ORIGINAL ARTICLE

Comparative *In Vitro* Efficacy of Doripenem and Imipenem Against Multi-Drug Resistant *Pseudomonas aeruginosa*

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Abstract

Objective: To compare the *in vitro* efficacy of doripenem and imipenem against multi-drug resistant (MDR) *Pseudomonas aeruginosa* from various clinical specimens.

Study Design: Descriptive cross-sectional study.

Place and Duration of Study: Department of Microbiology, Armed Forces Institute of Pathology, Rawalpindi, from November 2012 to November 2013.

Methodology: MDR *Pseudomonas aeruginosa* isolates from various clinical samples were included in the study. Susceptibility of *Pseudomonas aeruginosa* against doripenem and imipenem was performed by E-test strip and agar dilution methods. The results were interpreted as recommended by Clinical Laboratory Standard Institute (CLSI) guidelines.

Results: The maximum number of *Pseudomonas aeruginosa* were isolated from pure pus and pus swabs. *In vitro* efficacy of doripenem was found to be more effective as compared to imipenem against MDR *Pseudomonas aeruginosa* with both E-test strip and agar dilution methods. Overall, p-values of 0.014 and 0.037 were observed when susceptibility patterns of doripenem and imipenem were evaluated with E-test strip and agar dilution methods.

Conclusion: *In vitro* efficacy of doripenem was found to be better against MDR *Pseudomonas aeruginosa* as compared to imipenem when tested by both E-test and agar dilution methods.

Key Words: *Agar dilution. Doripenem. Multi-drug resistant (MDR). Pseudomonas aeruginosa. Imipenem.*

INTRODUCTION

Pseudomonas aeruginosa (*P. aeruginosa*) is an adaptable pathogen associated with a broad range of infections in humans. This organism is one of the most important pathogens causing infections in susceptible individuals in hospital settings.[1] Treatment of infections becomes more problematic as *P. aeruginosa* strains from clinical isolates show intrinsic resistant to many antimicrobials.[2] Moreover, treatment becomes increasingly difficult due to the emergence and dissemination of resistance leaving only few antibacterial agents as therapeutic options.[1]

Hospital associated *P. aeruginosa* isolates are multi-drug resistant, which may be due to continuous exposure to antimicrobials in the hospital. The other risk factors for acquiring MDR Pseudomonal infection may vary from individual risk factors to the number of carriers in the same ward, transfer of infection from staff to patient and failure of fulfillment of infection control measures.[3] There are several mechanisms of developing multi-drug resistance in *P. aeruginosa*. These involve enzyme production, outer membrane protein loss, and target site alteration.[4] Acquired resistance is mediated through multi-drug efflux pump system that plays a key role to the development of multi-drug resistance in *P. aeruginosa*.[5] An expanding population of multi-resistant and pan-resistant bacteria is also alarming. Treatment failure in more invasive pseudomonas infection is common. Carbapenems are considered as of the potent antipseudomonal beta-lactam agents that inhibit bacterial wall synthesis. These bind to and inactivate penicillin-binding protein PBP1 and PBP2 causing elongation and lysis of cell wall. Imipenem is a carbapenem and has a better affinity for PBP1a and PBP1b in *P. aeruginosa,* but lower affinity for PBP2 and PBP3.[6] Doripenem is a new parenteral carbapenem with greater affinity for PBP2 and PBP3 of *P. aeruginosa*.[7] A serious challenge with the wide spread use of carbapenems against *P. aeruginosa* is the emergence of resistance during treatment. However, the selection of resistant mutants in *P. aeruginosa* is less likely with the use of doripenem as compared to other carbapenems.[8] Doripenem has an advantage over imipenem as it does not need the augmentation with cilastatin for protection from the (Dehydropeptidase) DHP-I enzyme because of the presence and stability of a 1-β-methyl side chain. This is in contrast to imipenem which is massively broken down by DHP-I.[9]

To the best of authors' knowledge, there is no present data available in Pakistan regarding the susceptibility and comparison of doripenem against MDR *P. aeruginosa*. The rationale of this study was primarily

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to diversify the treatment options against MDR *P. aeruginosa* infections, quite rampant in our set up. The objective of the study was to compare *in vitro* efficacy of doripenem and imipenem against MDR *P. aeruginosa* from various clinical specimens.

METHODOLOGY

The descriptive cross-sectional study was conducted at Department of Microbiology, Armed Forces Institute of Pathology (AFIP), Rawalpindi. Non-probability consecutive sampling was done from November 2012 to November 2013. All ethical considerations and obligations were duly addressed and the study was conducted after approval from ethical committee.

