Original Article

First-line anti-tubercular drug resistance of mycobacterial strains from re-treatment cases that were smear-positive at 4th month onwards under the Revised National Tuberculosis Control Program

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ABSTRACT

Background: Programmatic management of drug-resistant TB (PMDT) under the RNTCP is being implemented in West Bengal in a phased manner since 2011. During the initial years MDR-TB cases were identified based on criteria A. This study examines the first line anti-tubercular drug resistance pattern of mycobacteria cultured from sputum samples of MDR suspects who were retreatment cases smear positive from 4th month onwards. **Materials and Methods:** In the following retrospective record based study, data on Drug Sensitivity Testing (DST) of sputum samples of MDR suspects between September 2011 and August 2012 were collected from the IRL Kolkata and analysed. Sputum samples, collected in the districts maintaining adequate aseptic containment measures, were decontaminated and centrifuged and the sediment inoculated on LJ medium. Probable M. tuberculosis colonies were identified by typical colony characteristics and Ziehl-Neelsen (ZN) staining. Sensitivity of the four 1st line drugs (Streptomycin, Isoniazid, Ethambutol and Rifampicin) was deduced by the economic variant of the proportion method. **Results:** Of all the 917 MDR suspects whose sputum was examined, 64 mycobacteria culture positive strains (6.98%) were mono-resistant to any of the four first line anti-tubercular drugs. Among the mono-resistant strains 43 (4.69%) were resistant to Rifampicin while 12 (1.31%) were resistant to INH. There were a total 78 (8.51%) poly drug-resistant strains. MDR-TB strains were seen in 741 (80.81%) samples. **Conclusion:** The magnitude of drug resistance were very high among retreatment patients that were smear positive from 4th months onwards probably because of repeated courses of anti-tubercular drugs prior to drug sensitivity testing (DST). The decision of the PMDT to enlist all retreatment patients as MDR suspects at initiation will result in early identification and treatment of MDR-TB patients.

KEY WORDS: Multidrug resistance, *mycobacterium tuberculosis*, programmatic management of drug-resistant tuberculosis, re-treatment cases

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INTRODUCTION

Programmatic management of drug-resistant tuberculosis (PMDT) under the Revised National Tuberculosis Control Program (RNTCP) is being implemented in West Bengal in a phased manner since 2011. Before the introduction of PMDT, there was no consistent policy for treatment of drug-resistant cases and patients who failed a re-treatment regimen were either diagnosed and treated with second-line antitubercular drugs through the support of nongovernment organizations (NGOs) or received repeated courses of category II under the RNTCP. During the initial years of the PMDT in the district, multidrug-resistant (MDR) tuberculosis suspects were identified based on criteria A of the program (Annexure 1)[1] and sputum samples sent for culture and Drug Sensitivity Testing (DST). Most of

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these MDR suspects were re-treatment cases, who were smear-positive at four months of treatment.

OBJECTIVES

The present study was undertaken to analyze the pattern of resistance among strains of mycobacteria obtained by the culture from patients under a re-treatment course, who were smear-positive from the fourth month onward, under the RNTCP (criteria A).

MATERIALS AND METHODS

Study population

This retrospective record-based study included records of culture and DST of sputum samples obtained from all smear-positive previously treated patients, who remained smear-positive from the fourth month onward. These samples were received at the Intermediate Reference Laboratory (IRL) in Kolkata, between September 2011 and August 2012.

Laboratory methods

Sputum collection and decontamination

One morning sample and one supervised spot sputum sample (5–10 ml each) were collected at the districts, in sterile 50 ml McCartney's bottles containing 2 ml of equal volumes of 1% cetylpyridinium chloride (CPC) (wt/vol) and 2% sodium chloride (wt/vol) (CPC-NaCl), in distilled water, and transported to the Intermediate Reference Laboratory (IRL), within seven days. The bottles were shaken until the specimens were liquefied and kept at room temperature in the walk-in-incubator for four days, maintaining adequate aseptic containment measures. After the decontamination period of four days, the bottles were filled with sterile distilled water, up to the brim, capped, and then centrifuged at 3000 × g for 15 minutes using 50 ml polypropylene tube adaptors, to minimize the chances of contamination. The McCartney tubes were then carefully removed from the centrifuge without shaking. After discarding the supernatant fluid, 50 ml of sterile distilled water was added to the sediment and it was again centrifuged at 3000x g.[2]

