Original Article

DOI: 10.7860/JCDR/2016/20289.8846

First Line Anti-TB Drug Resistance in an Urban Area of Odisha, India

Microbiology Section

DASARATHI DAS[1], PRAKASINI SATAPATHY[2], BISWANATH MURMU[3]

ABSTRACT

Introduction: Drug resistance is a major cause for increasing the global burden of Tuberculosis (TB). However in countries with larger geographical areas and different climatic conditions like India the prevalence of drug resistance varies from place to place. Information on anti-TB drug resistance patterns particularly among newly diagnosed cases is crucial for planning an effective TB control program.

Aim: To determine the prevalence of resistance against first line anti-TB drugs and Multi Drug Resistance (MDR) in *Mycobacterium tuberculosis* isolates.

Materials and Methods: The prospective study was carried out in National Reference Laboratory (NRL) of Regional Medical Research Centre (RMRC), Bhubaneswar. During this period from January to September 2014, sputum specimens were collected from 850 suspected pulmonary TB patients attending Designated Microscopy Center (DMC) of Capital Hospital, Bhubaneswar, Odisha. Sputum specimens were subjected to Acid Fast Bacilli (AFB) smear microscopy and further processed for isolation on solid Lowenstein Jensen (LJ) medium. Drug Susceptibility Testing (DST) on isolates with first line anti-TB drugs was performed as per Revised National Tuberculosis Control Programe (RNTCP) guidelines.

Results: Out of all the 850 suspected pulmonary TB patients subjected to AFB microscopy and solid LJ culture, isolation of *Mycobacterium tuberculosis* was successful only in 161 (117 new and 44 previously treated) pulmonary TB patients. On DST by the RNTCP approved proportion method, prevalence of MDR-TB among 0.85% new and 4.54% previously treated cases was observed. Prevalence of mono resistance to streptomycin, isoniazid, rifampicin and ethambutol observed among new and previously treated cases were 3.41%, 2.56%, 0, 0.85% and 2.27%, 13.6%, 2.27%, 0 respectively. Only one patient from each category showed resistance to both streptomycin and isoniazid in previously treated as well as in new case.

Conclusion: The study reports an unchanged low level of MDR-TB prevalence among new cases in an urban area of Odisha over a decade. This could be due to the success of Directly Observed Treatment Short-course (DOTS) in effective treatment of drug-susceptible TB in the state and non-transmission from primary Drug Resistance (DR) TB cases.

Keywords: Acid fast bacilli, Isoniazid, Multi-drug resistant

INTRODUCTION

Globally the progress of Tuberculosis (TB) control activities have been hampered by the emergence of Multi Drug Resistance (MDR) and Extensively Drug Resistant (XDR) strains resulting in increased burden to low and middle income countries. It has been estimated that out of 300,000 MDR-TB cases, more than half of the cases were from countries like India, China and Russian federation [1]. 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 [2]. 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 [3]. 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 [4] Jammu and Kashmir [5] and Delhi [6] had recorded a prevalence of 24%, 5.7% and 4.0% MDR-TB among new pulmonary TB cases. Studies conducted in other parts of Odisha in the year 2003 and 2014 reported less than 1% MDR-TB prevalence in new cases [7,8]. Information on anti-TB drug resistance patterns particularly among newly diagnosed cases is crucial for planning an effective TB control program. Therefore, this prospective study was planned to determine the antimicrobial susceptibility to first line anti TB drugs among newly diagnosed pulmonary TB patients.

