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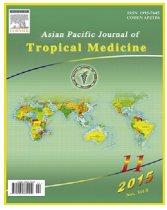

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Anti-TB drug resistance in Tanga, Tanzania: A cross sectional facility-base prevalence among pulmonary TB patients

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ABSTRACT

Objective: To determine the prevalence and risk factors associated with drug resistance tuberculosis (TB) at facility-base level in Tanga, Tanzania.

Methods: A total of 79 *Mycobacterium tuberculosis* (MTB) isolates included in the study were collected from among 372 (312 new and 60 previously treated) TB suspects self-referred to four TB clinics during a prospective study conducted from November 2012 to January 2013. Culture and drug susceptibility test of the isolates was performed at the institute of medical microbiology and epidemiology of infectious diseases, University hospital, Leipzig, Germany. Data on the patient's characteristics were obtained from structured questionnaire administered to the patients who gave informed verbal consent. Unadjusted bivariate logistic regression analysis was performed to assess the risk factors for drug resistant-TB. The significance level was determined at $P < 0.05$.

Results: The overall proportions of any drug resistance and MDR-TB were 12.7% and 6.3% respectively. The prevalence of any drug resistance and MDR-TB among new cases were 11.4% and 4.3% respectively, whereas among previously treated cases was 22.2% respectively. Previously treated patients were more likely to develop anti-TB drug resistance. There was no association between anti-TB drug resistances (including MDR-TB) with the risk factors analysed.

Conclusions: High proportions of anti-TB drug resistance among new and previously treated cases observed in this study suggest that, additional efforts still need to be done in identifying individual cases at facility-base level for improved TB control programmes and drug resistance survey should continuously be monitored in the country.

1. Introduction

Tuberculosis (TB) continues to be a major health challenge globally despite the efforts to combat the disease. Increased drug resistant strains in many parts of the world has worsen the situation [1]. Escalating human immunodeficiency virus (HIV) infection, increased prevalence of nontuberculous mycobacteria (NTM), poverty, and inadequacy public health infrastructure have also contributed greatly in worsening the situation [2].

During the last two decades, the World Health Organization and the International Union Against Tuberculosis and Lung Disease set up a global project to monitor the development of drug-resistant tuberculosis (DR-TB). Since that time approximately 60% of all countries in the world have implemented surveillance activities [3].

HIV epidemic and the emergence of drug resistant TB threaten the efforts to reduce the global burden of TB by 2015 that aims at ensuring that all TB patients benefit from universal access to high-quality diagnosis and patient-centred treatment [4]. Treatment of multi-drug resistant-TB (MDR-TB) is

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undoubtedly costly and requires longer treatment with more toxic drugs compared to drug susceptible TB [5,6]. Incorrect drug regimes, non-adherence to treatment, transmission in congregate settings, substandard drug quality, as well as erratic drug supply are key risk factors for drug resistance development [7,8]. Several studies have reported unacceptably high mortality rates among HIV-infected patients with MDR-TB [9,10].

Principally, drug resistance data are obtained through continuous surveillance by routine testing of all TB patients. However, in resource-poor settings like Tanzania, periodic drug resistance surveys (DRS) based on random, representative drug susceptibility testing (DST) among previously untreated smear-positive cases are seldom performed. Laboratories performing culture and DST are scarce and very often overburdened with divergent tasks for the TB control programme. Consequently, only phenotypic DST has been customarily performed; frequently faced with notable logistics and operational challenges [11], and therefore impeding regular DRS and precise surveillance. Generally, DST of *Mycobacterium tuberculosis* (*M. tuberculosis*) (MTB) demands the presence of bio-safety laboratories, which are rarely found outside reference centres in many resource-poor settings. Moreover, rapid transport of sputum from remote areas of the country to the reference laboratory is required to minimize losses due to contamination or growth failure. Poor infrastructure and unsustainable logistics in these settings humper this.

Tanzania is among the 22 high TB burden countries, with an estimated incidence (all forms) in 2007 and 2012 of 297 and 295 per 100 000 population respectively [12,13]. Available data on the prevalence of DR-TB and MDR-TB in Tanzania are still low owing to improved case management. Although the levels of anti-TB drug resistance in the country are still low, the need for continuous monitoring has always been emphasized [11,14]. The most effective strategies for limiting further spread of drug-resistant TB include rapid detection of drug resistance followed by prompt and effective therapy of each case. Routine surveillance linked to patient care, represents the best approach to monitor drug resistance [3].

