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ORIGINAL RESEARCH

Rifampicin-Resistant Multidrug-Resistant Tuberculosis Cases in Selected Hospitals in Western Oromia, Ethiopia: Cross-Sectional Retrospective Study

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Purpose: With the recommendation of World Health Organization (WHO) and as per the Ethiopian National Implementation Guideline, GeneXpert is used for rapid diagnosis of rifampicin (RIF)-resistant multidrug-resistant tuberculosis (MDR-TB) from the suspected TB patients; however, there were limited findings in Ethiopia particularly in the study area showing the magnitude of RIF-resistant MDR-TB and related factors among suspected TB cases. Hence, we aimed to assess resistance to RIF as a biomarker for the detection of MDR-TB cases from the suspected TB patients in selected hospitals, Western Oromia, Ethiopia.

Patients and Methods: We have conducted a cross-sectional review on 2300 registered GeneXpert data as clinically suspected TB cases in three governmental hospitals, Western Oromia, Ethiopia, between October 2015 and April 2016 to assess resistance to RIF as a biomarker for the detection of MDR-TB cases. Trained data collectors enumerated the data using pre-tested semi-structured questionnaires from the Gene Xpert records found in the registration logbook available at each hospital laboratories. Following checking the data for completeness, we have cleaned and entered our data into SPSS version 20 to compute different analyses. P-value of ≤0.05 was taken as statistically significant.

Results: A total of 2300 TB suspected cases were included in the study. The overall prevalence of TB diagnosed by the GeneXpert assay was 21.3% (491/2300). In all TB confirmed cases, RIF-resistant TB accounted for 25.9% (127/491) which expressed *rpoB* gene mutations. Sex (being male), age (within 16–30 age group), patient category (relapse, loss to follow-up, treatment failure and had MDR-TB contact) were significantly associated with rifampicin-resistant TB. Relapse patient was 20 times more likely to develop MDR-TB when compared to the new patient (P-value= 0.01, COR = 20.0, 95%C.I = 17.5–42.5).

Conclusion: The rifampicin-resistant TB is prevalent in all age groups. The strong association and high prevalence of RIF-resistant TB to failure after treatment in this study require more attention towards improving the treatment to minimize evolving of the MDR-TB cases.

Keywords: GeneXpert, *M. tuberculosis*, RIF-resistant MDR-TB, Oromia, Ethiopia

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Introduction

Tuberculosis (TB) remains a major public health problem in causing mortality and morbidity.[1] The emergence of drug-resistant isolates/strains has become an area of concern among scientists since it is too difficult to manage and control the disease. Since rifampicin resistance is an important indicator of multidrug-resistant TB

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detection, they help to know the magnitude of the problem and early management of multidrug-resistant TB.[2] GeneXpert MTB/RIF assay (Cepheid, Sunnyvale, CA, USA) is a real-time polymerase chain reaction (RT-PCR) that detects *Mycobacterium tuberculosis* as well as mutations that confer resistance to RIF in <2 hours.[1]

Extensive studies on the *rpoB* gene in rifampicin-resistant *M. tuberculosis* identified a variety of mutations and short deletion in the gene. More than 95% of the mutations are located in an 81 bp (1294–1375 bp) core region, Rifampicin Resistance Determining Region (RRDR) of the *rpoB* gene between codons 507–533 with the most common changes in codons*Se531Leu, His526Tyr*, and Asp*516Val*. These changes occur in more than 70% of rifampicin-resistant isolate.[3–6] The control of such drug-resistant disease is more difficult especially in endemic settings like Ethiopia due to having sub-standard laboratory services and lack of culture-based drug susceptibility testing at catchment areas, such a delay that results in an adverse effect.

Therefore, the implementation of molecular methods like GeneXpert assay for rapid detection of drug-resistant MDR-TB serves as an alternative to culture-based drug susceptibility testing (DST) as per the WHO recommendation in low-income countries like Ethiopia.[3] Studies conducted in different geographical locations indicated that the burden of MDR-TB and the mutations responsible for drug resistance vary from country to country and region to region.[7]

However, there is a scarcity of information concerning the magnitude of MDR-TB cases in the study area, western Oromia. Hence, to fill this gap, we have assessed all of the records of Gene Xpert results between November 2015 and April 2016 to know the magnitude of MDR-TB cases and rifampicin resistance in three hospitals, Western Oromia, Ethiopia.

