Aus der

Abteilung für Infektions- und Tropenmedizin

Klinikum der Ludwig-Maximilians-Universität München

Evaluation of the magnitude of Anti Tuberculosis Drug Resistance in Tanzania

Dissertation
zum Erwerb des Doctor of Philosophy (Ph.D.)
an der Medizinischen Fakultät der
Ludwig-Maximilians-Universität München

vorgelegt von

Beatrice Kemilembe Mutayoba

aus
Kibaha, Tanzania

Jahr
2023

Mit Genehmigung der Medizinischen Fakultät der
Ludwig-Maximilians-Universität München

Erstes Gutachten:	Prof. Dr. Michael. Hoelscher
Zweites Gutachten:	PD Dr. Norbert Heinrich
Drittes Gutachten:	PD Dr. Sophia Horster
Viertes Gutachten:	PD Dr. Sabine Hofmann- Thiel

Dekan: Prof. Dr. med. Thomas Gudermann

Tag der mündlichen Prüfung: 24.05.2023

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Dean's Office
Medical Faculty

MMRS MUNICH MEDICAL RESEARCH SCHOOL

Affidavit

Mutayoba, Beatrice Kemilembe

Surname, first name

Ministry of Health

Street

Not Applicable, Dodoma

Zip code, town

Tanzania

Country

I hereby declare, that the submitted thesis entitled

Evaluation of the magnitude of Anti Tuberculosis Drug Resistance in Tanzania

is my own work. I have only used the sources indicated and have not made unauthorised use of services of a third party. Where the work of others has been quoted or reproduced, the source is always given.

I further declare that the submitted thesis or parts thereof have not been presented as part of an examination degree to any other university.

Munich, 01.06.2023

Place, date

Beatrice Kemilembe Mutayoba

Signature doctoral candidate

LMU LUDWIG-MAXIMILIANS-UNIVERSITÄT MÜNCHEN

Dean's Office
Medical Faculty

MMRS MUNICH MEDICAL RESEARCH SCHOOL

Confirmation of congruency between printed and electronic version of the doctoral thesis

Beatrice Kemilembe Mutayoba

Surname, first name

Street

Zip code, town

Tanzania

Country

I hereby declare that the electronic version of the submitted thesis, entitled
Evaluation of the magnitude of Anti Tuberculosis Drug Resistance in Tanzania

is congruent with the printed version both in content and format.

Munich, 01.06.2023

Place, date

Beatrice Mutayoba

Signature doctoral candidate

Table of content

Key Words

Tuberculosis; Survey; MDR-TB; Drug resistance; Phylogenetic; Lineages; Mycobacterial isolates; Whole-genome sequencing; Tanzania

Abstract

Background: Development of multidrug resistant (MDR) and extensively drug resistant (XDR) tuberculosis (TB) has become a major threat to the control of TB globally. Anti-TB drug resistance survey (DRS) is an important assessment in providing critical information for effective planning, control and management of TB patients. The current study was conducted to estimate the magnitude of resistance to Tuberculosis medicines in people diagnosed with bacteriologically confirmed pulmonary Tuberculosis in Tanzania.

Methods: This was a nationally representative cross-sectional facility-based survey conducted between 2017 and 2018 involving 45 clusters selected based on probability proportional to size (PPS) sampling. It involved a sample of new smear-positive pulmonary TB (PTB) and previously treated individuals. Sputum samples were collected and transported to the Central TB Reference Laboratory in Dar es Salaam, Tanzania for smear microscopy, culture, strain identification and susceptibility testing. For whole genome sequencing (WGS), all culture positive isolates were shipped to the Supranational TB Reference Laboratory in Uganda (SNRL). Isolates were sub-cultured on selective Middlebrooks 7H11 agar. Genetic material was extracted by cetyltrimethylammonium bromide-technique. Genomic libraries were sequenced using the Illumina MiSeq V3 cartridge.

Results: This study enrolled 1557 of the 1714 eligible TB patients. Of the 1557 enrolled patients, 1408 (90.4%) were newly diagnosed and 149 (9.6%) previously treated patients. Overall, 1172 (78.5) were culture positive for *Mycobacterium tuberculosis (M. tuberculosis)*. A total of 1168 (99%) out of 1172 samples; 1060 (91%) new and 108 (9%) previously treated) had drug sensitivity test results. The overall prevalence of MDR-TB in this study was 14 (1.2%), of which 9 (0.9%) were newly diagnosed TB cases, and 5 (4.6%) had previously received treatment. Previous treatment for TB was the only significant factor for MDR-TB (OR=5.7, 95%CI: 1.9-17.2). For the isolates sent to SNRL for WGS, results for 191 were available for analysis whereby 169 (88.0%) were from newly treated PTB patients. Co-infection with HIV was observed in 33 (17.3%) TB patients. Out of the 191 isolates, 9 (4.7%) isolates were MDR-TB, 3 (1.6%) were resistant to all drugs, and 22 (11.5%) were resistant to one or more commonly used first line anti-TB drugs (FLD). Of the 191 MTB isolates, four main lineages were detected; Lineage 3 [Delhi Central Asia 81 (42.47%)], Lineage 4 [European American 74 (38.7%)], Lineage 1 [East Africa Indian Ocean 23 (12.0%)] and Lineage 2 [Beijing East Asia 13 (6.8%)]. Delhi Central Asia was

the most prevalent in both HIV positive 16 (47.0%) and HIV negative 66 (41.8%). Overall, 24 (12.6%) of the 191 MTB isolates had any resistance-conferring mutations. There were mutations for quinolone-determining mutation region of gyrA at positions Asp94Gly and Ala90Val of 4.2% each. There was an observed lineage specific variation in proportion to drug resistant- conferring mutations.

Conclusion: The second national anti-TB drug resistance survey revealed that the burden of MDR-TB is still relatively low with no evidence of XDR. This is a comparable finding to the first survey conducted in 2006. Delhi Central Asia lineage was the most prevalent in the counry. This study was able to isolate M.tuberculosis strains with resistance against second line drugs not in routine use in Tanzania. Resistance against first-line TB drugs was found in lineage 1 and 4 strains. Use of next generation sequencing may improve the basis for epidemiological conclusions.

List of figures

List of tables

List of abbreviations

BEA	Beijing East Asia
CDC	U.S. Centers for Disease Control and Prevention
CGH	Center for Global Health
CI	Confidence Interval
CO_2	Carbon dioxide
CTAB	Cetyltrimethylammonium bromide
CTRL	Central TB Reference Laboratory
DOTS	Directly Observed Treatment, Short-Course
DRS	Drug resistance surveys
DST	Drug susceptibility testing
E	Ethambutol
EA	European American
EAIO	East Africa Indian Ocean
FLD	First-line drugs
H	Isoniazid
HH	Household contact
iLED	Immunofluorescence light-emitting diode
INH	Isoniazid
LJ	Loewenstein Jensen
LPA	Line-probe assays
M.tb	*Mycobacterium tuberculosis*
MDR	Multi-drug resistant
MDR-TB	Multi-drug resistant TB
MTB	*Mycobacterium tuberculosis*
NatHREC	National Health Research Ethics Committee

NTLP	National Tuberculosis and Leprosy Program
NTM	Non-TB mycobacteria
OR	Odds Ratio
PLHIV	People living with HIV
PMDT	programmatic management of MDR-TB
PPS	Probability proportional to size
PTB	Pulmonary TB
R	Rifampicin
RIF	Rifampicin
S	Streptomycin
SLDs	Second line drugs
SNPs	Single nucleotide poly-morphisms
TB	Tuberculosis
WGS	Wide genome sequencing
WHO	World Health Organisation
XDR	Extensively drug resistant
XDR-TB	Extensively drug-resistant TB
ZTCLs	Zonal Tuberculosis Culture Laboratories

List of publications

Paper A

Mutayoba BK, Ershova J, Lyamuya E, Hoelscher M, Heinrich N, Kilale AM, et al. The second national anti-tuberculosis drug-resistance survey in Tanzania, 2017-18. Trop Med Int Heal. 2022 Sep 11;

Paper B

Mutayoba, B.K., Michael Hoelscher, Heinrich, N. et al. Phylogenetic lineages of tuberculosis isolates and their association with patient demographics in Tanzania. BMC Genomics 23, 561 (2022). https://doi.org/10.1186/s12864-022-08791-3

1. My contribution to the publications

1.1 Contribution to paper A

The first paper is titled “The second Tanzania national anti-tuberculosis drug resistance survey, 2016/2017”. I conceptualized the idea, advised the government on the need and appropriateness to conduct the study. After designing the study, I submitted the document to the National Health Research Ethics Committee (NatHREC) in Tanzania for review and ethical clearance. I conducted field supervision to the study sites as part of efforts to monitor and evaluate the implementation process. I organized and implemented a mid-term review and prepared a mid-term review report. I sought permit for shipment of isolates to Antwerp Supra National Reference Laboratory (SNRL). I supervised the shipment of the study isolates to Antwerp SNRL for quality control. I also conducted quarterly study meetings to monitor and evaluate the study. I prepared the analysis plan including dummy tables which was reviewed by my supervisors. I also took part in the analysis of data, prepared and reviewed the manuscript of which I am both the first and corresponding author. I shared the manuscript draft with co-authors including supervisors, incorporated their inputs before submission of the final version to the journal. I submitted the manuscript to the journal for publication and provided responses to review comments before its publication.

1.2 Contribution to paper B

My contribution to the second paper titled “Phylogenetic lineages of tuberculosis isolates and their association with patient demographics in Tanzania” included; conceptualizing and designing of the study. I applied for a permit to send isolates to Uganda SNRL for whole genome sequencing (WGS) then prepared and shipped the *Mycobacterium tuberculosis* (MTB) isolates. Before submitting the final version to the journal, I discussed the paper draft with co-authors, including my supervisors, and incorporated their suggestions. Prior to publication, I responded to reviewers' comments and submitted the work to the journal for publication of which I am the lead author.

2. Introductory summary

2.1 Background

All nations and all age categories are affected by tuberculosis (TB), according to the World Health Organization (WHO) **(WHO, 2021)**. An estimated 9.9 million persons worldwide suffered TB disease, of which 5.5 million were males, 3.3 million women, and 1.1 million children. Despite being treatable and preventable, TB is the world's second killer infectious diseases behind COVID-19 (before HIV/AIDS) and the 13th largest cause of death overall **(WHO, 2022)**. The most common cause of mortality for those with HIV continues to be TB. According to the WHO's global TB report, 1.5 million individuals worldwide passed away from TB in 2020. The analysis also demonstrates that throughout the same time frame, MDR-TB continued to be a public health emergency and a threat to health security. Only roughly one in three individuals with drug-resistant TB received treatment in 2020. In contrast to drug-sensitive TB treatment, where success rates are higher than 90%, MDR/RR TB patients' global treatment success rates in 2018 were 59% **(WHO, 2022).**

The United Republic of Tanzania is a low-income country with an estimated population of about 64 million people **(UN, 2022).** It is among the 30 high TB burden countries which are reported to account for 86% of the global TB incidence **([b]WHO, 2021).** The incidence of tuberculosis in Tanzania decreased from 306 per 100,000 in 2015 to 222 per 100,000 in 2020, this indicates a 27% decrease in the incidence rate of TB **(NTLP, 2021)**. According to the Tanzania TB and Leprosy Programme **(NTLP, 2021)**; in the year 2020, 85,597 cases of all forms of TB were notified of which 83,129 (97%) and 2,468 (3%) were new and previously treated **(NTLP, 2021).**

The WHO has classified *Mycobacterium tuberculosis* cases based on drug susceptibility testing (DST) of clinical isolates **(WHO, 2013)**. According to the WHO, multidrug resistance TB (MDR-TB) continues to be a public health problem as well as a health security threat in most countries. WHO also recommends where there is limited resources and infrastructure for continuous surveillance for TB drug resistance to all patients surveys should be done after every five years at minimum. Drug resistance surveys usually give insight to practical information to TB Programs on the burden of resistance to TB drugs including usual patient drug resistance profiles **([b]WHO, 2021)**. In addition, surveys can

improve the functioning of laboratory network, **(WHO, 2015)**. The first NationalTuberculosis B drug resistance survey **(Chonde et al., 2010)** was instrumental in setting up programmatic management of MDR-TB (PMDT) program within the NTLP in 2009 in Tanzania. The second survey was made possible through a grant from the Global Fund, who has incorporated such surveys in its TB control policies.

The global prevalence of MDR- or rifampicin-resistant TB was estimated to be 3.4 among newly diagnosed patients, and 18% among patients with history of treatment. Based on the Tanzania first National TB drug resistance survey of 2006, the prevalence of MDR-TB among new patients and among previously treated cases was 1.1% and 3.1%, respectively which is lower than the Global average for both treatment naive and thiose who have been treated for TB in tha past **(Chonde et al., 2010).** It has been reported that a second survey could provide insight whether the rate of MDR-TB is increasing or decreasing **(Cohen et al., 2014)**. Smaller absolute burdens of MDR-TB and more robust surveillance systems were associated with declining rates of MDR-TB among new notified cases **(Cohen et al., 2014, Dye and Williams, 2000)**. This survey was undertaken with the goal of determining the level of TB drug resistance in Tanzania and associated risk factors as a part of strengthening the national TB control efforts in the country.

2.2 Statement of the problem

Anti-TB drug resistance (primary and acquired) has important implications for control programs as well as for individual patient therapy. Acquired drug resistance is also considered a good indicator of poor treatment adherence and related practices in the community. Primary drug resistance on the other hand measures disease transmission in a community and indicate problems which TB control programs will encounter when administering chemotherapy **(Su et al., 2016)**.

The knowledge of resistance to Tuberculosis regimens is important for appropriate planning at National TB Programs and also to the laboratory experts and clinicians to detect and treat properly patients infected with drug-resistant strains. A few studies have been conducted to establish the anti-TB drug-resistance pattern in Tanzania. They include 1) TB drug resistance survey carried out in 1988; 2) TB/HIV study conducted in 1991-1993 (**Chum et al., 1996**); 3) study of impact of HIV on TB treatment outcomes (**van den Broek et al., 1998**); and 4) National anti-tuberculosis drug resistance study in 2006 (**Chonde et al., 2010**)**.** Although drug resistance surveillance is conducted routinely, the

surveillance system is weak and the collected data do not necessarily give a proper estimate of the burden of the disease. Even with the availability of molecular diagnostic technology such as Xpert MTB/RIF since 2012, its coverage in the country is still low and this rapid test can only detect rifampicin mono-resistance. In addition, the resistance occurrence and pattern to second line anti TB drugs is not known.

Bearing this in mind, the survey served as an indirect evaluation of TB diagnostics, control interventions and delivery of treatment within the framework of the NTLP. It also aimed to contribute to a better estimate of the national burden of DR-TB and to assess how mycobacterial genotypes relate to drug resistance, epidemiology and pathogenesis in order to provide information for proper planning of MDR-TB and XDR-TB treatment in the country. Furthermore, this national anti-TB drugs resistance survey supported the country to develop a stronger mechanism of surveillance.

2.3 Objectives

2.3.1 Broad Objective

To assess the proportion, and patterns of drug resistance against anti-TB drugs of first and second line, among newly diagnosed and previously treated patients with sputum smear positive TB and comprehensively understand the drug resistance profile as well as attempt to understand phylogenetic variations in Tanzania.

2.3.2 Specific Objectives

1. To determine the proportion of new and previously treated sputum smear positive pulmonary tuberculosis patients with resistance to rifampicin and isoniazid (MDR-TB) and other first line drugs.
2. To determine the proportion and patterns of drug resistance to second line drugs among confirmed MDR-TB patients.
3. To determine the risk factors associated with anti-TB drug resistance among smear positive pulmonary tuberculosis patients.
4. To determine the occurrence of phylogenetic lineages of *MTB* complex and their relationship with patient demographic characteristics and multidrug-resistant TB (MDR-TB).

2.4 Methods

2.4.1 Methods used in Paper A

This was a cross-sectional health facility-based survey conducted from 2017 to 2018 which included new and previously treated smear-positive pulmonary TB patients of all ages. Enrollin health facilities were considered as clusters with a design effect of 2 and a precision 0.8%. The sample size was 1,495 considering 15% losses. This calculation was based on indications from WHO technical guidance documents **(WHO, 2015)** All smear-positive previously treated patients who met eligibility criteria were enrolled during intake period. The selection of clusters was done using probability proportional to size (PPS) sampling; 45 clusters were selected. The number of clusters had advantages in terms of study logistics and corresponded to the management capacity of the programme. In each cluster a total of 34 new smear positive pulmonary TB patients and all previously treated smear positive pulmonary TB cases were enrolled. HIV status was obtained from the individual patients' files in the diagnosing health facilities. Sputum specimen were tested at the health facilty laboratory by LED microscope. The next processing was done at the Central TB Reference Laboratory (CTRL) in case of positive smear. At CTRL, Xpert MTB/RIF assay, smear by fluorescent microscopy, culture on Lowenstein Jensen media, and susceptibility testing following standard NTLP. Completed questionnaires were entered into an electronic Epi-Info/Access database by trained data entry personnel. TB laboratory network in Tanzania is organized into four main levels according to the type of services provided. The levels include: 1) the Central TB Reference Laboratory (CTRL) 2) Zonal tuberculosis culture laboratories (ZTCLs), 3) regional and district referral hospital laboratories and 4) health centers and dispensary laboratories. The CTRL routinely perform surveillance of resistant *Mycobacterium tuberculosis* (MTB) using first and/or second line DST for culture positive isolates. Currently, there are 1,750 diagnostic centers at different levels of the health system and GeneXpert ® MTB/RIF (Xpert) molecular technology has been scaled up to 336 GeneXpert machines countrywide. Sputum culture is done in the five ZTCLs while two zonal TB Reference laboratories at Mbeya Zonal Referral Hospital and Kibong'oto Infectious Diseases Hospital that perform identification and first line DST using molecular techniques [Xpert and line probe assays (LPA)].

2.4.2 Methods used in Paper B

For WGS, all positive cultures were taken to the TB Supranational TB Reference Laboratory in Uganda. The sub-cultures were done on Middlebrook agar media, incubated at 37^0C in a CO_2 incubator and monitored weekly for growth. High quality genomic DNA was extracted using an in-house cetyltrimethylammonium bromide (CTAB) technique. Genomic libraries were prepared using the Illumina Nextera XT library preparation kit following manufacturer's instruction. In total, 192 samples were sequenced. *De-novo* genome assembly of all samples was done using Unicycler v0.4.8. The assembled genomes were then annotated using Prokka (**Seemann, 2014**) to generate genomic feature files to be used as input for Roary v3.13.0 software which was then used to generate a core gene multiple sequence alignment. Using the GTR+G substitution model, a maximum likelihood phylogeny was constructed using RaxML-NG v1.0.3 software with 800 bootstrap replicates using H37Rv reference strain NC_000962.3 as the reference and *Mycobacterium canettii* NC_015848.1 as the out-group. The resulting trees were plotted, annotated and visualized using ggtree v2.0.4.

2.4.3 Ethical Considerations and related study procedures

The proposal for this survey was granted ethical clearance by the National Health Research Ethics Committee (NatHREC) at the National Institute for Medical Research (NIMR) in Tanzania. A written informed consent was obtained from each patient or guardian/caretaker enrolled in the study. The patients were given an information sheet to read and understand what the study was all about. The information sheet was prepared in Kiswahili (the national language). The relatives of the survey patients were read the information to the patients that are not able to read (illiterate patients). The study clinician or designated personnel answered any question asked by the patients. Patients who agreed to participate in the study were required to give their consent by signing the consent form or printing their thumbs in the respective forms. As recommended by the national guidelines, all cases of MDR-TB were referred to MDR-TB treatment initiating centres for appropriate management.