All MDR *P. aeruginosa* isolates from different clinical samples of urine, pus, pus swabs, ear swabs, tissue, throat, sputum, blood, bronchoalveolar lavage fluids and high vaginal swabs received at AFIP were considered for study. The identification of *P. aeruginosa* was done on the basis of colony morphology, pigment production, gram stain, motility and biochemical reactions.[10] All non-MDR samples were excluded from the study. In cases where there were two or more specimens yielding the same organism in same patient, the first isolate from each patient episode was also excluded.

European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria for defining MDR *P. aeruginosa* includes susceptibility to one or more than one agent in three or more than three antimicrobial categories which includes aminoglycosides, anti-pseudomonal penicillins with or without beta-lactamase inhibitors, anti-pseudomonal cephalosporins, anti-pseudomonal carbapenems, anti-pseudomonal floro-quinolones and polymyxins.[4] All MDR *P. aeruginosa* isolates were determined by Kirby Bauer disc diffusion method and were preserved in nutrient glycerol broth at -80°C as per standard protocol prior to E-test strip testing for doripenem and imipenem. American Type Control Cultures of *P. aeruginosa* ATCC 27853 was run with each batch of the test.[11]

E-test strip method was performed by first thawing and subculturing the stored micro-organisms on a non-inhibitory medium like blood agar (Oxoid, UK). One to two MDR *P. aeruginosa* colonies were emulsified into 5 ml of sterile normal saline to achieve a turbidity equivalent to 0.5 McFarland standard. A sterile swab was dipped into the inoculum suspension and the entire Mueller Hinton Agar (MHA) (Oxoid, UK) surface was swabbed 3 times to ensure an even distribution of inoculum. E-strips containing doripenem and imipenem (Oxoid, UK) were applied separately on the bacterial suspension of MHA and incubated at 35°C ±2 for 16 - 20 hours. The minimum inhibitory concentration (MIC) values were read where the respective inhibition ellipses intersected the strip (Figure 1).[12]

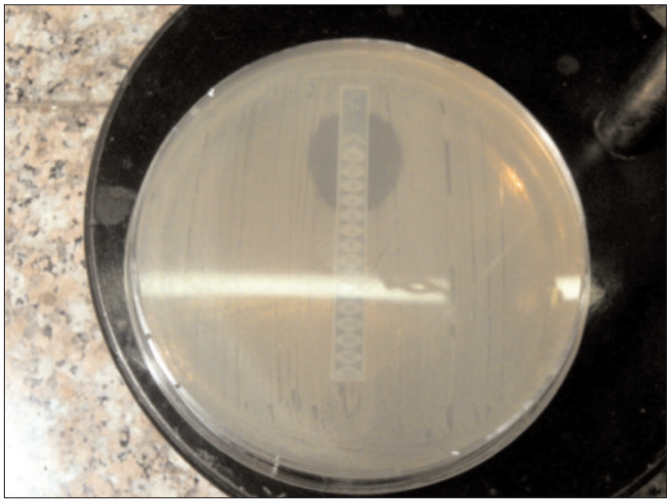

Figure 1: Inhibition ellipses intersecting the doripenem E-test strip.

The agar dilution method was performed by sterilizing the molten MHA by autoclaving it at 121°C for 15 minutes and allowing it to warm in a pre-heated water bath to 50°C.[10] For imipenem, sterile phosphate buffer of 0.01mole/litre with a pH of 7.2 was used as a solvent and also as a diluent. For doripenem, sterile 0.85% physiological saline was used as a solvent and also as a diluent. Stock solution was prepared by weighing 64 mg of base powders each for doripenem and imipenem (Sigma Aldrich, Germany) and dissolved in 100 ml of respective solvent which prepared a stock solution of 0.64 mg/ml or 640 μg/ml. Dilutions, each for imipenem and doripenem, were done separately in dilution bottles. One ml of each for doripenem and imipenem dilutions were added in 19 ml of molten MHA to prepare final concentrations of 32 μg/ml, 16 μg/ml, 8 μg/ml, 4 μg/ml, 2 μg/ml, 1 μg/ml, and 0.5 μg/ml. Mixing of the tube contents were done and then poured into 100 mm petri-dishes which were already labelled and allowed to set at room temperature. The control plates without antimicrobial drugs were also prepared. 0.5 McFarland suspension of different samples of this bacterium were added into the well of multipoint inoculator (Denley Instruments Limited) which transfers 1 to 2 μL of suspension to agar surface to give a final inoculum of 10^4 Colony Forming Unit per spot. The inoculation was done from lowest to highest antimicrobial concentration containing plates. Two control plates were also applied preinoculation and postinoculation of the test isolates. All plates were incubated at 35°C ±2 for 16 - 20 hours. The lowest concentration of antimicrobial drug that completely inhibited the visible growth of the organism judged by naked eye was taken as the required MIC.[11,13] Zones of inhibition for E-test strip and agar dilution methods were interpreted as per CLSI guidelines.[11]

The analysis was performed by using the Statistical Package for Social Sciences (SPSS) version 21. Pearson's chi-square test was applied to compare the

susceptibility pattern of imipenem and doripenem by the E-test strip and agar dilution methods. A p-value < 0.05 was considered as statistically significant difference of proportions between these two antimicrobials. Frequencies and percentages were calculated for susceptibility of antimicrobials and isolation of MDR *P. aeruginosa* from various clinical specimens.