Culture and sensitivity testing

Inoculation was done for each sample from the sediment, on two slopes of Löwenstein–Jensen (LJ) medium, using sterile, individually wrapped, disposable 10 mm inoculation loops. The cultures were read weekly for two months. Probable *Mycobacterium tuberculosis* colonies were identified by typical colony characteristics and Ziehl-Neelsen (ZN) staining. For further confirmation, *M. tuberculosis* colonies were subjected to sensitivity testing along with an inoculation on to a LJ slope containing para nitro benzoic acid, to rule out the nontubercular mycobacteria (NTM) simulating the *M. tuberculosis* colonies. Sensitivity of the four first-line drugs was deduced by the economic variant of the proportion method.[2]

The following drug media concentrations were used as the critical proportion for resistance: streptomycin (dihydrostreptomycin sulfate) 4 μg/ml 1%, isoniazid 0.2 μg/ml 1%, rifampicin 40 μg/ml 1%, and ethambutol 2 μg/ml 1%. Any strain with 1% (the critical proportion) of bacilli resistant to any of the four drugs – rifampicin, isoniazid, ethambultol, and streptomycin – is classified as resistant to that drug. For calculating the proportion of resistant bacilli, the highest count obtained on the drug-free and drug-containing medium was taken (regardless of whether both counts were obtained on the twenty-eighth day, both on the forty-second day, or one on the twenty-eighth day and the other on the forty-second day).[2]

Quality assurance of the IRL, Kolkata

During the study period, both Internal Quality Control (IQC) and External Quality Control (EQC) were in place. Control Strains H37RV (ATCC27294) were used for IQC. Growth characteristics of suspected MTBC colonies were matched with the growth characteristics of the control strains. Standard operating procedures for media preparation were also assessed by using this control strain. For each batch of drug media inoculation, a separate batch of H37RV (ATCC 27294) was used to check on the quality of the drug media. The EQC of the laboratory was done by retesting and panel culture testing by the National Tuberculosis Institute (NTI), Bangalore, an organization under the National Reference Laboratory (NRL), and in close collaboration with the World Health Organization (WHO). During the study period, IRL, Kolkata, recorded more than 90% proficiency in isoniazid and rifampicin and more than 85% proficiency in streptomycin and etambutol, which were within the acceptable limits of the quality assurance assessment. The contamination rate ranged from 3 to 4% annually.

Definitions

An MDR suspect in the present study was defined as a patient suspected of drug-resistant tuberculosis, based on RNTCP criteria for submission of specimens for drug-susceptibility testing [Annexure-1]. During the study period, in West Bengal, suspects were being identified based on criteria A.

Mono-resistance of mycobacterial strains was defined as resistance to any one of the four primary anti-TB drugs;

Annexure 1

Guidelines for MDR TB suspect case

Criteria A
- All failures of new TB cases (on CAT I),
- Sputum smear-positive re-treatment cases who remain smear-positive at four month or later (on CAT II),
- All Pulmonary TB cases, who are contacts of known MDR TB case

Criteria B
- All re-treatment smear-positive at diagnosis
- Any smear-positive follow-up of new or re-treatment cases

Criteria C
- Re-treatment smear-negative cases at diagnosis
- HIV TB coinfected cases in addition to the suspects in Criteria B

poly-resistance was defined as resistance to two or more first-line drugs, not including MDR[3], whereas, MDR was defined as resistance *in-vitro* to isoniazid and rifampicin, with or without other antitubercular drugs.[1] As rifampicin resistance is quite rare without isoniazid resistance, a great majority of DST results with rifampicin resistance are also considered to be isoniazid resistant, that is, MDR TB under the RNTCP, and they are managed as if they were MDR TB cases, even if they do not formally qualify as MDR TB cases, as per the above definition.

