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*Original Article*

# Rifampicin resistant tuberculosis in presumptive pulmonary tuberculosis cases in Dubti Hospital, Afar, Ethiopia

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## Abstract

Introduction: Ethiopia stood third in drug-resistant tuberculosis (TB) in Africa, and more than 5,000 MDR-TB patients are reported each year. Greater than 90% of rifampicin (RIF) resistant strains are resistant to isoniazid (INH) and hence the objective of this study was to determine the prevalence and risk factors of RIF resistant MTB among presumptive TB cases at Dubti General Hospital, Afar, Ethiopia.

Methodology: In this cross-sectional study, 384 presumptive TB cases were recruited and a structured questionnaire was used to collect socio-demographic and clinical data. Sputum samples were collected and examined using *X-pertMTB/RIF* assay. Bivariate, multivariate logistic regressions, and fishers' exact analysis were done to assess the associations between the prevalence of TB and MDR-TB with different socio-demographic and clinical variables.

Results: In the present study, the overall prevalence of pulmonary TB was 24.5% (94/384), of this 4 (4.3%) isolates were resistant to RIF. History of anti-TB treatment (AOR = 2.4, 95% CI: 1.3-4.4 and TB contact (AOR = 3.6, 95% CI: 2.1-6.2 were significantly associated with *gene X-pert* MTB/RIF positive TB. Moreover, resistance to rifampicin was statistically associated with the history of TB contact with multi-drug resistant TB (P = 0.027) and khat chewer cases (P = 0.04).

Conclusions: The overall prevalence of TB and its drug-resistant were relatively higher than that of in the general population in Ethiopia. History of anti-TB treatment and TB contact were significantly associated with *X-pert* MTB/RIF positive MDR-TB.

**Key words:** RIF resistant MTB; presumptive TB; risk factors.

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## Introduction

Tuberculosis (TB) is a bacterial infectious disease caused by mycobacterium tuberculosis (MTB) that most ordinarily affects the lungs [1]. In 2014, there were 9.6 million TB cases reported globally, of which 28% were from Africa [2]. The development of multidrug resistant TB (MDR-TB) is one the global threats of people living in both developed and undeveloped countries. The emergence and spread of drug-resistant MTB strain largely associated with inadequate treatment. MDR-TB is caused by MTB that is resistant to at least two of the most powerful first-line anti-TB drugs, isoniazid (INH), and rifampicin (RIF) [3,4]. It was estimated that from 480,000 new cases of MDR-TB in the worldwide, about 9% of them were advanced to extensively drug-resistant TB (XDR-TB) and approximately 190, 000 were dead [2,5-6].

Since more than 90% RIF resistant strains are also resistant to INH [7-10], determining the RIF resistant strains, using the gene X-pert assay a rapid and sensitive technique, is an alternative approach to detect MDR-TB [11,9,10], and this could be explained by rate of spontaneous mutation rate; in which INH target gene has 100 times higher rate of mutation than that of RIF target gene [12]. Therefore, in high MDR-TB burden countries, diagnosis of RIF resistant TB is regarded as MDR-TB [9]. Furthermore, almost one-tenth (9%) of new MDR-TB cases, are likely to convert to extensively drug-resistant TB (XDR-TB) [1,5,6]

In Sub-Saharan Africa, the estimated MDRT-TB incidence is very high (14%) [1]. Ethiopia, one of the sub-Saharan African countries, stands 15$^{th}$ out of the 27 high burden countries in the world and 3$^{rd}$ in Africa and expected he yearly burden of MDR-TB exceed 5,000 cases [12,13]. The very high burden of RIF resistant TB in Ethiopia could be attributed to four broad influential classes of risk factors: Limited coverage of rapid laboratory diagnosis for RIF resistant MTB, improper

treatment of drug-susceptible TB, contact with MDR-TB patients and low socioeconomic status [12-14]. History of previous TB treatment, history of TB [15-19], monthly income < 500 Ethiopian Birr and being urban resident [15], being male and contact with the known MDR TB patient [20] have been reported as significant risk factors for the emergence of MDR TB in Ethiopia [10,12].

