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Patterns of drug resistance in *Mycobacterium tuberculosis* from tuberculosis patients in Ibadan Nigeria

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ABSTRACT: The success of the global tuberculosis (TB) control program has been threatened with drug resistant strains emergence; especially the Multidrug Resistant Tuberculosis (MDR-TB). Despite that Nigeria is one of the countries with high tuberculosis burden, little is known on the magnitude of MDR-TB in the country. This study was to determine drug resistant patterns of *Mycobacterium tuberculosis* isolated from patients that attended Directly Observed Treatment Short-course (DOTS) centres in Ibadan, Nigeria. Sputum samples collected from confirmed TB patients were processed using the N-acetyl L-cysteine-sodium hydroxide decontamination method. Direct drug susceptibility test was carried out against rifampicin, isoniazid, ethambuthol and streptomycin. Out of the 319 samples collected, 149 (46.7%) were culture positive and susceptibility test was completed for 101 (67.8%) isolates, out of which any resistance and mono-resistance to rifampicin was 23.8% and 8.9% respectively. In all 11.9% MDR-TB was observed comprising 30.8% (acquired), and 8.3% (primary) while, 3.96% showed resistance to all tested drugs. The patterns of MDR-TB in this study indicates that active case findings as well as expansion of drug susceptibility testing is required to effectively control TB, drug resistant strains and to forestall the transmission and spread of the drug-resistant TB in the society.

Keywords: Tuberculosis; Multi-drug resistant tuberculosis; Directly observed treatment short-course; Antibiotics resistance.

1. INTRODUCTION

Tuberculosis (TB), a chronic infectious disease is responsible for majority of ill health all over the world. The disease can affect anyone; however, about 90% of those that develops the disease are adults while the male: female ratio is 2:1 [1]. Globally, TB is one of the leading causes of death, and highest number of causalities from a single infectious agent especially among people living with Human Immunodeficiency Virus (HIV) infection [2, 3]. Despite efforts to control TB epidemic, the estimated incident cases of TB in 2017 was 10 million in 133 per 100, 000 population and 1.57 million deaths which represented 1.8% and 3.9% declines from the 2016 incident cases respectively [4]. While, in 2018, 10 million fell ill with TB, 1.2 million (1.1-1.3 million) among HIV-negative people and 251000 among HIV infected individuals died of TB

[1]. The emergence of drug-resistant strains of *Mycobacterium tuberculosis*, especially the multidrug resistant TB (MDR-TB), extensively drug resistant TB (XDR-TB) and lately the total drug resistant TB (TDR-TB) is a threat to the control of TB program worldwide [5]. The report of drug resistant in TB was as soon as first TB drugs were made available for therapy in the late 1940's [6]. It was believed that there could be a reduction in the trend with quality-assured multidrug treatment; hence the introduction and implementation of the DOTS program in the early 1990's [7]. The global implementation of the strategy led to the cure of about 50 million patients, and prevention of about 7 million deaths in comparison with the pre-DOTS era. However, the strategy has been unable to stop the alarming increase of the resistant strains of TB [8, 9]. Patients infected with MDR strains are those that are resistant to rifampicin and isoniazid (the powerful anti-TB medications) with or without resistant to other drugs. This kind of infection complicates the length of treatment because they do not respond to the WHO DOTS therapy and require treating patients with second line drugs that are more toxic, more expensive, and less effective with longer time of treatment [3].

In Nigeria, TB remains a major infectious disease and for long years, the country has remained one of the highest TB burdened countries of the world. Currently, among the 30 high TB burden countries of the world, Nigeria is ranked 7th, while the country is ranked 2nd in Africa behind South Africa with a total of 429 (280-609) cases per 1000.000 populations in 2018 [1, 3]. Incidentally, Nigeria is not even among the eleven High Burden Countries (HBC) that were reported to be on track to reach the 2020 milestone of a 20% in the reduction rate of TB incidence [1]. The report of the WHO showed that Nigeria is among the high TB/HIV and MDR/RR-TB burden countries of the world. In 2017, the overall estimated rate of MDR/RR-TB in the country was 4.3% and 15% among new and previously treated cases respectively [3]. While in 2018, 4.3% (3.2-5.5) was the TB estimated proportion with MDR/RR-TB among the new and 15% (11-19) among the previously treated cases [1]. Although, it has been suggested that surveillance study should be continuous with regular drug resistance determination. The surveillance should be based on routine drug susceptibility testing especially, in countries with high burden the disease. Through these, the level and trends of antimicrobial resistance will be accessed [1]. However, reports of study on the determination of the prevalence of MDR-TB in Nigeria including Ibadan where the disease is on the rise are limited. Some of the few studies in Nigeria include the 16% and 11.7% reported in Jos and Calabar, respectively [10, 11]. In addition, 53% prevalence was reported in Ibadan in a study carried out at the University Teaching Hospital (UCH), a referral hospital [12]. While it was noted in the study that the report cannot be used to ascertain MDR-TB magnitude in the city, it has also been previously pointed out that reported figures from studies carried out at tertiary care hospitals and referral centres are usually high and may not represent the true state of the general population [13]. This study was therefore designed with the aim of determining the drug resistance in *Mycobacterium tuberculosis* isolated from TB patients that attended the DOTS centres in Ibadan, Nigeria.

