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Original Research Article

Evaluation of Drug Resistance Pattern of Mycobacterium Tuberculosis in a Tertiary Care Teaching Hospital

C. Senthil Vadivu[1*], R. Vinotha[2]

[1]Associate Professor, Department of Microbiology, Govt. Medical College, Krishnagiri
[2]Assistant Professor, Department of Microbiology, Government Ariyalur Medical College, Ariyalur

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Corresponding author: Dr. C. Senthil Vadivu
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Abstract:

Introduction: Nowadays with the greatly expanded efforts to strengthen tuberculosis prevention and control programmes worldwide, there is growing concern about the currently reported and potential future rates of drug-resistant tuberculosis. The resistant cases must be identified as swiftly as possible when they present at health care facilities so that they do not pose a threat to the community. Based on this aim of our study is to evaluate the drug sensitivitypattern of TB patients and correlate it with the socio demographic status of these patients, alsoto assess the influence of risk factors like socio demographic characteristics, DM, HIV status,smoking and previous treatment as a marker for the development of Drug Resistant TB in the study population.

Materials and Methods: The present study was conducted at the Department of Microbiology for a period of six months to assess the drug susceptibility profile TB patients registered under RNTCP. Sputum positive patients were included in the study. Socio demographic and clinical characteristics such as category, treatment details such as drug regularity, number of doses taken by the patients and reasons for default were obtained from patient. Early morning sputum specimens were collected in a sterile container from the study group who were smear positive by Ziehl Neelsen method. All the laboratory works were carriedout as per standard laboratory procedures and Bio-safety norms.

Results and Discussion: Age, diabetes were not risk factors for retreatment tuberculosis and multidrug resistant tuberculosis. Smoking was a significant risk factor amongrelapse, failure and MDR cases. Irregular and interrupted treatment was a risk factor among defaulters, failures and multidrug resistant cases. Ensuring adherence to a full course of treatment is the key to cure TB patients and prevent the emergence of drug resistance. Drug resistance was more among prior treatment failure cases, necessitating the need for timely culture and sensitivity testing for those who remain sputum positive during the course of treatment to curb the spread of multidrug resistant strains.

Keywords: Drug Susceptibility Profile, Pulmonary Tuberculosis.

Introduction

Tuberculosis (TB) is a treatable and preventable disease.[1] It is an infectious bacterial disease caused by Mycobacterium an acid fast bacilli.[2] The SAARC region accounts more than 29% of global burden of tuberculosis with 0.6 million deaths every year and 2 million new cases annually.[3] Tuberculosis is a socio-medical problem.[4] Ziehl-Neelson Staining or by Fluorochrome staining allows highly accurate diagnosis widely available, simple and multi- purpose equipment.[5] Culture increases the number of tuberculosis cases found, often by 30- 50% and detects cases earlier, often before they become infectious.[6] Most of the recent advances in the laboratory diagnosis of TB BY rapid culture, identification, and susceptibility systems.[7] Drug resistant tuberculosis is a case of tuberculosis (usually pulmonary) excreting bacilli resistant to one or more anti-tuberculosis drugs. MDR-TB is resistant to at least Isoniazide and Rifampicin, the main anti tuberculosis drugs.8 DOTS strategy as one of the mostcost-effective health interaction and recommends that effective TB treatment be a part of the essential clinical service package available in primary health care. As the programme strives hard to achieve its goals, it is at present facing the threatof drug resistance, a menace that would destabilize the tuberculosis control. Drug resistance as a limiting factor for success of chemotherapy was recognized immediately following the introduction of Streptomycin. Youmans et al[9] found that when Streptomycin was given alone,there was a rapid decrease in the number of bacilli in the sputum which however increased again. Pyle showed that during treatment with

Streptomycin alone, the proportion of drug- resistant bacilli increased progressively from about 1 in 88,750 organisms before therapy, to about 1 in 367 after 15 weeks of treatment. Studies by Crofton and Mitchison[10] showed that with mono therapy or inadequate therapy, the number of susceptible bacilli.

Drug resistance among new cases is defined as the presence of resistant isolates of M. tuberculosis in patients who, in response to direct questioning, deny having had any prior anti-TB treatment (for as much as 1 month). Drug resistance among previously treated cases is defined as the presence of resistant isolates of M.tuberculosis in patients who, in response todirect questioning, admit having been treated for tuberculosis for 1 month or more (Anti- tuberculosis drug resistance in the world, WHO Fourth Global Report 2008).