Limited anti-TB drug resistance surveys have been conducted in Tanzania, the last one being in 2007 as part of national representative sample of TB patients [11]. Since that time, no survey has been conducted outside the national survey. The present study aimed at assessing the magnitude of anti-TB drug resistance and associated risks among newly, and previously treated pulmonary TB patients at facility-base level in Tanga, Tanzania.

2. Materials and methods

2.1. Study design, area and study population

A total of 79 *M. tuberculosis* isolates collected from among 372 new and previously treated TB patients during a prospective study conducted in Tanga, Tanzania from November 2012 to January 2013 were eligible for this study. All patients with clinical signs and symptoms suggestive of TB self-referred to four TB clinics were eligible for the study. The clinics included Makorora and Ngamiani health centres, Bombo Regional referral hospital and Muheza designated District hospital. Demographic information and data were collected only after provision of informed consent. A careful cross examination of patient history for previous anti-TB treatment using a structured questionnaire was used to classify patients as 'new' or 'repeat' TB cases. A patient was considered as 'new case' if had not received anti-TB treatment for a period >1 month and was considered 'repeat' if had received anti-TB drugs in a period less or equal to 1 month. No restrictions on inclusion criteria regarding clinical symptoms and age of the patients.

2.2. Sputum and data collection

Demographic data was obtained by using structured questionnaire administered to the patients attending four TB clinics who provided informed verbal consent to the study. Two sputum samples (one spot during the initial visit to the clinic and one early morning) were collected into small autoclavable wide mouth glass bottles. The specimens were examined by direct smear microscopy at the respective clinics using either Ziehl Neelsen-stain (Makorora and Ngamian health centres) or fluorescence stain (Bombo referral hospital and MDHH). Diagnosis of smear-positive TB was performed based on the national tuberculosis and leprosy programme guidelines [15]. All morning sputum samples were kept at −20 °C at the respective clinics. No preservative was added until shipped to the Institute of Medical Microbiology and Epidemiology of Infectious Diseases, University hospital, Leipzig, Germany for culture and molecular analysis. HIV status of the patients was determined by rapid HIV screening method at the treatment and care centres of the respective clinics.

2.3. Sputum culture and identification of mycobacterial isolates

Sputum specimens were digested and decontaminated using N-acetyl-L-cysteine-sodium hydroxide method [16] and were re-examined for the presence of acid-fast bacilli by fluorescence stain in Leipzig. The isolates were cultured in BacT/Alert® 3D liquid culture system (bioMe'rieux) and on Löweinstein- Jensen and Gottsacker slants (Artelt-ENCLIT GmbH, Wyhra, Germany). Gottsacker slopes contains sodium pyruvate for isolation of *Mycobacterium bovis*. Cultures were incubated at 37 °C for up to 8 weeks. Confirmation of MTB was done based on presumptive phenotypic appearance of colonies and by line probe assay (GenoType®MTBC; Hain Life science, Nehren, Germany).

2.4. Phenotypic drug susceptibility testing by BacT/Alert® 3D system

M. tuberculosis isolates were tested for their resistance to rifampicin (RMP), isoniazid (INH), streptomycin (SM), ethambutol (EMB) and pyrazinamide (PZA) by a proportion method using BacT/Alert® 3D system. Critical concentrations of 1 μg/mL for RMP, INH, and SM; 2 μg/mL for EMB and 200 μg/mL for PZA were respectively used [17,18]. The PZA bottles were incubated in BacT/Alert® 3D automated system at 37 °C, as mycobacteria designed blood culture bottles to inactivate the delta-algorithm of the system for growth detection in order to avoid false drug-resistant results. Growth was monitored daily, and an isolate was considered resistant to a drug under test when the drug-containing bottle had a time to detection (TTD) that was less or equal to the TTD of the 1% control bottle.

Definitions: Any resistance was defined as resistance to one or more first-line anti-TB drugs. Mono-resistance was defined as

resistance to only one of the first line anti-TB drugs (INH, RMP, SM, EMB and PZA). MDR-TB was defined as *M. tuberculosis* isolate that is resistant to at least INH and RMP. Resistance among new cases was defined as patient with TB resistant to one or more anti-TB drugs, but who had never been previously treated for TB. Resistance among previously treated cases, was defined as patients diagnosed with TB who started anti-TB treatment and subsequently acquired resistance to one or more of the drugs used during treatment [19].

