Published in final edited form as:

Int J Tuberc Lung Dis. 2015 April ; 19(4): 420–427. doi:10.5588/ijtld.14.0488.

Phenotypic and Genotypic Diversity in a Multinational Sample of Drug-resistant *Mycobacterium Tuberculosis* Isolates

Richard S. Garfein^{1,*}, Donald G. Catanzaro², Timothy C. Rodwell¹, Elisea Avelos³, Lynn Jackson¹, Janice Kaping¹, Henry Evasco⁴, Camilla Rodriques⁵, Valeriu Crudu⁶, Shou-Yean Grace Lin⁷, Erik Gossel³, Naomi Hillery³, Andre Trollip⁸, Theodore Ganiats³, Thomas C. Victor⁸, Kathleen Eisenach⁹, Faramarz Valafar², Jessica Channick¹, Lishi Qian¹⁰, and Antonino Catanzaro¹

¹ Department of Medicine, University of California, San Diego, CA, USA

² University of Arkansas, Department of Biological Sciences, Fayetteville, AR, USA

³ Department of Family & Preventive Medicine, University of California, San Diego, CA, USA

⁴ Tropical Disease Foundation, Inc., Philippine Institute of Tuberculosis Building, Makati City, Philippines

⁵ Hinduja National Hospital, Mumbai, India

⁶ Microbiology and Morphology Laboratory, Institute of Phthisiopneumology, Chisinau, Moldova

⁷ California Department of Public Health, Richmond, CA, USA

⁸ Department of Biomedical Sciences, Stellenbosch University, Cape Town, South Africa

⁹ Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AK, USA

¹⁰ Department of Microbiology, University of Hawaii, Manoa, Honolulu, HI, US

Abstract

Background—To develop and evaluate rapid, molecular-based drug susceptibility tests (DST) for extensively drug resistant tuberculosis (XDR-TB), we assembled a phenotypically and genotypically diverse collection of *M. tuberculosis* (*Mtb*) isolates from patients evaluated for drug resistance in four high-burden countries.

Methods—*Mtb* isolates from India (n=111), Moldova (n=90), the Philippines (n=96), and South Africa (n=103) were selected from existing regional and national repositories to maximize phenotypic diversity for resistance to isoniazid, rifampin, moxifloxacin, ofloxacin, amikacin, kanamycin and capreomycin. MGIT-960 was performed on viable isolates in one laboratory using standardized procedures and drug concentrations. Genetic diversity within drug-resistance phenotypes was assessed.

Results—Nineteen distinct phenotypes were observed among 400 isolates with complete DST results. Diversity was greatest in the Philippines (14 phenotypes) and least in South Africa (9

* Corresponding author.

The authors have no conflicts of interest to disclose.

phenotypes). Nearly all phenotypes included multiple genotypes. All sites provided isolates resistant to injectable but susceptible to fluoroquinolone drugs. Many patients were taking antibiotics to which their current infection was resistant.

Discussion—Diverse phenotypes for XDR-TB-defining drugs, including resistance to fluoroquinolone and/or injectable drugs in rifampin-sensitive isolates indicate that rifampin-sensitivity does not ensure effectiveness of a standard four-drug regimen. Thus, rapid, low-cost DST assays for first- and second-line drugs are needed.

Keywords

Drug Susceptibility Testing; Diagnostics; Genotype; Epidemiology; Repository

INTRODUCTION

Worldwide implementation of acid-fast bacillus sputum smear microscopy and “directly observed therapy short course” have decreased tuberculosis (TB) incidence and mortality rates over the last 15 years.¹ Despite these strides, over two billion people remain infected with *Mycobacterium tuberculosis* (*Mtb*).² In 2012, there were an estimated 8.6 million new TB cases and 1.3 million deaths.³ The emergence of multi-drug resistant TB (MDR-TB); defined by the World Health Organization (WHO) as resistance to isoniazid (INH) and rifampin (RIF)), threatens to undermine the advances made in global TB control and rapid diagnosis of drug resistance is critical to avoid unnecessary costs and side effects of inappropriate therapy. MDR-TB strains that develop additional resistance to one fluoroquinolone (e.g., moxifloxacin [MOX], ofloxacin [OFX]) and at least one injectable drug (e.g., amikacin [AMK], kanamycin [KAN], capreomycin [CAP]) are classified by WHO as extensively drug resistant TB (XDR-TB).¹ In 2012, XDR-TB was detected in 92 countries with approximately 10% of all MDR-TB cases being XDR-TB.³ There is growing concern that regional increases in the prevalence of drug resistant TB (DR-TB),⁴ broad distribution of XDR-TB,⁴ and emergence of what is being called “totally drug-resistant TB”,⁵⁻⁷ will reverse recent gains in global TB control.¹