Multi-Drug Resistant Tuberculosis (MDR-TB) is defined as resistance to Isoniazid and Rifampicin with or without resistance to other first line drugs and Extensively Drug-ResistantTuberculosis (XDR TB) is defined as MDR-TB with added resistance to at least two (Fluoroquinolones & Injectable agent) of the six mainclasses of second line drugs.

Human error is the principal factor associated with the emergence of drug resistant strains of M. tuberculosis Prescription of inadequate chemotherapy, receiving improper treatment outside the National Programme from private qualified, or even unqualified practitioners, use of drugs of unproven bioavailability, patient's lack of knowledge of the treatment, difficulty experienced by poor patients due to lack of financial resources, shortages of drugs due to poor management and financial constraints in developing countries, poor case-management when the treatment is not directly observed are some of the reasons for the rise of multidrug resistance.

Today, with the greatly expanded efforts to strengthen tuberculosis prevention and control programmes worldwide, there is growing concern about the currently reported and potential future rates of drug-resistant tuberculosis. The resistant cases must be identified as swiftly as possible when they present at health care facilities so that they do not pose a threat to the community. To assess the extent of drug-resistant M. tuberculosis strains harbored among re-treatment pulmonary tuberculosis patients and analyze the factors that had contributed to it, the present study was undertaken at our tertiary care teaching hospital.

Based on this aim of our study is to evaluate the drug sensitivity pattern of TB patients and correlate it with the socio demographic status of these patients, also to assess the influence of risk factors like socio demographic characteristics, DM, HIV status, smoking and previous treatment as a marker for the development of Drug Resistant TB in the study population.

Material and Methodology

The present study was conducted at the Department of Microbiology, Government Medical College, Krishnagiri for a period of 6 months to assess the drug susceptibility profile of TB patients registered under RNTCP.

Early morning sputum specimens were collected in a sterile container from the study group who were smear positive by Ziehl Neelsen method. Surface decontamination was done by immersing the specimen container in Lysol solution before transferring to the laboratory. All the laboratory works were carried out as per standard laboratory procedures and Bio-safetynorms in Class II Bio safety cabinet. Acid fast staining was used to confirm infection. In Ziehl- Neelsen procedure, acid-fast organisms appear pink against a blue background.

Processing of sputum specimens was done by Modified Petroff's Method, When the sputum specimens could not be cultured on the same day, they were processed within a week by CPC method.

Tubercle bacilli do not grow in primary culture in less than one week and usually require two to four weeks to give visible growth from sputum specimens. All cultures were examined within 48-72 hours after inoculation to detect gross contaminants. Thereafter cultures are examined weekly, up to 8 weeks on a specified day of the week. During examination, slopes in which the surface has been completely contaminated or where the medium has been liquefied or discolored were discarded. The drug sensitivity testing for the positive cultures were carried out with inoculum prepared by using a representative sweep of the entire surface of the growth on the slope. The absolute concentration method uses a standardized inoculum grown on drug-free media and media containing graded concentrations of the drugs to be tested. Resistance is expressed in terms of the lowest concentration of the drug that inhibits growth; i.e., minimal inhibitory concentration (MIC).

The collected data were edited for completeness, consistency and accuracy. They were analyzed by the parameters like mean, median and percentages. The differences of above parameters were tested by the percentiles or whichever was applicable wherever. The statistical package used for analysis and interpretation is SPSS (version-13) with the level of significanceP=0.05.

Table 1: Minimum Inhibitory Concentration

Sl.No	Drug	MIC (conc. of drug)
1.	Streptomycin	8
2.	Isoniazid	5
3.	Rifampicin	128
4.	Ethambutol	8
5.	Kanamycin	64
6.	Ethionamide	114
7.	Ofloxacin	8

Results

A total of 100 tuberculosis cases were included for this study. The analysis shows that the mean age of female is 37.1 12.3 and of male is44.4 13.3. The numbers of male patients in each age group was proportionately higher than that of female patients except in the age group of 10-19 and 60-79. It was highest in the age group 30-59 years. In our study79 were male and 21 were female patients. Smoking Habit Patients who gave a history of habitual smoking during the previous treatment but hadstopped at present and who were currently smoking were considered smokers for the purpose of the analysis. In this study only male subjects had the habit of smoking, so the analysis is earmarked to male subjects. 74 were smokers in our study. In our study population, 30 patients were on treatment for diabetes. 9 patients were newly detected as diabetics. Treatment was considered as being on a regular basis if the medication was used as prescribed. Treatment was considered irregular if there was default in the use of medication forfive or more consecutive doses provided it did not reach 30 days a month. The regularity of treatment was assessed and analysed for the study subjects. Only 40% of the total cases had taken a regular treatment previously and the remaining 60% admitted that they had not strictlyadhered to the treatment schedule.

