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Drug Resistance Pattern of *Mycobacterium tuberculosis* in Eastern Amhara Regional State, Ethiopia

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Abstract

Background: Tuberculosis (TB) is a major public health problem in Amhara region, Ethiopia where the TB case detection rate is low (22%). This situation has been worsened by the emergence and spread of drug resistance strains which have been threatening efforts of TB control.

Objectives: The aim of this study was to assess the magnitude of drug resistance patterns of *M. tuberculosis* in Eastern Amhara region, Ethiopia.

Methods: A facility based cross sectional study was conducted among 230 (165 new and 65 retreated) smear positive TB patients (age ≥18 years old) from September 2010 to June 2011. Socio-demographic data of the study participants and possible factors for development of drug resistance were collected using pre-tested structured questionnaire. Smear positive sputum samples were processed and decontaminated by the modified Petrof method. Primary isolation and drug susceptibility testing (DST) were carried out on egg based Lowenstein –Jensen media (LJ). Data were entered and analyzed using SPSS version 16 software. Multivariate analysis using the logistic regression model was computed. P-values less than 0.05 were considered as statistically significant.

Results: The overall prevalence of drug resistance to at least a single drug was 77/230(33.5%). The prevalence of MDR-TB in all, new and re-treated patients was 15/230(6.5%), 3/165(1.8%) and 12/65 (18.5%), respectively. In the multivariate analysis previous exposure to anti-TB drugs and 1+ bacterial load were significantly associated with anti TB drug resistance (P<0.05).

Conclusion: A high rate of drug resistance for main anti-tuberculosis drugs was observed on new and previously treated cases. Previous exposure to anti-TB drugs and bacterial load were important determinants of development of drug resistance. So, patient's adherence to anti-TB drugs (especially re-treated cases) and scaling up of DST service at district hospital level will help to reduce the development of drug resistance in the study area.

Keywords: Drug resistance; MDR; Tuberculosis; Ethiopia

Background

Currently, tuberculosis (TB) is the second most common cause of death due to an infectious disease [1]. Globally, 9.4 million incidents and 14 million prevalent cases occurred in 2010 [2]. Africa, more specifically Sub-Saharan Africa, faces the worst TB epidemic [1].

According to 2012 the World Health Organization (WHO) report Ethiopia has been one of the 22 high TB burden countries [3] with an incidence and prevalence rate of 300 and 470 cases per 100,000 populations respectively. Among all new TB cases notified to federal ministry of health, 30% were smear positive. Among re-treatment cases 64%, 11% and 13% were relapse, treatment after failure, and treatment after default respectively [4,5]. The directly observed treatment short course (DOTS) detection rate remains low, at 34%, compared with WHO's target of 70% [4,5].

The emergence and spread of DR-TB strains on new and previously treated cases have been a global threat [1,6-28]. Worldwide, 3.7% of new cases and 20% of previously treated cases were estimated to have multi drug resistance-tuberculosis (MDR-TB). Extensively drug-resistant tuberculosis (XDR- TB) has been reported in more than 70 countries [29]. The proportion of MDR-TB patients who successfully completed treatment and cured were lowered than drug susceptible forms of TB. MDR-TB associated deaths were more worsen in the African Region (19%), where many of the diagnostic and treatment facilities were limited or totally absent [29].

In Ethiopia, the level of MDR- TB among new TB cases is estimated at 1.6% and 12% for previously treated cases [2,3]. The highest rate of drug resistance (DR) were reported to Streptomycin (S) (10.2%) and Isoniazid (H) (8.4%) [26].

Development of drug resistance has been associated with different risk factors such as being male/female [20], previous history of anti-TB drug exposure [16,18,20,21,27], treatment failure [20] and presence of cavitary condition [30].

Although few studies had conducted on drug resistance in Ethiopia [24,26-28], there are certain localities such as Eastern Amhara region, where epidemiological information regarding development of drug resistance TB and possible associated factors are lacking. Therefore,

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the aim of this study was to assess the prevalence of drug resistance patterns and possible associated factors among new and re-treatment cases in Eastern Amhara region, Ethiopia. The information obtained from this study will provide baseline data about the development of anti-TB drug resistance in the region.

Material and Methods

Study design and period

A facility based cross-sectional descriptive study was conducted among new and re-treated pulmonary TB patients (age ≥ 18 years old) attended different health institutions from September 2010 to June 2011 in Eastern Amhara region, Ethiopia.

