Original Research Article

Study of drug susceptibility pattern of mycobacterium tuberculosis samples from known cases of extrapulmonary tuberculosis samples in Mumbai city

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Abstract

Background: Extrapulmonary Tb constitutes 15-20% of all TB cases. Management of drug-resistant tuberculosis in EPTB cases represents a potential challenge for clinicians. Conventional DST (drug susceptibility testing), although time consuming, remains a gold standard method when it comes to testing of drug susceptibility for M.Tb. **Setting and Design:** The present study was a prospective, observational study conducted for 1-year duration in the Department of Microbiology, at a tertiary care hospital. **Material and Methods:** Patients of all ages and sexes registered at the outpatient department (OPD) / admitted with clinical suspicion of Extrapulmonary tuberculosis, willing to participate were considered for this study. **Results:** In the present study, 150 patients were included. The maximum number of cases i.e. 61 were in the age group of 21-30 years (40.6%), and male to female ratio was 1.14:1. The maximum sample received from patients was pus i.e. 46 (30.8%), followed by pleural fluid 24 (16%), CSF 23 (15.3%), tissue 18 (12%), lymph node biopsy 15 (10%). 31 (20.7%) samples stained positive on ZN stain , 25 (16.7%) patients were AFB culture positive while 125 were found negative. 23 (92%) were infected with M. tb while 2 (8%) were NTM's. Out of the 23 M. tuberculosis positive patients, 11 (47.8%) showed resistance to the first-line anti-tubercular drugs. Out of the 11 isolates showing resistance, 3 (13%) isolates were monoresistant to Rifampicin and 2 isolates (8.7%) to Streptomycin. MDR was observed in 5 (21.7%) isolates while polyresistance to Isoniazid, Ethambutol and Streptomycin was noted in a single (4.4%) isolate. Among the 11 resistance isolates, 9 were reported in the previously treated patients while 2 were new cases. **Conclusion:** Although time consuming, LJ culture DST remains a gold standard in the diagnosis of TB. The difficulty in the identification of the emerging heteroresistance is a matter of concern when we totally rely on molecular diagnostics. Also the paucibacillary nature of EPTB samples make it difficult to diagnose on smear microscopy (5000-10,000 bacilli/ml), so the culture positivity holds a great value as it can detect 10-100 bacilli of concentrated material.

Keywords: drug susceptibility pattern, Mycobacterium tuberculosis, extrapulmonary tuberculosis, rifampicin

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INTRODUCTION

Tuberculosis (TB) is a communicable disease caused by Mycobacterium tuberculosis. Primarily, Mycobacterium tuberculosis affects the lungs, causing pulmonary tuberculosis (PTB) but it may also be involved in causing extrapulmonary tuberculosis, in which sites of the body other than lungs are affected. Extra-pulmonary tuberculosis is defined as the disease that is not associated with lung involvement except for miliary tuberculosis. The

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other sites commonly involved are the skeletal and genitourinary systems. The diagnosis of extra-pulmonary tuberculosis poses a special challenge as it is often missed or misdiagnosed due to its atypical presentations. The first step in its diagnosis is its awareness and a high index of suspicion by the physicians. Even then, it is difficult to isolate Mycobacterium tuberculosis due to the small number of organisms present at these sites. As per the recent WHO estimates, 1/3rd of the world population is estimated to be infected with Mycobacterium tuberculosis (MTB), and 10.6 million new cases of TB arise each year. The global incident of TB is estimated to be 8.6 million cases and up to 80% cases are clustered in 22 countries in which, India ranked with highest-burden of disease.[1] Around 15-20% of all TB cases are constituted by extra-pulmonary tuberculosis (EPTB). The rural population constitutes the 70% population in the developing countries and EPTB diagnosis are still lacking in rural health facilities.[2] laboratories find it difficult to diagnose EPTB primarily because of the sampling difficulty and the paucibacillary nature of samples.[3] Limited studies have been available citing the prevalence of drug resistance in EPTB especially from high burden settings like India.[4] Management of drug-resistant tuberculosis represents a potential challenge for clinicians. Present study was conducted to study drug susceptibility pattern of Mycobacterium tuberculosis samples from known cases of extrapulmonary tuberculosis.

METHODOLOGY

The economic variant of the proportion method is recommended by the RNTCP guidelines for DST testing. Drug concentrations added to LJ Media and critical proportion for interpretation for the economic variant of proportion method were:

Table 1

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

One set of LJ media bottles for testing were taken. One culture consists of five LJ slopes, one for neat, two for 10^{-2} and two for 10^{-4}; eight LJ drug-containing slopes i.e. two each for drugs H, R, E and S (one each for 10^{-2} and 10^{-4} suspensions) and one for PNB slope, a total 14 LJ slopes were inoculated. The following procedure was adopted according to RNTCP guidelines (NTEP now);

MATERIAL AND METHODS

The present study was a prospective, observational study conducted for 1 year duration (September 2018 to August 2019), in the Department of Microbiology, at a tertiary care hospital. The study was approved by the Institutional Ethical Committee approval was taken prior to start of study. A written informed consent was obtained from the patient and in the case of the pediatric age group, by the guardian/relative.

