ORIGINAL ARTICLE

RESISTANCE TO QUINOLONES, AMINOGLYCOSIDES AND CAPREOMYCIN IN MULTI-DRUG RESISTANT (MDR-TB) M.TUBERCULOSIS

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ABSTRACT

Background: Tuberculosis is a major health issue of developing countries like Pakistan. Multi drug resistance (MDR) and Extensive drug resistance (XDR) in M. tuberculosis is on continuous rise in our region. Detection and prevalence of resistance in tuberculosis patients were discussed in few of the studies in Pakistan but pattern of resistance to second-line anti-tuberculosis drugs was still unknown and was a gap in the knowledge. This study was aimed to determine the pattern of resistance against Quinolones, Aminoglycosides and Capreomycin in Multi-drug resistant (MDR-TB) tuberculosis from a tertiary care hospital of Karachi.

Method: The cross-sectional study was carried out from January 2007 to October 2008 in the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Post Graduate Medical Centre, Karachi. Sixty MDR-M.tuberculosis strains of two groups were collected. Group 1 consisting of 30 samples from stock cultures of MDR-TB collected during previous 5 years (Laboratory of Microbiology, BMSI) and Group 2 consisting of 30 culture positive specimens from JPMC and Ojha Institute of Chest diseases, Karachi. These samples were further analysed for anti-microbial sensitivity against Quinolones (Ofloxacin), Aminoglycosides (Amikacin and Kanamycin) and Capreomycin. Indirect Proportion Method is used for drug susceptibility test (DST) following WHO guidelines 2001. For calculations & results, Quantitative analysis was done and percentages were calculated.

Results: From among 60 MDR-TB isolates, 3 (5%) were resistant to Ofloxacin; 1 (1.6%) resistant to Amikacin; 4 (6.6%) were resistant to Kanamycin and 60 (100%) were sensitive to Capreomycin. There was an increase in resistance in-group 2.

Conclusion: Our study confirmed the resistance against second-line anti-tuberculous drugs. Overall resistance to second-line anti-tuberculous is at low percentage but results clearly show the marked increase in resistance in Group 2. Immediate measures are surely required to detect and control drug resistance in our country.

KEYWORDS: MDR-TB, Drug resistance, Quinolones, Capreomycin, Aminoglycosides.

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INTRODUCTION

Tuberculosis (TB) is a global public health problem and a deadly infectious disease caused by Mycobacterium tuberculosis with an estimated death rate of 1-2 million of people per year. Approximately

9 million new cases were detected in a recent study [1]. M. tuberculosis (MTB) is an acid-fast rod bacterium, which usually infects the lung tissues. Transmission is mainly by droplet nuclei from untreated infected person, leading to the disease transmission. Progression of the disease occurs if human immune system is deficient, then the organisms start to multiply in lung tissues [2]. Sputum analysis confirms the positivity of infection in a person, followed by microscopy and culture [3].There has been advancement in technology for molecular genotyping and organism detection. Nonetheless, culture and DST (drug susceptibility tests) remain most sensitive and reliable methods for diagnosis of MTB drug resistance [4].

The treatment includes complete eradication of bacteria from the body using anti-microbial drugs in combinations.First line anti-mycobacterialagents include Isoniazid, Rifampin, Ethambutol, Pyrazinamide and Streptomycin. Resistance against them led to the development of second line of treatment including Quinolones, Amikacin, Caproeomycin, Kanamycin, Para Salicylic Acid, Ethionamide and Cycloserine. There has been also documentation of third line of treatment including Macrolides, Argenine, Linsolid and Rifabutin, but their efficacy and affectivity is not well-understood [5]. MDR-TB was reported in 1990s for the first time and is identified as TB resistant to at least isoniazid (INH) and rifampicin (RIF) [5,6]. Later on, many of the strains of Mycobacterium tuberculosis also showed extensively drug resistance that is XDR-TB (identified and declared by CDC and WHO in March 2006). XDR isdefined as MDR-TB plus resistance to any fluoroquinolones and one of the second-line injectible drugs, amikacin, kanamycin and capreomycin) [5, 7].

