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Short Communication

First Line Drug Resistance Study in Pulmonary Tuberculosis Patients from Mayurbhanj District of Odisha, India

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ABSTRACT

The study was conducted at Mayurbhanj district of Odisha, India, among pulmonary tuberculosis (PTB) patients to determine the resistance pattern to first-line drugs. Sputum samples were collected from 56 (42 new and 14 previously treated) patients and were tested at Regional Medical Research Centre, Bhubaneswar. Resistance to any anti-tubercular drug was observed to be 2.18% among new cases and 7.14% among previously treated patients, while multidrug-resistant tuberculosis (MDR-TB) was found to be 0% in new as well as in previously treated cases. The study reports an unchanged low level of MDR-TB prevalence among new cases in the district over a decade. Such a low level of resistance may be due to good adherence to the basic DOTS plus strategy and limited use of TB drugs outside the ongoing program.

Keywords: *Drug resistance, Mayurbhanj, MDR-TB*

INTRODUCTION

Tuberculosis (TB) is one of the most widespread infectious diseases in the world. In 2016, 0.4 million People fell ill with TB, and 1.7 million died from this disease globally, with over 95 % of the TB deaths occurring in low and middle- income countries. Seven countries account for 64% global TB burden of the total, with India leading the count. [1] TB has recently been dramatically expanding due to the HIV/AIDS epidemics and the emergence of multi-drug resistant *Mycobacterium tuberculosis* complex (MTC) strains. More than two million people die each year of TB, despite the fact that it is curable with early detection and prompt treatment. The currently available Directly Observed Treatment Short Course (DOTS) has helped enormously in combating the menace and providing treatment to a level of 85% cure rate. However the progress of TB control has suffered due to emergence of Multi Drug Resistant (MDR) and Extensively Drug Resistant (XDR) TB. [2] These drug resistances may vary in different geographical regions and settings and in the absence of TB culture may remain undetected for a long time. The major thrust of the TB control activity is the identification of drug resistance and rapid treatment to reduce the transmission. However in countries with larger geographical areas and different climatic conditions like India the prevalence of drug resistance may vary from place to place and

studies from Mumbai, Jammu and Kashmir [3] and Delhi [4] had recorded a prevalence of 24%, 5.7% and 4.0% MDR-TB among new pulmonary TB cases. Odisha comprises 31 tuberculosis districts, among them Mayurbhanj has repeatedly high tuberculosis incidence every year. [5] In 2003 a study in Mayurbhanj district reported 0.7% MDR-TB prevalence in new cases. As the TB incidence is repeatedly high in this district every year, MDR-TB prevalence may increases due to advancement of communication and globalization. Information on anti-TB drug resistance patterns particularly among newly diagnosed cases is crucial for planning an effective TB control program. So we planned a drug resistance study in Mayurbhanj a tribal dominated district of Odisha, which is mostly covered with forest and the people are mainly depends on cultivation for survival.

MATERIALS AND METHODS

The study protocol was review and approved by the institutional ethical committee of Regional Medical Research Centre (RMRC), Bhubaneswar. A written informed consent was obtained from all the study participants. Sputum samples are collected from PRM Medical College and Hospital, Mayurbhanj Odisha. Both morning and spot samples were collected from individual patients in 50 ml sterile plastic centrifuge tubes and transported to RMRC Bhubaneswar with triple packing.

The sputum samples were processed by NALC NaOH method described by Kent and Kubica. [6] Briefly the freshly prepared NALC solution (0.5gm NALC powder added to 100ml of equal proportion of 4% NaOH and 2.9% sodium citrate) was added to collected sputum samples in a 50ml sterile plastic centrifuge tube. Specimens were Vertex and allowed to stand up to 15 minutes at room temperature, the volume was brought to 50 ml with 0.067 M phosphate buffer (pH 6.8) and the contents were mixed by inversion. Bacteria were pelleted by centrifugation at 3,000 x g for 15 min. The supernatant was discarded, and the pelleted material was suspended in 1 ml of Phosphate buffer. From the pellet a loopful was inoculated in to two LJ and one LJ containing PNB slants and the smear was made for ZN staining. The culture slants were incubated at 37° C.

The *M. tuberculosis* isolates were confirmed by the rate of growth, optimum temperature of growth, colony morphology, pigmentation, growth in PNB, catalase and niacin test.

The drug susceptibility testing of the first line drugs was carried out by Proportion Sensitivity Test (PST) method. The PST method is currently the method of choice; in most of the laboratories. All strains of tuberculosis contain some subpopulation of bacilli that are resistant to anti-TB drugs. However, in resistant strains, the proportion of such bacilli is considerably higher than the sensitive strains. The proportion method calculates the proportion of resistant bacilli present in a strain. Two appropriate dilution of the bacilli, 10^{-2} and 10^{-4}dilutions (undiluted = 10^{6} to 10^{8} CFU/ml), are inoculated on drug-containing and drug-free media, to obtain countable colonies on both media. The ratio of number of colonies observed on the drug-containing media to drug-free medium indicates proportion of resistant bacilli present in the strain. The drug concentration used was Isoniazid (H) 0.2 µg/ml, Ethambutol (E) 2 µg/ml, Streptomycin (S, dihydrostreptomycin sulfate) 4 µg/ml, Rifampicin (R) 40 µg/ml.