The Global Consortium for Drug-resistant TB Diagnostics (GCDD) develops and tests diagnostic assays intended to reduce DST time from months to days. One goal of the GCDD is to characterize the diversity of *Mtb* drug resistance patterns among archived isolates obtained from four countries with a high burden of DR-TB, and to: 1) to assess the performance of molecular-based DST assays on isolates from distinct geographic locations; and 2) to fully characterize resistant organisms for future whole genome sequence analyses in order to discover novel mutations that could improve the sensitivity and specificity of these assays. Previous multisite studies have measured the prevalence and identified correlates of drug resistance,⁸ but to our knowledge, this is the first study to characterize resistance profiles to XDR-TB-defining antibiotics using standardized DST procedures on *Mtb* isolates from four high-burden countries. Furthermore, elucidating the range of phenotypic resistance patterns will help inform decisions about which drugs should be included in combination DST assays.

METHODS

Source of Isolates

In 2009, GCDD requested an inventory of stored DR-TB *Mtb* isolates from regional and national reference laboratory repositories in India, Moldova, the Philippines, and South Africa. We selected isolates with preference for maximizing XDR-TB strains and diversity of local phenotypic resistance patterns, rather than estimating phenotype prevalence. Since drug concentrations, drugs tested, and growth medium used were not uniform across sites, all isolates were sent to the University of California San Diego (UCSD) for standardized DST against seven target drugs (INH, RIF, MOX, OFX, CAP, AMK, KAN). Pan-susceptible isolates were obtained from the same repositories as comparator samples.

All *Mtb* isolates and patient clinical data were stripped of personal identifiers prior to acquisition for this collection; thus, a UCSD institutional review board deemed the study exempt from human subjects research.

Repository Descriptions

India—Samples were obtained from P.D. Hinduja National Hospital (PDHNNH (Mumbai, India). With a population of 12 million and TB incidence of 299/100,000,⁹ there were an estimated 35,880 TB cases in Mumbai in 2011 and MDR-TB prevalence was 24% and 41% among new and re-treatment cases.¹⁰ From January 2008 to December 2009, PDHNNH collected 215 consecutive XDR-TB isolates and 50 consecutive pan-susceptible, cultured them using MGIT and Löwenstein-Jensen (LJ) medium, and stored a subculture at -70°C.¹¹ All viable drug-resistant isolates, plus six randomly selected pan-susceptible isolates, were requested for this study.

Moldova—Samples were obtained from the Phthisiopneumology Institute (PPI) in Chisinau, which serves as the Moldovan National TB Control Program reference laboratory. Approximately 6,500 new TB cases were reported in 2011 among Moldova's estimated 3.5 million residents (TB incidence=277/100,000 pop.). In 2010, MDR-TB was detected in 20% of all new TB cases and in 51% of the retreatment TB cases.¹² Between June 2008 and April 2010, PPI collected 118 drug resistant *Mtb* isolates that were cultured and placed into storage at -70°C. All of these isolates, plus six pan-susceptible isolates, were requested for this study.

Philippines—The Tropical Disease Foundation (TDF) DOTS Clinic and TB Research Laboratory has been one of the National TB Program's most active partners for over 10 years. The Philippines has a population of 94 million with a TB incidence of 280/100,000 population.¹² MDR-TB was diagnosed in 4% of new TB cases and 21% of previously treated cases.¹³ The TDF patient population consisted of MDR-TB suspects referred from public and private facilities throughout the region; approximately 80% of whom came from the Metro Manila area (MG). TDF maintains a repository of all viable *Mtb* isolates cultured in its laboratory since 1999. All available M/XDR-TB isolates (n=125) obtained in 1999-2009, plus six pan-susceptible isolates, were requested for this study.

South Africa—The South African National Health Laboratory Service (NHLS) serves as a reference laboratory for the entire country. In 2011, South Africa had a population of approximately 48 million with a TB incidence of 795/100,000 pop. In a 2008 nationwide survey, 20.2% of notified TB cases were INH-resistant; nearly half of which (9.6% of all cases) were MDR-TB.¹⁴ An estimated 10.5% of MDR-TB cases tested for second-line drugs were XDR-TB.¹⁴ NHLS routinely retains drug resistant and other “interesting” isolates (GC), which included 758 DR-TB isolates from three provinces (KwaZulu-Natal [n=49], Gauteng [n=106], and Western Cape [n=603]). To obtain the most diverse sample of isolates by geographic region, we requested all viable isolates from KwaZulu-Natal and Gauteng, and a subset from the Western Cape. Western Cape isolates were selected as follows: all isolates with resistance to the seven study drugs (n=30); all isolates resistant to MOX (n=5); and a selection of isolates with resistance to INH and RIF plus at least 1 other drug that included all available DST patterns (n=76) (groups not mutually exclusive). Six pansusceptible isolates were also requested.

Sample Preparation at Sites

Sites revitalized frozen isolates by thawing them and inoculating LJ slants. When colonies formed, *Mtb* was transferred to sterile broth-containing cryovials. All but 14 isolates were prepared in this manner; the exceptions failed to grow on LJ slants, and were subsequently grown in MGIT tubes. Cryovials and unopened MGIT tubes were shipped to UCSD at ambient temperature.