2.5. M. tuberculosis genomic DNA extraction

Mycobacterial cells from positive BacT/Alert® bottles and from LJ slopes showing visible positive growth were used for DNA extraction. Briefly, bacterial DNA was extracted from heat-inactivated AFB isolates. A loopful of colony material was placed into a labelled screw caped eppendorf tube containing 500 µL sterile distilled water or by taking 500 µL from a positive BacT/Alert® bottles. Each specimen was incubated on a heat block at 95 °C for 20 min to inactivate the bacteria. Then centrifuged at 14 000 *g* for 15 min, the supernatant was discarded using pasture pipette, followed by addition of 200 µL distilled water to resuspend the pellets. This was followed by maximum vortexing for 10 s to homogenize the sediments. The tubes were incubated in an ultrasonic water bath at 95 °C for 15 min in order to rupture the inactivated mycobacterial cells to release the genomic DNA. The tubes were finally centrifuged at 14 000 *g* for 15 min. The supernatant was immediately used for PCR or transferred to a new sterile eppendorf for longer storage at −20 °C until used.

2.6. Genotypic drug susceptibility testing

Genotypic drug susceptibility testing for RMP and INH was performed using Genotype®MTBDR*plus* assay (Hain Life Science GmbH, Nehren, Germany). Genotypic detection of resistance to EMB was done by using Genotype®MTBDR*sl* assay (Hain Life Science GmbH, Nehren, Germany). PCR amplification, strip hybridization and interpretation of profiles were done by following manufacturer's instructions.

2.7. Data management and analysis

Data were first entered and cleared by using Ms Excel, then analysed using SPSS version 20 (SPSS Inc, Chicago, IL, USA). Unadjusted bivariate logistic regression analysis was performed to determine the risk factors for drug-resistant TB and the strength of the association was determined by odds ratio (*OR*) with 95% confidence interval (95% *CI*) and *P*-value of <0.05 was considered statistically significant.

The study was approved by the Ethical Review Committee of the National Institute for Medical Research, Dar es salaama, Tanzania. All patients gave verbal informed consent to participate in the study.

3. Results

3.1. M. tuberculosis culture results

From among 372 TB suspect patients enrolled in the study 312 (83.9%) were new and 60 (16.1%) were previously treated cases. Of the 312 new cases enrolled, 182 (58.3%) were culture negative, 33 (10.6%) contaminated and 97 (31.1%) had culture positive results. Of the 97 cases with culture positive results, 27 (27.8%) were excluded from the study because they were NTMs; only 70 (72.2%) cases from this category were eligible for DST. Of the 60 previously treated cases, 42 (70%) were culture negative, 5 (8.3%) were contaminated and 13 (21.7%) were culture positive. Four (30.8%) cases were excluded from the test because they were NTMs and nine (69.2%) were eligible for the DST. Overall, 79 (21.2%) out of 372 patients with positive *M. tuberculosis* isolates were eligible for phenotypic and genotypic DST as illustrated in Figure 1.

3.2. Demographic characteristics of the patients

The demographic characteristics of 79 (21.2%) patients who had positive MTB isolates were analysed for DST of the first line anti-TB drugs in this study. 70 (88.6%) were new and 9 (11.6%) were previously treated (repeat) cases. These included 55 (69.6%) males and 24 (30.4%) females with the mean age of (35.8 ± 12.6) years. Other demographic characteristics of the patients are shown in Table 1.

3.3. Drug resistance prevalence by phenotypic DST

Of the 79 patients with DST data, the overall proportion of any drug resistance was 12.7% (*n* = 10/79) and that of MDR-TB was 6.3% (*n* = 5/79). The proportion of any drug resistant anti-TB drug among new cases was 11.4% (*n* = 8/70) and the proportion of MDR-TB in this category was 4.3% (*n* = 3/70); whereas resistance to any drug and that of MDR-TB among previously treated cases were respectively 22.2% (*n* = 2/9). The proportions of any drug resistance and MDR-TB among HIV sero-positive patients were 30.8% (*n* = 4/13) and 23.1% (*n* = 3/13) respectively, while among the sero-negative group

