Antimicrobial activity of doripenem against Gram-negative pathogens: results from INVITA-A-DORI Brazilian Study

ABSTRACT

In vitro activity of doripenem and comparator antimicrobial agents was evaluated against Gram-negative bacilli recently isolated from Brazilian private hospitals that were enrolled in the INVITA-A-DORI Brazilian Study. A total of 805 unique Gram-negative bacilli were collected from patients hospitalized at 18 medical centers between May/08 and March/09. Each hospital was asked to submit 50 single Gram-negative bacilli isolated from blood, lower respiratory tract or intra-abdominal secretions. Bacterial identification was confirmed and antimicrobial susceptibility testing was performed using Clinical Laboratory Standards Institute (CLSI) microdilution method at a central laboratory. CLSI M100-S21 (2011) or US-FDA package insert criteria (tigecycline) was used for interpretation of the antimicrobial susceptibility results. Doripenem was as active as meropenem and more active than imipenem against *E. coli* and *K. pneumoniae* isolates. A total of 50.0% of *Enterobacter* spp. isolates were resistant to ceftazidime but 85.7% of them were inhibited at doripenem MICs ≤ 1 μg/mL. Polymyxin B was the only agent to show potent activity against *Acinetobacter* spp. ($MIC_{50/90}$, ≤ 0.5/1 μg/mL) and *P. aeruginosa* ($MIC_{50/90}$, 1/2 μg/mL). Although high rates of imipenem (53.1%) and meropenem (44.5%) resistance were detected among *P. aeruginosa*, doripenem showed MIC_{50} of 16 μg/mL against imipenem-resistant *P. aeruginosa* and inhibited a greater number of imipenem-resistant *P. aeruginosa* (10.5%) at MIC values of ≤ 4 μg/mL than did meropenem (0.0%). In this study, doripenem showed similar *in vitro* activity to that of meropenem and retained some activity against imipenem-resistant *P. aeruginosa* isolated from Brazilian medical centers.

Keywords: carbapenems; Gram-negative bacteria; Brazil.

INTRODUCTION

Doripenem is a novel parenteral 1-β-methyl-carbapenem with broad-spectrum activity against commonly isolated Gram-negative and Gram-positive pathogens.[1,2] Doripenem, like meropenem and ertapenem, is stable to renal dehydropeptidase-1 hydrolysis because of the presence of a 1-β-methyl constituent on the carbapenem nucleus. It also has a sulfamoylami noethyl-pyrrolidinylthio group in its side chain at position 2 that enhances its activity against non-fermentative Gram-negative bacilli.[1,2] Doripenem acts by inactivating multiple essential penicillin binding proteins (PBPs) for cell wall biosynthesis resulting in subsequent cell death. It has high affinity for PBP1, PBP2, and PBP4 of *Staphylococcus aureus* as well as for PBP2, PBP3 and PBP4 of *Escherichia coli* and *Pseudomonas aeruginosa*. The binding affinity of doripenem for PBP2 of *P. aeruginosa* was stronger than that documented for most cephalosporins.[3,4] In general, doripenem is less potent than imipenem, but more potent than meropenem, against Gram-positive bacteria. Against Gram-negative bacteria, the activity of doripenem is similar to that of meropenem but superior to that of imipenem.[1,5,6] None of the clinically available carbapenems are effective against *E. faecium*, methicilin-resistant *S. aureus* or coagulase-negative staphylococci due to poor binding affinity to PBP5 and PBP2a, respectively.[5,6] Doripenem is stable to hydrolysis by most β-lactamases, including penicillinases and cephalosporinases produced by Gram-positive and Gram-negative bacteria, but not to carbapenem hydrolyzing β-lactamases.[1,4,7] Thus, doripenem like other carbapenems is not active against *Stenotrophomonas maltophilia* as a result of its intrinsic metallo-β-lactamase production.[1,7]

The favorable spectrum, potency and pharmacokinetics for doripenem have led to successful clinical trial results for intra-abdominal

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infections, nosocomial pneumonias, and complicated urinary tract infections.[8] In October 2007, the doripenem clinical use was approved by the United States Food and Drug Administration (US-FDA) for treatment of complicated urinary tract and intra-abdominal infections.[8] In Europe it is also licensed for treatment of nosocomial pneumonia, including ventilator-associated pneumonia.