Drug Susceptibility Testing at UCSD Laboratory

Upon arrival at the UCSD laboratory, we performed standardized DST on all the isolates using MGIT 960 (BD Diagnostic Systems, Franklin Lakes, NJ, USA).¹⁵ Critical drug concentrations (Table 1) and testing procedures followed manufacturer's recommendations for INH and RIF, and WHO recommendations for AMK, CAP, MOX, OFX.¹⁶ We used a critical concentration of 2.5 µg/mL for KAN¹⁷ because WHO recommendations did not exist at the time of this study. When insufficient growth (X200 error) or contamination (X400 error) occurred in the drug-free tube, the test was considered invalid and DSTs were repeated.

Mtb Genotype and Lineage

We used spoligotype and 12 mycobacterial interspersed repetitive unit (MIRU-12) assays to determine whether isolates with the same phenotype differed genetically. Spoligotyping was performed at the University of Hawaii and MIRU-12 was performed at the California Department of Public Health using standardized methods.¹⁸⁻²⁰ Distinct genotypes were determined by combining spoligotype and MIRU-12 for each isolate. When results were obtained from only one assay (spoligotype or MIRU-12), we conservatively called a genotype distinct only if the available results differed from all other isolates within the same phenotype. To determine lineage, we used the Web-based TB-Lineage tool.²¹ SIVIT Clade (lineage subtypes) were predicted using KBBN²² for major phylogenetic lineages. All isolates were also sequenced at loci associated with drug resistance using Sanger and pyrosequencing and reported elsewhere.^{23,24}

Patient Clinical Data

Sociodemographic, behavioral and clinical data (Table 2) were obtained by trained site staff using records accompanying stored isolates and a common data abstraction form. Data were entered by sites using a secure Internet-based data collection system.

RESULTS

Drug Resistance Phenotypes

Of the 662 isolates requested, 143 could not be regrown by sites resulting in shipment of 519 isolates to the GCDD laboratory. Of these, 81 isolates could not be regrown by the GCDD laboratory and 22 isolates were determined to be non-*Mtb* bacteria, leaving a total of 416 isolates with DST results for at least one study drug and 400 isolates with DST results for all seven drugs (Table 1). Ability to grow isolates was not associated with patient socio-demographic or treatment variables; thus, the reduced sample size is unlikely to be biased.

We observed 19 distinct phenotypes ranging from complete resistance to complete susceptibility (Figure 1). The greatest phenotypic diversity was observed among isolates from the Philippines (14 phenotypes) compared with 9 phenotypes in South Africa. Only six phenotypes were common to all four sites. Of note, all four sites provided isolates that were resistant to one or more injectable drugs, but susceptible to both fluoroquinolone drugs. Similarly, all sites provided isolates that were resistant to INH plus one or more second-line drugs, but susceptible to RIF.

Across sites, 251 isolates were XDR-TB, 55 were MDR-TB with resistance to a fluoroquinolone or injectable drug (but not both); 36 were INH and RIF resistant only and 11 had mono-resistance to INH or RIF. One isolate was resistant to only MOX and OFX. Notably, all four sites had isolates that were RIF-sensitive and resistant to fluoroquinolone and/or injectable drugs.

Genotypic Diversity

Spoligotype and MIRU-12 was conducted on 395 isolates; 33 had only spoligotype, 4 had only MIRU, and 87 had neither. All but three phenotypic clusters included >1 genotype (Figure 1). Furthermore, clustering was rare across phenotypes in all four countries. The exceptions being 32 Moldovan isolates with a single phenotype represented by only five genotypes; and isolates resistant to all seven drugs in India (41 genotypes among 82 isolates) and South Africa (21 genotypes among 82 isolates). Overall, there were 213 (range: 38-72 per site) distinct genotypes, only nine of which were found in two or more sites, indicating the success of our efforts to obtain a diverse isolate collection.

The most common *Mtb* lineage observed was Beijing, followed by Euro-American, Indo-Oceanic, and East-African Indian (CAS) lineages (Figure 2). All four lineages were represented among the M/XDR-TB and pan-susceptible isolates. Notably, while the Beijing strain predominated, over one-third of XDR-TB and two-thirds of MDR-TB isolates were non-Beijing strains.

