



Original Research Article

Multidrug Resistant Tuberculosis in a Northern District of Karnataka, South India

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ABSTRACT

Background: Tuberculosis continues to be a major public health problem in most of the developing world. Further, emergence of multidrug-resistant tuberculosis and extensively drug-resistant tuberculosis has become a challenging problem in the management of the disease and tuberculosis control programmes. This study was undertaken to determine the prevalence rate of multi drug-resistance and analyze drug resistance pattern in new pulmonary tuberculosis cases.

Methodology: A total of 631 clinically suspected new adult pulmonary tuberculosis cases were evaluated for microbiological evidence of tuberculosis. Two hundred and eighty three *M. tuberculosis* isolates were subjected to antimicrobial susceptibility to isoniazid (INH), streptomycin (SM), ethambutol (EMB) and rifampicin (RIF).

Results: Amongst the 631 cases, direct microscopy was positive in 256 (40.57%) and *M. tuberculosis* was isolated in 283 cases (44.8%). Of the 283 isolates, 94 (33.21%) strains showed resistance to one or more drugs. Resistance to 1 drug, 2 drugs, 3 drugs and all the 4 drugs was seen in 23.32%, 5.65%, 1.76% and 2.47% of the isolates respectively. Multi drug resistance was seen in 12 (4.24%) cases. Maximum resistance was seen to streptomycin (28.97%).

Conclusion: For a better management of drug resistant cases, early detection of resistance pattern is extremely important so that effective treatment can be prescribed and further dissemination of drug-resistant strains in the community can be prevented.

Key words: New pulmonary tuberculosis cases; *Mycobacterium tuberculosis*; Multidrug resistance.

INTRODUCTION

Tuberculosis still remains a major cause of morbidity and mortality worldwide. According to WHO, in 2010, there were an estimated 8.8 million incident cases of tuberculosis globally with 2 million deaths annually. ^[1,2,3] Of these, more than half the

cases occurred in five South-East Asian countries - India, China, South Africa, Indonesia and Pakistan and India alone accounted for 26% of global tuberculosis cases. ^[1,4] The prevalence of multidrug-resistant tuberculosis (MDR-TB) is increasing throughout the world both in new

as well as previously treated cases. India has been designated as a 'high burden' country for multi-drug-resistant tuberculosis. [1,2,5,6] The magnitude of new tuberculosis cases with MDR varies from 0.5% to 5.3% in studies conducted in diverse locations in India. [1,7,8,9] Recently totally drug resistant cases have been reported from India. [10] The outcome of treatment in patients harboring MDR strains is poor with a high mortality rate. A prompt diagnosis and effective chemotherapy is the mainstay in the management of drug resistant tuberculosis. Comprehensive reports on MDR TB are not available from this part of India. Hence this study was undertaken to evaluate the rate of smear positive tuberculosis and delineate drug resistance pattern in new pulmonary tuberculosis cases in this area.

METHODOLOGY

This prospective, cross sectional study was carried out in the Department of Microbiology, B.L.D.E.U's Shri. B. M. Patil Medical College, Hospital and Research Centre, Bijapur, Karnataka, India. The entire experimental protocol was approved by the institutional ethical committee.

Source of data: Six hundred and thirty one pulmonary tuberculosis patients satisfying the inclusion and exclusion criteria and voluntarily offering to take part in the study were included.

Inclusion criteria: Clinically suspected new adult pulmonary tuberculosis cases were included in the study.

Exclusion criteria: Pulmonary tuberculosis patients receiving anti-tubercular treatment and relapse cases were excluded from the study.

Collection of specimen: Three early morning sputum samples, on 3 consecutive days were collected from each patient and processed on the same day.

Processing of sample: The specimens were processed according to the National

Tuberculosis Institute Manual, India. [11] A direct smear was prepared from each specimen and stained by Ziehl Neelsen staining for microscopic study. The sputa were then concentrated by modified Petroff's method and a loopful of the sediment was inoculated on to a pair of Lowenstein and Jensen media. The media were incubated at 37°C and observed for growth daily for the first week and then twice a week, up to 8 weeks.

Identification: The isolates were identified by growth rate, pigment production, growth in the presence of PNB, TCH and standard biochemical tests. [11]

