Mycobacterial Diseases
ISSN: 2161-1068

Nigus DM, J Mycobac Dis 2014, 4:3
DOI: 10.4172/2161-1068.1000152

Research Article Open Access

Prevalence of Multi Drug Resistant Tuberculosis among Presumptive Multi Drug Resistant Tuberculosis Cases in Amhara National Regional State, Ethiopia

Daniel Mekonnen Nigus[1*], Wondemagegn Mulu Lingerew[1], Bayeh Abera Beyene[1], Aschalew Admassu Tamiru[2], Martha Tibebu Lemma[1] and Mulat Yimer Melaku[1]

[1]*Bahir Dar University, College of Medicine and Health Sciences, Department of Medical Microbiology, Parasitology and Immunology, Bahir Dar, Ethiopia*

[2]*Bahir Dar Regional Health Research Laboratory Center, Department of Regional Mycobacteriology Laboratory, Bahir Dar, Ethiopia*

[*]**Corresponding author:** Daniel Mekonnen Nigus, Bahir Dar University, College of Medicine and Health Sciences, Ethiopia, Tel: +251-912 99 02 88; E-mail: nigusdaniel@gmail.com

Rec date: Mar 05, 2014; **Acc date:** Apr 26, 2014; **Pub date:** Apr 30, 2014

Abstract

Background: Multidrug resistant tuberculosis (MDR - TB) is becoming a major public health problem in Ethiopia. According to Ethiopian national drug resistance survey (2005), the prevalence of MDR-TB among new and retreatment cases was 1.6% and 12% respectively. So far there was no latest report on the prevalence of MDR-TB in Ethiopia, especially in the study area. The aim of this study was to determine the prevalence of MDR-TB among presumptive MDR-TB cases found in Amhara National Regional State, Ethiopia.

Methods: Across sectional study was done in Amhara National Regional State from May 2012 to May 2013. Samples processed using 2% N-acetyl-L-cysteine-sodium hydroxide for Lowenstein Jensen culture and Ziehl-Neelsen staining. Resistance to rifampicin and isoniazid was made using molecular line probe assay. Binary logistic regression analysis was done to compute P-value, odds ratio and confidence interval and P-value<0.05 was considered as statistically significant. Multivariate analysis was computed to identify the independently associated factors.

Results: A total of 606 Presumptive MDR-TB cases were took part in the study and screened for MDR-TB status. The overall prevalence of MDR-TB was 93(15.3%). Rifampicin (RMP) and isoniazid (INH) mono resistance were 17(2.8%) and 15(2.5%) respectively. Considering RMP mono resistance as surrogate marker for MDR TB, prevalence of MDR TB/RMP resistance was 110(18.2%). Moreover, the rate of MDR TB among smear and/or culture positive samples were 42.9% and together with RMP mono resistance, it increased to 50.7%. Age at a range of 21-30 years old, being female and TB history of defaulters were significantly associated with having MDR-TB.

Conclusions: MDR-TB is a major public health problem and mainly affects economically productive age group of the population and females. This is a threat to TB control programme in Ethiopia so that MDR-TB ward, diagnostic facility, and surveillance activities should be expanded.

Keywords: MDR-TB; Prevalence; Presumptive MDR-TB cases; Amhara National Regional State; Ethiopia

Abbreviations:

WHO: World Health Organization; MDR-TB: Multi Drug Resistance Tuberculosis; TB: Tuberculosis, NALC-Naoh: N-Acetyl-L-Cysteine-Sodium Hydroxide; L-J : Lowenstein Jensen; RMP: Rifampicin ; INH: Isoniziad; INH MR: Isoniziad Monoresitance; RMP MR: Rifampicin Monoresitance; Hbcs : High Burden Countries; ANRS : Amhara National Regional State; BRHRLC: Bahir Dar Regional Health Research Laboratory Center; CSA: Central Statistical Agency; ZN: Ziehl-Neelsen Staining ; LPA: Line Probe Assay Method; COR: Crude Odds Ratio; AOR: Adjusted Odds Ratio; R: Resistance

Introduction

Despite the availability of highly efficacious treatment for decades, tuberculosis (TB) remains a major global health problem and ever increasing [1]. The latest estimates showed that there were almost 9 million new TB cases in 2011 and 1.4 million TB deaths [2]. Most of the high burden countries (HBCs) have rates of around 150-300 cases per 100,000 population [3]. According to Ethiopian national prevalence survey 2010-2011, the prevalence of smear positive TB among the total population was 63 per 100,000 [4,5].

