

Drug resistance in *Mycobacterium tuberculosis* strains isolated from re-treatment cases of pulmonary tuberculosis in Ethiopia: susceptibility to first-line and alternative drugs

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SUMMARY

SETTING: Addis Ababa Tuberculosis Demonstration and Training Center, Ethiopia.

OBJECTIVES: To determine the pattern of drug resistance among re-treatment cases of pulmonary tuberculosis (TB), to determine the risk factors associated with multi-drug resistant (MDR) TB, and to propose re-treatment regimens based on the patterns of susceptibility to first-line and alternative drugs.

DESIGN: One hundred and seven *Mycobacterium tuberculosis* strains isolated from an equal number of re-treatment cases of pulmonary TB were included in the study. Drug susceptibility was determined by the Bactec method.

RESULTS: About 50% of the strains were resistant to one or more of the first-line drugs and 12% of the strains

were multi-drug resistant, i.e., resistant to both isoniazid and rifampicin. Previous treatment with rifampicin was the most important predictor of MDR-TB. All MDR strains were susceptible to amikacin, ciprofloxacin, ethambutol, ethionamide and clofazimine.

CONCLUSION: The WHO re-treatment regimen would theoretically be effective for the treatment of all non-MDR-TB patients in this study. A proposed 12-month re-treatment regimen for MDR-TB patients would include a fluoroquinolone in combination with streptomycin, pyrazinamide, isoniazid, ethambutol and clofazimine. There is an urgent need for more research to define safe and inexpensive treatment regimens for MDR-TB patients in low-income countries.

KEY WORDS: multidrug resistance; tuberculosis; Ethiopia

DRUG-RESISTANT TUBERCULOSIS (TB) is an increasing problem, especially in areas with the unfortunate combination of availability of drugs and inadequate control measures.¹ Rifampicin and isoniazid are the cornerstones in the treatment of TB, and resistance to both drugs makes the treatment of TB difficult even in industrialised countries. The spread of multi-drug resistant (MDR) TB (defined as resistance to both isoniazid and rifampicin at least) is a serious threat to TB control measures.² There are no standard regimens for the treatment of MDR-TB, and infection with an MDR strain in low-income countries is often a virtual death sentence.

Most low-income countries have limited capacity to perform culture and drug susceptibility testing of *Mycobacterium tuberculosis*, even from patients suspected of harbouring drug-resistant strains. Therefore, it is essential to identify predictors of drug-resistant TB, and to develop a strategy for the treatment of suspected cases.

The objectives of this study were: 1) to determine the pattern of drug resistance in re-treatment cases of pulmonary TB with the aim of finding an effective and affordable re-treatment regimen for patients at risk of harbouring drug-resistant TB, and 2) to determine the risk factors associated with MDR-TB.

MATERIALS AND METHODS

Specimens

Sputum samples were collected from 107 acid-fast smear-positive re-treatment patients attending the Addis Ababa Tuberculosis Demonstration and Training Center. Patients were referred to the center from health institutions in different regions of the country. Patients were classified as defaulters (i.e., patients who have discontinued treatment for at least one month), treatment failures (i.e., patients who remain smear positive after 5 months of treatment), relapse cases (i.e., patients who were cured in the past but

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again have active TB), and chronic cases (i.e., patients who remain acid-fast smear positive after completing a re-treatment regimen).^{3,4} A questionnaire was used to collect information on human immunodeficiency virus (HIV) status and drugs used in previous treatment for TB.

Isolation and identification of *M. tuberculosis*

The sodium lauryl sulphate method⁵ was used for the digestion and decontamination of sputum samples. The decontaminated samples were inoculated onto three Löwenstein-Jensen (LJ) culturing tubes, and the inoculated media were incubated at 37°C. Species identification was performed using standard biochemical tests⁶ and DNA-RNA hybridisation (Accu Probe®, GenProbe Inc., San Diego, CA, USA).

