ORIGINAL ARTICLE

In Vitro Efficacy of Doripenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* by E-Test

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ABSTRACT

Objective: To assess the *in vitro* efficacy of doripenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* using Epsilometer strips.

Study Design: Cross-sectional study.

Place and Duration of Study: Department of Microbiology, Army Medical College, Rawalpindi and National University of Sciences and Technology, Islamabad, from May 2014 to September 2014.

Methodology: A total of 60 isolates of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* collected from various clinical samples received from Military Hospital were included in the study. The specimens were inoculated onto blood, MacConkey and chocolate agars. The isolates were identified using Gram staining, motility, catalase test, oxidase test and API 20NE (Biomeriux, France). Organisms identified as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were included in the study. Bacterial suspensions equivalent to 0.5 McFarland turbidity standard of the isolates were prepared and applied on Mueller Hinton agar. Epsilometer strip was placed in the center of the plate and incubated for 18-24 hours. Minimum Inhibitory Concentration (MIC) was taken to be the point where the epsilon intersected the E-strip. MIC of all the isolates was noted.

Results: For *Pseudomonas aeruginosa* isolates, MIC_{50} was 12 µg/mL and MIC_{90} was 32 µg/mL. For *Acinetobacter baumannii* MIC_{50} and MIC_{90} was 32 µg/mL.

Conclusion: Doripenem is no more effective against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* in our setting.

Key Words: *Acinetobacter baumannii. Pseudomonas aeruginosa. Doripenem. Disk diffusion antimicrobial tests. Drug resistance. Minimum inhibitory concentration.*

INTRODUCTION

Despite efforts of 'antibiotic stewardship' globally, resistance to routinely used antimicrobials is continuously on the rise.[1] An ever growing challenge for physicians is the treatment of infections by Multidrug-Resistant (MDR) Gram negative bacilli. The most notable resistance problems are seen in *Pseudomonas aeruginosa*, Enterobacteriaceae, and *Acinetobacter* spp., with escalating resistance noted to all the major antibiotics against Gram-negative pathogens such as aminoglycosides, beta-lactams and fluoroquinolones.

Pseudomonas aeruginosa and *Acinetobacter baumannii* are opportunistic nosocomial pathogens, notorious for being multidrug and pandrug resistant. *Pseudomonas* readily acquires resistance to antibiotics and has the ability to survive in difficult conditions. *Acinetobacter* also has the ability of long-term survival in hospital environment and is becoming one of the most frequently isolated pathogens from intensive care units. Carbapenems, one of the β-lactam antibiotics, have the broadest spectrum of activity and greatest potency against both Gram positive and Gram negative bacteria. They are usually reserved as a last resort therapy but now they are being used more frequently because organisms having resistance to multiple antibiotics are being isolated increasingly.[2]

Doripenem, a carbapenem antibiotic, binds with penicillin-binding proteins (PBPs) to form acyl-enzymes. This inactivates the PBPs, preventing transpeptidation, leading to weakening of the cell wall, which eventually ruptures because of high osmotic pressure. It has been found to be equally, if not more, effective than other carbapenems (meropenem, imipenem) against various nosocomial pathogens.[3] When compared with other carbapenem antibiotics, doripenem has greater intrinsic activity against extended spectrum beta-lactamase (ESBL) producing enterobacteriaceae, Amp C producing *P. aeruginosa, Acinetobacter baumannii*, non-fermentive and anaerobic organisms.[4] In a bacterial population, doripenem has a weaker propensity to select for carbapenem resistant mutants.[5] In *Pseudomonas aeruginosa*, doripenem has high affinity for both PBP2 and PBP3.[6] Doripenem appeared to be more efficient

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with higher antimutant potential than imipenem against *P. aeruginosa*.[7]

The frequent isolation of *Pseudomonas aeruginosa* and *Acinetobacter baumannii* from various clinical samples in our department, of which many were multidrug resistant, prompted us to test new antimicrobials against them. More treatment options are needed when isolates with resistance to routinely used antibiotics are isolated. So we tested the *in vitro* efficacy of doripenem against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* by determining its minimum inhibitory concentration (MIC) using an Epsilometer strip.

METHODOLOGY

This cross-sectional study was conducted at the Department of Microbiology, Army Medical College, Rawalpindi and National University of Sciences and Technology, Islamabad, from May 2014 to September 2014.