In general, high resistance rates among key nosocomial pathogens have been reported in Brazil.[9] However, most of these reports originates from surveillance programs that have evaluated isolates collected from a few public/teaching hospitals.[10] Local surveillance studies are of crucial importance to establish the role that a new antimicrobial agent might play in a geographic region since local variations in the frequency distribution of pathogens and antimicrobial susceptibility profiles have been reported. Although doripenem has been tested *in vitro* against clinical isolates collected worldwide, it has been not tested against a large collection of pathogens isolated from distinct Brazilian medical centers. The main objective of this study was to evaluate the *in vitro* activity of doripenem and comparator agents against Gram-negative bacilli recently isolated from Brazilian private hospitals.

MATERIAL AND METHODS

Participant medical centers

Eighteen private hospitals participated of the INVITA-A-DORI Brazilian Study. The medical centers were located in the cities of Belo Horizonte (2 medical centers), Blumenau (1 medical center), Curitiba (1 medical center), Porto Alegre (2 medical centers), São Paulo (7 medical centers), Rio de Janeiro (3 medical centers), Salvador (1 medical center), and São Luís (1 medical center). The selection of the participant medical centers was primarily based on the criteria that they should have ≥ 200 beds, at least one adult intensive care unit and located in cities with more than one million inhabitants.

Bacterial isolates

A total of 806 consecutive Gram-negative bacilli were submitted between March 2008 and August 2009. By protocol, each medical center had to submit Gram-negative bacilli collected from patients with diagnosis of pneumonia (20 isolates; 10 of them from ventilated-associated pneumonia), bloodstream infections (20 isolates) and intra-abdominal infections (10 isolates) according to the Centers for Disease Control and Prevention (CDC) definitions.[11] Just a single isolate per patient was evaluated. All isolates were identified at the participating institution by routine methodologies of each laboratory. Upon receipt at the central laboratory (UNIFESP, São Paulo), isolates were subcultured to ensure viability and purity. Confirmation of species identification was performed with the BD Phoenix™ Automated Microbiology System (BD Diagnostics, MD, USA) or conventional methods, as required.

Susceptibility testing

Antimicrobial susceptibility testing was performed by the broth microdilution method, following recommendations of the Clinical and Laboratory Standards Institute (CLSI).[12] Antimicrobial powders were obtained from the respective manufacturers and microdilution plates were prepared by TREK Diagnostics (West Sussex, England). Susceptibility results were interpreted according to CLSI document M100-S21 for all comparison agents[13] except for doripenem[8] and tigecycline.[14] Quality control was performed by testing *E. coli* ATCC 25922 and *P. aeruginosa* ATCC 27853.

RESULTS

A total of 805 isolates were collected as part of the INVITA-A-DORI Brazilian Study. The bacterial isolates were collected mainly from patients diagnosed with bloodstream (40.9%), lower respiratory tract (39.1%) and intra-abdominal (15.4%) infections. The most frequent Gram-negative bacilli collected were *P. aeruginosa* (26.6%); *E. coli* (18.9%); *Klebsiella* spp. (18.3%); *Acinetobacter* spp. (13.9%); *Enterobacter* spp. (8.7%); and *Serratia* spp. (6.3%). They accounted for 92.7% of the total number of isolates collected as shown in Table 1.