Drugs

Ethambutol (Lederle, Wayne, NJ, USA), isoniazid (INH, Pharmacia, Uppsala, Sweden), streptomycin (Sigma Chemicals Co., St. Louis, MO, USA) and rifampicin (Ciba-Geigy AG, Basel, Switzerland) were tested on all 107 *M. tuberculosis* isolates. Amikacin (Bristol-Myers Squibb Co., New York, NY, USA), ciprofloxacin (Bayer AG, Leverkusen, Germany), clarithromycin (Abbott Laboratories, North Chicago, IL, USA), clofazimine (Ciba-Geigy), cycloserine (Eli Lilly & Co., Indianapolis, IN, USA), ethionamide (Sigma), paraminosalicylic acid (PAS, Ferrosan AB, Malmo, Sweden), pyrazinamide (Merck, Sharp & Dohme International, Rahway, NJ, USA), rifabutin (Farmitalia Carlo Erba, Milano, Italy) and thiacetazone (Sigma) were tested on all isolates resistant to two or more of the four first-line drugs. Multidrug resistance was defined as resistance to both isoniazid and rifampicin at least.²

A stock solution of each drug was prepared in a proper solvent. To each test vial containing 4 ml broth media, 0.1 ml of stock solution was added to give final concentrations of 5 µg/ml ethambutol, 0.2 µg/ml isoniazid, 4 µg/ml streptomycin, 2 µg/ml rifampicin, 4 µg/ml amikacin, 2 µg/ml ciprofloxacin, 2 µg/ml clarithromycin, 2 µg/ml clofazimine, 50 µg/ml cycloserine, 5 µg/ml ethionamide, 4 µg/ml PAS, 100 µg/ml pyrazinamide, 2 µg/ml rifabutin, and 3 µg/ml thiacetazone.

Susceptibility testing

The Bactec system with Bactec 7H12 medium (Becton-Dickinson, Sparks, MD, USA) was used for susceptibility testing to first-line and alternative drugs. A Bactec PZA test medium, a modified 7H12 broth of pH 6, was used for pyrazinamide susceptibility testing.

A 1 in 100 diluted bacterial suspension was used as a drug-free control in susceptibility testing of each isolate with drugs other than pyrazinamide. A 1 in 10 diluted bacterial suspension was used as a control for the pyrazinamide susceptibility test. The susceptibility

test and interpretation of the test results were performed following the manufacturers' recommendations.

RESULTS

Classification of patients

Of the 107 patients included in this study, 86 (80%) were males. The age of the patients ranged from 13 to 68 years, with a mean of 30 years; 96 patients (85%) were below 45 years of age. The majority (61%) came from Addis Ababa and the others were from different regions of the country (Shoa, Arsi, Gojam, Wello, Balle, Tigray, Gonder and Harrar). Fifty-seven (53%) were defaulters, 35 (33%) were relapse cases, 10 (9%) were chronic cases, and five (5%) were smear-positive after five months of treatment for tuberculosis. Twenty-six per cent (27/105) of patients tested were seropositive for HIV.

Four different treatment regimens had been used (for abbreviations see footnote, Table 1): 2SHT/10HT(E) (a 12-month standard regimen with streptomycin, isoniazid and thiacetazone for 2 months followed by isoniazid, thiacetazone or ethambutol for 10 months), 2SHE/10HE, 2SHR/4HR (or EHR), 3RHEZ/ 9RHE (or 2RHSZ/1RHZ/ 9RHE). Table 1 shows the treatment regimens used in the different groups of patients. For all patients, initial treatment included isoniazid and streptomycin. All defaulters had been treated for a minimum of 2 months. Fifty-five defaulters had had 3 months of treatment and two had received 2 months of treatment prior to defaulting. Only 27 patients had been treated with ethambutol, and only 13 had received rifampicin. Of those patients who had been treated with rifampicin, 11 had received treatment for 12 months or more, and two for 2 months. Only five patients had been treated with regimens containing pyrazinamide.

