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RESEARCH ARTICLE

Molecular Detection of Drug-Resistant *Mycobacterium tuberculosis* in Sputum Specimens from the New and Previously Treated Tuberculosis Cases at the National Reference Chest Diseases Laboratory in Lusaka, Zambia

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ABSTRACT

Background: Drug-Resistant Tuberculosis (DR-TB) is one of the major public health issues globally. Zambia is highly burdened by TB and multi-drug resistant TB. In this study, sputum samples obtained from the new and previously treated cases of TB were examined for drug-resistant *Mycobacterium tuberculosis* (MTB).

Methods: Sputum specimens were processed using the N-acetyl-L-cysteine-sodium hydroxide method, stained and examined using fluorescent technique and microscopy respectively. Mycobacterial DNA was extracted using the Genolyse kit, then subjected to multiplex polymerase chain reaction amplification and reverse hybridization. Drug-resistance and mutations in MTB genes were detected using the Genotype MTBDR*plus* VER 2.0 and MTBDR*sl* VER 2.0 assays.

Results: A total of 329 MTB-positive sputum specimens, 102 from the new TB cases and 227 from previously treated TB cases, were analysed for drug-resistance. Among the new TB cases, 3.9% had Rifampicin (RIF) mono-resistance, 12.8% Isoniazid (INH) mono-resistance, and 17.7% had Multi-Drug Resistance (MDR). For the previously treated TB cases, 10.1% had RIF mono-resistance, 6.6% INH mono-resistance, 33.0% MDR, 1.8% poly-drug resistance, and 0.8% had pre-Extensively Drug-Resistance (pre-XDR). Mutations identified were *rpoB* (Ser531Leu, His526Asp, Asp516Val, His526Tyr, and Glu510His), *kat*G (Ser315Thr 1 and Ser315Thr 2), *Inh*A (Cys15Thr), *gyr*A (Ala90Val and Asp94Gly), and *eis* (Cys14Thr), each with a varying frequency.

Conclusion: DR-TB is prevalent, especially MDR-TB, which is currently the most worrisome form of DR-TB and an emerging threat hampering efforts in the control of TB in Zambia. The early detection and effective treatment of TB cases are key in the control of DR-TB.

INTRODUCTION

Tuberculosis (TB) is one of the leading causes of death globally [1,2]. This airborne killer infectious disease causes high mortality and morbidity rates [3-5]. The *Mycobacterium tuberculosis* (MTB) is the major causative agent for human TB

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among the *Mycobacterium tuberculosis* Complex (MTBC), as it causes 97-99% of TB cases [6]. Globally 10 million individuals contract TB and from this number, 1.6 million individuals die from the disease [7]. The World Health Organization (WHO) global TB report of 2020 revealed that in 2019 there were 10 million people who got sick of TB, with 1,408,000 deaths globally [8].

Drug-resistant TB is a serious public health problem globally [9]. TB prevention, control, eradication and elimination strategies are being hampered by the successful transmission of drug-resistant MTB strains which are causing Multidrug-Resistant (MDR) and Extensively Drug-Resistant (XDR)-TB in the human population [10,11]. In 2020 the global prevalence of MDR/RR (Rifampicin-Resistance) was 3.3% among the new TB cases and 17.7% among the previously treated TB cases [8]. In 2019 was 3.4% and 18% among the new and previously treated TB cases respectively [12]. In 2017, 8.5% of TB patients diagnosed with MDR-TB progressed to developing XDR-TB [12]. XDR-TB is rapidly spreading throughout the world with 9.4 million new cases and 1.7 million reported deaths annually [13]. Globally in 2019 the prevalence of isoniazid mono-resistance among the new TB cases was 7.2% and 11.6% among the previously treated TB cases [8]. Drug-resistant TB is a serious public health threat in sub-Saharan Africa, especially for countries that have a high burden of both HIV and TB cases [14]. Drug-resistant TB is posing a huge obstacle for many countries to achieve the set WHO end TB targets [12,15].

Zambia is ranked among the top ten countries with a high burden of TB in the world [12,16,17]. TB is a major public health problem in the country, and is causing high mortality and morbidity rates [18-21]. Zambia is also among the top 10 countries in Africa with the highest cases of MDR/RR-TB [22]. MDR-TB is indeed a growing public health problem in the country [23]. In 2020, the MDR-TB prevalence rate in Zambia was 2.4% among the new and 18% previously treated TB cases [8]. While in 2019 was 2.8% and 18% among the new and previously treated TB cases respectively [12]. It has been projected that in 2021, Zambia will have a high burden of MDR and rifampicin-resistant TB cases [24].

Drug-resistant TB types include mono drug-resistant (resistance to any single anti-TB drug), poly drug-resistant (resistance to more than one anti-TB drug, other than both Rifampicin (RIF) and Isoniazid (INH) [25], MDR (resistance to at least INH and RIF), pre-XDR (resistance to INH, RIF, plus any one Fluoroquinolone (FLQ) (levofloxacin, ofloxacin, moxifloxacin or gatifloxacin) or any one of the Second-Line Injectable Drugs (SLIDs) (Kanamycin, Amikacin or Capreomycin), XDR (resistance to INH, RIF, plus any FLQ, plus any SLIDs) [26-30]. Poorly managed XDR-TB cases can progress to Totally Drug-Resistant TB (TDR-TB). TDR-TB is a type of DR-TB that is resistant to all first-and second-line anti-TB drugs. TDR-TB has been reported in India, Iran, Italy and South-Africa [31,32].

MTB develops resistance to anti-TB drugs due to a number of factors some of which include poor adherence, poor regimen selection, inadequate supply, and stock-out of anti-TB drugs [30,33]. However, research has shown that MTB resistance is mainly caused by mutations in the target genes and chromosomal replication errors [34,35].

Mutations in the *rpo*B gene, specifically in an 81 base pair region called the Rifampicin-Resistance Determining Region (RRDR) confer resistance to RIF [36,37]. The four most frequent mutations that confer resistance to RIF are Ser531Leu, His526Tyr, His526Asp, and Asp516Val [25]. Mutations in the *kat*G and/or *inh*A gene cause resistance to INH frequently [36]. However, mutations in the following genes *ahp*C, *oxy*R, *kas*A, *fur*A, *fab*G 1 and *ndh* infrequently cause resistance to INH [37]. The most frequent mutation in the *kat*G gene that confers resistance to INH is the Ser315Thr mutation [37]. The four most frequent mutations in the *inh*A gene that confer resistance to INH are Cys15Thr, Ala16Gly, Thr8Cys and Thr8Ala [25]. Mutations occurring in the *kat*G cause high-level resistance to INH, while those in the *inh*A cause low-level resistance [38].

Mutations occurring in the Quinolone Resistance Determining Region (QRDR) of both the *gyr*A and *gyr*B genes of resistant MTB strains cause resistance to the FLQs [39,40]. The most frequent mutations in the *gyr*A gene that confer resistance to the FLQs are Gly88Ala, Gly88Cys, Ala90Val, Ser91Pro, Asp94Ala, Asp94Asp, Asp94Tyr, Asp94Gly, and Asp94His. While those in the *gyr*B gene are Asp538Asp, Glu540Val [25]. It is worth noting that mutations in the *gyr*A gene confer a high-level resistance to FLQs, while those in the *gyr*B gene confer low-level resistance [41]. The frequency of mutations in the *gyr*A gene differ from one geographical location to another [41]. These differences are attributed to differences in treatment combinations containing FLQs used, and differences in geographic transmission environments for MTB [42]. Mutations in the *rrs*, *eis* and *tly*A genes cause resistance to the aminoglycosides and cyclic polypeptide antibiotics [40]. The most frequent mutations in the *rrs* gene are Ala1401Gly, Cys1402Thr and Gly1484Thr [25,39]. While those in the *eis* gene are Gly37Thr, Cys14Thr, Cys12Thr, Gly10Ala, Cys2Ala [25]. The *eis* (Cys14Thr) mutation is very specific for resistance to kanamycin than the *eis* (Gly10Cys) and *eis* (Cys12Thr) mutations [43].

The most effective strategy to halt the rise of DR-TB cases is early detection and effective treatment. Rapid molecular tools enable the early detection of DR-TB [44]. Line Probe Assays (LPAs) are molecular diagnostic assays that are used in detecting MTB and its resistance to anti-TB drugs [45]. The Genotype MTBDR*plus* assay is an LPA that is used for the qualitative identification of MTB and its resistance to RIF and INH, the key first-line anti-TB drugs [46]. While the Genotype MTBDR*sl* assay is used for the qualitative detection of MTB and its resistance to second-line anti-TB drugs [47]. These assays can use either clinical or cultivated specimens to identify MTB and its resistance [48]. LPAs also

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detect gene mutations in MTB that confer resistance to anti-TB drugs [25]. LPAs are recommended by the World Health Organization for diagnosis, guidance on empirical treatment and for surveillance of drug-resistant TB [15]. This study aimed to detect drug-resistant MTB in sputum specimens obtained from the new and previously treated TB cases.

MATERIALS AND METHODS

Study design and setting

This was a prospective laboratory-based, cross-sectional study that was carried out on TB positive sputum specimens from the new and previously treated TB cases. The study was conducted at the Chest Diseases Laboratory (CDL), in Lusaka, Zambia, from March 2020 to September 2020. CDL is a National Reference TB laboratory that is located at the National Institute for Scientific and Industrial Research (NISIR). CDL has the mandate to perform diagnosis and surveillance for both drug-susceptible and drug-resistant TB. The other key responsibilities for CDL are to conduct trainings in External Quality Assurance (EQA), biosafety, and diagnosis of TB. CDL performs EQA in TB with all provincial laboratories in the country as well as with the Supra-National TB Reference Laboratory in Kampala, Uganda. EQA is performed through blinded rechecking, proficiency testing, and on-site supervision. CDL is accredited by the Southern African Development Community Accreditation Services (SADCAS) of South-Africa.

