologically, following interruption of treatment for 2 months or more.

☐ **Other**. All cases that do not fit the above definitions. This group includes a chronic case, a subject who is sputum-positive at the end of a re-treatment regimen.

☐ **Unknown**

Comments:_____
_____

B. TB Drug History

List all drugs subject has taken for this current TB illness. Ignore treatment interruptions <2 weeks.

☐ No Drugs Documented

Drug	Currently Taking Yes No	Start Date	Unknown Start Date	Stop Date	Unknown Stop Date	Dose (mg)	Unknown Dose	Timing

C. CHEST X-RAY

1. Has the subject had a chest X-ray performed?

☐ Yes ☐ No ☐ Unknown

If yes, date of most recent X-ray: _____ (DD-MM-YYYY) ☐ Unavailable

Findings

1a. Abnormal: ☐ Yes ☐ No ☐ Unknown

If Abnormal, check all that apply:

I. Cavities ☐ Yes ☐ No ☐ Unknown

II. Infiltrate ☐ Yes ☐ No ☐ Unknown

III. Pleural effusion ☐ Yes ☐ No ☐ Unknown

IV. Bilateral ☐ Yes ☐ No ☐ Unknown

D. HIV STATUS

1. HIV Test Results ☐ Positive ☐ Negative ☐ Pending ☐ Not Tested

If HIV Positive or Negative:

Date of most recent test: _____ (DD-MM-YYYY) ☐ Unavailable

Source: ☐ Results report from laboratory or HIV testing site

☐ HIV status recorded in chart without other documentation

If HIV Positive:

1a. Most recent CD4 count (cells/mm3): _____ ☐ Unavailable

Date of most recent test: _____ (DD-MM-YYYY) ☐ Unavailable

1b. Viral load (copies/mm3): _____ ☐ Unavailable

Date of most recent test: _____ (DD-MM-YYYY) ☐ Unavailable

REFERENCES

1. WHO. Global Tuberculosis Report 2013. In. Geneva, Switzerland: World Health Organization; 2013.

2. Mathema B, Kurepina NE, Bifani PJ, Kreiswirth BN. Molecular epidemiology of tuberculosis: Current insights. *Clinical Microbiology Reviews* 2006,**19**:658-+.

3. Ndungu PW, Revathi G, Kariuki S, Ng'ang'a Z. Risk Factors in the Transmission of Tuberculosis in Nairobi: A Descriptive Epidemiological Study. In: *Advances in Microbiology*; 2013. pp. 160-165.

4. Kaufmann SHE. How can immunology contribute to the control of tuberculosis? *Nature Reviews Immunology* 2001,**1**:20-30.

5. Al-Mutairi NM, Ahmad S, Mokaddas E. First report of molecular detection of fluoroquinolone resistance-associated gyrA mutations in multidrug-resistant clinical Mycobacterium tuberculosis isolates in Kuwait. *BMC research notes* 2011,**4**:123.

6. Jeon CY, Calver AD, Victor TC, Warren RM, Shin SS, Murray MB. Use of fluoroquinolone antibiotics leads to tuberculosis treatment delay in a South African gold mining community. *International Journal of Tuberculosis and Lung Disease* 2011,**15**:77-83.

7. Li J, Gao X, Luo T, Wu J, Sun G, Liu Q, *et al.* Association of gyrA/B mutations and resistance levels to fluoroquinolones in clinical isolates of Mycobacterium tuberculosis. *Emerging Microbes & Infections* 2014,**3**.

8. Devasia R, Blackman A, Eden S, Li H, Maruri F, Shintani A, *et al.* High Proportion of Fluoroquinolone-Resistant M. tuberculosis Isolates with Novel Gyrase Polymorphisms and a gyrA Region Associated with Fluoroquinolone Susceptibility. *Journal of Clinical Microbiology* 2011.

9. van der Heijden YF, Maruri F, Blackman A, Mitchel E, Bian A, Shintani AK, *et al.* Fluoroquinolone susceptibility in Mycobacterium tuberculosis after pre-diagnosis exposure to older- versus newer-generation fluoroquinolones. *International Journal of Antimicrobial Agents* 2013,**42**:232-237.

10. Devasia RA, Blackman A, Gebretsadik T, Griffin M, Shintani A, May C, *et al.* Fluoroquinolone Resistance in Mycobacterium tuberculosis The Effect of Duration and Timing of Fluoroquinolone Exposure. *American Journal of Respiratory and Critical Care Medicine* 2009,**180**:365-370.

11. Zhang Z, Lu J, Wang Y, Pang Y, Zhao Y. Prevalence and Molecular Characterization of Fluoroquinolone-Resistant Mycobacterium tuberculosis Isolates in China. *Antimicrobial Agents and Chemotherapy* 2014,**58**:364-369.

12. Hillemann D, Ruesch-Gerdes S, Richter E. Feasibility of the GenoType MTBDRsl Assay for Fluoroquinolone, Amikacin-Capreomycin, and Ethambutol Resistance Testing of Mycobacterium tuberculosis Strains and Clinical Specimens. *Journal of Clinical Microbiology* 2009,**47**:1767-1772.

13. An DD, Duyen NTH, Lan NTN, Hoa DV, Ha DTM, Kiet VS, *et al.* Beijing Genotype of Mycobacterium tuberculosis Is Significantly Associated with High-Level Fluoroquinolone Resistance in Vietnam. *Antimicrobial Agents and Chemotherapy* 2009,**53**:4835-4839.

14. Pantel A, Petrella S, Veziris N, Brossier F, Bastian S, Jarlier V, *et al.* Extending the Definition of the GyrB Quinolone Resistance-Determining Region in Mycobacterium tuberculosis DNA Gyrase for Assessing Fluoroquinolone Resistance in M. tuberculosis. *Antimicrobial Agents and Chemotherapy* 2012,**56**:1990-1996.