Patient Characteristics

Patient data were limited and varied by site due to the fact that clinical characteristics were not systematically collected for all repository isolates. In particular, patient data for isolates from South Africa were limited to age, gender and race. Using available data (Table 2), the median patient age at time of specimen collection was 34.5 years (range: 4-88 years), with India having the youngest mean age. Males made up 54.5% of the sample, although gender was unknown for 47.6% of the patients in South Africa. Race/ethnicity varied little within sites, but varied greatly across sites. No patients had documented history of homelessness, injection drug use, or trading sex for money or other goods. Between 16.6% and 29.7% of the isolates came from patients who were close contacts of known TB cases. HIV status data were only available for patients from India and Moldova, of which only one-third (32.2%) had known HIV status and none were HIV-positive. In Moldova and the Philippines, nearly all patients had been previously treated for a prior TB diagnosis, compared with 22.5% in India. Among patients who had received treatment for a prior TB episode, the median duration was greatest in Moldova (27 months) and shortest in India (6 months). All patients in India and 83.9% of patients in Moldova were receiving TB treatment at the time of sputum collection, compared with 2.1% of patients in the Philippines. The median duration of treatment was 24 months in India compared with 6 months in Moldova. A large proportion of drug-resistant isolates came from patients who were taking the drug when the specimen was collected. For example, 94% and 90% of patients with KAN-resistant isolates in India and Moldova, respectively, were taking KAN (Table 3).

DISCUSSION

Phenotypic patterns of drug resistance varied considerably and several isolates were found to be resistant to first-line plus injectable drugs (AMK, KAN, CAP) that were sensitive to the fluoroquinolone drugs (OFX, MOX). Thus, assumptions about resistance to one drug should not be made solely on the basis of resistance to other drugs, and DST should be performed in panels rather than individually by drug. In India and Moldova, we found high proportions of patients receiving medications at the time of specimen collection to which their infections were resistant, indicating that those patients were on ineffective and potentially harmful medications longer than if their providers had access to rapid, molecular-based diagnostics.

M/XDR-TB phenotypes were found to include isolates from all the major *Mtb* lineages. Additionally, we found similar phenotypes across the four sites and most phenotypes contained multiple genotypes, highlighting the importance of acquired drug resistance in all four sites. We observed relatively little clustering by genotype as determined by spoligotyping and MIRU-12. These findings, combined with the fact that many patients were taking the drug(s) they were resistant to when their isolates were collected, emphasize the need for rapid, affordable DST assay.

Prior studies have found strong associations between the Beijing strain of *Mtb* and M/XDR-TB;²⁵ therefore, it was not surprising to find that Beijing was the predominant *Mtb* strain (44%). However, 51% of the M/XDR-TB isolates were non-Beijing strains. This finding supports the need to routinely perform DST regardless of *Mtb* lineage.

LIMITATIONS

Mtb isolates were obtained from existing repositories, which were not established uniformly within or across sites. While the sites routinely stored DR-TB specimens, their representativeness of all TB cases in those countries cannot be inferred. Thus, the GCDD collection should be viewed as representing the *diversity* of drug-resistance phenotypes in each country, not the *prevalence* of drug resistance. While each isolate collected for this study came from a single patient, it is possible that similar strains were obtained from multiple patients due to outbreaks within sites (e.g., XDR-TB in Tugula Ferry, KwaZulu Natal, South Africa).²⁶ We used spoligotype and MIRU-12 to genotype the isolates and identify clusters within phenotypes. Had we used the more specific MIRU-24 assay,²⁷ we might have seen less genotypic clustering. However, since the intent was to differentiate isolates within phenotypes, rather than to identify clusters, the resulting underestimation in the diversity of the isolates would tend to make our interpretations conservative. Analysis of patient factors associated with drug-resistance phenotypes was limited by reliance on existing data accompanying repository isolates which were not uniformly collected and retained across sites. Systematic collection and storage of patient characteristics (e.g., age, gender, location, TB risk factors, prior TB treatment and antibiotic use for non-TB illness, and clinical factors) should be conducted whenever possible when *Mtb* isolates are stored for future studies.

CONCLUSIONS

Our results, indicating that INH and/or RIF resistant strains from TB patients in these regions can also have additional resistance to injectable drugs or FQs independently, strongly support the 2104 WHO recommendation that “ideally, testing for drug resistance should be provided for all identified TB patients before the start of TB treatment”.²⁸ Based on these findings, we recommend that comprehensive, WHO recommended first and second-line DST be completed at the initiation of TB treatment for all TB patients, or at the very least, those TB patients considered at risk for DR-TB, rather than having DSTs to the different drug classes completed serially following chronic treatment failures. In addition, laboratories storing DR-TB isolates should maintain at least a limited set of epidemiological data to support further analyses. A major outcome of this study was the creation of a large, well-characterized, multinational collection of *Mtb* isolates with diverse phenotypic drug resistance patterns comprised of seven first- and second-line anti-TB drugs tested using standardized methods and drug concentrations in a single laboratory. The GCDD repository provides an invaluable isolate collection for developing and evaluating new technologies such as molecular-based DST assays to rapidly detect drug resistance.

ACKNOWLEDGEMENTS

The specimens, data, and funding for this project were provided by the Global Consortium for Drug-Resistant TB Diagnostics (GCDD [see <http://gcdd.ucsd.edu>]), funded by a grant (U01-AI082229) from National Institute of Allergy and Infectious Diseases (NIAID), US National Institutes of Health. T.C.R. was also supported by NIAID grant K01-AI083784. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health.