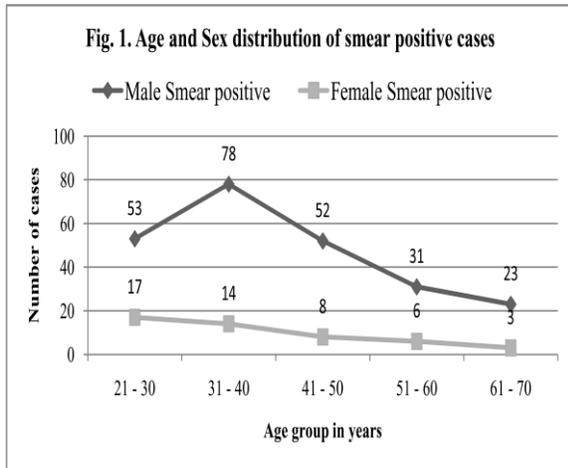
Drug susceptibility testing: All the isolates, along with standard strain H37Rv were subjected to antimicrobial susceptibility testing by indirect method using Lowenstein Jensen media with the recommended drug concentrations. The strains were tested for Isoniazid (INH), Rifampicin (RIF) and Ethambutol (EMB) by minimum inhibitory concentration method and by resistance ratio method for Streptomycin (SM). These media were incubated at 37°C and were examined for growth after 28 days. The concentration of the drug-containing medium in which there were < 20 colonies or no growth was taken as the MIC of the drug. [11]

Data analysis: Statistical analysis of the data was done using GrapPad InStat software.

RESULTS

In the present study out of 621 patients, 285 (45.1%) cases were positive by direct microscopy. The rate of smear positive pulmonary tuberculosis was more in males i.e. 237 (83.1%) as compared to 48 (16.8%) in females. Significant difference in the smear positivity rates between males and females was noted, with P= 0.0223. Amongst the smear positive cases in males, majority belonged to the age group of 31- 40 years and predominant age group affected in

females was 21-30 years (Fig.1). Of the 621 patients, *M. tuberculosis* was isolated from

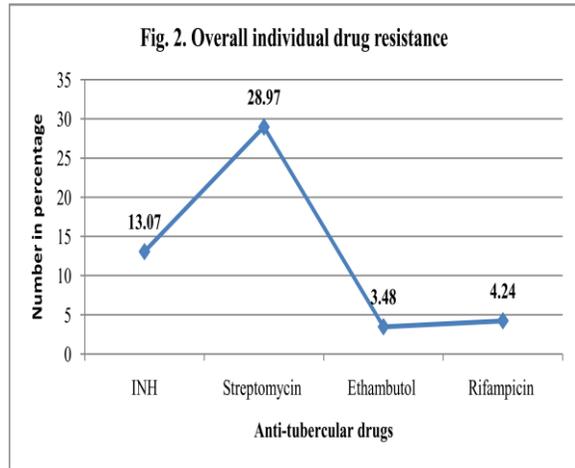


The relative results of direct microscopy and culture are given in the table (Table 1). The sensitivity and specificity of direct microscopy was 90.46% and 93.82% when compared to culture.

Of the 283 isolates tested for drug susceptibility, 189 (66.78%) strains were sensitive to all the 4 drugs and 94 (33.21%) were resistant to one or more drugs. Amongst the resistant cases, resistance to a single drug was seen in 66 (23.32%) cases, 2 drugs in 16 (5.65%), 3 drugs in 5 (1.76%) and all the 4 drugs in 7 (2.47%) cases (Table 2).

Of the 66 isolates showing resistance to a single drug, highest number was seen to streptomycin with 57 (20.14%) followed by

283 cases giving the isolation rate of 44.8%.



INH with 09 (3.18%) isolates. No single drug resistance was noted to rifampicin and ethambutol. Among the isolates showing resistance to 2 drugs, the combination of drugs involved were INH+SM in 13 (4.59%) and INH+RIF in 3 (1.06%). In strains exhibiting resistance to 3 drugs, the groups of drugs involved were INH+SM+RIF in 2 (0.70%) and INH+SM+EMB in 3 (1.06%).

Resistance to all the 4 drugs was seen in 7 (2.47%) cases (Table 3). Multidrug resistance was noticed in 12 strains i.e. 4.24%. Overall, amongst the resistant cases, maximum resistance was seen to streptomycin (28.97%) followed by isoniazid (13.07%), rifampicin (4.24%) and lastly ethambutol (3.48%) (Fig.2).

Table 1: Results of Direct microscopy and culture

Total cases	Micro +ve Culture +ve	Micro +ve Culture -ve	Micro -ve Culture +ve	Micro -ve Culture -ve	Contaminated
631	256 (40.57%)	21 (3.32%)	27 (4.27%)	319 (50.55%)	08 (1.26%)
Fisher's Exact test	P < 0.0001				

When compared to culture sensitivity and specificity of microscopy was found to be 90.46% and 93.82%.