Ethical approval for the study was obtained from the Ethical Committee of R.G. Kar Medical College and Hospital, Kolkata.

Data analysis

Patient data were recorded and analyzed using Microsoft Excel (Microsoft Inc, Version 2010).

RESULTS

Of all the 917 MDR suspects whose sputum was examined, 34 strains (3.71%) were sensitive to all four drugs. Sixty-four strains (6.98%) were mono-resistant to any of the four first-line antitubercular drugs. Among the mono-resistant strains, seven (0.76%) were resistant to streptomycin (S), 12 (1.31%) were resistant to Isoniazid (H), two (0.22%) were resistant to Ethambutol (E), and 43 (4.69%) were resistant to Rifampicin (R). There were a total of 78 (8.51%) poly-resistant strains [Table 1]. Of these three (0.33%), 25 (2.73%), and 15 (1.64%) strains showed resistance to RE, RS, and RES, respectively, whereas resistance to HE, HS, HES and SE was seen in seven (0.76%), nine (0.98%), 13 (1.42%), and six (0.65%), respectively. MDR TB strains were seen in 741 (80.81%) of the samples. Among them, 135 (14.72%) were resistant to only Rifampicin and INH. Seventy-eight (8.51%) strains were resistant to rifampicin, INH, and ethambutol, 98 (10.69%) were resistant to rifampicin, INH, and Streptomycin, and 430 (46.89%) were resistant to all four drugs (rifampicin, INH, streptomycin, and ethambutol). As the PMDT included all MDR- (resistance to at least Rifampicin and Isoniazid) and Rifampicin-resistant cases for treatment, with a drug regimen similar to MDR TB cases,[1] the proportion of patients that needed to be treated as MDR TB was 90.2% [Table 2].

Table 1: The sensitivity pattern of drugs (*n*=917)

Sensitivity pattern	Number	Percentage
Pan-sensitive	34	3.71
Mono-resistant	64	6.98
Streptomycin	7	0.76
Isoniazid	12	1.31
Ethambutol	2	0.22
Rifampicin	43	4.69
MDR resistant	741	80.81
RH only	135	14.72
RH+one more first-line drug	176	19.19
Resistant to all four first-line drugs	430	46.89
Poly-resistant	78	8.51
Rifampicin with Ethambutol or Streptomycin or both	43	4.69
Other drug resistant patterns	35	3.82

MDR: Multidrug-resistant, RH: Rifampicin and isoniazid

Table 2: Patients with resistance patterns who will receive category IV treatment under the PMDT (*n*=827)

Sensitivity pattern	Number	Percentage
Rifampicin	43	4.69
Rifampicin+Isoniazid	135	14.72
Rifampicin+Isoniazid+Ethambutol	78	8.51
Rifampicin+Isoniazid+Streptomycin	98	10.69
Rifampicin+Isoniazid+Ethambutol+Streptomycin	430	46.89
Rifampicin+Ethambutol	3	0.33
Rifampicin+Streptomycin	25	2.73
Rifampicin+Ethambutol+Streptomycin	15	1.64
Total	827	90.19

PMDT: Programmatic management of drug-resistant TB

DISCUSSION

Studies from around the world have shown significant variations in the prevalence of resistance to antitubercular drugs in patients previously treated for tuberculosis. Although mono-resistance to rifampicin among the re-treated cases attending tertiary care health facilities was not reported from north India, western India has reported a rifampicin mono-resistance of 9%.[4,5] Differences in the proportions of rifampicin mono-resistance in these cases were probably because of difference in patient selection. Although the north Indian study recruited re-treatment patients at the initiation of their re-treatment regimen, the other study recruited patients, who either had persistently positive sputum for acid fast bacilli (AFB) or had not clinically responded by the end of three months to antitubercular treatment (ATT). The difference in the proportion of rifampicin mono-resistance in the present study can again be because of the difference in the selection criteria of patients undergoing DST.