Drug-resistant and multidrug-resistant (MDR-TB); resistant to at least isoniazid [INH] and rifampin [RMP]) strains of *Mycobacterium tuberculosis* are man-made problems mainly related to poor case management and lack of quality drugs [6]. *M. tuberculosis* complex use several strategies to resist the action of antimicrobial agents [6]. Eighty five percent of MDR-TB occurs in 27 countries [7-9]. Globally, 3.7% of new cases and 20% of previously treated cases are estimated to have MDR-TB [3]. Ethiopian national drug resistance survey of 2005 estimated the prevalence of MDR-TB among new and retreatment cases was 1.6% and 12% respectively [1,2,5,10,11].

TB control activities require regular direct measurement of the absolute burden of disease to monitor trends and improve understanding of the epidemiology of TB in the target area [12]. In many HBCs, notification systems do not record all cases and vital registration systems are either absent or of such poor quality and

coverage that TB statistics is often unreliable [13]. So far there was no latest report on the prevalence of MDR-TB in Ethiopia, especially in the study area, Amhara National Regional State (ANRS). This study aimed to determine the prevalence and associated factors of MDR-TB among presumptive MDR-TB cases in ANRS.

Methods and Materials

Study design, period and area

A cross sectional study was conducted in ANRS from May/ 2012 through May/2013 among presumptive MDR-TB cases. Samples were collected from all zones of ANRS. The collected samples (Sputum, sterile body fluids, pus and tissue) were processed at Bahir Dar Regional Health Research Laboratory Center (BRHRLC). The region has an estimated area of 159,173.66 square kilometers and an estimated density of 108.2 people per square kilometer [14]. Based on the 2007 Census conducted by the Central Statistical Agency of Ethiopia (CSA), the ANRS has a population of 17, 221,976 [14]. At 91.47% of the local population, ANRS is predominantly inhabited by people from the Semitic-speaking Amhara ethnic group. Most other residents hail from other Afro-Asiatic language communities, including the Agaw/Awi, Oromo, and Argobba [14].

Simple size and sampling technique

Consecutively, a total of 606 presumptive MDR-TB cases living in ANRS were included in this study.

Data and specimen collection

Data on age, sex, HIV status, treatment history and type of TB were collected using standardized data collection sheet. Specimens were collected using 50 ml fulcon tube, packed and transported to RHRLC according to the international standards of WHO recommendation for transport of biological substance; category B, UN-3373 and arrived within 3 days of collection for processing within 7days of its collection.

Specimen processing

The collected samples processed by the conventional 2% N-acetyl-L-cysteine– NaOH (NALC-NaOH) method (final NaOH concentration, 1%). After decontamination, the concentrated sediment was suspended in 1.0 ml sterile phosphate buffer (pH 7.0) for inoculation to Lowenstein–Jensen (LJ) medium. Smears were prepared for Ziehl-Neelsen staining (ZN). For smear and /or culture positive samples, DNA extraction was carried out using mechanical and chemical methods alternatively for MDR-TB detection using molecular line probe assay method (LPA). For mechanical method of DNA extraction, 300 µl of colony suspension and 500 µl of sediment samples were centrifuged at 13,000 g for 15 min, the supernatant discarded, and the pellet was resuspended in 100 µl distilled water. Subsequently, the suspension was boiled for 20 minute at 90°C then sonication using Elma sonic for 15 minute. Finally it was centrifuged at 10,000 g for 5 min and the supernatant was transferred to a new 1.5 ml eppenddrof tube [15].

In Chemical method of DNA extraction, a loop full colony mixed with 300 ml DNA/RNA free molecular grade water or 500 µl sediment was centrifuged at 13,000 g for 15 min, the supernatant was discarded, and each pellet was resuspended in 100 µl of Lysis Buffer (A-LYS) and then incubated for 5 min at 90°C for further killing and lyses. Finally 100 ml of neutralization buffer (B-NB) was added and centrifuged at 10,000 g for 5 min then supernatant was aspirated in to sterile 1.5 ml of eppedroff tube [15].