2. MATERIALS AND METHODS

2.1. Study Area

The study was a cross sectional study and samples were collected from 11 DOTS in Ibadan, Nigeria between January 2010 and November 2011. The centres included: Chest Hospital, Jericho; TBL Health Centre, Moniya; TBL Health Centre, Iwo- Road; Oluyole TBL Health Centre Ibadan; Molete TBL clinic; Egbeda TBL Health Centre; Adeoyo Teaching hospital Maternity, Yemetu; Adifase TBL centre Apata; Alafara TBL centre; Atolu TBL centre Oremeji and Ido TBL centre Ido (Figure 1). Ibadan lies between Latitude 7^{o}32$^{`}$N and Longitude 3$^{o}$54$^{`}$E.

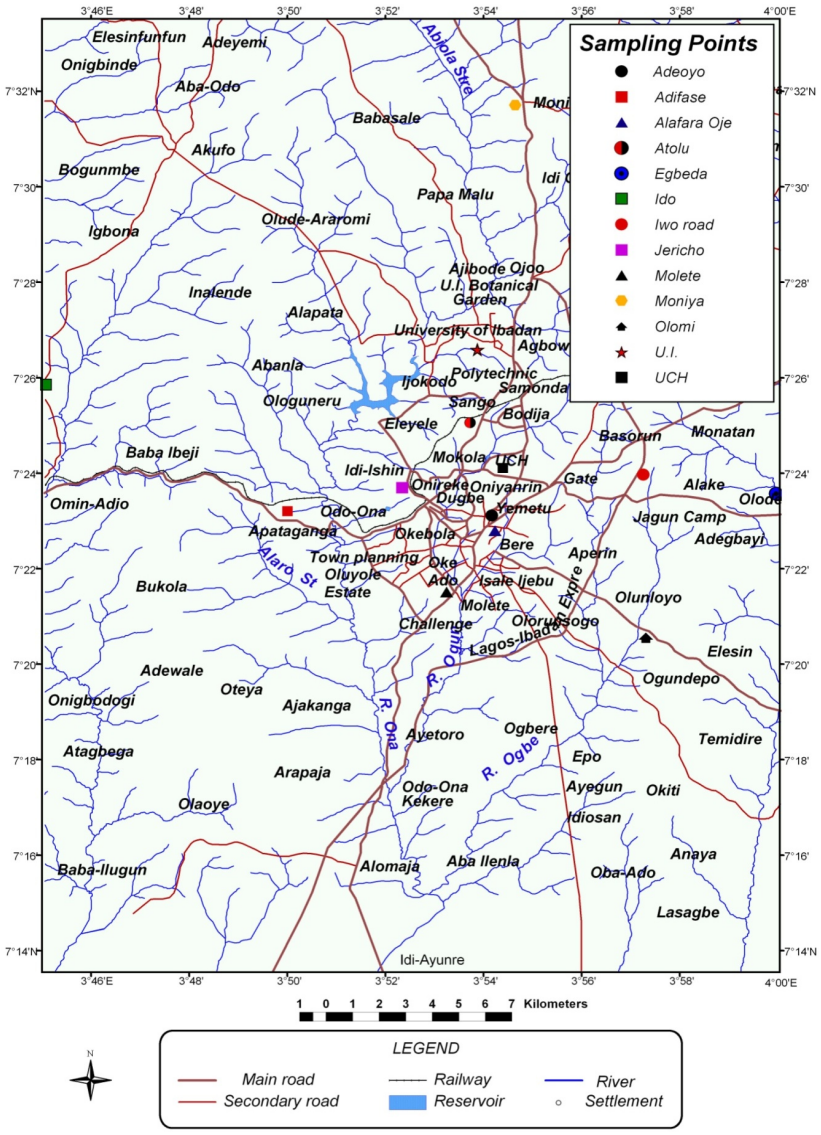

Figure 1. Map of Ibadan region showing the DOTS centers where samples were collected.

