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Prevalence of Fluoroquinolone Resistance among Pulmonary Tuberculosis Patients in the Union Territory of Puducherry

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Abstract

BACKGROUND

Fluoroquinolones have potent bactericidal activity against *Mycobacterium tuberculosis*. Unfortunately, the extensive use of fluoroquinolones(FQ) has led to the emergence of extensively drug resistant (XDR) and pre-extensively drug resistant (pre- XDR) TB, which have become areas of growing concern and are posing threat to global efforts of TB control.

AIMS & OBJECTIVES

1. To detect fluoroquinolone resistance among pulmonary tuberculosis patients
2. To Compare genotypic and phenotypic variations among (pre-XDR for Fluoroquinolone) Pulmonary Tuberculosis patients.

METHODOLOGY:

A prospective observational study conducted at Intermediate Reference Laboratory of a chest hospital in Pondicherry over a period of 2 months (May 2019 to July 2019). Patients fulfilling the inclusion criteria were subjected to phenotypic and genotypic evaluation. Drug sensitivity to first line and second line anti tuberculous drugs were evaluated. Statistical analysis was done using SPSS.

RESULTS:

A total of 258 patients were included , of which 42(16.3%) clinical isolates were identified as multi drug resistant tuberculosis. 16(6.25%) clinical isolates were resistant to second line drugs among which 1 was Extremely drug resistant, 14(5.4%) were fluoroquinolone resistant (pre-XDR) and 1 resistant to second line injectable drugs. Genotypic analysis of 14 pre XDR for Fluoroquinolone showed five to be truely resistant at codon 90(2),91(2) and 94(1) regions of gyrA and remaining nine are inferred.

CONCLUSION:

The study implies that the genotypic and phenotypic tests for detection of FQ resistant *Mycobacterium tuberculosis* has provided the advantage of rapid diagnosis which would greatly help in optimised treatment, thereby preventing the spread of resistant strains among the species.

KEYWORDS: Multidrug resistant Tuberculosis, Pre-XDR TB, Fluoroquinolones

INTRODUCTION

MDR-TB (Multi-drug resistance mycobacterium tuberculosis) is a known phenomenon which is defined as resistance to at least isoniazid (INH) and rifampicin (RIF) [1] . XDR-TB (Extensive drug resistance mycobacterium tuberculosis) is defined as resistance to at least isoniazid (INH) and rifampicin

(RIF) (i.e. MDR-TB), as well as further resistance to any fluoroquinolone (FQ) and a second-line injectable drug (kanamycin, amikacin or capreomycin. Pre-XDR-TB is defined as TB with resistance to isoniazid and rifampicin and either a FQ or a second- line injectable agent but not both [1,2] . Based on Revised National Tuberculosis Control Programme annual report 2017, India has the highest burden of both TB and MDR-TB [3,4]. Fluoroquinolones (FQs) are oral antibacterial agents that have potent bactericidal activity against Mycobacterium tuberculosis. Hence, FQs are recommended for the treatment of MDR-TB patients [5,6].

Fluoroquinolone resistance in Mycobacterium tuberculosis can develop as early as 13 days of fluoroquinolone therapy. Although fluoroquinolone resistance in Mycobacterium tuberculosis is not routinely assessed, the proportion of newly diagnosed (i.e., previously untreated) patients with tuberculosis with fluoroquinolone resistance has ranged from 0.15 to 3.6% in previous reports. Since FQs are broad-spectrum antibiotics, they are often prescribed as well as over-prescribed by clinicians for diverse infections. In many resource-limited countries, FQs are readily available as over-the-counter medications, leading to their increased misuse [3]. Such indiscriminate use has contributed to the increasing emergence of FQ-resistant Mycobacterium tuberculosis strains in India and worldwide [7,8]. Unfortunately, the extensive use of fluoroquinolones(FQ) has led to the emergence of extensively drug resistant (XDR) and pre-extensively drug resistant (pre- XDR) TB, which have become areas of growing concern and are posing threat to global efforts of TB control. There are no reports of isolated pre-XDR (fluoroquinolone resistance) in Pondicherry. Therefore we have done this study in our population.

AIMS

1. To detect fluoroquinolone resistance among pulmonary tuberculosis patients
2. To Compare genotypic and phenotypic variations among (pre-XDR for Fluoroquinolone) Pulmonary Tuberculosis patients.

METHODOLOGY

This is a prospective observational study conducted over a period of 2 months (May 2019 to July 2019) at the Intermediate reference laboratory, Government Chest Hospital, Puducherry. Institutional ethical clearance was obtained. Patients who were new smear positive for pulmonary tuberculosis and those who were already on anti tubercular therapy (ATT) were included in the study. Patients with extrapulmonary tuberculosis were excluded from the study.