The reasons for default are drug related problems like nausea, vomiting, giddiness were the leading causes for default. Migration to other distant places was a reason for default in 5%of cases. Domestic problems, too ill to attend, other illnesses, inconvenient DOT and dissatisfaction with treatment centers and DOT providers were the other reasons given for defaulting from treatment. Of the 100 samples processed, 79 sputum samples had given a positive culture result for M. tuberculosis 21 of the remaining samples (21.3%) had either given a negative culture orwas contaminated or had grown a Non-tuberculous mycobacteria. Drug sensitivity pattern was analysed. Among the 79 culture positive cases 50 were sensitive to the first line drugs. The remaining 29 were resistant to one or more drugs. The overview of resistance to the first line anti-tuberculosis drugs among the culture positive cases is enumerated in the Table 2.

Table 2: Percentage distribution of drug resistance

Particulars	Cases	
	N	%
Total patients recruited in the survey	108	100
Total patients with DST results	79	79
Total patients with susceptible isolates	50	63
Total patients with drug resistance	29	37
Any resistance to H	27	34
Any resistance to R	19	24
Any resistance to E	3	3.8
Any resistance to S	21	26.5
Total patients with MDR TB	18	22.8

Among the total culture positive cases cumulative drug resistance was most commonlyseen to Isoniazid (27) followed by Streptomycin (21), Resistance to Rifampicin was seen in 19patients and to Ethambutol in 3 patients. Mono drug resistance was noted in 14 patients. It was most commonly seen with Isoniazid and Streptomycin (6 patients each), followed by Rifampicin and Ethambutol (1 patient each). Among the 79 culture positive cases, 18 were MDR TB cases (22.8%).

Of the 18 MDR cases, 9 cases were sensitive to all the second line drugs tested. Of the 9 cases that were resistant to the drugs, resistance to Ethionamide was seen in 5 cases, to and Ofloxacin in 1 case. The Kanamycin and Ethionamide combination was resistant in 2 cases and Ethionomide plus Ofloxacin resistance was seen in 1 case. Kanamycin plus Ofloxacin resistance (XDR-TB) was not observed in the present study.

The regularity of treatment and the total courses of treatment they had undertaken has some effect on resistance, Among the 50 culture positive cases who had taken a single course of treatment 34 cases were sensitive to the drugs and 16 were resistant to the drugs. Among the29 culture positive cases who had taken more than a single course of treatment 14 cases were sensitive to the drugs and 15 were resistant to the drugs (57.6 %).

Among diabetic cases 10 cases were drug resistant of which 5 were multi-drug resistant of the culture positive male cases, 25 cases were drug resistant (41.5%) and among the drug resistant cases 13 were multi-drug resistant. Of the female cases, 8 cases were drug resistant and among the drug resistant cases 4 were multi-drug resistant cases.

Discussion

A total of 100 tuberculosis cases were included for this study. The analysis showed thatthe mean age of registering for re-treatment in female cases is 37.1 and in male cases is 44.4. This shows that women are registered for re-treatment in the younger age than the men. Both male and female cases had notified for retreatment at middle age than the extremes of age in the present study. Balasubramanian et al[11] also observed that the probability of notification decreased significantly with advancing age among both the sexes.

The number of male cases in the study group was more in age group 30-59 years, in which a large proportion of men are likely to be employed. Employed men may be unable to take leave from work to attend the health care settings and would have discontinued prior treatment. Better treatment compliance among women than men have been reported by Ngamvithayapong-Yanai et al[12] and Balasubramanian et al[11]. For men, being the head of the family, loss of job and fear of social isolation were reported as major reasons for discontinuation of the treatment.

Because of the incompliance noted, gender issues are significant for development of drug resistance. But an association was not found in the present study. An European study by Faustini et al[13] observed more MDR cases among men.

It was found that smoking was a significant risk factor among relapse cases in the present study. Studies by Joanna d'Arc et al [14] and Kolappan et al[15] support the observation that smokers are significantly more likely to relapse than nonsmokers. There was a significant association between smoking and multidrug resistant tuberculosis in the present study (62% vs. 17%). Barroso et al[16] identified that smoking was associated with MDR-TB in their analysis.