2.2. Study participants

The study participants included the consecutive patients seen at the DOTS clinics that were diagnosed for pulmonary TB either by ZN staining and/or X-ray with the help of the health officials.

2.3. Ethical consent and ethical approval

Consents were sought from adult participants who volunteered to participate in the study after adequate information on study objectives and implications were given. Consents were sought from parents/guardians of children included in the study. The study was approved by the University of Ibadan/University College Hospital Ethical committee (Approval number: NHREC/05/01/2008a).

2.4. Inclusion and exclussion criteria

Confirmed TB patients at the DOTS centres who gave consent and had not commenced anti-TB therapy were included. Those already on anti-TB treatment, those who declined to participate in the study and those that could not expectorate were excluded.

2.5. Sputum sample collection and processing

The samples were collected in leak-proof and clean sputum containers. With the help of the health officials, clear instructions were given to patients on how to produce the samples. Where feasible, three specimens were collected; on-the-spot, early morning, on-the-spot. The samples were properly labelled and transported in iced pack to the laboratory on the day of collection. The samples were stored in a refrigerator and processed within 48 hours of collection. Processing of the sputum samples were done using the BD BBL™ Mycoprep™ N-Acetyl L-Cysteine-sodium hydroxide (NALC-NaOH) decontamination method [14].

2.6. Quality control

Reference strains of fully susceptible H37RV (ATCC 27294), and MDR (ATCC 35838) *M. tuberculosis* obtained from the Division of Infectious Diseases, North-western University, Feinberg School of Medicine, Chicago, USA was used as control. These strains were sub-cultured freshly on Lowenstein Jensen (L-J) medium before use. For every batch of culture, the reference strains were inoculated and incubated alongside all test samples.

2.7. Direct drug susceptibility testing

This was done using L-J medium in accordance with the laboratory's standard procedure previously described [15, 16]. The critical concentration used for each drug was as recommended [16, 17]. Critical concentrations of 0.2 µg/ml (isoniazid) 40 µg/ml (rifampicin), 4.0 µg/ml (streptomycin) and 2.0 µg/ml (ethambuthol) were used. Preparation of the L-J medium with or without antimicrobials and inoculation of the media as well as interpretation of susceptibility test results was as previously descibed [18]. The tubes were incubated at 37°C. Final susceptibility results was reported after 40 days following the laboratory's standard procedure, but preliminary results were reported earlier for resistant strains (the earliest observation day was 14 days).

2.8. Definition of MDR-TB and Poly-drug resistance

The MDR-TB was defined according to the definition of W.H.O. (i.e resistance to rifampicin and isonoazid with or witout resistance to any other drugs). While poly-drug resistance was defined as resistance to more than one drug but not including both rifampicin and isoniazd at the same time.

2.9. Data analysis

The antibiotics resistance prevalence of the isolates was calculated and expressed in percentages. The association between the primary and acquired drug resistance was analysed using descripitive statistics and ANOVA at p=0.05

3. RESULTS

Out of the 319 samples collected, 149 (46.7%) were culture positive and drug susceptibility results was completed for 101 (67.8%), comprsising 60 (59.4%) new, 13 (12.9%) retreatment and 28 (27.7%) unclassified cases. *Mycobacterium tuberculosis* prevalence in relation to patient's age is shown in Table 1. The highest prevalent rate (47.5%) was between the age range 15-34 years followed by the age range 35-54. The least rate (0.99%) was within the age range of less than 15 years. Among the 74 (73.3%) patients with known HIV status, 51 (85.0%), 10 (76.9%), and 13 (46.4%) were new, retretament and unclassified cases, respectively (Table 1). The drug susceptibility patterns of the isolates showed that 22 (36.6%) out of the 60

new cases were susceptible to all the four drugs while, out of the 13 isolates from the retreatmet cases, 6 (46.2%) were susceptible to the four drugs, but 12 (42.9) were susceptible to the four drugs among the 28 isolates obtained from the unclassified cases (Table 2). Furthermore, any resistance to rifampicin was 14 (23.3%) among the new and 5 (38.5%) among the retreatment cases. Any resistance to ethambuthol was 9 (15.0%) and 3 (23.1%) among the new and the retreatment cases, respectively (Table 2).

