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HIGH PREVALENCE OF FLUOROQUINOLONE RESISTANCE AMONG DRUG-SENSITIVE PULMONARY TUBERCULOSIS PATIENTS: AN IMMINENT PERIL

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ABSTRACT

Background: Tuberculosis (TB), in its aggressively evolving drug-resistant form, draws enormous concern for the community. Poor treatment outcomes have been closely associated to Fluoroquinolone resistance, which in turn, have emerged to be of utmost threat for tuberculosis control programmes.

Methods and Materials: 100 non-duplicate sputum samples collected from diagnosed pulmonary tuberculosis (PTB) patients attending our hospital between October 2019 and March 2020 were studied. The samples were subjected to Xpert MTB-RIF® Assay and Genotype MTBDR*sl* VER 2.0® to detect the presence of Rifampicin and Fluoroquinolone resistance consecutively. Results were interpreted following standard guidelines and manufacturer's instructions.

Results: The Drug-senstitve (DS-TB) TB population of our study consisted of 91 patient samples, amidst which Genotype MTBDR*sl* VER 2.0® detected 27 isolates to be resistant to Fluoroquinolones. The prevalence of Fluoroquinolone resistance among Rifampicin-sensitive TB (RS-TB) patients was as high as 29.7%.

Conclusion: This study emphasized on the importance of performing second-line Drug Susceptibility Testing (DST) routinely in high TB endemic regions for both Drug-resistant TB(DR-TB) and DS-TB patients to evaluate the prevailing drug resistance in the community.

KEYWORDS : Fluoroquinolone Resistance, Line Probe Assay, Drug-sensitive Tuberculosis

INTRODUCTION:

Tuberculosis (TB), one of the leading causes of death due to a single infectious agent, bacillus *Mycobacterium tuberculosis*, ranks among one of the top 10 causes of death worldwide. [1] The Global Tuberculosis Report 2020, published by the World Health Organization, estimated that 10 million people were taken ill with TB in the year 2019 worldwide. [2] India happened to be the country with the highest TB burden in the world, with 26.9 lakh new TB cases recorded in the year 2019. [1,2] Furthermore, approximately half a million new cases of Rifampicin Resistant TB (RR-TB) were reported worldwide whereas India, accounting for the largest share of the global burden (27%), recorded an incidence of Multidrug-resistant TB (MDR-TB/RR-TB) estimated to be 124,000, distributed among 2.8% of the new TB cases and 14% of the previously treated ones. [1,2].

Fluoroquinolones, a group of broad-spectrum antibiotics, have excellent bactericidal activity against many Mycobacteria. Following oral administration, they achieve effective serum, tissue and intracellular levels and produce few adverse effects in the process. [3] These properties make them an essential component in the second-line antituberculosis regimens, reserved for treating patients with drug-resistant (DR) disease, yet occasionally recommended in treating drug-sensitive tuberculosis (DS-TB) patients intolerant to first-line drugs.[3] However, routine use of Fluoroquinolones (especially respiratory quinolones) should be strictly discouraged in undiagnosed tuberculosis patients, as resistance may develop, mostly when used as a monotherapy. Apart from the fact that injudicious use of Fluoroquinolones in high TB endemic regions has greatly contributed to the emergence of Fluoroquinolones-resistant *Mycobacterium tuberculosis* strains, existing evidence have also suggested that fluoroquinolone resistance can reach epidemic dimensions through circumstances other than fluoroquinolone exposure in treating tuberculosis.[4]

The Xpert® MTB/RIF assay (Cartridge-Based Nucleic Acid Amplification Test, **CBNAAT**) uses semi-quantitative nested real-time PCR for detection of *Mycobacterium tuberculosis* (MTB) complex DNA and rpoB gene core mutations associated with Rifampicin resistance, which can be used as a surrogate marker for MDR-TB.[5] The entire process is performed in a self-contained cartridge to minimize cross contamination between samples. The turn-around time is less than 2 hours, making it a very useful point-of-care test in a routine practice. [6,7]

The GenoTypeMTBDR*sl* VER 2.0 (Line probe assay, **LPA**) identifies the MTB complex and its resistance to Fluoroquinolones and Aminoglycosides/ Cyclic peptides from clinical specimens. LPAs are DNA–DNA hybridization assays that attempt to determine different mutations simultaneously using multiple probes. Following DNA extraction and target amplification of the specimen, the amplicon products are hybridized to specific oligonucleotide probes which are complementary to the target sequences. Several post-hybridization washes follow to remove the non-specific binding of the amplicon-probe hybrids. The results are finally visualized by naked eye as colored bands immobilized on the surface of a strip. [8] LPAs aid in early diagnosis of both MDR-TB and XDR-TB as results are available within 48-72 hours. [6,8]

AIMS AND OBJECTIVES:

Estimate Fluoroquinolone resistance among drug susceptible (DS) pulmonary tuberculosis patients by molecular Drug Susceptibility Testing (DST).