Patients and Methods

Study Design, Area and Period

A facility-based retrospective cross-sectional study was conducted between November 2015 and April 2016 to assess resistance to RIF as a biomarker for the detection of MDR-TB cases in three general hospitals, namely Nedjo, Gimbi, and Nekemte hospitals, Western Oromia, Ethiopia. These hospitals are governmental hospitals in the west part of Ethiopia, which are 500, 430, and 318 km, respectively, far away from the capital city, Addis Ababa. Besides this, these hospitals were too busy among the hospitals in western Ethiopia in providing referral services including TB diagnosis and treatment.

Study Population and Eligibility Criteria

All TB suspected (patients with clinical signs and symptoms suggestive of tuberculosis) who visited the study area during the study period were the study population. TB suspected patients who have submitted a sputum sample for GeneXpert analysis; whose variables like sex, age, HIV status, and Xpert MTB/RIF results have registered in the laboratory registration book were included in the study whereas, patients who had incomplete data of the mentioned above were excluded from the study.

Sampling Technique and Data Collection

Data were retrieved consecutively from the laboratory registration book of Gene Xpert result during the study period using the data extraction sheet.

Gene Xpert Assay Procedure

As per the standard operating procedure, the GeneXpert assay was performed on sputum specimens to detect MDR-TB cases.[8] Sputum samples are treated with sample reagent (SR) containing NaOH and isopropanol. The SR is added using a 2 to 1 ratio of the sputum sample, homogenized and incubated for 15 min at room temperature. The treated sample was then transferred into the GeneXpert cartridge and inserted into the real-time polymerase chain reaction (RT-PCR) machine for DNA extraction and amplification of 192 bp segments of the *rpoB* gene.[8]

Ethical Clearance

Ethical approval and clearance were received from the research and ethics review committee (RERC) of Wollega University, Institute of Health Science. A letter of request was officially written to the respective hospital administrator office to facilitate the data collection process. The data were not disclosed to the third body for confidentiality issues during and after data collection.

Data Analysis

Following checking the completeness of the data, we have entered and analyzed our secondary data into SPSS version 20 to see the association between dependent and outcome variables using OR with 95% confidence interval (C.I) and P-value ≤0.05 indicating statistical significance.

Results

Out of 2300 registered Gene Xpert result of RIF-resistant TB suspected cases, 1159 (50.4%) were males and the rest were females. The detection rate of GeneXpert for *M. tuberculosis* was 21.3% (491/2300) and from the positive TB cases, 25.9% (127/491) showed RIF-resistance by GeneXpert assay. From the MDR-TB identified, 15.7% and 42.5% were new and failed after completed the treatment, respectively (Table 1).

MTB^{+}/RIF^{+} detection by GeneXpert technique was 2 times more likely to be positive in males when compared to females [COR, 95% CI 2.01 (1.0, 2.9), P= 0.01] and showed statistically significant association in the age group of 16–30 (P-value = 0.001). MTB^{+}/RIF^{-}detection by molecular technique did not vary with the sex of the patients (P-value = 0.2). But it was statistically associated with the age group of 16–30 (P-value =0.01) (Table 2).

Mycobacterium tuberculosis diagnosis by the GeneXpert was statistically associated with the different patient categories when it was compared with a new patient (P-value ≤0.01). Relapse patient was 20 times more likely to develop MDR-TB by Gene Xpert MTB/RIF-resistance when compared to new patient (P-value ≤0.01, OR = 20.0, 95% CI = 17.5–42.5). Moreover, loss to follow-up and failure after treatment were statistically significant on MDR-TB diagnosed by GeneXpert/RIF-resistance (P-value ≤0.01) (Table 3).