Specimen processing

Sputum specimens were decontaminated and digested in a class II Biological Safety Cabinet (BSC II) using the N-Acetyl-L-Cysteine-Sodium Hydroxide (NALC-NaOH) method. Briefly, 0.25g of NALC (N-acetyl-L- cysteine) was added in clean falcon tubes, then 50 ml of 4% NaOH (Sodium Hydroxide) and 2.9 % $C_6H_5O_7$ (Citrate) mixture was added to the NALC. A physical examination of the sputum specimens was carried out and the volume of each sputum specimen in the falcon tube was recorded. An equal volume of the NALC-NaOH-citrate mixture was added to an equal volume of sputum, then allowed to stand for 15 minutes for digestion and decontamination of the sputum. 1 ml of phosphate buffer solution [pH 6.8] was added to each specimen mixture to neutralize the NaOH, dilute the homogenate and reduce its viscosity and specific gravity. The specimen mixtures were then centrifuged at 3,000 rpm for 20 minutes to concentrate the acid-fast bacilli. After discarding the supernatant into a disinfectant (5% phenol), the sediment was re-suspended in 3 ml phosphate buffer solution and used for fluorescent microscopy and line probe assay. Only confirmed MTB-positive sputum specimens were used for the detection of MTB resistance to the first-and second-line anti-TB drugs.

Detection of drug-resistant *Mycobacterium tuberculosis* complex

DNA extraction from MTB-positive sputum specimens was done using the GenoLyse kit (Hain Life Science, Germany) according to the manufacturer's instructions. Detection of drug-resistance genes in the DNA samples was achieved by two-line probe assays, Genotype MTBDR*plus* and MTBDR*sl* assays (Hain Lifescience, Germany), that utilise a Polymerase Chain Reaction (PCR)-based assay and a hybridization assay performed on an automated GT Blot 48 device (Hain Lifescience, Germany). Both assays were performed strictly according to the manufacturer's instructions. The Genotype MTBDR*plus* assay was used for detecting resistance against first-line ant-TB drugs, while the MTBDR*sl* assay was used for detecting resistance to second-line anti-TB drugs.

Statistical analysis

Data analysis was performed using STATA version 13.0 statistical software (StataCorp, Lakeway Drive, College Station, Texas, USA). Pearson's Chi-square test was used to find the association between the different types of drug-resistant TB to age and gender. A *p*-value of less than 0.05 was considered to be statistically significant at 95% confidence interval. Frequencies and percentages were used to determine the level of drug-resistant TB among the new and previously treated TB cases.

Ethics consideration

This was a laboratory-based study, with no direct contact with patients. Permission to conduct the study was obtained from the Zambia, Ministry of Health, as well the Lusaka Provincial Health Office and the Chest Diseases Laboratory in Lusaka, Zambia. Confidentiality was maintained using study-specific numbers to identify the samples. Integrity, professionalism, and Good Clinical Laboratory Practice (GCLP) standards were maintained throughout the study period. Ethics approval was sought from the University of Zambia, School of Health Sciences, Research and Ethics Committee (UNZA-HSREC) (Ethics Approval Number: 20203101001), while the final clearance and approval to conduct the study was obtained from the National Health Research Authority (NHRA).

RESULTS

Demographic characteristics of the study participants

In this study, a total of 329 sputum specimens were analysed, which translates to 329 cases for drug-resistance TB. The cases were categorized into two groups: the first group comprised 102 (31%) new TB cases, while the second group comprised 227 (69%) previously treated TB cases. Among the new TB cases, 80 (78.4%) were males and 22 (21.6%) females, with an age range of 1-90 years and a median age of 33 (Table 1).

In the previously treated TB cases, there were 166 (73.1%) males and 61 (26.9%) females, with an age range of

Table 1: Distribution of the new and previously treated TB cases by demographic characteristics.

New TB Cases (*N* = 102)			Previously Treated TB Cases (*N* = 227)		
Variables	**Number (n)**	**Percentage (%)**	**Variables**	**Number (n)**	**Percentage (%)**
Gender			**Gender**		
Females	22	21.6	**Females**	60	26.4
Males	80	78.4	**Males**	167	73.6
Age (years)			**Age (years)**		
1 - 19	5	4.9	**4 - 20**	8	3.5
20 - 39	65	63.7	**21 - 37**	128	56.4
40 - 59	26	25.5	**38 - 54**	70	30.8
60 - 79	5	4.9	**55 - 71**	17	7.5
80 - 99	1	1.0	**72 - 88**	4	1.8

Abbreviations: N: Total Number of Cases; n: Number of Cases; TB: Tuberculosis; Percentage = (n/N) x 100%.

4 -78 years (median age 34 years) (Table 1). The previously treated TB cases comprised treatment failure cases, lost to follow-up cases, relapse cases and defaulter cases. The classification of TB patients into the new and previously treated TB cases is important for surveillance of drug-resistance and treatment. The type of treatment for the new and previously treated TB cases differs, thus a great need for categorization of these cases.

Resistance profiles for first-and second-line anti-TB drugs

The prevalence of the different forms of drug-resistant TB among the new TB cases was as follows: 3.9% had rifampicin mono-resistance, 12.8% isoniazid mono-resistance, and 17.7% had both rifampicin and isoniazid resistance (Table 2). Drug-resistant TB was more prevalent in male cases than female cases, and the age range most affected with drug-resistance was 20-39 years among the new TB cases (Tables 2 & 3).

Table 2: Prevalence of drug-resistance among the new and previously treated TB cases.

Type of drug-resistance	New TB cases (*N* = 102) No. of cases (n) (%)	Previously tretaed TB cases (*N* = 227) No. of cases (n) (%)
Susceptible	67 (65.7%)	108 (47.6%)
Monodrug- resistance		
RIF mono-resistance	4 (3.9%)	23 (10.1%)
INH mono-resistance	13 (12.8%)	15 (6.6%)
Multidrug- resistance		
RIF + INH	18 (17.7%)	75 (33.0%)
Polydrug- resistance		
RIF + FLQs	0 (0%)	4 (1.8%)
Pre-extensively drug resistance		
RIF + INH + FLQs	0 (0%)	1 (0.4%)
RIF + INH + KAN	0 (0%)	1 (0.4%)

Abbreviations: N: Total number of cases; No: Number; RIF: Rifampicin; INH: Isoniazid; FLQs: Fluoroquinolones; KAN: Kanamycin; TB: Tuberculosis; Prevalence = (n/N) x 100%.

Table 3: Association between the different types of drug-resistant TB to demographic variables among the new TB cases.

Demographic variables	Mono-drug-resistance (*N* = 17)		Multidrug-resistance (*N* = 18)
	RIF (*N* = 4) n (%)	INH (*N* = 13) n (%)	RIF + INH n (%)
Gender Females	1 (25%)	2 (15.4%)	2 (11.1%)
Males	3 (75%)	11 (84.6%)	16 (88.9%)
Pearson's Chi-square (X^2)- value	1.4165	2.0583	1.8915
p-value	0.493	0.357	0.388
Age (years)			
1-19	0 (0%)	0 (0%)	1 (5.6%)
20-39	2 (50%)	11 (84.6%)	14 (77.8%)
40-59	1 (25%)	2 (15.4%)	3 (16.7%)
60-79	1 (25%)	0 (0%)	0 (0%)
80-99	0 (0%)	0 (0%)	0 (0%)
Pearson's Chi-square (X^2)- value	6.0982	6.9422	5.5664
p-value	0.636	0.543	0.696

Abbreviations: N: Total number of resistant cases; n: Number of resistant cases; RIF: Rifampicin; INH: Isoniazid; TB: Tuberculosis; Percentage = (n/N) X 100%; $p < 0.05$ statistically significant; $p > 0.05$ not statistically significant.

The prevalence of the different forms of drug-resistant TB among the previously treated TB cases was as follows: 10.1% had rifampicin mono-resistance, 6.6% isoniazid mono-resistance, 33.0% had both rifampicin and isoniazid resistance, 1.8% had poly-drug resistance, and finally, 0.8% had pre-extensively drug-resistant TB (Tables 2 & 4). The prevalence of drug-resistant TB was more common in males than in females. The age range most affected with drug resistance TB was 21-37 years among the previously treated TB cases (Table 4).

Association between drug-resistant TB types to age and gender among the TB cases

No association was found between the different types

Table 4: Association between the different types of drug-resistant TB to demographic variables among the previously treated TB cases.

