



## Baseline Resistance to Ofloxacin and Kanamycin Among Multi-Drug Resistant Strains of M.tuberculosis Isolated at an Intermediate Reference Laboratory in Delhi

### KEYWORDS

Baseline, Multi drug resistant, M. tuberculosis, Second line drugs, drug susceptibility testing

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**ABSTRACT** Prevalence of primary drug resistance serves as an epidemiological indicator to assess the success of the TB control programme. Thus early detection of Mycobacterium tuberculosis strains with primary resistance to antituberculosis drugs is important. We therefore conducted a study to estimate the prevalence of resistance at base line to two important second line anti-tuberculosis drugs i.e., Ofloxacin and Kanamycin among MDR-TB cases. 643 MDR strains were subject to drug susceptibility against Kanamycin and Ofloxacin using Bactec MGIT-960 system. DST results showed that 164 (29.63%) of these isolates were resistant to Ofloxacin, 3 (0.55%) resistant to kanamycin and 17 (3.17%) of these isolates were XDR. These results show that baseline resistance to second line antituberculosis drugs does occur and timely identification of such strains is important for formulating an effective treatment regimen.

### Introduction

Last decade witnessed emergence of extensively drug resistance tuberculosis (XDR-TB) defined as resistance to any fluoroquinolone (FQ) and at least one of second-line injectable agents: amikacin (AMK), kanamycin (KAN), and capreomycin (CAP) among MDR-TB [1]. Globally, 9.7% of MDR (MDR-TB is defined as tuberculosis caused by Mycobacterium tuberculosis isolates with resistance to isoniazid and rifampicin) cases are XDR and have already spread over 105 countries [2]. Through 2015, 105 countries reported cases of XDR-TB to the World Health Organization (WHO), composing just less than 10% of all patients diagnosed as having MDR-TB. [3]

Prevalence of primary drug resistance also serves as an

epidemiological indicator to assess the success of the TB control programme. With growing burden of DR-TB, such strains are transmitted increasingly from close contacts to normal population. Unfortunately there is paucity of studies on primary drug resistance from India. Therefore the aim of the present study was to estimate the prevalence of resistance at base line to two important second line anti-tuberculosis drugs i.e., Ofloxacin and Kanamycin among MDR-TB cases identified.

### Materials and Methods

The present study conducted at New Delhi Tuberculosis Centre which in addition of being a State Antituberculosis Training and Demonstration Centre (STDC) also serves as 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 & second line) by National Reference laboratory (NRL); National institute of Tuberculosis and Respiratory diseases: New Delhi India.

This study was conducted over a period of one and half year i.e., from April 2014 to October 2015. Sputum samples from suspected MDR-TB patients identified in RNTCP designated districts of Delhi state were sent to the IRL for determination of resistance to isoniazid (INH) and rifampicin (RIF) by Line Probe Assay (LPA). Samples identified as MDR and mono RIF resistant among these suspects were included in the study.

The second line drugs were obtained as powder form: ofloxacin (OFX) (Sigma-Aldrich Co.), kanamycin (KAN) (Sigma-Aldrich Co.) and the isolates were tested for susceptibility to these drugs using BACTEC MGIT960 system according to the manufacturer's instructions. The concentration of the each drug tested is 2.0 µg/mL for ofloxacin, 2.5 µg/mL for kanamycin. 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, 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. 100µl Drugs were added to the MGIT to have final concentrations of 2.0 µg/mL ofloxacin, 2.5 µg/mL of kanamycin. A growth control (GC) tube was prepared without antibiotic and 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 of 643 MDR-TB isolates were diagnosed and cultured on MGIT 960 during the study period. Of these 582 (90.51%) were found to be culture positive and subjected to drug susceptibility against Kanamycin and Ofloxacin. DST results showed that 164 (29.63%) of these isolates were resistant to Ofloxacin, 3 (0.55%) resistant to kanamycin and 17 (3.17%) of these isolates were XDR. (Table 1)

Among the 643 MDR patients, 524 (81.49%) patients belonged to 15-45 age group. 377 were male (58.63%) and 266 (41.36%) were female. Out of 377 male patients, 293 (77.71%) belonged to 15-45 age group and of the 266 female patients 231 (86.84%) belonged to 15-45 age group (Figure 1). Among the 17 XDR-TB patients, 11 (64.70%) belonged to 15-45 age group. This indicates more than half MDR-TB patient belongs to 15-45 age groups. (Figure 2)

**Discussion:**

The results of this study showed that resistance to second line anti-tuberculosis drugs does exist at baseline and if diagnosed early and accurately, proper treatment can be initiated. Thereby preventing the transmission of this deadly strain and avoiding the unnecessary delay in the treatment.

In present study 3.17% of XDR was diagnosed among MDR cases which are in concordance with other studies from India. Ramachandran et al reported 4% XDR among

MDR-TB isolates in Gujarat, [4] 4.3% of MDR-TB cases were XDR reported by Selvakumar et al in Tamil Nadu. [5] Chhavi et al reported 3.70% of MDR-TB isolates was XDR in Delhi [6]. As per WHO's reports, worldwide 9.7% XDR cases found among MDR. [7]

Another important finding of this study is that a substantial number of drug resistant cases were among women (41.36%). Supporting context comes from observational studies of drug-resistant TB in South Africa. In South African studies, higher percentages of XDR TB (50%–56%) than MDR TB patients (43%–53%) are women (8-11). In a study in the United States, few patients with MDR TB (36%) or XDR TB (38%) were female (12). Similarly, in cohorts from Latvia, Peru, and Russia, lower percentages of patients with MDR TB (17%–40%) and XDR TB (29%–35%) were female (13-15). Women with drug-resistant TB were more likely than men with drug-resistant TB to have XDR TB.

It has been established that Tuberculosis affects the economically most productive age group (2). Similarly in our study, 81.48% of the MDR and 64.70% XDR patients belonged to THE age group OF15-45 years. Similar studies across the world have confirmed these results (16, 17, 18, and 19).

In conclusion the results of this study showed that drug resistant tuberculosis does exist at base line and early detection of such strains is necessary not only for proper and timely treatments but also to curtail the chain of transmission.

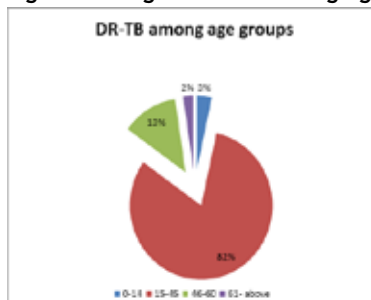
**Table 1: Resistance profile of XDR among MDR patients**

Resistance profile of XDR		
Resistance profile	Number	Percentage
Mono OFL	164	30.59
Mono KAM	3	0.55
XDR	17	3.17
Both Drug Sensitive	352	65.67
Total	536	100

**Figure 1: Drug resistance among male and female**



**Figure 2: Drug resistance among age groups.**



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