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DRUG RESISTANCE PATTERN IN NEW AND PREVIOUSLY TREATED CASES OF PULMONARY TUBERCULOSIS PATIENTS AT A TERTIARY CARE CENTRE IN MUMBAI

Microbiology

Disha Sharma — Resident doctor, Department of Microbiology, T.N. Medical College & B Y L Nair Ch. Hospital Mumbai 400008, India

Reena Set* — Professor (Additional), , Department of Microbiology, T.N. Medical College & B Y L Nair Ch. Hospital Mumbai 400008, India *Corresponding Author

Sheetal Bankar — Assistant Professor, Department of Microbiology, T.N. Medical College & B Y L Nair Ch. Hospital Mumbai 400008, India

Jayanthi Shastri — Professor and Head, Department of Microbiology, T.N. Medical College & B Y L Nair Ch. Hospital Mumbai 400008, India

ABSTRACT

Introduction: India has been identified as a hot spot region for multidrug resistant tuberculosis (MDR TB) infection. The objectives were to isolate and characterize Mycobacterium species isolated from cases of pulmonary TB, evaluate drug resistance patterns to first line drugs in isolates of Mycobacterium tuberculosis and to assess proportion of new and previously treated pulmonary TB patients with MDR TB.

Materials and methods: The present study was an open labelled prospective study. The study was conducted for a period of one year from December 2015 to November 2016 on 250 patients. Two early morning sputum samples were collected and subjected to Ziehl Neelson staining and culture on LJ media.

Results: The overall prevalence of pulmonary TB due to M. tuberculosis in the present study was 9.2%. The overall prevalence of drug resistance in the present study was 39.1%. The prevalence of MDR TB was 17.4%. Thirty percent of the previously treated cases were MDR TB, whereas none of the new cases were multidrug resistant.

KEYWORDS

Multidrug resistance, Polyresistance, new case, Previously treated

Introduction

India designated as a high burden country for tuberculosis (TB) has also been identified as a hot spot region for multidrug resistant tuberculosis (MDR TB) infection. Detecting antibiotic resistance in *Mycobacterium tuberculosis* (MTB) is becoming increasingly important with the global recognition of drug resistant strains. In WHO reports on global TB control, estimated percent of MDR TB in India continues to 2.2 % and 15 % amongst new TB and retreated patients respectively.[1] In contrast, key studies from a private tertiary care centre in Mumbai have reported initial drug resistance of 30% and acquired drug resistance of 67% to isoniazid and rifampicin in 2003 [2] and have shown increasing trends of MDR TB among new and retreated cases in 2006.[3] Desiree D Souza had determined drug resistance in Mumbai in newly diagnosed cases from April 2004 to January 2007 and found MDR TB in 24% of untreated and 41% in treatment failure cases.[4] A study done at J.J. Hospital, a government hospital in Mumbai, over a period of 5 years from 2005 to 2009 found MDR TB to be 47.5%. However, this study had not categorized patients into new and previously treated.[5]

Published literature from Mumbai, have consistently shown higher levels of MDR TB at 24-30% in new cases and 11-67% in treated cases, however the corresponding figures from other parts of the country are much lower. [4] Though studies have been done from many parts of Mumbai, the information on drug resistance in new and previously treated cases of pulmonary tuberculosis, in the geographic area served by our tertiary care centre are scanty. The objectives were to isolate and characterize Mycobacterium species isolated from cases of pulmonary TB, evaluate drug resistance patterns to first line drugs in isolates of *Mycobacterium tuberculosis* and to assess proportion of new and previously treated pulmonary TB patients with MDR TB.

Materials and Methods

The present study was an open labelled prospective study carried out in compliance with protocol in the department of Microbiology of a tertiary care hospital in south Mumbai, after approval from the Institutional Ethics Committee. The study was conducted for a period of one year from December 2015 to November 2016 on 250 patients. Two early morning sputum samples were collected. The inclusion criteria was clinically suspected cases of TB aged 18 years or above of either sex. Patients with only extrapulmonary TB and those who did not give consent were excluded from the study.