Table 1. Baseline characteristics of case-patients in a study of MDR TB, Ibadan, Nigeria.

Characteristics	New (%), n=60	Retreatment (%), n=13	Unclassified (%), n=28	Total (%), n=101
Age, y				
< 15	1 (1.7)	0	0	1 (0.99)
15-34	29 (48.3)	8 (61.5)	11 (39.3)	48 (47.5)
35-54	24 (40.0)	3 (23.1)	13 (46.4)	40 (39.6)
≥ 55	6 (10.0)	2 (15.4)	4 (14.3)	12 (11.9)
		Sex		
Male	31 (51.7)	6 (46.1)	11 (39.3)	4 (47.5)
Female	29 (48.3)	7 (53.8)	17 (60.7)	53 (52.5)
		Recruitment centres		
Ade Oyo	2 (3.3)	3 (23.1)	11 (39.3)	16 (15.8)
Adifase	9 (15.0)	5 (38.5)	2 (7.1	16 (15.8)
Alafara	7 (11.7)	1 (7.7)	2 (7.1)	10 (9.9)
Atolu	0	0	2 (7.1)	2 (1.98)
Egbeda	1 (1.7)	0	0	1 (0.99)
Ido	0	0	4 (14.3)	4 (3.96)
Iwo Road	3 (5.0)	0	0	3 (2.97)
Jericho	19 (31.7)	3 (23.1)	5 (17.9)	27 (26.7)
Molete	11 (18.3)	0	0	11(10.9)
Olomi	0	1 (7.1)	1 (3.6)	2 (1.98)
UCH	8 (13.3)	0	1 (3.6)	9 (8.9)
		HIV status known		
	51 (85.0)	10 (76.9)	13 (46.4)	74 (73.3)

Among the unclassified cases, any resistance to isoniazid was 19 (35.7%) while it was 4 (14.3%) for both streptomycin and ethambuthol. Monoresistance to rifampicin was highest (11.7%) among the new cases, while it was 5.0% to ethambuthol. Among the unclassified cases, the highest monoresistance was to ethambuthol, but the lowest was to streptomycin. In all, 12 (11.9%) were MDR-TB out of which 5 (8.3%) were from the new cases, 4 (30.8%) from retreatment cases and 3 (10.7%) from unclassified cases. The other drug resistance aside MDR-TB showed that 3 (5.0%) isolates among those from new cases showed resistance to both isoniazid and ethambuthol while, two (2) isolates each from the new cases were resistant to a combination of isoniazid and streptomycin and a combination of rifampicin and streptomycin respectively. Among the retreatment cases, only one isolate was resistant to a combination of rifampicin and streptomycin while, among the uncassified cases, three (10.7%) were resistant to three drugs (isoniazid, streptomycin and ethambuthol). Furthermore, while 4 (3.96) isolates were pan resistant (resistance to all the four drugs), 8.3%, 30.8% and 10.7% of the MDR-TB were primary, acquired and unclassified respectively. Moreover, 7 (58.3%) and 5 (41.7%) MDR-TB were from females and male patients respectively (Table 2). However, there was no

statistical significant difference in the pattern of resistance between the new and the retreatment cases (p = 0.87).

Table 2. Drug resistance pattern of the isolates to first line drug in new and previously treated cases.

	New, n=60	**Retreatment, n=13**	**Unclassified, n=28**	**Total, n=101**
	No (%)	No (%)	No (%)	No (%)
Resistance patterns				
Susceptible to all first line drugs	22 (36.6)	6 (46.2)	12 (42.9)	40 (39.6)
Any resistance				
RIF	14 (23.3)	5 (38.5)	5 (17.9)	24 (23.8)
INH	13 (21.7)	4 (30.8)	10 (35.7)	27 (26.7)
SM	12 (20.0)	3 (23.1)	4 (14.3)	19 (18.8)
EMB	9 (15.0)	3 (23.1)	4 (14.3)	16 (15.8)
Mono resistance				
RIF	7 (11.7)	0	2 (7.1)	9 (8.9)
INH	3 (5.0)	0	4 (14.3)	7 (6.9)
SM	5 (8.3)	0	1 (3.6)	6 (5.9)
EMB	3 (5.0)	1 (7.7)	3 (10.7)	7 (6.9)
MDR				
RIF + INH	2 (3.3)	2 (15.4)	2 (7.1)	6 (5.9)
RIF +INH +EMB	1 (1.7)	0	0	1 (0.99)
RIF +INH +SM	1 (1.7)	0	0	1 (0.99)
RIF +INH +SM +EMB	1 (1.7)	2 (15.4)	1 (3.6)	4 (3.96)
Total MDR	5 (8.3)	4 (30.8)	3 (10.7)	12 (11.9)
Prevalence of MDR by sex				
Male	4 (33.3)	0	1 (8.3)	
Female	3 (25.0)	2 (16.7)	2 (7.1)	
Resistance patterns to other first line drugs aside MDR				
INH + SM	2 (3.3)	0	0	2 (1.98)
SM +EMB	1 (1.7)	0	0	1 (0.99)
RIF +SM	2 (3.3)	1 (7.7)	0	3 (2.98)
INH +EMB	3 (5.0)	0	0	3 (2.98)
INH +SM +EMB	0	0	3 (10.7)	3 (2.98)