The authors gratefully acknowledge the clinical and laboratory staff at each of the study sites for their assistance in acquiring and preparing *M. tuberculosis* isolates. Support from Thelma E. Tupasi and Janice C. Caoili at the TDF, and Kanchan Ajbani at the PDHNNH, was invaluable for collecting and processing the isolates from Manila and India, respectively.

REFERENCES

1. WHO. World Health Organization, Global tuberculosis control: WHO report, 2011. WHO/HTM/TB/2011.16. Geneva, Switzerland: WHO. 2011.
2. WHO. World Health Organization. Global tuberculosis control - surveillance, planning, financing: WHO Report, 2005. WHO/HTM/TB/2005.349. WHO; Geneva, Switzerland: 2005.
3. WHO. World Health Organization. Global tuberculosis report: WHO report, 2013. WHO/HTM/TB/2013.11. WHO; Geneva, Switzerland: 2013.
4. Kapp C. XDR tuberculosis spreads across South Africa. *Lancet*. 2007; 369:729–729. [PubMed: 17340679]
5. Udwadia ZF, Amale RA, Ajbani KK, et al. Totally drug-resistant tuberculosis in India. *Clin Infect Dis*. 2012; 54:579–581. [PubMed: 22190562]
6. Velayati AA, Masjedi MR, Farnia P, et al. Emergence of new forms of totally drug-resistant tuberculosis bacilli: super extensively drug-resistant tuberculosis or totally drug-resistant strains in Iran. *Chest*. 2009; 136:420–425. [PubMed: 19349380]
7. Migliori GB, De Iaco G, Besozzi G, et al. First tuberculosis cases in Italy resistant to all tested drugs. *Euro Surveill*. 2007; 12:E070517 070511. [PubMed: 17868596]
8. Dalton T, Cegielski P, Akksilp S, et al. Prevalence of and risk factors for resistance to second-line drugs in people with multidrug-resistant tuberculosis in eight countries: a prospective cohort study. *Lancet*. 2012; 380:1406–1417. [PubMed: 22938757]
9. WHO. World Health Organization. Global tuberculosis control: surveillance, planning, financing: WHO report, 2008. WHO/HTM/TB/2008.393. WHO; Geneva, Switzerland: 2008.
10. Isaakidis P, Cox HS, Varghese B, et al. Ambulatory multi-drug resistant tuberculosis treatment outcomes in a cohort of HIV-infected patients in a slum setting in Mumbai, India. *PLoS One*. 2011; 6:e28066. [PubMed: 22145022]
11. Ajbani K, Rodrigues C, Shenai S, et al. Mutation detection and accurate diagnosis of extensively drug-resistant tuberculosis: report from a tertiary care center in India. *J Clin Microbiol*. 2011; 49:1588–1590. [PubMed: 21289142]
12. WHO. World Health Organization. Multidrug and extensively drug-resistant TB (M/XDR-TB): 2010 global report on surveillance and response: WHO report, 2010. WHO/HTM/TB/2010.3. WHO; Geneva, Switzerland: 2010.
13. Philippine Nationwide Tuberculosis Drug Resistance Survey T. Nationwide drug resistance survey of tuberculosis in the Philippines. *Int J Tuberc Lung Dis*. 2009; 13:500–507. [PubMed: 19335957]
14. Khanna A, Raj VS, Tarai B, et al. Emergence and molecular characterization of extensively drug-resistant *Mycobacterium tuberculosis* clinical isolates from the Delhi Region in India. *Antimicrob Agents Chemother*. 2010; 54:4789–4793. [PubMed: 20713679]
15. Siddiqi, S.; Rusch-Gerdes, S.; Alexander, H., et al. MGIT Procedure Manual For BACTEC™ MGIT 960™ TB System: FIND. 2006.
16. WHO. World Health Organization. Policy guidance on drug-susceptibility testing (DST) of second-line antituberculosis drugs: WHO report, 2008. WHO/HTM/TB/2008.392. WHO; Geneva, Switzerland: 2008.
17. Rodrigues C, Jani J, Shenai S, et al. Drug susceptibility testing of *Mycobacterium tuberculosis* against second-line drugs using the Bactec MGIT 960 System. *Int J Tuberc Lung Dis*. 2008; 12:1449–1455. [PubMed: 19017456]
18. Kamerbeek J, Schouls L, Kolk A, et al. Simultaneous detection and strain differentiation of *Mycobacterium tuberculosis* for diagnosis and epidemiology. *J Clin Microbiol*. 1997; 35:907–914. [PubMed: 9157152]