Fluoroquinolones, have potent bactericidal activity against M. tuberculosis (MTB) thus are a key component of the treatment of multidrug-resistant tuberculosis [8].The aminoglycosides; Amikacin (AK) and Kanamycin (KM) bind to 16S rRNA in the 30S small ribosomal subunit and inhibit protein synthesis [9]. Mutations in the rrs gene encoding 16S rRNA are associated with high-level drug resistance in M. tuberculosis. Collectively, rrsA1401G mutation is the most frequently reported mutation and is identified in 30 to 90% of KM resistant M. tuberculosis strains [10, 11].

This upcoming enigma remains a global health issue that hinders the prevention, treatment, and control of TB in many of developing countries[12].Early detection of all forms of drug resistance in TB is a key factor to reduce the spread of these resistant strains [13]

Thus, it leads to press demands for appropriate diagnosis of the resistant cases, and to find out the magnitude of problem not only for individual case management but also for drug resistance surveillance in Pakistan.

Keeping this in view, this study was designed to detect the Ofloxacin, Aminoglycoside and Capreomycin resistance in multidrug resistant isolates from multiple tuberculosis patients coming to a tertiary care hospital.

METHODS

This study was completed in the Department of Microbiology, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, Karachi, in collaboration with chest ward, JPMC Karachi and Ojha Institute of Chest Diseases. After obtaining approval from institutional ethical committee, samples were collected.

Total Sixty (60) samples were categorized in two groups. **Group 1** included thirty (30) samples of MDR-TB isolates from stock cultures, Department of Microbiology, BMSI. **Group 2** included thirty (30) Sputum samples with $\geq$ 10/HPF acid-fast bacilli. These were collected from patients with diagnosis of MDR-TB admitted in public sector hospitals like Chest Ward of JPMC, MDR-TB Clinic, JPMC and Ojha Institute. These were transported to Microbiology Department, Basic Medical Sciences Institute, Jinnah Postgraduate Medical Centre, and Karachi. Inclusion criteria for sample collection for two groups were the isolates resistant to INH and RIF and Sputum samples (Sputum smear +ve$\geq$ 10 AFB/HPF) from already diagnosed cases of MDR-TB. Lowenstein-Jensen (LJ) medium was prepared and used for culture and antibiotic susceptibility test (AST) [14]. For group 1, stock cultures were sub-cultured on L-J medium to get growth of young viable bacilli. For group 2, samples of sputum were collected from the fore-mentioned patients in sterile containers. All the details regarding patients were recorded on a pre-designed proforma. Each sample was treated by Petroff Method [14]. A small portion of sediment was used for preparation of microscopic slides, which were then stained with Zeihl –Neelson Technique (Z-N).The remaining sediment is used to inoculate one 5mm loopful on each of two slopes of plain L-J medium for each sample [15].

Drug susceptibility test (DST) was done by Indirect Proportion Method of Drug Susceptibility Testing. Following concentrations were incorporated in LJ medium for AST, before adding egg solution, distributed and inspissated in same manner as plain LJ medium. Critical concentrations of the anti-tuberculous drugs used in the medium were, 0.2µg/ml INH, 40µg/ml RIF, 2 µg/ml for OFX, 40µg/ml for CPM, 30.0µg/ml for KM and 40.0 µg/ml for AMK . The critical proportion values were 1% for INH, OFX, AMK, KM and CPM and 10% for RIF [16]. For each isolate from both the groups, a standard suspension of 0.05

MacFarland was prepared and two dilutions of 10 -3 and 10 -5 were inoculated in both drug- containing and drug-free L-J media. The cultures were incubated at 37° C for 8 weeks or until growth of colonies were observed [15, 16].

As a control, a drug free slope is set up for each strain tested and **H37 Rv**, a strain of Mycobacterium tuberculosis, which is sensitive to all anti-tuberculosis drugs, was inoculated with each set of tests and again within each set if the batch of medium is changed. A reading was made at 2 weeks to give a preliminary indication of the presence of resistant strains, but the definitive reading was done at 4 weeks. The colonies were counted only on the slopes seeded with the inoculum that has produced exact readable counts or actual counts (up to 100 colonies on the slope). The ratio of number of colonies obtained on drug containing medium to the number of colonies obtained on drug free medium indicates the proportion of resistant bacilli present in the strain. Below the critical proportion value, the strain is classified as sensitive and above as resistant. Final susceptibility results were reported after 40 days following the laboratory's standard procedure [15-17]. Collected results were then analyzed statistically.