15. Chakravorty S, Aladegbami B, Thoms K, Lee JS, Lee EG, Rajan V, *et al.* Rapid Detection of Fluoroquinolone-Resistant and Heteroresistant Mycobacterium tuberculosis by Use of Sloppy Molecular Beacons and Dual Melting-Temperature Codes in a Real-Time PCR Assay. *Journal of Clinical Microbiology* 2011,**49**:932-940.

16. Kam KM, Yip CW, Cheung TL, Tang HS, Leung OC, Chan MY. Stepwise decrease in moxifloxacin susceptibility amongst clinical isolates of multidrug-resistant Mycobacterium tuberculosis: Correlation with ofloxacin susceptibility. *Microbial Drug Resistance-Mechanisms Epidemiology and Disease* 2006,**12**:7-11.

17. van Klingeren B, Dessens-Kroon M, van der Laan T, Kremer K, van Soolingen D. Drug susceptibility testing of Mycobacterium tuberculosis complex by use of a high-throughput, reproducible, absolute concentration method. *Journal of Clinical Microbiology* 2007,**45**:2662-2668.

18. Umubyeyi AN, Rigouts L, Shamputa IC, Fissette K, Elkrim Y, de Rijk PWB, *et al.* Limited fluoroquinolone resistance among Mycobacterium tuberculosis isolates from Rwanda: results of a national survey. *The Journal of antimicrobial chemotherapy* 2007,**59**:1031-1033.

19. Bwanga F, Hoffner S, Haile M, Joloba ML. Direct susceptibility testing for multi drug resistant tuberculosis: A meta-analysis. *Bmc Infectious Diseases* 2009,**9**.

20. Zhao L-L, Xia Q, Lin N, Liu Z-G, Zhao X-Q, Wan K-L. Multiplex allele-specific PCR combined with PCR-RFLP analysis for rapid detection of gyrA gene fluoroquinolone resistance mutations in Mycobacterium tuberculosis. *Journal of microbiological methods* 2012,**88**:175-178.

21. Ali A, Hasan R, Jabeen K, Jabeen N, Qadeer E, Hasan Z. Characterization of Mutations Conferring Extensive Drug Resistance to Mycobacterium tuberculosis Isolates in Pakistan. *Antimicrobial Agents and Chemotherapy* 2011,**55**:5654-5659.

22. Pantel A, Petrella S, Matrat S, Brossier F, Bastian S, Reitter D, *et al.* DNA Gyrase Inhibition Assays Are Necessary To Demonstrate Fluoroquinolone Resistance Secondary to gyrB Mutations in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 2011,**55**:4524-4529.

23. Lau RWT, Ho PL, Kao RYT, Yew WW, Lau TCK, Cheng VCC, *et al.* Molecular Characterization of Fluoroquinolone Resistance in Mycobacterium tuberculosis: Functional Analysis of gyrA Mutation at Position 74. *Antimicrobial Agents and Chemotherapy* 2011,**55**:608-614.

24. Leung KL, Yip CW, Yeung YL, Wong KL, Chan WY, Chan MY, *et al.* Usefulness of resistant gene markers for predicting treatment outcome on second-line anti-tuberculosis drugs. *Journal of Applied Microbiology* 2010,**109**:2087-2094.

25. Yam WC, Tam CM, Leung CC, Tong HL, Chan KH, Leung ETY, *et al.* Direct detection of rifampin-resistant mycobacterium tuberculosis in respiratory specimens by PCR-DNA sequencing. *Journal of clinical microbiology* 2004,**42**:4438-4443.

26. Kolappan C, Gopi PG, Subramani R, Narayanan PR. Selected biological and behavioural risk factors associated with pulmonary tuberculosis. *International Journal of Tuberculosis and Lung Disease* 2007,**11**:999-1003.

27. Yu GP, Hsieh CC, Peng J. Risk-factors associated with the prevalence of pulmonary tuberculosis among sanitary workers in shanghai. *Tubercle* 1988,**69**:105-112.

28. Macedo R, Antunes AF, Villar M, Portugal I. Multidrug and extensively drug-resistant tuberculosis in Lisbon and Vale do Tejo, Portugal, from 2008 to 2010. *International Journal of Mycobacteriology*,**1**:131-136.

29. Choi JC, Lim SY, Suh GY, Chung MP, Kim H, Kwon J, *et al.* Drug resistance rates of Mycobacterium tuberculosis at a private referral center in Korea. *Journal of Korean Medical Science* 2007,**22**:677-681.

30. Greenwood CMT, Fujiwara TM, Boothroyd LJ, Miller MA, Frappier D, Fanning EA, *et al.* Linkage of tuberculosis to chromosome 2q35 loci, including NRAMP1, in a large aboriginal Canadian family. *American Journal of Human Genetics* 2000,**67**:405-416.

31. Nava-Aguilera E, Andersson N, Harris E, Mitchell S, Hamel C, Shea B, *et al.* Risk factors associated with recent transmission of tuberculosis: systematic review and meta-analysis. *International Journal of Tuberculosis and Lung Disease* 2009,**13**:17-26.

32. Mulligan CJ, Hunley K, Cole S, Long JC. Population genetics, history, and health patterns in native Americans. *Annual Review of Genomics and Human Genetics* 2004,**5**:295-315.

33. Waitt CJ, Squire SB. A systematic review of risk factors for death in adults during and after tuberculosis treatment. *International Journal of Tuberculosis and Lung Disease* 2011,**15**:871-885.

34. Balasubramanian R, Garg R, Santha T, Gopi PG, Subramani R, Chandrasekaran V, *et al.* Gender disparities in tuberculosis: report from a rural DOTS programme in South India. *International Journal of Tuberculosis and Lung Disease* 2004,**8**:323-332.

35. Duarte EC, Bierrenbach AL, da Silva JB, Jr., Tauil PL, Duarte EdF. Factors associated with deaths among pulmonary tuberculosis patients: a case-control study with secondary data. *Journal of Epidemiology and Community Health* 2009,**63**:233-238.