The antimicrobial susceptibility profile of the most frequent Enterobacteriaceae isolates is shown in Table 2. Doripenem (MIC_{50}, ≤ 0.12 μg/mL; 97.4% susceptible) was as potent as meropenem (MIC_{50}, ≤ 0.12 μg/mL; 98.0% susceptible) and at least two-fold more potent than imipenem (MIC_{50}, 0.25 μg/mL; 94.7% susceptible) against *E. coli*. Amikacin (MIC_{50}, ≤ 4 μg/mL; 97.4% susceptible), tigecycline (MIC_{50}, 0.5 μg/mL; 97.4% susceptible), and polymyxin B (MIC_{50}, ≤ 0.5 μg/mL; 96.7% susceptible) also showed good *in vitro* activity against *E. coli*. Doripenem (MIC_{50}, ≤ 0.12 μg/mL; 85.0% susceptible) and meropenem (MIC_{50}, ≤ 0.12 μg/mL; 83.7% susceptible) were the most active compounds against *Klebsiella* spp. followed by imipenem (MIC_{50}, 0.5 μg/mL; 78.2% susceptible), polymyxin B (MIC_{50}, ≤ 0.5 μg/mL; 91.2% susceptible) and tigecycline (MIC_{50}, 1 μg/mL; 95.2% susceptible). Ceftazidime (MIC_{50}, 2 μg/mL; 53.7% susceptible) was four- and sixteen-fold more potent than cefepime (MIC_{50}, 8 μg/mL; 51.7% susceptible) and ceftriaxone (MIC_{50}, 32 μg/mL; 39.5% susceptible) against *Klebsiella* spp. isolates, respectively. Against the 70 *Enterobacter* spp. isolates tested, doripenem (MIC_{50}, 0.25 μg/mL) and meropenem (MIC_{50}, 0.12 μg/mL) were at least eight-fold more potent than imipenem (MIC_{50}, 2 μg/mL) as shown in Table 2. Although cefepime (MIC_{50}, 2 μg/mL) was four- and eight-fold more potent than ceftazidime (MIC_{50}, 8 μg/mL) and ceftriaxone (MIC_{50}, 16 μg/mL), respectively,

Table 1. Distribution of the most frequent pathogens tested by the INVITA-A-DORI Brazilian Study

Bacterial genus/species	Amount (%)
Pseudomonas spp.[a]	214 (26.6)
Escherichia coli	152 (18.9)
Klebsiella spp.[b]	147 (18.3)
Acinetobacter spp.[c]	112 (13.9)
Enterobacter spp.[d]	70 (8.7)
Serratia spp.[e]	51 (6.3)
Proteus spp.[f]	21 (2.6)
Citrobacter spp.[g]	12 (1.5)
Morganella morgannii	12 (1.5)
Burkholderia cepacea	5 (0.6)
Salmonella spp.[h]	5 (0.6)
Alcaligenes xylosoxidans	1 (0.1)
Delfia acidovorans	1 (0.1)
Hafnia spp.	1 (0.1)
Pantoea spp.	1 (0.1)
Providencia stuartii	1 (0.1)
Total	806 (100.0)

[a] *P. aeruginosa* (209), *P. fluorescens* (02), *P. putida* (01), *P. stutizeri* (01), *Pseudomonas* spp. (01).
[b] *K. pneumoniae* (141), *K. oxytoca* (06).
[c] *Acinetobacter baumannii* (103), *A. lwoffii* (02), *Acinetobacter* spp. (07).
[d] *E. cloacae* (45), *E. aerogenes* (19), *Enterobacter* spp. (05), *E. amnigenus* (01).
[e] *S. marcescens* (48), *Serratia* spp. (02), *S. liquefaciens* (01).
[f] *P. mirabilis* (20), *P. vulgaris* (01).
[g] *C. freundii* (05), *C. koseri* (03), *Citrobacter* spp. (02), *C. braaki* (01), *C. amalonaticus* (01).
[h] *Salmonella* spp. (04), *S. enterica* (01).