Patients of all ages and sexes registered at the outpatient department (OPD) / admitted with clinical suspicion of Extrapulmonary tuberculosis, willing to participate were included in the study. Patients positive for pulmonary tuberculosis and those not willing to participate were excluded from the study. Clinically relevant EPTB samples were collected depending on history and clinical findings with all aseptic precautions in a sterile container and properly labelled. Samples included were Pus, Tissue, Pleural fluid, Ascitic fluid, Cerebrospinal fluid (CSF), Lymph node biopsy, Urine and Knee aspirate. Specimens like Body fluids, such as CSF, pleural fluid, ascitic fluid, and knee aspirate were collected aseptically and thus inoculated without decontamination. While pus, other mucopurulent specimens and urine were decontaminated and then processed, LJ medium was inoculated. Drug susceptibility testing using 1% proportion method was used for determination of drug susceptibility pattern towards first-line antitubercular drugs in cases of extrapulmonary tuberculosis.

1 ml SDW (Sterile Distilled Water) with six 3 mm glass beads + 1 loop-full (3 mm loop) of culture

↓

Was Vortexed for 20 – 30 seconds

↓

Added 4 ml of SDW to the above suspension of culture isolate

↓

Turbidity matched with McFarland std.1

↓

S2-10^{-2} two loop-full of neat + 2ml of SDW

↓

S4-10^{-4} two loop-full of S2 + 2ml of SDW

LJ media slopes were then labelled and inoculated respectively. The inoculated slopes were incubated at 37^0C. The growth was seen at 28 days and again at 42 days. Dividing the number of colonies in drug-containing slopes by that in drug-free slopes gave the proportion of resistant bacilli existing in the strain. Below 1%, the critical proportion, the strain was classified as sensitive; above that value, it was classified as resistant. If the result of the reading made on the 28th day was "resistant", no further reading of the test for that drug was required: the strain was classified as resistant. If the result at the 28th day was "sensitive", a second reading was made on the 42nd day only for the sensitive strain. The final definitive results for all the four drugs were reported on the 42nd day. If the strain was resistant to all the four drugs on the 28th day, then the report was given on the same day. Otherwise, incomplete reports were not be given before the 42nd day. Growth was interpreted as : Confluent growth = 3+ , More than 100 colonies = 2+ ,Actual number of colonies = 1-100 cols. When the number of colonies on a given dilution was less than 5, the number of colonies was counted with the next larger inoculum or estimated if more than 100. No attempt was made to estimate the number of colonies if the growth is 3+. The data obtained was subjected to statistical analysis using computer software (SPSS version 23; Chicago Inc., USA). The qualitative data were expressed in proportion and percentages and the quantitative data expressed as mean and standard deviations. Statistical analysis was done using descriptive statistics.

RESULTS

All 150 patient samples were included in the present study. 21-30 years (40.6%) age was the most common age group, followed by 31-40 years (22.7%). The mean age among the distribution of cases was 28.16 ±10.12 yrs. There was a higher Male (53.3%) preponderance observed in 21-30 yrs age and male to female ratio was 1.14:1. The maximum sample collected from patients was pus (30.8%), followed by pleural fluid (16%), CSF (15.3%), tissue (12%), lymph node biopsy (10%), ascitic fluid (9.3%), urine (3.3%) and Knee aspirate/Synovial fluid (3.3%). AFB smear positivity by ZN staining was seen in 31 specimens (20.7%). Using LJ medium. growth on culture was observed in 25 (16.7%) specimens whereas 125 did not show any growth. The sensitivity of ZN staining was 48% specificity and 78.7% accuracy. On comparing smear and culture positivity, in 25 culture positive specimens AFB smear positivity on ZN staining was seen in 12 specimens whereas 13 were smear negative. Five specimens being smear positive did not show any growth on LJ and 125 were smear as well as culture negative. Out of 25 culture positive specimens, 2 were NTM's while 23 were confirmed M. tb when tested on Mpt64 rapid antigen detection kit. Among the 23 M. tuberculosis positive patients, 11 (47.8%) showed resistance to the four first-line anti-tubercular drugs, 3 (13.0%) isolates showed monoresistance to Rifampicin and 2 (8.7%) to Streptomycin. MDR was observed in 5 (21.7%) isolates while polyresistance to HES was noted in a single isolate (table given below).

