ORIGINAL ARTICLE

In Vitro Evaluation of Linezolid and Meropenem Against Clinical Isolates of Multi Drug Resistant Tuberculosis By Mycobacterial Growth Indicator Tube (MGIT) 960

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ABSTRACT

Objective: To evaluate the *in vitro* effectiveness of multiple breakpoint concentrations of newer antituberculosis agents (Linezolid and Meropenem) against Multi Drug-Resistant Tuberculosis (MDR-TB) isolates.

Study Design: A descriptive cross-sectional study.

Place and Duration of Study: Microbiology Department, Armed Forces Institute of Pathology (AFIP), Rawalpindi, from September 2011 to August 2013.

Methodology: A total of 100 MDR-TB isolates recovered during the study period were subjected to susceptibility testing against multiple breakpoint concentrations of Linezolid (LZD) and Meropenem (MER). The breakpoint concentration used for LZD were 0.5, 1.0 and 2.0 μg/ml, while for MER were 4.0, 8.0 and 16 μg/ml. Mycobacterial Growth Indicator Tube (MGIT) 960 system was used to carry out drug susceptibility testing as per recommended protocol.

Results: At break point concentration of 0.5 μg/ml, 80 out of 100 (80%) MDR-TB isolates were susceptible to LZD while at breakpoint concentration of 1.0 μg/ml and 2.0 μg/ml, 96/100, (96%) of MDR-TB isolates were susceptible. For MER, at breakpoint concentrations of 4.0 μg/ml no MDR-TB isolate was susceptible, while at 8.0 μg/ml 3/100, (3%) and at 16.0 μg/ml 11/100, (11%) of MDR-TB isolates were susceptible.

Conclusion: LZD was found to have excellent *in vitro* efficacy as 96% of MDR-TB isolates were susceptible at breakpoint concentration of 1.0 μg/ml or more. In case of MER it was found that *in vitro* susceptibility improved as the break point concentrations were increased.

Key Words: *Multi drug resistant tuberculosis. Linezolid. Meropenem. MGIT 960.*

INTRODUCTION

One-third of world population is infected with *M. tuberculosis* and hence has increased risk for development of active tuberculosis.[1] Approximately 8.8 million people are diagnosed with active tuberculosis that causes 1.7 million deaths per year. Pakistan presently ranks fifth among the high TB laden countries with incidence rate of 181/100,000 and prevalence of 359/100,000 population.[2]

Tuberculosis (TB) can be treated with first line primary drugs when drug resistance is not defined. Nevertheless global increase in the incidence of MDR and extensively drug resistant tuberculosis (XDR-TB) has posed serious challenges to the ongoing efforts to combat tuberculosis.[3] MDR-TB is defined as disease caused by strains of *M. tuberculosis* that are at least resistant to treatment with isoniazid and rifampicin while XDR-TB refers to disease caused by multidrug resistant strains that are also resistant to treatment with fluoroquinolones and any of the injectable second line antituberculosis drugs (Amikacin, Capreomycin and Kanamycin).[4,5] Second line drugs used in the treatment of MDR-TB are not only costly but have many side effects and require long period of treatment that can limit their usage.[6] The increase in MDR-TB rates has led to pressing demands for appropriate treatment with second line anti-tuberculosis drugs and need to find newer compounds with potential *in vitro* activity against MDR-TB. A number of antimicrobial compounds i.e. Linezolid, Levofloxacin, Moxifloxacin, Carbepenems and Amoxicillin/clavulanic acid have been considered as potentially active agents against MDR-TB.[7-9]

LZD belongs to oxazolidinone group of antimicrobials which acts by inhibition of protein synthesis. It has been found to have good activity against MDR-TB isolates *in vitro* and animal studies.[10] Recently this antimicrobial was tested against XDR-TB and Pre-XDR-TB isolates from Pakistan with excellent *in vitro* efficacy.[11] Beta-lactams are not considered useful therapeutic agents against *M. tuberculosis* isolates due to microorganism's natural resistance to such class of antibiotics.[12] During recent times MER which is a member of carbapenem group has generated interest because it has low affinity

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substrate for the beta-lactamase with hydrolysis five times lower than other beta-lactams antibiotics. Carbapenems are thus thought to have potential in overcoming beta-lactamase resistance of *M. tuberculosis*.[13,14]

The rationale of the study was to look beyond the second line antituberculosis drugs and check for effectiveness of newer compounds like LZD and MER against MDR- TB in our own set up. The clinicians can then have choice of choosing the newer antituberculosis compound in case they encounter resistant case of TB. This study was aimed to evaluate the *in vitro* effectiveness of multiple breakpoint concentrations of newer compounds such as LZD and MER against MDR-MTB isolates in our setup.