19. Cowan LS, Diem L, Monson T, et al. Evaluation of a two-step approach for large-scale, prospective genotyping of *Mycobacterium tuberculosis* isolates in the United States. *J Clin Microbiol.* 2005; 43:688–695. [PubMed: 15695665]
20. Mazars E, Lesjean S, Banuls AL, et al. High-resolution minisatellite-based typing as a portable approach to global analysis of *Mycobacterium tuberculosis* molecular epidemiology. *Proc Natl Acad Sci U S A.* 2001; 98:1901–1906. [PubMed: 11172048]
21. Shabbeer A, Ozcaglar C, Yener B, et al. Web tools for molecular epidemiology of tuberculosis. *Infect Genet Evol.* 2012; 12:767–781. [PubMed: 21903179]
22. Aminian M, Couvin D, Shabbeer A, et al. Predicting *Mycobacterium tuberculosis* complex clades using knowledge-based Bayesian networks. *BioMed Res Int.* 2014;11. 2014, Article ID 398484.
23. Rodwell TC, Valafar F, Douglas J, et al. Predicting extensively drug-resistant *Mycobacterium tuberculosis* phenotypes with genetic mutations. *J Clin Microbiol.* 2014; 52:781–789. [PubMed: 24353002]
24. Ajbani, K.; Grace Lin, SY.; Rodrigues, C., et al. Evaluation of pyrosequencing for detecting extensively drug-resistant tuberculosis (XDR-TB) in clinical isolates from four high-burden countries *Antimicrob Agents Chemother.* 2014.
25. European Concerted Action on New Generation Genetic Markers and Techniques for the Epidemiology and Control of Tuberculosis. Beijing/W genotype *Mycobacterium tuberculosis* and drug resistance. *Emerg Infect Dis.* 2006; 12:736–743. [PubMed: 16704829]
26. Gandhi NR, Moll A, Sturm AW, et al. Extensively drug-resistant tuberculosis as a cause of death in patients co-infected with tuberculosis and HIV in a rural area of South Africa. *Lancet.* 2006; 368:1575–1580. [PubMed: 17084757]
27. Supply P, Allix C, Lesjean S, et al. Proposal for standardization of optimized mycobacterial interspersed repetitive unit-variable-number tandem repeat typing of *Mycobacterium tuberculosis*. *J Clin Microbiol.* 2006; 44:4498–4510. [PubMed: 17005759]
28. WHO. World Health Organization. Companion handbook to the WHO guidelines for the programmatic management of drug-resistant tuberculosis, 2014. WHO/HTM/TB/2014.11. WHO; Geneva, Switzerland: 2014.

TOTAL	India								Moldova								Philippines								South Africa								
No. of Isolates (genotypes)	INH	RIF	MOX	OFX	AMK	CAP	KAN	No. of Isolates (genotypes)	INH	RIF	MOX	OFX	AMK	CAP	KAN	No. of Isolates (genotypes)	INH	RIF	MOX	OFX	AMK	CAP	KAN	No. of Isolates (genotypes)	INH	RIF	MOX	OFX	AMK	CAP	KAN	No. of Isolates (genotypes)	
185 (68)	I	R	M	O	A	C	K	82 (41)	I	R	M	O	A	C	K	19 (10)	I	R	M	O	A	C	K	2 (2)	I	R	M	O	A	C	K	82 (21)	
3 (3)																																3 (3)	
3 (3)	I	R		O	A	C	K	2 (2)	I	R			A	C	K	3 (3)	I	R			A	C	K	6 (2)	I	R			A	C	K	3 (1)	
14 (8)	I	R			A	C	K	2 (2)	I	R	M	O	A		K	4 (2)	I	R	M	O	A		K	1 (1)	I	R			A	C	K	3 (1)	
13 (10)	I	R	M	O	A		K	6 (5)	I	R			A		K	3 (3)	I	R			A		K	2 (2)									2 (2)
5 (5)									I	R			A		K	6 (4)																	
6 (4)									I	R	M	O		C	K	1 (1)																	
1 (1)									I	R	M	O		C		32 (5)																	
40 (11)	I	R	M	O			K	5 (5)	I	R					K	6 (2)	I	R					K	1 (1)	I	R	M	O			K	3 (2)	
7 (3)									I	R						3 (3)	I	R	M	O				22 (18)									1 (1)
29 (23)	I	R	M	O				3 (2)	I	R	M	O				2 (1)	I	R						32 (21)									
36 (24)	I	R						2 (2)	I		M	O	A	C	K	2 (1)	I		M	O	A	C	K	1 (1)	I								
6 (5)	I		M	O	A	C	K	2 (2)									I						K	1 (1)	I		M	O	A	C	K	1 (1)	
1 (1)																	I		M	O				3 (3)									
3 (3)																	I							8 (8)									
9 (9)																		R						1 (1)									
2 (2)		R						1 (1)												M	O			1 (1)									
1 (1)																								15 (10)									
36 (29)								6 (6)								8 (7)								15 (10)									7 (6)
19	Total Phenotypes							10	Total Phenotypes							13	Total Phenotypes							14	Total Phenotypes							9	
400 (213)*	Total Isolates (genotypes)							111 (68)	Total Isolates (genotypes)							90 (43)	Total Isolates (genotypes)							96 (72)	Total Isolates (genotypes)							103 (38)	

Resistant to drug

Sensitive to drug

Phenotype not observed at the site

Resistant to drug
 Sensitive to drug
 Phenotype not observed at the site

Figure 1.