A total of 60 isolates (30 *Acinetobacter baumannii* and 30 *Pseudomonas aeruginosa*) from various clinical samples were included in the study. The specimens were inoculated onto blood, MacConkey and chocolate agars. (Biomeriux, France). *Acinetobacter* isolates were identified as Gram negative coccobacilli on Gram staining, oxidase negative, catalase positive, non-motile organisms having non-lactose fermenting colonies. *Pseudomonas* isolates were identified as oxidase positive, catalase positive, motile, Gram negative rods, having non- lactose fermenting colonies on MacConkey agar. Analytical profile index (API 20NE) (Biomeriux, France) was used to confirm the identification of isolates. Organisms identified as *Acinetobacter baumannii* and *Pseudomonas aeruginosa* were included in study.

Bacterial suspensions of the isolates equivalent to 0.5 McFarland turbidity standard were prepared and applied on Mueller Hinton agars. Doripenem Epsilometer strips (AB Biodisk, Solna, Sweden) were placed in the centre of the plates and were lightly pressed with sterile forceps to remove any air bubbles. The plates were then incubated at 37°C for 18 - 24 hours. MIC was taken to be the point where the epsilon intersected the E-strip as per manufacturer's instructions. Susceptibility to routinely used antibiotics against both organisms was determined by modified Kirby-Bauer disc diffusion method according to CLSI guidelines.[8]

For both *Pseudomonas aeruginosa* and *Acinetobacter* spp., the isolates having MIC ≤ 2 μg/ml were taken to be sensitive, MIC ≥ 8 μg/ml were resistant and MIC of 4 μg/ml to be intermediate according to CLSI guidelines.[8] The concentration of each antimicrobial agent, that inhibited 50% (MIC_{50}) and 90% (MIC_{90}) of the strains, was calculated.[9] Qualitative variables like susceptibility and resistance of *Acinetobacter baumannii* and *Pseudomonas aeruginosa* was measured by frequency and percentages. The data was entered and analyzed using Statistical Package for Social Sciences (SPSS) version 17.0.

RESULTS

From a total of 30 isolates of *Pseudomonas aeruginosa*, the highest percentage of isolates were from blood , i.e. 40% (n=12); followed by pus swabs 33.3% (n=10); endotracheal tube tips 10% (n=3); sputum, 6% (n=2) and 3.33% (n=1) each of urine, cerebrospinal and pleural fluid. The antibiogram of routinely used antibiotics against *Pseudomonas* is given in Table I.

From a total of 30 isolates of *Pseudomonas aeruginosa*, 10 (33.33%) were found to be sensitive to doripenem, 1 (0.03%) isolate was intermediately sensitive while the remaining 19 (63.33%) were found to be resistant. The MIC by E-test had a range of 0.125 - 32 μg/ml, as shown

Table I: Antibiogram of routinely used antibiotics against *Pseudomonas aeruginosa*.

Antibiotics	Percentage susceptible (%)
Colistin	79
Cefoperazone / Sulbactam	75
Cefoperazone	73
Piperacillin / Tazobactam	71
Meropenem	66.7
Ceftazidime	65
Amikacin	61.5
Aztreonam	52
Gentamicin	50
Ciprofloxacin	46.4

Table III: Antibiogram of routinely used antibiotics against *Acinetobacter baumannii*.

Antibiotics	Percentage susceptible (%)
Tigecycline	100
Cefoperazone / Sulbactam	82.4
Trimethoprim / Sulfamethoxazole	47
Minocycline	41.2
Meropenem	16.7
Amikacin	16.7
Piperacillin / Tazobactam	16.7
Gentamicin	16.7
Ciprofloxacin	16.7
Amoxicillin / Clavulanic acid	16.7
Ceftriaxone	6.7
Ampicillin	0.0

Table II: Percentages (%) of MDR *Pseudomonas aeruginosa* isolates with different Minimum Inhibitory Concentration (MIC) values alongwith MIC_{50} and MIC_{90}.

MIC (μg/ml)	0.125	0.19	0.25	0.38	1.0	1.5	3	8	12	24	32	MIC range	MIC_{50}	MIC_{90}
Percentage of isolates(%)	3.33	3.33	3.33	13.3	6.7	3.33	3.33	6.7	13.3	3.33	40	0.125-32	12	32

Table IV: Percentages (%) of MDR *Acinetobacter baumannii* isolates with different Minimum Inhibitory Concentration (MIC) values alongwith MIC_{50} and MIC_{90}.

MIC (μg/ml)	0.016	0.064	0.094	0.5	1.0	8	32	MIC range	MIC_{50}	MIC_{90}
Percentage of isolates(%)	3.33	3.33	6.7	3.33	6.7	3.33	73	0.016-32	32	32

in Table II. The MIC_{50} for *Pseudomonas aeruginosa* was 12 μg/mL and MIC_{90} was 32 μg/mL.