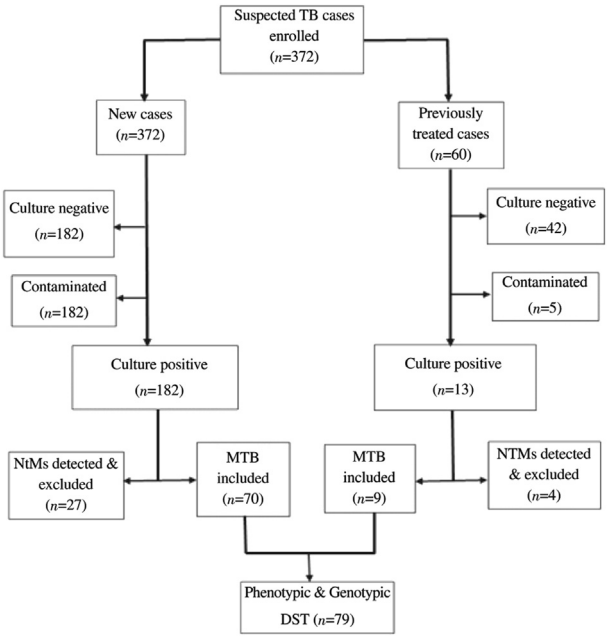

Figure 1. Flow diagram showing patients enrolled for drug susceptibility test (DST) of first-line anti-TB drugs in Tanga, Tanzania; November 2012–January 2013.

Table 1
Characteristics of patients enrolled for anti-TB drug susceptibility test in Tanga, Tanzania, November 2012–January 2013.

Characteristic		New (n = 70) (%)	Repeat (n = 9) (%)
Sex	Male	47 (67.1)	8 (88.9)
	Female	23 (32.9)	1 (11.1)
Age	<35 years	37 (52.9)	4 (44.4)
	≥35 years	33 (47.1)	5 (55.6)
Residence	Rural	36 (51.4)	3 (33.3)
	Urban	34 (48.6)	6 (66.7)
Site	Makorora HC	5 (7.1)	0
	Ngamian HC	20 (28.6)	6 (66.7)
	Bombo RH	14 (20.0)	0
	MDHH	31 (44.3)	3 (33.3)
Disease type	$Smear^+$ $Culture^+$	48 (68.6)	6 (66.7)
	$Smear^-$ $Culture^+$	22 (31.4)	3 (33.3)
HIV status	Positive	11 (15.7)	2 (22.2)
	Negative	59 (84.3)	7 (77.8)

were 9.1% (n = 6/66) and 3.0% (n = 2/66) respectively (Table 2).

3.4. Analysis of risk factors associated with any drug resistance and MDR-TB

Using unadjusted logistic regression model risk factors associated with any drug resistance and MDR-TB were determined. The patients were grouped into seven groups with the following characteristics in each group: Sex: males 55 (69.6%) cases, females 24 (30.4%) cases; age <35years: 41 (51.9%) cases; age ≥35 years: 38 (48.1%). With respect to residence, 40 (50.6%) were from urban and 39 (49.4%) from rural. Seventy (88.6%) were new and 9 (11.4%) were previously treated cases. With respect to HIV status 13 (16.5%) were HIV sero-positive, and 66 (83.5%) were sero-negative. With respect to disease type 55 (69.4%) were both smear and culture positive, while 24 (30.4%) were smear negative but culture positive. Regarding disease type, we interestingly detected 8.0% (n = 2/25) cases with any drug resistance and 4.0% (n = 1/25) case of MDR-TB among smear negative but culture positive patients. There was no significance difference among all variables analysed with development of any drug resistance or with MDR-TB in this population as shown in Table 2.

3.5. Resistance patterns of M. tuberculosis isolates

Table 3 shows different resistance patterns among new and previously treated (repeat) cases. Overall 87.3% of all cases were susceptible to all first line anti-TB drugs. Among the newly registered patients, any resistance to INH and RMP was each found in four (5.7%) isolates, resistance to SM in three (4.3%), any resistance to EMB and PZA was each found in 2 (2.9%) isolates and 4 (5.7%) isolates showed MDR-TB. Among the previously treated cases, any resistance to INH, SM and EMB were each found in 2 (22.2%) cases, any resistance to RMP and PZA was each found in one (11.1%) case and MDR-TB was found in one (11.1%) case, which was resistant to all first line ant-TB drugs. The overall prevalence of any resistance and MDR-TB when new and previously treated cases were combined was 12.7% and 6.3% respectively. Mono-resistance was only observed for SM and PZA in 1.4% each, all being from newly registered cases as shown in Table 3.

