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To Study the Genetic Diversity of the Circulating Mycobacterium Tuberculosis Complex (MTBC) Strains Isolated from Previously Treated Patients

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Abstract

Background & Methods: The aim of the study is to study the genetic diversity of the circulating Mycobacterium tuberculosis complex (MTBC) strains isolated from previously treated patients. All strains of tuberculosis contain some subpopulation of bacilli that are resistant to anti-TB drugs. However, in resistant strains, the proportion of such bacilli is considerably higher than the sensitive strains.

Results: There were 2 isolates resistant to all the four first line antituberculosis drugs (INH, RIF, SM and EMB), 4 isolates were resistant to INH, RIF, and EMB while 18 isolates were resistant to SM, RIF and INH.

Conclusion: In order to understand the transmission and epidemiology of TB globally as well as locally, the use of molecular typing methods has become imperative not only for understanding genetic diversity and population structure of MTBC but also for carrying out supervision and monitoring of TB control programs and for understanding TB epidemic. Spoligotyping is a PCR-based method that amplifies the spacer; the presence and absence of spacers will result in different polymorphisms. A total of 100 patients with sputum smear positive pulmonary tuberculosis from whom MTBC was grown in pure culture were included in this study. Among the 100 patients with TB, were sensitive to all four drugs while drug resistant isolates.

Keywords: Genetic, Mycobacterium, Tuberculosis, Strains & Isolated.
Study Design: Observational Study.

1. INTRODUCTION

Tuberculosis (TB) is a communicable disease caused by infection by Mycobacterium tuberculosis complex species. TB has been a major cause of illness and one of the leading causes of death worldwide from time immemorial[1]. Until the coronavirus (COVID-19) pandemic, TB was the leading cause of death from a single infectious agent, ranking above HIV/AIDS (1). It is an ancient disease that currently presents an immense global health challenge. It is known that more than one

third of the world‟s populations are infected with the tubercle bacilli (approximately 2 billion people)[2].

The severity of national TB epidemics, in terms of the number of incident TB cases per 1,00,000 population per year, varies widely among countries, from less than five to more than 500 new and relapse cases per 1,00,000 population per year. Fifty-seven countries had a low incidence of TB in 2020 (<10 cases per 1,00, 000 population per year) spread mostly in the WHO Region of the Americas and the WHO European Region, plus a few countries in the WHO Eastern Mediterranean and Western Pacific regions. Countries with a low incidence are well placed for target TB elimination[3-5].

The German physician Robert Koch discovered this organism in 1882 and concluded this microorganism responsible for the deadly pulmonary Tuberculosis (19). Subsequently, in 1883, the organism was named Mycobacterium tuberculosis as a causative agent of TB[6]. The organism is aerobic, non-motile, non-spore forming, slowly reproducing, rod shaped organism belonging to genus Mycobacterium. Slow cell division, resistance to detergents and certain antimicrobial agents, persistence in the environment and an acid/alcohol fast nature are due to the characteristic hydrophobic cell wall. The lipid rich cell wall does not take up stain readily and when it does so, it resists decolorization when de-stained with an acid-alcohol wash. It is classified as a gram-positive bacterium due to lack of outer membrane[7].

2. MATERIAL AND METHODS

The study was conducted at VIMSAR, Burla, Odisha for 01 Year on 100 cases. The Mycobacterium tuberculosis complex (MTBC) strains were collected from the accredited RNTCP Tuberculosis Culture and Drug Susceptibility Testing laboratory at the institute. These strains have been isolated by culture of previously treated, sputum smear positive pulmonary tuberculosis patients attending this hospital as well as from the other hospitals.

Inclusion Criteria

1. Only those isolates which have been grown from smear positive sputum samples of pulmonary tuberculosis patients.
2. All isolates obtained from previously treated patients with Pulmonary TB.

Exclusion Criteria

1. Smear negative, culture positive isolates.
2. New cases with Pulmonary TB.

3. RESULT

Table No.1: Age vs MDR isolates

S. No.	Age	No. of Isolated	Percentage
1	21-30	03	03
2	31-40	04	04

3	41-50	04	04
4	51-60	05	05
5	61-70	05	05
6	71-80	03	03

The highest number of isolated individuals is in the 51–70 age range (5 individuals each in 51–60 and 61–70). The lowest numbers are found in the youngest (21–30) and oldest (71–80) groups, each with 3 isolated individuals. The percentages mirror the count values, suggesting either:

- The total sample size is 100 (making the % directly equal to the number), or
- The percentages were not calculated from a base total and might need verification.