In a global survey conducted by the World Health Organization (WHO) and the International Union Against Tuberculosis and Lung Disease (IUATLD) in 35 countries, in the five subcontinents, resistance to four first-line drugs (excluding Streptomycin) among the previously treated patients was 0–17%, with a median of 4.4%.[6] A study conducted by the Indian Council of Medical Research (ICMR) in nine centers in India, found MDR TB ranging from 6 to 30% in respect to acquired drug resistance.[7] The prevalence of any drug resistance among first-time re-treatment patients with relapse, treatment after default, and treatment after failure was 33.3, 42.1, and 69.7%.[8] Among category II treatment failure TB cases from three Directly Observed Treatment, Short-Course (DOTS) clinics in Kolkata, Gupta *et al*. reported MDR in 68.2% cases.[9] Very high levels of MDR prevalence, of 60%, have also been reported among samples received from re-treated cases. from a tertiary care center in Mumbai, treating outpatients.[10]

Among the previously treated patients, the pattern of resistance identified by Maurya *et al*.[11] to HR, HRE, HRS, and HRES was 12.2, 7.6, 7.6, and 15.7%, respectively, whereas, in another study by Sangaré *et al*.[12], the different resistance patterns to HR, HRE, HRS, and HRES among the previously treated cases were 8.6, 5.4, 1.1, and 35.5%, respectively. Similar to the present study, in both cases, the proportion of samples with resistance to all four drugs was the highest.

Prior to the initiation of PMDT, there was no consistent treatment policy for patients failing a re-treatment regimen. Some patients shifted over to MDR treatment from non-governmental sources. Others who remained with the program received repeated courses of category II treatment. During the initial phase of the PMDT in West Bengal, many of these patients were identified as MDR suspects based on the smear-positive results at four months of treatment. Patients who reported in the present study included patients who had received two or more courses of the re-treatment regimen under the RNTCP. Use of standardized short course chemotherapy in patients diseased with MDR TB strains failed to cure a significant proportion of such cases and could create even more resistance to the drugs in use. This was termed as the 'amplifier effect of short course therapy' and it implied that the resistant strains in the bacterial population were selected repeatedly when a regimen was used continuously over a long period and these became the dominant strains.[13]

Repeated inadequate courses of therapy in patients with relapsing TB generate incremental increases in the degree of drug resistance.[14] This is probably the reason for the very high levels of drug resistance noted in these patients, compared to the first-time re-treatment patients. A study by Kandi S. among the re-treatment patients who were positive at four months, reported nearly 42% of the samples to be sensitive to all the four drugs, even as 28% of the samples were MDR-positive. Fourteen percent were declared as drug-resistant TB other than MDR. About 39% of the cases were resistant to INH. However, these patients included only relapse and treatment after the default cases (TAD).[15] Inclusion of re-treatment failure patients would have increased the prevalence of MDR among the cohort, as is seen in the present study.

Categorization of re-treatment patients who have remained positive from the fourth month onward, as MDR suspects, as per criteria A of the PMDT, delays the initiation of MDR treatment by several months, as a large number of these patients will already be harboring MDR-resistant strains at initiation. The present guideline of the PMDT, to include all re-treatment patients at diagnosis (as per criteria C) will result in the initiation of treatment with second-line drugs of drug-resistant cases at the earliest.

Limitations

1. The study was limited to patients who were being examined at the IRL during the period, and therefore, was not representative of the total MDR pool in the state
2. Many of the patients included in the study had repeated courses of antitubercular drugs. However, details of the courses were not available
3. The human immunodeficiency virus (HIV) serological status of the patients was not recorded in the records at the IRL, and hence, the HIV status of the patients was unknown in the study.

CONCLUSION

The study found a high level of drug resistance in people who received multiple courses of anti-TB drugs. Repeated antitubercular drug courses increased the level of resistance, including MDR, among patients with tuberculosis. Categorization of the re-treatment patients as MDR suspects, after they remained smear-positive at four months delayed the initiation of MDR treatment by several months. The decision of the PMDT to include all re-treatment patients at diagnosis would result in the initiation of treatment with second-line drugs of drug-resistant cases at the earliest.

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