against *Enterobacter* spp., only 58.6% of strains were susceptible to this compound. Amikacin inhibited 67.1% of *Enterobacter* spp. In contrast, tigecycline (MIC_{50}, 1μg/mL) showed a reasonable activity against *Enterobacter* spp. inhibiting 91.4% of strains at concentrations ≤ 2 μg/mL. Doripenem ($MIC_{50/90}$, 0.25/4 μg/mL) and meropenem ($MIC_{50/90}$, 0.25/2 μg/mL) were also very potent against cefepime non-susceptible *Enterobacter* spp. and eight-fold more potent than imipenem ($MIC_{50/90}$, 2/8 μg/mL). The highest MIC values for doripenem and meropenem against non-susceptible *Enterobacter* spp. were 8 μg/mL and ≥ 16 μg/mL, respectively. Doripenem (MIC_{50}, 0.25 μg/mL) and meropenem (MIC_{50}, 0.25 μg/mL) exhibited a similar potency to that of levofloxacin (MIC_{50}, ≤ 0.5 μg/mL) against *S. marcescens* isolates. The highest susceptibility rates were observed for the carbapenems followed by amikacin (76.5%), tigecycline (74.5%), and cefepime (70.6%) as shown in Table 2.

P. aeruginosa and *Acinetobacter* spp. showed high resistance rates to all compounds tested except for polymyxin B (Table 3). This compound was the most active antimicrobial agent against *P. aeruginosa* (MIC_{50}, 1 μg/mL; 98.6% susceptible) and *Acinetobacter* spp. (MIC_{50}, ≤ 0.5 μg/mL; 98.2% susceptible). Against *P. aeruginosa*, piperacillin/tazobactam (57.4%) showed the second highest susceptibility rate. Doripenem and meropenem were equally potent against *Acinetobacter* spp. (MIC_{50}, > 8 μg/mL) and *P. aeruginosa* (MIC_{50}, 8 μg/mL); however, doripenem inhibited a greater number of *P. aeruginosa* isolates (47.3%) at MIC values ≤ 4 μg/mL than did meropenem (39.1%) or imipenem (38.6%; Table 4). Doripenem (MIC_{50}, 16 μg/mL) was more active than meropenem (MIC_{50}, > 8 μg/mL) against imipenem-resistant *P. aeruginosa* isolates, and inhibited 10.5% of imipenem-resistant *P. aeruginosa*, while meropenem inhibited none of these strains at MIC values of 4 μg/mL (Table 4). Polymyxin B was the only agent active against 97.8% imipenem-resistant *Acinetobacter* spp. ($MIC_{50/90}$, ≤0.5/1 μg/mL) and 99.1% imipenem-resistant *P. aeruginosa* ($MIC_{50/90}$, 1/2 μg/mL). Its potency was slightly affected by the carbapenem-resistant phenotype since 100% of imipenem-susceptible *Acinetobacter* spp. and 98.8% of imipenem-susceptible *P. aeruginosa* were inhibited at polymyxin B concentrations of 1 and 2 μg/mL, respectively. Only 1.8% of *Acinetobacter* spp. isolates showed polymyxin B MIC results ≥ 4 μg/mL and were classified as resistant by CLSI criteria. Full polymyxin B resistance was not detected among *P. aeruginosa* isolates.

DISCUSSION

The emergence of antimicrobial resistance represents one of the greatest public health threats. An area of particular concern involves Gram-negative pathogen resistance, where few agents are effective or in late-stage of development.[15] ESBL-producing Enterobacteriaceae have been established as a major cause of nosocomial-acquired infections. Usually, ESBL-producing Enterobacteriaceae also carry genes encoding mechanisms of resistance to other antimicrobial classes such as quinolones, aminoglycosides, and tetracyclines.[15,16] Enterobacteriaceae resistant to broad-spectrum cephalosporins due to AmpC derepression have also become more frequent in the nosocomial setting.[15] In addition, ESBL encoding genes have spread out to other bacterial species than *E. coli* and *Klebsiella* spp. and reached the community setting.[17,18] In this manner, carbapenems have become the drug of choice for treatment of serious ESBL and derepressed AmpC infections.[15]

Doripenem represents an attractive option among the carbapenems since (I) it has potent *in vitro* activity against many Gram-negative and -positive pathogens;[1,4,19,20] (II) lower propensity to select for resistance;[21-23] (III) safe tolerability profile with lower seizure potential than imipenem;[4,8,24] and