3.6. Comparison between phenotypic and genotypic drug susceptibility testing

Congruent results between phenotypic DST performed by BacT/Alert® 3D system and Genotype®MTBDR*plus* assay were obtained in 72 (91.1%) of the 79 subjects. Of 72 isolates identified as being susceptible to both INH and RMP by BacT/Alert® 3D, 66 (91.7%) cases were congruent by Genotype®MTBDR*plus*. 3 (4.2%) isolates were detected as MDR-TB by both methods and all with mutations in the *rpoß* gene and *katG* gene as shown by Genotype®MTBDR*plus*. Two (40.0%) isolates detected by BacT/Alert® 3D as MDR-TB, were detected by the

Table 2
Unadjusted bivariate logistic regression analysis of risk factors associated with any drug resistance and MDR-TB.

Variable		n	Susceptible n (%)	Any DR n (%)	OR, 95% *CI*	P value	MDR-TB n (%)	OR, 95% *CI*	P value
Sex	Male	55	48 (87.3)	7 (12.7)	0.44 (0.87–2.22)	0.32	3 (5.5)	0.37 (0.1–2.7)	0.33
	Female	24	21 (87.5)	3 (12.5)	Ref		2 (8.3)	Ref	
Age	<35 years	41	36 (87.8)	5 (12.2)	0.98 (0.21–4.57)	0.97	3 (7.3)	1.66 (0.2–4.9)	0.61
	≥35 years	38	33 (86.8)	5 (13.2)	Ref		2 (5.3)	Ref	
Residence	Rural	39	35 (89.7)	4 (10.3)	0.29 (0.012–7.36)	0.46	3 (7.7)	0.34 (0.004–28.73)	0.64
	Urban	40	34 (85.0)	6 (15.0)	Ref		2 (5.0)	Ref	
Treatment	New case	70	62 (88.6)	8 (11.4)	0.38 (0.05–2.97)	0.36	3 (4.3)	0.44 (0.03–7.42)	0.57
history	Previously treated	9	7 (77.8)	2 (22.2)	Ref		2 (22.2)	Ref	
Site	Makorora HC	5	5 (100.0)	0	–		0	–	–
	Ngamian HC	26	22 (86.4)	4 (15.4)	0.25 (0.01–6.62)	0.41	1 (3.9)	0.9 (0.001–9.11)	0.31
	Bombo RH	14	13 (92.9)	1 (7.1)	0.15 (0.03–7.96)	0.35	0 (0)	0.19 (0.001–29.1)	0.52
	MDHH	34	29 (85.3)	5 (14.7)	Ref		4 (11.8)	Ref	
Disease	$Smear^+$ $Culture^+$	54	46 (85.2)	8 (14.8)	1.61 (0.27–9.53)	0.60	4 (7.4)	1.94 (0.18–21.03)	0.59
Type	$Smear^-$ $Culture^+$	25	23 (92.0)	2 (8.0)	Ref		1 (4.0)	Ref	
HIV status	Sero-positive	13	9 (69.2)	4 (30.8)	0.42 (0.08–2.35)	0.33	3 (23.1)	0.21 (0.03–1.43)	0.11
	Sero-negative	66	60 (90.9)	6 (9.1)			2 (3.0)	Ref	
Total		79		10 (12.7)			5 (6.3)		

DST, drug susceptibility testing; DR, drug resistance; MDR-TB, multidrug-resistant tuberculosis; OR, odds ratios.

Table 3
Drug resistance patterns to first line anti-TB drugs in Tanga, Tanzania (new *vs* repeat cases).