For children under 18 years (6 to 18 years), in addition to consent by their parents/guardians an assent to participate was sought. For children below 6 years of age, the parents or guardians consented to participate in the study for them.

Protection of confidentiality: All survey forms had patient identifying information on the cover sheet, as the clinician needed this information when referring the patient. After entering the data into the database, a survey identification number and specimen laboratory number was served as identification numbers for each suspected specimen, and subsequent TB isolates. All names were electronically removed from the final TB database at the end of the survey.

Data safety/protection: Data was stored in the Access format with password protection and was only accessible to authorized personnel. All names were removed from the database after data cleaning prior to data analysis. Backup data was stored on the external hard drive in a separate site in case of any fault in the primary computers.

2.5 Results

2.5.1 Result of publication A

The survey was conducted in 45 clusters. A total of 1557 (91%) TB patients were enrolled in the survey out of 1714 who were eligible. Among them, 1408 (90%) were newly diagnosed and 149 (10%) previously treated patients. In total, during the intake period 157 (9%) eligible cases were not enrolled in the survey due to several reasons that are described in the discussion section. Culture on LJ media was performed for 1493 (93%) specimens of the enrolled patients; 64 (4%) patients did not have culture results including 14 whose specimens were rejected at the CTRL due to sputum leakage. Among enrolled cases with culture performed, 1172 (78.5%) were MTB positive, 278 (19%) were MTB negative, 10 (0.5%) had non-TB mycobacteria, and 33 (2%) were contaminated. Among MTB cases, 1063 (92%) were new and 109 (9%) previously treated patients. The distribution of 1172 patients who were MTB positive by age and sex is shown in **Figure 2.1.**

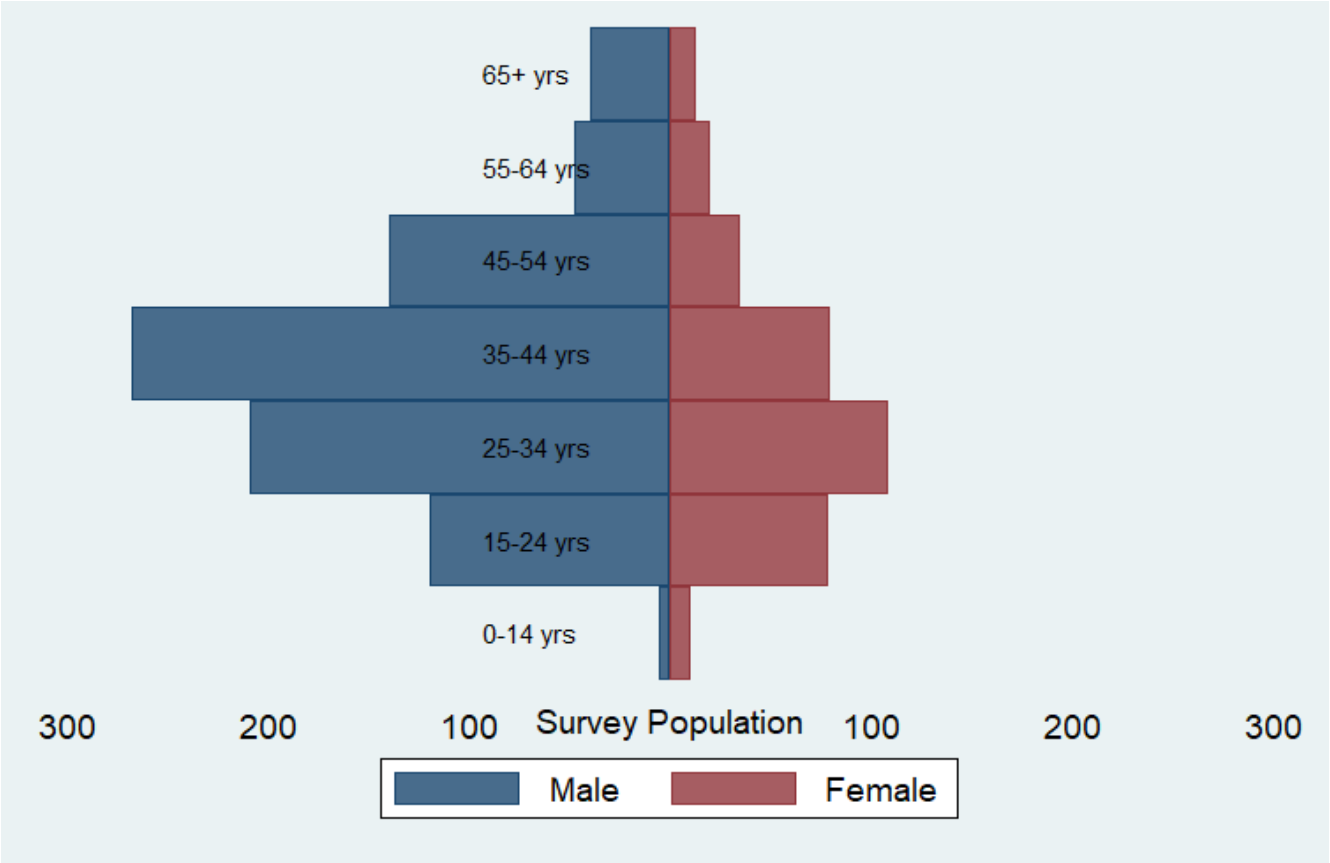

Figure 2.1. Distribution of TB patients by age and gender (n=1,172)

The estimated total prevalence of multi-drug resistant TB (MDR-TB) in this survey was 14 (1.2) out of which was 9 (0.9%) were in newly diagnosed patients, and 5 (4.6%) in patinets who had previous treatment. Resitance against any of the first-line anti-TB drugs (streptomycin (S), isoniazid (H), rifampicin (R), and ethambutol (E) was estimated at 24 (1.7%) among new and 10 (6.5%) among previously treated TB patients. Drug resistance to all first-line drugs was similar to 0.1% in new and previously treated patients. No poly-resistance (other than MDR) or extensive resistance (XDR-TB) was detected in any isolate. History of prior TB treatment was identified as the only risk factor for MDR-TB (OR=5.7, 95%CI: 1.9-17.2) (**Table 2.1**).

Table 2.1. Factors associated with MDR-TB in Tanzania

Risk Factors	MDR n (%)	Non-MDR n (%)	OR	95%CI	p-value
Patient classification					
New	9 (0.9)	1,051			

Previously treated	5 (4.6)	103	5.7	1.9-17.2	0.002
Gender					
Male	8 (1.0)	815(99)			
Female	6 (1.7)	339(98.3)	1.8	0.6-5.2	0.3
Age groups			1.1	0.7-1.6	0.7
0-14	0 (0)	16 (100)			
15-24	0 (0)	198 (100)			
25-34	8 (2.5)	308 (97.5)			
35-44	2 (0.6)	344 (99.4)			
45-54	3 (1.7)	171 (98.3)			
55-64	0 (0)	67 (100)			
65+	1 (2.0)	50 (98)			
Age groups					
0-34	8 (1.5)	522 (98.5)			
35+	6 (0.9)	632 (99.1)	0.6	0.2-1.8	0.4
Age groups					
0-44	10 (1.1)	866 (98.9)			
45+	4 (1.4)	288 (98.6)	1.2	0.4-3.9	0.8
HIV status					
Yes	2 (0.7)	282 (99.3)	0.5	0.1-3.2	0.4
No	12 (1.4)	872 (98.6)			
Alcohol use					
Yes	4 (1.6)	245 (98.4)	1.9	0.6-6.7	0.3
No	7 (0.8)	834 (99.2)			
Missing	3 (3.8)	75 (96.2)			
Ever smoked					
Yes	2 (1.0)	200 (99)	0.97	0.2-4.5	0.9
No	9 (1.0)	870 (99)			
Missing	3 (3.4)	84 (96.6)			
Diabetes mellitus					
Yes	0 (0)	16 (100)	N/A		
No	11 (1.0)	1058 (99)			
Missing	3 (3.6)	80 (96.4)			
HH Contact with MDR-TB case					
Yes	0 (0)	57 (100)	N/A		
No	9 (1.0)	910 (99)			
Missing	5 (2.6)	187 (97.4)			

MDR = multi-drug resistance; HH = Household contact

2.5.2 Result of publication B

A total of 627 *Mycobacterium tuberculosis* isolates were shipped from Tanzania and only 617 isolates were sub-cultured at the NTRL Uganda. Ten isolates were rejected for inability to meet the requirements for sub-culture. Of the 617 isolates, 265 (43%) yielded either no growth, contaminated or non-tuberculous mycobacteria (NTM) therefore, could

not be processed for WGS. The remaining 352 isolates yielded a positive TB culture, however, only 191 (54%) of 352 isolates were sequenced due to financial constraints. Therefore, the current results are a summary and analysis of the 191 isolates processed for WGS and their association with patient demographics.

Of the 191 isolates, 169 (88.0%) were from newly treated PTB patients. Thirty-three (17.3%) of isolates were collected from individuals who were TB/HIV co-infected. Of 191 isolates, in 22 (11.5%), resistance to one or more first line anti-TB drugs (FLD) was found. 3 (1.6%) were resistant to all drugs examined, and 9 (4.7%) were MDR-TB.

Four main lineages were identified; Lineage 3 [Delhi Central Asia, 81 (42.7%)], Lineage 4 [European American, 74 (38.5%)], Lineage 1 [East Africa Indian Ocean, 23 (11.9%)] and Lineage 2 [Beijing East Asia, 13 (6.8%)]. Delhi Central Asia was the most prevalent among the 22 isolates from previously treated TB, as compared to isolates among newly treated patients 9 (47.4%) versus 72 (41.9%), respectively. Delhi Central Asia was the most prevalent in both HIV positive 15 (47.0%) and HIV negative 66 (41.8%). The same was true for European American, which had 15 (45.5%) isolates from HIV-positive patients and 59 (37.3%) isolates from HIV-negative individuals. **(Table 2.2).**

Table 2.2. *M. tuberculosis* lineages and their correlation with anti-TB drug resistance

Drug resistance	**Total**	***M. tuberculosis* lineages**				
		Lineage 1	Lineage 2	Lineage 3	Lineage 4	p-value
Isoniazid						
Sensitive	178	20 (87.0)	13 (100)	77 (95.1)	68 (91.9)	0.411
Resistant	13	3 (13.0)	0 (0)	4 (4.9)	6 (8.1)	
Rifampicin						
Sensitive	181	22 (95.7)	12 (92.3)	76 (93.8)	71 (96.0)	0.867
Resistant	10	1 (4.4)	1 (7.7)	5 (6.2)	3 (4.1)	
Ethambutol						
Sensitive	183	22 (95.7)	12 (92.3)	79 (97.5)	70 (94.6)	0.454
Resistant	8	1 (4.4)	1 (7.7)	2 (2.5)	4 (5.4)	
Pyrazinamide						

Sensitive	183	22 (95.7)	13 (100)	81 (100)	67 (90.5)	0.02
Resistant	8	1 (4.4)	0 (0)	0 (0)	7 (9.5)	
Streptomycin						
Sensitive	185	21 (91.3)	13 (100)	81 (100)	70 (94.6)	0.46
Resistant	6	2 (8.7)	0 (0)	0 (0)	4 (5.4)	
Ciprofloxacin						
Sensitive	189	23 (100)	12 (92.3)	80 (98.8)	74 (100)	0.146
Resistant	2	0 (0)	1 (7.7)	1 (1.2)	0 (0)	
Moxifloxacin						
Sensitive	189	23 (100)	12 (92.3)	89 (98.8)	74 (100)	0.146
Resistant	2	0 (0)	1 (7.7)	1 (1.2)	0 (0)	
Ofloxacin						
Sensitive	189	23 (100)	12 (92.3)	80 (98.8)	74 (100)	0.146
Resistant	2	0 (0)	1 (7.7)	1 (1.2)	0 (0)	
Ethionamide						
Sensitive	189	21 (91.3)	13 (100)	81 (100)	74 (100)	0.018
Resistant	2	2 (8.7)	0 (0)	0 (0)	0 (0)	

Note: No resistance was reported in Amikacin, Capreomycin, kanamycin, Cycloserine, Clofazimine, PAS, Delanamid, Bedaquiline, and linezolid

The Delhi Central Asia (Lineage 3) is represented with 9 (10.7%) isolates resistant to first line drugs, of which 3 (3.6%) were MDR-TB, compared to the Beijing East Asian (Lineage 2) lone (10.0%) isolate that showed resistance to rifampicin and ethambutol. Out of the 22 isolates for the East Africa Indian Ocean (Lineage 1), three (13.6%) were first line drug resistant and two (9.1%) were MDR-TB. Out of 74 isolates from the European American (Lineage 4) population, 9 (12.2%) were first line drug -resistant and 3 (4.1% were MDR-TB). There was M. tuberculosis strains resistant to some second line drugs mutation at Ala90Val and Asp94Gly in the gyrA's conserved quinolone resistance determining regions (QRDR), two (8.3%) of the isolates had fabG1 and inhA mutations that made them resistant to ethionamide, mutations at the inhA and fabG1 loci that confer resistance. Each Ser94Ala was recorded as 1 (4.2%). (**Table 3 on the manuscript)**. There was an observed lineage specific variation in proportion to drug resistant- conferring mutations. **(Table 2.3)**

Table 2.3 The pattern of drug resistance mutations by phylogenetic lineages

Drug-resistant mutations	**Total, n (%)**	**Type of Lineage**			
		Lineage 1, n (%)	Lineage 2, n (%)	Lineage 3, n (%)	Lineage 4, n (%)

Isoniazid, n=13					
CTG607CTA	1 (7.7)	0 (0)	-	1 (20.0)	0 (0)
Ser315Thr	9 (69.3)	0 (0)	-	4 (80.0)	5 (83.3)
Ser94Ala	1 (7.7)	1 (50.0)	-	0 (0)	0 (0)
c.-15C>T	1 (7.7)	1 (50.0)	-	0 (0)	0 (0)
c.-8T>A	1 (7.7)	0 (0)	-	0 (0)	1 (16.7)
Rifampicin, n=11					
Ser441Gln	1 (10.0)	0 (0)	0 (0)	1 (20.0)	0 (0)
Gln432Glu	3 (30.0)	0 (0)	0 (0)	0 (0)	3 (100)
His445Asn	1 (10.0)	1 (100)	0 (0)	0 (0)	0 (0)
Leu430Pro	1 (10.0)	0 (0)	0 (0)	1 (20.0)	0 (0)
Ser441Gln	2 (20.0)	0 (0)	0 (0)	1 (20.0)	0 (0)
Ser450Leu	3 (30.0)	0 (0)	1 (100)	2 (40.0)	0 (0)
Ethambutol, n=8					
Asp1024Asn	1 (12.5)	-	0 (0)	1 (33.3)	0 (0)
Gln497Arg	2 (25.0)	-	0 (0)	1 (33.3)	1 (25.0)
Leu359Ile	1 (12.5)	-	0 (0)	1 (33.3)	0 (0)
Met306Ile	4 (50.0)	-	1 (100)	0 (0)	3 (75.0)
Pyrazinamide, n=8					
Ala30Val	2 (25.0)	0 (0)	-	-	2 (28.6)
E111$	1 (12.5)	0 (0)	-	-	1 (14.3)
GAG331TAG	2 (25.0)	0 (0)	-	-	2 (28.6)
Leu172Pro	1 (12.5)	0 (0)	-	-	1 (14.3)
Phe106Leu	1 (12.5)	1 (100)	-	-	0 (0)
Thr160Ala	1 (12.5)	0 (0)	-	-	1 (14.3)
Streptomycin, n=6					
Lys88Met	4 (66.7)	0 (0)	-	-	4 (100)
Pro93Leu	1 (16.7)	1 (50.0)	-	-	0 (0)
Ser172Cys	1 (16.7)	1 (50.0)	-	-	0 (0)
Ethionamide, n=2					
Ser94Ala	1 (50.0)	1 (50.0)	-	-	-
c.-15C>T	1 (50.0)	1 (50.0)	-	-	-
Fluoroquinolones, n=2					
Ala90Val	1 (50.0)	-	1 (100)	0 (0)	-
Asp94Gly	1 (50.0)	-	0 (0)	1 (100)	-

2.6 Discussion

The overall prevalence of MDR-TB reported in this study was 1.2%, 0.9% among new and 4.6% in previously treated sputum smear-positive TB patients. This represents a slight decline in new and an increase among previously treated patients compared to the MDR-TB prevalence from the previous national survey conducted in 2006 **(Chonde et al., 2010)**. Like in the previous survey, no XDR cases were reported. The reported prevalence of MDR-TB in our survey was lower compared to other recently reported findings in Ghana **(Sylverken et al., 2021)**, South Africa **(Ismail et al., 2018),** Namibia **(Ruswa et al., 2019)** and Zimbabwe **(Timire et al., 2019).**This finding shows that resistance to anti-TB drugs has remained fairly low over the years in Tanzania.

Although HIV has been reported to be associated with TB drug-resistance **(Pavlenko et al., 2018, Kamolwat et al., 2021, Bykov et al., 2022)**, this was not the case in our study. The current finding of not having association between HIV and TB drug resistance is in line with those reported in Uganda **(Lukoye et al., 2013)** and Namibia **(Ruswa et al., 2019)** where they also found that HIV was not a driving factor for MDR-TB. History of previous TB treatment was the only factor significantly associated with MDR-TB in Tanzania. This finding has also been reported in most recent surveys **(Tembo and Malangu, 2019, Ismail et al., 2018, Ruswa et al., 2019, Sylverken et al., 2021, Kamolwat et al., 2021)**. Although the magnitude of MDR-TB among previously treated cases is not as high as in other high burden countries, there still needs to be some programmatic strategies to closely monitor the treatment in TB patients.

In this study we report isolation of four lineages; Delhi Central Asia (CAS), European American, East Africa Indian Ocean (EAI) and Beijing East Asia. Lineage 3 (CAS) was the most prevalent followed by Lineage 4 (European American) similar to the findings reported in earlier studies conducted in the country **(Rutaihwa et al., 2019, Mfinanga et al., 2014).** Previous studies elsewhere have also reported high prevalence of CAS **(Elegail et al., 2018, Couvin et al., 2019)**. This finding was also found in a database geographical assessment of the distribution of CAS and East African Indian Ocean lineages globally **(Couvin et al., 2019).** Beijing East Asia (Beijing) presented the lowest prevalence in the current study. Several studies have shown that the Beijing East Asia and its modern sub-types to be associated with high occurrence of MDR-TB **(Niemann et al., 2010, Panossian et al., 2018)**. Also, a study conducted in Lebanon documented that

Beijing lineage was driving the massive spread of MDR TB in Eurasia **(Panossian et al., 2018).** The lineage was reported to be associated with large MDR-TB outbreaks **(Munsiff et al., 2003)** and appeared to be rapidly expanding in population **(Cowley et al., 2008).**

Due to the small sample size that was examined, there was no compelling evidence of a relationship between any specific lineage and drug-resistant mutations. As a result, more work needs to done to define which Mycobacterium tuberculosis lineages are more prone to develop drug resistance in Tanzania including on the functioning of the resistance genes. Due to the low burden of MDR in Tanzania, further rersearch focusing in areas of high MDR prevalence such as Dar es Salaam where direct person to person transmision is more likely to provide more locally relevant data.

