

Comparison of *in Vitro* Activity of Doripenem versus Old Carbapenems against *Pseudomonas Aeruginosa* Clinical Isolates from both CF and Burn Patients

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ABSTRACT

Purpose: The antimicrobial activity of doripenem in comparison of imipenem, meropenem and ertapenem among *Pseudomonas aeruginosa* isolated from burn and Cystic Fibrosis (CF) patients were determined. **Methods:** Metallo- β -lactamase (MBL) genes in imipenem non susceptible *P. aeruginosa* isolates were detected using PCR method. The *in vitro* susceptibilities of doripenem, imipenem, meropenem and ertapenem were determined by Etests. MIC₅₀ and MIC₉₀ for corresponding antibiotics were determined individually in burn and CF isolates. **Results:** Among isolates which were resistant to imipenem, 16 isolates were positive for the *bla*_{IMP} gene. All isolates had no *bla*_{VIM} gene. All MBL producing isolates were excluded. MIC₅₀/MIC₉₀ of doripenem in CF and burn isolates were 0.75/>32 and >32/>32 mg/L respectively. The corresponding values for imipenem in CF and burn isolates were 2/>32 and >32/>32 mg/L, respectively. **Conclusion:** The susceptibility rate of doripenem is higher than that of imipenem and meropenem among *P.aeruginosa* isolated from CF patients, whereas, there is no difference between the efficiency of doripenem and old carbapenems in non MBL producing *P.aeruginosa* isolates in burn patients.

Introduction

The synthesis of new carbapenem remain an area of intense research because of the broad-spectrum antibacterial activity of this chemical class.¹⁻³ Doripenem is a recently released antibiotic with significant potential for use in *Pseudomonas aeruginosa* infections occur in CF and burn patients.⁴ The *In vitro* antimicrobial activity of Doripenem, is generally comparable to that of meropenem and imipenem although it is more active against Gram-negative organisms than imipenem.⁵ The activity of doripenem against *P. aeruginosa* isolates is slightly better than that of other carbapenems. However, development of carbapenem resistance may significantly compromise their efficacy.⁶ Resistance to carbapenems including doripenem resulted from the complex interaction of several mechanisms including loss of the OprD porin, overexpression of efflux systems (MexAB-OprM, MexEF-OprN) and

production of carbapenemase activity, usually a metallo- β -lactamase (MBL).⁷⁻¹⁰ It should be noted that doripenem is no less susceptible to hydrolysis by MBL than are the other carbapenems and none of them is active against *P. aeruginosa* isolates harboring various MBL genes.¹¹ Since, there is no CLSI guideline for doripenem MIC breakpoint until now, so the results of MIC susceptibility pattern obtained from different geographical regions from different clinical isolates could be helpful in this regard.

Since, *P. aeruginosa* is one of the most frequently isolated pathogens from both CF and burn patients, we designed the study to determine susceptibility patterns of all the isolates and to compare the *in vitro* antibacterial activity of doripenem with that of imipenem, and meropenem among non MBL *P. aeruginosa* isolates from both CF and burn patients.

Materials and Methods

Bacterial Strains

From June to December 2011, a total number of 92 non repetitive *P. aeruginosa* isolates was enrolled in this study. Sixty three burns isolates were recovered from hospitalized patients in a level one burn care center and 29 isolates were collected from CF patients admitted to a children's medical center. This collection of bacteria was identified by conventional biochemical tests.

Antimicrobial Susceptibility Testing

The Kirby-Bauer disk diffusion method was employed to evaluate susceptibility of the following antimicrobial agents: piperacillin/tazobactam, aztreonam, ticarcillin, trimetoprim and tobramycin (MAST, UK). MIC values of the imipenem, meropenem, ertapenem (AB BIODISK, Solna, Sweden), doripenem, ceftazidime, cefepime, ciprofloxacin, amikacin, gentamicin (Liofilchem, Italy) were determined by Etests. Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria, where applicable¹². FDA interpretive criteria were applied to doripenem results (susceptible ≤ 2 mg/l for *P. aeruginosa*).¹³ The results were examined to ensure that reported MICs were within acceptable standards set by CLSI based on a comparator agent and the following ATCC quality control strain, ATCC 25922 (*E. coli*).