METHODOLOGY

This laboratory based descriptive cross-sectional study was carried out at the Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from September 2011 to August 2013. Prior permission was obtained from institutional ethical review board for the study. A total of 100 MDR-TB isolates were included in the study. Non-probability convenience sampling was adopted. All the isolates were confirmed as MDR on gold standard Bactec MGIT 960 TB system. Duplicate specimens were excluded from the study. In the protocol we followed, all MDR-TB isolates were separately subjected to susceptibility testing against two newer antituberculosis compounds LZD and MER. LZD as pure substance ca.100% Catalog No. 165800-03-3 with storage recommendation at room temperature while MER Catalog No 119478-56-7 with storage temperature at -20°C was used. These drugs were dissolved in deionized water. The stock solutions of LZD and MER were prepared in sterile water as per instructions provided by the manufacturer. A critical concentration of 1.0 μg/ml and 4.0 μg/ml for LZD and MER was used. Two additional breakpoint concentrations of the two drugs i.e. 0.5 and 2.0 μg/ml for LZD and 8.0 and 16.0 μg/ml for MER were also tested on MGIT 960 system. The susceptibility testing on MGIT is based on the fluorescence detection of the mycobacterial growth in the tube containing modified Middle brook 7H9 liquid medium along with fluorescence quenching based oxygen sensor incorporated at the bottom of the tube.[15] A strain of *M. tuberculosis*, H37Rv (ATCC 27294), was used as a Quality Control (QC) strain and was tested with each batch of Drug Susceptibility Test (DST) at the critical concentration of each drug.

The statistical analysis of the data was done by the software Statistical Package for Social Sciences (SPSS) version 19. Descriptive statistics was calculated in terms of mean, standard deviation, minimum, maximum and range. Mean and standard deviation was calculated for quantitative variable like age of patients. Frequency and percentage was calculated for susceptibility and resistance of MDR-TB isolates to LZD and MER.

RESULTS

Out of 100 MDR-TB isolates included in the study, 64 were from men and 36 from women. The mean age was 34.9 ± 13.99 years ranging from 15 to 71 years. The maximum number of MDR-TB isolates (32%) were recovered from patients belonging to age group 15 - 25, while only (11%) of the isolates belonged to age group ≥ 56 years of age.

Susceptibilities of MDR-TB isolates to different concentrations of LZD and MER is shown in Tables I and II.

Table I: Susceptibilities of MDR-TB isolates to different concentrations of LZD (n=100).

Critical concentration	No. of susceptible isolates	Susceptible percentage
0.5 μg/ml	80	80%
1.0 μg/ml	96	96%
2.0 μg/ml	96	96%

Table II: Susceptibilities of MDR-TB isolates to different concentrations of MER (n=100).

Critical concentration	No. of susceptible isolates	Susceptible percentage
4.0 μg/ml	0/100	0%
8.0 μg/ml	3/100	3%
16.0 μg/ml	11/100	11%

DISCUSSION

The World Health Organization has acknowledged the fact that despite following the guidelines regarding programmatic management of drug resistant tuberculosis the treatment success rate is still less than 50%. This confession has thus thrown challenge to researchers all over the world to try and evaluate newer antituberculosis drugs to combat MDR-TB.[16]

The reliable drug susceptibility testing method provides with detailed knowledge on quantitative drug resistance pattern which ultimately paves the way for empirical treatment of drug resistant tuberculosis. During the last decade or so MGIT 960 system has been extensively studied and validated for susceptibility testing of first line antituberculosis drugs.[17] The multicentre laboratory validation of the Bactec MGIT 960 technique for testing susceptibilities of *M. tuberculosis* to classical second line drugs and newer antimicrobials has provided us with a guideline for resource poor countries like Pakistan to endeavor testing such compounds against our local isolates.[15] The main purpose and idea behind testing and evaluating multiple concentrations instead of one breakpoint concentration was to firstly assess and compare our isolates with those reported around the globe and secondly to present it as benchmark for further large scale studies to be conducted in Pakistan.

The protocol and multicentre laboratory validation of the Bactec MGIT 960 technique for testing susceptibilities

against LZD was first developed by Rusch-Gerdes *et al*.[15] This study was conducted in three phases, multiple concentrations 0.5, 1.0 and 2.0 μg/ml of linezolid were tested at three sites. The authors concluded that MIC of 1.0 μg/ml be used as critical concentration of LZD for testing isolates by Bactec MGIT 960 technique. The guidelines provided in that study were followed here as a benchmark to test the isolates against LZD by broth based Bactec MGIT 960 technique. This study revealed that for LZD at breakpoint concentration of 0.5. μg/ml, 80% of isolates were susceptible, while at MIC's of 1.0 and 2 μg/ml, 96% of isolates were susceptible. Hence only 4% of MDR-TB isolates from our study period were resistant at MIC ≥ 1 μg/ml. Similar breakpoint concentration of 1.0 μg/ml has also been followed and applied for checking the susceptibilities of 28 MDR-TB isolates recovered from clinical samples of patients in Netherlands.[18] The results of their study carried out at National Tuberculosis Reference Laboratory in Netherlands concluded that DST performed on MGIT 960 method was advantageous over previous standard 7H10 agar dilution method on account of shorter turn around time.