The reported low prevalence of MDR can be attributed to a consistently well performing National TB program with strict regulation of TB medications in the private sector over the years, even as the TB program is decentralizing treatment of MDR-TB. It is also evident that the lack of dominant strains with resistance conferring mutations as also found in a recent study in the country **(Mbelele et al., 2022)** could be an additional factor in the low rate of drug resistance. Contrary to what was recommended by WHO **(Cox and Mizrahi, 2018, ed, 2018)** due to the limited resources there is strong justification for continuing to implement universal DST using more practical and the less expensive rapid molecular techniques such as Gene Xpert technology and reserving the resource heavy Whole Genome Sequencing for periodic epidemiologic Surveys and cases requiring special management.

2.7 Conclusion

The second national anti-TB drug resistance survey revealed that the burden of MDR-TB is still relatively low with no evidence of XDR. This is a comparable finding to the first survey conducted in 2006. Delhi Central Asia lineage was the most prevalent in the country. Overall, our present study demonstrated that M. tuberculosis strains occurred that displayed resistance against second line drugs, that are not in frequent use in Tanzania. In patients with previous TB treatment and in TB patients living with HIV, lineage 3 was the most frequently identified lineage. Lineage 1 and 4 were associated with resistance

against first-line drugs. Next generation sequencing may enhance the database generated from anti-TB drug resistance surveys and help in decision making.

3. References

aWHO 2021. WHO Fact sheets; Tuberculosis, key facts. https://www.who.int/news-room/fact-sheets/detail/tuberculosis [Accessed on 20 September 2022.

bWHO 2021. WHO global lists of high burden countries for TB, multidrug/rifampicin-resistant TB (MDR/RR-TB) and TB/HIV, 2021–2025. Geneva: World Health Organization; 2021. Licence: CC BY-NC- SA 3.0 IGO); https://cdn.who.int/media/docs/default-source/hq-tuberculosis/who_globalhbcliststb_2021-2025_backgrounddocument.pdf?sfvrsn=f6b854c2_9 [Accessed on 22 September 2022

Bykov, I., Dyachenko, O., Ratmanov, P., Liu, H., Liang, L. & Wu, Q. 2022. Factors contributing to the high prevalence of multidrug-resistance/Rifampicin-resistance in patients with tuberculosis: an epidemiological cross sectional and qualitative study from Khabarovsk krai region of Russia. *BMC Infectious Diseases,* 22**,** 612.

Chonde, T. M., Basra, D., Mfinanga, S. G., Range, N., Lwilla, F., Shirima, R. P., van Deun, A., Zignol, M., Cobelens, F. G., Egwaga, S. M. & van Leth, F. 2010. National anti-tuberculosis drug resistance study in Tanzania. *Int J Tuberc Lung Dis,* 14**,** 967-72.

Chum, H. J., O'Brien, R. J., Chonde, T. M., Graf, P. & Rieder, H. L. 1996. An epidemiological study of tuberculosis and HIV infection in Tanzania, 1991-1993. *Aids,* 10**,** 299-309.

Cohen, T., Jenkins, H. E., Lu, C., McLaughlin, M., Floyd, K. & Zignol, M. 2014. On the spread and control of MDR-TB epidemics: an examination of trends in anti-tuberculosis drug resistance surveillance data. *Drug Resist Updat,* 17**,** 105-23.

Couvin, D., Reynaud, Y. & Rastogi, N. 2019. Two tales: Worldwide distribution of Central Asian (CAS) versus ancestral East-African Indian (EAI) lineages of Mycobacterium tuberculosis underlines a remarkable cleavage for phylogeographical, epidemiological and demographical characteristics. *PLOS ONE,* 14**,** e0219706.

Cowley, D., Govender, D., February, B., Wolfe, M., Steyn, L., Evans, J., Wilkinson, R. J. & Nicol1, M. P. 2008. Recent and Rapid Emergence of W-Beijing Strains of Mycobacterium tuberculosis in Cape Town, South Africa. *Clinical Infectious Diseases,* 47**,** 1252-1259.

Cox, H. & Mizrahi, V. 2018. The Coming of Age of Drug-Susceptibility Testing for Tuberculosis. *New England Journal of Medicine,* 379**,** 1474-1475.

Dye, C. & Williams, B. G. 2000. Criteria for the control of drug-resistant tuberculosis. *Proceedings of the National Academy of Sciences,* 97**,** 8180-8185.

ed 2018. 'Prediction of Susceptibility to First-Line Tuberculosis Drugs by DNA Sequencing'. *New England Journal of Medicine,* 379**,** 1403-1415.

Elegail, A., Ibrahim Mohamed, N., Mohamed Nour, E., Hoffner, S. & Haile, M. 2018. Molecular characterization of Mycobacterium tuberculosis isolates from

pulmonary tuberculosis patients in Khartoum, Sudan. *International Journal of Mycobacteriology,* 7**,** 236-241.

Ismail, N. A., Mvusi, L., Nanoo, A., Dreyer, A., Omar, S. V., Babatunde, S., Molebatsi, T., van der Walt, M., Adelekan, A., Deyde, V., Ihekweazu, C. & Madhi, S. A. 2018. Prevalence of drug-resistant tuberculosis and imputed burden in South Africa: a national and sub-national cross-sectional survey. *Lancet Infect Dis,* 18**,** 779-787.

Kamolwat, P., Nateniyom, S., Chaiprasert, A., Disratthakit, A., Mahasirimongkol, S., Yamada, N. & Smithtikarn, S. 2021. Prevalence and associated risk factors of drug-resistant tuberculosis in Thailand: results from the fifth national anti-tuberculosis drug resistance survey. *Tropical Medicine & International Health,* 26**,** 45-53.

Lukoye, D., Adatu, F., Musisi, K., Kasule, G. W., Were, W., Odeke, R., Kalamya, J. N., Awor, A., Date, A. & Joloba, M. L. 2013. Anti-tuberculosis drug resistance among new and previously treated sputum smear-positive tuberculosis patients in Uganda: results of the first national survey. *PLoS One,* 8**,** e70763.

Mbelele, P. M., Utpatel, C., Sauli, E., Mpolya, E. A., Mutayoba, B. K., Barilar, I., Dreyer, V., Merker, M., Sariko, M. L., Swema, B. M., Mmbaga, B. T., Gratz, J., Addo, K. K., Pletschette, M., Niemann, S., Houpt, E. R., Mpagama, S. G. & Heysell, S. K. 2022. Whole genome sequencing-based drug resistance predictions of multidrug-resistant Mycobacterium tuberculosis isolates from Tanzania. *JAC-Antimicrobial Resistance,* 4.

Mfinanga, S. G., Warren, R. M., Kazwala, R., Kahwa, A., Kazimoto, T., Kimaro, G., Mfaume, S., Chonde, T., Ngadaya, E., Egwaga, S., Streicher, E. M., Van Pittius, G. N., Morkve, O. & Cleaveland, S. 2014. Genetic profile of Mycobacterium tuberculosis and treatment outcomes in human pulmonary tuberculosis in Tanzania. *Tanzan J Health Res,* 16**,** 58-69.

Munsiff, S. S., Nivin, B., Sacajiu, G., Mathema, B., Bifani, P. & Kreiswirth, B. N. 2003. Persistence of a Highly Resistant Strain of Tuberculosis in New York City during 1990–1999. *The Journal of Infectious Diseases,* 188**,** 356-363.

Niemann, S., Diel, R., Khechinashvili, G., Gegia, M., Mdivani, N. & Tang, Y. W. 2010. Mycobacterium tuberculosis Beijing lineage favors the spread of multidrug-resistant tuberculosis in the Republic of Georgia. *J Clin Microbiol,* 48**,** 3544-50.

NTLP 2021. TB Prevalence in Tanzania. https://www.ntlp.go.tz/tuberculosis/tb-prevalence-in-tanzania/ [Accessed on 22 September 2022].

Panossian, B., Salloum, T., Araj, G. F., Khazen, G. & Tokajian, S. 2018. First insights on the genetic diversity of MDR Mycobacterium tuberculosis in Lebanon. *BMC Infectious Diseases,* 18**,** 710.

Pavlenko, E., Barbova, A., Hovhannesyan, A., Tsenilova, Z., Slavuckij, A., Shcherbak-Verlan, B., Zhurilo, A., Vitek, E., Skenders, G., Sela, I., Cabibbe, A. M., Cirillo, D. M., de Colombani, P., Dara, M., Dean, A., Zignol, M. & Dadu, A. 2018. Alarming levels of multidrug-resistant tuberculosis in Ukraine: results from the first national survey. *The International Journal of Tuberculosis and Lung Disease,* 22**,** 197-205.

Ruswa, N., Mavhunga, F., Roscoe, J. C., Beukes, A., Shipiki, E., van Gorkom, J., Sawadogo, S., Agolory, S., Menzies, H., Tiruneh, D., Makumbi, B., Bayer, B., Zezai, A., Campbell, P., Alexander, H., Kalisvaart, N. & Forster, N. 2019. Second nationwide anti-tuberculosis drug resistance survey in Namibia. *Int J Tuberc Lung Dis,* 23**,** 858-864.

Rutaihwa, L. K., Sasamalo, M., Jaleco, A., Hella, J., Kingazi, A., Kamwela, L., Kingalu, A., Malewo, B., Shirima, R., Doetsch, A., Feldmann, J., Reinhard, M., Borrell, S., Brites, D., Reither, K., Doulla, B., Fenner, L. & Gagneux, S. 2019. Insights into the genetic diversity of Mycobacterium tuberculosis in Tanzania. *PLOS ONE,* 14**,** e0206334.

Seemann, T. 2014. Prokka: rapid prokaryotic genome annotation. *Bioinformatics,* 30**,** 2068-9.

Su, V. Y., Yen, Y. F., Pan, S. W., Chuang, P. H., Feng, J. Y., Chou, K. T., Chen, Y. M., Chen, T. J. & Su, W. J. 2016. Latent Tuberculosis Infection and the Risk of Subsequent Cancer. *Medicine (Baltimore),* 95**,** e2352.

Sylverken, A. A., Kwarteng, A., Twumasi-Ankrah, S., Owusu, M., Arthur, R. A., Dumevi, R. M., Adu-Amoah, L., Addofoh, N., Okyere, P. B., Dzata, F., Bonsu, F., Adusi-Poku, Y., Kranzer, K., Siroka, A., Gemert, W. V., Dean, A. & Owusu-Dabo, E. 2021. The burden of drug resistance tuberculosis in Ghana; results of the First National Survey. *PLoS One,* 16**,** e0252819.

Tembo, B. P. & Malangu, N. G. 2019. Prevalence and factors associated with multidrug/rifampicin resistant tuberculosis among suspected drug resistant tuberculosis patients in Botswana. *BMC Infectious Diseases,* 19**,** 779.

Timire, C., Metcalfe, J. Z., Chirenda, J., Scholten, J. N., Manyame-Murwira, B., Ngwenya, M., Matambo, R., Charambira, K., Mutunzi, H., Kalisvaart, N. & Sandy, C. 2019. Prevalence of drug-resistant tuberculosis in Zimbabwe: A health facility-based cross-sectional survey. *International Journal of Infectious Diseases,* 87**,** 119-125.

UN 2022. Department of Economic and Social Affairs, Population Division (2022). World Population Prospects 2022, Online Edition. World Population Prospects - Population Division - United Nations; https://population.un.org/wpp/ [Accessed on 22 September 2022].

van den Broek, J., Mfinanga, S., Moshiro, C., O'Brien, R., Mugomela, A. & Lefi, M. 1998. Impact of human immunodeficiency virus infection on the outcome of treatment and survival of tuberculosis patients in Mwanza, Tanzania. *Int J Tuberc Lung Dis,* 2**,** 547-52.

WHO 2013. World Health Organization (2013) Definitions and reporting framework for tuberculosis – 2013 revision (updated December 2014 and January 2020). WHO/HTM/TB/2013.2. ISBN 978 92 4 150534 5. https://apps.who.int/iris/bitstream/handle/10665/79199/9789241505345_eng.pdf [Accessed on 22 September 2022].

WHO 2015. *World Health Organization (c). Guidelines for surveillance of drug resistance in tuberculosis 5th Edition. ISBN 978 92 4 154913 4.*

https://apps.who.int/iris/bitstream/handle/10665/174897/9789241549134_eng.pdf [Accessed on 24 September 2022].

WHO 2022. World Health Organization. (2022). Global tuberculosis report 2021: supplementary material. World Health Organization. https://apps.who.int/iris/handle/10665/360605. License: CC BY-NC-SA 3.0 IGO [Accessed on 23 September 2022].

4. Publications

4.1 Paper A (*appended as pdf file*)

Mutayoba BK, Ershova J, Lyamuya E, Hoelscher M, Heinrich N, Kilale AM, et al. The second national anti-tuberculosis drug-resistance survey in Tanzania, 2017-18. Trop Med Int Heal. 2022 Sep 11;

DOI: 10.1111/tmi.13814

RESEARCH ARTICLE

The second national anti-tuberculosis drug resistance survey in Tanzania, 2017–2018

Beatrice Kemilembe Mutayoba[1,2] | **Julia Ershova**[3] | **Eligius Lyamuya**[4] | **Michael Hoelscher**[2] | **Norbert Heinrich**[2] | **Andrew Martin Kilale**[5] | **Nyagosya Segere Range**[5] | **Benard James Ngowi**[6] | **Nyanda Elias Ntinginya**[7] | **Saidi Mwinjuma Mfaume**[5] | **Emmanuel Nkiligi**[8] | **Basra Doulla**[9] | **Johnson Lyimo**[8] | **Riziki Kisonga**[10] | **Amri Kingalu**[8] | **Yakobo Lema**[5] | **Zuwena Kondo**[8] | **Michel Pletschette**[2]

[1]Department of Preventive Services, Ministry of Health National AIDS Control Program, Dodoma, Tanzania

[2]Department of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich, Munich, Germany

[3]Division of Global HIV and TB, Global TB Branch, US Centers for Disease Control and Prevention, Atlanta, Georgia, USA

[4]Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences, Dar es Salaam, Tanzania

[5]Muhimbili Medical Research Centre, National Institute for Medical Research, Dar es Salaam, Tanzania

[6]Mbeya College of Health and Allied Sciences, University of Dar es Salaam, Mbeya, Tanzania

[7]National Institute for Medical Research, Mbeya Medical Research Centre, Mbeya, Tanzania

[8]National Tuberculosis and Leprosy Program, Department of Preventive Services, Ministry of Health, Dodoma, Tanzania

[9]National Tuberculosis and Leprosy Program, Central Tuberculosis Reference Laboratory, Dar es Salaam, Tanzania

[10]Kibong'oto Infectious Diseases Hospital, Kilimanjaro, Tanzania

Correspondence
Beatrice Kemilembe Mutayoba, Ministry of Health, P.O. Box 743, 40478 Dodoma, Tanzania.
Email: bmutayoba@gmail.com

Funding information
The Global Fund to Fight AIDS, Tuberculosis and Malaria

Abstract

Objective: To determine the levels and patterns of resistance to first- and second-line anti-tuberculosis (TB) drugs among new and previously treated sputum smear positive pulmonary TB (PTB) patients.

Methods: We conducted a nationally representative cross-sectional facility-based survey in June 2017–July 2018 involving 45 clusters selected based on probability proportional to size. The survey aimed to determine the prevalence of anti-TB drug resistance and associated risk factors among smear positive PTB patients in Tanzania. Sputum samples were examined using smear microscopy, Xpert MTB/RIF, culture and drug susceptibility testing (DST). Logistic regression was used to account for missing data and sampling design effects on the estimates and their standard errors.

Results: We enrolled 1557 TB patients, including 1408 (90.4%) newly diagnosed and 149 (9.6%) previously treated patients. The prevalence of multidrug-resistant TB (MDR-TB) was 0.85% [95% confidence interval (CI): 0.4–1.3] among new cases and 4.6% (95% CI: 1.1–8.2) among previously treated cases. The prevalence of *Mycobacterium tuberculosis* strains resistant to any of the four first-line anti-TB drugs (isoniazid, rifampicin, streptomycin and ethambutol) was 1.7% among new TB patients and 6.5% among those previously treated. Drug resistance to all first-line drugs was similar (0.1%) in new and previously treated patients. None of the isolates displayed poly-resistance or extensively drug-resistant TB (XDR-TB). The only risk factor for MDR-TB was history of previous TB treatment (odds ratio = 5.7, 95% CI: 1.9–17.2).

Conclusion: The burden of MDR-TB in the country was relatively low with no evidence of XDR-TB. Given the overall small number of MDR-TB cases in this survey, it will be beneficial focusing efforts on intensified case detection including universal DST.

KEYWORDS
drug resistance, MDR-TB, survey, Tanzania, tuberculosis

Sustainable Development Goal: Good Health and Wellbeing

INTRODUCTION

Tuberculosis (TB) was the leading cause of death due to a single microorganism worldwide in the pre-COVID era [1–4]. According to the WHO Global TB Report 2020, almost half a million people developed rifampicin-resistant TB (RR-TB) in 2019, of which 78% had multidrug-resistant TB (MDR-TB) [1]. Most people with TB are cured using a 6-month drug regimen which is provided to patients with close monitoring and supervision. *Mycobacterium tuberculosis* (M. tb), the bacterium that causes TB, can develop resistance to the antimicrobial drugs during the long course of treatment [5]. The development of resistance could be due to inappropriate or incorrect use of antimicrobial drugs, or use of ineffective formulations of drugs, poor quality medicines or bad storage conditions and treatment interruption [5–7]. Pharmacokinetic variability due to genetic polymorphisms [8, 9] and spontaneous mutation of M. tb [10, 11] may also contribute to the development of drug-resistant TB. However, the ongoing transmission of drug-resistant TB strains, including MDR-TB and extensively drug-resistant (XDR) is the dominant mode of spread in many endemic countries [12, 13]. MDR-TB is the resistance to the two most powerful anti-TB drugs, isoniazid and rifampicin [12]. Treatment of MDR-TB is difficult as treatment options are limited and expensive, recommended medicines are not always available, and patients experience many adverse effects from the drugs [5]. Patients with MDR-TB require treatment with second-line treatment regimens which are more complex than those used to treat patients without drug-resistant TB. In some cases, even more severe drug-resistant TB may develop. Extensively drug-resistant TB (XDR-TB) is defined as TB caused by M. tb strains that fulfil the definition of MDR-TB/RR-TB and which are also resistant to any fluoroquinolone and at least one additional Group A drug [14].

The trends in incidence, prevalence and death of MDR-TB decreased globally from 2000 to 2017 with estimated annual percentage changes of −1.4%, −1.3% and −3.3%, respectively [13]. However, in 2019 more cases of MDR-TB/RR-TB were detected and notified globally than in the previous year, presenting a 10% increase from 186,883 to 206,030 cases [1].

According to WHO guidelines, detection of MDR-TB/RR-TB requires bacteriological confirmation of TB and testing for drug resistance [drug susceptibility testing (DST)] using rapid molecular tests, culture methods or sequencing technologies [1]. WHO estimated that only 44% of the estimated 465,000 MDR-TB/RR-TB incident cases in 2019 were notified [1]. The factors influencing detection of MDR-TB include suboptimal referral for DST, inadequate coverage of diagnostic DST, limited laboratory capacity and insufficient uptake of WHO-recommended rapid diagnostic tests. Furthermore, the global MDR-TB burden is underestimated by limiting the pool of patients considered to have MDR-TB to those with notified or incident TB [15–17].