Resistance pattern	New *n* (%)	Repeat *n* (%)	Total *n* (%)
Total patients	*n* = 70	*n* = 9	*n* = 79
Susceptible to all drugs	62 (88.6)	7 (77.8)	69 (87.3)
¥Any resistance	8 (11.4)	2 (22.2)	10 (12.7)
Any resistance to;			
INH	4 (5.7)	2 (22.2)	6 (7.6)
RMP	4 (5.7)	1 (11.1)	5 (6.3)
SM	3 (4.3)	2 (22.2)	5 (6.3)
EMB	2 (2.9)	2 (22.2)	4 (5.1)
PZA	2 (2.9)	1 (11.1)	3 (3.8)
All INH + RMP resistant (MDR-TB)	4 (5.7)	1 (11.1)	5 (6.3)
INH + RMP (only)	1 (1.4)	0	1 (1.3)
INH + RMP + SM	2 (2.8)	0	2 (2.5)
INH + RMP + EMB	1 (1.4)	0	1 (1.3)
INH + RMP + PZA	0	0	0
INH + RMP + SM + EMB + PZA	0	1 (11.1)	1 (1.3)
INH + Other resistance	0	2 (22.2)	2 (2.5)
INH + SM	0	1 (11.1)	1 (1.3)
INH + EMB	0	0	0
INH + PZA	0	0	0
INH + SM + EMB	0	1 (11.1)	0
INH + SM + EMB + PZA	0	0	1 (1.3)
RMP + Other resistance	0	1 (11.1)	1 (1.3)
RMP + SM	0	0	0
RMP + EMB	0	0	0
RMP + PZA	0	0	0
RMP + SM + EMB + PZA	0	1 (11.1)	1 (1.3)
*Mono-resistance to	2 (2.9)	0	2 (2.5)
INH	0	0	0
RMP	0	0	0
SM	1 (1.4)	0	1 (1.3)
EMB	0	0	0
PZA	1 (1.4)	0	1 (1.3)

INH, isoniazid; RMP, rifampicin; SM, streptomycin; EMB, ethambutol; PZA, pyrazinamide; ¥Any resistance: Resistance to any of the first-line anti-TB either in combination or as single; *Mono-resistance: Resistance to only one anti-TB drug.

Genotype®MTBDR*plus* assay as being RMP mono-resistant. Overall Genotype®MTBDR*plus* detected 3 (3.8%) isolates as RMP mono-resistant, whereas no isolate could be detected as RMP mono-resistant by the BacT/Alert® 3D. On the other hand, 2 (2.5%) isolates were detected by either methods as INH mono-resistant; with one (1.4%) having mutation in *katG* gene and one missed by the genotypic method as depicted in Table 4.

4. Discussion

Our key findings show that the prevalence of resistance to any anti-TB drug among new patients was 11.4% and that of MDR-TB was 4.3%, whereas among previously treated patients the resistance to any anti-TB drug and MDR-TB were respectively 22.2%. The overall resistance to any anti-TB drugs and MDR-TB when newly and previously treated cases were combined was 12.7% and 6.3% respectively. The overall prevalence to any resistance of 7.6% for INH; 6.3% for RMP and SM; 5.1% for EMB and 3.8% for PZA were high. The levels reported in our study are higher than those reported during the last national DST survey [11]. These proportions are alarming, hence calling for immediate intervention to reverse the trend, as it raises concern on the increased transmission of drug resistant MTB in the settings.

High proportion of resistance to any drug (22.2%) and MDR-TB among previously treated patients, also rises concerns on continual reliance on home-based supervision of TB treatment as previously advocated [20]; as patient adherence to treatment may be difficult to monitor, since the supervision of patients at home is made by non-medical professionals. Moreover, the existence of other resistance patterns with INH and or RMP among previously treated patients further reaffirms the concern over the home-based supervision of TB patients. Although no association to any anti-TB drug and or MDR-TB was found between newly and previously treated cases, our findings show that 22.2% (*n* = 2/9) of the previously treated cases were more likely to develop resistance to first-line anti-TB drugs. Lack of association between history of treatment with DR-TB and MDR-TB observed could be due to low number of previously treated patients enrolled during the study.

Observed mono-resistance to SM and PZA in 2.9% among new patients is plausibly an indication of growing use of these antibiotics in the treatment of other bacterial infections in the community. Generally, our findings are well in agreement with those of the nation-wide survey, which showed the prevalence of any resistance among new and previously treated patients in the range of 8.3% and 20% respectively [11]. The observed resistance to PZA in this study is, to the best of our understanding reported for the first time in Tanzania. The standard practice for drug resistance surveys has been to test for four first-line drugs (INH, RMP, EMB and SM); and for the purposes of surveys NTPs are required to perform, at minimum DST for INH and RMP on all cases included in the survey [21]. From the findings of this study, it may be worth including DST for PZA especially among HIV positive patients.