RESULTS

The present study involved analysis of 60 diagnosed MDR-TB samples. The drug susceptibility pattern of Mycobacterium tuberculosis tested showed that out of 60 isolates,03 (5%) were resistant to Ofloxacin, 01 (1.6%) was resistant to Amikacin, 4 (6.6%) showed resistance to Kanamycin, whereas 60 (100%) were sensitive to Capreomycin. **(Table 1, Fig 1)**

In Group 1, Out of 30 isolates, 1 sample (3.3%) were resistant to Ofloxacin, 1 (3.3%) cases were resistant to Kanamycin, 30 (100%) cases were sensitive to Amikacin and 30 (100%) were sensitive to Capreomycin. In Group 2; out of 30 isolates, 2 (6.6 %) were resistant to Ofloxacin, 3 (10%) cases were resistant to Kanamycin and 1 (3.3%) cases showed resistance to Amikacin whereas, 30 (100%) were sensitive to Capreomycin. **(Table 2, Fig 2)**.There was no cross-resistance between AMK and KM. **(Table 3)**

TABLE 2: COMPARISON OF DRUG SUSCEPTIBILITY PATTERN OF MYCOBACTERIUM TUBERCULOSIS OF STOCK CULTURES (GROUP1) & NEW CASES (GROUP2) BY PROPORTION METHOD (DPM)

	Stock cultures (Group 1) n = 30				New Cases (Group 2) n = 30			
Drug	**Sensitive**	**%**	**Resistance**	**%**	**Sensitive**	**%**	**Resistant**	**%**
Ofloxacin (OFX)	29	96.7	1	3.3	28	93.4	2	6.6
Amikacin (AK)	30	100	0	0	29	96.7	1	3.3
Kanamycin (KM)	29	96.7	1	3.3	27	90	3	10
Capreomycin (CPM)	30	100	0	0	100	100	0	0

TABLE 3 :DISCORDANCE OF AMIKACIN & KANAMYCIN RESISTANCE

Resistant to Amikacin (AK)	Resistant to Kanamycin (KM)	Resistant to both AK & KM
1	4	0