Discussion

The emergence of the drug-resistance poses a serious threat to global TB control. The rapid detection and identification of drug-resistant *Mycobacterium tuberculosis* is a challenge in Ethiopia due to limited laboratory facilities and the absence of molecular techniques. This might have indications of incorrect patient management, abusing the use of anti-TB drugs, and finally leading to emerging of drug-resistance strains.[9,10] Therefore, this molecular identification using the GeneXpert technique

Table 1 Distribution of Selected Socio-Demographic Characteristics and Their HIV-Status in Relation to GeneXpert Results Among MDR-TB Suspected Patients in Three Hospitals, West Oromia, Ethiopia, 2016 (n=2300)

Variables	Nekemte Hospital			Gimbi Hospital			Nedjo Hospital		
	GeneXpert Results			GeneXpert Results			GeneXpert Results		
	MTB^{+}/RIF^{-} n=70 (%)	MTB^{+}/RIF^{+} n=67 (%)	MTB^{-}/RIF^{-} n=590(%)	MTB^{+}/RIF^{-} n=205(%)	MTB^{+}/RIF^{+} n=37 (%)	MTB^{-}/RIF^{-} n=926(%)	MTB^{+}/RIF^{-} n=89 (%)	MTB^{+}/RIF^{+} n=23 (%)	MTB^{-}/RIF^{-} n=293(%)
Sex									
Male	40(57.1)	45(67.2)	331(56.1)	98(47.8)	24(64.9)	432(46.6)	38(42.7)	15(65.2)	136(46.4)
Female	30(42.9)	22(32.8)	259(43.9)	107(52.2)	13(35.1)	494(53.4)	51(57.3)	8(34.8)	157(53.4)
Age									
1–15	4(5.7)	2(3.0)	38(6.4)	27(13.2)	4(10.8)	165(17.8)	9(10.1)	2(8.7)	45(15.4)
16–30	24(34.3)	41(61.2)	203(34.4)	95(46.3)	19(51.4)	323(34.9)	46(51.7)	15(65.2)	91(31.1)
31–45	24(34.4)	18(26.9)	172(29.2)	49(23.9)	11(29.7)	236(25.5)	18(20.2)	5(21.7)	87(29.7)
46–60	14(20.0)	5(7.5)	134(22.7)	20(9.8)	3(8.1)	121(13.1)	11(12.4)	1(4.3)	48(16.4)
61–75	2(2.8)	1(1.5)	39(6.6)	8(3.9)	0(0.0)	62(6.7)	3(3.4)	0(0.0)	14(4.8)
≥76	2(2.8)	0(0.0)	4(0.1)	6(2.9)	0(0.0)	19(2.1)	2(2.2)	00(0.0)	8(2.7)
Patient category									
New	47(65.3)	10(14.9)	507(85.9)	106(51.7)	5(13.5)	697(75.3)	55(64.0)	5(21.7)	209(71.3)
Relapse	21(29.2)	8(11.9)	68(11.5)	84(40.9)	5(13.5)	198(21.4)	25(28.1)	2(8.7)	68(23.2)
Loss to follow-up	0(0.0)	4(5.97)	5(0.85)	7(3.4)	7(18.9)	12(1.3)	4(4.5)	4(17.4)	4(1.4)
Failure after Rx	2(2.8)	30(44.8)	10(1.7)	4(1.9)	15(40.5)	19(2.1)	2(2.2)	9(39.1)	12(4.1)
MDR-TB contact	0(0.0)	15(22.4)	0(0.0)	4(1.9)	5(13.5)	0(0.0)	3(3.4)	3(13.0)	0(0.0)
HIV-status									
Positive	3(4.2)	15(22.4)	57(9.7)	14(6.8)	10(27.0)	95(10.3)	14(15.7)	5(21.7)	24(8.2)
Negative	11(15.3)	51(76.1)	79(13.3)	57(27.8)	26(70.3)	120(13.0)	28(31.5)	16(69.6)	21(7.2)
Unknown	56(77.8)	1(1.5)	454(77)	134(65.4)	1(2.7)	711(76.7)	47(52.8)	2(8.7)	248(84.6)

Abbreviations: MTB^{+}/RIF^{-}, *M. tuberculosis* detected; MTB^{+}/RIF^{+}, *M. tuberculosis* detected as well as RIF resistance; MTB^{-}/RIF^{-}, *M. tuberculosis* no detected as well as RIF resistance.