RESULTS

Out of the total 100 isolates, maximum number of *P. aeruginosa* isolates were recovered from pure pus and pus swabs followed by urine and tissue and least from bronchoalveolar lavage fluids, sputum and high vaginal swabs as shown in Figure 2.

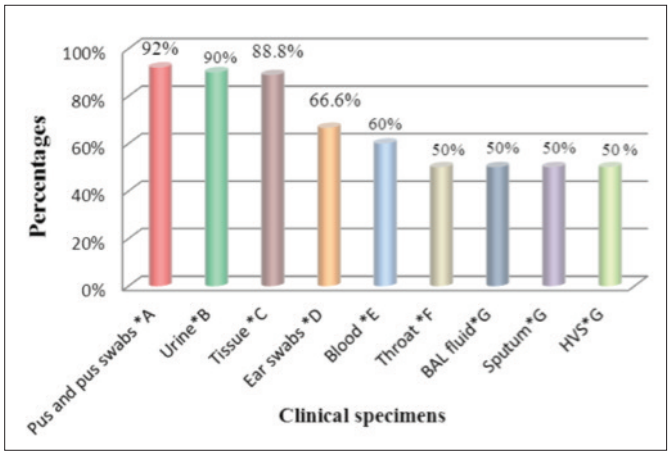

Figure 2: Distribution of MDR *P. aeruginosa* from various clinical specimens (n=100).
Key: Total number of isolates n=100
*A: %age of MDR *P. aeruginosa* calculated from total No. of pus and pus swabs (n= 50)
*B: %age of MDR *P. aeruginosa* calculated from total No. of urine isolates (n= 20)
*C: %age of MDR *P. aeruginosa* calculated from total No. of tissue isolates (n=09)
*D: %age of MDR *P. aeruginosa* calculated from total No. of ear swabs (n=6)
*E: %age of MDR *P. aeruginosa* calculated from total No. of blood isolates (n=5)
*F: %age of MDR *P. aeruginosa* calculated from total No. of throat isolates (n=4)
*G: %age of MDR *P. aeruginosa* calculated from total No. of bronchoalveolar lavage fluid (n=2); Sputum (n=2); High vaginal swab (n=2).

Table I: Susceptibility results of imipenem and doripenem by E-test and agar dilution methods.

Antimicrobials	Susceptible Number Percentage	Intermediate Number Percentage	Resistance Number Percentage	p-value
E-test method:				
Imipenem	20 (23.8%)	14 (16.7%)	50 (59.5%)	0.014
Doripenem	37 (44%)	14 (16.7%)	33 (39.3%)	
Agar dilution method:				
Imipenem	24 (28.6%)	17 (20.2%)	43 (51.2%)	0.037
Doripenem	40 (47.6%)	11 (13.1%)	33 (39.3%)	

Percentage calculated from total No. of MDR P. aeruginosa isolates (n=84)

Table II: MIC ranges of doripenem and imipenem against MDR *P. aeruginosa*.

Antimicrobials	MIC Range (µg/ml)
E-test method:	
Imipenem	0.19-32
Doripenem	0.032-16
Agar dilution method:	
Imipenem	1-32
Doripenem	0.5-16

**A: Percentage calculated from total No. of MDR P. aeruginosa isolates (n=84)*

By E-test strip method, it was found that 37 (44%) of MDR *P. aeruginosa* isolates were susceptible to doripenem compared to 20 (23.8%) with imipenem. Similarly, with agar dilution method, 40 (47.6%) of isolates were susceptible to doripenem and only 24 (28.6%) to imipenem. Overall, p-values of 0.014 and 0.037 were observed when susceptibility patterns of doripenem and imipenem were evaluated with E-test strip and agar dilution methods, respectively. A significant statistical difference between 2 antimicrobials was noted when both imipenem and doripenem were tested with E-test strip and agar dilution methods (Table I).

MIC ranges of doripenem were lower as compared to imipenem by both E-test strip and agar dilution methods are shown in Table II.