4. DISCUSSION

The pattern of drug resistance of TB isolates from patients in DOTS centres in Ibadan, Nigeria was examined in the present study. The 59.4%, 12.9% and 27.8% patients that were new, retreatment, and unclassified TB cases differs from the report of a similar study in the northern part of the country where 37.2%, 40.7% and 22.1% new, re-treatment and unclassified cases respectively were reported [10]. The observed resistance to anti-TB drugs in this study is high with 60.4% resistant to at least one drug compared to the 35.7% and 15.3% reported in India and the 48.6% from Kenya [20-22]. These countries are also among the 30 TB high burdened countries like Nigeria. The observation was also higher than the 7.8% reported in USA [22], but is comparably similar to the 68.1% of resistance to at least one drug reported in Lima, Peru [23].

Of the 60.4% resistant to at least one drug in this study, 6.6% were 'pan resistant' which is lower compared to 11.4% previously reported [23]. Furthermore, the 39.6% that were observed to be fully susceptible in this study is lower compared to the reported 76.0% in Northern Nigeria [11], 76.5% in Ghana, a neighbouring West African country [24], 73.8% in Kenya [21] and 92.2% in USA [22]. The mono-resistance, 8.9% (RIF), 6.9% (INH), 5.0% (SM) and 4.0% (EMB) observed in this study is lower compared to 3.0% (RIF), 0% (INH), 11.0% (SM) and 1.0% (EMB) reported in Jos except for SM [10]. The observation is also similar to the report of previous studies in Ghana and USA where lower mono-resistance was also reported [22, 24]. Furthermore, the mono-resistant observed in this study is lower compared to the 5.3% (INH), 4.3% (RIF), 0.3% (EMB) and 4.3% (SM) as well as 6.3% (INH) and 0.2% (RIF) reported from studies in Italy and USA respectively [22, 25]. The reason for the disparity may be because drug-resistant TB is not a major health problem in countries with good national TB control programs like Western Europe, except for the increasing immigrants [25, 26]. In addition, the mono-resistance of the isolates in this study among the new patients and the previously treated cases are not in agreement with the report from a similar study in Kenya. Isoniazid (6.9%) and rifampicin (8.9%) mono-resistance in this study also differs from the 16% (isoniazid) and 0% (rifampicin) reported in Russia [27].

Any resistance of the isolates to the four drugs in this study that were 23.3%, 21.7%, 20.0% and 15.0% among the new cases were higher than the 8.0%, 13.4%, 8.2% and 13.4% respectively to RIF, INH, SM and EMB in another study conducted in Swaziland [28]. Also, while the mono-resistant of the isolates from the new cases in this study was 11.7% (RIF), 5.0% (INH), 8.3% (SM) and 5.0% (EMB), it was 0.3% (RIF), 1.4% (INH), 1.7% (SM) and 0% (EMB) in the latter study. The reason for this disparity may be due to the burden of TB in both countries. While Nigeria has for a long time remained among the high burden countries, Swaziland is not. Moreover, any resistance to RIF (23.8%), INH (26.7%), SM (18.8%) and EMB (15.8%) observed in this study is lower compared to 78.1% (RIF), 92.7% (INH), 61.0% (SM) and 91.5% (EMB) reported from another study in the southern part of the country [29]. This might be due to the fact that the TB control program in the location of the present study is better compared to that of the latter study. The 11.9% MDR-TB in this study is comparably similar to 12.0%, 13.7% and 10.6% reported from similar studies in Pune (India), Cotonou (Republic of Benin) and China, respectively [19, 30, 31]. The observation is however higher than the reported 2.0% in Ghana [24], 6.6% in Cameroun [32], 4.8% in Kenya [21], 5.7% and 5% in India [20, 33], 4.0% in Mongolia [34], 1.2% in USA, 8.9% in Somalia [35] and 1.1% in Italy [25]. However, the observed MDR-TB in this study is lower compared to the 24.0% reported among high risk groups in Zimbabwe, another African country [36].

