Brief communication

Drug susceptibility of *Mycobacterium tuberculosis* isolates from smear negative pulmonary tuberculosis patients, Addis Ababa, Ethiopia

Kassu Desta[1], Daniel Asrat[2], Eshetu Lemma[3], Mekdes Gebeyehu[3], Beniam Feleke[4]

Abstract

Drug resistance tuberculosis threatens the National Tuberculosis Control Programme in several countries. A cross-sectional study was conducted during the period between November 2004 and October 2005 to determine drug susceptibility pattern of *Mycobacterium tuberculosis* (n=37) isolated from smear negative pulmonary tuberculosis patients (PTB), and to access whether these patients are at risk of harbouring drug resistant strains. Of the 37 *M. tuberculosis* isolates, 21/37 (29.8%) showed resistance to any of the drugs tested. No MDR-TB strains (resistant to INH and Rifampicin) were observed in this study. No statistically significant differences appeared in the frequency and pattern of resistance between isolates from smear positive and negative cases. This study provides potentially valuable information of the value of culture in the diagnosis of smear-negative cases to certain extent in untreated newly diagnosed PTB patients. Smear negative TB patients can harbor drug resistant strains like their smear positive counterparts. [*Ethiop.J.Health Dev.* 2008;22(2):212-215]

Introduction

Drug resistance tuberculosis threatens the National Tuberculosis Control Programme in several countries, and the major problem is multidrug resistance TB (MDR-TB) (1). MDR-TB is defined as *M. tuberculosis* strains that are resistant to at least isoniazid and rifampicin, the two key first line drugs in short course TB-chemotherapy. Resistance to any single TB drug is close to 10% in all African countries surveyed (2). Recently, extensively drug-resistant (XDR) *M. tuberculosis* (defined as resistant to at least isoniazid, rifampin, and fluoroquinolone, and either aminoglycosides [amikacin, kanamycin or capreomycin or both) is emerging (3). The problem of drug resistant TB exists in different parts of Ethiopia, and data on patterns of resistance among Ethiopian isolates is ranging from 2%-21% for isoniazid, 2%-20% for streptomycin and 14%-15% for any of the drugs tested (4, 5, 6). MDR-TB was also reported in about 1.2% of new cases and 12% of re-treatment cases (5). Little information is available in Ethiopia related to drug susceptibility assay on *M. tuberculosis* isolates from smear negative and culture positive sputum samples (7). This study was undertaken to determine drug susceptibility pattern of *Mycobacterium tuberculosis* isolates with special emphasis from smear negative and culture positive TB patients in order to access whether smear negative TB patients poses risk of harbouring drug resistant strains.

Methods

Three consecutive sputum samples (spot, early morning, spot) were collected from 297 informed, consented untreated and newly diagnosed adult patients (15 or more years of age) with suspected PTB. All the samples were screened for using Ziehl-Neelsen (ZN) staining method at St. Peter's Tuberculosis Specialized Hospital, Addis Ababa, Ethiopia.

All smear negative and positive sputum samples from each patient were pooled separately and processed for mycobacterial culture using conventional Löwenstein-Jensen (LJ) egg slant medium (BBL, Sparks, MD, USA) containing 0.6% sodium pyruvate and glycerol. All positive cultures obtained from conventional methods were examined by ZN staining to confirm the presence of AFB. Mycobacterial identification was performed using colonial morphology, growth time, and standard biochemical tests (8).

Drug susceptibility testing was performed on *M. tuberculosis* isolates from smear negative (n=37) and positive cases (n =36) using LJ slant media based on indirect proportionate method as described by Canetti *et al*. (9). Isoniazid (0.2 μg/ml), Rifampicin (2μg/ml); Ethambutol (5μg/ml) and Streptomycin (4μg/ml) (Sigma Chemicals, St Louis, USA) were used for susceptibility testing. Drug susceptibility results were interpreted on the same day that distinct growth was visualized in control media as described by Kent and Kubica (8). *M. tuberculosis* $H_{37}Rv$ (ATCC 27294) reference strain was used as a quality control for both culture and susceptibility testing.