DISCUSSION

Multi-drug resistance in *P. aeruginosa* is on the rise worldwide and restricts the use of optimal antimicrobials. The agents of last resort for MDR pathogens include the aminoglycosides and polymyxins. These agents may or may not be as effective as the first-line agents due to the adverse effects, i.e. nephrotoxicity, ototoxicity and neurotoxicity.[14]

In this study, MDR *P. aeruginosa* isolates were mainly recovered from pus, pus swabs followed by urine. A number of earlier studies showed that maximum number of *Pseudomonas* isolates were recovered from pus.[15,16] On the contrary, a study conducted in Karachi showed that the highest number of such recovered isolates were from urine and ear swabs.[17]

In vitro efficacy of doripenem was found to be better (44%) as compared with imipenem (24%) when susceptibilities were performed by E-test strip method. These results were similar to a study conducted in Riyadh in 2012 whereby doripenem was found out to be the most potent antimicrobial among the 3 carbapenems tested against MDR *P. aeruginosa* by E-test strip method. In that study, 60.6% of *P. aeruginosa* isolates were susceptible to doripenem and only 9.1% to imipenem.[18]

A study conducted in Poland against *P. aeruginosa* by Drzewiecki *et al*. revealed that 71.43% of *P. aeruginosa* isolates were susceptible to doripenem and 52.38% to imipenem by E-test strip method.[19] Similarly, a study conducted by comparative activity of Carbapenem Testing (COMPACT) study centres in Turkey studied susceptibility pattern of doripenem and imipenem against *P. aeruginosa*. It was found that the *in vitro* efficacy of doripenem was better than imipenem when tested by E-strip method.[20] Similar concordant results of better susceptibility of doripenem as compared to

imipenem with the present results were found in different studies from Korea and Spain.[21,22] In this study, *P. aeruginosa* isolates were found to be much more resistant as compared to the studies carried out in European and Middle-East countries.

A study conducted in California, USA revealed that doripenem had better *in vitro* efficacy than imipenem when agar dilution method was carried out. This finding is similar to results obtained in this study. In this study, MIC range of doripenem (0.5 - 16 μg/ml) was lower than imipenem (1 - 32 μg/ml) with agar dilution method. These results were similar to a study conducted in USA by agar dilution method which revealed low MIC range of doripenem (≤ 0.015 - 0.06 μg/ml) as compared to imipenem (0.125 - 1 μg/ml).[23] Similarly, when the *in vitro* efficacy of the 2 carbapenems were performed by E-test strip method in this study, it was found that MIC ranges of doripenem (0.032 - 16 μg/ml) were lower than imipenem (0.19 - 32 μg/ml). This finding of low MIC for doripenem was comparable to a study conducted in Turkey, where MIC ranges of doripenem and imipenem were 0.03 - ≥ 64 μg/ml and 0.12 - ≥ 64 μg/ml, respectively.[20] The possible reason of low MICs of doripenem may be due to the fact that it is highly stable to the Amp C enzyme of *P. aeruginosa*. It interacts in an altered way with this organism regarding OprD down regulation which results in lower MIC shifts, in at least 50% of cases.[24]

Doripenem has a broad spectrum of activity against variety of bacteria including MDR Gram-negative bacteria. It is indicated as a single antimicrobial for the treatment of problematic intra-abdominal infections and urinary tract infections including pyelonephritis caused by *P. aeruginosa*. The most important difference between doripenem and the other carbapenems is the superior activity noted in several *in vitro* studies for doripenem against *P. aeruginosa*. It is also observed that doripenem has the greatest potency against *P. aeruginosa* as compared to other carbapenems.[25] Clinical trials are required to support these *in vitro* results.

Doripenem may prove as an excellent choice in treatment of MDR *P. aeruginosa* infections. This is due to the fact that doripenem has low MICs and its favourable pharmacologic properties allow for optimal administration as the only carbapenem in continuous infusion.[26]

Doripenem is not available in Pakistan and is more expensive as compared to imipenem. Whereas, imipenem is available and is being widely used in tertiary care hospital settings. The therapeutic use of imipenem and doripenem for treatment of *P. aeruginosa* should be reserved only for severe infections. This is particularly true where the infection is polymicrobial, anaerobic or *Pseudomonas* is resistant to other antimicrobials. There is a need to foresee the capability of emergence of resistance while selecting for these carbapenems. In suitable conditions, routine culture and susceptibility testing should be carried out for early detection of development of resistance in *P. aeruginosa*.

CONCLUSION

Doripenem was found to have better *in vitro* activity as compared to imipenem against MDR *P. aeruginosa* producing isolates. The older carbapenems, such as imipenem and meropenem, are almost becoming ineffective because of emerging resistance. Doripenem seems to be a promising antimicrobial agent in treating patients with serious infections who require broad-spectrum antimicrobial coverage.

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