Sampling method and procedure

Forty three public health facilities (4 hospitals, 39 health centers) in Eastern Amhara, Ethiopia were selected randomly. A stratified random sampling method was used to create different strata (referral hospital, district hospital and health centers). A separate sample unit was selected from each stratum using proportionate to size. The sample size was determined using single population proportion formula with the prevalence of smear positive *M. tuberculosis* 286/100,000 [2], 95% CI, that give a final sample size of 230.

Culture and drug susceptibility testing procedure

Smear was prepared from morning sputum of each study participants to diagnose Acid Fast Bacilli (AFB) using the national TB and leprosy guideline [5]. About 5 ml of sputum sample were transported using ice pack to Ethiopian Health and Nutrition Research Institute (EHNRI), the national TB reference laboratory of Ethiopia.

Only AFB positive samples were subjected for digestion and decontamination by modified petrof method [31]. Then 0.2 ml of the processed sputum was inoculated on to Lowenstein –Jensen media (L.J) slants. All inoculated media were incubated at 37°C. The inoculated solid L.J media was inspected two times per day for the first two weeks while 3 times per weeks for the remaining 2-4 weeks [31]. The *M. tuberculosis* isolates were confirmed by colony morphology and different biochemical tests (nitratase activities, catalase and niacin test) [31].

Indirect proportional method was used to test the drug susceptibility patterns of culture positive isolates. Colonies from surface of L.J medium were transferred into sterile test tubes containing 6-8 glass beads. The suspension was adjusted to 1 McFarland standard. The final concentration of drugs were; 0.2 µg/ml for Isoniazid (H), 40 µg/ml for Rifampicin (R), 2 µg/ml for Ethambutol (E) and 4 µg/ml for Streptomycin (S) [31].

H37Rv were used as positive control while Start and end control as internal quality control. All activities like reagent and media preparation were carried out as standard operating procedure describe by Kent and Kubica [30].

Definitions

The following definitions were used based on the National TB guide line of Ethiopian federal ministry of health [5]. Defaulter is a patient whose anti TB treatment was interrupted for 2 consecutive months or more. New case means a patient who has never had treatment of TB or has been on treatment for less than four weeks. Re-treatment case is a patient previously treated for TB undergoing treatment for a new episode, usually of bacteriological active positive tuberculosis. Primary drug resistance is a drug resistant strain from a patient without a history of previous treatment. Any resistance refers to resistance to at least one anti-TB drug, while mono-resistance is a resistance to only one anti-TB drug.

Statistical analysis

Data were entered, cleaned and analyzed using SPSS (Statistical Package for Social Science) version 16 by a trained data encoder. Multivariate analysis using logistic regression model was computed. P values <0.05 were statistically significance.

Ethical considerations

Ethical clearance was obtained from Institution of Review Board (IRB) of Medical faculty, Addis Ababa University. Informed consent was obtained from each study participant. All the data were recorded using codes. The laboratory results were communicated to treating physicians for better management of the patients.

Results

Characteristics of study participants

A total of 230 smear positive respondents were enrolled in this study. Of whom 137/230 (59.6%) were males with mean age of 32.9 years. Most of the study participants were married, rural resident and literate 174 (75.7%), 261 (57.8%) and 117 (51%) respectively. One hundred and sixty five (71.7%) were new cases. While 65 of the study participants (36 relapse, 26 failure and 3 defaulter cases) had previous history of anti-TB treatment (Table 1).

Mycobacterium tuberculosis drug resistance profile of study participants

The prevalence of resistance to at least single anti- TB drug was 77 (33.5%). Among 230 isolates the highest rate of drug resistance was observed to streptomycin (S) 62 (27%). The overall prevalence of MDR-TB was 15 (6.5%). Drug resistance for one or more drugs in new cases and re-treated cases was 39/165 (23.6%) and 38/65 (58.5%) respectively. The prevalence of MDR-TB among new and re-treated

Characteristics	Frequency	Percent
Sex		
Male	137	59.6
Female	93	40.4
Age (Years)		
18 – 30	128	55.7
31 – 40	51	22.2
41 – 50	31	13.5
>50	20	8.7
Educational status		
Literate	128	55.7
Illitrate	102	44.3
Residence		
Urban	97	42.2
Rural	133	57.8
Category		
New	165	71.7
Re-treated cases	65	28.3
Overall	230	100.0

Table 1: Characteristics of study participants (n=230), Eastern Amhara region, Ethiopia, September 2010 - June 2011.

patients was 3/165 (1.8%) and 12/65 (18.5%), respectively (Table 2). Within the re-treated cases the failure cases had the highest MDR-TB prevalence 6/65 (9.23%).