Table 2: Results of antimicrobial susceptibility test

Isolates tested	Sensitive	Resistant	Resistance to 1 drug	Resistance to 2 drugs	Resistance to 3 drugs	Resistance to 4 drugs
283	189 (66.78%)	94 (33.21%)	66 (23.32%)	16 (5.65%)	5 (1.76%)	7 (2.47%)

Table 3: Drug resistance pattern

Resistance to 1 drug				Resistance to 2 drugs		Resistance to 3 drugs		Resistance to 4 drugs
INH	STR	ETH	RIF	INH + STR	INH + RIF	INH + STR + RIF	INH + STR + ETH	INH + STR + ETH + RIF
09 (3.18%)	57 (20.14%)	00 (00%)	00 (00%)	13 (4.59%)	3 (1.06%)	2 (0.70%)	3 (1.06%)	7 (2.47%)
66 (23.32%)				16 (5.65%)		5 (1.76%)		7 (2.47%)

DISCUSSION

Tuberculosis has been for many centuries the most important of human infections in its global prevalence. Of all the forms of the disease, pulmonary tuberculosis is the commonest, constituting about 85%. From a public health perspective, smear positive patients bear greater significance than smear negative patients in terms of the bacillary load as well as their potential to spread the organisms in the community. In our study, of the 631 cases, 285 (45.1%) showed smear positive tuberculosis which is lower compared to studies by Ramachandran R et al. (98.5%) and Jain A et al. (87.3%) [12,13] but higher to that of Ghatole M et al. (23.5%). [14] Significant difference in the smear positivity rates between males and females was noted, rates being 83.1% in males and 16.8% in females. Similar findings have been reported by Yeboah-Manu D et al., Mahadev B et al. and Abdul Wadud ABM et al. [15,16,17] The commonest age group affected in males was 31-40 years and in females the major group affected was 21-30 years. Similar findings have been noted by Abdul Wadud ABM et al. [17]

M. tuberculosis was isolated in 283 (44.8%) cases in our study. Higher isolation rates ranging from 57.7% - 95% have been

reported by Jain A et al., Mahadev B et al., Abdul Wadud ABM et al., Acharya S et al. and Malhotra B et al. [13,16-19]

Of the 283 isolates tested in this study, 94 (33.21%) showed resistance to one or more drugs. Similar findings have been reported by Jain A et al. and Datta BM et al. [13,20]

Lower resistance rates have been reported by Mahadev B et al. and Al-Rubaish AM et al. [16,21] and very high resistance has been noted in studies by A B M Abdul Wadud et al. and S Acharya et al. [17,18]

Resistance to a single drug was observed in 66 (23.32%) cases and the highest number was seen to streptomycin with 57 (20.14%) cases followed by INH with 09 (3.18%) cases. Our findings correlate well with studies by Ramachandran R et al. and Yeboah-Manu D et al. [12,15] However in studies by Abdul Wadud ABM et al. and Nagaraja C et al. maximum number of resistance was seen to INH. [17,22]

No single drug resistance was seen to rifampicin and ethambutol in our study which is similar to findings of Ramachandran R et al. and Sharma SK et al. [12,23] Two drug resistant cases were seen in 16 (5.65%) cases and the combination of drugs involved were INH+SM in 13 (4.59%) and INH+RIF in 3 (1.06%) cases in our study. INH+SM was also the most frequent

combination observed in studies by Yeboah-Manu D et al and Mahadev Bet al. [15,16] while INH+RIF combination was the commonest in studies by Abdul Wadud ABM et al. and Nagaraja C et al. [17,22]

The groups of drugs involved in 3 drug resistance were INH+SM+RIF in 2 (0.70%) and INH+SM+EMB in 3 (1.06%) cases. Similar findings have been noted by Sharma SK et al. [23] INH+SM+RIF and INH+RIF+EMB were the commonest groups of drugs in studies by Datta BS et al. and Nagaraja C et al. [20,22] The lone group of 3 drugs seen in studies by Ramachandran R et al. and Mahadev B et al. was INH+SM+EMB. [12,16]

In this study resistance to all the 4 drugs was seen in 7 (2.47%) cases. A lower rate of 0.6% and 0.8% has been noted by Ramachandran R et al. and Sharma SK et al. [12,23] while Datta BS et al. has reported a higher rate of 5.7%. [20]

Overall, amongst the resistant cases, we noted maximum resistance to streptomycin (28.97%) followed by isoniazid (13.07%), rifampicin (4.24%) and lastly ethambutol (3.48%). Our findings are similar to studies by Ramachandran R et al. and Yeboah-Manu D et al. [12,15] In studies by Jain A et al., Abdul Wadud ABM et al., Al-Rubaish AM et al. and Sharma SK et al. highest number of resistance was seen to isoniazid, [13,17,21,23] while maximum resistance for rifampicin was noted by Ani AE et al. [24]

Incidence of multidrug resistance in our study was 4.24% which is similar to that of Malhotra B et al. (4.5%). [19] Diverse MDR rates from 1.2% to 29.9% have been documented in studies by many authors: Mahadev B et al., S Acharya et al., Al-Rubaish AM et al., Agatha E. Ani AE et al., Chakraborty N et al. and Poojary A et al. [16,18,21,24-26]

CONCLUSION

Multidrug resistance and extensively drug-resistant tuberculosis have caused significant concern among health care practitioners. Early detection of resistance is extremely important so that effective treatment can be prescribed and further dissemination of drug-resistant strains in the community can be prevented. In this study multidrug resistance rate was 4.2% and the maximum resistance was seen to streptomycin.

Competing interests: No author has conflicts of interest related to this work.

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