The United Republic of Tanzania is among the 30 high TB and TB/HIV burden countries and had an estimated annual TB incidence rate of 237 per 100,000 population in 2019 [1]. Understanding the burden of TB drug resistance is critical to inform the development of appropriate treatment regimens, guiding resources for diagnosis and treatment and control of the disease. In settings without capacity for continuous surveillance of anti-TB drug resistance based on routine DST, WHO recommends surveys on new TB cases to be conducted at least every 5 years. The surveys can provide critical information for the TB program on the burden of drug resistance and common patient resistance profiles [18]. Surveys can also strengthen laboratory capacity, transportation and referral systems, as well as evaluate the accuracy of classification of patients by treatment history and risk factors for drug resistance [18]. The first anti-TB drug resistance survey (DRS) in Tanzania was conducted in 2006 [19]. The prevalence of MDR-TB among new patients and previously treated TB cases reported in that survey was 1.1% and 3.1%, respectively [19, 20]. We conducted the second nationwide anti-TB DRS in 2017–2018 to determine the levels and patterns of resistance to first and second-line anti-TB drugs among new and previously treated sputum smear positive pulmonary TB (PTB) patients.

METHODS

Survey design

We conducted a nationally representative cross-sectional health facility-based survey during June 2017–July 2018. The study was designed to conform to WHO guidelines for periodic DRSs [18].

Survey population

The survey population included newly diagnosed and previously treated smear positive PTB patients of all ages including children. All enrolled patients signed an informed consent form. Parents/guardians signed informed consent form for children younger than 18 years old. Children 15–18 years also signed an Assent Form. Patients whose previous and subsequent MDR-TB treatment course(s) have failed based on WHO guidelines (multiple episodes of TB treatment failures or more than one previously known episode of MDR-TB) were not eligible for the survey [18].

Sample size determination

The sample size was calculated according to the WHO Guidelines for Surveillance of Drug Resistance in Tuberculosis [18]. Taking into account correlation between individuals within a cluster with design effect of 2, the desired absolute precision of the estimate of 0.8%, and 15% of expected loses due to culture contaminations and other issues, the sample size of 1495 new smear positive PTB patients was required for the survey. All smear positive previously treated patients who met eligibility criteria were enrolled during the survey intake period.

Sampling strategy and selection of clusters

The unit of sampling was represented by a diagnostic facility that notified at least eight smear positive TB cases in 2015; whereas facilities with less than eight smear positive TB cases were excluded from the selection as they represented only 5% of all diagnosed smear positive cases in 2015. A cluster could include one or several diagnostic facilities depending on the number of notified cases in the selected health facility in 2015. Clusters were selected by probability proportional to size; 45 clusters were selected. In each cluster, a total of 34 new smear positive PTB patients and all previously treated smear positive PTB cases diagnosed during the intake period for the survey were enrolled into the study (Appendix A).

Training of survey staff

A 2-day training was conducted by zones before the start of the survey using the developed standardised training materials. The training was done on enrolment of study clients, sputum collection and transportation. At peripheral laboratory level, laboratory personnel were trained on use of light-emitting diode (LED) microscopy, specimen preparation, mixing of sputum with OmniGene-sputum transport solution, storage and transport of specimen. At the Central TB Reference Laboratory (CTRL) 1-day training was conducted for CTRL personnel and data management.

Data collection

Enrollment of patients

The study was conducted for a period of 12 months and/or until the required sample size of new smear positive cases was reached at each cluster. For persons suspected of having TB, two sputum samples (at the time of diagnostic workup and early morning the following day) were collected and tested at the cluster level using immunofluorescence LED smear microscopy in accordance with the national guidelines. All patients with smear positive results were eligible for enrollment after providing informed consent. Demographic information and previous TB treatment history was obtained from enrolled individuals during interview using a standardised questionnaire. HIV status was obtained from patient records available at the treatment facilities. Two additional sputum samples were collected from the enrolled individuals: one sputum sample at the time of enrollment and second one on the next morning.

Laboratory procedures

The sputum samples were transported to the CTRL in Dar es Salaam for Xpert MTB/RIF testing, smear examination with fluorescent microscopy, culture on Lowenstein Jensen solid media, and phenotypic DST to first- and second-line drugs following standard NTLP procedures. All culture positive isolates were identified by Capilia MPT64 test, an immunochromatographic test for the rapid identification of M. tb from solid cultures, before processed for DST [21]. Isolates that were positive on MPT64 test were subjected for DST. MPT64 negative results indicated the presence of non-tuberculous mycobacteria (NTM); such isolates were not tested for DST. The following critical concentrations for the first-line DST were used: 0.2 μg/ml for isoniazid, 40 μg/ml for rifampicin, 4 μg/ml for streptomycin and 2 μg/ml for ethambutol. For the second-line DST the concentration for kanamycin was 30 μg/ml, for ofloxacin 2.0 μg/ml and for capreomycin 40 μg/ml.

Survey monitoring

To ensure the quality of enrolment of patients and specimen collection regular supervision and monitoring of the field sites were conducted. A checklist was used to assess compliance to the survey procedures in line with the protocol. Observations and recommendations made during supervisory visits were immediately relayed to the clusters for action. A mid-term review was done in September 2017 came up with pertinent recommendations that were also implemented to improve the survey.

Quality assurance

All laboratory procedures adhered to the internal quality control procedures in accordance with international standards [18]. Handling of specimens for culture and DST was carried out in a high-risk TB (P3) laboratory, as defined in WHO's Tuberculosis Laboratory Biosafety Manual [22]. To ensure reliability and comparability of the Tanzania survey results, internal and external quality control of susceptibility testing was performed during the survey. All RIF-resistant specimens and 10% of randomly selected susceptible specimens identified were shipped to Antwerp Supra-national Laboratory (SRL) for EQA testing. Re-checking of strains at the SRL was conducted to validate the survey results. No changes in patient care were implemented based on the SRL results.

Data management and analysis

Completed questionnaires were entered into an electronic Epi Info database by trained data entry personnel. Entered data were stored in Access format, and data were double-entered and cleaned before analysis. The analysis was fully accounted for the cluster survey design. Missing laboratory results for 101 cases were imputed based on a probability model of the complete data for age, gender, treatment history, rifampicin, isoniazid and multidrug resistance. To address over/under-enrolment by facility, weights against calculated cluster size were included in the regression model. Different approaches (with imputation, without imputation,

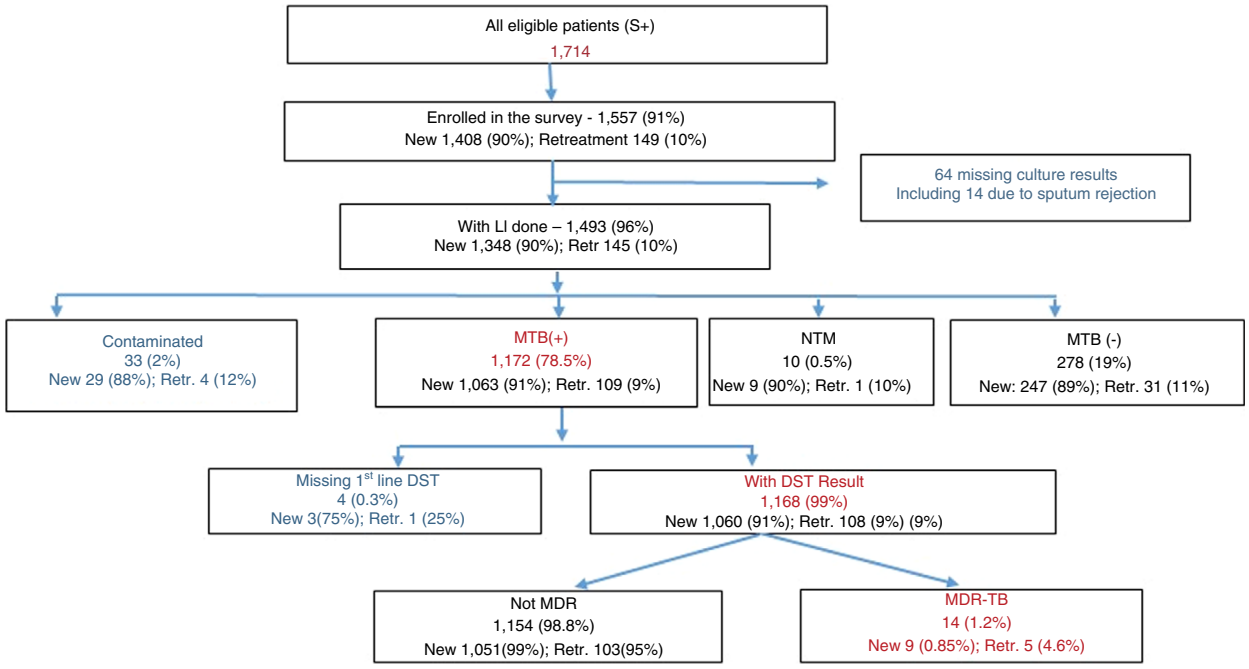

FIGURE 1 Culture/drug susceptibility testing flowchart for drug resistance survey in Tanzania

with weight and without weight) were used to estimate the prevalence of MDR-TB. As no significant differences were observed between the results from different methods, the results received without imputation of missing values were accepted as official DRS results in Tanzania.

Logistic regression was used to analyse association between possible risk factors and MDR-TB in Tanzania. Analysis was carried out using Stata version 15 (StataCorp. 2017. Stata Statistical Software: Release 15. College Station, TX: StataCorp LLC).

Ethical considerations

The study was approved by the National Health Research Ethics Committee of Tanzania and the Center for Global Health at the U.S. Centers for Disease Control and Prevention (CDC). The study was reviewed in accordance with the U.S. CDC human research protection procedures and determined to be research, but CDC investigators did not interact with human subjects or have access to identifiable data or specimens for research purposes. Written informed consent was obtained from all participants or their legal guardian; assent was also obtained from children 15–17 years old.

RESULTS

Demographic characteristics of the survey participants

A total of 1714 smear positive PTB cases were notified in the selected facilities during the survey period, thus were eligible for the survey. All 1714 were treated according to routine NTLP guidelines, and 1557 were enrolled for the survey; 1493 (96%) of them sent samples for investigation. Out of all samples received 1172 (78%) grew MTB and 10 (0.6%) grew NTM. There was no growth in 278 samples (Figure 1). Among MTB cases, 1063 (91%) were new and 109 (9%) were previously treated.

Among the 1172 enrolled patients with confirmed TB, the majority [825 (70.4%)] were males (Table 1). The proportion of males was higher among previously treated (80.7%) than new cases (69.3%). The mean age of the participants was 37 years (36.7 years for new and 40.5 years for previously treated patients); most of the patients [665 (56.7%)] were aged 25–44 years. Among MTB positive patients, 286 (24.4%) were infected with HIV. Almost one third (82/286) of all HIV-positive MTB individuals were diagnosed in Dar es Salaam. The proportion of HIV-positive individuals was higher among previously treated patients 39 (35.8%) than new patients [247 (23.2%)].

Resistance to first-line anti-TB drugs

Among 1172 MTB isolates, 1168 (99%) had DST results for all drugs (Figure 1). Results for resistance to first-line anti-TB drugs are summarised in Table 2. Of the 1168 TB patients with DST results 25 (2.1%) patients (including 18 new and 7 previously treated) had any resistance to the first- and second-line anti-TB drugs. Seventeen (1.5%) M. tb isolates were resistant to rifampicin (R), the same number of isolates (17 or 1.5%) were resistant to isoniazid (H). Fourteen (1.2%) MTB isolates were resistant to both R and H, meaning they were MDR-TB, including nine (0.8%) among new cases and five (4.6%) among previously treated cases.

TABLE 1 Profile of participants in the national anti-tuberculosis drug resistance survey, 2017–2018

Characteristic	New *n* (%)	Previously treated *n* (%)	Total *n* (%)
Total	1063 (91%)	109 (9%)	1172
Sex			
Male	737 (69.3)	88 (80.7)	825 (70.4)
Female	326 (30.7)	21 (19.3)	347 (29.6)
Age group (years)			
0–14	16 (1.5)	0	16 (1.4)
15–24	189 (17.8)	9 (8.2)	198 (16.9)
25–34	301 (28.3)	17 (15.6)	318 (27.1)
35–44	299 (28.1)	48 (44.0)	347 (29.6)
45–54	153 (14.4)	21 (19.3)	174 (14.9)
55–64	57 (5.4)	10 (9.2)	67 (5.7)
65+	48 (4.5)	4 (3.7)	52 (4.4)
Mean age	36.7	40.5	37
Median age (IQR)	36 (19)	40 (14)	37 (19)
Contact with MDR-TB case			
Yes	55 (5.2)	2 (1.8)	57 (4.9)
No	833 (78.4)	90 (82.6)	923 (78.7)
Missing	175 (16.4)	17 (15.6)	192 (16.4)
HIV status			
Positive	247 (23.2)	39 (35.8)	286 (24.4)
Negative	816 (76.8)	70 (64.2)	886 (75.6)
Smoking			
Yes	175 (16.5)	27 (24.8)	202 (17.4)
No	811 (76.3)	72 (66.0)	883 (75.3)
Missing	77 (7.2)	10 (9.2)	87 (7.4)
Alcohol use			
Yes	220 (20.7)	31 (28.4)	251 (21.4)
No	774 (72.8)	69 (63.3)	843 (71.9)
Missing	69 (6.5)	9 (8.3)	78 (6.7)
Diabetes			
Yes	15 (1.4)	1 (0.9)	16 (1.4)
No	975 (91.7)	98 (89.9)	1073 (91.5)
Missing	73 (6.9)	10 (9.2)	83 (7.1)

Note: Numbers rounded to make percentages sum to 100%.
Abbreviations: IQR, interquartile range; MDR-TB, multidrug-resistant tuberculosis.

TABLE 2 Results from the national drug resistance survey in Tanzania, 2017–2018

Drug resistance ($n = 1168$)	New *n* (%)	Previously treated *n* (%)	All TB patients *n* (%)
Total	1060 (100)	108 (100)	1168 (100)
Any resistance			
H	12 (1.1)	5 (4.6)	17 (1.5)
R	10 (0.9)	7 (6.5)	17 (1.5)
E	2 (0.2)	1 (0.9)	3 (0.3)
S	5 (0.5)	1 (0.9)	6 (0.5)
Total any resistance	18 (1.7)	7 (6.5)	25 (2.1)
Mono-resistance			
H only	3 (0.3)	0	3 (0.3)
R only	1 (0.1)	2 (1.9)	3 (0.3)
E only	1 (0.1)	0	1 (0.1)
S only	4 (0.4)	0	4 (0.3)
Total mono-resistance	9 (0.8)	2 (1.9)	11 (1.0)
Multidrug resistance			
Any H + R (MDR)	9 (0.8)	5 (4.6)	14 (1.2)
H + R only	8 (0.7)	4 (3.7)	12 (1.0)
H + R + E only	0	0	0
H + R + S only	0	0	0
H + R + E + S	1 (0.1)	1 (0.9)	2 (0.2)

Abbreviation: TB, tuberculosis.

The resistance pattern to individual first-line drugs (FLD) shows highest resistance to H [any 17 (1.5%), mono 3 (0.3%)] and to R [any 17 (1.5%), mono 3 (0.3%)] followed by resistance to streptomycin (S) [any 6 (0.5%), mono 4 (0.3%)] and ethambutol (E) [any 3 (0.3%), mono 1 (0.1%)]. Eleven (1.0%) cases, including nine (0.8%) new and two (1.9%) previously treated had mono-resistance to at least one FLD. Mono-resistance to FLD among new TB patients was highest to S [4 (0.4%)] followed by H [3 (0.3%)]. In contrast, mono-resistance among the previously treated patients was only observed for R [2 (1.9%)]. Drug resistance to all FLDs was seen in one new and one previously treated patient. None of the isolates displayed poly-resistance (other than MDR) or XDR-TB (Table 2).

The socio-demographic characteristics of the 14 identified MDR-TB patients are shown in Table 3. Of the 14 MDR-TB patients, 9 (64.3%) were new and 5 (35.7%) were previously treated. Eight (57.1%) MDR-TB patients were males and six (42.9%) were females. The age of the MDR-TB patients ranged from 25 to 54 years. The proportion of HIV-positive cases among patients with MDR-TB was 14.3%.

The distribution of MDR-TB cases by region is shown in Table 4. Only 6 (29%) of all the 21 regions participated in the study had MDR-TB cases. Of the 14 MDR-TB cases, majority [8, (57.1%)] were from Dar es Salaam. Kilimanjaro had 4 (28.6%) cases, while Mbeya, Mtwara, Songwe and Unguja regions each had 1 (1.7%) case.

Estimated prevalence of MDR-TB in Tanzania

Logistic regression was used to account for missing data and sampling design effects on the estimates the prevalence of

TABLE 3 Characteristics of patients with multidrug resistance in the national drug resistance survey in Tanzania, 2017–2018

Characteristics	New *n* (%)	Previously treated *n* (%)	Total *n* (%)
Total # MDR-TB patients	9 (64.3)	5 (35.7)	14 (100)
Sex			
Male	4 (44.4)	4 (80.0)	8 (57.1)
Female	5 (55.6)	1 (20.0)	6 (42.9)
HIV			
Negative	8 (88.9)	4 (80.0)	12 (85.7)
Positive	1 (11.1)	1 (20.0)	2 (14.3)
Age group (years)			
0–14	0	0	0
15–24	0	0	0
25–34	6 (66.7)	2 (40.0)	8 (57.1)
35–44	2 (22.2)	0	2 (14.3)
45–54	0	3 (60.0)	3 (21.4)
55–64	0	0	0
65+	1 (11.1)	0	1 (7)
Contact with MDR-TB case			
No	7 (77.8)	2 (40.0)	9 (64.3)
Yes	0	0	0
Unknown	2 (22.2)	3 (60.0)	5 (35.7)
Alcohol			
No	6 (66.7)	1 (20.0)	7 (50.0)
Yes	2 (22.2)	2 (40.0)	4 (28.6)
Unknown	1 (11.1)	2 (40.0)	3 (21.4)
Smoking			
No	7 (77.8)	2 (40.0)	9 (64.3)
Yes	1 (11.1)	1 (20.0)	2 (14.3)
Unknown	1 (11.1)	2 (40.0)	3 (21.4)
Diabetes			
No	8 (88.9)	3 (60.0)	11 (78.6)
Yes	0	0	0
Unknown	1 (11.1)	2 (40.0)	3 (21.4)

Note: Numbers rounded to make the percentages sum to 100.0%.
Abbreviation: MDR-TB, multidrug-resistant tuberculosis.

MDR-TB and their standard errors. Missing laboratory results for 101 cases were imputed based on probability model of the complete data for age, gender, treatment history, rifampicin, isoniazid and MDR-TB. To address over/under-enrolment by facility, weights against notification data (total number of patients with positive smear per cluster compared with the enrolled patients) were included in the regression model. Different approaches (with imputation, without imputation, with weight and without weight) were used to estimate the prevalence of MDR-TB (Table 5).

After comparing the results from different methods, the results received without imputation of missing values were accepted as official DRS results in Tanzania, namely estimated prevalence of MDR-TB among new cases is 0.85% [95% confidence interval (CI): 0.4–1.3], among previously treated cases is 4.6% [95% CI: 1.1–8.2] and overall is 1.2% [95% CI: 0.6–1.8].