First LZD resistant clinical isolates of *M. tuberculosis* were reported by Richter *et al*. in Germany when they found that 4 out of 210 (1.9%) strains tested at German National Laboratory for Mycobacteria from 2003 to 2005 at MIC of ≥ 1.0 μg/ml were resistant.[19] The researchers in that study too used Bactec 460 and Bactec 960 system for LZD keeping 1.0 μg/ml as breakpoint concentration.

In a study carried out recently at the Aga Khan University Hospital (AKUH), Karachi, Pakistan, LZD was tested against XDR and Pre-XDR isolates. The results of their study revealed that 94% of their tested isolates were susceptible to LZD at breakpoint concentration of 0.5 μg/ml. The present results are thus in concordance with those reported in this study. Most of our MDR-TB isolates were recovered from patients belonging to Northern Pakistan, whereas AKUH is major tertiary care treatment and diagnostic centre in the South of the country. The results of these two studies thus give very encouraging news as far as therapeutic potential of LZD is concerned in the treatment of drug resistant tuberculosis.[11]

The *in vitro* susceptibility results of LZD have been found to have meaningful correlation with the *in vivo* behavior of the drug as reported by Zhang *et al*.[20] According to their results, those MDR-TB patients whose isolates had higher MIC range than the breakpoint concentration had adverse clinical outcome compared to those patients who had MIC in the susceptible range. During the recent past a multi-centre study conducted in eight developed countries has revealed very encouraging results of therapeutic trial with LZD for the treatment of drug resistant tuberculosis.[21] It was highlighted in this multi-centre study that true benefits of using LZD in the treatment of drug resistant tuberculosis will ultimately depend on how judiciously the antimicrobial has been prescribed in such patients.

Beta-lactam antibiotics have not been widely used against *M. tuberculosis* mainly due to lack of efficacy. Recently, there has been activity to reinvestigate this phenomenon and some important developments have taken place which indicates deletion or inhibition of major beta-lactamase enzyme of MTB, BLaC.[22] These studies have created significant interest in the usage of beta-lactam agents against MTB. We tested Meropenem because it has been found to have low affinity substrate for the beta-lactamase enzyme produced by *M. tuberculosis* with hydrolysis many times lower than other beta-lactam antimicrobial like Ampicillin. Combination of this compound with clavulanic acid has shown to have good *in vitro* activity against MDR-TB including non-replicative strains and is able to sterilize cultures in 14 days.[14]

MER was selected and was tested against QC strain at three breakpoint concentrations of 1.0, 2.0 and 4.0 μg/ml. The QC strain was susceptible at 4.0 μg/ml, hence three breakpoint concentrations 4.0, 8.0 and 16 μg/ml of Meropenem were prepared and tested against MTB. The main purpose of using two additional concentrations of MER was basically to see the *in vitro* efficacy of this compound as we increase the concentration. The results revealed that none of the MTB isolate was susceptible at 4.0 μg/ml while 3% of isolates were susceptible at 8.0 μg/ml and 11% isolates at 16.0 μg/ml. As the reports of efficacy of Meropenem with clavulanic acid had started to pour in, future studies can be performed by combining the MER with beta-lactamase inhibitor like clavulanic acid. The main limitation of this study was that we used MER without adding clavulanic acid which would have given us more information with regard to *in vitro* efficacy of this compound. There are plenty of reports in literature where combination of MER and clavulanic acid has resulted in the cure of patients suffering from drug resistant tuberculosis.[23,24] The World Health Organization has already included MER in group-V of their classification of drugs list with daily adult dosage of 1000 mg twice or thrice daily to be administered by intravenous route.[25]

As this was a single centre study performed at centre with facility to perform drug susceptibility testing against first and second line antituberculosis drugs so results of this study can provide a base and platform for other researchers and centres in Pakistan to test and try newer antimicrobials for TB. As this study has revealed excellent results with regard to the *in vitro* susceptibility of *M. tuberculosis* against Linezolid so both the diagnosticians and clinicians can think of trying this compound in

their respective areas with sufficient level of confidence. Although the results of *in vitro* efficacy of MER alone against MDR-TB as found in this study can not be termed satisfactory but it must be kept in mind that MER has great potential and future specially when combined with Clavulanic acid for managing cases of drug resistant tuberculosis.

CONCLUSION

The results of this study conclude that Linezolid has an excellent *in vitro* activity against multidrug resistant *M. tuberculosis* isolates. However, meropenem has showed poor activity against these isolates alone but by serially increasing the break point concentrations the more number of isolates became susceptible. This compound thus has potential to become effective anti-tuberculosis drug if it is combined with beta-lactamase inhibitor agent to act as an effective antituberculosis drug.

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