Resistance to RMP and INH were done molecularly using line probe assay. Master mix preparation, amplification and hybridization were performed as recommended by the manufacturer [15].

Data quality assurance and analysis

Both the solid culture and LPA laboratory were validated by national TB reference laboratory and the procedure was done by trained microbiologist. The laboratory also participated in external quality assurance network by national and global laboratory initiative. Both positive and negative controls were used during each batch of specimen processing and DNA extraction. Lot to lot quality assurance system was used for verification of new LPA reagents. Randomly selected 200 specimens were sent to national TB laboratory for validation of the BRHRLC MDR-TB laboratory results.

Data entered, cleared, and analyzed using the SPSS statistical software package, Version 20 (SPSS Inc., Chicago, IL, USA). Variables descriptively expressed by number and percent. Comparisons between MDR-TB and /or RMP mono resistance (MR) and non MDR were done using odds ratio and 95% confidence interval (CI). Uni-variate and multi-variate logistic regression used to assess various factors that were potentially associated with the risk of MDR-TB.

Ethical considerations

Ethical clearance was obtained from Bahir Dar University Research Ethical Review Committee (RERC). Support letter was written to BRHRLC, MDR-TB treatment initiation sites, Hospitals and Health centres. The respective facilities accept and facilitate the data collection.

Informed consent was obtained from all study participants though their respective health facility authority and BRHRLC. Confidentiality of results was maintained. All results were given to regional MDR-TB department for further diagnosis and treatment.

Results

Sociodemographic characteristics

A total of 606 presumptive MDR-TB cases, of whom 363 (59.9%) male, 243 (40.1%) females, with female to male ratio of 1:1.5 were participated in the study. The mean age of the participants was 35.6 years, ranging from 2-80 years old. Majority of the participants (32.8%) were in the age group 21-30, followed by 31-40 (23.9%) (Table1). Majority of patients, 218 (36%) were from North Gondar zone where the first MDR-TB treatment initiation centre was found followed by North Wollo zone dwellers 64 (10.1%). More than half of participants notified from health centres 351 (57.9%) followed by hospitals 253 (41.7%). However, only 2 patients were notified from private health facilities (Table 1).

Variables	Frequency	Percent
Sex		
Male	363	59.9
Female	243	40.1

Age (in year)		
1-10	6	1
11-20	76	12.5
21-30	199	32.8
31-40	145	23.9
41-50	93	15.3
≥51	87	14.4
Residency by zone		
North Gonder	218	36
South Gonder	61	10.1
North Wollo	64	10.6
Bahir Dar	61	10.1
Awi zone	18	3
West Gojjam	23	3.8
East Gojjam	56	9.2
North Shewa	40	6.6
Oromiya	6	1
South Wollo	59	9.7
Referring health facility		
Government Hospitals	253	41.7
Government Health centres	351	57.9
Private health facilities	2	0.3
Total	606	100

Table 1: Sociodemographic characteristics of presumptive MDR-TB cases in ANRS, 2013

Levels of MDR-TB

Of the 606 specimens analyzed, 190 (31.4%) and 416 (68.6%) were smear positive and negative respectively. When concentrated sediment prepared by NALC-NaOH method and inoculated on LJ medium, 163 (26.9%) positive by both culture and smear microscopy. Moreover, 370 (61.1%) and 211 (34.8%) were negative and positive by solid culture respectively. The remaining, 20 (3.3%) and 5 (0.8%) were contaminated and non *M. tuberculosis* (NTM) respectively.

The prevalence of MDR, RMP-MR and INH-MR were 93 (15.3%), 17 (2.8%) and 15 (2.5%) respectively. Ninety two (15.2%) of participants were susceptible to both RMP, and INH. According to WHO recommendation, considering RMP MR as surrogate marker for MDR TB, prevalence of MDR TB/RMP resistance was 110 (18.2%). The prevalence of INH resistance was 108 (17.8%). Moreover, the rate of MDR TB among smear and/or culture positive samples were 93/217 (42.9%) and together with RMP mono resistance, it increased to 110/217 (50.7%). However, the prevalence of INH resistance was 108 (49.8%) (Table 2).

