INTRODUCTION
Tuberculosis (TB) remains a major health challenge in many developed countries but more so in the developing world. One-third of the world population is suffering from tuberculosis. Majority of the cases (95%) of TB infection occurs in underdeveloped countries where diagnostic and treatment facilities are inadequate.1 Multidrug Resistant Tuberculosis (MDR-TB) strains are resistant to both rifampicin and isoniazid (INH).1 Extensively Drug Resistant Tuberculosis (XDR-TB) is caused by strains resistant to rifampicin, isoniazid, any fluoroquinolone and any of the second line anti-TB injectable drugs like CAP, kanamycin and AMK.1 Worldwide, 3.6% of incident TB cases appear as MDR-TB, out of which 50% occur in China and India.1 In the year 2012, there were 450,000 estimated new cases of MDR-TB cases worldwide with estimated mortality of 170,000.2

As per WHO statistics of 2012, Pakistan stands at fifth position among 22 high TB burden countries.3 In Pakistan, incidence of TB is 231/100,000 population, prevalence 376/100,000 population while percentage of MDR-TB ranges from 3.5% to 32%.2 One of the causes for this high prevalence may be poor coverage and difficulties in applying DOTS Programme.4 Most of the second line anti-TB drugs are available in Pakistan. Worldwide most of the high burden TB and MDR-TB countries do not have the recommended capacity of one laboratory per 5 million population to perform culture and DST.3 The situation is more grave in developing countries like Pakistan, where only 1187 notified laboratories are capable of smear microscopy accounting 0.7 laboratory per 100,000 population and only 10 laboratories are capable of performing DST accounting 0.3 laboratory per 5 million population.3 BACTEC MGIT 960 system is among rapid diagnostic systems for TB culture with capacity to provide sensitivity for first and second line anti-TB drugs. This study was aimed to determine the sensitivity pattern of MDR-TB clinical isolates against second line anti-tuberculosis drugs in our setup.

METHODOLOGY
This cross-sectional study was carried out in the Department of Microbiology, AFIP, Rawalpindi, Pakistan, 2011 to April 2013. Samples received during the study period were processed on BACTEC MGIT 960 system for Mycobacterium tuberculosis (MTB) culture followed by first line drugs susceptibility testing of culture proven MTB isolates. On the basis of resistance to rifampicin and isoniazid, 100 clinical isolates of MDR-TB were further subjected to susceptibility testing against amikacin (AMK), capreomycin (CAP), ofloxacin (OFL) and ethionamide (ETH) as per standard BACTEC MGIT 960 instructions.

RESULTS
Out of 100 MDR-TB isolates, 62% were from male patients and 38% from female patients. 97% were sensitive to AMK, 53% to OFL, 87% to CAP; and 87% were sensitive to ETH.

CONCLUSION
The majority of the MDR-TB isolates showed excellent sensitivity against AMK, CAP and ETH. However, sensitivity of MDR-TB isolates against fluoroquinolones like OFL was not encouraging.

from November 2011 through April 2013. Sampling technique was non probability consecutive sampling. All the culture positive MDR-TB isolates from clinical specimens like sputum, C.S.F, pleural fluid and peritoneal fluid etc., submitted at AFIP, Rawalpindi, for culture of *Mycobacterium tuberculosis* were included in the study while non MDR-TB clinical specimens, blood, urine, stool and improper samples like saliva and pus swab were excluded from the study.

Permission of the Institutional Ethical Committee was obtained and guidance was sorted out from Institutional Biosafety Committee. This laboratory receives samples from tertiary care military hospitals as well as civil hospitals of Rawalpindi/Islamabad and northern areas of the country.

All the samples (except sterile body fluids) were subjected to standard N-Acetyl-L-Cystine (NALC), NaOH digestion-decontamination method as described by Kent and Kubica. To each MGIT tube already containing 7 ml of 7H9 broth, 0.8 ml of growth supplement and a combination of 5 antibiotics Polymyxin-B, Amphotericin-B, Nalidixic acid, Trimethoprim and Azlocillin (PANTA) were added. Then 0.5 ml of decontaminated specimen was added to this tube, which was then inoculated in MGIT 960 after barcode scanning. Growth yielded on MGIT 960 System was confirmed as MTB by ZN staining and commercially available immunochromatographic kit (BD MGIT™TBc identification test). Confirmed culture positive isolates were subjected to DST to first line anti-tuberculosis drugs namely isoniazid, rifampicin, streptomycin and ethambutol. On the basis of DST results, MDR-TB isolates were segregated.

A total of 100 culture confirmed MDR-TB isolates (71 sputum, 14 endobronchial washings, 6 tissues and 9 pus) were dealt during the study period; 85% pulmonary and 15% extra pulmonary. MDR-TB isolates were processed on BACTEC MGIT 960 system for DST against AMK, CAP, OFL and ETH in recommended concentrations. All drugs were procured from Sigma (St. Louis, MO, USA). The stock solutions of AMK (83 µg/ml), CAP (184 µg/ml) and OFL (166 µg/ml) were prepared in sterile water as per instructions provided in leaflets of respective drugs. These stock solutions were filtered through 0.22 µm pore size Millex-GS filter units (Millipore, Bedford, MA), aliquoted and stored at -70°C. Stock solution of ETH (415 µg/ml) was prepared in ethylene glycol and incubated at 37°C overnight. The critical concentrations of AMK, CAP, OFL, ETH used for BACTEC MGIT 960 system were 1.0 µg/ml, 2.5 µg/ml, 2.0 µg/ml, and 5.0 µg/ml respectively.