Factors associated with MDR-TB

The proportion of MDR-TB cases was higher among females (6, or 1.7%) than males (8, or 1.0%), but this association was not statistically significant ($p = 0.3$). In this study, the only risk factor found to be significantly associated with MDR-TB was history of previous TB treatment (odds ratio = 5.7, 95% CI: 1.9–17.2; $p = 0.002$) (Table 6). Due to the small number of MDR-TB cases, using multivariate logistic regression model to adjust for other factors was not possible.

DISCUSSION

The findings of the second nationwide anti-TB DRS in Tanzania demonstrate the presence of M. tb strains that are resistant to the commonly used first-line anti-TB drugs. The overall prevalence of MDR-TB was 1.2%, being higher among previously treated TB patients (4.6%) than new cases (0.8%). The proportion of survey participants with MDR-TB was higher among male than female TB patients. History of previous TB treatment was the only risk factor for MDR-TB in this study. According to the old WHO definition for XDR-TB, none of the cases were identified in the survey. It is of interest to note that most of the MDR-TB cases were new rather than previously treated patients, suggesting that primary transmission of MDR-TB strains takes place among newly infected patients. This suggestion was also confirmed geographically: the majority of new MDR-TB cases were localised in Dar es Salaam (5/9, 56%).

The current findings shows that there was no increase in MDR-TB rates compared to the previous survey conducted in 2006 [15]. This finding is in line with the WHO conclusion that the burden of MDR-TB or RR-TB as a share of the number of TB cases remains stable globally during a few pre-COVID years [23].

The estimate of the prevalence of MDR-TB in Tanzania is still among the lowest in the recently reported DRS conducted in other African countries and globally (3.3% among new cases) [17, 18]. None of the patients in this survey had any resistance to fluoroquinolones or second-line injectable TB drugs.

In low- and middle-income countries TB prevalence is significantly higher among men than women, with strong evidence that men are less forthcoming in seeking and/or accessing TB care in many settings [24–26]. In the current survey we report a slightly higher proportion of MDR-TB among female TB patients than among male TB patients, but this difference was not statistically significant. Similar

TABLE 4 Multidrug resistance in the national tuberculosis drug resistance survey in Tanzania by regions, 2017–2018

Region	New *n*	MDR *n* (%)	Previously treated *n*	MDR *n* (%)	Total *n*	MDR *n* (%)
Dar es Salaam	377	5 (1.3)	45	3 (6.7)	422	8 (1.9)
Kilimanjaro	65	1 (1.5)	9	3 (33.3)	74	2 (2.7)
Mbeya	29	1 (3.4)	0	0 (0)	29	1 (3.4)
Mtwara	59	0 (0)	3	1 (33.3)	62	1 (1.6)
Songwe	61	1 (1.6)	6	0 (0)	67	1 (1.5)
Unguja	38	1 (2.6)	4	0 (0)	42	1 (2.4)

Note: Only regions with at least one MDR-TB case were included.
Abbreviation: MDR-TB, multidrug-resistant tuberculosis.

TABLE 5 Estimated prevalence of MDR-TB in Tanzania

Method	New	Previously treated	All
Individual level no imputation			
Simple random sampling	0.85 [0.4–1.6]	4.6 [1.5–10.5]	1.2 [0.7–2.0]
Cluster design, no weights	0.85 [0.5–1.5]	4.6 [2.1–10.0]	1.2 [0.7–2.1]
LR: no weights, no clustering	0.85 [0.3–1.4]	4.6 [0.7–8.6]	1.2 [0.6–1.8]
LR: weights, no clustering	0.74 [0.2–1.2]		
Robust standard errors no weights	0.85 [0.4–1.3]	4.6 [1.1–8.2]	1.2 [0.6–1.8]
Robust standard errors and weights	0.74 [0.3–1.2]		
Individual level with imputation			
Robust standard errors no weights	1.1 [0.4–1.8]	5.0 [1.0–8.9]	1.4 [0.7–2.2]
Robust standard errors and weights	0.97 [0.2–1.7]		

Abbreviations: LR, likelihood ratio; MDR-TB, multidrug-resistant tuberculosis.

findings were observed from the first national drug-resistant survey conducted in Ukraine, where the proportion of MDR-TB was higher among female TB patients than among male TB patients and this difference was statistically significant [27].

Different factors, such as HIV, have been reported elsewhere [28–30] to be associated with MDR-TB. We also investigated possible risk factors such as alcohol, smoking, diabetes and HIV but none of these was found to be statistically significant. However, our findings of not identifying an association between HIV and MDR-TB were in line with those reported by Lukoye and others in Uganda [31] and elsewhere [32–34]. It is also important to note that in this survey the lack of statistically significant association between MDR-TB and HIV may be due to the small number of MDR patients. History of previous TB treatment was the only factor significantly associated with MDR-TB in Tanzania. While transmission of MDR-TB strains seems to be the most common mechanism of getting MDR-TB, none of the 57 survey participants who reported to be household contacts of an MDR-TB case had MDR-TB [13, 35]. On the other hand, household contact studies by Fox et al. [34] in Vietnam showed that under 2% of household contacts of a TB case developed TB disease. This corresponds with the findings reported by earlier studies that previous exposure to anti-TB treatment was the most common risk factor for MDR-TB [37]. We also speculate that if MDR-TB was missed at the first diagnosis, especially when diagnoses were done only via smear microscopy, patients were likely to fail on the first-line TB treatment.

Assessment of risk factors of MDR-TB should be conducted regionally to develop the most effective strategy for MDR-TB control in each country. Across all regions, previous TB disease and treatment are essential factors associated with MDR-TB, indicating necessity of timely diagnosis, appropriate treatment and thorough monitoring [38, 39].

Survey limitations

Several limitations during the study were encountered starting from prolonged specimen collection over the planned period of time due to different reasons such as lack of reagents along the way which meant losing some eligible clients. Some clusters repeated enrollment due to inconsistent enrolling and missing eligible patients. As such, 9% of all eligible individuals were not enrolled in the survey. However, despite this limitation, the survey results were consistent with the results from the previous survey in Tanzania [20] as well as with the results that have been reported in neighbouring countries [26, 27]. Due to the high prevalence of HIV in Tanzania, and the fact that individuals living with HIV/AIDS were more likely to have smear negative TB than those without HIV, inclusion of smear negative specimens

TABLE 6 Factors associated with MDR-TB

Risk factors	MDR *n* (%)	Non-MDR *n* (%)	OR	95% CI	*p* Value
Patient classification					
New	9 (0.8)	1051 (99.2)	Reference		
Previously treated	5 (4.6)	103 (95.4)	5.7	1.9–17.2	0.002
Sex					
Male	8 (1.0)	815 (99)	Reference		
Female	6 (1.7)	339 (98.3)	1.8	0.6–5.2	0.3
Age groups					
0–14	0 (0)	16 (100)	N/A		
15–24	0 (0)	198 (100)	N/A		
25–34	8 (2.5)	308 (97.5)	4.5	0.9–21.2	0.06
35–44	2 (0.6)	344 (99.4)	Reference		
45–54	3 (1.7)	171 (98.3)	3.0	0.5–18.2	0.2
55–64	0 (0)	67 (100)	N/A		
65+	1 (2.0)	50 (98)	3.4	0.3–38.6	0.3
Age groups					
0–34	8 (1.5)	522 (98.5)	Reference		
35+	6 (0.9)	632 (99.1)	0.6	0.2–1.8	0.4
Age groups					
0–44	10 (1.1)	866 (98.9)	Reference		
45+	4 (1.4)	288 (98.6)	1.2	0.4–3.9	0.8
HIV status					
Yes	2 (0.7)	282 (99.3)	0.5	0.1–3.2	0.4
No	12 (1.4)	872 (98.6)	Reference		
Alcohol use					
Yes	4 (1.6)	245 (98.4)	1.9	0.6–6.7	0.3
No	7 (0.8)	834 (99.2)	Reference		
Missing	3 (3.8)	75 (96.2)			
Ever smoked					
Yes	2 (1.0)	200 (99)	0.97	0.2–4.5	0.9
No	9 (1.0)	870 (99)	Reference		
Missing	3 (3.4)	84 (96.6)			
Diabetes mellitus					
Yes	0 (0)	16 (100)	N/A		
No	11 (1.0)	1058 (99)			
Missing	3 (3.6)	80 (96.4)			
Contact with MDR-TB case					
Yes	0 (0)	57 (100)	N/A		
No	9 (1.0)	910 (99)			
Missing	5 (2.6)	187 (97.4)			

Note: Tanzania national anti-tuberculosis drug resistance survey, 2017–2018.
Abbreviations: CI, confidence interval; MDR-TB, multidrug-resistant tuberculosis; OR, odds ratio.

and the use of molecular techniques could be considered for future survey [36, 37]. There may have been misclassification bias due to reporting/transcription errors of previous TB history of enrolled patients. Nevertheless, efforts to minimise this bias were undertaken by including additional questions regarding previous TB history and checking the history of previous TB treatment in hospital TB registers.

In addition, there were some laboratory challenges including storage of specimens and delays in shipping of specimens from the facilities to CTRL leading to loss of viability of possible drug-resistant strains in the specimens [24]. Despite these challenges, external quality assessment of DST of the isolates demonstrated consistency with the survey results.

CONCLUSION

The second TB DRS in Tanzania confirmed that the burden of MDR-TB in the country was relatively low. The findings show no evidence of XDR. Given the overall small number of MDR-TB cases in this survey, efforts should be aimed at improving case detection by including universal DST ensuring that all patients with presumptive DR-TB have access to DST for all anti-TB medicines, timely initiation of treatment and enhancing measures to prevent transmission of the disease to assure that levels of TB drug resistance remain low.

ACKNOWLEDGEMENTS

Special thanks are extended to the Global Fund for providing funding for this survey through the NTLP. The authors are grateful to all patients who participated in this study.

FUNDING INFORMATION

This study was funded by the Global Fund to Fight AIDS, Tuberculosis and Malaria. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript. The findings in this manuscript are those of the authors, and do not necessarily represent the official position of the funding agencies.

REFERENCES

1. World Health Organization. Global tuberculosis report 2020. Geneva, Switzerland: World Health Organization; 2020.
2. Lange C, Chesov D, Heyckendorf J, Leung CC, Udwadia Z, Dheda K. Drug-resistant tuberculosis: an update on disease burden, diagnosis and treatment. Respirology. 2018;23(7):656–73.
3. Sallin MA, Kauffman KD, Riou C, Bruyn ED, Foreman TW, Sakai S, et al. Host resistance to pulmonary *Mycobacterium tuberculosis* infection requires CD153 expression. Nat Microbiol. 2018;3:1198–205.
4. Conradie F, Diacon AH, Ngubane N, Howell P, Everitt D, Crook AM, et al. Treatment of highly drug-resistant pulmonary tuberculosis. N Engl J Med. 2020;382:893–902. https://doi.org/10.1056/NEJMoa1901814
5. World Health Organization. Tuberculosis: Multidrug-resistant tuberculosis (MDR-TB). 2018. https://www.who.int/news-room/questions-and-answers/item/tuberculosis-multidrug-resistant-tuberculosis-(mdr-tb). Accessed 13 Mar 2021.
6. Dheda K, Gumbo T, Maartens G, Dooley KE, McNerney R, Murray M, et al. The epidemiology, pathogenesis, transmission, diagnosis, and management of multidrug-resistant, extensively drug-resistant, and incurable tuberculosis. Lancet Respir Med. 2017;5:291–360.
7. Shi R, Itagaki N, Sugawara I. Overview of anti-tuberculosis (TB) drugs and their resistance mechanisms. Mini Rev Med Chem. 2007;7(11):1177–85.
8. Naidoo A, Chirehwa M, Ramsuran V, McIlleron H, Naidoo K, Yende-Zuma N, et al. Effects of genetic variability on rifampicin and isoniazid pharmacokinetics in South African patients with recurrent tuberculosis. Pharmacogenomics. 2019;20(4):225–40. https://doi.org/10.2217/pgs-2018-0166
9. Singh R, Dwivedi SP, Gaharwar US, Meena R, Rajamani P, Prasad T. Recent updates on drug resistance in *Mycobacterium tuberculosis*. J Appl Microbiol. 2020;128(6):1547–67. https://doi.org/10.1111/jam.14478
10. Kempker RR, Kipiani M, Mirtskhulava V, Tukvadze N, Magee MJ, Blumberg HM. Acquired drug resistance in *Mycobacterium tuberculosis* and poor outcomes among patients with multidrug-resistant tuberculosis. Emerg Infect Dis. 2015;21(6):992–1001. https://doi.org/10.3201/eid2106.141873
11. Colijn C, Cohen T, Ganesh A, Murray M. Spontaneous emergence of multiple drug resistance in tuberculosis before and during therapy. PLoS One. 2011;6(3):e18327. https://doi.org/10.1371/journal.pone.0018327
12. World Health Organization. Drug resistance: tuberculosis. https://www.who.int/drugresistance/tb/en/. Accessed 13 Mar 2021.
13. Ou ZJ, Yu DF, Liang YH, He WQ, Li YZ, Meng YX, et al. Trends in burden of multidrug-resistant tuberculosis in countries, regions, and worldwide from 1990 to 2017: results from the global burden of disease study. Infect Dis Poverty. 2021;10(1):24. https://doi.org/10.1186/s40249-021-00803-w
14. World Health Organization. Meeting report of the WHO expert consultation on the definition of extensively drug-resistant tuberculosis, 27–29 October 2020. Geneva: World Health Organization; 2021.
15. Centers for Disease Control and Prevention (CDC). Tuberculosis: multidrug-resistant tuberculosis (MDR TB). https://www.cdc.gov/tb/publications/factsheets/drtb/mdrtb.htm. Accessed 13 Mar 2021.
16. Seung KJ, Keshavjee S, Rich ML. Multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis. Cold Spring Harb Perspect Med. 2015;5(9):a017863. https://doi.org/10.1101/cshperspect.a017863
17. Nourzad S, Jenkins HE, Milstein M, Mitnick CD. Estimating the global burden of multidrug-resistant tuberculosis among prevalent cases of tuberculosis. Int J Tuberc Lung Dis. 2017;21:6–11.
18. World Health Organization. Guidelines for surveillance of drug resistance in tuberculosis. 5th ed. Geneva: World Health Organization; 2015. https://www.who.int/tb/publications/2015/drs_guidelines/en/
19. Chonde TM, Doulla B, van Leth F, Mfinanga SGM, Range N, Lwilla F, et al. Implementation of a national anti-tuberculosis drug resistance survey in Tanzania. BMC Public Health. 2008 Dec;8:427. https://doi.org/10.1186/1471-2458-8-427
20. Chonde TM, Basra D, Mfiinanga SGM, Range N, Lwilla F, Shirima RP, et al. National anti-tuberculosis drug resistance study in Tanzania. Int J Tuberc Lung Dis. 2010;14(8):967–72.
21. BRUKER. TBCheck MPT64 VER 1.0 – Rapid detection of MTB complex. https://www.hain-lifescience.de/en/products/microbiology/mycobacteria/tuberculosis/tbcheck-mpt64.html
22. World Health Organization. Tuberculosis laboratory biosafety manual. Geneva: World Health Organization; 2012.
23. World Health Organization. Global TB Report 2020. 2020. https://www.who.int/publications/i/item/9789240013131. Accessed 8 July 2021.
24. Abouyannis M, Dacombe R, Dambe I, Mpunga J, Faragher B, Gausi F, et al. Drug resistance of *Mycobacterium tuberculosis* in Malawi: a cross-sectional survey. Bull World Health Organ. 2014;92:798–806.
25. Horton KC, MacPherson P, Houben RMGJ, White RG, Corbett EL. Sex differences in tuberculosis burden and notifications in low- and middle-income countries: a systematic review and meta-analysis. PLoS Med. 2016;13(9):e1002119. https://doi.org/10.1371/journal.pmed.1002119
26. Asgedom SW, Teweldemedhin M, Gebreyesus H. Prevalence of multidrug-resistant tuberculosis and associated factors in Ethiopia: a systematic review. J Pathog. 2018;2018:7104921. https://doi.org/10.1155/2018/7104921
27. Ruswa N, Mavhunga F, Roscoe JC, Beukes A, Shipiki E, van Gorkom J, et al. Second nationwide anti-tuberculosis drug resistance survey in Namibia. Int J Tuberc Lung Dis. 2019;23(7):858–64.
28. Pavlenko E, Barbova A, Hovhannesyan A, Tsenilova Z, Slavuckij A, Shcherbak-Verlan B, et al. Alarming levels of multidrug-resistant tuberculosis in Ukraine: results from the first national survey. Int J Tuberc Lung Dis. 2018;22(2):197–205.
29. 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(5):e5561. https://doi.org/10.1371/journal.pone.0005561
30. van den Hof S, Tursynbayeva A, Abildaev T, Adenov M, Pak S, Bekembayeva G, et al. Converging risk factors but no association between HIV infection and multidrug-resistant tuberculosis in Kazakhstan. Int J Tuberc Lung Dis. 2013;17(4):526–31. https://doi.org/10.5588/ijtld.12.0703

31. Elduma AH, Mansournia MA, Foroushani AR, Ali HMH, Elegail AMA, Elsony A, et al. Assessment of the risk factors associated with multidrug-resistant tuberculosis in Sudan: a case-control study. Epidemiol Health. 2019;41:e2019014. https://doi.org/10.4178/epih.e2019014
32. Lukoye D, Adatu F, Musisi K, Kasule GW, Were W, Odeke R, et al. Anti-tuberculosis drug resistance among new and previously treated sputum smear-positive tuberculosis patients in Uganda: results of the first national survey. PLoS One. 2013 Aug 1;8(8):e70763. https://doi.org/10.1371/journal.pone.0070763
33. Ahmed S, Shukla I, Fatima N, Varshney SK, Shameem M, Tayyaba U. Profile of drug-resistant-conferring mutations among new and previously treated pulmonary tuberculosis cases from Aligarh region of northern India. Int J Mycobacteriol. 2018;7(4):315–27.
34. Fox GJ, Nhung NV, Sy DN, Britton WJ, Marks GB. Household contact investigation for tuberculosis in Vietnam: study protocol for a cluster randomized controlled trial. Trials. 2013;14:342. https://doi.org/10.1186/1745-6215-14-342
35. Quispe N, Asencios L, Obregon C, Velásquez GE, Mitnick CD, Lindeborg M, et al. The fourth national anti-tuberculosis drug resistance survey in Peru. Int J Tuberc Lung Dis. 2020;24(2):207–13.
36. Harries AD, Banda HT, Boeree MJ, Welby S, Wirima JJ, Subramanyam VR, et al. Management of pulmonary tuberculosis suspects with negative sputum smear and normal or minimally abnormal chest radiographs in resource-poor settings. Int J Tuberc Lung Dis. 1998;2:999–1004.
37. Mtei L, Matee M, Herfort O, Bakari M, Horsburgh RC, Waddell R, et al. High rates of clinical and subclinical tuberculosis among HIV-infected ambulatory subjects in Tanzania. Clin Infect Dis. 2005;40(10):1500–7. https://doi.org/10.1086/429825
38. Smith RL, Yew K, Berkowitz KA, Aranda CP. Factors affecting the yield of acid-fast sputum smears in patients with HIV and tuberculosis. Chest. 1994;106:684–6.
39. Pradipta IS, Forsman LD, Bruchfeld J, Hak E, Alffenaar JW. Risk factors of multidrug-resistant tuberculosis: a global systematic review and meta-analysis. J Infect. 2018;77(6):469–78. https://doi.org/10.1016/j.jinf.2018.10.004