Table 2. **In vitro** *activity of doripenem in comparison to selected antimicrobial agents tested against the main Enterobacteriaceae pathogens collected by the INVITA-A-DORI Brazilian Study*

Organism (no. tested)/ Antimicrobial agent	MIC (µg/mL)			% by category[a]	
	50%	90%	Range	Susceptible	Resistant
E. coli (152)					
Doripenem	≤ 0.12	0.25	≤ 0.12 to > 4	97.4	1.3
Meropenem	≤ 0.12	0.25	≤ 0.12 to 8	98.0	1.4
Imipenem	0.25	1	≤ 0.12 to 8	94.7	2.7
Piperacillin/tazobactam	2	> 64	≤ 2 to > 64	83.6	11.2
Ceftriaxone	≤ 0.25	> 32	≤ 0.25 to > 32	69.7	24.9
Ceftazidime	0.25	16	≤ 0.12 to > 16	82.2	13.1
Cefepime	≤ 0.12	> 16	≤ 0.12 to > 16	83.6	14.5
Cefoxitin	≤ 4	> 16	≤ 4 to > 16	72.4	17.1
Aztreonam	≤ 1	> 16	≤ 1 to > 16	78.9	18.4
Levofloxacin	4	> 4	≤ 0.5 to > 4	48.0	48.0
Amikacin	≤ 4	8	≤ 4 to > 32	97.4	1.3
Polymyxin B	≤ 0.5	1	≤ 0.5 to > 4	96.7	1.3[b]
Tigecycline	0.5	1	0.12 to > 4	97.4	0.7
Klebsiella spp. (147)					
Doripenem	≤ 0.12	4	≤ 0.12 to > 16	85.0	1.3
Meropenem	≤ 0.12	4	≤ 0.12 to > 8	83.7	10.9
Imipenem	0.5	4	≤ 0.12 to > 8	78.2	10.2
Piperacillin/tazobactam	8	64	≤ 2 to > 64	57.1	36.1
Ceftriaxone	32	> 32	≤ 0.25 to > 32	39.5	57.8
Ceftazidime	2	> 16	≤ 0.12 to >16	53.7	36.0
Cefepime	8	> 16	≤ 0.12 to >16	51.7	47.6
Cefoxitin	8	> 16	≤ 4 to > 16	52.4	29.9
Aztreonam	8	> 16	≤ 1 to > 16	46.9	47.0
Levofloxacin	2	> 4	≤ 0.5 to > 4	52.4	40.1
Amikacin	≤ 4	32	≤ 4 to > 32	87.8	4.1
Polymyxin B	≤ 0.5	2	≤ 0.5 to > 4	91.2	4.8[b]
Tigecycline	1	2	0.12 to 4	95.2	0.0
Enterobacter spp. (70)					
Doripenem	0.25	1	≤ 0.12 to > 16	90.0	5.7
Meropenem	0.12	0.5	≤ 0.12 to > 8	94.3	2.8
Imipenem	2	4	0.25 to > 8	28.6	25.7
Piperacillin/tazobactam	64	> 64	≤ 2 to > 64	42.9	38.6
Ceftriaxone	16	> 32	≤ 0.25 to > 32	34.3	61.5
Ceftazidime	8	> 16	≤ 0.12 to > 16	47.1	50.0
Cefepime	2	> 16	≤ 0.12 to > 16	58.6	37.1
Cefoxitin	> 16	> 16	≤ 8 to > 16	1.4	92.9
Aztreonam	16	> 16	≤ 1 to > 16	45.7	52.9
Levofloxacin	1	> 4	≤ 0.5 to > 4	54.3	45.7
Amikacin	≤ 4	> 32	≤ 4 to > 32	67.1	27.1
Polymyxin B	≤ 0.5	> 4	≤ 0.5 to > 4	77.1	20.0[b]
Tigecycline	1	2	0.25 to 4	91.4	0.0

cont.