Anti TB drugs	Resistance pattern	All suspects	Culture and/ or smear
		(N=606), No (%)	Positive (N=217), No (%)
RMP	R	110(18.2)	110(50.7)
	S	107(17.7)	107(49.3)
INH	R	108(17.8)	108(49.8)
	S	109(18)	109(50.2)
MDR	R	93(15.3)	93(42.9)
RMP and INH	S	92(15.2)	92(42.4)
RMP mono	R	17(2.8)	17(7.8)

Table 2: Resistance pattern of *M. tuberculosis* against RMP and INH among presumptive MDR-TB and smear and/or culture positive cases, ANRS, 2014

Prevalence of MDR-TB among females and males were 53 (21.8%) and 57 (15.7%) respectively [AOR (95%CI) = 0.7 (0.44- 1.0)]. Prevalence of MDR-TB was higher with age range of 21-30 years (23.6%), HIV positive co-infection 18 (19.4%), patients with TB history of defaulter 7 (24.1%) and patients with retreatment history 97 (19.4%). Age at range of 21-30 years, females and history of defaulter were the only variable that showed statistical significant association (Tables 3 and 4).

Variables	MDR-TB and/or RMP-MR	Non MDR	Total		
	N (%)	N (%)	N (%)	COR(95%CI)	AOR (95% CI)
Sex				0.7 (0.44-1.0)	0.7(0.44-1.0)
Male	57 (15.7)	306 (84.3)	363 (59.9)		
Female	53 (21.8)	190 (78.2)	243 (40.1)		
Age					
>51	9 (10.3)	78 (89.7)	87 (14.4)	1	1
41-50	13 (14)	80 (86)	93 (15.3)	1.4 (0.6-3.5)	0.7 (0.3-1.7)

31-40	25 (17.2)	120 (82.8)	145 (23.9)	1.8 (0.8-4.07)	0.5 (0.23-1.2)
21-30	47 (23.6)	152 (76.4)	199 (32.8)	2.7 (1.3-5.8)	0.4 (0.2-0.8)
Nov-20	16 (21.1)	60 (78.9)	76 (12.5)	2.3 (0.95-5.59)	0.5 (0.2-1.1)
02-Oct	0 (0)	6 (100)	6 (1)	-	-
HIV status					
Negative	65 (18.9)	279 (81.1)	344 (56.8)	1	1
Positive	18 (19.4)	75 (80.6)	93 (15.3)	0.8 (0.4-1.5)	0.95 (0.5-1.7)
Un known	27 (16)	142 (84)	169 (27.9)	0.97(0.5-1.7)	1.3 (0.7-2.2)
Treatment history					
New	13 (16.9)	64 (83.1)	77 (12.7)	1.1 (0.6-2.0)	0.24 (0.04-1.4)
Retreatment	97 (18.3)	432 (86.6)	529 (87.3)		
TB history					
New	15 (19.2)	63 (80.8)	78 (12.9)	1	1
Relapse	40 (15.9)	212 (84.1)	252 (41.6)	0.79 (0.41-1.5)	4.5 (0.8-25.5)
Failure	46 (21.7)	16 9(78.3)	215 (35.5)	1.14 (0.6-2.2)	2.9 (0.5-16)
Defaulter	7 (24.1)	22 (75.9)	29 (4.9)	0.35 (0.7-1.6)	10.6 (1-99)
Other	0 (0)	6 (100)	6 (1.00)	-	-
Unknown	2 (7.7)	24 (92.3)	26 (4.3)	1.33 (0.5-3.7)	2.5 (0.4-16.7)
Total	110 (18.2)	496 (81.8)	606 (100)		

Table 3: Logistic regression analysis for assessing association of MDR-TB and/or RMP-MR with socio demographic and clinical history among presumptive MDR-TB cases, ANRS, 2013