For *Acinetobacter baumannii*, 15 (50%) isolates were from blood followed by nasobronchial lavage 7 (23.33%); endotracheal tube tips 4 (13.3%), pus swabs 2 (7%) and 1 (3.3%) each of urine and bronchoalveolar lavage. Most of the isolates, 16 (53.33%), were received from intensive care wards (neonatal as well as medical intensive care units), while the remaining 14 (46.67%) were from other wards like female medical, male medical and pediatric wards. The antibiogram of routinely used antibiotics against *Acinetobacter baumannii* is given in Table III.

Only 7 (23.33%) isolates of *Acinetobacter baumannii* were sensitive while the remaining 23 (76.7%) showed resistance to doripenem. MIC had a range of 0.01 - 32 μg/ml as shown in Table IV. For *Acinetobacter baumannii* MIC_{50} and MIC_{90} were 32 μg/mL.

DISCUSSION

Pseudomonas aeruginosa and *Acinetobacter baumannii* are increasingly acquiring resistance to routinely used antibiotics. We need newer and reliable treatment options against them. Many studies conducted world-wide have shown doripenem to be an effective option.

In this study, 66.67% of *Pseudomonas aeruginosa* and 76.67% of *Acinetobacter baumannii* were resistant to doripenem. A study conducted by Mustafa *et al*. showed that 29% of *P. aeruginosa* isolates and 83% of *A. baumannii* were not susceptible to doripenem. The MIC_{90} was 32 μg/ml.[2] Another Comparative Activity of Carbapenem Testing (COMPACT) II surveillance study found that carbapenem resistance among *P. aeruginosa* and *A. baumannii* isolates was 30% and 73% respectively. The MIC_{50} and MIC_{90} for *P. aeruginosa* were 0.38 μg/ml and 8 μg/ml and for *A. baumannii* were 32 μg/ml and 64 μg/ml respectively.[10] Doripenem was not effective against 77.4% of *A. baumannii* and 39.4% of *Pseudomonas aeruginosa* in a study conducted in Riyadh, KSA.[11] A study conducted in Iran on *P. aeruginosa* from patients of cystic fibrosis and burns found the susceptibility rates to be 89.3% and 10.6% respectively. The MIC_{50} and MIC_{90} of isolates from cystic fibrosis patients was > 32 μg/ml and MIC_{50} and MIC_{90} of isolates from burn patients was found to be 0.75 μg/ml and > 32 μg/ml.[9] In Taiwan, a study showed 87% of *Pseudomonas aeruginosa* and 56% of *Acinetobacter baumannii* to be susceptible to doripenem. The MIC_{50}/MIC_{90} for *P. aeruginosa* and *A. baumannii* were 0.25/6 and 0.38/32 μg/ml respectively.[12]

Previously, studies advocated the use of doripenem because they found it to be slightly more efficacious against *Pseudomonas aeruginosa* when compared with other carbapenems[13-16] and also to be stable against renal dehydropeptidase-I (DHP-I) and thus not needing the administration of cilastatin with it.[17] Another study found the efficacy of doripenem to be equal to mero-penem but greater than imipenem and ertapenem.[18] In Taiwan, a study by Shao-Xing *et al*. found meropenem, doripenem and imipenem to be equally efficacious.[12] However, this study showed that among *Pseudomonas aeruginosa* resistant to doripenem, 14 (73.7%) of the isolates were multi drug resistant i.e. organisms showing resistance to at least 1 agent from 3 antimicrobial groups. All the isolates resistant to meropenem were also resistant to doripenem. However, 6 isolates (31.5%) resistant to doripenem and 1 isolate intermediately sensitive to doripenem were sensitive to meropenem.

According to Marti *et al*., *Acinetobacter baumannii* was found to be more sensitive to doripenem as compared to meropenem and imipenem.[19] Among the 23 doripenem resistant *Acinetobacter baumannii* isolates in this study, 22 (95.6%) were resistant to meropenem while 1 isolate (0.04%) was sensitive to meropenem. All the *Acinetobacter baumannii* resistant to meropenem were also resistant to doripenem, thus showing the superior efficacy of meropenem when compared with doripenem.

Our susceptibility results of doripenem against *Acinetobacter* are similar to other studies. Most of the studies conducted have found a high percentage of *Acinetobacter* isolates to be resistant to doripenem with very high MIC_{50} and MIC_{90}. Most of the studies found *Pseudomonas aeruginosa* to be susceptible to doripenem with low MIC_{50} and MIC_{90}. Thus, the results for *Pseudomonas aeruginosa* are very different from others except for the study conducted on burn patients in Iran.[9]

CONCLUSION

Doripenem, which was once considered to be a new option for treatment of infections due to multi-drug resistant organisms, is not efficacious in our setting anymore. The rate of *in vitro* doripenem resistance in *Pseudomonas aeruginosa* and *Acinetobacter baumannii* was high in this study.

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