Demographic variables	Monodrug-resistance (*N* = 38)		MDR (*N* = 75)	Poly-drug resistance (*N* = 4)	Pre-XDR (*N* = 1)	Pre-XDR (*N* = 1)
	RIF (*N* = 23) n (%)	INH (*N* = 15) n (%)	RIF + INH n (%)	RIF + FLQs n (%)	RIF + INH + FLQs n (%)	RIF + INH + KAN n (%)
Gender Females	6 (26.1%)	5 (33.3%)	23 (30.7%)	1 (25%)	1 (100%)	0 (0%)
Males	17 (73.9%)	10 (66.7%)	52 (69.3%)	3 (75%)	0 (0%)	1 (100%)
Pearson's Chi-square (X^2) value	4.3554	5.0371	1.0331	0.0043	2.7956	0.3609
p- value	0.499	0.284	0.309	0.948	0.095	0.548
Age (years)						
4 - 20	1 (4.3%)	0 (0%)	2 (2.7%)	0 (0%)	0 (0%)	0 (0%)
21 - 37	18 (78.3%)	9 (60%)	45 (60%)	1 (25%)	1 (100%)	0 (0%)
38 - 54	4 (17.4%)	4 (26.7%)	22 (29.3%)	2 (50%)	0 (0%)	0 (0%)
55 - 71	0 (0%)	2 (13.3%)	6 (8.0%)	1 (25%)	0 (0%)	1 (100%)
72 - 88	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Pearson's Chi-square (X^2) value	26.0315	18.5882	2.5878	3.0787	0.7769	12.4076
p- value	0.165	0.291	0.629	0.545	0.942	0.125

Abbreviations: N: Total number of resistant cases; n: number of resistant cases; MDR: Multidrug-Resistance; Pre-XDR: pre-extensively drug resistance; RIF: Rifampicin; INH: Isoniazid; FLQs: Fluoroquinolones; KAN: Kanamycin; Percentage = (n/N) x 100%; $p < 0.05$ statistically significant; *p-value* > 0.05 not statistically significant.

of drug-resistant TB to age and gender among the new or previously treated TB cases. All the p-values were above the significant level of 0.05 at 95% confidence interval (Tables 3 and 4).

Frequency of mutations conferring drug-resistance in M. tuberculosis resistant strains

Mutations were detected in the *rpo*B, *kat*G, *Inh*A, *gyr*A, and *eis* genes of MTB resistant strains. Overall, 34.3% of the cases had mutations detected among the new TB cases and 52.4% among the previously treated TB cases.

Mutations conferring resistance to Rifampicin

From a total of 4 RIF mono-resistant TB cases detected among the new TB cases, the most frequent mutation conferring resistance to RIF was the *rpo*B MUT 3 (Ser531Leu), with a frequency of 75% (Table 5). Among the previously treated TB cases, a total of 23 RIF mono-resistant cases were detected, and the most frequent mutation was the *rpo*B MUT 3 (Ser531Leu), which had a frequency of 56.5% (Table 6).

Mutations conferring resistance to isoniazid

From a total of 13 INH mono-resistant TB cases detected among the new TB cases, the most frequent mutation conferring resistance to INH was the *Inh*A MUT 1 (Cys15Thr), with a frequency of 76.9% (Table 5), while a total of 15 INH mono-resistance cases were detected among the previously treated TB cases, with the most frequent mutation being *kat*G MUT 1 (Ser315Thr1), 53.3% (Table 6). MTB resistance to INH was detected in the *inh*A and/or *kat*G gene(s).

Mutations conferring resistance to both rifampicin and isoniazid

From a total of 18 multi-drug resistance cases detected among the new TB cases, the most frequent mutation conferring resistance to both RIF and INH was a combination of *rpo*B MUT 3 (Ser531Leu) and *kat*G MUT 1(Ser315Thr 1), with a frequency of 27.8% (Table 5). From a total of 75 multi-drug resistant cases detected among the previously treated TB cases, the most frequently detected mutation was a combination of *rpo*B MUT 3 (Ser531Leu) and *kat*G MUT 1 (Ser315Thr 1), with a frequency of 29.3% (Table 6).

Mutations conferring resistance to both rifampicin and fluoroquinolones

From a total of 4 poly-drug resistant TB cases detected among the previously treated TB cases, the most frequent mutation conferring resistance to both RIF and fluoroquinolones was a combination of the *rpo*B MUT 2A (His526Tyr) and *gyr*A MUT 1(Ala90Val), with a frequency of 50% (Table 6).

Mutations conferring resistance to both rifampicin and isoniazid plus fluoroquinolones or kanamycin

Two pre-extensively drug-resistant cases were detected among the previously treated TB cases. One of the cases had a mutation profile of *rpo*B MUT 2B (His526Asp), *kat*G MUT 2 (Ser315Thr 2), and *eis* MUT 1 (Cys14Thr). While the other case had a mutation profile of *rpo*B MUT 2A (His526Tyr), *kat*G MUT 1 (Ser315Thr), and *gyr*A MUT 3C (Asp94Gly). Each of these profiles had a frequency of 50% (Table 6).

Table 5: Frequency of mutations detected in *M. tuberculosis* resistant isolates among the new TB cases.

Genes implicated in drug-resistance	Genotypic profile of drug-resistance	Mutations detected	No. of MTB isolates	Frequency (%)
	Mono drug resistance (*n* = 17)			
	Rifampicin (*n* = 4)			
***rpo*B**	*rpo*B MUT 1	Asp516Val	1	1/4 (25%)
	***rpo*B MUT 3/Δ *rpo*B WT 8**	**Ser531Leu**	**3**	**3/4 (75%)**
	Isoniazid (*n* = 13)			
***Inh*A *or kat*G**	***Inh*A MUT 1/Δ *Inh*A WT 1**	**Cys15Thr**	**10**	**10/13 (76.9%)**
	*kat*G MUT 1	Ser315Thr 1	3	3/13 (23.1%)
	Multidrug-resistance (*n* = 18)			
***rpo*B + *kat*G *or Inh*A**	Δ *rpo*B WT 8 Δ *kat*G WT	Ser531Leu C-315	1	1/18 (5.6%)
	***rpo*B MUT 3/Δ *rpo*B WT 8** ***kat*G MUT 1/Δ *kat*G WT**	**Ser531Leu** **Ser315Thr 1**	**5**	**5/18 (27.8%)**
	Δ *rpo*B WT 2 *Inh*A MUT 1	Glu510His Cys15Thr	1	1/18 (5.6%)
	*rpo*B MUT 3 *Inh*A MUT 1	Ser531Leu Cys15Thr	1	1/18 (5.6%)
	Δ *rpo*B WT 2 *Inh*A MUT 1/Δ *Inh*A WT 1	Glu510His Cys15Thr	1	1/18 (5.6%)
	*rpo*B MUT 2B/Δ *rpo*B WT 7 *kat*G MUT 1/Δ *kat*G WT	His526Asp Ser315Thr 1	1	1/18 (5.6%)
	Δ *rpo*B WT 3/Δ *rpo*B WT 4 *kat*G MUT 1/Δ *kat*G WT	Asp516Val Ser315Thr 1	2	2/18 (11.1%)
	Δ *rpo*B WT 1 Δ *rpo*B WT 2 Δ *rpo*B WT 8 *Inh*A MUT 1/Δ *Inh*A WT 1	C 505-509 Glu510His Ser531Leu Cys15Thr	1	1/18 (5.6%)
	Δ *rpo*B WT 8 *kat*G MUT 2	Ser531Leu Ser315Thr 2	1	1/18 (5.6%)
	*rpo*B MUT 2B *rpo*B MUT 3 *kat*G MUT 1	His526Asp Ser531Leu Ser315Thr 1	1	1/18 (5.6%)
	*rpo*B MUT 2B *kat*G MUT 1	His526Asp Ser315Thr 1	1	1/18 (5.6%)
	*rpo*B MUT 2A /Δ *rpo*B WT 7 *kat*G MUT 1/Δ *kat*G WT	His526Tyr Ser315Thr 1	1	1/18 (5.6%)
	*rpo*B MUT 3/Δ *rpo*B WT 8 *Inh*A MUT 1/Δ *Inh*A WT 1	Ser531Leu Cys15Thr	1	1/18 (5.6%)

Abbreviations: Δ: deletion; WT: Wild Type; MUT: Mutation; ΔWT/MUT: deletion of the wild-type probe and presence of mutation; C: Codon; TB: Tuberculosis; MTB: *Mycobacterium tuberculosis*; Ala: Alanine; Asp: Aspartic acid; Cys: Cysteine; Glu: Glutamic acid; Gly: Glycine; His: Histidine; Leu: Leucine; Ser: Serine; Thr: Threonine; Tyr: Tyrosine; Val: Valine. Mutations with the highest frequency are bold in black.

DISCUSSION

This study highlights the growing threat posed by the emergence of resistant MTB strains responsible for causing the different types of drug-resistant TB in Zambia. The majority of patients identified with drug-resistant TB had a history of being treated for TB previously and interrupted first-line anti-TB therapy. These factors are predictors of the emergence of drug-resistant TB [49-51]. In this study, drug-resistant-TB was found to be high among the previously treated TB patients than among the new TB patients. Drug-resistant TB was high among adult males than females, because of social stigma, cultural habits and poor health-seeking behaviour among males [52].

The prevalence of Rifampicin Resistant (RR)-TB in our study was 3.9% among the new TB cases and 10.1% among the previously treated TB cases. These findings are consistent with the findings reported elsewhere. A previous study conducted in Zambia reported a prevalence for RR at 5.9% among the previously treated TB cases [20]. A similar study conducted in Zimbabwe found the prevalence of RR to be 4.0% among the new TB cases and 14.2% among the previously treated TB cases [53], while a South-African found the burden of RR to be 8.8% among the previously treated TB cases [54]. In Burkina Faso, the prevalence of RR was found at 2.0% and 14.5% among the new and previously treated TB cases, respectively [55]. RR-TB is associated with a history of being treated for TB previously. Therefore, improvement in adherence to treatment halts the emergence or re-emergence of MTB and RR-TB cases [56]. The differences in the reported prevalence for RR-TB from other countries can

Table 6: Frequency of mutations detected in M. tuberculosis resistant isolates among the previously treated TB cases.