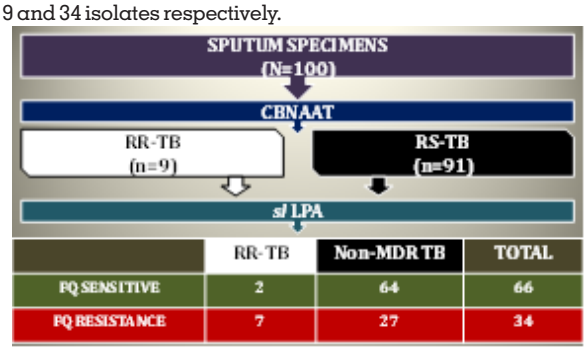
MATERIALS AND METHODS:

An observational cross-sectional study was conducted in the Department of Bacteriology, Calcutta School of Tropical Medicine, Kolkata, over a period of six months (October 2019 to March 2020). 100 non-duplicate sputum samples from diagnosed pulmonary tuberculosis patients were studied. Patient samples were collected in pre-sterilized falcon tubes

after thorough rinsing of the oral cavity. Direct smears were prepared from sputum samples for Z-N staining and examined under brightfield microscope. One part of the sputum sample (2-5 ml), subjected to CBNAAT, was diluted by normal saline with three times the reagent, incubated at room temperature and was soon after loaded into the cartridge. These samples were processed according to the standard guidelines set by Xpert MTB/RIF® (Cepheid, Sunnyvale, US) for subsequent detection of Mycobacteria and Rifampicin resistance in the same setting. [9] Another part of the sputum, with the intention of molecular determination of Fluoroquinolone resistance, was subjected to GenoType MTBDR*sl* VER 2.0® (Hain Lifescience GmbH, Nehren, Germany). The sputum was decontaminated using 1% N-acetyl-L-cysteine/ NaOH method; DNA extraction was carried out of the decontaminated sputum samples directly by Genolyse (Hain Lifescience, Germany), followed by multiplex PCR amplification and reverse hybridization, as per manufacturer's instructions. [10]

RESULTS:

All the patient samples (N=100) analyzed by Xpert MTB/RIF® and GenoTypeMTBDR*sl* VER 2.0® showed presence of *Mycobacterium tuberculosis*. 64 sputum samples were found to be sensitive to all anti-tuberculosis drugs tested whereas any drug resistance (Rifampicin and/or Fluoroquinolones) was detected in 36 samples. GeneXpert/ RIF® assay and GenoTypeMTBDR*sl* VER 2.0® detected presence of Rifampicin resistance (RR-TB/MDR-TB) and Fluoroquinolone resistance in 9 and 34 isolates respectively.



	RR-TB	Non-MDR TB	TOTAL
FQ SENSITIVE	2	64	66
FQ RESISTANCE	7	27	34

Fig. 1- Study Design

In the present study, the non-MDR TB population constituted of 91 patient samples. Higher percentage of the study sample included patients in their third (28.6%) and fifth (23%) decades. Predominant population in our study consisted of male patients (62.6%). The study population included higher number of patients who were treatment-naïve (72.5%) as compared to their previously-treated counterparts (27.5%). Sputum microscopy (Zeihl-Neelson staining) detected the presence of acid-fast bacilli (AFB) in the majority of the population (70.3%).