TB case-finding strategies for RIF resistant TB cases in Ethiopia focus on previously treated TB patients and the following groups of patients (HIV positive persons, contacts of patients with MDR-TB cases, refugees, prisoners and persons aged less than 15 years) [1,14,21,22]. Therefore, the present study was designed to assess the prevalence and assess the risk factors for RIF resistant TB at Dubti general hospital, Afar, north Ethiopia.

## Methodology

*Study design and setting*

A hospital-based cross-sectional study was conducted in Dubti general hospital, Afar region, Ethiopia, between November 2016 and May 2017.Afar region, which is located northeast of Addis Ababa in the lower Awash valley. The study site is a Dubti General Hospital, which is located 574 km northeast of Addis Ababa and 490 Km south east of Mekelle city. The hospital has 300 staff members and 200 beds. Moreover, this hospital is the only MDR TB treatment center in the region.

*Study population and sampling techniques*

Presumptive TB patients who visit Dubti General Hospital were considered as the source of population and these TB diagnosed patients willing to participate and able to give their consent were involved in this study. Critically ill patients were excluded from this study. Patients who were unable to provide sputum specimen were also excluded. Using the consecutive convenience sampling technique, 384 presumptive TB patients who visited Dubti hospital during the study period were included in the study.

*Data Collection*

Sociodemographic characteristics, clinical data and other factors associated with TB were collected using a structured questionnaire. Two to four milliliter (mL) of spot sputum specimen was collected by trained laboratory professionals in a 50 mL falcon tube and tested for the genotype of MTB by *gene X-pert* MTB/RIF assay [23]. Delayed samples were stored in the deep freezer -20°C until processed.

*X-pert MTB/RIF Assay*

Based on the manufacturer's manual, one mL spot sputum samples were treated with 2 mL of sample reagent containing sodium hydroxide (NaOH) and isopropanol. Then shake gently, homogenized and incubated for 15 minutes at room temperature. Two mL of the treated sample was transferred into the cartridge and then loaded into the gene *X-pert* instrument (Cepheid, Sunnyvale, CA, USA).

*Data Quality Assurance*

Demographic, behavioral and clinical data were collected through a structured questionnaire by trained data collector from the participants. Patient's mouth was rinsed twice with water before 2-4 mL spot sputum was collected in a sterile container (50 mL falcon tube).

*Laboratory Quality assurance*

All laboratory tests were performed by standard operating procedures of the host laboratory to ensure the reliability and validity of test results (see annex 10). Gram stains on sputum specimen were done to assure quality based on the numbers of polymorph nuclear leukocytes and squamous epithelial cells present. Sputum with polymorph nuclear leucocytes > 10/ low power field and squamous epithelial cells < 25/low power field per 10 fields was considered as good quality [21].

*Gene X-pert MTB/RIF Quality Assurance*

This was done through reagent control and sample processing control. Reagent control (probe check): Probe check was performed on every cartridge before initiation of polymerase chain reaction (PCR). During probe check, fluorescence readings in the reaction tube for each probe were compared to default settings established by Cepheid Company. If the readings did not match the default settings, the assay was terminated.

*Sample Processing Control*

This was used to verify the integrity of extracting nucleic acids, favorable reaction conditions for PCR performance and absence of excess PCR inhibitors. Using an analytic-positive test, the sample processing control was read as either negative or positive. If sample processing control was negative in an analyst-negative test, an INVALID result was reported.

*Data Management*

Data was entered and analyzed using the SPSS statistical package version 22 software for analysis.

Descriptive statistical analysis was performed for socio-demographic and clinical characteristics and bivariate and multivariate logistic regressions was performed to compute the effect of independent variables on the dependent variable and those with p-value < 0.05 at 95% confidence interval were considered as statistically significant.

*Ethical Considerations*

Ethical approval was obtained from Mekelle University, College of Health Science, Ethical Review Committee prior to data collection. An official letter was obtained from Afar regional health bureau. Permission was also obtained from administrative of Dubti General Hospital. Written for those who able to read or verbal for these didn't able to read consent and was obtained from the study participants.