Genes implicated in drug-resistance	Genotypic profile of drug-resistance	Mutations detected	No. of MTB isolates	Frequency (%)
	Mono drug resistance (*n* = 38)			
***rpo*B**	**Rifampicin (*n* = 23)**			
	***rpo*B MUT 3/Δ *rpo*B WT 8**	**Ser531Leu**	**13**	**13/23 (56.5%)**
***Inh*A *and/or kat*G**	**Isoniazid (*n* = 15)**			
	*Inh*A MUT 1/Δ *Inh*A WT 1	Cys15Thr	5	5/15 (33.3%)
	***kat*G MUT 1/Δ *kat*G WT**	**Ser315Thr 1**	**8**	**8/15 (53.3%)**
	*kat*G MUT1 *Inh*A MUT 1/Δ *Inh*A WT 1	Ser315Thr 1 Cys15Thr	1	1/15(6.7%)
	Multidrug-resistance (*n* = 75)			
***rpo*B + *kat*G &/or *Inh*A**	***rpo*B MUT 3/Δ *rpo*B WT 8 *kat*G MUT 1/Δ *kat*G WT**	**Ser531Leu Ser315Thr 1**	**22**	**22/75 (29.3%)**
	*rpo*B MUT 2B/Δ *rpo*B WT 7 *kat*G MUT1/Δ *kat*G WT	His526Asp Ser315Thr 1	20	20/75 (26.7%)
	*rpo*B MUT 1 *kat*G MUT1/Δ *kat*G WT	Asp516Val Ser315Thr 1	1	1/75 (1.3%)
	*rpo*B MUT 2B *rpo*B MUT 3 *kat*G MUT1 *Inh*A MUT 1	His526Asp Ser531Leu Ser315Thr 1 Cys15Thr	1	1/75 (1.3%)
	*rpo*B MUT 2A *kat*G MUT1/Δ *kat*G WT	His526Tyr Ser315Thr 1	1	1/75 (1.3%)
	*rpo*B MUT 1 Δ *rpo*B WT 3/Δ *rpo*B WT 4 *kat*G MUT 2/Δ *kat*G WT	Asp516Val Ser315Thr 2	1	1/75 (1.3%)
	*rpo*B MUT 3/Δ *rpo*B WT 8 *Inh*A MUT 1/Δ *Inh*A WT 1	Ser531Leu Cys15Thr	2	2/75 (2.7%)
	*rpo*B MUT 2B *Inh*A MUT 1	His526Asp Cys15Thr	1	1/75 (1.3%)
	Poly drug- resistance (*n* = 4)			
***rpo*B + *gyr*A**	*rpo*B MUT 2 A Δ *gyr*A WT 2 Δ *gyr*A WT 3	His526Tyr C 89-93 C 92-96	1	1/4 (25)
	***rpo*B MUT 2 A/Δ *rpo*B WT 7 *gyr*A MUT 1/Δ *gyr*A WT 2**	**His526Tyr Ala90Val**	**2**	**2/4 (50%)**
	*rpo*B MUT 2 A/Δ *rpo*B WT 7 Δ *gyr*A WT 2	His526Tyr C 89-93	1	1/4 (25)
	Pre- extensively drug-resistance (n=2)			
***rpo*B + *kat*G + *gyr*A**	*rpo*B MUT 2A/Δ *rpo*B WT 7 *kat*G MUT 1 /Δ *kat*G WT *gyr*A MUT 3C/Δ *gyr* A WT 3	His526Tyr Ser315Thr 1 Asp94Gly	1	1/2 (50%)
rpo*B + *kat*G + *eis	*rpo*B MUT 2B/Δ *rpo*B WT 7 *kat*G MUT 2/Δ *katG* WT *eis* MUT 1	His526Asp Ser315Thr 2 Cys14Thr	1	1/2 (50%)

Abbreviations: Δ deletion; WT: Wild Type; MUT: Mutation; ΔWT/MUT: deletion of the wild-type probe and presence of mutation; C: Codon; TB: Tuberculosis; MTB: *Mycobacterium Tuberculosis*; Ala: Alanine; Asp: Aspartic acid; Cys: Cysteine; Glu: Glutamic acid; Gly: Glycine; His: Histidine; Leu: Leucine; Ser: Serine; Thr: Threonine; Tyr: Tyrosine; Val: Valine. Mutations with the highest frequency are bold in black.

be attributed to differences in the sample size used, study settings, study design, the method employed, differences in socio-economic factors, differences in case management of drug-resistant TB, and many other factors.

The prevalence of Isoniazid Mono-Resistance (INHr)-TB in our study was 12.8% and 6.6% among the new and previously treated TB cases, respectively. These findings are consistent with other regions of the world. A low prevalence of INHr among the new TB cases was reported in Tanzania (7.6%) [57], and Ethiopia (9.5%) [58]. Similar studies in South-Korea and Pakistan reported 11% and 9.8%, respectively, for INHr-TB among new TB cases [59,60]. INHr is more common than RR and is a growing public health problem globally because research and policy directions are only focused on RR as a marker for MDR-TB. However, this growing problem of INHr is being ignored globally [61].

MDR-TB is one of the most serious public health problems in the world [55,62]. Its prevalence is increasing in every part of the world today, for both new and previously treated TB cases [63]. In the current study, the prevalence of MDR-TB was high, 17.7% among the new TB cases and 33.0% among the previously treated TB cases. These

findings were consistent with those reported in India in which the prevalence of MDR-TB was found to be 11.4% and 36.4% among the new and the previously treated TB cases, respectively [64]. Another study conducted in Ethiopia reported 11.6% and 32.7% MDR prevalence among the new and previously treated cases, respectively [65], while that in China reported the prevalence of MDR-TB at 30.4% among the previously treated cases [66]. MDR-TB is a growing public health threat in Zambia, and the WHO has projected that by 2021 the country will have a high burden of the disease [23,24]. DR-TB is not caused by a single factor, but by several factors which come into play and some of these include: poor adherence to TB treatment, irregular supply and stock-out of anti-TB drugs, inappropriate TB therapy, delayed diagnosis and initiation of ineffective therapy as well as spontaneous chromosomal mutations [1,30,33,58]. Liang and colleagues in their study found that inappropriate treatment was the most common cause of MDR-TB [66]. Failure to implement effective TB prevention and control measures also facilitates the emergence of the different forms of DR-TB including MDR-TB [67-69]. The key factors above including treatment failure, TB relapse, treatment defaulting, MDR contacts, loss to follow-up cases, and misdiagnosis could be contributing to the reasons for the observed rise in the number of MDR-TB cases in Zambia. MDR-TB must be managed effectively to reduce mortality, morbidity, and the eventual transmission of MTB resistant strains [70].

In this study, the prevalence of poly drug-resistant TB was found to be low (1.8%) among the previously treated TB cases. This finding was similar to studies conducted in Ethiopia (2.3%) and Canada (0.4%) [71,72]. However, studies elsewhere reported a high prevalence of poly drug-resistance among the previously treated: India reported 12.0% prevalence [64], Bangladesh 8.6% [72] and Sudan 6.4% [72]. Poor case management of mono-drug resistant TB results in poly drug-resistant TB.

The prevalence of pre-XDR TB in our study was low (0.8%) among the previously treated TB cases. This result is similar to that reported in Ethiopia, 5.7% [73]. Studies elsewhere reported a high prevalence of pre-XDR: India (56%), China (34%), Nepal (28%), Zimbabwe (27%, Nigeria (17%) and Bangladesh (16.2%) [73-75]. The high prevalence of pre-XDR TB can indicate poor management of MDR-TB cases. Poorly managed MDR-TB cases progress to pre-XDR TB, which eventually get to XDR-TB.

In this study, no association was found between the different types of Drug-Resistance (DR) to age and gender among the new and previously treated TB cases. This finding was similar to other countries such as Sudan [76], Nigeria [77], Ethiopia [78], and Egypt [79]. Age and gender were not associated with drug-resistance because these variables are not significant risk factors. Studies have shown that the key risk factors associated with the development of drug-resistant TB include: history of previous treatment for TB, poor adherence to TB treatment, inadequate supply and stock-out of anti-TB drugs, poor management of TB, and contact with DR-TB patients [30,33,58,78-79].

Drug-resistant MTB strains use various complex mechanisms to inactive or resist anti-TB drugs. Among these are point mutations in chromosomal genes, which can be an insertion, deletion or missense [80-82]. Molecular assays detect mutations that confer resistance to anti-TB drugs. In this study the *rpo*B MUT 3 (Ser531Leu) mutation was the most frequently detected (75%), conferring resistance to rifampicin, among the new TB cases. This result is similar to that reported in Brazil (75%) [83], and in Ethiopia (74.2% and 77.1%) [84,85]. In contrast, other studies elsewhere reported a low frequency of the mutation, with Canada reporting 47.8% [86], India reporting 46.9% and 19.5% in two separate studies [82,87], and Uganda reporting 40% [37]. Based on these results it suffices to say, mutation frequencies vary from one geographical location to another due to differences in the epidemiology of TB, differences in geographic transmission environments for MTB, as well as differences in treatment combinations used in the management of cases.