Sample size was calculated to be 236 ($4pq/d^2$, where p-prevalence of pre-XDR [9] for tuberculosis 18%, q=1-p, d = 5). Two sputum samples were collected from the study subjects after getting consent and screened for acid fast bacilli (AFBs) using Fluorescence (FM) microscopy. The smear positive sputum samples in Fluorescence microscopy were directly processed by GenoType MTBDRplus V.2.0 assay. The smear negative samples were subjected to liquid culture using the BACTEC MGIT 960 system (Becton Dickinson Microbiology Systems, Cockeysville, MD, USA) under stringent conditions [10]. The culture positive samples from the MGIT system were in turn subject to the GenoType MTBDRplus V.2.0 assay**.** The GenoType MTBDRplus V.2.0 assay was performed according to the manufacturer's protocol. The test was based on DNA strip technology and had three steps: DNA extraction, multiplex PCR amplification, and reverse hybridization [11]. All three steps were performed as per the WHO recommendations.

All the laboratory bench works related with potentially infectious specimens were performed in a Class II biosafety cabinet placed at BioSafety Level III facility. All processed specimens were stored at -20°C for the duration of the study to allow re-testing of specimens giving discrepant results [12,13]. Statistical analysis was carried out using SPSS v.18.

RESULTS

In Between May 2019 and July 2019, 258 clinical isolates of Mycobacterium tuberculosis were isolated from patient's samples in Intermediate Reference Laboratory at Government Hospital for Chest Diseases, Puducherry Southern India. The study included a total of 258 patients with Genotype MTBDRplus confirmed TB and whose DST results were available. Overall, 188 (72.9%) strains were

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isolated from male patients and 70 (27.1%) from female patients.

A total of 42 (16.3%) of 258 clinical isolates were identified as multi drug resistant tuberculosis, 200 (77.5%) and 9 (3.5%) were identified as isoniazid and rifampicin mono resistant respectively as shown in table 1. There are 16 (6.2%) second line drug resistant strains among the screened samples. Fourteen (14) isolates were fluoroquinolone resistant (pre-XDR), the prevalence being 5.4%. One was extremely drug resistant and one was resistant to second line injectable drugs. Among the multi drug resistant clinical isolates, fluoroquinolone resistant (pre-XDR) was 9.5% (4/42) as shown in table 2.

All 258 isolates were subjected to Genotype MTBDR sl assay and results are interpreted as per the guidance and the results are tabulated as shown in Figure 1. Among the 14 pre-XDR, five is true resistant at codon 90(2), 91(2) and 94(1) regions of gyrA gene and remaining nine are inferred in the gyrB gene. Asp94 mutations led to high-level MICs of LVX (Levofloxacin), MFX (Moxifloxacin) and GAT (Gatifloxacin) for most strains. Their exact positions and amino acid changes were not known and it needs to be subjected for sequencing to know the changes.

DISCUSSION

Drug resistance poses a severe challenge to tuberculosis control, as it raises not only the possibility of a condition that can no longer effectively be treated with anti-tubercular drugs but also further transmission among the public [14]. This situation of MDR-TB highlights the urgent need for rapid and accurate drug susceptibility testing (DST) to optimize the treatment regimen and reduce the risk of acquired resistance.

Our study included more male patients as compared to that of females which was similar to the study done by Advani et al [15]. The prevalence of Pre XDR of Fluoroquinolone in our study was 5.4% which was in concordance with the study conducted by Porwal et al [16] in the capital of Delhi where the prevalence was 7.5%.

The prevalence of Fluoroquinolone Pre-XDR in MDR TB in our clinical isolates is 9.5%. This has yielded similar results to studies done by Sharma et al [17]. which showed 10% prevalence of Fluoroquinolone Pre XDR in MDR patients. But few studies have shown a higher prevalence of Fluoroquinolone Pre XDR in MDR TB isolates such as Advani et al [15] where the prevalence was 55.95% and Agrawal et al [3] where it was 35% which is discordant with our findings which might be due to the fact that these studies are conducted in big states where there is more population per meter square and hence more chances of using Fluoroquinolones, when compared to a small union territory like Pondicherry.