## Results

*Socio-Demographic Characteristics*

A total of 384 study participants were enrolled; of whom 195 (50.8%) were females. Their ages ranged from 8 to 78 years (median of 29 (± 21)). Moreover, 216 (56.3%) were rural and 168 (43.8%) were urban residents. Majority 283 (73.7%) of them were illiterates. One hundred eighty-seven (48.7%) of the participants were unemployed and 81 (21.1%) were pastoralists. About (n = 211; 54.9%) of the study participants had a family size of 6 or fewer members. The majority (n = 216; 56.3%) were rural residents and illiterate (n = 283; 73.7%). (Table1).

*The prevalence of TB*

The overall prevalence of pulmonary TB was 24.5% (94/384); the majority of them (n = 44; 23.3%) and n = 51; 23.6%) were male and rural residents respectively. Moreover, the prevalence of TB among unable to read and write, elementary school and high school were (n = 70; 24.5%), n = 20; 27.4%) and n = 4; 23.5%) respectively. Moreover, MTB was also detected from (n = 12; 38.7%) HIV positive individuals, (n = 67; 20.9%) new TB patients and (n = 27; 42.2%) previously treated patients. Moreover, 34 (31.5%) MTB was detected among khat chewers (Table 2).

**Table 1.** Socio-demographic characteristics of presumptive pulmonary TB patients (n = 384) in Dubti general hospital, February to April 2017.

| Characteristics | | Total TB suspects | Percent |
|---|---|---|---|
| **Gender** | | | |
| | Male | 189 | 49.2 |
| | Female | 195 | 50.8 |
| **Age(in years)** | | | |
| | ≤ 25 | 152 | 39.6 |
| | 26-35 | 87 | 22.7 |
| | 36-45 | 70 | 18.2 |
| | ≥ 46 | 75 | 19.5 |
| **Residence** | | | |
| | Urban | 168 | 43.8 |
| | Rural | 216 | 56.3 |
| **Ethnicity** | | | |
| | Afar | 292 | 76 |
| | Amara | 80 | 20.8 |
| | Others* | 12 | 3.1 |
| **Education** | | | |
| | Illiterate | 283 | 73.7 |
| | Elementary school | 73 | 19.0 |
| | High school | 20 | 5.2 |
| | Diploma and above | 8 | 2.1 |
| **Occupation** | | | |
| | Pastoralist | 81 | 21.1 |
| | Self employed | 46 | 12.0 |
| | Farmer | 27 | 7.0 |
| | Unemployed | 187 | 48.7 |
| | Government employed | 43 | 11.2 |
| **Family size** | | | |
| | ≤ 6 | 211 | 54.9 |
| | > 6 | 173 | 45.1 |

*Tigray, Oromo, Somali.

*Prevalence of Rifampicin Resistant TB*

The overall prevalence of RIF resistance among the *gene X-pert* positive TB was 4(4.3%), and all were isolated from those who were unable to read and write. Moreover, 3 (16.6%) of the RIF resistant MTB cases were revealed from pastoralists. In a multivariate analysis, history of anti-TB treatment (AOR = 2.4, 95% CI: 1.3-4.4) and history of contact with TB cases (AOR = 3.6, 95% CI: 2.1-6.2) were significantly associated with *X-pert MTB/RIF* positive TB. Whereas, sex, age, residence, khat chewing, cigarette smoking, imprisonment, HIV status and alcohol consumption were not significantly associated with *X-pert MTB/RIF* positive TB (Table 3).

## Discussion

In the present study, 24.5% of the pulmonary TB suspected study participants were positive for gene *X-pert MTB/RIF* positive TB. This was lower than the previous study done in the same region Afar (32%) [24] but higher than studies done in other parts of Ethiopia [16,25]. The possible explanation for this might be these studies were community-based studies. High prevalence of *gene X-pert MTB/RIF positive* TB found in were HIV seropositive persons which is similar to previous studies done in other parts of Ethiopia [1,23].