In Nigeria, previous MDR-TB reported from similar studies was a bit higher, though comparable to the findings of the present study. In Jos (Northern Nigeria) and Abeokuta (Southwestern Nigeria), 16% and 17.6% prevalence were reported respectively [1, 37]. However, the MDR-TB (11.9%) observed in this study, is higher than the 5.8% reported from a study carried out in three hospitals in Abeokuta [38]. Furthermore, the observation is far lower compared to the 53.0% previously reported from another study in the same city [12]; the reason for the disparity might be due to the study centers. While the latter study was in a referral hospital that receives patients with chronic medical conditions from different parts of the country; the participants for the present study were drawn from the DOTS centers most of which are in primary health care centres. There could therefore have been selection bias in the latter study and some cases that might have previously failed TB treatment may be regarded as new cases [10]. This is coupled with the fact that figures of tertiary care hospitals and referral centers are usually high compared to what is obtainable among the general population

[13]. The total drug resistance observed in this study was also high especially for RIF (23.3%), regarded as a surrogate marker for MDR-TB alongside that of INH (21.7%).

The primary MDR-TB (8.3%) observed in this study was lower than the acquired MDR-TB (30.8%) which is also in agreement with the report of another study in Jaipur with 4.5% primary and 24.3% acquired MDR-TB (24.3%). Also in Somalia, 5.2% and 40.8% primary and acquired MDR-TB respectively was reported [36, 30]. However, the acquired resistance in the present study is a bit lower compared to 38.6% and 43.8% previously reported from other studies in Russia and China respectively [27, 31]. But, the current observation is not in agreement with the 51.7% and 34.9% primary and acquired MDR-TB, respectively reported in Thailand, another HBC [40]. Similarly, the primary and acquired MDR-TB in this study is not in line with a lower prevalence of 1.7% and 12.0% respectively, reported in India [20]; as well as the 2% (primary) and 29.4% (acquired) reported in Kenya [21]. Moreover, this observation is also higher than the 4% (primary) and 18% (acquired) from Jos, a city in the Northern part of Nigeria [10]. That there was no statistical significance difference ($p = 0.87$) in the MDR-TB cases among HIV-negative and HIV-positive patients in this study agrees with previous reports in Argentina and USA [22, 41].

The patterns of MDR in this study, RIF-INH (5.9%), RIF-INH-EMB (0.99%), RIF-INH-SM (0.99%) and RIF-INH-SM-EMB (3.96%) is not in agreement with the RIF-INH (4.9%), RIF-INH-EMB (1.2%), RIF-INH-SM (23.2%) and RIF-INH-SM-EMB (42.7%) previously reported [29]. Consequently, the observed poly-drug resistance (INH-SM, 8.5%; INH-SM-EMB, 11.0%) reported in another study [29] was higher than the patterns (INH-SM, 1.8%; INH-SM-EMB, 2.98%) in this study. The reason for the disparity may be as a result of geographical locations of the study sites and more importantly as a result of better control program in our study area which enjoy supports from a Belgian non-Governmental organization (Daimen Foundation). Nonetheless, this study has some limitations; first, the sample size was small. Second, patients already on anti-TB treatment at the time of recruitment were excluded; hence some MDR cases could have been screened out. Third, it was only the population of those that presented at the health care centres that were included and transport costs could have limited access to care of some other patients.

In conclusion, a high MDR-TB prevalence was observed in the study. It is however not as alarming as was previously reported over a decade ago. The study also showed that primary MDR-TB is common in the overall burden of the MDR-TB in the city. Hence, active case findings in addition to expanding the drug susceptibility testing especially for the new TB patients is highly required to effectively control TB and drug resistant strains and to forestall the transmission and spread of the drug-resistant TB in the society.

Authors' Contributions: The study was designed by OIF and SIC. OIF managed literature search and data acquisition/analysis and wrote the first draft. OEF and SIBC supervised the work and read the draft. All authors read and approved the final manuscript.

Conflict of Interest: The author declares no conflicts of interest.

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