The drugs panel was consisted of five MGIT tubes, one for growth control and four MGIT tubes for the drugs (one for each drug i.e. AMK, CAP, OFL and ETH). Each 7ml MGIT tube was checked for any contamination or turbidity and labelled properly. After mixing the growth supplement (OADC), 0.1 ml of each antibiotic stock solution was added in respective MGIT tubes. 0.5 ml of culture proven MDR-TB sample was added to all these four MGIT tubes while 0.5 ml of 1:100 diluted sample was added to control tube. After bar code scanning all the inoculated tubes were entered in the instrument and incubated at a temperature of 37°C. An un-inoculated MGIT tube was used as a negative control and MTB ATCC 25177 was used as positive control strain.

Once positivity was indicated by the system, respective tube was removed and the result was interpreted as sensitive or resistant to respective second line drug. Descriptive statistics were calculated for both qualitative and quantitative variables. SPSS version 17 was used for quantitative variables like age, mean was calculated. Frequency and percentages were calculated for qualitative variables like gender and drug sensitivity, while qualitative variables were presented as tables and charts.

**RESULTS**

During the study period, a total of 100 culture proven MDR-TB isolates were subjected to DST against AMK, CAP, OFL and ETH. Out of 100 MDR-TB specimens, 85 (85%) were pulmonary (71 sputum samples, 14 endobronchial washings) while 15 (15%) were extra-pulmonary (6 tissues; 9 pus samples).

![Figure 1: Age-wise distribution of MDR TB cases (n=100).](image1)

![Figure 2: Sensitivity pattern of MDR-TB isolates against 2nd line anti-TB drugs (n=100).](image2)
Sixty two (62%) MDR-TB isolates were from male patients and 38 (38%) from female patients; male to female ratio of 3.1:1.9. The mean age of the patients was 35.4 years, ranging from 15 to 71 years (Figure 1).

Out of 100 culture proven MDR-TB isolates, 44 (44%) were sensitive to all four tested second-line anti-TB drugs, 97 (97%) were sensitive to AMK, 53 (53%) to OFL, 87 (87%) to CAP and also 87 (87%) were sensitive to ETH (Figure 2).

DISCUSSION

TB is a disease of low socioeconomic groups especially residing in ill-ventilated and overcrowded places. Despite of all the medical and public health developments, TB incidence is still scary with a daily addition of 5000 new cases and loss of two lives every third minute. Developing countries bear a very high burden (98%) of deaths due to TB. As estimated, nearly 70 million people will die from this risk group-3 pathogen within next 20 years if appropriate timely steps are not ensured.

WHO reported globally 310,000 MDR-TB cases among notified pulmonary TB patients. According to Pakistan National Tuberculosis Programme (NTP) report, 413,450 cases of TB were reported in year 2011.

A number of studies have been conducted worldwide to determine the sensitivity pattern of different second-line anti-TB drugs against MDR-TB isolates. This study focused on four second-line anti-tuberculosis drugs i.e. AMK, OFL, CAP and ETH. The present results regarding OFL and ETH were similar to a Pakistani study done by Khurram et al. which also shows 53.3% isolates sensitive to OFL and 86.7% to ETH. These results regarding ETH were also in concordance with another study from Pakistan, which depicted 13.4% MDR-TB isolates resistant to ETH, but the percentage of resistance to OFL was less than that of this study results. A study conducted in India showed 67.3% isolates sensitive to ETH and 83.6% to OFL as compared to our results of 87% and 53% respectively. A study by Ying-Cheng et al. revealed 99.2% isolates sensitive to CAP, 94% to AMK and 74% to fluoroquinolones. A study from Thailand showed 94.9% isolates susceptible to AMK and around 79% to ETH, comparable to the present results.

A study done in northern region of South Africa demonstrated 4.5% resistance to CAP, less than the present results. A study from Rwanda showed 99% MDR-TB isolates sensitive to ETH and 95.7% to OFL. Results of a multicenter international study from Russia, Estonia and UK showed lesser sensitivities to these; 81.2% isolates sensitive to AMK and 78.9% to CAP. Results of a study by Skrahina et al. from Minsk (Belarus) showed higher resistance to AMK as well as CAP, however, more MDR-TB isolates were sensitive to OFL in that study than the present. Higher percentage of resistance against ETH and CAP has been reported by Ozkotok et al. while sensitivity against AMI is comparable to these results.

MDR as well XDR-TB are posing a real challenge in resource constrained countries where diagnostic facilities are sparse along with poor infection control practices. Awareness among the population about this disease, preventive measures to avoid the spread of disease in community along with early diagnosis and effective treatment play a vital role in the management of this disease. There is an urgent need to improve the techniques for second-line DST and to configure screening as well as diagnostic algorithms for MDR-TB. Though the facilities are available at the study place, a huge area remains without forcing the clinicians to resort to blind therapy.

CONCLUSION

The sensitivity pattern results showed higher OFL resistance against MDR-TB isolates, which is alarming and a serious challenge to manage MDR-TB cases. On the other hand, AMK, ETH and CAP are still very effective against MDR-TB. It is critical to have accurate detection of drug resistance along with quality-assured DST against first and second line anti-TB drugs for subsequent decisions to promptly manage TB and MDR-TB patients. Though the results of this study will be beneficial to clinicians for prescribing second line anti-TB drugs to MDR-TB patients, more centers are required to establish DST facilities.

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REFERENCES