How to cite this article: Mutayoba BK, Ershova J, Lyamuya E, Hoelscher M, Heinrich N, Kilale AM, et al. The second national anti-tuberculosis drug resistance survey in Tanzania, 2017–2018. Trop Med Int Health. 2022. 1–11. https://doi.org/10.1111/tmi.13814

APPENDIX A: LIST OF CLUSTERS FOR ANTI-TB DRUG RESISTANCE SURVEY; 2016–2017

Region	District	Name of diagnostic Centre	Cluster #
Arusha	Karatu District Council	Karatu Health Centre	11
Arusha	Arusha City East	Mount Meru Hospital	26
Arusha	Arusha City West	Levolosi Health Centre	16
Dar es Salaam	Dar Ilala I	Chanika Dispensary	5
Dar es Salaam	Dar Ilala I	Buguruni Health Centre	3
Dar es Salaam	Dar Ilala I	Kiwalani Dispensary	15
Dar es Salaam	Dar Kinondoni	Kimara Dispensary	14
Dar es Salaam	Dar Ilala I	Ukonga Dispensary	44
Dar es Salaam	Dar Ilala II	Infectious Disease Clinic (IDC)	8
Dar es Salaam	Dar Kinondoni	Gati Dispensary	7
Dar es Salaam	Temeke TB/LP Region	Kigamboni Health Centre	13
Dar es Salaam	Dar Ilala I	Amana Hospital	1
Dar es Salaam	Dar Kinondoni	Sinza Hospital	38
Dar es Salaam	Dar Kinondoni	Magomeni Health Centre	18
Dar es Salaam	Temeke TB/LP Region	Rangitatu Hospital	35
Dar es Salaam	Dar Kinondoni	Mwananyamala Hospital	29
Dodoma	Dodoma Municipal Council	DDRRH	6
Geita	Geita District Council	Nyarugusu Dispensary	34
Iringa	Kilolo District Council	Ilula Hospital	9
Kagera	Bukoba Municipal Council	Buyekela Dispensary	4
		Kashai[a] Dispensary	4
Kagera	Missenyi District Council	Mugana District Designated Hospital (DDH)	27
Kagera	Kyerwa District Council	Nkwenda Health Centre	32
Kilimanjaro	Same District Council	Same Designated Hospital	36
Kilimanjaro	Moshi Municipal Council	Mawenzi Referral Hospital	21
Manyara	Simanjiro District Council	Mererani Health Centre	23

(Continues)

Region	District	Name of diagnostic Centre	Cluster #
Mara	Rorya District Council	Barak Health Centre	2
		Shirati Hospital[a]	2
Mara	Tarime Town Council	Tarime Hospital	42
Mbeya	Mbozi District Council	Mbozi Mission Hospital	22
Mbeya	Rungwe District Council	Tukuyu District Hospital	43
Mbeya	Mbozi District Council	Vwawa District Hospital	45
		Mlowo Dispensary[a]	45
Morogoro	Ulanga District Council	Lugala Hospital	17
Mtwara	Mtwara District Council	Nanguruwe Health Centre	30
Mtwara	Newala District Council	Newala Hospital	31
Mtwara	Tandahimba Dist. Council	Tandahimba Hospital	41
Mwanza	Kwimba District Council	Mwamashimba Hospital	28
Mwanza	Mwanza Urban North	S'Toure Hospital	40
Njombe	Makambako Town Council	Makambako Hospital	19
Pemba	South Pemba	Abdalla Mzee Hospital	34
	North Pemba	Wete Hospital[a]	34
Pwani	Kibiti District Council	Kibiti Hospital	12
Pwani	Mkuranga District Council	Mkuranga Hospital	24
Ruvuma	Songea Municipal Council	Songea Regional Hospital	39
Shinyanga	Shinyanga Municipal Council	Shinyanga Regional Hospital	37
Shinyanga	Kahama Town Council	Kahama Hospital (Government)	10
Tanga	Mkinga District Council	Maramba Health Centre	20
Unguja	Town and West	Mnazi Mmoja Hospital	25

[a] Complementary health facility.

4.2 Paper B (*appended as pdf file*)

Mutayoba, B.K., Michael Hoelscher, Heinrich, N. et al. Phylogenetic lineages of tuberculosis isolates and their association with patient demographics in Tanzania. BMC Genomics 23, 561 (2022). https://doi.org/10.1186/s12864-022-08791-3

BMC Genomics

RESEARCH

Open Access

Phylogenetic lineages of tuberculosis isolates and their association with patient demographics in Tanzania

Beatrice Kemilembe Mutayoba[1,2*], Michael Hoelscher[2], Norbert Heinrich[2], Moses L. Joloba[3,4], Eligius Lyamuya[5], Andrew Martin Kilale[6], Nyagosya Segere Range[6], Bernard James Ngowi[6,7], Nyanda Elias Ntinginya[8], Saidi Mwinjuma Mfaume[6], Amani Wilfred[6], Basra Doulla[9], Johnson Lyimo[10], Riziki Kisonga[10], Amri Kingalu[9], Jupiter Marina Kabahita[3], Ocung Guido[3], Joel Kabugo[3], Isa Adam[3], Moses Luutu[4], Maria Magdalene Namaganda[4], Joanitah Namutebi[3], George William Kasule[3], Hasfah Nakato[3], Henry Byabajungu[3], Pius Lutaaya[3], Kenneth Musisi[3], Denis Oola[3], Gerald Mboowa[11] and Michel Pletschette[2]

Abstract

Background: *Mycobacterium tuberculosis* presents several lineages each with distinct characteristics of evolutionary status, transmissibility, drug resistance, host interaction, latency, and vaccine efficacy. Whole genome sequencing (WGS) has emerged as a new diagnostic tool to reliably inform the occurrence of phylogenetic lineages of *Mycobacterium tuberculosis* and examine their relationship with patient demographic characteristics and multidrug-resistance development.

Methods: 191 *Mycobacterium tuberculosis* isolates obtained from a 2017/2018 Tanzanian drug resistance survey were sequenced on the Illumina Miseq platform at Supranational Tuberculosis Reference Laboratory in Uganda. Obtained fast-q files were imported into tools for resistance profiling and lineage inference (Kvarq v0.12.2, Mykrobe v0.8.1 and TBprofiler v3.0.5). Additionally for phylogenetic tree construction, RaxML-NG v1.0.3(25) was used to generate a maximum likelihood phylogeny with 800 bootstrap replicates. The resulting trees were plotted, annotated and visualized using ggtree v2.0.4

Results: Most [172(90.0%)] of the isolates were from newly treated Pulmonary TB patients. Coinfection with HIV was observed in 33(17.3%) TB patients. Of the 191 isolates, 22(11.5%) were resistant to one or more commonly used first line anti-TB drugs (FLD), 9(4.7%) isolates were MDR-TB while 3(1.6%) were resistant to all the drugs. Of the 24 isolates with any resistance conferring mutations, 13(54.2%) and 10(41.6%) had mutations in genes associated with resistance to INH and RIF respectively. The findings also show four major lineages i.e. Lineage 3[81 (42.4%)], followed by Lineage 4 [74 (38.7%)], the Lineage 1 [23 (12.0%)] and Lineages 2 [13 (6.8%)] circulaing in Tanzania.

Conclusion: The findings in this study show that Lineage 3 is the most prevalent lineage in Tanzania whereas drug resistant mutations were more frequent among isolates that belonged to Lineage 4.

Keywords: Phylogenetic, Lineages, Mycobacterial isolates, Whole-genome sequencing, Tanzania

*Correspondence: beatricemutayoba@yahoo.com

[2] Department of Infectious Diseases and Tropical Medicine, Medical Center of the, University of Munich, Munich, Germany
Full list of author information is available at the end of the article

Background

Collective tuberculosis (TB) drug resistance analysis studies from Sub-Saharan African countries report the prevalence of multi-drug resistant tuberculosis (DR-TB)

in new cases to be 2.1%. This low prevalence is however likely to be due to under reporting and lack of intensive access to drug resistance testing (DST) [1]. Phylogenetic analysis has been revolutionary in understanding the evolutionary development and diversification of pathogenic organisms and is useful in understanding their distribution. Seven major lineages of *Mycobacterium tuberculosis (M. tuberculosis)*, have been globally documented each exhibiting distinct characteristics from another in terms of evolutionary status, transmissibility, drug resistance, host interaction, latency, and vaccine efficacy [2]. These major lineages have been further subdivided into sub-lineages for example lineage 2 (East Asian) and lineage 4 (Euro-American) comprise the Beijing and Haarlem genotypes respectively. These show variation in virulence and pathogenicity with high association for tuberculosis outbreaks and drug-resistance [3]. Understanding TB transmission is key in disease control and prevention and the later highly depends upon rapid case detection. Rapid case detection should incorporate timely accurate drug susceptibility testing (DST) of *Mycobacterium tuberculosis* (*M. tuberculosis*) isolates. Several testing methods have been endorsed by the World Health Organisation (WHO) to test and confirm *M. tuberculosis*, revealing its phenotypic and genotypic characteristics. The most widely used phenotypic method i.e., culture and drug susceptibility testing are notoriously challenging and require stringent biosafety requirements to obtain the actual diagnosis [4]. These conventional methods are slow for comprehensive understanding of the *M. tuberculosis* infections to administer appropriate treatment. The molecular methods which include line-probe assays (LPAs) and Xpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) tend to overcome some of these challenges but fall short on covering the entire genomic understanding of the *M. tuberculosis* strains [5]. New molecular diagnostic methods based on genomic DNA sequencing have increasingly expounded TB genomics characteristically describing phylogeny of *M. tuberculosis* [6]. These include IS6110-RFLP methodology necessitating Southern blotting, spoligotyping, mycobacterial interspersed repetitive and whole genome sequencing (WGS) [7–10]. These have greatly improved the understanding of detection of unsuspected transmission and discrimination between re-infection, relapse and phylogeographical variations of the *M. tuberculosis* [11, 12].

Tanzania ranks among the seven TB high burden countries worldwide [13] with a total of 75,845 cases notified and incidence of 253 per 100,000 in 2018. The regional distribution of the cases in the country ranks Dar es Salaam city as the major contributor of TB cases notification at 20% contribution of all cases [13] with the rest in other regions of Mwanza, Arusha, Geita, Dodoma, Manyara and Mbeya but less has been done to understand the phylogenetic distribution.

Worldwide, vast numbers of sequences of *M. tuberculosis* strains have been generated with several libraries of single nucleotide poly-morphisms (SNPs) and other variants generated for comparative purposes. The research in low- and middle-income countries where Tanzania falls still lags in this area and more work needs to be done to guide accurate clinical decisions and provide more evidence of the prevailing strains in the country. To comprehensively understand the phylogeographical variations in Tanzania, we performed WGS on the drug resistance survey (DRS) isolates sourced all around Tanzania. Findings from this work should inform intervention strategies and future MDR-TB monitoring tactics. The sequence data will also help to understand the genomic characteristics of *M. tuberculosis* isolates and their resistant mutations prevalent among pulmonary TB patients enrolled during the second national anti-TB drug resistance survey in Tanzania.

Methods

Study design, population and sampling

This was a cross sectional national drug resistance survey conducted from June 2017 to July 2018. A cluster sampling strategy was used and the unit of sampling was a diagnostic center that notified 8 and more smear positive cases in 2015. Based on this, a total of 45 clusters were selected and in each cluster, a total of 34 new smear positive pulmonary TB patients and all previously treated smear positive pulmonary TB cases diagnosed during the intake period for the survey were enrolled. Sputum samples were collected and forwarded to the Central TB Reference Laboratory (CTRL) in Dar es Salaam for smear microscopy, culture, strain identification and susceptibility testing following standard NTLP procedures. For WGS, a total of 627 culture positive isolates were shipped to the National TB Reference Laboratory/Supranational Tuberculosis Reference Laboratory- Uganda.

Sub-culture and DNA extraction for whole-genome sequencing

All isolates were sub-cultured on selective Middlebrook 7H11 agar (Becton and Dickson, USA), incubated at 37^0C in a CO_2 incubator (Panasonic, Osaka, Japan) and monitored weekly for growth. Once sufficient bacterial colonies were observed, these were harvested into a 15 ml Falcon tube with 1.0 ml of sterile water, followed by a thirty-minute heat inactivation at 85^0C. High quality genomic DNA was extracted using an in-house cetyltrimethylammonium bromide (CTAB) method previously described [14]. Integrity of the extracted DNA was assessed using the TapeStation 4150 (Agilent USA) with

the Agilent Genomic DNA ScreenTape and reagents. Purity of the bacterial DNA was assessed using the NanoDrop 2000c (ThermoFisher Scientific).

Library preparation and sequencing

Genomic libraries were prepared using the Illumina Nextera XT library preparation kit following manufacturer's instructions [15]. Quality of the prepared libraries was assessed with the Agilent 4150 using the D1000 High sensitivity ScreenTape and reagents. Libraries were sequenced on the MiSeq (Illumina, San Diego, CA, USA) using the Illumina MiSeq V3 cartridge at the Supranational Tuberculosis Reference Laboratory in Uganda.

Bioinformatics analysis

Resistance and lineage determination

A total of 191 samples were sequenced. Quality of reads was assessed using FastQC [16] v0.11.8 and MultiQC [17] v1.0. Bad quality bases were trimmed off using Trimmomatic v0.39 [18]. Three tools for resistance profiling and lineage inference namely Kvarq [19] v0.12.2, Mykrobe [20] v0.8.1 and TBprofiler [21] v3.0.5 were run.

Phylogenetic tree construction

De-novo genome assembly of all samples was done using Unicycler v0.4.8[22]. The assembled genomes were then annotated using Prokka [23] to generate genomic feature files to be used as input for Roary v3.13.0 [24] which was then used to generate a core gene multiple sequence alignment. Using the GTR + G substitution model, a maximum likelihood phylogeny was constructed using RaxML-NG v1.0.3 [25] with 800 bootstrap replicates with H37Rv reference strain NC_000962.3 as the reference and *Mycobacterium canettii* NC_015848.1 as the out-group. The resulting trees were plotted, annotated and visualized using ggtree v2.0.4 [26].

Ethical considerations

The study was approved by the National Health Research Ethics Committee of Tanzania and the Department of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich, Munich, Germany. Written informed consent or assent was obtained from all participants.

Results

Demographic characteristics of TB patients from whom the isolates were collected

Of the 627 samples received at the NTRL-Uganda, 10 were rejected and only 617 were sub-cultured. Of these 265 (43%) yielded either no growth (negative), contaminated or NTM and could not be processed further for WGS. Of the 352 samples that yielded a positive TB culture, 191 (54%) were sequenced due to resource constraints. Of these, 133 (70.0%) were from male TB patients. The mean age (standard deviation) of the TB patients from whom the isolates were collected was 37.5 (± 13.8) years. Most (107; 55.8%) of the TB patients were aged 25–44 years. Most [169 (88.0%)] of the isolates were from newly treated pulmonary TB patients. Coinfection with HIV was observed in 33 (17.3%) of the 191 TB patients. Of the 191 isolates, 22 (11.5%) were resistant to one or more commonly used first line anti-TB drugs (FLD). While 3 (1.6%) were resistant to all the drugs, 9 (4.7%) isolates were MDR-TB (Supplementary data Table 1).

Phylogenetic analysis

From the 191 M. *tuberculosis* isolates, four main lineages were identified at different frequencies (Table 1). The dominant lineage was Lineage 3 [81 (42.4%)], followed by Lineage 4 [74 (38.7%)], then Lineage 1 [23 (1209%)] and Lineage 2 [13 (6.8%)] (Table 1). Lineage 3 was the most prevalent among isolates from previously treated TB cases 9 (47.4%) as compared to 72 (41.9%) among isolates from newly treated patients. Lineage 4 dominated 7 (36.8%) those previously treated as compared to 67 (39.0%) of the newly treated. Lineage 1 was reported in 2 (10.5%) of the previously treated as compared to 21 (12.1%) of the newly treated patients. Lineage 2 was isolated in 1 (5.3%) of the previously treated TB case while the newly treated patients harboured 12 (6.9%) of these isolates. Lineage 3 was the most prevalent in both HIV positive 15 (5.5%) and HIV negative 66 (41.8%). This was

Table 1 Patients' history of previous TB treatment and HIV status by *M. tuberculosis* lineages

M. tuberculosis lineages	Total n (%)	History of TB, n (%)		HIV Status, n (%)	
		Previously treated	Newly treated	HIV positive	HIV negative
Lineage 2	13 (6.8)	1(5.3)	12 (7.0)	1 (3.0)	12 (7.6)
Lineage 3	81 (42.4)	9 (47.4)	72 (41.9)	15 (45.5)	66 (41.8)
Lineage 1	23 (12.0)	2 (10.5)	21 (12.1)	2 (6.1)	21 (13.3)
Lineage 4	74 (38.7)	7 (36.8)	67 (39.0)	15 (45.5)	59 (37.3)
Total	**191**	**19 (10.0)**	**172 (90.0)**	**33 (17.3)**	**158 (82.7)**

also the case for Lineage 4 with 59 (37.3%) isolates from HIV negative and 15 (45.5%) from HIV positive patients (Table 1).

M. tuberculosis Lineages and their correlation with drug resistance conferring mutation

While the Lineage 2 had 1 (7.7%) isolate that showed resistance to rifampicin and ethambutol, Lineage 3 had 7 (8.6%) isolates resistant to FLDs, out of which 3 (3.7%) were MDR-TB. For Lineage 1, out of the 23 isolates, 5 (21.7%) were resistant to FLDs and 2 (8.7%) were MDR-TB. Out of 74 isolates for Lineage 4, 9 (12.2%) were resistant to FLDs and 3 (4.1%) were MDR-TB (Table 2, Fig. 1 and Supplementary data Table 2).

Frequency of drug resistant mutations

The most prevalent Isoniazid conferring mutation was *KatG.*Ser315Thr [9 (37.5%)]. The *inhA*.Ser94Ala and *fabG1 c.-15C>T*, *c.-8 T>A*, CTG607CTA had 1 (4.2%) mutation each. All Isoniazid conferring mutations were classified as common with a high resistance level observed in *fabG1 c.-15C>T* and *KatG. Ser315Thr* while the promoter regions of *inhA*.Ser94Ala, *fabG1.*CTG607CTA and *fabG1 c.-8 T>A*. All had a low detected resistance level (Table 3).

The most prevalent Rifampicin resistance-conferring mutation were *rpoB.*Gln432Glu and *rpoB.*Ser450Leu with each accounting for a total of 3 (12.5%), while the remaining mutations were as follows: *rpoB.*Ser441Gln was found twice (8.3%), *rpoB.*His445Asn 1 (4.2%), and *rpoB.Leu430Pro* as well only 1 (4.2%). Rifampicin resistance-conferring mutation *rpoB.*His445Asn and *rpoB.*Ser441Gln were classified as rare with an equally low observed resistance level, while *rpoB.*Gln432Glu, *rpoB.*Leu430Pro and *rpoB.*Ser450Leu were classified as commonly occurring mutation with a high resistance level observed (Table 3).