36. Lienhardt C, Fielding K, Sillah JS, Bah B, Gustafson P, Warndorff D, *et al.* Investigation of the risk factors for tuberculosis: a case-control study in three countries in West Africa. *International Journal of Epidemiology* 2005,**34**:914-923.

37. Gustafson P, Gomes VF, Vieira CS, Rabna P, Seng R, Johansson P, *et al.* Tuberculosis in Bissau: incidence and risk factors in an urban community in sub-Saharan Africa. *International Journal of Epidemiology* 2004,**33**:163-172.

38. Pang PTT, Leung CC, Lee SS. Neighbourhood risk factors for tuberculosis in Hong Kong. *International Journal of Tuberculosis and Lung Disease* 2010,**14**:585-592.

39. Wingfield T, Schumacher SG, Sandhu G, Tovar MA, Zevallos K, Baldwin MR, *et al.* The seasonality of tuberculosis, sunlight, vitamin D, and household crowding. *The Journal of infectious diseases* 2014,**210**:774-783.

40. Baussano I, Williams BG, Nunn P, Beggiato M, Fedeli U, Scano F. Tuberculosis Incidence in Prisons: A Systematic Review. *Plos Medicine* 2010,**7**.

41. Kong PM, Tapy J, Calixto P, Burman WJ, Reves RR, Yang ZH, *et al.* Skin-test screening and tuberculosis transmission among the homeless. *Emerging Infectious Diseases* 2002,**8**:1280-1284.

42. Narasimhan P, Wood J, Macintyre CR, Mathai D. Risk factors for tuberculosis. *Pulmonary medicine* 2013,**2013**:828939-828939.

43. Loennroth K, Castro KG, Chakaya JM, Chauhan LS, Floyd K, Glaziou P, *et al.* Tuberculosis control and elimination 2010-50: cure, care, and social development. *Lancet* 2010,**375**:1814-1829.

44. Oxlade O, Murray M. Tuberculosis and Poverty: Why Are the Poor at Greater Risk in India? *Plos One* 2012,**7**.

45. Younis MZ, Rivers PA, Fottler MD. The impact of HMO and hospital competition on hospital costs. *Journal of health care finance* 2005,**31**:60-74.

46. Bates MN, Khalakdina A, Pai M, Chang L, Lessa F, Smith KR. Risk of tuberculosis from exposure to tobacco smoke - A systematic review and meta-analysis. *Archives of Internal Medicine* 2007,**167**:335-342.

47. Slam K, Chiang CY, Enarson DA, Hassmillert K, Fanning A, Gupta P, *et al.* Tobacco and tuberculosis: a qualitative systematic review and meta-analysis. *International Journal of Tuberculosis and Lung Disease* 2007,**11**:1049-1061.

48. Arcavi L, Benowitz NL. Cigarette smoking and infection. *Archives of Internal Medicine* 2004,**164**:2206-2216.

49. Loennroth K, Williams BG, Stadlin S, Jaramillo E, Dye C. Alcohol use as a risk factor for tuberculosis - a systematic review. *Bmc Public Health* 2008,**8**.

50. Winetsky DE, Almukhamedov O, Pulatov D, Vezhnina N, Dooronbekova A, Zhussupov B. Prevalence, Risk Factors and Social Context of Active Pulmonary Tuberculosis among Prison Inmates in Tajikistan. *Plos One* 2014,**9**.

51. Ribeiro Macedo L, Reis-Santos B, Riley LW, Maciel EL. Treatment outcomes of tuberculosis patients in Brazilian prisons: a polytomous regression analysis. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2013,**17**:1427-1434.

52. Telisinghe L, Fielding KL, Malden JL, Hanifa Y, Churchyard GJ, Grant AD, *et al.* High Tuberculosis Prevalence in a South African Prison: The Need for Routine Tuberculosis Screening. *Plos One* 2014,**9**.

53. Wei G, Moss J, Yuan CS. Opioid-induced immunosuppression: is it centrally mediated or peripherally mediated? *Biochemical Pharmacology* 2003,**65**:1761-1766.

54. Story A, Murad S, Roberts W, Verheyen M, Hayward AC, London Tuberculosis Nurses N. Tuberculosis in London: The importance of homelessness, problem drug use and prison. *Thorax* 2007,**62**:667-671.

55. Deiss RG, Rodwell TC, Garfein RS. Tuberculosis and Illicit Drug Use: Review and Update. *Clinical Infectious Diseases* 2009,**48**:72-82.

56. Zetola NM, Macesic N, Modongo C, Shin S, Ncube R, Collman RG. Longer hospital stay is associated with higher rates of tuberculosis-related morbidity and mortality within 12 months after discharge in a referral hospital in Sub-Saharan Africa. *Bmc Infectious Diseases* 2014,**14**.

57. Andrews JR, Shah NS, Weissman D, Moll AP, Friedland G, Gandhi NR. Predictors of Multidrug- and Extensively Drug-Resistant Tuberculosis in a High HIV Prevalence Community. *Plos One* 2010,**5**.

58. Jeon CY, Murray MB. Diabetes mellitus increases the risk of active tuberculosis: A systematic review of 13 observational studies (vol 5, pg e152, 2008). *Plos Medicine* 2008,**5**:1298-1298.

59. Alisjahbana B, van Crevel R, Sahiratmadja E, den Heijer M, Maya A, Istriana E, *et al.* Diabetes mellitus is strongly associated with tuberculosis in Indonesia. *International Journal of Tuberculosis and Lung Disease* 2006,**10**:696-700.

60. Martens GW, Arikan MC, Lee J, Ren F, Greiner D, Kornfeld H. Tuberculosis susceptibility of diabetic mice. *American Journal of Respiratory Cell and Molecular Biology* 2007,**37**:518-524.

61. Dooley KE, Chaisson RE. Tuberculosis and diabetes mellitus: convergence of two epidemics. *Lancet Infectious Diseases* 2009,**9**:737-746.