Variables	RMP-R	RMP-S	Total	COR (95%CI)	AOR (95%CI)
Sex					
Male	57 (44.1)	72 (55.8)	129 (59.4)	1.9 (1.1-3.3)	2.2 (1.2-3.9)
Female	53 (60.2)	35 (39.8)	88 (40.6)		
Age					
>51	9 (36)	16 (64)	25 (11.5)	1	1
41-50	13 (39.4)	20 (60.6)	33 (15.2)	1.2 (0.4-3.2)	0.8 (0.3-2.6)
31-40	25 (54.3)	21 (45.7)	46 (21.2)	2.1 (0.78-5.8)	0.4 (0.2-1.2)
21-30	47 (58)	34 (42)	81 (37.3)	2.5 (1.0 6.2)	0.4 (0.2-1.1)
Nov-20	16 (51.6)	15 (48.4)	31 (14.3)	1.9 (0.7-5.6)	0.5 (0.2-1.7)
02-Oct	0 (0)	1 (100)	1 (0.5)	-	-
HIV status					
Negative	65 (53.3)	57 (46.7)	122 (56.2)	1	1
Positive	18 (54.5)	15 (45.5)	33 (15.2)	1 (0.5-2.3)	1 (0.5-2.4)
Unknown	27 (43.5)	35 (56.5)	62 (28.6)	0.7 (0.4-1.3)	1.7 (0.8-3.4)

Treatment history					
New	13 (41.9)	18 (58.1)	31 (14.3)	1.5 (0.7-3.3)	0.4 (0.03-5.6)
Retreatment	97 (52.2)	89 (47.8)	186 (85.7)		
TB history					
New	15 (44.1)	19 (55.9)	34 (15.7)	1	1
Relapse	40 (47.6)	44 (52.4)	84 (38.7)	1.2 (.5-2.6)	2 (0.04-91)
Failure	46 (51.1)	34 (37.8)	80 (36.9)	1.7 (0.8-3.9)	2.2 (0.2-30)
Defaulter	2 (28.6)	5 (71.4)	7 (3.22)	0.5 (0.1-3.0)	1.2 (0.9-17)
Other	0 (0)	2 (100)	2 (0.9)	-	3 (0.14-69)
Unknown	7 (70)	3 (30)	10 (4.6)	3 (0.7-13.4)	-
Total	110 (50.7)	107(49.3)	217 (100)		

Table 4: Logistic regression analysis showing the associated factors with MDR-TB and/or RMP-MR with socio demographic and clinical history among TB positive, presumptive MDR-TB cases, ANRS, 2013

Discussion

It is known that MDR-TB is the major public health problem in Ethiopia and in particular, ANRS. In this study we have determined the prevalence of MDR-TB and its association with sex, age, type of treatment, HIV status and TB history of the participants.

This prevalence report was the first high report in Ethiopia. It is expected that the prevalence of MDR-TB is higher than this report, because as you can see from the Table 2 above, (606-217=389(64.2%)) participants were negative for *M. tuberculosis* complex (MTBC), indicating that selection criteria was non-specific; probably hindering the actual prevalence.

Comparing to other studies, the present finding was higher than studies in Germany (4%) [16], Iran (12.2%) [17], China (5.6%) [18]. The present study's higher prevalence might be due to difference in study population, time of the study and also geography.

In this study, RMP –MR was slightly higher than INH-MR; this might be due to the method that we used, GenoType MTBDRPlus rapid molecular LPA. In GenoType MTBDRPlus, all INH resistance genes were not included in the Strip, making the method less sensitive for INH resistance detection.

The prevalence of MDR and/or RMP resistance among newly suspected and previously treated cases was 16.9% and 18.3% respectively which is higher than Ethiopian drug resistance survey 2005 (DRS-2005) that reported 1.6 and 12% among new and retreatment TB cases respectively [2,5,11]. This might be difference in time that, now due to natural selection, resistance strains are increasing than before and also due to difference in study population that DRS 2005 of Ethiopia were done among smear positive TB patients unlike our study which was done among MDR-TB suspected patients making our study prevalence higher. Other study in Ethiopia St. Peter's TB Specialized hospital among previously treated 376 culture positive patients; the prevalence of MDR-TB was 46.3% [19]. Our report from smear and/or culture positive previously treated cases were 97/186 (52.2%) still higher, this difference might be difference in time that MDR-TB is now alarmingly increasing each year in Ethiopia and also difference in target population.