Data entry and analysis were performed using SPSS version 10 statistical package. Chi-square or Fisher's exact test was applied to test whether differences

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[1]School of Medical Laboratory Technology, Faculty of Medicine Addis Ababa University, P.O.Box. 11331, Addis Ababa, Ethiopia; [2]Department of Medical Microbiology, Immunology and Parasitology, Faculty of Medicine, Addis Ababa University, P.O.Box.9086, Tel.: +251-11-552-87- 26, Fax: +251-11-551-30-99, E-mail asratdaniel@ethionet.et Addis Ababa, Ethiopia; [3]Ethiopian Health Nutrition and Research Institute, P.O. Box. 1242, Addis Ababa, Ethiopia; [4]St. Peter's Tuberculosis Specialized Hospital, Addis Ababa, CDC-Ethiopia

significantly exist between values. *P* values <0.05 was considered statistically significant

The research proposal was approved and ethically cleared by Medical Faculty Ethical Review Committee and endorsed by the Faculty Academic commission, Addis Ababa University.

Results
Of the 37 *M. tuberculosis* isolates from smear negative PTB patients, 11/37 (29.8%) showed resistance to any of the drugs tested (Table 1). Mono-resistance was found

only for streptomycin in 9 (24.3%) isolates. Resistance to streptomycin was observed in 11 (29.7%) of the strains tested. Resistance to isoniazid, ethambutol and rifampicin accounted for 1 (2.7%) each. Multi-drug resistance (resistance to two or more drugs) was observed in 5/37(13.5%) strains. No MDR-TB strains (resistant to INH and Rifampicin) were observed in this study. No statistically significant differences were observed in the frequency and pattern of resistance between *M. tuberculosis* isolates from smear positive and negative cases (Table 1).

Table 1: **Drug resistance patterns of *Mycobacterium tuberculsis* isolates from smear negative and positive cases at St. Peter's Tuberculosis Specialized Hospital, Addis Ababa**

Drug resistance	*Mycobacterium* *tuberculosis* isolates		Total n=73 No. (%)	P-value
	Smear negative cases (n=37) No. (%)	Smear Positive cases (n=36) No. (%)		
Resistance to any drug	11 (29.8)	9 (25.0)	20 (27.4)	0.92
Resistance to one drug only				
Isoniazed	-	-	-	
Rifampicin	-	-	-	
Ethambutol	-	1 (2.8)	1 (1.4)	1.00
Streptomycin	9 (24.3)	5 (13.9)	14 (19.2)	0.52
Resistance to any of the following				
Isoniazed	1 (2.7)	3 (8.3)	4 (5.5)	0.62
Rifampicin	1 (2.7)	-	1 (1.4)	0.97
Ethambutol	1 (2.7)	1 (2.8)	2 (2.7)	0.48
Streptomycin	11 (29.7)	8 (22.2)	19 (26.0)	0.76
Multidrug resistance				
Isoniazed & Streptomycin	4 (10.8)	3 (8.3)	7 (9.6)	0.94
Isoniazed, Streptomycin & Ethambutol	1 (2.7)	-	1 (1.4)	0.97

Discussion
The overall resistance rate (27.4%) involving one or more drugs observed in this study is higher than those in the previous studies done in Ethiopia (14-22.3%) (4, 6, 10, 11). Reports from other developing countries resistance to one or more anti-tuberculosis drugs ranges from 3.4 to 37.0%; for instance 18.7% in Korea (12), 7.3% in South Africa (13), 5.2% in India (14), 30.5% in Taiwan (15) and 30.5% in Central Asia (16). A comparable level of drug resistance has also been reported from Latin American countries (17). This may reflect the variations in the studied population.

The resistance rate observed for isoniazid in this study was 5.5%. Previous studies in Ethiopia showed that the frequency of resistance to isoniazid was within a range of 4.1%-21% (4, 6, 10, 18, 19), and a study conducted in Bangladesh showed a similar rate of resistance (5.4% (20).