62. Hicks RM, Padayatchi N, Shah NS, Wolf A, Werner L, Sunkari VB, *et al.* Malnutrition associated with unfavorable outcome and death among South African MDR-TB and HIV co-infected children. *International Journal of Tuberculosis and Lung Disease* 2014,**18**:1074-1079.

63. Moran-Mendoza O, Marion SA, Elwood K, Patrick D, FitzGerald JM. Risk factors for developing tuberculosis: a 12-year follow-up of contacts of tuberculosis cases. *International Journal of Tuberculosis and Lung Disease* 2010,**14**:1112-1119.

64. Patel KB, Belmonte R, Crowe HM. Drug malabsorption and resistant tuberculosis in hiv-infected patients. *New England Journal of Medicine* 1995,**332**:336-337.

65. Suchindran S, Brouwer ES, Van Rie A. Is HIV Infection a Risk Factor for Multi-Drug Resistant Tuberculosis? A Systematic Review. *Plos One* 2009,**4**.

66. Joshi R, Reingold AL, Menzies D, Pai M. Tuberculosis among health-care workers in low- and middle-income countries: A systematic review. *Plos Medicine* 2006,**3**:2376-2391.

67. Fox GJ, Barry SE, Britton WJ, Marks GB. Contact investigation for tuberculosis: a systematic review and meta-analysis. *European Respiratory Journal* 2013,**41**:140-156.

68. Verdier JE, Jan de Vlas S, Kidgell-Koppelaar ID, Richardus JH. Risk factors for tuberculosis in contact investigations in Rotterdam, the Netherlands. *Infectious disease reports* 2012,**4**:e26-e26.

69. Faustini A, Hall AJ, Perucci CA. Risk factors for multidrug resistant tuberculosis in Europe: a systematic review. *Thorax* 2006,**61**:158-163.

70. Espinal MA, Laserson K, Camacho M, Fusheng Z, Kim SJ, Tiali E, *et al.* Determinants of drug-resistant tuberculosis: analysis of 11 countries. *International Journal of Tuberculosis and Lung Disease* 2001,**5**:887-893.

71. Chen S, Huai P, Wang X, Zhong J, Wang X, Wang K, *et al.* Risk factors for multidrug resistance among previously treated patients with tuberculosis in eastern China: a case-control study. *International Journal of Infectious Diseases* 2013,**17**:E1116-E1120.

72. Hillery N, Groessl E, Trollip A, Catanzaro D, Jackson L, Rodwell T, *et al.* The Global Consortium for Drug-resistant Tuberculosis Diagnostics (GCDD): design of a multi-site, head-to-head study of three rapid tests to detect extensively drug-resistant tuberculosis. In: Trials; 2014.

73. Ando H, Mitarai S, Kondo Y, Suetake T, Kato S, Mori T, *et al.* Evaluation of a line probe assay for the rapid detection of gyrA mutations associated with fluoroquinolone resistance in multidrug-resistant Mycobacterium tuberculosis. *Journal of Medical Microbiology* 2011,**60**:184-188.

74. Kontsevaya I, Mironova S, Nikolayevskyy V, Balabanova Y, Mitchell S, Drobniewski F. Evaluation of Two Molecular Assays for Rapid Detection of Mycobacterium tuberculosis Resistance to Fluoroquinolones in High-Tuberculosis and -Multidrug-Resistance Settings. *Journal of Clinical Microbiology* 2011,**49**:2832-2837.

75. Soudani A, Hadjfredj S, Zribi M, Messaoud T, Masmoudi A, Majed B, *et al.* First report of molecular characterization of fluoroquinolone-resistant Mycobacterium tuberculosis isolates from a Tunisian hospital. *Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases* 2010,**16**:1454-1457.

76. Mokrousov I, Otten T, Manicheva O, Potapova Y, Vishnevsky B, Narvskaya O, *et al.* Molecular characterization of ofloxacin-resistant Mycobacterium tuberculosis strains from Russia. *Antimicrobial agents and chemotherapy* 2008,**52**:2937-2939.

77. Bravo LTC, Tuohy MJ, Ang C, Destura RV, Mendoza M, Procop GW, *et al.* Pyrosequencing for Rapid Detection of Mycobacterium tuberculosis Resistance to Rifampin, Isoniazid, and Fluoroquinolones. *Journal of Clinical Microbiology* 2009,**47**:3985-3990.

78. Cui Z, Wang J, Lu J, Huang X, Hu Z. Association of mutation patterns in gyrA/B genes and ofloxacin resistance levels in Mycobacterium tuberculosis isolates from East China in 2009. *Bmc Infectious Diseases* 2011,**11**.

79. Duong DA, Nguyen THD, Nguyen TNL, Dai VH, Dang TMH, Vo SK, *et al.* Beijing genotype of Mycobacterium tuberculosis is significantly associated with high-level fluoroquinolone resistance in Vietnam. *Antimicrobial agents and chemotherapy* 2009,**53**:4835-4839.

80. Takiff HE, Salazar L, Guerrero C, Philipp W, Huang WM, Kreiswirth B, *et al.* Cloning and nucleotide sequence of Mycobacterium tuberculosis gyrA and gyrB genes and detection of quinolone resistance mutations. *Antimicrobial agents and chemotherapy* 1994,**38**:773-780.

81. Maruri F, Sterling TR, Kaiga AW, Blackman A, van der Heijden YF, Mayer C, *et al.* A systematic review of gyrase mutations associated with fluoroquinolone-resistant Mycobacterium tuberculosis and a proposed gyrase numbering system. *Journal of Antimicrobial Chemotherapy* 2012,**67**:819-831.

82. Alangaden GJ, Manavathu EK, Vakulenko SB, Zvonok NM, Lerner SA. Characterization of fluoroquinolone-resistant mutant strains of Mycobacterium tuberculosis selected in the laboratory and isolated from patients. *Antimicrobial agents and chemotherapy* 1995,**39**:1700-1703.