Distribution of phenotypic drug resistance patterns and genotypic diversity among isolates with complete drug susceptibility testing to seven drugs by country. Rows represent distinct resistance phenotypes. Phenotypes not detected in each country are represented by white boxes. The number of distinct genotypes (based on spoligotype and MIRU-12) observed within phenotypes is presented in parentheses beside the number of isolates with each phenotype. *The total number of distinct genotypes added across the four countries (221) was greater than the number of distinct genotypes overall (213), because 9 genotypes were found in more than one site.

Figure 2.

Sankey diagram showing the relationships between the drug susceptibility test results and *M. tuberculosis* lineages. Isolate counts in each phenotype and genotype group are shown in parentheses. Only isolate counts >10 for linkages between phenotype and genotype are shown in figure. All isolates in the East Asian lineage belong to the Beijing sub-lineage. XDR = extensively drug resistant; MDR = multiple drug resistant; RIF^R = rifampicin resistant; INH^R = isoniazid resistant.

Table 1

Resistance to first and second-line anti-tuberculosis drugs by site

Drug (concentration) *	Total (n=519) Resistant/Tested	India (n=176) Resistant/Tested	Moldova (n=112) Resistant/Tested	Philippines (n=125) Resistant/Tested	S. Africa (n=106) Resistant/Tested
INH (0.1µg/mL)	370/414 (89.4%)	104/111 (93.7%)	83/91 (91.2%)	87/109 (79.8%)	96/103 (93.2%)
RIF (1.0µg/mL)	356/416 (85.6%)	103/111 (92.8%)	80/91 (87.9%)	78/111 (70.2%)	95/103 (92.2%)
MOX (0.25µg/mL)	292/414 (70.5%)	98/111 (88.2%)	68/91 (74.7%)	34/109 (31.2%)	92/103 (89.3%)
OFX (2.0µg/mL)	292/414 (70.5%)	100/111 (90.1%)	67/90 (74.4%)	35/110 (31.8%)	90/103 (87.4%)
AMK (1.0µg/mL)	230/416 (55.3%)	94/111 (84.7%)	31/91 (34.1%)	13/111 (11.7%)	92/103 (89.3%)
CAP (2.5µg/mL)	219/405 (54.1%)	88/111 (79.3%)	32/91 (35.2%)	9/100 (9.0%)	90/103 (87.4%)
KAN (2.5µg/mL)	286/416 (68.8%)	99/111 (89.2%)	76/91 (83.5%)	16/111 (14.4%)	95/103 (92.2%)
Isolates with DST results for all drugs	400	111	90	96	103

* Isolates screened for phenotypic resistance on a MGIT 960 platform using WHO critical concentration. Policy Guidance on Drug Susceptibility Testing (DST) of second-line anti-tuberculosis drugs. Geneva, WHO, 2008 (WHO/HTM/TB/2008.392). The critical concentration for KAN was based on published literature because this drug was not included in the WHO document (Rodrigues, et al., 2008. *Int J Tuberc Lung Dis* 12: 1449-1455.).

Table 2

Characteristics of patients from whom *M. tuberculosis* isolates were obtained (n=400)