The *kat*G MUT 1 (Ser315Thr 1) mutation was the most frequently (53.3%) encountered mutation conferring resistance to INH among the previously treated TB cases in this current study. This finding is consistent with studies in Taiwan (50.4%), Myanmar (57.3%) and India (57.8%) [1,88]. Studies have shown that 50-95% of MTB resistant strains have mutations in the *kat*G gene [44]. A high frequency of the *kat*G Ser315Thr 1 mutation is associated with countries with a high prevalence of TB, such as Zambia [44]. The *Inh*A MUT1 (Cys15Thr) mutation was also frequently (76.9%) detected among the new TB cases in this current study and this finding is consistent with findings from similar studies in other countries such as Ethiopia (77.5%) [85], South-Africa (70.1%) [89], and India (85.9%) [1]. It has been shown that the Ser315Thr 1 mutation is the most frequent in the *kat*G gene that confers resistance to INH, while the Cys15Thr mutation is the most frequent in the *Inh*A gene that confer resistance to INH [90].

Mutations conferring resistance to both RIF and INH were detected in our study. These mutations are responsible for causing MDR-TB. The *rpo*B MUT 3 (Ser531Leu) and the *kat*G MUT1 (Ser315Thr 1) mutations were the most frequent (27.8%) conferring resistance to both RIF and INH, among the new TB cases. This same mutation was the most frequent among the previously treated TB cases. These findings are similar to results obtained in Thailand (36.4%) [25].

This study also detected other mutations such as the *rpo*B MUT 2A (His526Tyr) and *gyr*A MUT 1 (Ala90Val) with a frequency of 50%, that confer resistance to both RIF and FLQs among the previously treated TB cases and was consistent

with findings reported in Ethiopia where the mutational frequency was found to be 50% [65]. The Ala90Val mutation is associated with high-level resistance to FLQs [41]. Studies have shown that most mutations associated with FLQ resistance occur in the *gyr*A gene on codon 90-94 of MTB resistant strains [65]. This is in agreement with our findings. Misuse of FLQ antibiotics has contributed to resistance in MTB strains, associated with mutations in the *gyr*A gene [41,42].

In this study other multiple mutations detected were associated with resistance in the *rpo*B, *kat*G, *gyr*A and *eis* genes. These combined mutation profiles were responsible for causing pre-XDR TB and each had a frequency of 50%. One of these combination mutations had a profile: *rpo*B MUT 2B (His526Asp), *kat*G MUT 2 (Ser315Thr 2), and *eis* MUT 1 (Cys14Thr). The other had *rpo*B MUT 2A (His526Tyr), *kat*G MUT 1 (Ser315Thr), and *gyr*A MUT 3C (Asp94Gly). Unlike the high frequency of these mutations in this study, studies elsewhere, such as Morocco and India, reported low mutational frequency in these genes [3,91]. Mismanagement of DR-TB cases result in resistance associated with multiple mutations in MTB genes.

CONCLUSION

Drug-resistant TB is prevalent in Zambia, especially MDR-TB, is hampering efforts in the control of TB. DR-TB is mainly caused by mutations in the target genes of resistant MTB strains. Mutations identified in this study were the *rpo*B (Ser531Leu, His526Asp, Asp516Val, His526Tyr, and Glu510His), *Inh*A (Cys15Thr), *gyr*A (Ala90Val and Asp94Gly), *kat*G (Ser315Thr 1 and Ser315Thr 2), and *eis* (Cys14Thr), each with a varying frequency. Zambia needs to scale-up on the number of laboratory sites performing genotypic drug-susceptibility testing, effectively treat DR-TB, especially MDR-TB, and implement effective TB control strategies to combat DR-TB.

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References

1. Polu GP, Mohammad Shaik J, Kota NMK, Karumanchi D, Allam US. Analysis of drug resistance mutations in pulmonary Mycobacterium tuberculosis isolates in the Southern coastal region of Andhra Pradesh, India. Braz J Infect Dis. 2019 Sep-Oct;23(5):281-290. doi: 10.1016/j.bjid.2019.07.002. Epub 2019 Aug 14. PMID: 31421108.

2. Kar SS, Bhat VG, Shenoy VP, Bairy I, Shenoy GG. Design, synthesis, and evaluation of novel diphenyl ether derivatives against drug-susceptible and drug-resistant strains of Mycobacterium tuberculosis. Chem Biol Drug Des. 2019 Jan;93(1):60-66. doi: 10.1111/cbdd.13379. Epub 2018 Nov 28. PMID: 30118192.

3. Oudghiri A, Karimi H, Chetioui F, Zakham F, Bourkadi JE, Elmessaoudi MD, Laglaoui A, Chaoui I, El Mzibri M. Molecular characterization of mutations associated with resistance to second-line tuberculosis drug among multidrug-resistant tuberculosis patients from high prevalence tuberculosis city in Morocco. BMC Infect Dis. 2018 Feb 27;18(1):98. doi: 10.1186/s12879-018-3009-9. PMID: 29486710; PMCID: PMC5830342.

4. Baya B, Achenbach CJ, Kone B, Toloba Y, Dabitao DK, Diarra B, Goita D, Diabaté S, Maiga M, Soumare D, Ouattara K, Kanoute T, Berthe G, Kamia YM, Sarro YDS, Sanogo M, Togo ACG, Dembele BPP, Coulibaly N, Kone A, Akanbi M, Belson M, Dao S, Orsega S, Siddiqui S, Doumbia S, Murphy RL, Diallo S. Clinical risk factors associated with multidrug-resistant tuberculosis (MDR-TB) in Mali. Int J Infect Dis. 2019 Apr;81:149-155. doi: 10.1016/j.ijid.2019.02.004. Epub 2019 Feb 14. PMID: 30772470; PMCID: PMC6481646.

5. Ukwamedua H, Omote V, Etaghene J, Oseji ME, Agwai IC, Agbroko H. Rifampicin resistance among notified pulmonary tuberculosis (PTB) cases in South-Southern Nigeria. Heliyon. 2019 Jul 16;5(7):e02096. doi: 10.1016/j.heliyon.2019.e02096. PMID: 31360790; PMCID: PMC6639537.

6. Guler SA, Bozkus F, Inci MF, Kokoglu OF, Ucmak H, Ozden S, Yuksel M. Evaluation of pulmonary and extrapulmonary tuberculosis in immunocompetent adults: a retrospective case series analysis. Med Princ Pract. 2015;24(1):75-9. doi: 10.1159/000365511. Epub 2014 Oct 17. PMID: 25341702; PMCID: PMC5588178.

7. Sakamoto H, Lee S, Ishizuka A, Hinoshita E, Hori H, Ishibashi N, Komada K, Norizuki M, Katsuma Y, Akashi H, Shibuya K. Challenges and opportunities for eliminating tuberculosis - leveraging political momentum of the UN high-level meeting on tuberculosis. BMC Public Health. 2019 Jan 16;19(1):76. doi: 10.1186/s12889-019-6399-8. PMID: 30651096; PMCID: PMC6335677.

8. World Health Organization (WHO). Global tubeculosis report. Geneva, Switzerland. 2020;9-11. https://bit.ly/3w83swW

9. Li D, Song Y, Zhang CL, Li X, Xia X, Zhang AM. Screening mutations in drug-resistant Mycobacterium tuberculosis strains in Yunnan, China. J Infect Public Health. 2017 Sep-Oct;10(5):630-636. doi: 10.1016/j.jiph.2017.04.008. Epub 2017 Jun 13. PMID: 28623123.

10. Pholwat S, Stroup S, Heysell S, Ogarkov O, Zhdanova S, Ramakrishnan G, Houpt E. eis Promoter C14G and C15G Mutations Do Not Confer Kanamycin Resistance in Mycobacterium tuberculosis. Antimicrob Agents Chemother. 2016 Nov 21;60(12):7522-7523. doi: 10.1128/AAC.01775-16. PMID: 27736762; PMCID: PMC5119041.

11. Nguyen TNA, Anton-Le Berre V, Bañuls AL, Nguyen TVA. Molecular Diagnosis of Drug-Resistant Tuberculosis; A Literature Review. Front Microbiol. 2019 Apr 16;10:794. doi: 10.3389/fmicb.2019.00794. PMID: 31057511; PMCID: PMC6477542.

12. World Health Organization (WHO). Global tuberculosis report. Geneva, Switzerland. 2019;1- 4. https://bit.ly/3sph2tA

13. Takawira FT, Mandishora RSD, Dhlamini Z, Munemo E, Stray-Pedersen B. Mutations in rpoB and katG genes of multidrug resistant Mycobacterium tuberculosis undetectable using genotyping diagnostic methods. Pan Afr Med J. 2017 Jun 28;27:145. doi: 10.11604/pamj.2017.27.145.10883. PMID: 28904673; PMCID: PMC5567934.

14. Mekonnen F, Tessema B, Moges F, Gelaw A, Eshetie S, Kumera G. Multidrug resistant tuberculosis: prevalence and risk factors in districts of metema and west armachiho, Northwest Ethiopia. BMC Infect Dis. 2015 Oct 26;15:461. doi: 10.1186/s12879-015-1202-7. PMID: 26503269; PMCID: PMC4624367.

15. Namburete EI, Tivane I, Lisboa M, Passeri M, Pocente R, Ferro JJ, Harrison LH, Bollela VR. Drug-resistant tuberculosis in Central Mozambique: the role of a rapid genotypic susceptibility testing. BMC Infect Dis. 2016 Aug 17;16:423. doi: 10.1186/s12879-016-1766-x. PMID: 27534745; PMCID: PMC4989517.

16. Mulenga C, Chonde A, Bwalya IC, Kapata N, Kakungu-Simpungwe M, Docx S, Fissette K, Shamputa IC, Portaels F, Rigouts L. Low Occurrence of Tuberculosis Drug Resistance among Pulmonary Tuberculosis Patients from an Urban Setting, with a Long-Running DOTS Program in Zambia. Tuberc Res Treat. 2010;2010:938178. doi: 10.1155/2010/938178. Epub 2010 Jun 30. PMID: 22567261; PMCID: PMC3335559.