83. Wang J-Y, Lee L-N, Lai H-C, Wang S-K, Jan IS, Yu C-J, *et al.* Fluoroquinolone resistance in Mycobacterium tuberculosis isolates: associated genetic mutations and relationship to antimicrobial exposure. *The Journal of antimicrobial chemotherapy* 2007,**59**:860-865.

84. Malik S, Willby M, Sikes D, Tsodikov OV, Posey JE. New Insights into Fluoroquinolone Resistance in Mycobacterium tuberculosis: Functional Genetic Analysis of gyrA and gyrB Mutations. *Plos One* 2012,**7**.

85. Anand RS, Somasundaram S, Doble M, Paramasivan CN. Docking studies on novel analogues of 8 methoxy fluoroquinolones against GyrA mutants of Mycobacterium tuberculosis. *BMC structural biology* 2011,**11**:47.

86. Aubry A, Veziris N, Cambau E, Truffot-Pernot C, Jarlier V, Fisher LM. Novel gyrase mutations in quinolone-resistant and -hypersusceptible clinical isolates of Mycobacterium tuberculosis: Functional analysis of mutant enzymes. *Antimicrobial Agents and Chemotherapy* 2006,**50**:104-112.

87. Prammananan T, Chaiprasert A, Leechawengwongs M. 8-years experience of fluoroquinolone susceptibility testing of multidrug-resistant Mycobacterium tuberculosis isolates from Siriraj Hospital, Thailand. *International journal of antimicrobial agents* 2011,**37**:84-85.

88. WHO. Anti-Tuberculosis Drug Resistance in the World. In. Geneva, Switzerland: World Health Organization; 2008.

89. Georghiou SB, Magana M, Garfein RS, Catanzaro DG, Catanzaro A, Rodwell TC. Evaluation of Genetic Mutations Associated with Mycobacterium tuberculosis Resistance to Amikacin, Kanamycin and Capreomycin: A Systematic Review. *Plos One* 2012,**7**.

90. Sun Z, Zhang J, Zhang X, Wang S, Zhang Y, Li C. Comparison of gyrA gene mutations between laboratory-selected ofloxacin-resistant Mycobacterium tuberculosis strains and clinical isolates. *International journal of antimicrobial agents* 2008,**31**:115-121.

91. Tahmasebi P, Farnia P, Sheikholslami F, Velayati A. Rapid identification of extensively and extremely drug resistant tuberculosis from multidrug resistant strains; using PCR-RFLP and PCR-SSCP. *Iranian journal of microbiology* 2012,**4**:165-170.

92. Nosova EY, Bukatina AA, Isaeva YD, Makarova MV, Galkina KY, Moroz AM. Analysis of mutations in the gyrA and gyrB genes and their association with the resistance of Mycobacterium tuberculosis to levofloxacin, moxifloxacin and gatifloxacin. *Journal of medical microbiology* 2013,**62**:108-113.

93. Zhu C, Zhang Y, Shen Y, Siu GKH, Wu W, Qian X, *et al.* Molecular characterization of fluoroquinolone-resistant Mycobacterium tuberculosis clinical isolates from Shanghai, China. *Diagnostic microbiology and infectious disease* 2012,**73**:260-263.

94. Post FA, Willcox PA, Mathema B, Steyn LM, Shean K, Ramaswamy SV, *et al.* Genetic polymorphism in Mycobacterium tuberculosis isolates from patients with chronic multidrug-resistant tuberculosis. *The Journal of infectious diseases* 2004,**190**:99-106.

95. Hu Y, Mathema B, Wang W, Kreiswirth B, Jiang W, Xu B. Population-based investigation of fluoroquinolones resistant tuberculosis in rural eastern China. *Tuberculosis* 2011,**91**:238-243.

96. Chernyaeva E, Fedorova E, Zhemkova G, Korneev Y, Kozlov A. Characterization of multiple and extensively drug resistant Mycobacterium tuberculosis isolates with different ofloxacin-resistance levels. *Tuberculosis (Edinburgh, Scotland)* 2013,**93**:291-295.

97. Huang TS, Kunin CM, Lee SSJ, Chen YS, Tu HZ, Liu YC. Trends in fluoroquinolone resistance of Mycobacterium tuberculosis complex in a Taiwanese medical centre: 1995-2003. *Journal of Antimicrobial Chemotherapy* 2005,**56**:1058-1062.

98. Streicher EM, Bergval I, Dheda K, Bottger EC, Gey van Pittius NC, Bosman M, *et al.* Mycobacterium tuberculosis population structure determines the outcome of genetics-based second-line drug resistance testing. *Antimicrobial agents and chemotherapy* 2012,**56**:2420-2427.

99. Chen J, Chen Z, Li Y, Xia W, Chen X, Chen T, *et al.* Characterization of gyrA and gyrB mutations and fluoroquinolone resistance in Mycobacterium tuberculosis clinical isolates from Hubei Province, China. *The Brazilian journal of infectious diseases : an official publication of the Brazilian Society of Infectious Diseases* 2012,**16**:136-141.

100. Yin X, Yu Z. Mutation characterization of gyrA and gyrB genes in levofloxacin-resistant Mycobacterium tuberculosis clinical isolates from Guangdong Province in China. *The Journal of infection* 2010,**61**:150-154.

101. Antonova OV, Gryadunov DA, Lapa SA, Kuz'min AV, Larionova EE, Smirnova TG, *et al.* Detection of mutations in Mycobacterium tuberculosis genome determining resistance to fluoroquinolones by hybridization on biological microchips. *Bulletin of Experimental Biology and Medicine* 2008,**145**:108-113.

102. Poudel A, Maharjan B, Nakajima C, Fukushima Y, Pandey BD, Beneke A, *et al.* Characterization of extensively drug-resistant Mycobacterium tuberculosis in Nepal. *Tuberculosis (Edinburgh, Scotland)* 2013,**93**:84-88.

103. El Sahly HM, Teeter LD, Jost KC, Jr., Dunbar D, Lew J, Graviss EA. Incidence of Moxifloxacin Resistance in Clinical Mycobacterium tuberculosis Isolates in Houston, Texas. *Journal of Clinical Microbiology* 2011,**49**:2942-2945.