The resistance rate to streptomycin in this study (26.0%) has increased when compared with previous studies done in Ethiopia (4.9%-20%) (4, 10, 18, 19, 21). This can be explained as; streptomycin is widely used in the treatment of other bacterial infections and patterns of

inadequate treatment of tuberculosis patients, either due to lack of drugs or poor compliance by patients (defaulters); both in turn selecting drug resistant mutant strains. Streptomycin resistance must be seriously considered since this drug is core components of the standard and DOTs regimens. It is relatively more affordable drug with a vital role in the treatment of tuberculosis in developing counties. Losing the effectiveness of this drug may mean changing the treatment regimen to a more expensive ones. Currently, it is almost replaced by rifampicin, and even the current standard regimen considered to be relatively cheap, is unaffordable for many developing countries.

Although rifampicin is currently used for the treatment of many other infectious diseases and sold all over Ethiopia, the level of resistance was still very low. Only 1 out of 73 (1.4%) isolates was found to be resistant to rifampicin in this study. The rate is slightly higher than in the previous studies done in Ethiopia (0-1.8%) (4, 10, 21) and in Bangladesh (0.5%) (20).

The resistance rate observed for ethambutol in this study was 2.7%. Previous studies conducted in Ethiopia have shown that ethambutol resistance is low (below 0.5%)

(5). This is an advantage that should be exploited in order to develop a regimen for the management of MDR-TB. Ethambutol is a drug that enhances the effect of many other drugs including beta lactam drugs on different mycobacterial species (22).

Multiple drug resistance (MDR) involving isoniazid and rifampicin was not observed in this study. However, there were reports from the earlier studies conducted in Ethiopia indicating that the prevalence MDR-TB is about 1.2% in new cases and 3.5-12% in re treatment cases of PTB (5, 19). In other sub-Saharan Africa, routine reports indicate that, MDR-TB prevalence in new TB cases is 0.8%, 0.9-2.6% and 1.8% in Botswana, South Africa and Zambia, respectively (23). However, among previously treated cases in high-HIV-burden African countries, MDR prevalence is estimated at 6.3% (23). Relatively, a lower frequency of MDR-TB has been reported in Bangladesh (0.23% for new cases and 5.56% for previously treated patients) (20). It should be noted that the present investigation dealt with newly diagnosed TB patients had never been treated for TB and by excluding patients who had previously treated may underestimate the prevalence of resistance rates particularly for MDR-TB.

In conclusion, this study provides potentially valuable information on the value of culture in the diagnosis of smear-negative cases to certain extent in untreated newly diagnosed PTB patients. Smear negative TB patients can harbor drug resistant strains like their smear positive counterparts.