There was no evidence for an increased risk of retreatment and MDR TB among peoplewith diabetes in the present study. A systematic review of 13 observational studies on the relation between diabetes and tuberculosis by Christie et al[17] found consistent evidence for an increased risk of TB among people with diabetes.

Sophia Vijay et al[18] observed that higher default rates were associated with irregular treatment as in the present study. However Thomas et al 2005 found that the patients who were irregular on treatment were twice as likely to relapse as those who were regular.

There is virtually a consensus among researchers regarding the fact that the number of previous treatments is a risk factor for MDR TB and our study confirmed this association. Pande et al[19] observed the prevalence of MDR-TB in patients with past history of ATT at twocenters in Delhi to be 44.7% and 20% which was statistically significant in their study.

Of the 100 samples processed, 79 showed a positive growth for M. tuberculosis. 14% of the total samples processed were culture negative, 3% were contaminated and 4% had a growth of Non Tuberculous Mycobacteria. A negative culture result with the specimen containing tubercle bacilli may be due to several reasons. In patients receiving treatment, the organisms may have lost their ability to grow on a culture media and be practically dead.. The sputum specimens exposed to heat, stored too long, dried out or contaminated also yield a negative culture. Excessive decontamination procedures before inoculation, over heating before centrifugation, inadequate culture media and deficient incubation also result in a negative culture. Positive smears may be caused by non- tuberculous mycobacteria.

The study group patients claimed to have taken anti-tuberculosis therapy without improvement; however, 50 isolates were sensitive to all four first-line drugs (INH, RIF, SM and EMB) that were tested.

Among all the culture positive patients (n=79) resistance to two drugs in 9 patients, to three drugs in 10 patients and to all four drugs in 2 patients. The similar pattern of resistance was observed by Sophia Vijay et al[18] except for all four drugs which was 15.21% in the former and 4% in the latter.

The present study shows high degree of resistance to Streptomycin similar to study of Sophia Vijay et al[18] which showed resistance to Streptomycin in 9.3%, followed by Isoniazid in 8.4%, Rifampicin in 1.8% and Ethambutol in 0 cases. This is in contrast to study of Shah et al[20], which showed less resistance to Streptomycin, where the pattern of resistance was Isoniazid in 7.5%, followedby Streptomycin in 1.4%, Rifampicin in 0.97% and Ethambutol in 0.4%. Resistance to Isoniazid plus Rifam-

picin alone was similar to study of Shah et al[20] which showed 9.2 % but 4.9% by Sophia Vijay et al[18].

Most of the Rifampicin resistant cases were also resistant to Isoniazid. This has also been observed in studies of Shah et al[20].

Ethambutol was the least resistant drug in all the studies as in the present study, although the percentage was very less (3.5%) compared to 6.6% in Bangalore (Sophia Vijay et al[18]),Chennai 28.7% (Deivanayagam et al [21]), and Jodhpur 39.39% (Mathur et al [22]).

A high degree (22.8 %) of MDR-TB was observed among the study group. This was inaccordance with most of the studies in India. Proportion of MDR- TB in re-treatment cases varied from Jodhpur 38.2% (Mathur et al [22]), Bangalore 12.8% (Sophia Vijay et al 18).

Of the 18 MDR cases, 9 cases were sensitive to all the second line drugs tested. XDR- TB cases were not seen in the present study. Rajesh Mondal et al [23], reported 7.4 % of XDR TB cases, the first ever report from India. A limitation to accurate detection of XDR TB is because; the existing tests for resistance to second line drugs is not yet standardized and are less reproducible than results for first line drugs. Only then second-line treatment can be individualized, based on in-vitro drug resistance, or can be standardized.

Conclusion

Age, diabetes were not risk factors for retreatment tuberculosis and multidrug resistant tuberculosis. Smoking was a significant risk factor among relapse, failure and MDR cases. There is a need to devise effective strategies for counselling patients about the impact ofsmoking on their cure. Irregular and interrupted treatment was a risk factor among defaulters, failures and multidrug resistant cases. Ensuring adherence to a full course of treatment is the key to cure TB patients and prevent the emergence of drug resistance.

Though registered for retreatment, most of the isolates were sensitive to all the first line drugs and hence can be successfully treated with a category II regimen if they are compliant enough. Drug resistance was more among prior treatment failure cases, necessitating the need for timely culture and sensitivity testing for those who remain sputum positive during the course of treatment to curb the spread of multidrug resistant strains.

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