Ethical Standards

Ethical approval to perform the study was obtained from the institutional review board of Tabriz University of Medical Sciences. Written informed consent was obtained from all patients included in the study.

MIC₅₀ and MIC₉₀ Calculation

The concentration of each antimicrobial agent, that inhibited 50% (MIC₅₀) and 90% (MIC₉₀) of the strains, was calculated for each of the antibiotics singly. The formula of geometric means was used as follows:¹⁴

$$\text{MIC}_{50} = (M < 50) + \frac{(n - X) \times [(M > 50) - (M < 50)]}{Y}$$

Where $M < 50$ is the MIC of the highest cumulative percentage below 50%, $M > 50$ is the MIC of the lowest cumulative percentage above 50%; n is 50% of the number of organisms tested, X is the number of organisms in the group at $M < 50$, and Y is the number of organism in the group at $M > 50$.

Screening for Metallo B-Lactamase (Mbl) Production

In order to identify MBL producing isolates, we detected non susceptible isolates against imipenem by Kirby-Bauer disk diffusion method using imipenem disk 10 mg/L. Imipenem non susceptible isolates were selected for detection of IMP and VIM metalloenzymes. Total genomic DNA of the isolates which were resistant to imipenem, was extracted as described previously.¹⁵ Genes encoding class B carbapenemases were detected by PCR using specific primers for *bla*_{IMP} and *bla*_{VIM} metalloenzyme genes.

The sequences of primers were as follows: IMP-F1, (CATGGTTTGGTGGTTCTTGT), IMP-R1, (GTAAGTTTCAAGAGTGATGC), VIM-F1, (GTTTGGTCCGATATCGCAAC) and VIM-R1 (CTACTCGGCGACTGAGCGAT). The generated PCR products were 524 and 623 base pairs, respectively.

Results

Screening MBL Production

PCR Screening of isolates which were non susceptible to imipenem indicated the presence of 17 *P. aeruginosa* isolates harboring *bla*_{IMP} gene. Only one isolate among CF and 16 isolates among burn patients were detected as MBL positive isolates. There was no *bla*_{VIM} carrying isolate detected in our study. All MBL producing isolates were excluded to remove the effect of one of the most interfering factors involved in carbapenem resistance. To the best of our knowledge, our study was the first report of Iran that evaluated the *in vitro* activity of doripenem in comparison with that of previously FDA approved carbapenems.

Antimicrobial Susceptibility Testing

As shown in Table 1, the Kirby-Bauer disk diffusion method was performed in 75 non MBL *P. aeruginosa* which comprised of 47 burn and 28 CF isolates. Table 2, summarizes the MIC's of some antimicrobial agents other than those mentioned in Table 1. The tables showed that among the tested comparators, piperacillin/tazobactam (14.9% and 93.1% susceptible, respectively) provided the greatest activity in both burn and CF isolates, followed by tobramycin (12.8%) in burn isolates. The susceptibility rates of amikacin, imipenem, meropenem and doripenem were the same among burn isolates (10.6%). However, the susceptibility rate of doripenem among CF isolates was similar to that of amikacin (89.3%) and higher than that of old carbapenems (imipenem & meropenem). The greatest differences in the susceptibility rate between burn and CF strains were observed with doripenem (10.6% versus 89.3%), amikacin (10.6% versus 89.3%) and piperacillin/tazobactam (14.9 % versus 93.1%). According to the Table 2, among CF isolates, at any given MIC concentration from ≤ 0.5 to 1.5 mg/L, doripenem (MIC₅₀, 0.75 mg/L) inhibited a slightly greater proportion of isolates than meropenem (MIC₅₀, 0.75 mg/L) and notably greater than imipenem (MIC₅₀, 2 mg/L). However, higher MIC levels of doripenem at 2 and 4 mg/L, provided the same coverage as meropenem, inhibiting 85.7% and 89.3% of isolates, respectively. Table 2 showed that ertapenem was the least efficacious carbapenem (susceptibility rate, 66.7%) that could inhibit only 25% of CF isolates at the MIC level of 4 mg/L.