Table 2 Diagnostic Characteristics of Study Population by Sex and Age in Three Hospitals, 2016

Variables	GeneXpert Results								
	MTB^+/RIF^- n=491(%)	**COR[95% CI]**	**P-value**	**MTB^+/RIF^+ n=127(%)**	**COR[95% CI]**	**P-value**	**MTB^-/RIF^- n=1808(%)**	**COR[95% CI]**	**P-value**
Sex									
Male	260(53.0)	0.9[0.72–1.01]	0.2	84(66.1)	2.0[1.4–2.9]	0.01	899(49.7)	1.2[0.9–1.4]	0.22
Female	231(47.0)	1.0		43(33.9)	1.0		909(50.3)	1.0	
Age									
1–15	48(9.9)	1.0		8(6.3)	1.0		248(13.7)	1.0	
16–30	236(48.5)	0.5[0.35–0.70]	0.01	75(59.1)	0.3(0.14–0.64)	0.001	616(34.1)	2.0[1.4–2.8]	0.981
31–45	125(25.7)	0.8[0.5–1.10]	0.154	34(26.8)	0.5(0.22–1.05)	0.065	495(27.4)	1.3[0.9–1.9]	0.245
46–60	53(10.9)	1.1[0.70–1.7]	0.703	9(7.1)	1.1(0.41–2.82)	0.885	303(16.8)	0.9[0.6–1.4]	0.903
61–75	14(2.9)	1.6[0.80–3.00]	0.152	1(0.8)	3.6(0.44–28.73)	0.234	115(6.4)	0.6[0.3–1.2]	0.651
≥76	10(2.0)	0.6(2.8–1.30)	0.198	0(0.0)	0(0)	-	31(1.7)	1.7[0.8–3.6]	0.453

Abbreviations: MTB^+/RIF^-, *M. tuberculosis* detected; MTB^+/RIF^+, *M. tuberculosis* and resistance to Rif detected; MTB^-/RIF^-, *M. tuberculosis* and resistance to RIF not detected.

Table 3 GeneXpert MTB/RIF Assay in Different Patient Category in Three Hospitals, 2016

Variables	GeneXpert Results					
	MTB^+/RIF^- n=491(%)	**COR [95% CI]**	**P-value**	**MTB^+/RIF^+ n=127(%)**	**COR [95% CI]**	**P-value**
Patient category						
New	228(46.4)	1.0		20(15.7)	1.0	
Relapse	145(29.5)	0.4[0.3–0.5]	0.01	15(11.8)	20[17.5,42.5]	0.01
Loss to follow-up	26(5.3)	0.1[0.07–0.2]	0.01	15(11.8)	12[9.8,24.1]	0.01
Failure after Rx	62(12.6)	0.1[0.06–0.16]	0.01	54(42.5)	5[4.7,15.2]	0.01
MDR-TB contact	30(6.1)	0.00	0.997	23(18.1)	3[1.2,7.8]	0.01

Abbreviations: MTB^+/Rif^-, *M. tuberculosis* detected; MTB^+/Rif^+, *M. tuberculosis* detected as well as resistance to RIF.

offers an opportunity for early detection and timely management of MDR-TB cases in endemic areas.[11–13] Our report indicated that the prevalence of *M. tuberculosis* diagnosed by GeneXpert was 21.3% (491/2300). From these, the prevalence of RIF-resistant MDR-TB cases detected using GeneXpert was 25.8% (127/491). Our finding was comparable with previous reports from similar findings reported in Ethiopia and elsewhere in the world (19.4–45.3%).[14–16] But it was higher than similar findings reported in Southern, Northern Eastern, and Northwest part of Ethiopia with the prevalence of 3.4%, 2.5%, and 1.7%, and 9.3[17–20] respectively. This difference might be attributed to unorganized patient diagnosis, treatment, and follow-up that may contribute to the higher prevalence of rifampicin resistance in the study area. Besides this, variation in the design of the study, study population, sample-related factors, access to health care facilities, geography, and effectiveness of TB control program might be possible reasons for the differences in GeneXpert test results.