RIF resistant TB was found in 4.3% of the study participants and was comparable to a study done in northwest Ethiopia (5.7%) [13] and Saudi (5.3%) [26]. On the other hand, it was lower (7.5%) than study in

**Table2**. Prevalence of TB among presumptive pulmonary TB cases (n = 384) in Dubti general hospital, February to April 2017.

| Characteristics | | Gene XPert MTB result | | Total |
|---|---|---|---|---|
| | | Detected n (%) | Not detected n (%) | |
| **Gender** | | | | |
| | Male | 44 (23.3) | 145 (76.7) | 189 |
| | Female | 50 (25.6) | 145 (74.4) | 195 |
| | Total | 94 (24.5%) | 290 (75.5%) | 384 |
| **Residence** | | | | |
| | Urban | 43 (25.6) | 125 (74.4) | 168 |
| | Rural | 51 (23.6) | 165 (76.4) | 216 |
| **Education** | | | | |
| | Illiterate | 70 (24.5) | 216 (75.5) | 286 |
| | Elementary school | 20 (27.4) | 53 (72.6) | 73 |
| | High school | 4 (23.5) | 13 (76.5) | 17 |
| | Diploma and above | 0 | 8 (100) | 8 |
| **Occupation** | | | | |
| | Pastoralist | 18 (22.2) | 63 (77.8) | 81 |
| | Self employed | 9 (19.6) | 37 (80.4) | 46 |
| | Farmer | 8 (29.6) | 19 (70.4) | 27 |
| | Unemployed | 51 (27.3) | 136 (72.7) | 187 |
| | Government employed | 8 (18.6) | 35 (81.4) | 43 |
| **HIV status** | | | | |
| | Positive | 12 (38.7) | 19 (61.3) | 31 |
| | Negative | 82 (23.2) | 271 (76.8) | 353 |
| **History of anti TB treatment** | | | | |
| | Yes | 27 (42.2) | 37 (57.8) | 63 |
| | No | 67 (20.9) | 253 (79.1) | 321 |
| **Chewing khat** | | | | |
| | Yes | 34 (31.5) | 74 (68.5) | 108 |
| | No | 60 (21.7) | 216 (78.3) | 276 |
| **Family size** | | | | |
| | ≤ 6 | 48 (22.7) | 163 (77.3) | 211 |
| | > 6 | 46 (26.6) | 127 (73.4) | 173 |
| **Household monthly income (ETB)** | | | | |
| | < 500 | 21 (30.9) | 47 (69.1) | 68 |
| | 501-1500 | 43 (23.0) | 144 (77.0) | 187 |
| | 1501-2000 | 11 (22.9) | 37 (77.1) | 48 |
| | > 2001 | 19 (23.5) | 62 (76.5) | 81 |

central Ethiopia [16] and Bahir Dar [27]. This variation might be due to the method variation used to diagnose MDR-TB. Moreover, our result was much lower than the study reported from Sudan (22.5%) [28,29]. These variations might be due to the study participants were patients on anti-TB treatment. However, it was higher than reports from Kenya [30,31] and Uganda [32]. Might be due to only new pulmonary TB (PTB) cases were enrolled and differences in TB control and prevention program among countries. Moreover, in our study RIF resistant MTB among the new cases was found 2.6%, which is comparable to studies reported from Ethiopia (3.38%) [33,34], Saudi (2.6%) [26] and Uganda [32]. This might be due to the difference in TB control and prevention program among the countries.

On the other side, the prevalence of RIF resistant MTB among the previously treated TB cases was 11.1%, which was comparable to studies from Uganda [32] and much lower than that of Addis Ababa, Ethiopia [35] and Saudi [26].The possible reason for the differences could be because these studies were conducted among TB treatment failure TB group. All the RIF resistant TB cases, 4(7.8%) were among rural residents. This was comparable to the findings from other places in Ethiopia [16].The most likely reason could be because of low socioeconomic status and long

**Table 3.** Bivariate and multivariate logistic regression analysis for the assessment of factors associated with MTB among presumptive pulmonary TB cases (n = 384).