All the samples were screened for acid fast bacilli (AFB) after staining the smear by Ziehl Neelsen (Z-N) method. A loopful of sediment was inoculated on a Lowenstein Jenson (LJ) medium slope in a certified biosafety cabinet type 2 after concentration by N-acetyl L cysteine NaOH (NALC-NaOH) method. All slopes were incubated at 37^{0}C and reported weekly for 8 weeks. Speciation was done by the niacin accumulation test, nitrate reduction test and susceptibility to paranitrobenzoic acid (PNB) method (All kits for biochemical analysis and LJ with drugs were provided by Hi Media.)

Antitubercular sensitivity testing was done by Economic variant of Proportion Method.[7]

Drug media	Concentration	Critical proportion to determine resistance
Isoniazid	0.2 µg/ml	1%
Rifampicin	40 µg/ml	1%
Ethambutol	2 µg/ml	1%
Streptomycin	4 µg/ml	1%

Results and Observation

Out of 250 patients, 144 were males and 106 were females. The mean age of study population was 40 years with a range of 18-58 years. Forty nine percent of the patients were in the age group of 18-38 years. Of these, males constituted 58% and 42% were females. In the present study, majority of the patients (75%) presented with cough. Fever was the second most common symptom (64%) followed by weight loss, dyspnoea, chest pain and hemoptysis.

Out of a total 250 clinically suspected cases of pulmonary tuberculosis, mycobacterial growth was seen in 27 samples (10.8%), 17 (6.8%) got contaminated and 206 (82.4%) did not show any growth. Out of the 27 culture positive samples, 23 were positive for *Mycobacterium tuberculosis* and 4 were non-tuberculous mycobacteria. The overall prevalence of pulmonary TB due to *M. tuberculosis* in the present study was 9.2%.

Table No I: Grading of smears in culture positive cases of tuberculosis (n=23)

Grade	No.	Percentage
Scanty	0	0
1+	12	52.3%
2+	5	21.7%
3+	6	26%

*All 23 patients who were culture positive were also smear positive.

Table II: History of household contacts in positive cases (n=23)

	No.	Percentage
H/o Household contacts	16	69.6%
No H/O Household contacts	7	30.4%

Table No III. Treatment history in culture positive cases (n=23)

	Susceptible	95% CI	Resistant	95% CI	Total	Percentage
New case	9	0.04-0.38	1	0.04-0.12	10	43.4%
Previously Treated	5	0.04-0.45	8	0.15-0.54	13	56.6%

Table No. IV Drug susceptibility pattern in culture positive patients (n=23)

	Total	Percentage
Susceptible to all primary line drugs	14	60.9%
Resistant to RIF only	2	8.7%
Resistant to S only	2	8.7%
Polyresistant cases	1	4.3%
Multi drug resistant	4	17.4%

Out of the 23 cases of pulmonary tuberculosis, 9 were resistant to one or more drugs. The overall prevalence of drug resistance in the present study was 39.1%. Monoresistance i.e.resistance to any one drug was observed in 4 out of 23 cases (17.4%). There was no case of resistance to Isoniazid and ethambutol. All the 4 cases of MDR TB were previously treated cases.

Table No. V: Drug susceptibility pattern of M. tuberculosis to first line anti TB drugs (n=23) in new and previously treated cases

Resistance status	New case(n=10)	Previously treated(n=13)	Total (N=23)
Total susceptible	9	5	14
Resis to S only	1	1	2
Resis to R only	0	2	2
Resis to RES	0	1	1
Resis to HR	0	1	1
Resis to HRS	0	1	1
Resis to HRSE	0	2	2

Drug resistant TB was seen in 9 cases of which 8 were previously treated and only 1 patient was a new case of TB. Thirty percent of the previously treated cases were MDR TB, whereas none of the new cases were multidrug resistant. Monoresistance to rifampicin was seen in 15.3% (2/13) of previously treated cases. Ten per cent (10%) of new cases and 7.6% of previously treated cases were resistant to Streptomycin.

Discussion

Multidrug resistant tuberculosis is increasing alarmingly. Pattern of drug resistance varies between geographical locations and at different time periods. Therefore, it is important to know the drug resistance pattern of the area where our tertiary care center is situated to formulate an effective drug regimen.