Table 1 Treatment regimens used in different groups of patients

Groups of patients	Treatment regimens used	Number of patients
Defaulters	2SHT/1HT (or HE)	48
	2SHE/1HE	7
	2SHR	2
Treatment failures	2SHT/>4HT	4
	2SHE/9HE	1
Relapse cases	2SHT/10HT	27
	2SHE/10HE	7
	2RHES/10RHE	1
Chronic cases	2SHT/10HT and 2RHE(S)Z/RHEZ/>9RHE or 3RHEZ/>9RH (or RHE)	10

S = streptomycin; H = isoniazid; T = thiacetazone; E = ethambutol; R = rifampicin; Z = pyrazinamide.

The numbers indicate the number of months. All chronic cases initially received 2SHT/10HT, followed by the rifampicin-containing re-treatment regimen.

Susceptibility to first-line drugs

All 107 mycobacterial isolates were identified as *M. tuberculosis*. Forty-four per cent (47/107) of isolates were resistant to isoniazid, 28% (30/107) to streptomycin, 12% (13/107) to rifampicin and 2% (2/107) to ethambutol. The susceptibility pattern to four first-line drugs is shown in Table 2. Resistance to at least one first-line drug was seen in 18/57 (32%) isolates from defaulters, 20/35 (57%) isolates from relapse cases, 9/10 (90%) isolates from chronic cases, and all five isolates from cases who were smear-positive after five months of treatment. The difference in resistance patterns between defaulters and relapse or chronic cases was statistically significant ($P < 0.05$). There was no statistically significant difference in the resistance patterns of isolates from relapse cases and those from chronic cases ($P > 0.05$).

Susceptibility to alternative drugs

Thirty-five *M. tuberculosis* isolates were tested for susceptibility to 10 other drugs. These 35 isolates were selected because they were resistant in vitro to at least two of the four first-line drugs. Ninety-four per cent (33/35) were resistant to clarithromycin, 83% (29/35) to thiacetazone, 71% (25/35) to cycloserine, 69% (24/35) to PAS, 31% (11/35) to rifabutin and 11% (4/35) to pyrazinamide. All (35/35), including the MDR strains, were susceptible to amikacin, ciprofloxacin, clofazimine and ethionamide. The susceptibility pattern of all MDR strains to first-line and alternative drugs is shown in Table 3.

Risk factors associated with drug-resistant TB

Resistance to first-line drugs was not related to age ($P > 0.05$), gender ($P > 0.05$), or HIV status ($P > 0.05$). All patients had received isoniazid in their treatment, and in almost 50% of the cases the infecting bacilli was resistant to this drug. MDR-TB was seen more in patients who had received a rifampicin-containing regimen ($P < 0.01$). Moreover, all isolates that were resistant to rifampicin were also resistant to isoniazid, indicating rifampicin resistance as a strong predictor of MDR-TB. Of the 13 MDR strains, seven (54%) were isolated from chronic cases, four (30%) from relapse cases, one (8%) from a defaulter and one (8%) from a case who was smear-positive after 5 months of treatment.

Table 2 *M. tuberculosis* strains resistant to single or combination of first-line drugs ($n = 107$)

Drug(s)	Number of resistant strains (%)
Isoniazid only	12 (11)
Streptomycin only	4 (4)
Isoniazid + streptomycin	20 (19)
Isoniazid + rifampicin	9 (8)
Isoniazid + streptomycin + rifampicin	4 (4)
Isoniazid + streptomycin + ethambutol	2 (2)

Table 3 Susceptibility pattern of MDR *M. tuberculosis* isolates ($n = 13$)