Acknowledgements

References
1. WHO/IUATLD. Anti-tuberculosis drug resistance in the world: the WHO/IUATLD Global Project on Anti-tuberculosis Dug Resistance Surveillance. Report 2: Prevalence and Trends. World Health Organization, Geneva, WHO/CDS/TB/2000.278.
2. Aziz MA, Wright A, Laszlo A, De Muynck A, Portaels,F, Van Deun A, Wells C, Nunn P, Blanc L, Raviglione M. Epidemiology of antituberculosis drug resistance (the Global Projecton Anti-tuberculosis Drug Resistance Surveillance): an updated analysis. Lancet 2006;368:2142-2154.
3. World Health Organization. Laboratory extensively drug-resistant (XDR-TB) definitions. Meeting of the Global XDR-TB Task Force. World Health Organization, Geneva, Switzerland; 2006.
4. Demissie M, Gebeyehu M, Berhane Y. Primary resistance to anti-tuberculosis drugs in Addis Ababa, Ethiopia. Int J Tuberc Lung Dis 1997;1:64-67.
5. Abate G. Review: Drug resistance tuberculosis in Ethiopia Problem scenarios and recommendation. Ethiop Med J; 2002;40:79-86.
6. Bruchfeld J, Aderaye G, Berggren Palme I, Bjorvatan B, Gebremichael S, Lindquist L. Molecular epidemiology and drug resistance of *Mycobacterium tuberculosis* isolates from Ethiopian pulmonary TB patients with and without HIV infection. J Clin Microbiol 2002;40:1636-43.
7. Ejigu GS, Woldeamanuel Y, Shah NS, Gebeyehu M, Selassie A, Lemma E. Microscopic observation drug susceptibility assay provides rapid and reliable identification of MDR-TB. Int J Tuberc Lung Dis 2008;12:332-337.
8. Kent PT and Kubica GP. Public health mycobacteriology a guide for the level III laboratory. U.S. department of health and human services. Public health service. CDC, Atlanta, Georgia; 1985.
9. Canetti G, Fox W, Khomenko A, Mahler HT, Menon NK, Mitchison DA, Rist N, Smeley NA. Advances in techniques of testing mycobacterial drug sensitivity, and the use of sensitivity tests in tuberculosis control programmes. Bull World Health Organ 1969;41:21-43.
10. Wolde K, Lemma E, Abdi A. Primary resistance to the major anti-tuberculosis drugs in Ethiopia. Ethiop Med J. 1986;24:15-8.
11. Eyob G, Guebrexabher H, Lemma E,Wolday D, Gebeyehu M, Abate G, Rigouts L, van Soolingen D, Fontanet A, Sanders E, Dorigo-Zetsma JW. Drug susceptibility of *Mycobacterium tuberculosis* in HIV-infected and uninfected Ethiopians and its impact on outcome after 24 months of follow-up. Int J Tuberc Lung Dis 2004;8:1388-1391.
12. Lee JH, Chang JH. Drug resistance tuberculosis in a tertiary referral teaching hospital of Korea. Korean J Intern Med 2001;16:173-179.
13. Churchyard GJ, Corbett EL, Kleinschmidt I, Mulder D, DeCock KM. (2000). Drug resistance tuberculosis in South African gold miners: Incidence and associated factors. Int J Tuberc Lung Dis 2000; 4:433-440.
14. Dhingra VK, Rajpal S, Bhalla P, Yadav A, Jain SK, Hanif M. (2003). Prevalence of initial drug resistance in new sputum positive revised national tuberculosis control programme (RNTCP) patients. J Commun Dis 2003;35:82-89.
15. Liaw YS, Hsueh PR, YU CJ, Wang SK, Yang PC, Luh KT. Drug resistance pattern of *Mycobacterium tuberculosis* in a University hospital in Taiwan 1998-2002. J Formos Med Assoc 2004;103:671-677.
16. Hellen S, Juan D, Roy M, Sabine R, Dennis F, Ian S, Darebay D, Yared K, Mohammed A. Multidrug resistance in Central Asia . Emerg Infec Dis 2004; 10:1210-1215.

17. Laszlo A, de Kantor IN. Random sample survey of initial drug resistance among tuberculosis cases in Latin America. Bull World Health Organ 1994; 72:603-610.
18. Lemma E, Valdivia Alvarez JA, Gebre-Tsadik G, Echemendia Font M. Drug sensitivity patterns of *Mycobcterium tuberculosis* isolated in Addis Ababa. Ethiop Med J 1984;22:93-96.
19. Mitike G, Kebede D, Yeneneh H. Prevalence of antituberculosis drug resistance in Harar Tuberculosis Center, Ethiopia. East Afr Med J 1997; 74:158-61.
20. Van Deun A, Aung KJ, Chowdhury S, Saba S, Pankaj A, Ashraf A, Rigouts L, Fissette K, Portaels F. Drug susceptibility of *Mycobacterium tuberculosis* in a rural area of Bangladesh and its relevance to the national treatment regimens. Int J Tuberc Lung Dis 1999;3:143-148.
21. Gebeyehu M, Lemma E, Eyob G. Prevalence of drug resistant tuberculosis in Arsi zone, Ethiopia. Ethiop J Health Dev 2001;15:11-6.
22. Abate G, Miorner H. Susceptibility of multidrug-resistant strains of *Mycobacterium tuberculosis* to amoxycillin in combination with clavulanic acid and ethambutol. J Antimicrob Chemother 1998;42:735-40.
Zignol M, Hosseini MS, Wright A, Weezenbeek CL, Nunn P, Watt CJ, Williams, BG, Dye C. Global incidence of multidrug-resistant tuberculosis. J Infect Dis 2006;194:479-485.