Table 2. **In vitro** *activity of doripenem in comparison to selected antimicrobial agents tested against the main Enterobacteriaceae pathogens collected by the INVITA-A-DORI Brazilian Study* **(Cont.)**

Organism (No. tested)/ Antimicrobial agent	MIC (µg/mL)			% by category[a]	
	50%	90%	Range	Susceptible	Resistant
Serratia spp. (51)					
Doripenem	0.25	8	≤ 0.12 to > 16	78.4	15.7
Meropenem	0.25	4	≤ 0.12 to > 8	84.3	11.8
Imipenem	2	8	0.12 to > 8	47.1	11.8
Piperacillin/tazobactam	16	> 64	≤ 2 to > 64	54.2	35.3
Ceftriaxone	32	> 32	≤ 0.25 to > 32	45.1	52.9
Ceftazidime	2	> 16	≤ 0.12 to > 16	62.7	25.5
Cefepime	4	> 16	≤ 0.12 to > 16	72.5	23.5
Aztreonam	2	> 16	≤ 1 to > 16	58.8	35.3
Levofloxacin	≤ 0.5	> 4	≤ 0.5 to > 4	68.6	25.5
Amikacin	≤ 4	32	≤ 4 to > 32	76.5	9.8
Polymyxin B	> 4	> 4	0.5 to > 4	7.8	90.2[b]
Tigecycline	2	4	0.5 to > 4	74.5	9.8

[a] Breakpoint criteria established by CLSI document M100-S21, except for doripenem and tigecycline, where FDA breakpoints were applied.
[b] According to breakpoints established by CLSI for *P. aeruginosa* (≤ 2 µg/mL for susceptibility and ≥ 8 µg/mL for resistance).

Table 3. **In vitro** *activity of doripenem in comparison to selected antimicrobial agents tested against non-fermentative Gram-negative pathogens collected by the INVITA-A-DORI Brazilian Study*

Organism (No. tested)/ Antimicrobial agent	MIC (µg/mL)			% by category[a]	
	50%	90%	Range	Susceptible	Resistant
P. aeruginosa (209)					
Doripenem	8	> 16	≤ 0.12 to > 16	39.7	_[b]
Meropenem	8	> 8	≤ 0.12 to > 8	40.7	44.5
Imipenem	> 8	> 8	≤ 0.5 to > 8	40.2	53.1
Piperacillin/tazobactam	64	> 64	≤ 2 to > 64	57.4	42.6
Ceftazidime	16	> 16	≤ 1 to > 16	44.5	45.5
Cefepime	16	> 16	≤ 1 to > 16	45.0	43.5
Aztreonam	> 16	> 16	≤ 1 to > 16	26.8	54.5
Levofloxacin	> 4	> 4	≤ 0.5 to > 4	43.1	52.2
Polymyxin B	1	2	≤ 0.5 to > 4	98.6	0.0
Acinetobacter spp. (112)					
Doripenem	> 16	> 16	≤ 0.12 to > 16	12.5	_[b]
Meropenem	> 8	> 8	≤ 0.12 to > 8	15.2	82.1
Imipenem	> 8	> 8	0.5 to > 8	16.1	82.1
Piperacillin/tazobactam	> 64	> 64	≤ 2 to > 64	10.7	87.5
Ceftriaxone	> 32	> 32	≤ 0.25 to >32	6.3	87.5
Ceftazidime	> 16	> 16	≤ 0.25 to > 16	15.2	83.0
Cefepime	> 16	> 16	≤ 0.12 to > 16	13.4	83.0
Levofloxacin	> 4	> 4	≤ 0.5 to > 4	10.7	83.9
Polymyxin B	≤ 0.5	1	≤ 0.5 to > 4	98.2	1.8
Tigecycline	1	4	0.012 to > 4	86.6	3.6

[a] Breakpoint criteria established by CLSI document M100-S21, except for doripenem and tigecycline, where FDA breakpoints were applied.
[b] Interpretative criteria not established by CLSI or FDA.