FQ resistance in Mycobacterium tuberculosis occurs due to mutations mostly in the gyrA gene, rarely in the gyrB gene [18-21]. In this study, gyrA mutations were predominantly found to occur in codons 90, 91, and 94, corroborating the findings of other studies ie Zhang et al [22], Zhao et al [23] which are in agreement with previous studies showing that fluoroquinolone resistance of Mycobacterium tuberculosis is mostly attributed to the mutations of the gyrA gene. In our study, all inferred mutations have occurred in the gyrB gene. Asp94 mutations led to high-level MICs of LVX, MFX and GAT for most strains.

Thus, knowledge of the specific mutation may help to understand the level of FQ resistance, which can, in turn, inform decisions regarding the selection of OFL or newer FQs, such as MOX (Moxifloxacin) or levofloxacin (LVX). This information can also be used to determine whether phenotypic testing should be extended from OFL (Ofloxacin) to MOX and LVX. In the present study, we therefore aim to characterize mutations in the gyrA and gyrB genes of Mycobacterium tuberculosis isolates from patients suspected of having MDR-TB.

CONCLUSION

The study implies that the genotypic and phenotypic tests for detection of FQ resistant *Mycobacterium tuberculosis* have provided the advantage of rapid diagnosis. The results of our study suggest that in all newly diagnosed cases of pulmonary tuberculosis, genotype and phenotype tests for detection of resistance to Fluoroquinolones should be carried out and on the basis of these results individualized treatment can be initiated, leading to better outcomes and less chances of treatment failure. This would be a

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positive step in our aim of tuberculosis elimination by 2025 in India.

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Tables

TABLE 1: Distribution of Resistant Strains Among the Clinical Isolates

S.No	CLINICAL ISOLATES	NUMBER OF CASES	PERCENTAGE (%)
1	MDR TB	42	16.3
2	ISONIAZID MONORESISTANCE	200	77.5
3	RIFAMPICIN MONORESISTANCE	9	3.5
4	NON RESISTANT	7	2.7
	TOTAL	258	100

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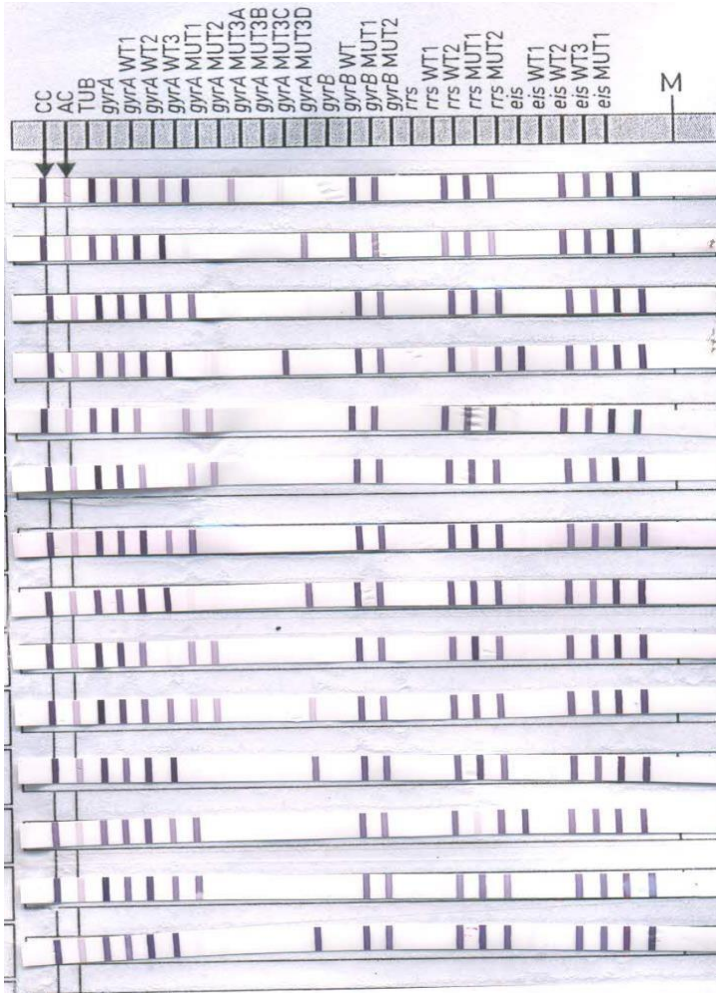
Table 2: Distribution of XDR and Pre XDR among the resistant samples

Clinical Isolates	XDR	Pre XDR for Fluoroquinolones	Pre XDR for Second line Injectables	Total
MDR (42)	1	4	Nil	5
Isoniazid Monoresistance (200)	Nil	8	1	9
Rifampicin Monoresistace (9)	Nil	2	Nil	2
Total	1	14	1	16

Figure 1: Interpreta



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