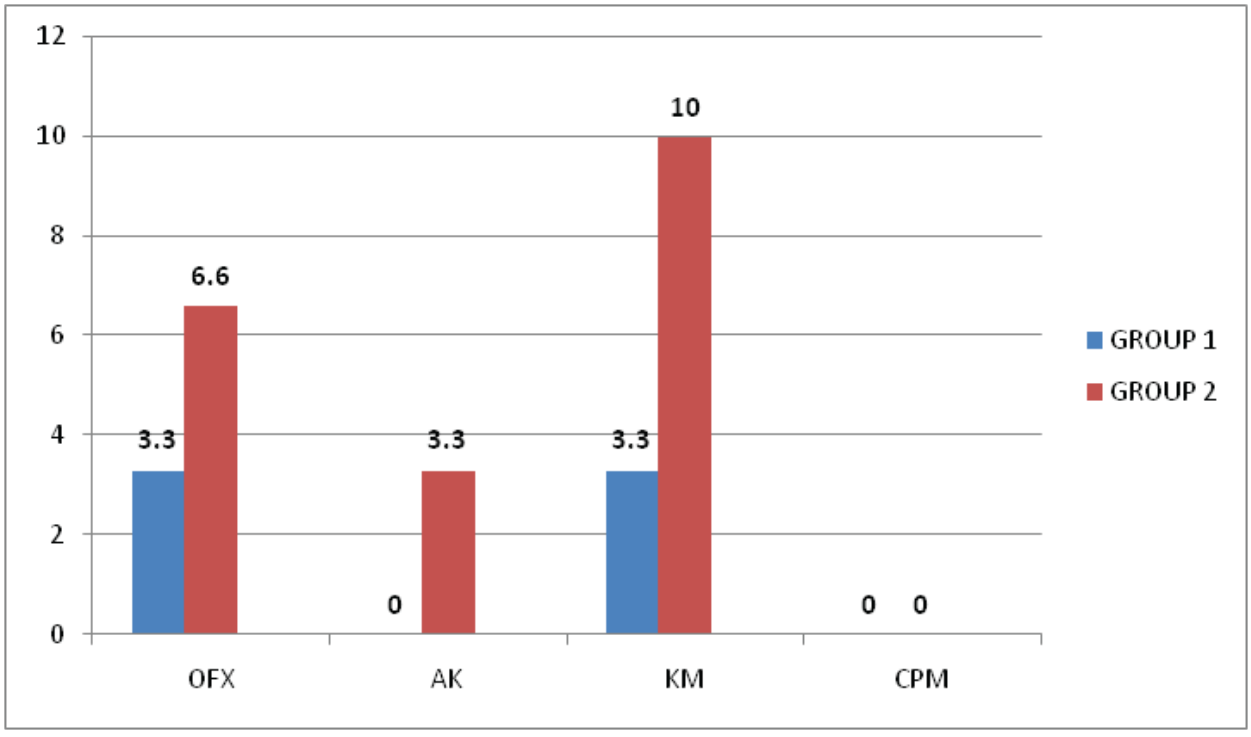

FIGURE 1: COMPARISON OF RESISTANCE PATTERN IN GROUP 1 (n=30) AND GROUP 2 (n=30)

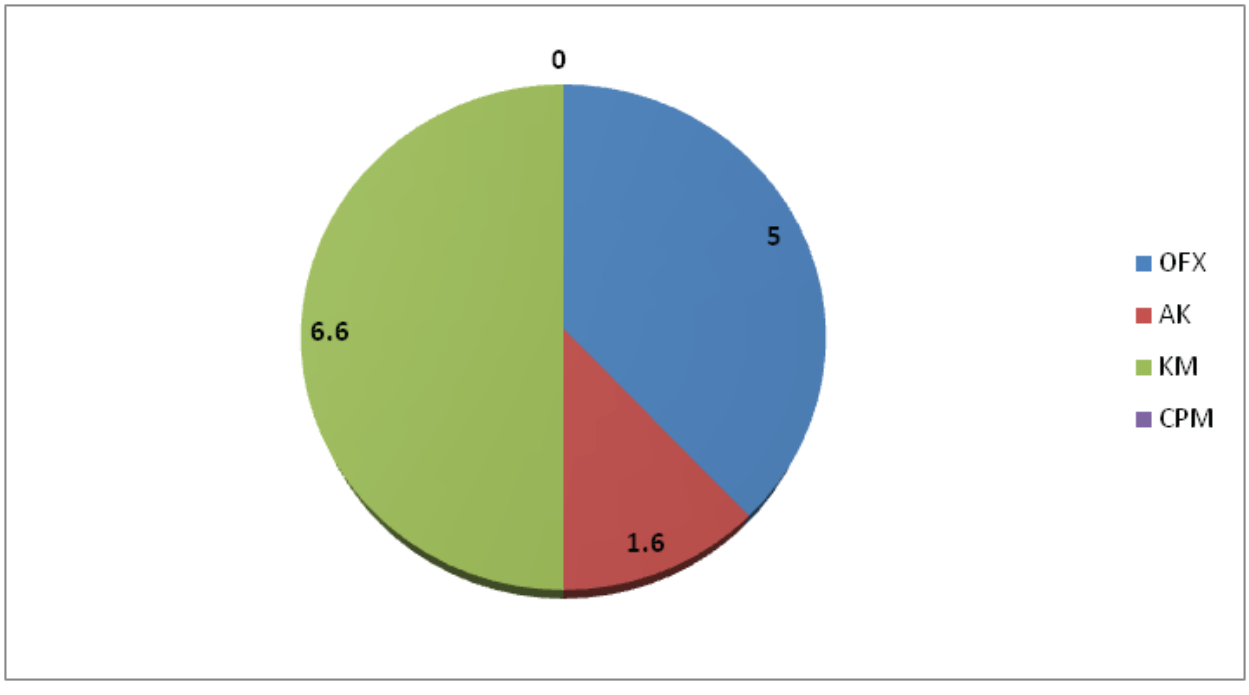

FIGURE 2: SECOND LINE DRUG RESISTANCE PATTERN IN MDR-TB ISOLATES

DISCUSSION

Increasing frequency of drug resistant TB is a major concern to human health as it significantly limits the treatment options for this highly contagious, low ID50%, chronic, debilitating and life-threatening disease [18]. The transition of 100% sensitive organism to MDR, then XDR and now TDR (Totally Drug Resistant) TB is alarming [5].