| Variables | | MTB | | COR (95% CI) | AOR | |
|---|---|---|---|---|---|---|
| | | Detected n (%) | Not detected n (%) | | (95% CI) | p-value |
| **Gender** | | | | | | |
| | Male | 44 (23.3) | 145 (76.7) | 0.88 (0.71-1.81) | N/A | N/A |
| | Female | 50 (25.6) | 145 (74.4) | 1 | 1 | 1 |
| **Age (in years)** | | | | | | |
| | ≤ 25 | 34(22.4) | 118 (77.6) | 1 | 1 | 1 |
| | 26-35 | 22 (25.3) | 65 (74.7) | 0.90 (0.50-1.9) | N/A | N/A |
| | 36-45 | 21 (30) | 49 ( (70) | 1.15 (0.55-2.38) | N/A | N/A |
| | ≥ 46 | 17 (22.7) | 58 (77.3) | 1.45 (0.7-3.10) | N/A | N/A |
| **Residence** | | | | | | |
| | Urban | 43 (25.6) | 125 (74.4) | 1.11 (0.65-1.67) | N/A | N/A |
| | Rural | 51 (23.6) | 165 (76.4) | 1 | 1 | 1 |
| **Khat chewing** | | | | | | |
| | Yes | 34 (31.5) | 74 (68.5) | 1.60 (1.0-2.71) | 1.5 (0.8-2.4) | 0.153 |
| | No | 60 (21.7) | 216 (78.3) | 1 | 1 | 1 |
| **Cigarettes smoking** | | | | | | |
| | Yes | 10 (30.3) | 23 (69.7) | 1.40 (0.63-3.0) | N/A | N/A |
| | No | 84 (23.9) | 267 (76.1) | 1 | 1 | 1 |
| **Imprisonment** | | | | | | |
| | Yes | 39 (30) | 7 (70) | 1.33 (0.33-5.26) | N/A | N/A |
| | No | 91 (24.3) | 283 (75.7) | 1 | 1 | 1 |
| **HIV status** | | | | | | |
| | Positive | 12 (38.7) | 19 (69.2) | 2.10 (0.9-4.48) | 1.37 (0.6-3.2) | 0.458 |
| | Negative | 82 (23.8) | 271 (76.8) | 1 | 1 | 1 |
| **History of anti-TB treatment** | | | | | | |
| | Yes | 27 (42.2) | 37 (57.8) | 2.75 (1.56-4.84) | 2.4 (1.3-4.4) | 0.004* |
| | No | 67 (20.9) | 253 (79.1) | 1 | 1 | 1 |
| **History of contact with TB case** | | | | | | |
| | Yes | 35 (47.3) | 39 (52.7) | 3.8 (2.2-6.5) | 3.6 (2.1-6.2) | 0.00* |
| | No | 59 (19) | 251 (81) | 1 | 1 | 1 |
| **Alcohol consumption** | | | | | | |
| | Yes | 4 (30.8) | 9 (69.2) | 1.38 (0.41-4.61) | N/A | N/A |
| | No | 90 (24.3) | 281 (75.7) | 1 | 1 | 1 |

MTB: *Mycobacterium tuberculosis*, COR: Crude Odds ratio, AOR: Adjusted Odds ratio, N/A: not available.

distance from health facilities which may affect the treatment adherence. Similarly, all RIF resistant TB were from participants who can't read and write. This was comparable to previously reported studies in different parts of Ethiopia [16,34,36]. In our study, participants those who had a previous history of anti-TB treatment were 2.4 times more likely to develop RIF resistance than did not have. Moreover, participants those who was the history of contact with TB cases 3.6 times more likely to detect MTB compared to those who had no history of contact with TB cases (AOR = 3.6; 95% CI = 2.1-6.2). This might be due to the patients might have poor adherence.

In our study, though not statistically significant, MTB detection was higher among khat chewers than non-khat chewers. The possible explanation for this could be the daily chewing khat leads to poor treatment adherence. In addition, the MTB detection rate was higher among those who had a history of imprisonment and alcohol consumption than those who did not have. The higher rate (16.6%) of RIF resistance was also reported among HIV positive participants, though did not reach the statistical significance. This might be due to the low number of known previous history of anti-TB treatment case involved in the study.

## Conclusions

The overall prevalence of TB and its drug-resistant were relatively higher than that of in the general population in Ethiopia. History of anti-TB treatment and TB contact were significantly associated with *X-pert* MTB/RIF positive MDR-TB.

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