Variable	Value	India	Moldova	Philippines	S. Africa	Total
Age, years – median (range)		28 (13-78)	37 (18-72)	39 (16-88)	36 (5-55)	34.5 (4-88)
Gender – n (%)						
Male		68 (61.3)	71 (78.9)	55 (57.3)	24 (23.3)	218 (54.5)
Female		43 (38.7)	19 (21.1)	40 (41.7)	30 (29.1)	132 (33.0)
Unknown		0 (0)	0 (0)	1 (1.0)	49 (47.6)	50 (12.5)
Race – n (%)						
White		0 (0)	90 (100)	0 (0)	0 (0)	90 (22.5)
Black/Colored		0 (0)	0 (0)	0 (0)	49 (47.6)	49 (12.3)
Asian/Indian		111 (100)	0 (0)	95 (99.0)	0 (0)	206 (51.5)
Pacific Islander		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Other		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Unknown		0 (0)	0 (0)	1 (1.0)	54 (52.4)	55 (13.6)
Ethnicity – n (%)						
Dravidian		25 (22.5)	0 (0)	0 (0)	0 (0)	25 (6.3)
Ino-Aryan		86 (77.5)	0 (0)	0 (0)	0 (0)	86 (21.5)
Bulgarian		0 (0)	2 (2.2)	0 (0)	0 (0)	2 (0.5)
Gagauz		0 (0)	2 (2.2)	0 (0)	0 (0)	2 (0.5)
Moldovan		0 (0)	85 (94.4)	0 (0)	0 (0)	85 (21.3)
Ukrainian		0 (0)	1 (1.1)	0 (0)	0 (0)	1 (0.3)
Bikol		0 (0)	0 (0)	10 (10.4)	0 (0)	10 (2.5)
Bisaya		0 (0)	0 (0)	14 (14.6)	0 (0)	14 (3.5)
Cebuano		0 (0)	0 (0)	1 (1.0)	0 (0)	1 (0.3)
Tagalog		0 (0)	0 (0)	56 (58.3)	0 (0)	56 (14.0)
Other		0 (0)	0 (0)	14 (14.5)	0 (0)	14 (3.5)
Unknown		0 (0)	0 (0)	1 (1.0)	103 (100)	104 (26.0)
Homeless – n (%)						
Yes		0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
No		108 (97.3)	86 (95.6)	95 (99.0)	0 (0)	289 (72.3)
Incarcerated – n (%)						
Unknown		3 (2.7)	4 (4.4)	1 (1.0)	103 (100)	111 (27.7)
Yes		0 (0)	13 (15.2)	0 (0)	0 (0)	13 (3.3)
No		108 (97.3)	74 (81.3)	95 (99.0)	0 (0)	277 (69.3)
Unknown		3 (2.7)	3 (3.6)	1 (1.0)	103 (100)	110 (27.5)

Variable	Value	India	Moldova	Philippines	S. Africa	Total
Close contact of a known TB case – n (%)		33 (29.7)	24 (26.7)	16 (16.6)	0 (0)	73 (18.3)
	Yes					
	No	78 (70.3)	48 (53.3)	16 (16.6)	0 (0)	142 (35.5)
HIV status – n (%)						
	Unknown	0 (0)	18 (20.0)	64 (66.7)	103 (100)	185 (46.3)
	Positive	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	Negative	49 (44.1)	78 (86.7)	0 (0)	0 (0)	167 (32.2)
	Unknown	62 (55.9)	12 (13.3)	96 (100)	103 (100)	352 (67.8)
Ever treated for prior TB diagnosis – n (%)						
	Yes	25 (22.5)	87 (96.7)	82 (85.4)	0 (0)	194 (48.5)
	No	0 (0)	2 (2.2)	5 (5.2)	0 (0)	7 (1.8)
	Unknown	86 (77.5)	1 (1.1)	9 (9.4)	103 (100)	199 (49.8)
Months on treatment for prior TB diagnosis – median (range) N=number treated		6 (6-6) N=1	27 (1-84) N=75	9 (1-60) N=77	-	18 (1-84) N=153
On TB treatment when specimen collected – n (%)						
	Yes	111 (100)	75 (83.9)	2 (2.1)	0 (0)	188 (47.0)
	No	0 (0)	7 (8.0)	78 (81.3)	0 (0)	85 (21.3)
	Unknown	0 (0)	8 (8.0)	16 (16.7)	103 (100)	127 (31.8)
Months on treatment for current TB diagnosis – median (range) N=number treated		24 (1-120) N=111	6 (1-25) N=65	13 (13-13) N=1	-	12 (1-120) N=177

Table 3

Drug resistance by history of drugs taken at time of specimen collection

Was patient taking drug at time of specimen collection?	India (n=64) ^a		Moldova (n=67) ^a	
	Resistant N (%)	Sensitive N (%)	Resistant N (%)	Sensitive N (%)
Isoniazid: Yes	2 (67)^b	1 (33)	4 (40)	6 (60)
No	59 (97)	2 (3)	56 (98)	1 (2)
Rifampicin: Yes	1 (50)	1 (50)	4 (40)	6 (60)
No	60 (97)	2 (3)	55 (96)	2 (4)
Moxifloxacin: Yes	9 (82)	2 (18)	44 (81)	10 (19)
No	48 (91)	5 (9)	4 (31)	9 (69)
Ofloxacin: Yes	9 (82)	2 (18)	44 (81)	10 (19)
No	49 (92)	4 (8)	4 (31)	9 (69)
Amikacin: Yes	8 (80)	2 (20)	0 (0)	2 (100)
No	45 (83)	9 (17)	22 (34)	43 (66)
Capreomycin: Yes	9 (69)	4 (31)	23 (41)	33 (59)
No	42 (82)	9 (18)	1 (9)	10 (91)
Kanamycin: Yes	31 (94)	2 (6)	44 (90)	5 (10)
No	27 (87)	4 (13)	11 (61)	7 (39)

^a Sample sizes were less than the number of patients who were on tuberculosis treatment at the time of sputum collection because the drugs taken were unknown for some patients.^b Bolded figures indicate that at least half of the patients taking the drug were infected with *M. tuberculosis* strains that were resistant to the drug.