Table 4
Comparison of resistance patterns of 79 *M. tuberculosis* isolates to INH and RMP by phenotypic and genotypic method.

Genotypic resistance pattern	Phenotypic resistance pattern				
	INH^{s}/RMP^{s} *n* (%)	INH^{r}/RMP^{s} *n* (%)	INH^{s}/RMP^{r} *n* (%)	MDR-TB *n* (%)	Total *n* (%)
INH^{wt}/RMP^{wt}	65 (91.7)	1 (50.0)	0	2 (40.0)	68 (86.1)
INH^{mut}/RMP^{mut}	3 (4.2)	0	0	3 (60.0)	6 (7.6)
INH^{mut}/RMP^{wt}	1 (1.4)	1 (50.0)	0	0	2 (2.5)
INH^{wt}/RMP^{mut}	3 (4.2)	0	0	0	3 (3.8)
Total	72 (91.1)	2 (2.5)	0	5 (6.3)	79 (100)

INH^{s}, isoniazid sensitive; INH^{r}, isoniazid resistance; RMP^{s}, rifampicin sensitive; RMP^{r}, rifampicin resistance; INH^{wt}, isoniazid wild type gene without mutation; INH^{mut}, isoniazid with mutation band detected; RMP^{wt}, rifampicin wild type gene without mutation; RMP^{mut}, rifampicin with mutation band detected; MDR-TB, multidrug-resistant tuberculosis.

The findings of any drug resistance of 8.0% and of MDR-TB 4.0% among smear-negative but culture-positive patients in this study need to be addressed with special concern, as this suggests the inadequacy of peripheral microscopy performance. Several studies have shown that peripheral smear microscopy in Tanzania has frequently more problems in false-negative slides than in false-positive [22].

Although several reports from other countries, have documented that HIV positivity is an important risk factor associated with primary MDR-TB [23–26], and that HIV infection has been associated with MDR-TB outbreaks in institutional settings, such as hospitals and prisons [27,28]. Our findings showed lack of association between anti-TB drug resistance in patients with or without HIV and these results are in agreement with studies conducted in Mwanza, Tanzania [14]. This lack of association could be explained by the fact that majority of HIV infected TB patients are likely to be smear negative and tend to have lower rate of sputum smear positivity [29].

Of the 5 (6.3%) MDR isolates detected in our study, 2/5 isolates showed discordant results by BacT/Alert® 3D system and Genotype®MTBDR*plus*. While BacT/Alert® 3D detected all isolates as being INH resistant, the later detected one of the isolate as INH mono-resistant and the other as INH susceptible. Possible explanation of this disparity could be due to presence of mutations outside the 81-bp "hot-spot" of the *rpo*B gene, though this does occur less frequently [30,31]. Such mutations may occur for example at codon 490 CAG to CAG [32], codon 534 (GGG to GAG), codon 535 (CCC to CAC) [33] and at codon 572 (ATC to TTC) [34]. Another possible explanation could be due to changes occurring in genes whose products participate in antibiotic permeation or metabolism [35]. Several studies have also indicated presence of high discordance in RMP susceptibility testing [36,37]. Since the Genotype®MTBDR*plus* assy we used is not very robust; such methods like DNA sequencing may be considered for isolates with discordant results.

Limitations: The small sample size may have limited the generalization of the observed results in our study. High rates of culture negative and contaminated specimens may be attributed to such factors as: (i) inclusion of specimens with very low bacilli load resulting to loss of viability during culture, (ii) understaffed and overburdened of the clinics with other non-TB patients might have led to delayed sample processing, (iii) frequent power interruption may have resulted into inappropriate cold chain maintenance at the clinic level, (iv) some patients failing to deliver early morning samples within the specified period, due to among other factors long distance and costs of travel to and from the clinics. Despite the limitations of the study, it has provided important information regarding the resistance profiles at the facility-base level where majority of the patients may not have opportunity to be covered during the national drug resistance surveys. High proportions of anti-TB drug resistance among new and previously treated cases, gives an insight of DR-TB situation at facility levels in the country. Therefore, it provides for a better planning of the drug resistance surveys including improving smear microscopy performance for case detection and improvement of TB control programmes. Frequent drug resistance survey need to be done in order to monitor the situation of DR-TB in the country.

Conflict of interest statement

We declare that we have no conflict of interest.

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