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Additional Resistance to Moxifloxacin and Levofloxacin among MDR-TB Patients with Base Line Resistance to Ofloxacin at a Reference Laboratory

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Abstract

Fluoroquinolones are among the most promising antibiotic drugs for Tuberculosis treatment. Although high levels of fluoroquinolone resistance have been detected among many common bacterial pathogens, little is known about the fluoroquinolone resistance of *M. tuberculosis* especially at the baseline. The present study was thus aimed at determining the profile of resistance against two newer generation fluoroquinolones-Moxifloxacin and Levofloxacin in MDR-TB isolates with baseline resistance to ofloxacin. A total number of 65 isolates (4 XDR and 61 pre-XDR) were subjected to susceptible testing against levofloxacin and two (higher and lower) concentrations of moxifloxacin. 72.3% in addition to being resistant to ofloxacin were also resistant to levofloxacin and lower concentration of moxifloxacin. The increasing use of FQs for the treatment of other bacterial infections has led to increasing resistance to these antimicrobials. Newer generation FQs are promising drugs in the treatment of drug-resistant Tuberculosis but care should be taken regarding the rationale use of these drugs for the treatment of other diseases especially when other drugs are available.

Keywords: *Mycobacterium tuberculosis;* XDR; Fluoroquinolones; Baseline resistance; Drug susceptibility testing

Introduction

Fluoroquinolones (FQs) are a critical component of antituberculous drug regimens and these along with the second-line injectable drugs amikacin (AMK), kanamycin (KAN) and capreomycin (CAP) are used for the treatment of multi-drug resistant tuberculosis (MDR-TB) which is defined as tuberculosis caused by Mycobacterium tuberculosis isolates with resistance to isoniazid and rifampicin [1,2]. FQs directly inhibit DNA topoisomerases which solve topological problems associated with DNA replication, transcription, recombination, and chromatin remodelling by introducing temporary single- or double-strand breaks in the DNA. The FQ forms a complex with the DNA and the topoisomerase and generates double-strand DNA breaks, which is lethal for the bacteria [3].

Having broad antimicrobial activity FQs are also widely used to treat a variety of other bacterial infections [4]. This exuberant use of FQs in the treatment of community-acquired bacterial infections has thus led to the marked emergence of fluoroquinolone resistant MTB (FQ r-MTB) in many countries. It has been established that exposure to fluoroquinolones prior to the diagnosis of TB has been associated with fluoroquinolone resistance, particularly when fluoroquinolone exposure occurs >60 days before TB diagnosis and for longer than 10 days [5,6]. Furthermore, use of FQs complicates diagnosis of pulmonary tuberculosis (TB) by reducing smear positivity and delaying access to treatment [7,8]. Thus in high burden tuberculosis countries where FQs are also used to treat a variety of other bacterial infections besides tuberculosis, it may be useful to know the prevalence of resistance to FQs in *Mycobacterium tuberculosis* isolates particularly at the base line [9]. Unfortunately limited data on FQ resistance among MDR-TB isolates at baseline is available worldwide. The present study was thus aimed at determining the profile of resistance against two newer generation fluoroquinolones-Moxifloxacin and Levofloxacin in MDR-TB isolates with baseline resistance to ofloxacin.

Materials and Methods

The present study was conducted at New Delhi Tuberculosis Centre which is an Intermediate Reference laboratory (IRL) for Delhi State. The laboratory has high work load, processing over 15,000 samples per year and is going through regular rounds of proficiency testing for culture and drug susceptibility testing (First and second line) by National Reference laboratory (NRL).

This study was conducted over a period of one year, i.e., from January 2015 to December 2015 after due clearance from Institute's ethics committee. XDR and pre-XDR cases among MDR and Mono rifampicin resistance cases identified at baseline were included in the study. These samples were subjected to drug susceptibility testing against Levofloxacin (LVX) at a concentration of 1.5 µg/mL and two concentrations of Moxifloxacin (MOX) - 0.5 µg/mL and 2.0 µg/mL.

The FQs were obtained as powder form M/s Sigma-Aldrich Co. The isolates were tested for susceptibility to these drugs using BacTec MGIT-960 system according to the manufacturer's instructions. The drug susceptibility testing was performed according to the standard 1% proportionate method as per the manufacturer's instructions (Becton Dickinson, Sparks, MD).

Briefly, MGIT BBL tubes were supplemented with 0.8 ml of oleic acid-albumin-dextrose-catalase. Culture suspension for drug,

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inoculation was diluted 1:5 with sterile saline from 3-5 days old positive tube and direct inoculation was done for 0-2 days old positive tube. 100 µl Drugs were added to the MGIT to have final concentrations of 2.0 µg/mL ofloxacin, 1.5 µg/mL of levofloxacin, 0.5 µg/mL and 2.0 µg/mL of moxifloxacin. A growth control (GC) tube was prepared without antibiotic; culture suspension for GC tube was diluted to 1:100 with sterile saline from drug inoculum. The drug tubes were inoculated with 0.5 ml of the inoculum diluted 1:5 and for GC tube, 0.5 ml of the inoculums diluted 1:100.