The prevalence of TB was 9.2% in the present study. Our study is similar to a study done in north India where, 10.4% MTB was isolated from pulmonary TB cases.[8] *M.tuberculosis* had been isolated from 16.22% of specimens in a 5 year study done on solid media at JJ Hospital in Mumbai but they had included both pulmonary and extra pulmonary tuberculosis samples.[5]

A total of 23 patients were found to be culture positive for *Mycobacterium tuberculosis*, 70% of which were males. The median age of patients with culture positivity was 42 years with maximum patients (49%) in 38-58 years age group. Majority of the cases had a 1+ grading (Table I). In Das et al's study, the predominant grading was 1+ in the smear positive cases.[9]

In the present study, 69.5% (16/23) of culture positive cases gave a history of household contact with a TB patient (Table II). In Gaude et al's study, 14.6% of culture positive cases had a contact history of TB.[10] The history of contact with TB was 44.2% among MDR TB cases in a study from North India.[11] Hence, it may be concluded that contact with a patient suffering from pulmonary TB constitutes an important risk factor for the development of TB.

The prevalence of drug resistance in the present study was 39.1% (9/23), where 4 out of 9 cases were MDR (Table IV). Rao et al[14] reported an overall drug resistance of 33.4% from southern India and Sethi et al[12] in 2012 observed 48.9% drug resistance from northern India. Das et al[9] recorded a drug resistance of 13.04% from eastern India. Rokade et al[15] reported a drug resistance of 30% in 2012 in Mumbai. All these studies were done using solid media. Thus, the prevalence of overall drug resistance in the present study is within range of other studies. Table IV shows overall sensitivity to all four anti TB drugs to be 60.8%. This correlates with a study done in Lucknow where overall sensitivity was 78.6%.[16] In the present study, 10% of new cases and 61.5% of previously treated were found to be drug resistant. Jain et al[16] reported 64% drug resistance among previously treated cases.

The overall prevalence of monoresistance was 17.4% in this study. This is in accordance to study by Sethi et al which showed overall monoresistance of 15.5 % [12]. The study conducted by Menon et al in Mumbai found an overall monoresistance of 14.11% .[5] Monoresistance was not seen for isoniazid in this study, similar to findings by Gaude et al.[10]

Ninety percent of the new cases and 38.46% of the previously treated group were sensitive to the four first line drugs. Sethi et al[8] found 27% and Maurya et al[13] found 43.2% of previously treated cases to be susceptible to first line drugs. Monoresistance was seen in 10% of new and 23% of previously treated cases.

Monoresistance was seen in 10% of new and 23% of previously treated cases (Table V). D'souza et al reported 16% monoresistance in new and 21% in previously treated cases.[4] Fifteen percent of previously treated cases were monoresistant to rifampicin. Ten percent (10%) of new cases and 7.6% of previously treated cases were resistant to Streptomycin in our study (Table V). Sethi et al reported 12.4% resistance to Streptomycin in new cases and 11.2% in previously treated cases.[12]

In the present study, the prevalence of MDR TB was observed to be 17.4% which correlated with Sethi et al[12] who reported a multi drug resistance of 21% and Jain et al[16] observed multidrug resistance in 20% cases of pulmonary TB. Menon et al[5] reported a multidrug resistance of 25% in 2009 in Mumbai. Another study conducted in Mumbai by Rokade et al[15] observed 15% of MDR TB in pulmonary TB cases. In the present study, 44.4 % (4/9) were MDR TB among drug resistant cases (Table no.IV). Maurya et al reported 39.9% drug resistant *M.tuberculosis* isolates to be MDR [13]. Many factors lead to MDR TB.

MDR TB was seen in 30.7% of the previously treated cases (Table V). A study in Mumbai showed 41% MDR in previously treated cases[4]. In a study by Sethi et al[8] in 2013, 28% of the previously treated group had MDR TB which correlates with our study. Maurya et al[13] reported 43.2% MDR amongst previously treated cases

The present study has a limitation in not knowing whether the source of infection in previously treated cases are exogenous or endogenous. This is due to the lack of application of molecular techniques due to financial constraints and also, the isolates from previous episode and the present episode have not been genotyped

Conclusion

This study gives the insight of the increasing rate of multidrug resistance in pulmonary TB patients in Mumbai. The study significantly highlights the resistance pattern in new and previously treated patients. Area specific studies as this one, compliment the overall estimates and can help in directing resources and prioritizing interventions.

Monoresistance is also increasing in the mycobacterial isolates, however, more studies need to be conducted to study the pattern. This

further signifies the importance of performing sensitivity tests in culture positive isolates for initiating proper treatment regime for the patients.

The past history of treatment constitutes an important risk factor for the development of drug resistance.

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