17. Malama S, Muma JB, Godfroid J. A review of tuberculosis at the wildlife-livestock-human interface in Zambia. Infect Dis Poverty. 2013 Jul 9;2(1):13. doi: 10.1186/2049-9957-2-13. PMID: 23849550; PMCID: PMC3734204.

18. Kapata N, Chanda P, Michelo C. The social determinants of tuberculosis and their association with TB/ HIV co-infection in Lusaka. Medical Journal of Zambia. 2013;40(2):1-7. https://bit.ly/3rfYScl

19. Monde N, Munyeme M, Malama S. A review of tuberculosis in Ndola District of Zambia. Journal of Tuberculosis Research. 2016;4:1-8. doi: 10.4236/jtr.2016.41001

20. Masenga SK, Mubila H, Hamooya BM. Rifampicin resistance in Mycobacterium tuberculosis patients using GeneXpert at Livingstone Central Hospital for the year 2015: a cross sectional explorative study. BMC Infect Dis. 2017 Sep 22;17(1):640. doi: 10.1186/s12879-017-2750-9. PMID: 28938874; PMCID: PMC5610453.

21. Munthali T, Chabala C, Chama E, Mugode R, Kapata N, Musonda P, Michelo C. Tuberculosis caseload in children with severe acute malnutrition related with high hospital based mortality in Lusaka, Zambia. BMC Res Notes. 2017 Jun 12;10(1):206. doi: 10.1186/s13104-017-2529-5. PMID: 28606173; PMCID: PMC5468953.

22. Ismail N, Ismail F, Omar SV, Blows L, Gardee Y, Koornhof H, Onyebujoh PC. Drug resistant tuberculosis in Africa: Current status, gaps and opportunities. Afr J Lab Med. 2018 Dec 6;7(2):781. doi: 10.4102/ajlm.v7i2.781. PMID: 30568900; PMCID: PMC6295755.

23. Kasapo CC, Chimzizi R, Simwanza SC, Mzyece J, Chizema E, Mariandyshev A, Lee HY, Harries AD, Kapata N. What happened to patients with RMP-resistant/MDR-TB in Zambia reported as lost to follow-up from 2011 to 2014? Int J Tuberc Lung Dis. 2017 Aug 1;21(8):887-893. doi: 10.5588/ijtld.16.0933. PMID: 28786797.

24. Ministry of Health (MOH). National tuberculosis and leprosy control report, Lusaka, Zambia. 2017;3:12-13. https://bit.ly/3w0LUT9

25. Charoenpak R, Santimaleeworagun W, Suwanpimolkul G, Manosuthi W, Kongsanan P, Petsong S, Puttilerpong C. Association Between the Phenotype and Genotype of Isoniazid Resistance Among Mycobacterium tuberculosis Isolates in Thailand. Infect Drug Resist. 2020 Feb 24;13:627-634. doi: 10.2147/IDR.S242261. PMID: 32158238; PMCID: PMC7047971.

26. Jabeen K, Shakoor S, Hasan R. Fluoroquinolone-resistant tuberculosis: implications in settings with weak healthcare systems. Int J Infect Dis. 2015 Mar;32:118-23. doi: 10.1016/j.ijid.2015.01.006. PMID: 25809767.

27. Tiberi S, Scardigli A, Centis R, D'Ambrosio L, Muñoz-Torrico M, Salazar-Lezama MÁ, Spanevello A, Visca D, Zumla A, Migliori GB, Caminero Luna JA. Classifying new anti-tuberculosis drugs: rationale and future perspectives. Int J Infect Dis. 2017 Mar;56:181-184. doi: 10.1016/j.ijid.2016.10.026. Epub 2016 Nov 3. PMID: 27818361.

28. Mamatha HG, Shanthi V. Baseline resistance and cross-resistance among fluoroquinolones in multidrug-resistant Mycobacterium tuberculosis isolates at a national reference laboratory in India. J Glob Antimicrob Resist. 2018 Mar;12:5-10. doi: 10.1016/j.jgar.2017.08.014. Epub 2017 Sep 5. PMID: 28887289.

29. Muzondiwa D, Mutshembele A, Pierneef RE, Reva ON. Resistance Sniffer: An online tool for prediction of drug resistance patterns of Mycobacterium tuberculosis isolates using next generation sequencing data. Int J Med Microbiol. 2020 Feb;310(2):151399. doi: 10.1016/j.ijmm.2020.151399. Epub 2020 Jan 17. PMID: 31980371.

30. Iacobino A, Fattorini L, Giannoni F. Drug-Resistant Tuberculosis 2020 : Where We Stand. Journal of Applied Sciences. 2020;10:1-17. https://bit.ly/3tW6FgT

31. Migliori GB, Centis R, D'Ambrosio L, Spanevello A, Borroni E, Cirillo DM, Sotgiu G. Totally drug-resistant and extremely drug-resistant tuberculosis: the same disease? Clin Infect Dis. 2012 May;54(9):1379-80. doi: 10.1093/cid/cis128. PMID: 22492321.

32. Velayati AA, Farnia P, Masjedi MR. The totally drug resistant tuberculosis (TDR-TB). Int J Clin Exp Med. 2013 Apr 12;6(4):307-9. PMID: 23641309; PMCID: PMC3631557.

33. Okethwangu D, Birungi D, Biribawa C, Kwesiga B, Turyahabwe S, Ario AR, Zhu BP. Multidrug-resistant tuberculosis outbreak associated with poor treatment adherence and delayed treatment: Arua District, Uganda, 2013-2017. BMC Infect Dis. 2019 May 7;19(1):387. doi: 10.1186/s12879-019-4014-3. PMID: 31064332; PMCID: PMC6503550.

34. Seifert M, Catanzaro D, Catanzaro A, Rodwell TC. Genetic mutations associated with isoniazid resistance in Mycobacterium tuberculosis: a systematic review. PLoS One. 2015 Mar 23;10(3):e0119628. doi: 10.1371/journal.pone.0119628. PMID: 25799046; PMCID: PMC4370653.

35. Liu J, Shi W, Zhang S, Hao X, Maslov DA, Shur KV, Bekker OB, Danilenko VN, Zhang Y. Mutations in Efflux Pump Rv1258c (Tap) Cause Resistance to Pyrazinamide, Isoniazid, and Streptomycin in M. tuberculosis. Front Microbiol. 2019 Feb 19;10:216. doi: 10.3389/fmicb.2019.00216. PMID: 30837962; PMCID: PMC6389670.

36. Damtie D, Woldeyohannes D, Mathewos B. Review on Molecular mechanism of first-line antibiotic resistance in Mycobacterium tuberculosis. Journal of Mycobacterial Diseases. 2014;4(6):4-7. doi: 10.4172/2161-1068.1000174

37. Kigozi E, Kasule GW, Musisi K, Lukoye D, Kyobe S, Katabazi FA, Wampande EM, Joloba ML, Kateete DP. Prevalence and patterns of rifampicin and isoniazid resistance conferring mutations in Mycobacterium tuberculosis isolates from Uganda. PLoS One. 2018 May 30;13(5):e0198091. doi: 10.1371/journal.pone.0198091. PMID: 29847567; PMCID: PMC5976185.

38. Dean AS, Zignol M, Cabibbe AM, Falzon D, Glaziou P, Cirillo DM, Köser CU, Gonzalez-Angulo LY, Tosas-Auget O, Ismail N, Tahseen S, Ama MCG, Skrahina A, Alikhanova N, Kamal SMM, Floyd K. Prevalence and genetic profiles of isoniazid resistance in tuberculosis patients: A multicountry analysis of cross-sectional data. PLoS Med. 2020 Jan 21;17(1):e1003008. doi: 10.1371/journal.pmed.1003008. PMID: 31961877; PMCID: PMC6974034.

39. Zimenkov DV, Antonova OV, Kuz'min AV, Isaeva YD, Krylova LY, Popov SA, Zasedatelev AS, Mikhailovich VM, Gryadunov DA. Detection of second-line drug resistance in Mycobacterium tuberculosis using oligonucleotide microarrays. BMC Infect Dis. 2013 May 24;13:240. doi: 10.1186/1471-2334-13-240. PMID: 23705640; PMCID: PMC3671172.

40. Chen C, Weng J, Huang H, Yen W, Tsai Y. A new oligonucleotide array for the detection of multidrug and extensively drug-resistance tuberculosis. Nature Journal. 2019;9:1-11. https://go.nature.com/39gksqK

41. Kabir S, Tahir Z, Mukhtar N, Sohail M, Saqalein M, Rehman A. Fluoroquinolone resistance and mutational profile of gyrA in pulmonary MDR tuberculosis patients. BMC Pulm Med. 2020 May 11;20(1):138. doi: 10.1186/s12890-020-1172-4. PMID: 32393213; PMCID: PMC7216623.

42. Avalos E, Catanzaro D, Catanzaro A, Ganiats T, Brodine S, Alcaraz J, Rodwell T. Frequency and geographic distribution of gyrA and gyrB mutations associated with fluoroquinolone resistance in clinical Mycobacterium tuberculosis isolates: a systematic review. PLoS One. 2015 Mar 27;10(3):e0120470. doi: 10.1371/journal.pone.0120470. PMID: 25816236; PMCID: PMC4376704.