The present study showed that prevalence of MDR-TB among new cases was higher but lower among previously treated cases as compared to WHO (2013) (3.7% and 20%) [3], Swaziland national survey (7.7% and 33.8%) [20], WHO European region (13.7 and 48.7 %) [21], and 2 studies in China (5.4% and 25.6%) [22] and (7.63% and 33.07%) [23] respectively. All these studies have lower prevalence of MDR-TB among new cases than our even in countries with highest prevalence of MDR and Extensively drug resistance like China, the possible reason might be time frame difference and study population but our prevalence among previously treated cases was lower than above mentioned studies. This difference can be explained with difference in patient selection that in our cases all retreated cases were considered as suspects of MDR-TB regardless of their previous or current smear status. Most retreated cases were smear and culture negative indicating that most of these participants were not eligible for MDR-TB screening.

Statistical significant association was found only on the age range of 21-30 years (AOR=0.4,CI=0.2 0.8) and TB history of defaulter (AOR=10.6, CI=1 99) (Table 3). When logistic regression was done among smear and/or culture positive participants, being female (AOR (95% CI) =2.2 (1.2 3.9) was again significantly associated (Table 4). In the contrary, a study done by Abebe et al., showed the absence of statistically significant difference in the proportion of any resistance by sex, age, HIV status and history of being imprisoned [24]. This might be because of their small sample size and difference in the study population.

Similar to our study, in Georgia being female showed statistically higher MDR-TB. This might be due to socio economic factors and health seeking behavior, with prolonged delays in female patients (probably due to lack of control of financial resources at household levels), higher rate of transient and permanent immune suppression with pregnancy, lactation and high rate of HIV.

Unlike our, previous TB treatment were associated with risk for having MDR-TB in Georgian study [25]. The possible reason that

failed to associate might be selection of retreatment cases. In our country empirical TB treatment is higher despite repeatedly negative by smear microscopy. Clinicians consider these patients as smear negative pulmonary TB. These patients did not respond to first line anti-TB drugs and then will considered them as retreatment cases without having previously confirmed TB nor current smear and/or culture positive result. Due to this reason the number of participants with smear and/or culture negative was higher hindering the actual prevalence as well expected associations.

A recent study in Nigeria, MDR-TB was found to be significantly associated with HIV seropositive patients having 32% rate when compared to HIV seronegative rate of 2.2% ($P<0.05$) [26]. Unlike this, ours failed to get association of MDR-TB and HIV; this is because many of participants in our cases were with unknown history of HIV making our finding imprecise. However, the prevalence of MDR-TB was higher among HIV positive participants than negative even in our study.

Conclusions

MDR-TB is a major public health problem and mainly affects economically productive age group of the population and females. This finding also depicts that the prevalence of MDR-TB was increasing time to time. This is a threat to TB control programme in ANRS, Ethiopia so that MDR-TB ward, diagnostic facility, and surveillance activities should be expanded and also specimen referral linkage should be established to diagnose more patients.

Competing Interests

The authors declare that they have no competing interests.

Authors contributions

DM was involved in the design, participates in laboratory procedure, implementation of the study, performed statistical analysis, and drafted the manuscript. WM conceived and designed the study, involved in the implementation of the study, performed statistical analysis and critically revised the manuscript. AA performed the laboratory activities, critically revised the manuscript and BA was involved in the writing and critically revised the manuscript. MT and MY critically revised the manuscript. All the authors read and approved the final manuscript.

Acknowledgement

The authors thank Bahir Dar University and Bahir Dar Regional Health Research Laboratory Center, Regional Health Bureau for their help in many ways. Our appreciation also goes to study participants.