Resistance-conferring mutations to Ethambutol in the *embCAB* loci were found in 8 (33.3%) isolates, with *embB.*Met306Ile being the most prevalent in 4 (16.7%), followed by *embB.*Gln497Arg at 2 (8.4%) while *embB.*Asp1024Asn and *embB.*Leu359Ile each had 1 (4.2%) mutation prevalence. All Ethambutol driven mutations were classified as common with a high resistance level. Resistance to Pyrazinamide at the *pncA* locus was identified in 8(33.3%) isolates and none with *rpsA*. The most prevalent Pyrazinamide resistance-conferring mutation *pncA*.Ala30Val and GAG331TAG with each accounting for 2 (8.4%), while the remaining mutations of *pncA*.Leu172Pro, *pncA*.Phe106Leu, *pncA.*Thr160Ala, and *pncA*.E111$ all had 1 (4.2%) mutation each. Resistance conferring mutation at pncA.Phe106Leu was classified as rare while *pncA*.Leu172Pro, *pncA.*Ala30Val, *pncA*.GAG331TAG, *pncA.*Thr160Ala and *pncA*.E111 were considered common (Table 3).

For Streptomycin resistance, mutation in the *rspL.*Lys88Met was reported at 4 (16.7%) and were more frequent followed by resistance-conferring mutation in *rrs*. *Ser172Cys* at 1 (4.2%) while mutations in the *gidB* promoter region of *Pro93Leu* accounted for 1 (4.2%). Resistance to Ethionamide due to mutations in *fabG1* and *inhA* were found in 2(8.3%) of the isolates. Resistance-conferring mutation at loci *fabG1 c.-15C>T* and *inhA*.Ser94Ala each Ser94Ala were each reported at 1 (4.2%). Mutations in the conserved quinolone resistance-determining region (QRDR) of *gyrA* at position *Ala90Val* at 1 (4.2%) as well as *Asp94Gly* at 1 (4.2%) and classified as common (Table 3 and Supplementary data Table 3).

Discussion

This study reports the existence of heterogeneity among MTBC lineages circulating in Tanzania. Central Asian Lineage (L3) was the most predominant followed by Euro American (L4), Indo-Oceanic (L1) and East-Asian [2] lineage respectively. This is contrary to an earlier study done in the same setting that reported L4 to be the more widely distributed lineage as compared to L3 [27]. Previous studies have also highlighted that the East Asian lineage has only been recently circulating within the African continent which is consistent to findings in this study [28]. Furthermore, L3 was reported to be widely distributing among the newly treated population in this study as compared to the population with a previous history of TB treatment which may be suggestive of a high TB

Table 2 Anti-TB drug resistance stratified by M. tuberculosis lineages, *N* = 191

M. tuberculosis lineages	Total n	Anti-TB drugs resistance (row %)					
		INH n (%)	RMP n (%)	EMB n (%)	PZA n (%)	MDR n (%)	RFLD[a] n (%)
Lineage 2	13	0 (0)	1 (7.7)	1 (7.7)	0 (0)	1 (7.7)	1 (7.7)
Lineage 3	81	4 (4.9)	5 (6.1)	2 (2.4)	0 (0)	3 (3.7)	7 (8.6)
Lineage 1	23	3 (13.0)	1 (4.4)	1 (4.4)	1 (4.4)	2 (8.7)	5 (21.7)
Lineage 4	74	6 (8.1)	3 (4.1)	4 (5.4)	7 (9.5)	3 (4.1)	9 (12.2)

RFLD[a] Resistant to first line drugs, *INH* Isoniazid, *RMP* Rifampicin, *EMB* Ethambutol, *PZA* Pyrazinamide, *MDR* Multi-drug resistance

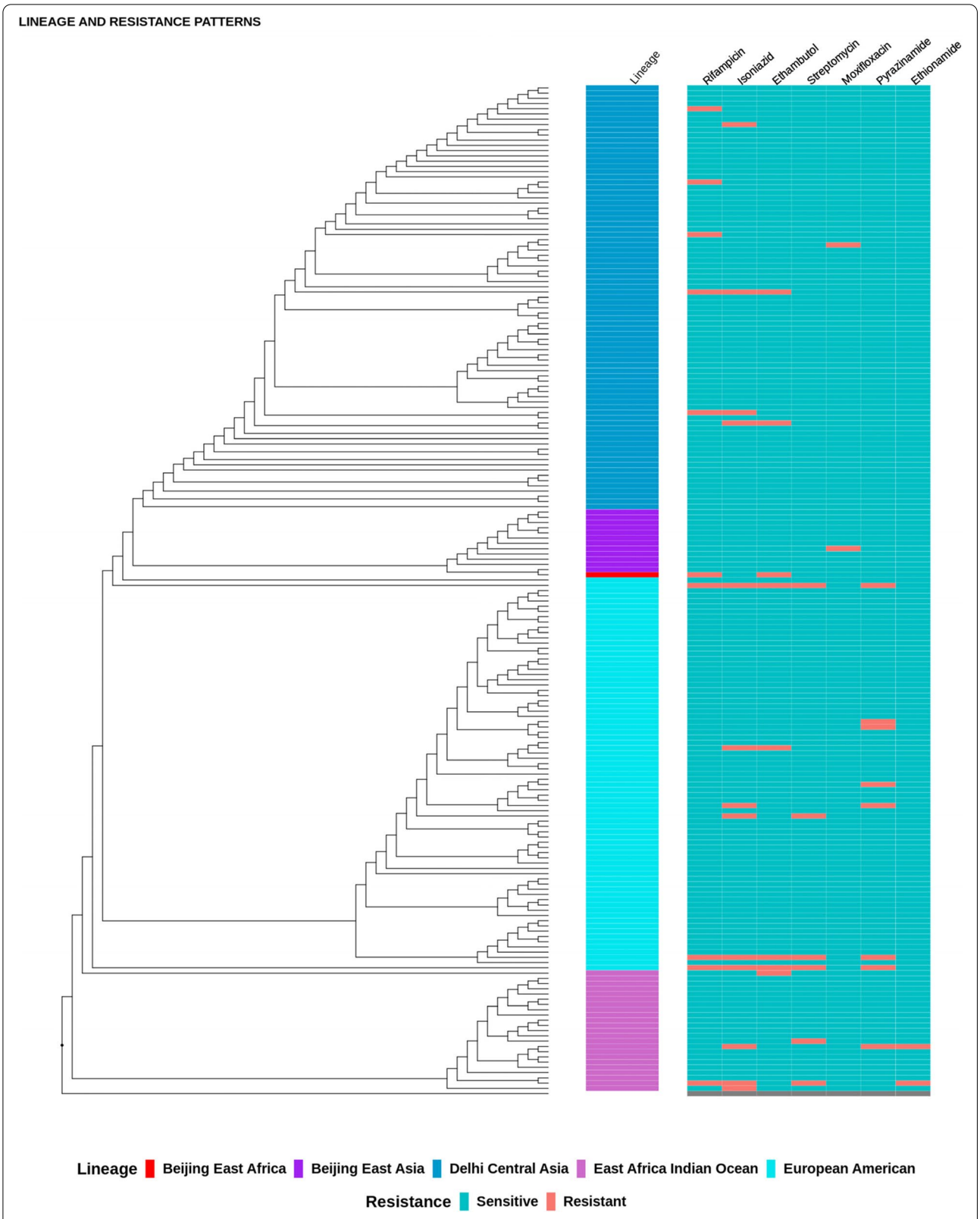

Fig. 1 Phylogenetic tree showing association between Mycobacterium tuberculosis lineages and drug resistance

Table 3 Frequency of drug resistance mutations, $N = 24$

Drug	Gene	Mutation	Resistant (n/N (%))	Classification of the mutation	Resistance level
Isoniazid	fabG1	c.-15C > T	1/24 (4.2)	Common	High
	fabG1	CTG607CTA	1/24 (4.2)	Common	Low
	fabG1	c.-8 T > A	1/24 (4.2)	Common	Low
	inhA	Ser94Ala	1/24 (4.2)	Common	Low
	katG	Ser315Thr	9/24 (37.5)	Common	High
Rifampicin	rpoB	Gln432Glu	3/24 (12.5)	Common	High
	rpoB	His445Asn	1/24 (4.2)	Rare	Low
	rpoB	Leu430Pro	1/24 (4.2)	Common	High
	rpoB	Ser441Gln	2/24 (8.3)	Rare	Low
	rpoB	Ser450Leu	3/24 (12.5)	Common	High
Ethambutol	embB	Asp1024Asn	1/24 (4.2)	Common	High
	embB	Gln497Arg	2/24 (8.4)	Common	High
	embB	Leu359Ile	1/24 (4.2)	Common	High
	embB	Met306Ile	4/24 (16.7)	Common	High
Pyrazinamide	pncA	Ala30Val	2/24 (8.4)	Common	High
	pncA	E111$	1/24 (4.2)	Common	Low
	pncA	GAG331TAG	2/24 (8.4)	Common	High
	pncA	Leu172Pro	1/24 (4.2)	Common	High
	pncA	Phe106Leu	1/24 (4.2)	Rare	Low
	pncA	Thr160Ala	1/24 (4.2)	Common	High
Streptomycin	gid	Pro93Leu	1/24 (4.2)	-	
	rpsL	Lys88Met	4/24 (16.7)	-	
	rrs	Ser172Cys	1/24 (4.2)	-	
Ethionamide	fabG	c.-15C > T	1/24 (4.2)	-	
	inhA	Ser94Ala	1/24 (4.2)	-	
Fluoroquinolones	gyrA	Ala90Val	1/24 (4.2)	Common (LEV, MOX CC)	
	gyrA	Asp94Gly	1/24 (4.2)	Common	

Note: Indicates missing information on classification

transmission pattern of the widely transmitting L3 in Tanzania.

In this study, we show that East Asian lineage and Euro American lineages were largely found in TB patients living with HIV. This is a rare finding in Tanzania since no previous study has demonstrated no such association between TB drug resistance and HIV infection [29, 30]. However, our findings are in line with the findings from a recent study conducted in Haiti that reported the same MTB lineages harbouring MDR-TB resistance patterns as well as the higher risk of MDR-TB infection in people living with HIV (PLHIV) [31].

Although previous treatment for TB is the strongest risk factor for development of DR-TB [32–35], treatment-naïve patients may also acquire drug resistance due to either transmission of resistant strains or spontaneous mutations. In our study we report strains resistant to some SLDs which are not being used to treat TB in Tanzania. However, similar findings were reported in a study conducted in India to determine the antimicrobial susceptibility to first-line and second line anti-TB drug resistance among newly diagnosed pulmonary TB (PTB) cases, primary multi-drug resistance (MDR) and extensively drug resistance (XDR) were reported [36]. Prevalence of primary drug resistance serves as an epidemiological indicator to assess the success of the national TB control programme. Based on these findings, there is a need to give emphasis on appropriate screening of TB cases, effective and rational use of second line drugs for newly diagnosed MDR-TB patients to prevent the emergence of pre-XDR/XDR-TB strains.

Resistance to anti-TB drugs in *M. tuberculosis* arises as a result of spontaneous gene mutations that reduce the bacterium's susceptibility to the most commonly used anti-TB drugs[37]. Several previous studies have identified different genes that encode anti-TB drug targets and have briefly described different mechanisms of resistance both to RIF and INH [37, 38].

The genes can encode drug targets or drug metabolism mechanisms and influence the efficacy of anti-TB treatment [13, 39, 40]. INH resistance appears more complex and has been associated with multiple genes, most commonly *katG* and the promoter region of the *inhA* gene [27]. In the current study, we report that the most prevalent INH conferring mutation was *KatG.*Ser315Thr [9 (37.5%)]. Other studies have also shown that molecular diagnostic tests for INH resistance rely on detection of the 'canonical' mutations in codon 315 of *katG* and position 15 in the *inhA* promoter region. Also, many earlier studies have identified highly variable frequencies of these mutations, with *katG*315 mutations accounting for 42–95% and *inhA*–15 mutations accounting for 6–43% of phenotypic INH resistance [38, 40]. Reta and colleagues [27] found a prevalence of 95.8% for the katG315 mutation and 5.9% for the inhA promoter area mutation in patients with INH-resistant M. tuberculosis in a systematic evaluation of gene variants related with RIF and INH resistant M. tuberculosis in Ethiopia.

According to the World Health Organization (WHO), Next- Generation Sequencing is an important technique for drug-resistant tuberculosis (TB) (DR-TB) surveillance [41]. Whole Genome Sequencing offers more accurate and complete results for both first-line and second-line anti-TB medications, as well as useful insights into molecular epidemiology, such as phylogenetics, strain evolution, and transmission, than the traditional phenotypic drug susceptibility test (DST) [41]. Despite the fact that our study did not set out to compare the performance of conventional phenotypic DST and WGS, we found higher levels of MDR-TB and resistance to one or more commonly used first-line anti-TB drugs than those found in Tanzania's first national anti-TB drug resistance survey and the main survey from which the current isolates were derived. Other studies (not including national anti-TB surveys) [7, 32] have found that WGS testing of anti-TB drugs has the potential to provide comprehensive resistance detection much faster, with improved turnaround times, allowing for prompt appropriate treatment and associated patient and health-care benefits. [33].

Our study was limited to a small sample size, therefore findings of the phylogenetic distribution and association between lineages with patient demographic characteristics and drug resistance patterns may not be representative of the entire country profile. Furthermore, unavailability of data from conventional phenotypic DST methods in this study still limits our current understanding of the comparison of such methods with next generation sequencing approaches such as WGS in this setting.

Conclusion

The findings in this study shows existence of *M. tuberculosis* strains resistant to some second line drugs which were not routinely used to treat TB in Tanzania. Lineage 3 was the most prevalent among previously treated TB cases and in TB patients living with HIV. Lineage 1 and 4 were found to be prevalent in cases that were resistant to first line anti-TB drugs. The use of next generation sequencing tools such as WGS at a national anti-TB drug resistance survey is recommended as it may improve the epidemiological findings for appropriate interventions.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12864-022-08791-3.

Additional file 1: Supplementary data Table 1. Socio demographics, clinical characteristics and drug resistance among study subjects *N*=191. **Supplementary data Table 2**. M. tuberculosis lineages and their correlation with anti-TB drug resistance, *N*=191. **Supplementary data Table 3**. Pattern of drug resistance mutations by phylogenetic lineages, *N*=24.

Acknowledgements

We acknowledge the support rendered to the Global Fund Round New Funding Model 2 for the financial support that enabled the enrolment of the study patients and collection of sputum samples which were later used as sources of the isolates for the current study. We sincerely acknowledge the NTRL through the East, Central & Southern Africa Health Community (ECSA-HC) project that financed the procurement of the MiSeq sequencing machine, supporting equipment and reagents that were used to run the samples. We also thank the Nurturing Genomics and Bioinformatics Research Capacity in Africa (BReCA) project, award number 1U2RTW010672-01 for the Bioinfomatics training provided to Jupiter Marina Kabahita and Maria Magdalene Namaganda. We also acknowledge the Gilead research support provided to Jupiter Marina Kabahita via the Infectious Diseases Institute, Makerere University. Kabahita Jupiter Marina was also was supported by the Fogarty International Center of the National Institutes of Health, U.S. Department of State's Office of the U.S. Global AIDS Coordinator and Health Diplomacy (S/GAC), and President's Emergency Plan for AIDS Relief (PEPFAR) under Award Number 1R25TW011213. The content is solely the responsibility of the authors and does not necessarily represent the official views of the National Institutes of Health." We would also like to acknowledge the technical support and assistance provided by Mr. Edgar Kigozi and Mr. Fred Ashaba to the team that carried out WGS.

Authors' contributions

BKM AMK MH NH EL NSR BJN NEN SMM JL RK and MP: Contributed to the conception of the study. BKM AMK MLJ MH NH EL NSR BJN NEN SMM JL RK and MP: Contributed to the design of the work. BKM MLJ DO AW KM AK BD SMM AK JK IA HB GWK PL JMK OG JN HN ML MMN GM: Contributed to the acquisition and analysis of data. BKM MLJ AMK DO AW KM AK BD SMM AK JK IA HB GWK PL JMK OG JN HN ML MMN GM MH NH EL NSR BJN NEN SMM JL RK and MP: Contributed to the interpretation of data. BKM MLJ AMK DO AW KM AK BD SMM AK JK IA HB GWK PL JMK OG JN HN ML MMN, GM MH NH EL NSR BJN NEN SMM JL RK and MP: Drafted the work and substantively revised it. BKM MLJ AMK DO AW KM AK BD SMM AK JK IA HB GWK PL JMK OG JN HN ML MMN GM MH NH EL NSR BJN NEN SMM JL RK and MP: Approved the submitted version. BKM MLJ AMK DO AW KM AK BD SMM AK JK IA HB GWK PL JMK OG JN HN ML MMN, FA EK GM MH NH EL NSR BJN NEN SMM JL RK and MP: Agreed both to be personally accountable for the author's own contributions and to ensure that questions related to the accuracy or integrity of any part of the work, even ones in which the author was not personally involved, ere appropriately investigated, resolved, and the resolution documented in the literature. The author(s) read and approved the final manuscript.

Authors' information
Not applicable.

Funding
This study was funded by the Global Fund Round New Funding Model 2. The funders had no role in study design, data collection and analysis, decision to publish, or preparation of the manuscript.

Availability of data and materials
The datasets generated and/or analysed during the current study are available at the SRA under the study BioProject ID: PRJNA807440 and at the Zenodo open access repository https://doi.org/10.5281/zenodo.6271274

Declarations

Ethics approval and consent to participate
The main study was approved by the National Health Research Ethics Committee (NatHREC) of the Medical Research Coordinating Committee in Tanzania (Certificate No. NIMR/HQ/R.8a/Vol. IX/2341 of 7th November 2016) and the Center for Global Health (CGH) at the U.S. Centers for Disease Control and Prevention (CDC). It was reviewed in accordance with the U.S. CDC human research protection procedures and determined to be research. Written informed consent was obtained from all participants or their legal guardians; assent was also obtained from children aged 15–17 years from whom the source sputum samples were collected. All methods were performed in accordance with the national guidelines and regulations.

Consent for publication
Not Applicable.

Competing interests
The authors declare that they have no competing interests.

Author details
[1]Department of Preventive Services, Ministry of Health, Dodoma, Tanzania. [2]Department of Infectious Diseases and Tropical Medicine, Medical Center of the, University of Munich, Munich, Germany. [3]National Tuberculosis Reference Laboratory/Supranational Reference Laboratory, Luzira, Uganda. [4]Department of Immunology and Molecular Biology, College of Health Sciences, Makerere University, Kampala, Uganda. [5]Department of Microbiology and Immunology, Muhimbili University of Health and Allied Sciences (MUHAS), Dar es Salaam, Tanzania. [6]Muhimbili Research Centre, National Institute for Medical Research (NIMR), Dar es Salaam, Tanzania. [7]University of Dar Es Salaam, Mbeya College of Health and Allied Sciences, Mbeya, Tanzania. [8]Mbeya Medical Research Centre, NIMR, Mbeya, Tanzania. [9]Central Tuberculosis Reference Laboratory, Ministry of Health, National TB and Leprosy Programme, Dar es Salaam, Tanzania. [10]Department of Preventive Services, Ministry of Health, National Tuberculosis and Leprosy Programme, Dodoma, Tanzania. [11]Africa Centres for Disease Control and Prevention, African Union Commission, Addis Ababa, Ethiopia.