On the other hand, among burn isolates, all carbapenems except ertapenem had the same activity (MIC₅₀ and MIC₉₀, >32 mg/L). The proportion of isolates inhibited at MIC level ≥ 1 mg/L of doripenem

and meropenem was similar (10.6%), however, the inhibition rates for doripenem at MIC levels of ≤ 0.5 and 0.75 mg/L were slightly higher than that of meropenem. At any given concentration from ≤ 0.5 to > 32 mg/L, doripenem inhibited a remarkably greater

proportion of isolates than imipenem (Table 2). Similar to CF isolates, ertapenem identified as the least potent agent in burn isolates which inhibited 6.4% of burn isolates in comparison with 10.6% inhibition by other carbapenems.

Table 1. Results of disk diffusion method on non-MBL *P. aeruginosa* isolates.

Antibiotic	PTZ		ATM		TN		TM		TC	
	S n(%)	NS n(%)	S n(%)	NS n(%)	S n(%)	NS n(%)	S n(%)	NS n(%)	S n(%)	NS n(%)
Burn(47)	7(14.9)	40(85.1)	4(8.5)	43(91.5)	6(12.8)	41(87.2)	0	47(100)	4(8.5)	43(91.5)
CF(28)	26(93.1)	2(6.9)	9(31)	19(69)	22(79.3)	6(20.7)	2(6.9)	26(93.1)	23(82.8)	5(17.2)

PTZ: piperacillin/tazobactam, ATM: aztronam, TN: tobramycin, TM: trimetoprim, TC: ticarcillin, S: susceptible, NS: non susceptible.

According to the susceptibility rates, the MIC levels of imipenem, meropenem and doripenem were completely in line with each other except for 3 isolates; one burn

and 2 CF isolates which showed the MIC level of imipenem of >32 mg/L but doripenem and meropenem MIC levels of ≤ 1 mg/L.

Table 2. *In vitro* activities of doripenem and comparators against non MBL *P. aeruginosa* isolates in burn and CF patients.

		MIC ₅₀	MIC ₉₀	Range (mg/L)	Susceptibility (%)	Cumulative % inhibited at MIC (mg/L)						
						≤ 0.5	0.75	1	1.5	2	4	>32
Amikacin	CF	5.5	>256	1->256	89.3	-	-	-	-	-	-	-
	Burn	256	256	3->256	10.6	-	-	-	-	-	-	-
Gentamicin	CF	6.83	>256		60.7	-	-	-	-	-	-	-
	Burn	128	>128	3->256	8.5	-	-	-	-	-	-	-
Cefepime	CF	12.25	>256	2->256	57.1	-	-	-	-	-	-	-
	Burn	256	256	0.75->256	6.4	-	-	-	-	-	-	-
Ceftazidime	CF	2	>256	0.5->256	75.0	-	-	-	-	-	-	-
	Burn	256	256	2->256	8.5	-	-	-	-	-	-	-
Ciprofloxacin	CF	0.19	6.25	0.047->32	78.6	-	-	-	-	-	-	-
	Burn	>32	>32	0.047->32	8.5	-	-	-	-	-	-	-
Imipenem	CF	2	>32	0.75->32	85.7	0	7.1	14.3	39.3	60.7	85.7	100
	Burn	>32	>32	0.75->32	10.6	0	2.2	2.2	6.5	8.7	8.7	100
Meropenem	CF	0.75	>32	0.125->32	85.7	28.6	50.0	78.6	82.1	85.7	89.3	100
	Burn	>32	>32	0.15->32	10.6	8.5	8.5	10.6	10.6	10.6	10.6	100
Doripenem	CF	0.75	>32	0.094->32	89.3	39.3	75.0	82.1	85.7	85.7	89.3	100
	Burn	>32	>32	0.125->32	10.6	10.6	10.6	10.6	10.6	10.6	10.6	100
Ertapenem	CF	32	>32	0.094->32	66.7	3.6	3.6	3.6	7.1	10.7	25.0	100
	Burn	>32	>32	3->32	6.4	0	0	0	0	0	8.5	100