Associated factors

In the multivariate logistic analyses previous drug exposure (AOR=6.4, 95 CI: 2.76-15.06) and bacterial load of 1+ (AOR=6.3, 95% CI: 2.10-18.87 were significantly associated with the development of drug resistance (Table 3). However, factors such as sex, age, residence and educational status were not significantly associated with the development of drug resistance (P>0.05) (Table 3).

Discussion

The overall drug resistance rate for at least one anti-TB drug was 33.5% (Table 2). This result was comparable with studies done in South Africa (30.2%) [21], in Kenya (30%) [32] and in Ethiopia (29.4%) [28].

In our study mono drug resistance was 17.4% (Table 2). This result was comparable with reports from Nigeria (15%) [22]. However, it was higher than a previous study done by Desta et al. [27] in Ethiopia (7.9%). This difference might be due to variations in the study population and load of drug resistance strains in different geographical location.

In the present study, a majority of single drug resistance cases were occurred by S followed by H (Table 2). This study was comparable with Demissie et al. in Ethiopia [25]. However, there were different reports regarding the frequency of different first line drugs such as Githui et al. [32] in Kenya who depicted that single drug resistance was most common to H, S and Rifampicin (R) respectively. In addition, Green et al. [20] in South Africa proved that a majority of single drug resistant cases were occurred by R. Possibly this variation might be due to utilization of different first line drugs as mono therapy, poor quality of drugs, utilization for bacterial infections other than TB, lack of pharmaceutical control, and geographical variation on load of drug resistance TB strains.

The prevalence of MDR-TB in the present study, 6.5% (Table 2) was higher than those studies conducted in Tanzania (0.4%), in Kenya (0.7%) [32,33] and in Ethiopia (0.6%) [25] While lower than in studies conducted in South Africa [34], in Burkinafaso [31], in Ethiopia (12%) [27]. This variation might be explained by difference in methods used to detect resistance strains and geographical variation on load of drug resistance TB strains.

In the present study, drug resistance for one or more drugs in new cases was 23.6% (Table 2). This finding was in line with a study conducted by Meskel et al. [23] in Ethiopia (22.4%). However it is higher than a study conducted by Demisse et al. [25] in Ethiopia (15.6%). Among new patients 3(1.8%) were MDR-TB cases (Table 2). This result was higher than previous studies done in Kenya (0.54%) [33], in Tanzania (0.4%) [35] and in Ethiopia (1.2%) [27]. The high rates of resistance among new cases in the present study might be due to lack of appropriate control and prevention systems, including delay in diagnosis that favor transmission of drug resistance strains circulating in the community.

Similarly, in the present study, drug resistance for one or more drugs observed in previously treated case was (58.5%) (Table 2), which was higher than previous studies in Ethiopia (50-53.6%) [24,27]. Moreover, 18.46% MDR-TB cases were observed in previously treated cases (Table 2). A comparable level of MDR-TB cases was observed in Nigeria (18%) [21]. However lower level of resistance reported in Kenya (8.54%) [33]. A high load of resistance among re-treated cases might be indicated that resistant mutants naturally occurring in the mycobacterial population were selected and multiplied by inadequate or interrupted treatment with anti-tuberculosis agents.

In this study, previous history of anti-TB treatment was showed statistically significant association with development of drug resistance (P<0.05). Previously anti- TB treated patients had 6.4 times more likely to develop drug resistance TB than new cases (Table 3).This result was supported by many studies in different parts of the world [16,18,20,21,23].