Results

A total number of 65 isolates (4 XDR and 61 pre-XDR) were identified during this period and subjected to susceptible testing against levofloxacin and two (higher & lower) concentrations of moxifloxacin. Of these 65 isolates tested 47 (72.3%) in addition to being resistant to ofloxacin were also resistant to levofloxacin and lower concentration of moxifloxacin while 10 (15.3%) isolates were sensitive to levofloxacin and both concentration of moxifloxacin. Out of 47 resistant isolates 45/47 (95.8%) were sensitive to higher concentration of moxifloxacin and only 2 (3%) were resistant. 8 (12%) of the isolates that were resistant to ofloxacin were also resistant to levofloxacin but sensitive to moxifloxacin. No isolate sensitive to ofloxacin and levofloxacin was resistant to moxifloxacin at any concentration 9 (Table 1).

Discussion

Drug resistance has become a problem in the management of TB, with an urgent need for research into new drugs as well as the development of efficacious combinations and regimens. Newer generation Fluoroquinolones have the potential to become part of treatment regimen against TB especially the drug resistant TB. However, these drugs being broad-spectrum antibiotics are also used in the management of a variety of community-acquired infections. A study of out-patient prescriptions for the treatment of community-acquired pneumonia conducted in the United States between November 2000 and January 2001 found that FQs accounted for 43% of prescriptions [10]. A similar audit in India was conducted by ORG IMS, a joint venture between ACNielsen ORG-MARG and IMS Health in India, which conducts prescription audits, attitudinal surveys and disease-specific studies. They reported that the two most frequently prescribed antibiotics were CFX and OFX, with gatifloxacin and levofloxacin being respectively the sixth and the eighth most frequently prescribed drug [11].

The increasing use of FQs has led to increasing resistance to these antimicrobials. There is also cross-resistance within the FQ class, such that reduced susceptibility to one quinolone likely confers reduced susceptibility to all FQs [12]. Similar results were obtained in this study where 72.3% of MDR isolates resistant to ofloxacin at base line were also resistant to levofloxacin and moxifloxacin.

Resistance profile	Number	Percentage
All sensitive	10	15.4
LVX	8	12.4
MOX (0.5)	0	0
MOX (2.0)	0	0
LVX+MOX (0.5)	45	69.2
LVX+MOX (0.5)+MOX (2.0)	2	3
Total	**65**	**100**

Table 1: Resistance profile of XDR and Pre XDR isolates of *M. tb* to newer generation FQs.

Resistance to levofloxacin (LVX) in this study was 12.4 % (8/64). This is in contrast to earlier studies reporting 54.3% (19/35) LVX resistance in XDR isolates from Peru [13] and 28% resistance among MDR-TB cases from Taiwan [14]. The possible reason could be the use of lower cut-off of 1 µg/mL in comparison to 1.5 µg/mL used in this study for determination of resistance to LVX.

In this study two concentrations of moxifloxacin (0.5 and 2.0 µg/mL) were tested. While none of the ofloxacin resistant isolates tested was resistant to any concentration of moxifloxacin, 69.2% (45/65) of isolates resistant to ofloxacin and levofloxacin were also resistant to moxifloxacin when tested at a concentration of 0.5 µg/mL. Only a negligible percentage (3%) of ofloxacin and levofloxacin resistant isolates were also resistant to moxifloxacin at the higher concentration of 2.0 µg/mL. The results demonstrate that moxifloxacin is active against strains with low levels of resistance (MIC, 0.5 µg/mL) and reduces the mortality associated with strains with intermediate resistance (MIC, 2.0 µg/mL). Thus these results provide data which support the current WHO recommendation to use moxifloxacin when there is resistance to early-generation fluoroquinolones, such as ofloxacin [15].

Several studies across India have reported FQ resistance among drug resistant TB isolates at varying rates [16-18]. Since India has the largest absolute number of MDR-TB patients in the world, and thus by inference must have the largest XDR population [19,20]. Therefore it could be postulated that FQ resistance is a more prevalent problem than actually reported.

In conclusion, newer generation FQs are promising drugs in the treatment of drug-resistant TB but care should be taken regarding the rationale use of these drugs for the treatment of other diseases especially when other drugs are available. We also support the adoption of a FQ-restriction policy in India as has been adopted by other countries like Canada; otherwise the alarming increase in FQ resistance globally could be a threat to TB control programs.

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