Worldwide, the proportion of new cases with MDR-TB is 3.5% higher among people previously treated for TB, at 20.5% in 2013.[21] In the present study, of the total resistance cases, 15.7% (20/127) was new patients and 42.5% (54/127) in the failure after treatment cases. The Rif resistance among new and TB treatment failure was high when it was compared with word wide. The high level of drug resistance among new cases in this study might be due to the exposure of patients to anti-TB drugs before they visited health institutions or exposure to drug-resistant *M. tuberculosis* strain in the community. According to the different sources, the association between TB treatment failure and MDR-TB might be related to unsatisfactory compliance by patients, lack of supervision of treatment, improper drug regimens, and inadequate or irregular drug supply that may potentiate drug resistance.[22–26] Also, this may indicate that whether the patients are not strictly following the Directly Observed Therapy; a short course (DOTS) program or the gene of the bacteria may mutate and form resistance to the first

lines of anti-TB drugs. Relapse patient was 20 times more likely to develop MDR-TB by GeneXpert MTB/RIF when compared to new patient [P-value ≤0.01, OR = 20.0, 95% CI = 17.5–42.5]. The association between relapse and MDR-TB might be due to poor adherence to patients that may potentiate secondary drug resistance.

The increased incidence of MDR-TB cases affects its management and increases the motility rate in high burden countries like Ethiopia. Culture is a better diagnosis for tuberculosis although it consumes more resources like time and human power.[27] Thus, the implementation of molecular methods like GeneXpert for the rapid detection of drug-resistant *mycobacterium* species is crucial. Recently, WHO and the Ethiopian National Implementation Guideline for GeneXpert strongly recommend the use of GeneXpert for the initial diagnosis of individuals suspected of MDR-TB for rapid screening of MDR-TB.[28–30] Moreover, the identification of *tubercle bacilli* by culture is required for the ultimate proof of Mycobacterial infection. However, due to the unavailability of laboratory equipment and safety procedures, the method is not practiced in resource-poor settings.[31,32]

Molecular methods in the specific target like GeneXpert employed for rapid identification of *mycobacteria* from clinical specimens.[33] This indicated that the result obtained from a rapid molecular method like the GeneXpert test in our present study has contributed to early management of TB patients, which avoids adverse drug reaction, incorrect use of TB treatments and emerging of drug-resistant strains. In addition to this, the GeneXpert assay helps for early detection of TB cases and prompt treatment that ensures better treatment outcomes and reduced TB transmission to contacts.[34]

Limitation of the Study

Here are limitations that include the use of the small-scale sampling and retrospective study design to assess RIF-resistant MDR-TB infection status, which may have probably underestimated the finding. We also lacked doing culturing to identify isolates.

Conclusion

Our study indicated the high prevalence of rifampicin-resistant TB was detected in the study area. Sex (being male), age (16–30 age group), patients who lost follow-up, failed treatment and on relapse were significantly associated with MDR-TB cases. Besides this, RT-PCR helps to diagnose both TB and RIF-resistant TB cases simultaneously.

Data Sharing Statement

The data and material set supporting the results of this article are included in the article. In case needed, it can be accessed upon formally requesting the corresponding author.

Ethical Consideration

Prior to conducting the study, we have received ethical approval and clearance from the research and ethics review committee (RERC) of Wollega University. Official letters to the respective hospitals were obtained from the Wollega University. The hospital administrative officers permitted us to collect data. Then after, the questionnaire was given to laboratory professionals based on the informed consent.

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Author Contributions

All authors contributed to data analysis, drafting or revising the article, have agreed on the journal to which the article will be submitted, gave final approval of the version to be published, and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest for this work.

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