43. Bablishvili N, Tukvadze N, Shashkina E, Mathema B, Gandhi NR, Blumberg HM, Kempker RR. Impact of gyrB and eis Mutations in Improving Detection of Second-Line-Drug Resistance among Mycobacterium tuberculosis Isolates from Georgia. Antimicrob Agents Chemother. 2017 Aug 24;61(9):e01921-16. doi: 10.1128/AAC.01921-16. PMID: 28630205; PMCID: PMC5571295.

44. Solo ES, Nakajima C, Kaile T, Bwalya P, Mbulo G, Fukushima Y, Chila S, Kapata N, Shah Y, Suzuki Y. Mutations in rpoB and katG genes and the inhA operon in multidrug-resistant Mycobacterium tuberculosis isolates from Zambia. J Glob Antimicrob Resist. 2020 Sep;22:302-307. doi: 10.1016/j.jgar.2020.02.026. Epub 2020 Mar 10. PMID: 32169686.

45. Lynn SZ. The World Health Organization recommended tuberculosis diagnostic tools. 2018;73(7):72-90. https://bit.ly/3lMODet

46. Ombura IP, Onyango N, Odera S, Mutua F, Nyagol J. Prevalence of Drug Resistance Mycobacterium tuberculosis among Patients Seen in Coast Provincial General Hospital, Mombasa, Kenya. PLoS One. 2016 Oct 6;11(10):e0163994. doi: 10.1371/journal.pone.0163994. PMID: 27711122; PMCID: PMC5053611.

47. Schön T, Miotto P, Köser CU, Viveiros M, Böttger E, Cambau E. Mycobacterium tuberculosis drug-resistance testing: challenges, recent developments and perspectives. Clin Microbiol Infect. 2017 Mar;23(3):154-160. doi: 10.1016/j.cmi.2016.10.022. Epub 2016 Nov 1. PMID: 27810467.

48. Eddabra R, Ait Benhassou H. Rapid molecular assays for detection of tuberculosis. Pneumonia (Nathan). 2018 May 25;10:4. doi: 10.1186/s41479-018-0049-2. PMID: 29876241; PMCID: PMC5968606.

49. Parida SK, Axelsson-Robertson R, Rao MV, Singh N, Master I, Lutckii A, Keshavjee S, Andersson J, Zumla A, Maeurer M. Totally drug-resistant tuberculosis and adjunct therapies. J Intern Med. 2015 Apr;277(4):388-405. doi: 10.1111/joim.12264. Epub 2014 Jun 18. PMID: 24809736.

50. Asgedom SW, Teweldemedhin M, Gebreyesus H. Prevalence of Multidrug-Resistant Tuberculosis and Associated Factors in Ethiopia: A Systematic Review. J Pathog. 2018 Apr 3;2018:7104921. doi: 10.1155/2018/7104921. PMID: 29850257; PMCID: PMC5903304.

51. Elduma AH, Mansournia MA, Foroushani AR, Ali HMH, Elegail AMA, Elsony A, Holakouie-Naieni K. Assessment of the risk factors associated with multidrug-resistant tuberculosis in Sudan: a case-control study. Epidemiol Health. 2019;41:e2019014. doi: 10.4178/epih.e2019014. Epub 2019 Apr 20. PMID: 31010280; PMCID: PMC6545493.

52. Adejumo OA, Olusola-Faleye B, Adepoju V, Bowale A, Adesola S, Falana A, Owuna H, Otemuyiwa K, Oladega S, Adegboye O. Prevalence of rifampicin resistant tuberculosis

and associated factors among presumptive tuberculosis patients in a secondary referral hospital in Lagos Nigeria. Afr Health Sci. 2018 Sep;18(3):472-478. doi: 10.4314/ahs.v18i3.2. PMID: 30602977; PMCID: PMC6307017.

53. Timire C, Metcalfe JZ, Chirenda J, Scholten JN, Manyame-Murwira B, Ngwenya M, Matambo R, Charambira K, Mutunzi H, Kalisvaart N, Sandy C. Prevalence of drug-resistant tuberculosis in Zimbabwe: A health facility-based cross-sectional survey. Int J Infect Dis. 2019 Oct;87:119-125. doi: 10.1016/j.ijid.2019.07.021. Epub 2019 Jul 27. PMID: 31357057.

54. Diriba G, Kebede A, Tola HH, Alemu A, Tadesse M, Tesfaye E, Mehamed Z, Meaza A, Yenew B, Molalign H, Dagne B, Sinshaw W, Amare M, Moga S, Abebaw Y, Sied G. Surveillance of drug resistance tuberculosis based on reference laboratory data in Ethiopia. Infect Dis Poverty. 2019 Jun 14;8(1):54. doi: 10.1186/s40249-019-0554-4. PMID: 31200748; PMCID: PMC6567428.

55. Diandé S, Badoum G, Combary A, Zombra I, Saouadogo T, Sawadogo LT, Nébié B, Gnanou S, Zigani A, Ouédraogo SM, Diallo A, Kaboré S, Sangaré L. Multidrug-Resistant Tuberculosis in Burkina Faso from 2006 to 2017: Results of National Surveys. Eur J Microbiol Immunol (Bp). 2019 Feb 6;9(1):23-28. doi: 10.1556/1886.2018.00029. PMID: 30967972; PMCID: PMC6444799.

56. Araya S, Negesso AE, Tamir Z. Rifampicin-Resistant Mycobacterium tuberculosis Among Patients with Presumptive Tuberculosis in Addis Ababa, Ethiopia. Infect Drug Resist. 2020 Oct 6;13:3451-3459. doi: 10.2147/IDR.S263023. PMID: 33116664; PMCID: PMC7547769.

57. Hoza AS, Mfinanga SGM, König B. Anti-TB drug resistance in Tanga, Tanzania: A cross sectional facility-base prevalence among pulmonary TB patients. Asian Pac J Trop Med. 2015 Nov;8(11):907-913. doi: 10.1016/j.apjtm.2015.10.014. Epub 2015 Oct 9. PMID: 26614989.

58. Seyoum B, Demissie M, Worku A, Bekele S, Aseffa A. Prevalence and Drug Resistance Patterns of Mycobacterium tuberculosis among New Smear Positive Pulmonary Tuberculosis Patients in Eastern Ethiopia. Tuberc Res Treat. 2014;2014:753492. doi: 10.1155/2014/753492. Epub 2014 Apr 16. PMID: 24834351; PMCID: PMC4009208.

59. Jhun BW, Koh WJ. Treatment of Isoniazid-Resistant Pulmonary Tuberculosis. Tuberc Respir Dis (Seoul). 2020 Jan;83(1):20-30. doi: 10.4046/trd.2019.0065. PMID: 31905429; PMCID: PMC6953491.

60. Tahseen S, Khanzada FM, Rizvi AH, Qadir M, Ghazal A, Baloch AQ, Mustafa T. Isoniazid resistance profile and associated levofloxacin and pyrazinamide resistance in rifampicin resistant and sensitive isolates/from pulmonary and extrapulmonary tuberculosis patients in Pakistan: A laboratory based surveillance study 2015-19. PLoS One. 2020 Sep 23;15(9):e0239328. doi: 10.1371/journal.pone.0239328. PMID: 32966321; PMCID: PMC7511002.

61. Sulis G, Pai M. Isoniazid-resistant tuberculosis: A problem we can no longer ignore. PLoS Med. 2020 Jan 21;17(1):e1003023. doi: 10.1371/journal.pmed.1003023. PMID: 31961857; PMCID: PMC6974036.

62. Olson G, Nathavitharana RR, Lederer PA. Diagnostic Delays and Treatment Implications for Patients with Isoniazid-Resistant Tuberculosis: A Case Report and Review of the Literature. Open Forum Infect Dis. 2019 May 14;6(6):ofz222. doi: 10.1093/ofid/ofz222. PMID: 31211162; PMCID: PMC6559270.

63. Muthaiah M, Shivekar SS, Cuppusamy Kapalamurthy VR, Alagappan C, Sakkaravarthy A, Brammachary U. Prevalence of mutations in genes associated with rifampicin and isoniazid resistance in Mycobacterium tuberculosis clinical isolates. J Clin Tuberc Other Mycobact Dis. 2017 Jun 20;8:19-25. doi: 10.1016/j.jctube.2017.06.001. PMID: 31723707; PMCID: PMC6850230.

64. Singh A, Prasad R, Balasubramanian V, Gupta N. Drug-Resistant Tuberculosis and HIV Infection: Current Perspectives. HIV AIDS (Auckl). 2020 Jan 13;12:9-31. doi: 10.2147/HIV.S193059. PMID: 32021483; PMCID: PMC6968813.

65. Welekidan LN, Skjerve E, Dejene TA, Gebremichael MW, Brynildsrud O, Agdestein A, Tessema GT, Tønjum T, Yimer SA. Characteristics of pulmonary multidrug-resistant tuberculosis patients in Tigray Region, Ethiopia: A cross-sectional study. PLoS One. 2020 Aug 14;15(8):e0236362. doi: 10.1371/journal.pone.0236362. PMID: 32797053; PMCID: PMC7428183.

66. Liang L, Wu Q, Gao L, Hao Y, Liu C, Xie Y, Sun H, Yan X, Li F, Li H, Fang H, Ning N, Cui Y, Han L. Factors contributing to the high prevalence of multidrug-resistant tuberculosis: a study from China. Thorax. 2012 Jul;67(7):632-8. doi: 10.1136/thoraxjnl-2011-200018. Epub 2012 Mar 8. PMID: 22403070.