104. Sirgel FA, Wiid IJF, van Helden PD. Measuring minimum inhibitory concentrations in mycobacteria. *Methods in molecular biology (Clifton, N.J.)* 2009,**465**:173-186.

105. Long Q, Li W, Du Q, Fu Y, Liang Q, Huang H, *et al.* gyrA/B fluoroquinolone resistance allele profiles amongst Mycobacterium tuberculosis isolates from mainland China. *International Journal of Antimicrobial Agents* 2012,**39**:486-489.

106. Yuan X, Zhang T, Kawakami K, Zhu J, Li H, Lei J, *et al.* Molecular characterization of multidrug- and extensively drug-resistant Mycobacterium tuberculosis strains in Jiangxi, China. *Journal of clinical microbiology* 2012,**50**:2404-2413.

107. Singh M, Jadaun GPS, Ramdas, Srivastava K, Chauhan V, Mishra R, *et al.* Effect of efflux pump inhibitors on drug susceptibility of ofloxacin resistant Mycobacterium tuberculosis isolates. *Indian Journal of Medical Research* 2011,**133**:535-540.

108. Williams KJ, Chan R, Piddock LJ. gyrA of ofloxacin-resistant clinical isolates of Mycobacterium tuberculosis from Hong Kong. *The Journal of antimicrobial chemotherapy* 1996,**37**:1032-1034.

109. Sekiguchi J-i, Disratthakit A, Maeda S, Doi N. Characteristic Resistance Mechanism of Mycobacterium tuberculosis to DC-159a, a New Respiratory Quinolone. *Antimicrobial Agents and Chemotherapy* 2011,**55**:3958-3960.

110. Lee ASG, Tang LLH, Lim IHK, Wong SY. Characterization of pyrazinamide and ofloxacin resistance among drug resistant Mycobacterium tuberculosis isolates from Singapore. *International journal of infectious diseases : IJID : official publication of the International Society for Infectious Diseases* 2002,**6**:48-51.

111. Jnawali HN, Hwang SC, Park YK, Kim H, Lee YS, Chung GT, *et al.* Characterization of mutations in multi- and extensive drug resistance among strains of Mycobacterium tuberculosis clinical isolates in Republic of Korea. *Diagnostic microbiology and infectious disease* 2013,**76**:187-196.

112. Xu C, Kreiswirth BN, Sreevatsan S, Musser JM, Drlica K. Fluoroquinolone resistance associated with specific gyrase mutations in clinical isolates of multidrug-resistant Mycobacterium tuberculosis. *The Journal of infectious diseases* 1996,**174**:1127-1130.

113. Sulochana S, Narayanan S, Paramasivan CN, Suganthi C, Narayanan PR. Analysis of fluoroquinolone resistance in clinical isolates of Mycobacterium tuberculosis from India. *Journal of chemotherapy (Florence, Italy)* 2007,**19**:166-171.

114. Chan RCY, Hui M, Chan EWC, Au TK, Chin ML, Yip CK, *et al.* Genetic and phenotypic characterization of drug-resistant Mycobacterium tuberculosis isolates in Hong Kong. *Journal of Antimicrobial Chemotherapy* 2007,**59**:866-873.

115. Brossier F, Veziris N, Aubry A, Jarlier V, Sougakoff W. Detection by GenoType MTBDRsl Test of Complex Mechanisms of Resistance to Second-Line Drugs and Ethambutol in Multidrug-Resistant Mycobacterium tuberculosis Complex Isolates. *Journal of Clinical Microbiology* 2010,**48**:1683-1689.

116. Huang W-L, Chi T-L, Wu M-H, Jou R. Performance Assessment of the GenoType MTBDRsl Test and DNA Sequencing for Detection of Second-Line and Ethambutol Drug Resistance among Patients Infected with Multidrug-Resistant Mycobacterium tuberculosis. *Journal of Clinical Microbiology* 2011,**49**:2502-2508.

117. Siddiqi N, Shamim M, Hussain S, Choudhary RK, Ahmed N, Prachee, *et al.* Molecular characterization of multidrug-resistant isolates of Mycobacterium tuberculosis from patients in North India. *Antimicrobial agents and chemotherapy* 2002,**46**:443-450.

118. Kiet VS, Lan NTN, An DD, Dung NH, Hoa DV, van Vinh Chau N, *et al.* Evaluation of the MTBDRsl test for detection of second-line-drug resistance in Mycobacterium tuberculosis. *Journal of clinical microbiology* 2010,**48**:2934-2939.

119. Perdigao J, Macedo R, Malaquias A, Ferreira A, Brum L, Portugal I. Genetic analysis of extensively drug-resistant Mycobacterium tuberculosis strains in Lisbon, Portugal. *The Journal of antimicrobial chemotherapy* 2010,**65**:224-227.

120. Suzuki Y, Nakajima C, Tamaru A, Kim H, Matsuba T, Saito H. Sensitivities of ciprofloxacin-resistant Mycobacterium tuberculosis clinical isolates to fluoroquinolones: role of mutant DNA gyrase subunits in drug resistance. *International Journal of Antimicrobial Agents* 2012,**39**:435-439.

121. Escribano I, Rodriguez C, Llorca B, Garcia-Pachon E, Ruiz M, Royo G. Importance of the efflux pump systems in the resistance of Mycobacterium tuberculosis to Fluoroquinolones and linezolid. *Chemotherapy* 2007,**53**:397-401.

122. van Doorn HR, An DD, de Jong MD, Lan NTN, Hoa DV, Quy HT, *et al.* Fluoroquinolone resistance detection in Mycobacterium tuberculosis with locked nucleic acid probe real-time PCR. *The international journal of tuberculosis and lung disease : the official journal of the International Union against Tuberculosis and Lung Disease* 2008,**12**:736-742.