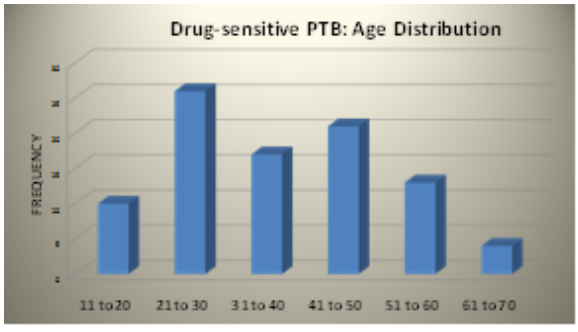


Fig. 2- Age Distribution: Non-MDR PTB patients

Molecular DST (second-line LPA) was conducted on this population to evaluate the prevalence of baseline resistance to Fluoroquinolones in the community. It was estimated that existing Fluoroquinolone resistance, as detected by GenoTypeMTBDR*sl* VER 2.0®, among non-MDR patients in the study population stood at 29.7% (27/91). It was the previously-treated PTB patients who developed higher (15/27; 55.6%) degree of Fluoroquinolone resistance as compared to their treatment-naïve (12/27; 44.4%) DS-TB counterparts. All 27 phenotypic Fluoroquinolone-resistant isolates were further studied to evaluate the presence of precise mutant genes. It was observed that the most common single mutation in *gyrA* gene was D94G in 10 (37%) isolates. Other predominant phenotypic susceptibility pattern observed was that of the 'hetero-resistant' type in 11 isolates (40.7%), where the tested specimens either contained a strain that has developed hetero-resistance or contained a wild type and a resistant strain simultaneously due to mixed infection and in either case, the resistance-causing mutation was not covered by the mutation probes. [10]

MISSING WILD TYPE (WT) PROBE	DEVELOPING MUTATION PROBE	PHENOTYPIC SUSCEPTIBILITY	MUTATION	FREQUENCY (n=27)
gyrA WT3	*gyrA* MUT 3C	RESISTANT	D94G	10
gyrA WT3	*gyrA* MUT 3A	RESISTANT	D94A	1
NONE	*gyrA* MUT 3C	HETERO-RESISTANT	-	11
Any one wild type probe (WT1/WT2/WT3)	NONE	RESISTANT	A90V D94A D94G G88A G88C	5

Fig. 3: The frequency and mutation-conferring resistance to Fluoroquinolones among Drug-sensitive PTB patients

DISCUSSION:

As indicated in the Global Tuberculosis Report 2019, among countries with high TB or MDR-TB burden, the proportion of MDR-TB/RR-TB cases with resistance to any fluoroquinolones, when tested, was 20.1%. [1] Yet, there is indeed limited information available on the frequency of Fluoroquinolone resistance among DS-TB patients globally, due to the infrequent practice of DST for Fluoroquinolones in routine and periodic surveillance programmes. [11] In surveys conducted in several countries to assess effectiveness of Levofloxacin in different patient groups, it was observed that among patients who were susceptible to both Isoniazid and Rifampicin, the prevalence of Levofloxacin resistance was approximately 4.3% and 9% in Bangladesh and Pakistan respectively. [12] In the Indian scenario, a long-term retrospective study that aimed at reviewing DST reports of all TB culture-positive samples in Mumbai reported that Fluoroquinolone resistance increased steadily yet exponentially from 8% in 1998 to 35% in 2004. [13] Years later, a study conducted by Nair, *et al* observed Ofloxacin resistance in first-line drug susceptible patients to be 14.9%. [14] Another study conducted by A. Jain *et al* showed resistance to Ofloxacin among non-MDR isolates stood at 20.7%. [15] In our present study, the estimated prevalence of Fluoroquinolone resistance among the DS-TB patients was reportedly 29.7%, a figure considerably high to be concerned, yet in line with the increasing burden of Fluoroquinolone resistance observed by multiple studies conducted over the years. [16] This study thus justified the importance of performing second-line DST routinely for both RR-TB/MDR-TB and DS-TB/non-MDR TB patients to evaluate the prevailing Fluoroquinolone susceptibility pattern in the community at an initial stage itself.

CONCLUSION:

In high-burden regions, it is imperative to ensure judicious use of Fluoroquinolones in non-TB infections, by minimising prolonged or repeated use, or if feasible, avoidance of Fluoroquinolones altogether in patients at risk of having

active TB. Increased surveillance activities for identifying Fluoroquinolone-resistant MTB in TB endemic regions and adherence to strict implementation of Fluoroquinolone DST prior to the initiating Fluoroquinolone-based antituberculosis drug regimens can effectively control this menace of antimicrobial resistance. Our study highlighted that high prevalence of Fluoroquinolone resistance among treatment-naïve non-MDR TB patients is an understated yet serious issue of concern which needs to be addressed urgently to control the increasing burden of drug-resistant TB as well as prevent the potential cause for treatment non-response or failure among DS-TB patients.

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