Discussion

Infections caused by *P. aeruginosa* in burn and CF patients often treated with difficulty due to the emergence of resistance and lack of effective antibiotics.¹⁶ Doripenem as a new carbapenem offers potentially enhanced carbapenem activity but does not expand the spectrum of activity of this class.⁴ Like other carbapenems, doripenem has stability against many β -lactamases, but remains labile to class B enzymes, known as metallo- β -lactamases.⁵ Therefore,

in the present work, we attempted to assess the *in vitro* activity of doripenem among non MBL *P. aeruginosa* isolated from CF and burn patients, in comparison with other carbapenems.

Despite the higher carbapenem MIC rates in our CF isolates as compared with similar studies,^{15,16} it can be concluded that doripenem has much greater potency than imipenem. Although the MIC₅₀ of both doripenem and meropenem was similar, the more inhibition rate of 25% of this recently approved carbapenem indicated

that doripenem could be considered as a good alternative therapeutic agent in CF patients. In a recent similar study,¹⁶ antibiotic susceptibility of *P. aeruginosa* isolated from CF patients, doripenem showed as the most active antibiotic in the absence of piperacillin/tazobactam. Totally, it seems that doripenem can be considered as the most potent carbapenems against *P. aeruginosa* infections in CF patients.

Since, MBLs were the most important mechanisms in high level of resistance against all carbapenems, we decided to exclude the MBL positive isolates to explore the probable difference in doripenem MIC's versus old carbapenems. Although the susceptibility rates against doripenem in burn isolates showed no superiority to old carbapenems, the greater population of isolates were inhibited at any concentration of doripenem as compared with imipenem and meropenem. Among burn isolates, all carbapenems have the same activity except for ertapenem which has the least efficiency. We found 3 imipenem resistant isolates which were susceptible to meropenem and doripenem. This phenomenon occurred to those isolates with nonenzymatic resistance involving loss of porin OprD and up-regulation of efflux pumps¹³ which we intend to explore in a further study. Conversely, other researchers declared that this could be the exception other than a rule with no reason.¹⁷

Although ertapenem is not a representative of carbapenems with the consideration of broad spectrum activity which can not be used to treat infections due to non-fermentative Gram-negative bacteria,¹³ we intended to investigate the *in vitro* activity of this antimicrobial agent for the first time in Iran. Our results are consistent with the results of other investigators¹⁸ which showed the lowest susceptibility rate among all used carbapenems in both burn and CF isolates (6.4% and 66.7%). Our results corroborated by the results of the study conducted by Quale *et al.* They found only 18% of *P. aeruginosa* isolates that were susceptible against ertapenem while imipenem and meropenem were more potent, inhibiting 55% and 64% of isolates compared to ertapenem.

Conclusion

Although doripenem is more active than imipenem and meropenem against *P. aeruginosa* isolated from CF patients, no superiority of doripenem is observed to old carbapenems in non MBL producer *P. aeruginosa* isolates in burn patients. In terms of MIC level of doripenem, this antibiotic is the most active but this advantage is partly offset by lower regulatory breakpoints. Ertapenem is the least potent agent against *P. aeruginosa* isolates.

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Conflict of interest

There is no conflict of interest in this study.

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