Table No.2: Composition of Master mix for PCR

S. No.	Components	Volume	Total Volume
1	2x Master Mix	12.5µl	437.5µl
2	DH20	2.5µl	87.5µl
3	Template	2µl	NA
4	Total Reaction	25µl	-

The table outlines the PCR mix preparation for 35 reactions, with exact component volumes per reaction and the total volumes for bulk preparation (excluding the template). The template is typically added individually to each reaction tube.

Table No.3: Combination Resistance among MDR Isolates in This Study

S. No.	Variable	Previously Treated Cases No.	Percentage
	Sensitivity (Any)		
1	Streprtomycin	63	63
2	Isonizide	51	51
3	Rifampicin	71	71
4	Ethambutol	92	92
	Resistance (Any)		
1	Streprtomycin	33	33
2	Isonizide	47	47
3	Rifampicin	28	28
4	Ethambutol	04	04

Ethambutol:

- Highest sensitivity (92%) and lowest resistance (4%).

- Suggests it remains highly effective in most previously treated cases.

Rifampicin:

- Sensitivity: 71%
- Resistance: 28%
- Indicates that nearly a third of patients have developed resistance to this drug, which is critical since Rifampicin is a key anti-TB medication.

Isoniazid:

- Moderate sensitivity (51%) and relatively high resistance (47%).
- Indicates a high level of resistance, reducing its effectiveness.

Streptomycin:

- Sensitivity: 63%
- Resistance: 33%
- Still somewhat effective, but a significant resistance rate is present.

Table No. 4: Drug susceptibility testing results of the culture positives

S. No.	Variable	Previously Treated Cases No.	Percentage
	Sensitivity (Any)		
1	Streprtomycin	63	63
2	Isonizide	51	51
3	Rifampicin	71	71
4	Ethambutol	92	92
	Resistance (Any)		
1	Streprtomycin	33	33
2	Isonizide	47	47
3	Rifampicin	28	28
4	Ethambutol	04	04

There were 2 isolates resistant to all the four first line antituberculosis drugs (INH, RIF, SM and EMB), 4 isolates were resistant to INH, RIF, and EMB while 18 isolates were resistant to SM, RIF and INH.

4. DISCUSSION

MDR-TB is caused by Mycobacterium tuberculosis and is defined as TB that is resistant to at least isoniazid and rifampicin, with or without ethambutol resistance [8]. As reported by WHO, a global total of 150,359 people with MDR/RR-TB were enrolled on treatment in 2020. In India, the estimated proportion of TB cases with RR/MDR in 2018 was 14%, sudden reduction was noticed in 2020 due to Covid-19 pandemic. The present study showed prevalence of drug resistant TB in previously treated patients to be 58%. Among them Multidrug resistant TB (MDR TB) was 23%. These results are

comparable to some previous studies in India[9]. A study from Gujarat in the year 2009 reported 17.4% MDR-TB. A study from Madhya Pradesh., in the year 2015 reported it to be 8.2%. A study from Delhi in 2011 reported a higher prevalence of 33% MDR-TB in previously treated patients. High levels of MDR-TB among previously treated patients can be due to inadequate understanding of the end-user about the drug regimens, poor patient adherence to treatment or substandard quality of the drug[10].

The detection of 33 different spoligotype lineages reflects the high rate of secondary or acquired resistance in this population. East African Indian (EAI), the most ancient M. tuberculosis lineage, was first described in Guinea-Bissau, which is prevalent in African and Indian subcontinent. EAI is characterized by absence of spacers 29–32, 34 and presence of spacer 33 and a low copy number of IS6110. In our study EAI family prevalence was 33%, of which EAI3-Ind were 20 (19.23%) EAI5- 13 (12.50%) and EAI7-BGD2-1 (1%). Similar study in North India in 2017 reported 19.10% EAI prevalence. Among the 34 (35%) EAI family isolates, 24 (70.6%) were males and 10 (29.4%) were females. Males were higher compared to females. Similar result was arrived at in a study from Vellore, India where males were higher in EAI family compared to females[11]. The higher number of males may be due to their higher number in the study population. Among 34 EAI family isolates, 15 (44.1%) isolates were sensitive to all drugs while the remaining 19 (55.9%) were drug resistant strains.

5. CONCLUSION

In order to understand the transmission and epidemiology of TB globally as well as locally, the use of molecular typing methods has become imperative not only for understanding genetic diversity and population structure of MTBC but also for carrying out supervision and monitoring of TB control programs and for understanding TB epidemic. Spoligotyping is a PCR-based method that amplifies the spacer; the presence and absence of spacers will result in different polymorphisms. A total of 100 patients with sputum smear positive pulmonary tuberculosis from whom MTBC was grown in pure culture were included in this study. Among the 100 patients with TB, were sensitive to all four drugs while drug resistant isolates.

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