Pakistan is among the countries endemic for TB with reported cases of XDR-TB. The true incidence, prevalence and pattern of drug resistance for second line anti-tuberculous drugs are not known due to limited researches done regarding this issue. A limitation to accurate detection of resistance to drugs is also a fact as existing tests for resistance to second line drugs (SLD) are not standardized and are less accurate and precise for first-line drugs[19].- Due to paucity of community based data, hospital based local studies provide information about the levels of drug resistance particularly of rising resistance trends over the years. [20]

The current study has contributed to the analysis of the pattern of resistance in SLD for tuberculosis. Review of DST has highlighted the severity of the drug resistant TB in Pakistan.Quinolones are potent agents and mainstay in treatment of cases resistant or intolerant to first line of ATT [21]. Easy and unregulated availability of Quinolones and its usage for treating infections other than TB has significant contribution for resistance development especially if given in the pre-diagnostic period (within 12 months prior to diagnosis of TB) [8].In this study, resistance to OFX is 3.3% in stock cultures which increased to 6.6% in new cases (overall:5%); for KM, AMK & CPM resistance is 6.6%, 1.6% & 0% respectively. There is no cross-resistance identified among aminoglycosides and CPM. The two recent studies conducted in Pakistan reported 11% (5/45) resistance to FQ (Fluoroquinolone) and 07% (40/577) MDR plus Quinolone resistant isolates, with 100% sensitivity to Capreomycin. Resistance against Amikacin and Kanamycin was not tested in that study [22, 23]. Our result is closer to these studies. It is noticeable that as CPM is an expensive drug and is not available or prescribed in Pakistan; therefore no resistance can be spotted against it. In comparison, Quinolones and Aminoglycosides are readily available over-the-counter drugs. In another study, Jabeen reported resistance to Ciprofloxacin (quinolone) was 23% during 1998-2004. This resistance has been fluctuating from 63% in1998, to 48% in1999,39% in 2000, 40% in 2001, 28% in 2002,10% in 2003 and13% in 2004 [24]. This mentioned study show complete cross-resistance between quinolones. The results are much higher than ours. Increase in Sample size and duration of study might be a factor for variation in the results.

Ho and his colleagues found 11% FQ resistance in MDR-TB isolates in Australia. He conducted a year surveillance study on FQ resistance in MTB isolates and compared his results with 10 potentially relevant studies conducted in different parts of the world. Conclusion of the study was that there is strong association between resistance to FQs and their unregulated use as a broad-spectrum agent to cover simple bacterial infections. [8] Falzon reported resistance to FQ as 1% (83/6724) , AK and / or KM as 21% (1425/6724), AK and/ or KM plus CPM as 13% (393/6724), and CPM as 16% (503/6724) in MDR-TB isolatesconducting a large scale study. In this study, AK and KM were not tested for resistance separately in all the isolates assuming complete cross-resistance between the two aminoglycosides [25]. Our results were not comparable to these results. Reason might be the large sample size and change in the dynamics of the population.

A study conducted in Georgia, on prisoners with pulmonary tuberculosis, reported resistance to OFX as 5.1% (2/39) in MDR-TB isolates. Finally, 43.6% (17/39) resistance to KM and CPM both was also noticed [26].Our results for Quinolones are similar but resistance to aminoglycosides and CPM is much higher in this study.

Resistance to OFX is 20% and 9% for AMK in a study conducted in Hong Kong [27]. Ozkutukin reported 25% resistance to CPM, and 5% to KM and AMK in Turkey [28]. In the present study, cross–resistance between AMK and KM was not found out, which is against the general concept that both drugs show complete cross-resistance. Kruuner found 43 out of 79 KM resistant isolates were sensitive to AMK indicating the need to test the resistance of M. tuberculosis isolates against both the drugs [29].

Drug resistant T.B is spreading very fast. Resistance against the Quinolones and Aminoglycisides leaves a little margin for treatment for resistant tuberculosis. Drugs such as Capreomycin and other third-line drugs are not only expensive; but are also less effective. No cross-resistance between AMK and KM surely provides us an opportunity for using them as one of the treatment option. It also draws attention to the fact that resistance should be verified against both the drugs. Similar were the cases of resistance against Comparison of the two groups: Group 1, stock cultures collected in previous 5 years and Group 2, samples collected during our study period, also revealed the fact that resistance has almost doubled within few years.

CONCLUSION

Although low resistance is detected, overall against second-line anti-tuberculous drugs (Fluoroquinolones and aminoglycosides) small sample size might be the reason of it. No cross-resistance between AMK, KM and Capreomycin provides us margin of using them as one of the option even if one is resistant. So resistance should be checked against

all of them. Comparison of the two groups of our study also revealed the fact that resistance almost doubled within few years

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