MATERIALS AND METHODS

This study was a subcomponent of a prospective microscopy study undertaken in Bhubaneswar, the state capital of Odisha from January 2014 to September 2014 in collaboration with state and district TB department, Government of Odisha. During this period, suspected TB patients attending Designated Microscopy Center (DMC) of Capital hospital, Bhubaneswar were interviewed with the standard questionnaire to determine their history of anti-TB drug therapy and were classified as new or old cases based on treatment history. Patients unwilling to take part in the study or denying signing the consent form were not included in the study. Sputum specimens of 850 suspected TB patients were collected from the DMC after the DMC laboratory technician finished his/her smearing for Ziehl-Neelsen (ZN) microscopy. The sputum specimens were transported same day from DMC to the NRL of RMRC, Bhubaneswar for AFB microscopy by Ziehl-Neelsen (ZN) and Auramin staining. All the sputum specimens irrespective of their AFB status were processed for solid culture by modified Petroff's method up to May 2014 after which they were processed by N-acetyl-L-cysteine-Sodium hydroxide (Nalc-NaOH) method due to introduction of Line Probe Assay (LPA) in the laboratory. After processing, a loopfull of deposit was inoculated on two LJ slopes and one LJ slope with p-Nitrobenzoic Acid (PNB). The culture slants were incubated at 37°C. All the slopes were observed for occurrence of growth daily for first week and then at weekly intervals for eight weeks. 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. Drug susceptibility was carried out as per RNTCP guidelines following proportion method [9]. Briefly colonies from surface of LJ medium were transferred into sterile test tubes containing 6-8 glass beads. The suspension was adjusted to 1 McFarland standard. The final concentration of drugs were; 0.2µg/ml for Isoniazid (H), 40µg/ ml for Rifampicin (R), 2µg/ml for Ethambutol (E) and 4µg/ml for Streptomycin (S). H37Rv was used as internal control for drug susceptibility testing. During this period the laboratory passed external quality testing by Supra National Reference Laboratory, National Institute for Research in Tuberculosis (NIRT), Chennai. All the activities like reagent and media preparation were carried out as per standard operating procedures. The ethical committee of RMRC Bhubaneswar approved the study protocol.

RESULTS

Out of the 850 cases, 161 patients showed positive growth on LJ medium. Out of these culture positives, 53 were smear negative and 10 were scanty and rest were having higher grades of AFB load in the specimen [Table/Fig-1]. The age of the 161 patients ranged between 10 and 82 years (Mean ± SD, 39.84 ± 15.05, Median 40). Among them 72.0% were males and 72.7% were newly diagnosed cases [Table/Fig-2]. The age of newly diagnosed cases (n=117) ranged between 10 and 82 years (Mean ± SD, was 40.45 ± 16.09, Median 40), and previously treated cases (n=44) ranged between 19 and 63 years (Mean ± SD, 38.20 ± 11.85, Median 40). On drug susceptibility testing of these 161 cases, three cases showed resistance to rifampicin and isoniazid. The single MDR-TB case detected among the 117 newly diagnosed cases was an 80 year male, who showed resistance to Streptomycin (S), Isoniazid (H), Rifampicin (R), and Ethambutol (E). Thus, the prevalence of MDR-TB among new sputum positive pulmonary TB patients was 0.85%. The prevalence of MDR-TB observed among 44 previously treated cases were 4.54%. Prevalence of mono resistance to streptomycin, isoniazid, rifampicin and ethambutol in new cases were 3.41%, 2.56%, 0 and 0.85% respectively [Table/Fig-3]. While mono resistance to streptomycin, isoniazid, rifampicin and ethambutol in previously treated cases were 2.27%, 13.6%, 2.27% and 0 respectively and one showed resistance to both streptomycin and isoniazid in previously treated as well as in new case.

Smear grade	No. of smear positive (%)
Negative	53 (32.9)
Scanty	10 (6.2)
1+	38 (23.6)
2+	30 (18.6)
3+	30 (18.6)

[Table/Fig-1]: ZN smear status of culture positives on LJ medium (n=161).
ZN: Ziehl-Neelsen.

Characteristics	Frequency	Percentage (%)
Sex		
Male	116	72
Female	45	28
Age		
<=15	3	1.9
16-30	49	30.4
31-45	53	32.9
46-60	43	26.7
>60	13	8.1
Category		
New	117	72.7
Retreated cases	44	27.3
Overall	161	100

[Table/Fig-2]: Characteristics of study participants.