Received: 9 February 2022 Accepted: 12 July 2022
Published online: 05 August 2022

References

1. Musa BM, Adamu AL, Galadanci NA, Zubayr B, Odoh CN, Aliyu MH. Trends in prevalence of multi drug resistant tuberculosis in sub-Saharan Africa: a systematic review and meta-analysis. PLoS ONE. 2017;12(9): e0185105. https://doi.org/10.1371/journal.pone.0185105.
2. Jones RC, Harris LG, Morgan S, et al. Phylogenetic analysis of Mycobacterium tuberculosis strains in Wales by use of core genome multilocus sequence typing to analyze whole-genome sequencing data. J Clin Microbiol. 2019;57:e02025-e2118. https://doi.org/10.1128/JCM.02025-18.
3. Marais BJ, Victor TC, Hesseling AC, et al. Beijing and Haarlem genotypes are overrepresented among children with drug resistant tuberculosis in the western cape province of South Africa. J Clin Microbiol. 2006;44:3539–43. https://doi.org/10.1128/JCM.01291-06.
4. Tania T, Sudarmono P, Kusumawati RL, et al. Whole-genome sequencing analysis of multidrug-resistant Mycobacterium tuberculosis from Java. Indonesia J Med Microbiol. 2020;69(7):1013–9. https://doi.org/10.1099/jmm.0.001221.
5. Wilson ML. Rapid diagnosis of Mycobacterium tuberculosis infection and drug susceptibility testing. Arch Pathol Lab Med. 2013 Jun;137(6): 812e9. PubMed PMID: 23721277.
6. Gygli SM, Keller PM, Ballif M, et al. Whole-genome sequencing for drug resistance profile prediction in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2020;69(7):1013–9. https://doi.org/10.1128/AAC.02175-18.
7. Doyle RM, Burgess C, Williams R et al. Direct whole-genome sequencing of sputum accurately identifies drug-resistant mycobacterium tuberculosis faster than MGIT culture sequencing. J Clin Microbiol. 2018;56(8). https://doi.org/10.1128/jcm.00666-18. PMID: 29848567; PMCID: PMC6062781.
8. 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. 2007;44:4498–510. https://doi.org/10.1128/JCM.01392-06.
9. Cronin WA, Golub JE, Magder LS et al. Epidemiologic usefulness of spoligotyping for secondary typing of mycobacterium tuberculosis isolates with low copy numbers of IS6110. J Clin Microbiol. 2020;39(10). https://journals.asm.org/doi/full/ https://doi.org/10.1128/JCM.39.10.3709-3711.2001.
10. Alland D, Kalkut GE, Moss AR, et al. Transmission of tuberculosis in New York city – an analysis by DNA fingerprinting and conventional epidemiologic methods. N Engl J Med. 1994;330:1710–6. https://doi.org/10.1056/NEJM199406163302403.
11. Lagos J, Couvin D, Arata L, et al. Analysis of mycobacterium tuberculosis genotypic lineage distribution in Chile and neighboring countries. PLoS ONE. 2016;11(8).https://doi.org/10.1371/journal.pone.0160434
12. Couvin D, Rastogi N. The establishment of databases on circulating genotypes of Mycobacterium tuberculosis complex and web tools for an effective response to better monitor, understand and control the tuberculosis epidemic worldwide. EuroReference - Les Cahiers de la Référence, ANSES. 2014;2014(12):36–48. ⟨pasteur-02954167⟩
13. Global tuberculosis report 2019. Geneva: World Health Organization; 2019. Licence: CC BY-NC-SA 3.0 IGO.
14. Kigozi E, Kasule GW, Musisi K, Lukoye D, Kyobe S, Katabazi FA, et al. Prevalence and patterns of rifampicin and isoniazid resistance conferring mutations in Mycobacterium tuberculosis isolates from Uganda. PLoS ONE. 2018;13(5):e0198091. https://doi.org/10.1371/journal.pone.0198091.
15. Illumina. Nextera-xt-library-prep-reference-guide-15031942–05.pdf. Document # 15031942 v05 May 2019. [Online]. Available: https://support.illumina.com/content/dam/illumina-support/documents/documentation/chemistry_documentation/samplepreps_nextera/nextera-xt/nextera-xt-library-prep-reference-guide-15031942-05.pdfAccessed: 21 Sep 2021
16. Babraham Bioinformatics. FastQC A Quality Control tool for High Throughput Sequence Data." https://www.bioinformatics.babraham.ac.uk/projects/fastqc/ (accessed Sep. 22, 2021).
17. Ewels P, Magnusson M, Lundin S, Käller M. MultiQC. Summarize analysis results for multiple tools and samples in a single report, Bioinformatics. 2016;32(19):3047–8. https://doi.org/10.1093/bioinformatics/btw354.
18. Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. Bioinformatics. 2014;30(15):2114–20. https://doi.org/10.1093/bioinformatics/btu170.
19. Steiner A, Stucki D, Coscolla M, Borrell S, Gagneux S. KvarQ: targeted and direct variant calling from fastq reads of bacterial genomes. BMC Genomics. 2014;15:881. https://doi.org/10.1186/1471-2164-15-881.
20. Hunt M, Bradley P, Lapierre SG et al. Antibiotic resistance prediction for Mycobacterium tuberculosis from genome sequence data with Mykrobe. Wellcome Open Res. 2019, 4:191 (https://doi.org/10.12688/wellcomeopenres.15603.1)
21. Phelan J, O'Sullivan DM, Machado D, et al. Integrating informatics tools and portable sequencing technology for rapid detection of resistance to anti-tuberculous drugs. Genome Med. 2019;11:41. https://doi.org/10.1186/s13073-019-0650-x.
22. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads. PLOS

Comput Biol. 2017;13(6): e1005595. https://doi.org/10.1371/journal.pcbi.1005595.
23. Prokka ST. Rapid prokaryotic genome annotation". Bioinformatics. 2014;30(14):2068–9. https://doi.org/10.1093/bioinformatics/btu153.
24. Page AJ, Cummins CA, Hunt M, et al. Rapid large-scale prokaryote pan genome analysis. Bioinformatics. 2015;31(22):3691–3. https://doi.org/10.1093/bioinformatics/btv421.
25. Kozlov AM, Darriba D, Flouri T, Morel B, Stamatakis A. RAxML-NG: a fast, scalable and user-friendly tool for maximum likelihood phylogenetic inference. Bioinformatics. 2019;35(21):4453–5. https://doi.org/10.1093/bioinformatics/btz305.
26. Yu G, Smith DK, Zhu H, Guan Y, Lam TTY. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol. 2017;8(1):28–36. https://doi.org/10.1111/2041-210X.12628.
27. Vilchèze C, Wang F, Arai M, et al. Transfer of a point mutation in Mycobacterium tuberculosis inhA resolves the target of isoniazid. Nat Med. 2006;12:027–1029.
28. Reta MA, Alemnew B, Beletew BA, Fourie PB. Prevalence of drug resistance-conferring mutations associated with isoniazid and rifampicin-resistant Mycobacterium tuberculosis in Ethiopia: a systematic review and meta-analysis. J Glob Antimicrob Resist. 2021;26:207–18. https://doi.org/10.1016/j.jgar.2021.06.009.
29. Kremer K, van Soolingen D, Frothingham R, et al. Comparison of methods based on different molecular epidemiological markers for typing of Mycobacterium tuberculosis complex strains: interlaboratory study of discriminatory power and reproducibility. J Clin Microbio. 1999;37(8):2607–18.
30. Umubyeyi AN, Gasana M, Basinga P, et al. Results of a national survey on drug resistance among pulmonary tuberculosis patients in Rwanda. Int J Tuberc Lung Dis. 2007;11(2):189–94.
31. Chum HJ, O'Brien RJ, Chonde TM, Graf P, Rieder HL. An epidemiological study of tuberculosis and HIV infection in Tanzania, 1991–1993. AIDS. 1996;10:299–309. https://doi.org/10.1097/00002030-199603000-00009.
32. Votintseva AA, Bradley P, Pankhurst L, et al. Same-day diagnostic and surveillance data for tuberculosis via whole-genome sequencing of direct respiratory samples. J Clin Microbiol. 2017;55(5):1285–98. https://doi.org/10.1128/JCM.02483-16.
33. Zhang Y, Zhao R, Zhang Z, et al. Analysis of factors influencing multidrug-resistant tuberculosis and validation of whole-genome sequencing in children with drug-resistant tuberculosis. Infect Drug Resist. 2021;24(14):4375–93. https://doi.org/10.2147/IDR.S331890.PMID:34729015;PMCID:PMC8554314.
34. Zignol M, Wright A, Jaramillo E, Nunn P, Raviglione MC. Patients with previously treated tuberculosis no longer neglected. Clin Infect Dis. 2007;44(1):61–4. https://doi.org/10.1086/509328.
35. Chioma KN, Isaac AA, Bamidele IO, et al. Multidrug-resistant tuberculosis in HIV-negative patients in Lagos. Nigeria Afr J Bacteriol Res. 2020;12(2):12–9. https://doi.org/10.5897/JBR2020.0319.
36. Myneedu VP, Singhal R, Khayyam KU, Sharma PP, Bhalla M, Behera D, Sarin R. First and second line drug resistance among treatment naïve pulmonary tuberculosis patients in a district under revised national tuberculosis control programme (RNTCP) in New Delhi. J Epidemiol Glob Health. 2015;5(4):365–73. https://doi.org/10.1016/j.jegh.2015.04.002 Epub 2015 May 2. PMID: 25944154; PMCID: PMC7320499.
37. Torres JN, Paul LV, Rodwell TC, et al. Novel katG mutations causing isoniazid resistance in clinical M. tuberculosis isolates. Emerg Microbes Infect. 2015;4(7):e42. https://doi.org/10.1038/emi.2015.42.
38. Zhang Y, Yew WW. Mechanisms of drug resistance in Mycobacterium tuberculosis. Int J Tuberc Lung Dis. 2009;13:1320–30.
39. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PLoS One. 2015;10:e0119628. https://doi.org/10.1371/journal.pone.0119628.
40. Marahatta SB, Gautam S, Dhital S, et al. katG (SER 315 THR) gene mutation in isoniazid-resistant Mycobacterium tuberculosis. Kathmandu Univ Med J. 2011;9:19–23. https://doi.org/10.3126/kumj.v9i1.6256.
41. World Health Organization. Global tuberculosis report 2020. Geneva: World Health Organization; 2020.

Publisher's Note

Acknowledgements

I would like to thank my supervisors; Dr Michel Pletschette, Dr. Norbert Heinrich, Dr. Nyanda Elias Ntinginya and Prof. Dr. Michael Hoelscher for their immense academic and professional guidance during the entire period of my studies. Special thanks go to the management of the Department of Infectious Diseases and Tropical Medicine, Medical Center of the University of Munich, for accepting my placement and admission as a student. I also thank my husband, children and family for their understanding and support which facilitated my completion of this PhD. I am also grateful to the Global Fund for providing funding for this survey through the Tanzania NTLP and the Ministry of Health Tanzania for supporting the logistics for all teh field work. Last but not least, my sincere thanks to all the health facility staff patients who participated in this study.

Complete list of my publications

1. **Beatrice Kemilembe Mutayoba**, Michael Hoelscher, Norbert Heinrich, Moses Joloba, Eligius Lyamuya, Andrew Martin Kilale, Nyagosya Segere Range, Benard James Ngowi, Nyanda Elias Ntinginya, Saidi Mwinjuma Mfaume, Amani Wilfred, Basra Doulla, Johnson Lyimo, Riziki Kisonga, Amri Kingalu, Jupiter Marina Kabahita, Ocung Guido, Joel Kabugo, Isa Adam, Moses Luutu, Maria Magdalene Namaganda, Joanitah Namutebi, George William Kasule, Hasfah Nakato, Henry Byabajungu, Pius Lutaaya, Kenneth Musisi, Denis Oola, Gerald Mboowa, Michel Pletschette. Phylogenetic lineages of tuberculosis isolates and their association with patient demographics in Tanzania. *BMC Genomics*. 2022; 23:561, 2022 August 05.

2. Beatrice Kemilembe Mutayoba, Julia Ershova, Eligius Lyamuya, Michael Hoelscher, Norbert Heinrich, Andrew Martin Kilale, Nyagosya Segere Range,Benard James Ngowi, Nyanda Elias Ntinginya, Saidi Mwinjuma Mfaume, Emmanuel Nkiligi, Basra Doulla, Johnson Lyimo,Riziki Kisonga, Amri Kingalu, Yakobo Lema, Zuwena Kondo, Michel Pletschette. The second national anti-tuberculosis drug resistance survey in Tanzania, 2017–2018. TMIH. 2022 August 17 https://doi.org/10.1111/tmi.13814)

3. Peter M. Mbelele, Christian Utpatel, Elingarami Sauli, Emmanuel A. Mpolya, **Beatrice K. Mutayoba,** Ivan Barilar, Viola Dreyer, Matthias Merker, Margaretha L. Sariko, Buliga M. Swema, Blandina T. Mmbaga, Jean Gratz, Kennedy K. Addo, Michel Pletschette, Stefan Niemann, Eric R. Houpt, Stellah G. Mpagama, Scott K. Heysell. Whole genome sequencing-based drug resistance predictions of multidrug-resistant *Mycobacterium tuberculosis* isolates from Tanzania. *JAC-Antimicrobial Resistance*, Volume 4, Issue 2, April 2022.

4. Andrew Martin Kilale, Andrea Pantoja, Bhavin Jani, Nyagosya Range, Bernard James Ngowi, Charles Makasi, Melkisedeck Majaha, Chacha Dionis Manga, Sylvia Haule, Amani Wilfred, Pudensiana Hilary, Vishnu Mahamba, Emmanuel Nkiligi, Wilbard Muhandiki, Emmanuel Matechi, **Beatrice Mutayoba,** Nobuyuki Nishkiori and Julia Ershova. Economic burden of tuberculosis in Tanzania: a national survey of costs faced by tuberculosis-affected households. BMC Public Health. (2022) 22:600. https://doi.org/10.1186/s12889-022-12987-3)

5. Basra Esmail Doulla, Stephen Bertel Squire, Eleanor MacPherson, Esther Stanslaus Ngadaya, **Beatrice Kemilembe Mutayoba** and Ivor Langley. Reducing delays to multidrug-resistant tuberculosis case detection through a revised routine surveillance system. (BMC Infectious Diseases (2020) 20:594. 2020 August 12.

6. Basra Esmail DoullaID, Stephen Bertel Squire, Eleanor MacPherson, Esther Stanslaus Ngadaya, **Beatrice Kemilembe Mutayoba**, Ivor Langley. Routine surveillance for the identification of drug resistant Tuberculosis in Tanzania: A cross- sectional study of stakeholders' perceptions. PLoS ONE 14(2): e0212421. 2019 Feb 22.

7. Puck T. Pelzer, **Beatrice Mutayoba,** Frank G.J. Cobelens. BCG vaccination protects against infection with Mycobacterium tuberculosis ascertained by tuberculin skin testing. Journal of Infection. 2018 May 18.77 (2018) 335–340.

8. Jerry Hella, Carl Morrow, Francis Mhimbira, Samuel Ginsberg, Nakul Chitnis, Sebastien Gagneux, **Beatrice Mutayoba,** Robin Wood, Lukas Fenner. Tuberculosis transmission in public locations in Tanzania: A novel approach to studying airborne disease transmission. Journal of Infection. 2017; 75, 191e197. 2017 July 1.

9. Francis Mhimbira, Jerry Hella, Thomas Maroa, Shadrack Kisandu, Magreth Chiryamkubi, Khadija Said, Grace Mhalu, Abdallah Mkopi, **Beatrice Mutayoba,** Klaus Reither, Sébastien Gagneux, Lukas Fenner. Home-Based and Facility-Based Directly Observed Therapy of Tuberculosis Treatment under Programmatic Conditions in Urban Tanzania. (PLoS One 2016 11;11(8):e0161171. Epub 2016 Aug 11.)

10. Grace Mhalu, Jerry Hella, Basra Doulla, Francis Mhimbira, Hawa Mtutu, Helen Hiza, Mohamed Sasamalo, Liliana Rutaihwa, Hans L Rieder, Tamsyn Seimon, **Beatrice Mutayoba,** Mitchell G Weiss, Lukas Fenner. Do Instructional Videos on Sputum Submission Result in Increased Tuberculosis Case Detection? A Randomized Controlled Trial. PLoS One. 2015; 29;10(9):e0138413. Epub 2015 Sep 29)

11. Sayoki G Mfinanga, Bruce J Kirenga, Dr Duncan M Chanda, **Beatrice Mutayoba,** Thuli Mthiyane, Getnet Yimer, Oliver Ezechi, Cathy Connolly, Vincent Kapotwe, Catherine Muwonge, Alex Pym, Prof Moses Joloba, Roxana Rustomjee, Prof Alimuddin Zumla, Philip Onyebujoh et al. Early versus delayed initiation of highly active

antiretroviral therapy for HIV-positive adults with newly diagnosed pulmonary tuberculosis (TB-HAART): a prospective, international, randomised, placebo-controlled trial. (Lancet Infect Dis 2014 Jul 5;14(7):563-71. Epub 2014 May 5)

12. Mfinanga GS., **Mutayoba B.**, Mbogo G., Kahwa A., Kimaro G., Mhame P. P., Mwangi C., Malecela M.N. and Kitua A.Y. Quality of HIV laboratory testing in Tanzania: a situation analysis. Tanzania Health Research Bulletin. 2007; 9(1). pp. 44-47.

13. Sayoki G Mfinanga, **Beatrice K Mutayoba**, Amos Kahwa, Godfather Kimaro, Rugola Mtandu, Esther Ngadaya, Said Egwaga, Andrew Y Kitua. The magnitude and factors associated with delays in management of smear positive tuberculosis in Dar es Salaam, Tanzania. BMC Health Serv Res. 2008 Jul 27;8:158. Epub 2008 Jul 27.

14. Mfinanga G.S, **Mutayoba B**, Ngadaya E, Mtandu R, Doulla B and Kimaro, G. The Quality of Sputum Smear microscopy in the diagnosis of TB in Dar es Salaam. Tanzania Health Research Bulletin. 2007; 9 (2).

15. Mfinanga SG, Kahwa A, Kimaro G, Kilale A, Kivuyo S, Senkoro M, Ngowi B, Mtandu R, **Mutayoba B,** Ngadaya E, Mashoto K. Dissatisfaction with the laboratory services in conducting HIV related services among public and private medical personel in Tanzania. BMC Health Services Research 2008, 8:171 doi: 10.1186/1472-6963-8-171

16. A. Kahwa, G.S. Mfinanga, G.D. Kimaro, **B. Mutayoba,** E. Ngadaya, G. Mbogo, J.W. Ogoniek, A.M. Mdemu, V.R. Nyigo, L.Lema, S.Kivuyo and G.Mubyazi. Exploring the costs of laboratory testing for HIV and major sources of funding for Tanzania. Research Journal of Medical Sciences Vol 2 (1), 2008, p 33-37

17. Mlozi M.R.S., Shayo E.H., Senkoro K.P., Mayala B.K., Rumisha S.F., **Mutayoba B.**, Senkondo E., Maerere A and Mboera L.E.G.. Participatory involvement of farming communities and public sectors in determining malaria control strategies in Mvomero District, Tanzania. Tanzania Health Research Bulletin, Vol. 8, No. 3, 2006, pp. 134-140.

18. Andrew Martin Kilale, Charles Makasi, Melkisedeck Lolakeha Majaha , Chacha Dionis Manga, Sylvia Haule, Pudensiana Hilary, Omari Kimbute, Stephen Kitua,

Bhavin Jani, Nyagosya Range, Bernard James Ngowi, Emmanuel Nkiligi, Emmanuel Matechi, Wilbard Muhandiki, Vishnu Mahamba, **Beatrice Kemilembe Mutayoba**, Julia Ershova. Implementing tuberculosis patient cost surveys in resource-constrained settings: lessons from Tanzania. Research Square, DOI: https://doi.org/10.21203/rs.3.rs-1329772/v. (2022 April 13)