Table 4. Cumulative frequency distributions of doripenem, meropenem and polymyxin B against imipenem-resistant* Acinetobacter *spp. and* P. aeruginosa *isolated from Brazilian patients (INVITA-A-DORI Brazilian Study)

Organism (no. of isolates)	Cumulative % inhibited at MIC (µg/mL)															
	Doripenem						Meropenem					Polymyxin B				
	≤ 1	2	4	8	16	> 16	≤ 1	2	4	8	> 8	≤ 0.5	1	2	4	> 4
Acinetobacter spp. (112)																
Imipenem-resistant (92)	0.0	0.0	1.1	1.1	5.4	100.0	0.0	0.0	0.0	0.0	100.0	88.0	96.7	97.8	100.0	
Intermediate to imipenem (2)	0.0	0.0	0.0	50.0	100.0		0.0	0.0	50.0	100.0		50.0	50.0	100.0		
Imipenem-susceptible (18)	77.8	94.4	100.0				83.3	88.9	88.9	100.0		77.8	100.0			
P. aeruginosa (209)																
Imipenem-resistant (111)	0.0	0.0	10.8	40.5	64.0	100.0	0.0	0.0	0.0	20.7	100.0	3.6	77.5	99.1	100.0	
Intermediate to imipenem (14)	21.4	35.7	71.4	85.7	92.9	100.0	21.4	28.6	57.1	71.4	100.0	14.3	71.4	92.9	100.0	
Imipenem-susceptible (84)	73.8	92.9	96.4	98.8	100.0		76.2	84.5	91.7	98.8	100.0	14.3	75.0	98.8	100.0	

(IV) extended solution stability at room temperature.[4,8,25] Sakyo et al.[21] have shown that the potency of carbapenems in preventing the growth of the carbapenem-resistant *P. aeruginosa* mutants differed for doripenem, imipenem, and meropenem. Mutants were not selected on agar plates containing ½ or ¼ MIC doripenem at a frequency of greater than 10^{-9} per cell per generation, whereas mutants of each *P. aeruginosa* strain were selected on agar containing meropenem or imipenem at frequencies of 10^{-7} to 10^{-9} per cell per generation. Other resistance selection experiments have reported similar results.[26] Furthermore, a clinical study evaluating doripenem pre- and post-treatment of urinary tract infections showed that microbiological failures were more frequently associated with the acquisition of a new pathogen than by emergence of resistant isolates.[27]

Although a considerable number of studies have reported the potent *in vitro* activity of doripenem against Gram-negative pathogens, scarce studies have reported the activity of this compound against bacterial pathogens isolated from Brazilian hospitals.[19] Since the susceptibility rates may vary from distinct hospitals located within the same geographic area due to epidemiological factors, we aimed to evaluate the activity of this agent against Gram-negative pathogens isolated from Brazilian private hospitals. Interestingly, the antimicrobial resistance rates observed among Gram-negative bacilli isolates collected from private hospitals were higher than those previously reported by other surveillance studies that evaluated bacterial isolates mostly collected from public/teaching hospitals.[10,19] It was expected that the resistance rates would have been lower in private institutions since compliance to infection control policies is assumed to be higher in these institutions than in the public hospitals that sometimes experiment scarcity of resources. The high resistance rates observed in this study could also be partially attributed to the high percentage of Gram-negative pathogens causing nosocomial-acquired bloodstream or lower respiratory tract infections. Our data also reinforce the concept that the level of patient's assistance probably impacts more in the antimicrobial resistance rates than hospital's administrative category, public or private.

The increased MIC_{90} values for carbapenems against Enterobacteriaceae, especially *K. pneumoniae*, noted in this study might be related to the sporadic occurrence of metallo-beta-lactamase or mainly KPC (*Klebsiella pneumoniae* carbapenemase) producers, which has been described in some Brazilian medical centers.[28-30] *P. aeruginosa* and *Acinetobacter* spp. showed high resistance rates to all compounds tested except polymyxin B. Doripenem also demonstrated potent activity against wild type *Acinetobacter* spp. and *P. aeruginosa*, and was more active than meropenem against imipenem-resistant *P. aeruginosa* isolates. Doripenem retained a degree of activity against imipenem- and/or meropenem-resistant *P. aeruginosa*. Specifically, among those *P. aeruginosa* with imipenem MIC values ≥ 8 µg/mL, 10.8% were susceptible to