DISCUSSION

The emergence of MDR-TB is a challenge to TB control programmes globally. India is one of the highest MDR-TB burden countries in the world with an estimated 99,000 incidence of MDR-TB cases. In order to plan effective control measures for MDR-TB, data from various areas across the country are needed. The prevalence of MDR-TB varies between 0.07 to 5.7% across India [2,3,5-8,10] and our findings of 0.85% also falls in this range. However few areas like urban Mumbai reported very high prevalence of 24% of MDR-TB in new cases [4]. In the present study it was observed that out of 161 symptomatic pulmonary TB patients found positive by LJ culture, 18.9% were smear negatives and 6.2% were having very low levels of mycobacteria (scanty smears) proving the superiority of culture over microscopy. The study reported mono resistance of 3.41%, 2.56%, 0 and 0.85% in new and 2.27%, 13.6%, 2.27% and 0 in previously treated TB cases to streptomycin, isoniazid, rifampicin and ethambutol respectively. The observed 0.85% prevalence of MDR TB in new cases was slightly higher than the previous studies [7,8] that reported 0.7% and 0 prevalence in Odisha. Prevalence of 4.1% MDR-TB in the present study of previously treated individuals was also lower than the earlier study in Odisha that reported 8.1% MDR-TB [8]. It was advocated that, in areas reporting high MDR- TB prevalence may be due to high transmission from primary DR-TB cases rather than failure of Directly Observed Treatment Short-course (DOTS) [11]. A study carried out in Sahariya tribe of Madhya Pradesh showed that in spite of high TB prevalence (3000/1,00,000 population), MDR-TB prevalence was 2.2% in newly diagnosed cases [12]. In order to maintain the low levels of MDR-TB, it will be necessary to diagnose DR-

Resistance status	New cases, n=117		Re-treated cases, n=44		Total, n=161	
	N	%(95% CI)	N	%(95% CI)	N	%(95% CI)
Total Susceptible	107	91.4 (84.7-95.3)	33	75.0 (59.9-85.7)	140	86.9(80.7-91.3)
Any Resistance	10	8.5(4.6-15.2)	11	25.0 (14.2-40.0)	21	13.04 (8.6-19.2)
Resistance to S	4	3.41 (1.27-8.85)	1	2.27 (0.30-14.9)	5	3.10 (1.28-7.3)
Resistance to H	3	2.56 (0.81-7.74)	6	13.6 (6.1-27.5)	9	5.59 (2.9-10.4)
Resistance to R	0	0	1	2.27 (0.30-14.9)	1	0.06 (0.008-4.3)
Resistance to E	1	0.85 (0.11-5.94)	0	0	1	0.06 (0.008-4.3)
Resistance to HR	0	0	2	4.54 (1.10-16.8)	2	1.24 (0.03-4.8)
Resistance to HS	1	0.85 (0.11-5.94)	1	2.27 (0.3-14.9)	2	1.24 (0.03-4.8)
Resistance to SHRE	1	0.85 (0.11-5.94)	0	0	1	0.06(0.008-4.3)

[Table/Fig-3]: Drug susceptibility pattern of *M. tuberculosis* to first line anti TB drugs
S: Streptomycin, H: Isoniazid, R: Rifampicin, E: Ethambutol, HR: Isoniazid and Rifampicin, HS: Isoniazid and Streptomycin, SHRE: Streptomycin, Isoniazid, Rifampicin and Ethambutol, CI: Confidence Interval, N: Number of Samples.

TB cases early and render DOTS Plus treatment promptly. In the present scenario with the availability of Cartridge Based Nucleic Acid Amplification Tests (CB- NAAT) to district level hospitals, the diagnosis of MDR-TB will be accelerated resulting in early DOTS plus treatment initiation and blocking of DR-TB transmission.

LIMITATION

The major limitation of the present study is the small sample size and therefore, it is not representative of the population at large. In fact, this limitation was observed in most previous studies on MDR-TB.

CONCLUSION

Our findings suggest, the persistence of low level of MDR-TB prevalence in the state and good adherence to the basic DOTS. This low level can be maintained by early diagnosis of MDR-TB cases by CB NAAT in district level and prompt initiation of DOTS Plus regimen in the state of Odisha.

ACKNOWLEDGEMENTS

We thank the State TB Officer, Odisha and District Tuberculosis Officer, Bhubaneswar who permitted the collection of sputum samples for this study. The technical help of Mr. Sisir Kumar Barik, TA, Ms Lucy Parija, M Sc dissertation student and NRL, Bhubaneswar staff has been acknowledged. This work was supported by the Indian Council of Medical Research (ICMR) under an extramural research grant which is acknowledged.