123. Giannoni F, Iona E, Sementilli F, Brunori L, Pardini M, Migliori GB, *et al.* Evaluation of a new line probe assay for rapid identification of gyrA mutations in Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 2005,**49**:2928-2933.

124. Louw GE, Warren RM, van Pittius NCG, McEvoy CRE, Van Helden PD, Victor TC. A Balancing Act: Efflux/Influx in Mycobacterial Drug Resistance. *Antimicrobial Agents and Chemotherapy* 2009,**53**:3181-3189.

125. Sun G, Luo T, Yang C, Dong X, Li J, Zhu Y, *et al.* Dynamic Population Changes in Mycobacterium tuberculosis During Acquisition and Fixation of Drug Resistance in Patients. *Journal of Infectious Diseases* 2012,**206**:1724-1733.

126. Surcouf C, Heng S, Pierre-Audigier C, Cadet-Daniel V, Namouchi A, Murray A, *et al.* Molecular detection of fluoroquinolone-resistance in multi-drug resistant tuberculosis in Cambodia suggests low association with XDR phenotypes. *Bmc Infectious Diseases* 2011,**11**.

127. Barnard M, Warren R, Van Pittius NG, van Helden P, Bosman M, Streicher E, *et al.* GenoType MTBDRsl Line Probe Assay Shortens Time to Diagnosis of Extensively Drug-Resistant Tuberculosis in a High-Throughput Diagnostic Laboratory. *American Journal of Respiratory and Critical Care Medicine* 2012,**186**:1298-1305.

128. Feng Y, Liu S, Wang Q, Wang L, Tang S, Wang J, *et al.* Rapid Diagnosis of Drug Resistance to Fluoroquinolones, Amikacin, Capreomycin, Kanamycin and Ethambutol Using Genotype MTBDRsl Assay: A Meta-Analysis. *Plos One* 2013,**8**.

129. Theron G, Peter J, Richardson M, Barnard M, Donegan S, Warren R, *et al.* The diagnostic accuracy of the GenoType(r) MTBDRsl assay for the detection of resistance to second-line anti-tuberculosis drugs. In: Cochrane Database of Systematic Reviews; 2014.

130. WHO. The Use of Molecular Line Probe Assay for the Detection of Resistance to Second-Line Anti-Tuberculosis Drugs. In. Geneva, Switzerland: World Health Organization; 2013.

131. Rodwell TC, Valafar F, Douglas J, Qian L, Garfein RS, Chawla A, *et al.* Predicting Extensively Drug-Resistant Mycobacterium tuberculosis Phenotypes with Genetic Mutations. *Journal of Clinical Microbiology* 2014,**52**:781-789.

132. Campbell PJ, Morlock GP, Sikes RD, Dalton TL, Metchock B, Starks AM, *et al.* Molecular Detection of Mutations Associated with First- and Second-Line Drug Resistance Compared with Conventional Drug Susceptibility Testing of Mycobacterium tuberculosis. *Antimicrobial Agents and Chemotherapy* 2011,**55**:2032-2041.

133. WHO. Global Tuberculosis Report 2014. In. Geneva, Switzerland: World Health Organization; 2014.

134. Porwal C, Kaushik A, Makkar N, Banavaliker JN, Hanif M, Singla R, *et al.* Incidence and Risk Factors for Extensively Drug-Resistant Tuberculosis in Delhi Region. *Plos One* 2013,**8**.

135. Sagonda T, Mupfumi L, Manzou R, Makamure B, Tshabalala M, Gwanzura L, *et al.* Prevalence of Extensively Drug Resistant Tuberculosis among Archived Multidrug Resistant Tuberculosis Isolates in Zimbabwe. *Tuberculosis research and treatment* 2014,**2014**:349141-349141.

136. Isaakidis P, Das M, Kumar AMV, Peskett C, Khetarpal M, Bamne A, *et al.* Alarming Levels of Drug-Resistant Tuberculosis in HIV-Infected Patients in Metropolitan Mumbai, India. *PloS one* 2014,**9**:e110461-e110461.

137. Dalal A, Pawaskar A, Das M, Desai R, Prabhudesai P, Chhajed P, *et al.* Resistance Patterns among Multidrug-Resistant Tuberculosis Patients in Greater Metropolitan Mumbai: Trends over Time. *Plos One* 2015,**10**.

138. Jenkins HE, Crudu V, Soltan V, Ciobanu A, Domente L, Cohen T. High risk and rapid appearance of multidrug resistance during tuberculosis treatment in Moldova. *European Respiratory Journal* 2014,**43**:1132-1141.

139. Crudu V, Arnadottir T, Laticevschi D. Resistance to anti-tuberculosis drugs and practices in drug susceptibility testing in Moldova, 1995-1999. *International Journal of Tuberculosis and Lung Disease* 2003,**7**:336-342.

140. Bhembe NL, Nwodo UU, Govender S, Hayes C, Ndip RN, Okoh AI, *et al.* Molecular detection and characterization of resistant genes in Mycobacterium

tuberculosis complex from DNA isolated from tuberculosis patients in the Eastern Cape province South Africa. *Bmc Infectious Diseases* 2014,**14**.

141. Klopper M, Warren RM, Hayes C, van Pittius NCG, Streicher EM, Mueller B, *et al.* Emergence and Spread of Extensively and Totally Drug-Resistant Tuberculosis, South Africa. *Emerging Infectious Diseases* 2013,**19**:449-455.

142. Ruddy M, Balabanova Y, Graham C, Fedorin I, Malomanova N, Elisarova E, *et al.* Rates of drug resistance and risk factor analysis in civilian and prison patients with tuberculosis in Samara Region, Russia. *Thorax* 2005,**60**:130-135.

143. Shah NS, Pratt R, Armstrong L, Robison V, Castro KG, Cegielski JP. Extensively Drug-Resistant Tuberculosis in the United States, 1993-2007. *Jama-Journal of the American Medical Association* 2008,**300**:2153-2160.