Drugs	Strain number												
	1	7	11	12	49	58	63	70	75	99	111	140	147
Ethambutol	S	S	S	S	S	S	S	S	S	S	S	S	S
Isoniazid	R	R	R	R	R	R	R	R	R	R	R	R	R
Pyrazinamide	S	S	S	S	S	S	S	S	S	S	R	R	S
Rifampicin	R	R	R	R	R	R	R	R	R	R	R	R	R
Streptomycin	R	R	S	S	S	S	R	S	S	S	S	R	S
Amikacin	S	S	S	S	S	S	S	S	S	S	S	S	S
Ciprofloxacin	S	S	S	S	S	S	S	S	S	S	S	S	S
Clarithromycin	R	R	R	R	R	S	R	R	S	R	R	R	R
Clofazimine	S	S	S	S	S	S	S	S	S	S	S	S	S
Cycloserine	S	S	S	R	S	R	R	R	R	R	R	R	S
Ethionamide	S	S	S	S	S	S	S	S	S	S	S	S	S
PAS	R	R	S	R	S	S	S	R	R	R	S	R	S
Rifabutin	R	R	S	R	R	R	R	R	R	S	R	R	R
Thiacetazone	R	R	R	R	R	R	R	R	R	R	R	R	R

S = susceptible; R = resistant; PAS = para-aminosalicylic acid.

DISCUSSION

This study revealed a high percentage of drug-resistant *M. tuberculosis* strains among re-treatment cases in Addis Ababa. These patients were either infected with drug-resistant bacilli, or the resistance developed because of inadequate treatment. A recent survey on primary drug resistance in Ethiopia (Addis Ababa) showed 16% resistance to one or more first-line drugs.⁷ In our study, about 50% of the strains were resistant to one or more of the first-line drugs, and 12% of the strains were MDR. An equally high percentage of resistance to isoniazid and streptomycin (46% to each drug) in re-treatment cases was reported in a similar study in Addis Ababa in 1979.⁸ History of previous treatment for tuberculosis is a good indicator of development of resistance,^{9,10} and this was also clearly shown in our study.

These results reflect the impact of the lack of a well maintained control program in Ethiopia. Since patients from only one center were included in the present study, our findings may not reflect the real drug resistance problem in Ethiopia. However, since almost 40% of patients were referred from different regions of the country, our results indicate a problem of drug resistance in general, and an emerging problem of MDR-TB in particular.

In patients other than those with MDR-TB, the re-treatment regimen recommended by the World Health Organization (WHO)³ (i.e., 2SRHEZ/1RHEZ/5RHE) could be effective. Relatively cheap alternative drugs such as clofazimine and ethionamide should also be considered in some cases infected with isolates resistant to ethambutol, isoniazid and streptomycin. In another study (unpublished) we found that only 13 out of 50 isoniazid-resistant strains showed a high level of resistance (minimum inhibitory concentration [MIC] $> 4 \mu\text{g/ml}$). The MIC of isoniazid for the majority of INH-resistant strains is well below the serum

achievable concentration of the drug (i.e., below 3-5 µg/ml). Therefore, it is our opinion that isoniazid should be considered in a re-treatment regimen regardless of the in vitro resistance of the strain to 0.2 µg/ml of the drug.

All MDR isolates in our study were susceptible to ciprofloxacin, clofazimine, amikacin, ethambutol and ethionamide, and most (11/13) of the strains were susceptible to pyrazinamide. The susceptibility of only 2/13 (15%) MDR isolates in this study to rifabutin is low compared to a previous report where 31% of rifampicin-resistant isolates were susceptible to rifabutin.¹¹ Eight of the 13 MDR strains in this study were resistant to cycloserine. This is in contrast to previous reports recommending that cycloserine be included in the re-treatment regimen for MDR-TB.^{12,13} All four streptomycin-resistant MDR isolates were found to be susceptible to amikacin, which is in agreement with earlier observations on *M. tuberculosis* resistance to aminoglycosides.¹⁴

There is no standard regimen for the treatment of MDR-TB patients, but different treatment regimens with first-line and alternative drugs have been previously suggested.^{10,15} All MDR isolates in this study were susceptible to five alternative drugs, but it would still not be possible to recommend a low-cost re-treatment regimen since any theoretically effective regimen would have to include an expensive fluoroquinolone. A re-treatment regimen with streptomycin (or amikacin) for the first 2 months, pyrazinamide for 3 months and a fluoroquinolone for at least 12 months, in combination with isoniazid, ethambutol and possibly clofazimine, would theoretically be effective for all MDR-TB patients in this study. The susceptibility of MDR isolates to ethambutol is important, since ethambutol potentiates the effect of other new drugs and increases the possibility of finding effective and affordable re-treatment regimens for this group of patients.¹⁶

We conclude that there is an urgent need for more research to define inexpensive, safe and effective re-treatment regimens for use in program conditions in low-income countries, and that there is a need to increase the support to the National TB Control Program to prevent further development of drug-resistant TB.