Table 2: Patterns of resistance of MTB isolates to the first-line anti-tubercular drugs:(n= 23)

Resistance		No. of Patients	Percentage
Mono resistance	INH only	00	-
	RIF only	03	13.0%
	EMB only	00	-
	SM only	02	8.7%
MDR	INH+RIF	05	21.7%
	INH+RIF+EMB	00	-
	INH+RIF+SM	00	-
	INH+RIF+EMB+SM	00	-
Polyresistance*	INH+EMB+SM	01	4.4%
	INH+SM	00	-
	RIF+SM	00	-
	EMB+SM	00	-
Total Resistance		11	47.8%

{*Resistant to two or more drugs but not both isoniazid and rifampicin; EMB=Ethambutol; INH=Isoniazid; MDRTB=Multidrug-resistant tuberculosis (resistant to at least both isoniazid and rifampicin with or without resistance to other drugs); RIF=Rifampicin; SM=Streptomycin} Overall, the distribution of samples showing resistance were as; 6 (54.5%) pyogenic samples (Pus) which showed resistance i.e. 4 with MDR and 2 with monoresistance to Streptomycin, followed by 2 tissue samples (18.2%) i.e. 1 MDR and 1 with monoresistance to Rifampicin. Two lymph node biopsy (18.2%) showing polyresistance and monoresistance respectively. Single pleural fluid sample (9.1%) reported monoresistance to Rifampicin. Among 12 new cases, only 2 (16.7%) cases showed monoresistance to Streptomycin each and the rest 10 were susceptible to all drugs as compared to 9 (81.8%) in previously treated cases which showed resistance to HR (5 isolates; 45.5%) followed by Rifampicin monoresistance (3 isolates; 27.3%); and HES (1 isolate; 9.1%).

DISCUSSION

Isolation of mycobacteria from clinical specimens is considered to be the best method for the diagnosis of extrapulmonary tuberculosis (EPTB). Culture isolation followed by reliable and accurate DST is essential to determine an effective treatment regimen and to avoid further development of drug resistance. In the present study, 21-30 years (40.6%) was the most common age group, followed by 31-40 years (22.7%) and male to female ratio was 1.14:1. Sharma *et al.*[6] reported the mean age similar to the present study and 15-41 years age group was most common (71%) in a study by Sethi *et al.*[7] In the present study the sensitivity of ZN staining was 48% specificity and 78.7% accuracy, similar findings were observed by Ozkutuk *et al.*[8] while Munir *et al.*[9] presented the findings which were discordant with present study. These differences in observations are quite likely due to the paucibacillary nature of the sample and large inter-observer variability. We observed the culture positivity in 25 (16.7%) isolates. Comparable results were reported by Dusthackeer *et al.*[10] and Maurya *et al.*[11] Patients with drug-resistant tuberculosis are increasing rapidly due to high population density, high prevalence of TB and an overstretched public, coupled with an unregulated private health sector. The overall prevalence of drug resistance was found to be 47.8%. Dusthackeer *et al.*[10] reported an overall drug resistance of 41% from southern India. Overall sensitivity to all four anti TB drugs was 52.17% in the present study. Maurya *et al.*[11] reported similar results. The overall prevalence of monoresistance in the present study was observed to be 21.7%, 13 % to only Rifampicin and 8.7% to Streptomycin respectively. Menon *et al.* noted overall monoresistance concordant with the present study. No case of monoresistance to Isoniazid was recorded in the present study. The emergence of MDR TB has been attributed to factors such as nonadherence to treatment, inappropriate treatment regimens, drug malabsorption, poor drug quality and poor health infrastructure for effective delivery of treatment. Overall, the prevalence of MDR TB (resistance to Isoniazid and Rifampicin) was observed to be 21.7% in the present study. Menon *et al.*[14] reported a similar outcome of multidrug resistance. In the present study, it was observed that among 12 new cases only 2 (16.7%) cases showed resistance, i.e. to Streptomycin only as compared to 9 (81.1%) in previously treated cases which showed resistance to HR (45.5%) followed by Rifampicin only (27.7%) and HES (9.1%). Similarly, D'Souza *et al.*[17] and Sethi *et al.*[7] reported concordant results. The present study had a limitation of including a small number of cases since the results may vary with a larger sample size. Also, the clinical outcomes of the drug-resistant TB cases were not studied.

CONCLUSION

In a city like Mumbai, with increasing population density and poor living conditions, Tb burden is on the rise. Although time consuming, LJ culture DST remains a gold standard in the diagnosis of TB. The difficulty in the identification of the emerging heteroresistance is a matter of concern when we totally rely on molecular diagnostics. Also the paucibacillary nature of EPTB samples make it difficult to diagnose on smear microscopy (5000-10,000 bacilli/ml), so the culture positivity holds a great value as it can detect 10-100 bacilli of concentrated material. The expensive set-ups and the requirement of an expertise hand is a drawback in the molecular diagnostics compared to culture DST, therefore its cost-effectiveness is an advantage which cannot be ignored especially in the developing countries**.** Also the studies with larger subsets of population should be undertaken.

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