67. Calver AD, Falmer AA, Murray M, Strauss OJ, Streicher EM, Hanekom M, Liversage T, Masibi M, van Helden PD, Warren RM, Victor TC. Emergence of increased resistance and extensively drug-resistant tuberculosis despite treatment adherence, South Africa. Emerg Infect Dis. 2010 Feb;16(2):264-71. doi: 10.3201/eid1602.090968. PMID: 20113557; PMCID: PMC2958014.

68. Guo Q, Yu Y, Zhu YL, Zhao XQ, Liu ZG, Zhang YY, Li GL, Wei JH, Wu YM, Wan KL. Rapid detection of rifampin-resistant clinical isolates of Mycobacterium tuberculosis by reverse dot blot hybridization. Biomed Environ Sci. 2015 Jan;28(1):25-35. doi: 10.3967/bes2015.003. PMID: 25566860.

69. Vashistha H, Hanif M, Chopra KK, Shrivastava D, & Khanna A. Genetic polymorphism of rare mutations in Mycobacterium tuberculosis-infected patients in Delhi. Journal of Biomedical and Biotechnology Research Journal. 2018;2(1):74-81. https://bit.ly/2QBMIOi

70. Pires GM, Folgosa E, Nquobile N, Gitta S, Cadir N. Mycobacterium tuberculosis resistance to antituberculosis drugs in Mozambique. J Bras Pneumol. 2014 Mar-Apr;40(2):142-7. doi: 10.1590/s1806-37132014000200007. PMID: 24831398; PMCID: PMC4083649.

71. Lobie TA, Woldeamanuel Y, Asrat D, Beyene D, Bjørås M, Aseffa A. Genetic diversity and drug resistance pattern of Mycobacterium tuberculosis strains isolated from pulmonary tuberculosis patients in the Benishangul Gumuz region and its surroundings, Northwest Ethiopia. PLoS One. 2020 Apr 8;15(4):e0231320. doi: 10.1371/journal.pone.0231320. PMID: 32267877; PMCID: PMC7141659.

72. Kundu S, Marzan M, Gan SH, Islam MA. Prevalence of Antibiotic-Resistant Pulmonary Tuberculosis in Bangladesh: A Systematic Review and Meta-Analysis. Antibiotics (Basel). 2020 Oct 17;9(10):710. doi: 10.3390/antibiotics9100710. PMID: 33080862; PMCID: PMC7602942.

73. Shibabaw A, Gelaw B, Gebreyes W, Robinson R, Wang SH, Tessema B. The burden of pre-extensively and extensively drug-resistant tuberculosis among MDR-TB patients in the Amhara region, Ethiopia. PLoS One. 2020 Feb 13;15(2):e0229040. doi: 10.1371/journal.pone.0229040. PMID: 32053661; PMCID: PMC7018133.

74. Sagonda T, Mupfumi L, Manzou R, Makamure B, Tshabalala M, Gwanzura L, Mason P, Mutetwa R. Prevalence of Extensively Drug Resistant Tuberculosis among Archived Multidrug Resistant Tuberculosis Isolates in Zimbabwe. Tuberc Res Treat. 2014;2014:349141. doi: 10.1155/2014/349141. Epub 2014 May 20. PMID: 24967101; PMCID: PMC4054961.

75. Tasnim T, Tarafder S, Alam FM, Sattar H, & Kamal SMM. Pre-extensively drug-resistant tuberculosis (pre-XDR-TB) among pulmonary multidrug-resistant tuberculosis (MDR-TB) patients in Bangladesh. Journal of Tuberculosis Research. 2018;6:199-206. doi: 10.4236/jtr.2018.63018

76. Ali MH, Alrasheedy AA, Hassali MA, Kibuule D, Godman B. Predictors of Multidrug-Resistant Tuberculosis (MDR-TB) in Sudan. Antibiotics (Basel). 2019 Jul 9;8(3):90. doi: 10.3390/antibiotics8030090. PMID: 31323935; PMCID: PMC6783989.

77. Uzoewulu NG, Ibeh IN, Lawson L, Goyal M, Umenyonu N, Ofiael RO, & Okonkwo R. Drug-resistant Mycobacterium tuberculosis in Tertiary Hospital South East, Nigeria. Journal of Medical Microbiology & Diagnosis. 2014;3(2):1-5. DOI: 10.4172/2161-0703.1000141

78. Brhane M, Kebede A, Petros Y. Molecular detection of multidrug-resistant tuberculosis among smear-positive pulmonary tuberculosis patients in Jigjiga town, Ethiopia. Infect Drug Resist. 2017 Mar 9;10:75-83. doi: 10.2147/IDR.S127903. PMID: 28331348; PMCID: PMC5352243.

79. Hosny AM, Shady HMA, & Essawy AKE. rpo B, kat G and inh A Genes : The mutations associated with resistance to rifampicin and isoniazid in Egyptian Mycobacterium tuberculosis clinical isolates. Journal of Microbial and Biochemical Technology. 2020;12(428):1-11. https://bit.ly/39eskcj

80. Desikan P, Kharate A, Panwalkar N, Khurana J, Mirza SB, Chaturvedi A, Varathe R, Chourey M, Kumar P, Doshi N, Pandey M. Frequency of mutations in rifampicin and isoniazid resistant isolates of M. tuberculosis: an analysis from Central India. Germs. 2016 Dec 2;6(4):125-131. doi: 10.11599/germs.2016.1096. PMID: 28053915; PMCID: PMC5187753.

81. Zaw MT, Emran NA, Lin Z. Mutations inside rifampicin-resistance determining region of rpoB gene associated with rifampicin-resistance in Mycobacterium tuberculosis. J Infect Public Health. 2018 Sep-Oct;11(5):605-610. doi: 10.1016/j.jiph.2018.04.005. Epub 2018 Apr 26. PMID: 29706316.

82. Eddabra R, Neffa M. Mutations Associated with Rifampicin Resistance in Mycobacterium tuberculosis Isolates from Moroccan Patients: Systematic Review. Interdiscip Perspect Infect Dis. 2020 Oct 9;2020:5185896. doi: 10.1155/2020/5185896. PMID: 33133185; PMCID: PMC7568785.

83. Feliciano CS, Nascimento MM, Anselmo LM, Pocente RH, Bellissimo-Rodrigues F, Bollela VR. Role of a GenoType MTBDRplus line probe assay in early detection of multidrug-resistant tuberculosis at a Brazilian reference center. Braz J Med Biol Res. 2015 Aug;48(8):759-64. doi: 10.1590/1414-431X20154458. Epub 2015 Jun 30. PMID: 26132094; PMCID: PMC4541697.

84. Meaza A, Kebede A, Yaregal Z, Dagne Z, Moga S, Yenew B, Diriba G, Molalign H, Tadesse M, Adisse D, Getahun M, Desta K. Evaluation of genotype MTBDRplus VER 2.0 line probe assay for the detection of MDR-TB in smear positive and negative sputum samples. BMC Infect Dis. 2017 Apr 17;17(1):280. doi: 10.1186/s12879-017-2389-6. PMID: 28415989; PMCID: PMC5392973.

85. Reta MA, Alemnew B, & Abate BB. Prevalence of drug-resistance-conferring mutations associated with Isoniazid and Rifampicin resistant Mycobacterium tuberculosis in Ethiopia: A systematic review and meta-analysis. Journal of MedRxiv and BioRxiv. 2020;1-33. doi: 10.1101/2020.06.07.20124958

86. Spinato J, Boivin É, Bélanger-Trudelle É, Fauchon H, Tremblay C, Soualhine H. Genotypic characterization of drug resistant Mycobacterium tuberculosis in Quebec, 2002-2012. BMC Microbiol. 2016 Jul 26;16(1):164. doi: 10.1186/s12866-016-0786-4. PMID: 27459848; PMCID: PMC4962473.

87. Shenoy VP, Kumar A, Chawla K. Rapid detection of multidrug resistant tuberculosis in respiratory specimens at a tertiary care centre in south coastal Karnataka using Genotype MTBDR plus assay. Iran J Microbiol. 2018 Oct;10(5):275-280. PMID: 30675322; PMCID: PMC6339998.

88. Tseng ST, Tai CH, Li CR, Lin CF, Shi ZY. The mutations of katG and inhA genes of isoniazid-resistant Mycobacterium tuberculosis isolates in Taiwan. J Microbiol Immunol Infect. 2015 Jun;48(3):249-55. doi: 10.1016/j.jmii.2013.08.018. Epub 2013 Nov 1. PMID: 24184004.

89. Click ES, Kurbatova EV, Alexander H, Dalton TL, Chen MP, Posey JE, Ershova J, Cegielski JP. Isoniazid and Rifampin-Resistance Mutations Associated With Resistance to Second-Line Drugs and With Sputum Culture Conversion. J Infect Dis. 2020 Jun 11;221(12):2072-2082. doi: 10.1093/infdis/jiaa042. PMID: 32002554; PMCID: PMC7289556.

90. Jian J, Yang X, Yang J, Chen L. Evaluation of the GenoType MTBDRplus and MTBDRsl for the detection of drug-resistant Mycobacterium tuberculosis on isolates from Beijing, China. Infect Drug Resist. 2018 Oct 1;11:1627-1634. doi: 10.2147/IDR.S176609. PMID: 30319279; PMCID: PMC6171507.

91. Rufai SB, Umay K, Singh PK, Singh S. Performance of Genotype MTBDRsl V2.0 over the Genotype MTBDRsl V1 for detection of second line drug resistance: An Indian perspective. PLoS One. 2020 Mar 4;15(3):e0229419. doi: 10.1371/journal.pone.0229419. PMID: 32130233; PMCID: PMC7055869.

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