Quality assurance and quality control

Annual proficiency testing was carried out by the Supra National Reference Laboratory (National Institute for Research in Tuberculosis, Chennai) and National Tuberculosis Institute, Bangalore for first line anti TB drugs. Internal quality assurance for DST results was performed using two strains, one susceptible; H37Rv strain and one fully resistant strain of *M. Tuberculosis.*

RESULTS

Out of 56 culture positives on LJ, 5 of each were smear negative and scanty and rest were having higher grades of AFB load in the specimen [Table-1]. The age of the 56 patients ranged between 14 and 75 years. Among them 76.79% were males and 75% were newly diagnosed cases [Table -2]. The age of newly diagnosed cases (n=42) ranged between 14 and 72 years, and previously treated cases (n=14) ranged between 30 and 75 years. On drug susceptibility testing of these 56 cases, only one cases showed resistance to streptomycin in each new and retreated cases [Table-3]. There is no MDR-TB case was detected among the 42 newly diagnosed cases and 14 retreated cases. Thus, the prevalence of MDR-TB among new sputum positive pulmonary TB patients was 0%. When each drug was considered separately, S resistance was in one patient each for new and in one patient each for previously treated cases respectively

Table 1: ZN smear status and culture status of the study participants (N=56)

Smear grade	No of smear positive (%)
Negative	5 (8.93)
Scanty	5 (8.93)
1+	15 (26.79)
2+	11 (22.67)
3+	20 (30.30)
Total	56 (100)

Table 2: Socio-demographic Characteristics of study participants (N=56)

Characteristics	Frequency	Percentage (%)
Sex		
Male	43	76.79
Female	13	23.21
Category		
New cases	42	75
Retreated cases	14	25
Age		
0-25years	4	7.14
26-51years	35	62.5
Above 51years	17	30.36

Table 3: Drug susceptibility patterns of *M. tuberculosis* among new and retreatment cases (n=56) of the *M tuberculosis* isolates

Resistance status	New cases (n=42)	Re-treated cases (n=14)	Total (n=56)
Total Susceptible	41(97.61)	13(92.86)	54 (96.43)
Any Resistance	1(2.38)	1(7.14)	2 (3.57)
Resistance to S	1	1	2(3.57)
Resistance to H	0	0	0
Resistance to R	0	0	0
Resistance to E	0	0	0
MDR	0	0	0

DISCUSSION

Sputum specimens collected from patients were mainly contaminated with other microbes. Decontamination with 4% sodium hydroxide may be detrimental to mycobacteria to some extent and the percentage of organisms killed varies according to the method used. It was reported that NALC coupled with 2% sodium hydroxide provided reasonable recovery of mycobacteria compared to 4% sodium hydroxide. [7] During culture one of the major concerns is to minimize contamination to save the loss of specimen, so we have decontaminated the sputum specimens by NALC- NaOH method.

In India, large population based studies conducted by RNTCP in states like Gujarat, Maharashtra, Andhra Pradesh have estimated the prevalence of MDR-TB to be 3% in newly diagnosed and 12-17% in retreatment cases. [8] A study conducted by WHO/International Union Against Tuberculosis and Lung Diseases in three states like Tamilnadu, Karnataka and Maharashtra had also reported 0.5 to 2.8% prevalence of MDR-TB among new cases during 1999-2002. [9]

The observed 0% prevalence of MDR TB in new cases was comparable with previous studies [10,11] that reported 0.66% and 0% prevalence in Odisha. Prevalence of 0 % MDR-TB in the new cases was also comparable with earlier study in 2003 from Mayurbhanj district, Odisha that reported 0.7% MDR-TB. [12] However few urban areas like Mumbai reported very high prevalence of 24% of MDR-TB in new cases. [13] In order to maintain the low levels of MDR-TB, it will be necessary to diagnose DR-TB cases early and render DOTS Plus treatment promptly. However in spite of high TB incidence, we found low level of drug resistance in the district, it may be due to: very less number of private practitioners in the district, patients are mainly depends upon RNTCP Govt hospitals for anti TB drugs; good adherence to the basic DOTS plus strategy; tribal dominated population mainly depends upon cultivation within the district.

CONCLUSION

Though in this study sample size is very low, our findings suggest, the persistence of low level of MDR-TB prevalence in the Mayurbhanj district. This low level can be maintained by early diagnosis of MDR-TB cases by CB NAAT in both district and TU (Tuberculosis Unit) level with improved case detection, treatment and awareness among TB patients.

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Conflict of Interest

The authors declare that they have no conflict of interest.

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