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RÉSUMÉ

CADRE : Centre de formation et de démonstration en tuberculose en Addis-Ababa, Ethiopie.

OBJECTIFS : Détermination du type de résistance aux médicaments dans les cas de retraitement de tuberculose, fixation des facteurs de risque associés à la tuberculose à germes multirésistants et proposition de régimes de retraitement basés sur le type de sensibilité à l'égard des médicaments de première ligne et des médicaments alternatifs.

SCHÉMA : L'étude a couvert 107 souches de *Mycobacterium tuberculosis* isolées d'un même nombre de cas de reprise de traitement de tuberculose pulmonaire. La sensibilité aux médicaments a été déterminée par la méthode Bactec.

RÉSULTATS : Environ 50% des souches étaient résistantes à l'égard d'un ou plusieurs médicaments de première ligne et 12% étaient multirésistantes (c'est à

dire résistantes à l'égard de l'isoniazide *et* de la rifampicine). Un traitement antérieur à la rifampicine fut le facteur prédictif le plus important d'une tuberculose multirésistante. Toutes les souches multirésistantes étaient sensibles à l'amikacine, la ciprofloxacine, l'éthambutol, l'éthionamide et la clofazimine.

CONCLUSION : Le régime de retraitement préconisé par l'OMS serait théoriquement efficace pour soigner tous les patients *non* multirésistants de cette étude. Nous pro-

posons un régime de retraitement de 12 mois pour les patients multirésistants : il comporterait une fluoroquinolone en combinaison avec la streptomycine, le pyrazinamide, l'isoniazide, l'éthambutol et la clofazimine. Il est urgent que l'on fasse plus de recherches pour définir un régime de traitement sûr et peu coûteux pour les patients tuberculeux multirésistants des pays à faibles revenus.

RESUMEN

MARCO DE REFERENCIA : Centro de demostración y de entrenamiento de tuberculosis en Addis Ababa, Etiopía.

OBJETIVOS : Determinar el modelo de farmacorresistencia entre los casos de retratamiento de tuberculosis pulmonar (TB), determinar los factores de riesgo asociados con la TB multirresistente y proponer esquemas de retratamiento basados en el modelo de sensibilidad a las drogas de primera línea y a las drogas alternativas.

MÉTODO : Se incluyeron en el estudio 107 cepas de *Mycobacterium tuberculosis* aisladas de igual número de casos de retratamiento de TB pulmonar. Se determinó la sensibilidad a las drogas por el método Bactec.

RESULTADOS : Alrededor del 50% de las cepas fueron resistentes a una o más de las drogas de primera línea y el 12% de las mismas fueron resistentes a múltiples drogas (RMD), es decir resistentes tanto a la isoniacida

como a la rifampicina. El tratamiento previo con rifampicina fue el factor predictivo más importante de la TB RMD. Todas las cepas RMD fueron sensibles a la amikacina, ciprofloxacina, etambutol, etionamida y clofazimine.

CONCLUSIÓN : Teóricamente, los esquemas de retratamiento de la OMS podrían ser efectivos para el tratamiento de los pacientes con TB no RMD en este estudio. Se propone un esquema de retratamiento de 12 meses para los pacientes con una TB RMD, que incluye una fluoroquinolona en combinación con estreptomycina, pirazinamida, isoniacida, etambutol y clofazimine. Existe una necesidad urgente de investigación para definir esquemas de tratamiento seguros y baratos para pacientes con TB RMD en los países de bajos ingresos.