144. WHO. Treatment of tuberculosis guidelines-4th ed. In. Geneva, Switzerland: World Health Organization; 2010.

145. Siddiqi S, Rusch-Gerdes S. MGIT Procedure Manual For BACTEC™ MGIT 960™ TB System. In. Geneva, Switzerland: Foundation for Innovative New Diagnostics (FIND); 2006.

146. WHO. Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs. In. Geneva, Switzerand: World Health Organization; 2008.

147. Rodrigues C, Jani J, Shenai S, Thakkar P, Siddiqi S, Mehta A. Drug susceptibility testing of Mycobacterium tuberculosis against second-line drugs using the Bactec MGIT 960 System. *International Journal of Tuberculosis and Lung Disease* 2008,**12**:1449-1455.

148. Lin SYG, Desmond E, Bonato D, Gross W, Siddiqi S. Multicenter Evaluation of Bactec MGIT 960 System for Second-Line Drug Susceptibility Testing of Mycobacterium tuberculosis Complex. *Journal of Clinical Microbiology* 2009,**47**:3630-3634.

149. Banerjee R, Schecter GF, Flood J, Porco TC. Extensively drug-resistant tuberculosis: new strains, new challenges. *Expert Review of Anti-Infective Therapy* 2008,**6**:713-724.

150. Blower S, Supervie V. Predicting the future of XDR tuberculosis. *Lancet Infectious Diseases* 2007,**7**:443-443.

151. Falzon D, Gandhi N, Migliori GB, Sotgiu G, Cox HS, Holtz TH, *et al.* Resistance to fluoroquinolones and second-Line injectable drugs: impact on multidrug-resistant TB outcomes. *European Respiratory Journal* 2013,**42**:156-168.

152. Wright A, Zignol M, Van Deun A, Falzon D, Gerdes SR, Feldman K, *et al.* Epidemiology of antituberculosis drug resistance 2002-07: an updated analysis of the Global Project on Anti-Tuberculosis Drug Resistance Surveillance. *Lancet* 2009,**373**:1861-1873.

153. Salvo F, Dorjee K, Dierberg K, Cronin W, Sadutshang TD, Migliori GB, *et al.* Survey of tuberculosis drug resistance among Tibetan refugees in India. *International Journal of Tuberculosis and Lung Disease* 2014,**18**:655-662.

154. Hu Y, Hoffner S, Wu L, Zhao Q, Jiang W, Xu B. Prevalence and Genetic Characterization of Second-Line Drug-Resistant and Extensively Drug-Resistant Mycobacterium tuberculosis in Rural China. *Antimicrobial Agents and Chemotherapy* 2013,**57**:3857-3863.

155. Von Groll A, Martin A, Jureen P, Hoffner S, Vandamme P, Portaels F, *et al.* Fluoroquinolone resistance in Mycobacterium tuberculosis and mutations in gyrA and gyrB. *Antimicrobial agents and chemotherapy* 2009,**53**:4498-4500.

156. Bloss E, Kuksa L, Holtz TH, Riekstina V, Skripconoka V, Kammerer S, *et al.* Adverse events related to multidrug-resistant tuberculosis treatment, Latvia, 2000-2004. *International Journal of Tuberculosis and Lung Disease* 2010,**14**:275-281.

157. Ahmed I, Jabeen K, Inayat R, Hasan R. Susceptibility testing of extensively drug-resistant and pre-extensively drug-resistant Mycobacterium tuberculosis against levofloxacin, linezolid, and amoxicillin-clavulanate. *Antimicrobial agents and chemotherapy* 2013,**57**:2522-2525.

158. Kim DH, Kim HJ, Park S-K, Kong S-J, Kim YS, Kim T-H, *et al.* Treatment Outcomes and Survival Based on Drug Resistance Patterns in Multidrug-resistant Tuberculosis. *American Journal of Respiratory and Critical Care Medicine* 2010,**182**:113-119.

159. Andersson T, Alfredsson L, Kallberg H, Zdravkovic S, Ahlbom A. Calculating measures of biological interaction. *European Journal of Epidemiology* 2005,**20**:575-579.

160. Rothman KJ, Greenland S, Rothman KJ, Greenland S. Modern epidemiology, Second edition. *Modern epidemiology, Second edition* 1998:xiii+737p-xiii+737p.

161. Jeon CY, Hwang SH, Min JH, Prevots DR, Goldfeder LC, Lee H, *et al.* Extensively drug-resistant tuberculosis in South Korea: Risk factors and treatment outcomes among patients at a tertiary referral hospital. *Clinical Infectious Diseases* 2008,**46**:42-49.

162. Cegielski JP, Dalton T, Yagui M, Wattanaamornkiet W, Volchenkov GV, Via LE, *et al.* Extensive Drug Resistance Acquired During Treatment of Multidrug-Resistant Tuberculosis. *Clinical Infectious Diseases* 2014,**59**:1049-1063.

163. Buu TN, Huyen MN, Lan NTN, Quy HT, Hen NV, Zignol M, *et al.* The Beijing genotype is associated with young age and multidrug-resistant tuberculosis in rural Vietnam. *International Journal of Tuberculosis and Lung Disease* 2009,**13**:900-906.

164. Kidenya BR, Webster LE, Behan S, Kabangila R, Peck RN, Mshana SE, *et al.* Epidemiology and genetic diversity of multidrug-resistant tuberculosis in East Africa. *Tuberculosis* 2014,**94**:1-7.

165. Leimane V, Dravniece G, Riekstina V, Sture I, Kammerer S, Chen MP, *et al.* Treatment outcome of multidrug/extensively drug-resistant tuberculosis in Latvia, 2000-2004. *European Respiratory Journal* 2010,**36**:584-593.

166. Reis-Santos B, Gomes T, Locatelli R, de Oliveira ER, Sanchez MN, Horta BL, *et al.* Treatment Outcomes in Tuberculosis Patients with Diabetes: A Polytomous Analysis Using Brazilian Surveillance System. *Plos One* 2014,**9**.