REFERENCES

[1] World Health Organisation (WHO), Global tuberculosis Report: 2014. Available:www.who.int/tb/publications/global_report/gtbr14-main-text.pdf. [Accessed at 15th January 2016].
[2] Ramachandran R, Nalini S, Chandrasekhar V, Dave PV, Sanghvi AS, Wares F, et al. Surveillance of drug–resistant tuberculosis in the state of Gujrat, India. *Int J Tubercl Lung Dis.* 2009;13:1154-60.
[3] Aziz MA, Wright A, Laszlo A, De Muynck A, Portaels F, Van Deun A, et al. Epidemiology of anti-tuberculosis drug resistance (the Global Project on Anti-tuberculosis Drug Resistance Surveillance): an updated analysis. *Lancet.* 2006;368: 2142-54.
[4] D'souza DT, Mistry NF, Vira TS, Dholakia Y, Hoffner S, et al. High levels of multidrug resistant tuberculosis in new and treatment-failure patients from the Revised National Tuberculosis Control Programme in an urban metropolis (Mumbai) in Western India. *BMC Public Health.* 2009;29:209–11.
[5] Datta BS, Hassan G, Kadri SM, Qureshi W, Kamili MA, Singh H, et al. Multidrug-resistant and extensively drug resistant tuberculosis in Kashmir, India. *J Infect Dev Countries.* 2010;4:19-23.
[6] Myneedu VP, Singhal R, Khayyam KU, Sharma PP, Bhalla M, Behera D, et al. First and second line drug resistance among treatment naive pulmonary tuberculosis patients in a district under Revised National Tuberculosis Control Programme (RNTCP) in New Delhi. *J of Epidemiol Global Health.* 2015;5:365-73.
[7] Mahadev B, Kumar P, Agarwal SP, Chauhan LS, Srikantaramu N. Surveillance of drug resistance to anti-tuberculosis drugs in Districts of Hoogli in West Bengal and Mayurbhanj in Orissa. *Ind J of Tuberculosis.* 2005;52:5-10.
[8] Das D, Dwibedi B, Kar SK. Low levels of anti TB drug resistance in Rayagada district of Odisha, India. *Int J of Mycobacteriology.* 2014;3:76-78.
[9] Revised National Tuberculosis Control Programme, Manual of standard operating procedures (SOPS) Culture of *Mycobacterium tuberculosis* and drug susceptibility testing on solid medium. Central Tuberculosis Division, New Delhi, India, 2009.
[10] Sharma SK, Kumar S, Saha PK, George N, Arora SK, Gupta D, et al. Prevalence of multidrug-resistant tuberculosis among Category II pulmonary tuberculosis patients. *Indian J Med Res.* 2011;133(3):312–15.
[11] Suen SC, Bendavid, E, Goldhaber-Fiebert JD, Disease control implications of India's changing multidrug-resistant tuberculosis epidemic *PLos One.* 2014;9(3):1-11.
[12] Bhat J, Rao VG, Yadav R, Muniyandi M, Sharma R, Karfarma C, Luke C. Situation of drug resistant tuberculosis in Saharia tribe of central India. *Indian J Med Res*. 2015;141(5):636–39.

PARTICULARS OF CONTRIBUTORS:
1. Scientist-E, Regional Medical Research Centre, Bhubaneswar, Odisha, India.
2. Senior Research Fellow, ICMR, Regional Medical Research Centre, Bhubaneswar, Odisha, India.
3. Technical Assistant (Research), Regional Medical Research Centre, Bhubaneswar, Odisha, India.

NAME, ADDRESS, E-MAIL ID OF THE CORRESPONDING AUTHOR:
Dr. Dasarathi Das,
Scientist-E, Tuberculosis Division, Regional Medical Research Centre, South Eastern Railways Complex,
Bhubaneswar-751023, Odisha, India.
E-mail: drdas60@rediffmail.com

FINANCIAL OR OTHER COMPETING INTERESTS: None.

Date of Submission: **Mar 22, 2016**
Date of Peer Review: **Apr 20, 2016**
Date of Acceptance: **Aug 24, 2016**
Date of Publishing: **Nov 01, 2016**