Sex didn't show any significant association with the development

Resistance status	New cases (n=165) n (%)	Re-treated cases (n=65) n (%)	Total (n=230) n (%)
Any resistance	39(23.6)	38(58.5)	77(33.5)
Multidrug resistance	3(1.8)	12(18.5)	15(6.5)
HR	1(0.6)	-	1(0.4)
HSR	1(0.6)	3(4.6)	4(1.7)
HSRE	1(0.6)	9(13.9)	10(4.4)
Resistance to 1 drug only	22(13.3)	18(27.7)	40(17.4)
H	7(4.2)	5(7.7)	12(5.2)
S	14(8.5)	12(18.5)	26(11.3)
R	1(0.6)	1(1.5)	2(0.9)
E	-	-	-
Resistance to 2 drugs	13(7.9)	7(10.8)	20(8.7)
HS	7(4.2)	4(6.2)	11(4.8)
SR	4(2.4)	2(3.1)	6(2.6)
SE	1(0.6)	1(1.5)	2(0.9)
HR	1(0.6)	-	1(0.4)
Resistance to 3 drugs	3(1.8)	4(6.2)	7(3.0)
HSE	2(0.12)	1(1.5)	3(1.3)
HSR	1(0.6)	3(4.6)	4(1.7)
Resistance to all drugs	1(0.6)	9(13.9)	10(4.4)

Key: H-Isoniazid, S- Streptomycin, R- Rifampicin , E-Ethambutol, MDR- TB -resistance to at least Isoniazid and Rifampicin , "_": indicates absence of resistance

Table 2: Susceptibility patterns of *M. tuberculosis* among new and retreatment cases (n=230) in Eastern Amhara regional state, Ethiopia, from September 2010 to June 2011.

Variable	Total examined No (%)	COR (95% CI)	P-value	AOR (95% CI)	P-value
Sex					
Male	137 (59.6)	0.44-1.36 (0.78)	0.320	0.25-1.67(0.43)	0.89
Female	93(40.4)	1		1	
Age (Years)					
18 – 30	128(55.7)	1			
31 – 40	51(22.2)	0.70-2.92(1.43)	0.320	0.58-34(0.97)	0.68
41 – 50	31(13.5)	0.56-3.12(1.33)	0.520	0.48-2.97(0.76)	0.62
>=51	20(8.7)	0.26-1.72(0.66)	0.400	0.31-3.45(0.45)	0.54
Educational status					
Literate	128(55.7)	1		1	
Illitrate	102(44.3)	0.8-2.42(1.395)	0.240	0.67-2.32(0.34)	0.47
Residence					
Urban	97(42.2)	0.51-1.55(0.888)	0.667	0.47-2.53(0.91)	0.72
Rural	133(57.8)	1		1	
Previous drug exposure					
New	165(71.7)	1			
Re-treated cases	65(28.3)	2.47-8.37(4.547)	0.000	2.76-15.07 (6.4)	0.000
Bacterial load					
Scanty	25(10.9)	1			
1+	89(38.7)	1.67-10.94(4.27)	0.002	2.10-18.87(6.3)	0.010
2+	76(33)	0.67-4.12(1.66)	0.270	0.66-5.25(1.86)	0.240
3+	40(17.4)	0.59-4.45(1.63)	0.340	0.64-6.51(2.04)	0.230
Overall	230				

Table 3: Drug susceptibility patterns of smear positive cases (n=230) with different variables in Eastern Amhara region, Ethiopia from September 2010 to June 2011.

of drug resistance (Table 3). WHO 2010 report also supported this finding, in which overall risk of harboring drug resistant strains was not influenced by sex [36].

In the present study, bacterial load (1+) independently contributed 6.3 times more for the development of drug resistance strains than those TB patients with scanty load (Table 3). This was inconsistent with the previous report suggest cavitary conditions and high bacillary load in the lungs contribute to the emergence of drug resistance strains [37]. The high drug resistance in those TB patients with 1+ bacterial load might be due to poor socio-economic status of the study participants. Large scale researches are needed to come to a definite conclusion.

Conclusions

High rate of drug resistance of main anti-tuberculosis drugs was observed on new and previously treated cases in this study. This situation might threaten efforts of TB control activities and further aggravate development of MDR-TB. Previous exposure to anti-TB drug and bacterial load were important determinants of development of drug resistance. Therefore, it is essential to address the problems of development of drug resistant strains of TB by establishing good TB control programs (DOTS and DOTS plus). Patient's adherence to anti-TB drugs (especially re-treated cases) and scaling up of DST service at district hospital level will help to reduce the development of drug resistance in the study area.

Competing Interests

This work was sponsored by USAID/TB CTA, Ethiopia. No future financial aid was received from any organization for publication or other interest. There is no any competing of interest.

Author's Contributions

AE: conception and initiation of the study, design, implementation, analysis and writing. KD: initiation, design, implementation, analysis and co-writing. IA and ME: design, implementation and co-writing. MA and MG: implementation, and analysis. All authors read and approved the final manuscript.

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