doripenem at ≤ 4 μg/mL (Table 4). Although this categorization is based on a MIC value above the current US-FDA breakpoints, pK/pD studies have shown a ≥ 90% probability of target attainment for isolates possessing doripenem MICs ≤ 8 μg/mL, when higher dosages in prolonged infusion (1 g tid in 4-h infusion) were given.[31] In addition, it has been reported that doripenem prolonged regimens, particularly at the 1-g dose with the 4-h infusion, achieved higher cell kill and resistance suppression rates against wild-type *P. aeruginosa*.[32] Since the current US-FDA breakpoints were established based on the licensed dosage (500 mg tid in 1-h infusion), modifications in the doripenem breakpoints for non-fermentative baciili are also expected in the near future. By contrast, doripenem showed little potential against imipenem-resistant *Acinetobacter* spp. isolates more than 90.5% showed MIC results at ≥ 8 μg/mL.

Doripenem is not hydrolyzed by ESBL and AmpC enzymes; however, like other carbapenems, doripenem remains labile to most carbapenemases.[26,33] Among carbapenemases, metallo-beta-lactamases (MβLs) are considered the most prevalent among non-fermentative bacilli. In Brazilian hospitals, the dissemination of an endemic clone *P. aeruginosa* producer of SPM-1, which is a MβL, has been reported.[34] In addition, carbapenems are generally hydrolyzed by OXA β-lactamases commonly reported in *Acinetobacter* spp. worldwide.[35] OXA-23-producing isolates have also been reported in Brazilian hospitals.[36] Although the production of OXA-23 by itself would not lead to high level resistance to carbapenems, OXA-23-producing isolates may possess other associated mechanisms of resistance since usually they show high resistance to all carbapenems.[35,36] Thus, the dissemination of SPM-1-producing *P. aeruginosa* or OXA-23-producing *Acinetobacter* spp. clones in Brazilian hospitals might justify the low susceptibility rates displayed by carbapenenems observed in this study that figure amongst the lowest ones observed and compared only to the rates of the Asian-Pacific region.[37]

Despite of the limited role of carbapenems for the treatment of non-fermentative infections in Brazilian hospitals, doripenem shows potent *in vitro* activity compared to that of meropenem. This compound could have its clinical benefit maximized by utilizing higher dosages in prolonged infusion and might exert a lower selective pressure in the hospital microbiota. In addition, doripenem use might have economic and clinical benefit to patients and healthcare delivery. Kollef et al.[38] assessed medical resource utilization in patients with ventilator-associated pneumonia through a pooled analysis of two prospective, randomized, open-label, multicenter, phase III studies, which also showed that doripenem was clinically non-inferior to comparator agents. The authors observed that in patients with *P. aeruginosa* at baseline, median durations of mechanical ventilation (7 *versus* 13 days; p = 0.03) and ICU stay (13 *versus* 21 days; p = 0.02) were shorter for doripenem. Recently, Kongnakorn et al.[39] using an economic model predicted that doripenem use for treatment of ventilator-associated pneumonia would save an average of approximately $7,000 per patient compared to imipenem, with 95% driven by reduction in hospital length of stay. The model predicted 63% less seizures, 52% less emerging *P. aeruginosa* resistance, and 15% shorter stays leading to 46% less transmission associated with doripenem.

As shown in this study, carbapenems may no longer represent an effective drug for empirical treatment of infections caused by non-fermentative and polymyxins represent the last resort drugs for treatment of carbapenem-resistant *P. aeruginosa* or *Acinetobacter* spp. infections. However, the emergence of KPC-producing isolates resistant to polymyxins and all clinical available drugs poses a clinical challenge.[40] Since the high rates of resistance are mainly due to spread of endemic clones, infection control measures must be strictly applied to restore the activity of carbapenems among non-fermentative isolates. At this moment, it is imperative the establishment of task-force groups to oppose this situation at a national level.

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