References

1. World Health Organization Report (2011) Global Tuberculosis Control. WHO/HTM/TB/2011;16.
2. World Health Organization (2012) Global tuberculosis control; WHO report 2012.
3. World Health Organization Report (2013) Global Tuberculosis Control. WHO/HTM/TB/2013.11. ISBN 978 92 4 156465 6
4. Ministry of Health of Ethiopia (2011) First Ethiopia National Population based Tuberculosis survey Tuberculosis. Addis Ababa: MOH.
5. Federal Democratic Republic of Ethiopia Ministry of Health (2013) Guidelines for Clinical and Programmatic Management of TB, Tb/HIV and Leprosy in Ethiopia.5th Ed.
6. Barnard M, Parsons L, Miotto P, Cirillo D, Feldmann K, et al (2012) Find for innovation for new diagnostics (FIND). Molecular Detection of Drug-Resistant Tuberculosis by Line Probe Assay. Laboratory Manual for Resource-Limited Settings.
7. Smith I (2003) Mycobacterium tuberculosis pathogenesis and molecular determinants of virulence. Clin Microbiol Rev 16: 463-496.
8. Parsons LM, Somoskövi A, Gutierrez C, Lee E, Paramasivan CN, et al. (2011) Laboratory diagnosis of tuberculosis in resource-poor countries: challenges and opportunities. Clin Microbiol Rev 24: 314-350.
9. Berhe G, Enqueselassie F, Hailu E, Mekonnen W, Teklu T, et al. (2013) Population-based prevalence survey of tuberculosis in the Tigray region of Ethiopia. BMC Infect Dis 13: 448.
10. Federal Democratic Republic of Ethiopia Ministry of Health (2012) Comprehensive Training Manual for Clinical and Programmatic Management of Tb, Leprosy and TB/HIV Participants' Manual.
11. WHO (2011) towards universal access to diagnosis and treatment of multidrug-resistant and extensively drug-resistant tuberculosis by 2015: WHO progress report.
12. World Health Organization (2011) Tuberculosis prevalence surveys: a handbook. Geneva, Switzerland: World Health Organization.
13. World Health Organization (2011) Tuberculosis prevalence surveys: a handbook. 1211 Geneva 27, Switzerland: WHO Press, World Health Organization, 20 Avenue Appia : 1–5.
14. Wikipedia The free encyclopedia (2013).
15. Hainlifescience GenoTypeMTBDRplusVER2.0 (2011) Instruction for use. IFU-304A-01 2011.
16. Eker B, Ortmann J, Migliori GB, Sotgiu G, Muetterlein R, et al. (2008) Multidrug- and extensively drug-resistant tuberculosis, Germany. Emerg Infect Dis 14: 1700-1706.
17. Metanat M, Sharifi-Mood B, Shahreki Sh, Dawoudi SH (2012) Prevalence of multidrug-resistant and extensively drug-resistant tuberculosis in patients with pulmonary tuberculosis in zahedan, southeastern iran. Iran Red Crescent Med J 14: 53-55.
18. Zhao M, Li X, Xu P, Shen X, Gui X, et al. (2009) Transmission of MDR and XDR tuberculosis in Shanghai, China. PLoS One 4: e4370.
19. Abate D, Taye B, Abseno M, Biadgilign S (2012) Epidemiology of anti-tuberculosis drug resistance patterns and trends in tuberculosis referral hospital in Addis Ababa, Ethiopia. BMC Res Notes 5: 462.
20. Sanchez-Padilla E, Dlamini T, Ascorra A, Rüsch-Gerdes S, Tefera ZD, et al. (2012) High prevalence of multidrug-resistant tuberculosis, Swaziland, 2009-2010. Emerg Infect Dis 18: 29-37.
21. Dara M, Dadu A, Kremer K, Zaleskis R, Kluge HH (2013) Epidemiology of tuberculosis in WHO European Region and public health response. Eur Spine J 22 Suppl 4: 549-555.
22. He GX, Zhao YL, Jiang GL, Liu YH, Xia H, et al. (2008) Prevalence of tuberculosis drug resistance in 10 provinces of China. BMC Infect Dis 8: 166.
23. Shao Y, Yang D, Xu W, Lu W, Song H, et al. (2011) Epidemiology of anti-tuberculosis drug resistance in a Chinese population: current situation and challenges ahead. BMC Public Health 11: 110.
24. Abebe G, Abdissa K, Abdissa A, Apers L, Agonafir M, et al. (2012) Relatively low primary drug resistant tuberculosis in southwestern Ethiopia. BMC Res Notes 5: 225.
25. Vashakidze L, Salakaia A, Shubladze N, Cynamon M, Barbakadze K, et al. (2009) Prevalence and risk factors for drug resistance among hospitalized tuberculosis patients in Georgia. Int J Tuberc Lung Dis 13: 1148-1153.
26. Oluwaseun E, Akinniyi P, Afolabi O (2013) Primary multi-drug resistant tuberculosis among HIV seropositive and seronegative patients in Abeokuta, Southwestern Nigieria. American Journal of Research Communication 2013 ISSN: 2325-4076.