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Activity of Doripenem Tested Against an International Collection of ESBL- and AmpC-Producing Enterobacteriaceae

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AMENDED ABSTRACT[a]

Background: Emerging resistance (R) among Enterobacteriaceae and nonfermentative bacilli is rendering many broad-spectrum agents useless. Doripenem (DOR), a new parenteral carbapenem (CARB) currently in late-stage clinical development, displays inherent stability to most β-lactamases. This study compares the activity of DOR against commonly occurring Amp-C and ESBL-producing Enterobacteriaceae (ENT).

Methods: Non-duplicate bacterial isolates (16,246) were collected in >60 medical centers participating in the global DOR surveillance program (2003-2005; see Table). All isolates were susceptibility tested using CLSI methods against DOR and comparator agents. ESBL production was confirmed using the CLSI disk approximation method; ceftazidime (CAZ)-R was a marker for stably de-repressed AmpC production.

Results: DOR results are in the Table.

	MIC (μg/mL)		Cum. % inhibited at MIC (μg/mL)		
Organism (no. tested)	**50%**	**90%**	**≤2**	**4**	**8**
E. coli (EC)					
All (8,527)	≤0.06	≤0.06	>99	100	
ESBL-confirmed (329)	≤0.06	≤0.06	100		
Klebsiella spp. (KSP)					
All (3,835)	≤0.06	≤0.06	98	99	>99
ESBL-confirmed (571)	≤0.06	0.12	>99	>99	100
Proteus mirabilis (PM)					
All (905)	0.12	0.25	>99	>99	>99
ESBL-confirmed (29)	0.12	0.25	100		
Enterobacter aerogenes (EA)					
CAZ-S (307)	≤0.06	0.12	100		
CAZ-R (116)	0.12	2	96	97	98
E. cloacae (ECL)					
CAZ-S (1,120)	≤0.06	≤0.06	100		
CAZ-R (419)	0.12	0.5	96	97	98
Citrobacter freundii (CF)					
CAZ-S (169)	≤0.06	≤0.06	100		
CAZ-R (56)	≤0.06	0.12	100		
Serratia marcescens (SM)					
CAZ-S (759)	0.12	0.25	>99	>99	>99
CAZ-R (31)	0.12	0.5	93	93	97

ESBLs were detected in 4%, 15%, and 3% of EC, KSP, and PM, respectively; AmpC-production was evident in 27%, 27%, 25%, and 4% of EA, ECL, CF, and SM. Overall, 99.7% and 99.4% of ESBL- and AmpC-producers were inhibited by ≤4 μg/mL of DOR. ESBLs in EC, KSP, and PM had little impact on DOR potency. Sporadic occurrence of Bush group 2f carbapenemases were detected among klebsiellae (KPC) and SM, and rare metallo-β-lactamases were detected among PM, EA, and ECL, producing elevated DOR MICs. Decreased DOR potency (MIC_{90} values) among CAZ-R subsets was noted, varying from 2- (CF, SM) to 16-fold (EA).

Conclusions: Among ENT strains expressing ESBL and AmpC enzymes, 99.7% were inhibited by ≤4 μg/mL of DOR. The dramatic increase in ESBL-producing ENT is changing empiric therapy, with greater reliance upon CARB; DOR may represent a significant new choice for broad-spectrum coverage of R phenotypes.

a. Amended to reflect a change in the number of isolates investigated.

INTRODUCTION

Dramatic increases in the prevalence of ESBL-producing Enterobacteriaceae (primarily *Escherichia coli* and *Klebsiella* spp.), constitutively expressed chromosomal AmpC (Bush group 1) enzymes, and multidrug-resistant nonfermentative Gram-negative bacilli are changing the face of empiric antimicrobial therapy in health care settings that deal with a high proportion of seriously ill patients, often with co-morbidities. Resistance to third- and fourth-generation cephalosporins, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones, and aminoglycosides has become commonplace in various geographic regions, requiring the utilization of carbapenems, glycylcyclines, or unproven antimicrobial "cocktails" or "agents of last resort," such as polymyxin B and colistin.

Doripenem, a new parenteral carbapenem in late-stage clinical development, displays inherent stability to most β-lactamases. This agent has been characterized as having broad activity against Gram-positive pathogens most similar to that of imipenem and against Gram-negative pathogens most similar to that of meropenem.[1-6]

The present study was conducted to further evaluate the activity and potency of doripenem when tested against an international collection of *E. coli*, *Klebsiella* spp., and *Proteus mirabilis* with documented ESBL-production and *Enterobacter* spp., *Citrobacter freundii*, and *Serratia marcescens* with constitutively expressed chromosomal AmpC enzymes.

MATERIALS AND METHODS

Bacterial Strain Collection

A total of 16,246 non-duplicate consecutive clinical isolates were submitted from medical centers (59 to 64) located in North America, South America, and Europe as part of a doripenem international surveillance program for the years 2003 to 2005. Isolates originated from patients with documented bloodstream, respiratory, skin and soft tissue, and urinary tract infections. The distribution of species and strains reported here included *E. coli* (8,527 isolates), *Klebsiella* spp. (3,835), *P. mirabilis* (905), *Enterobacter aerogenes* (423), *Enterobacter cloacae* (1,541), *C. freundii* (225), and *S. marcescens* (790).

Susceptibility Test Methods

All strains were tested by the Clinical and Laboratory Standards Institute (CLSI; formerly the NCCLS) broth microdilution method in validated dry-form panels (TREK Diagnostics, Cleveland, Ohio) using cation-adjusted Mueller-Hinton broth against a variety of antimicrobial agents representing the most common classes and examples of drugs used for the empiric or directed treatment of the indicated pathogen. Interpretation of MIC results was in accordance with CLSI criteria as published in the M100-S16 (2006) document.[7,8] Enterobacteriaceae with elevated MIC values (≥2 μg/mL) for ceftazidime or ceftriaxone or aztreonam were considered as ESBL-producing phenotypes; confirmatory testing was performed using cefotaxime and ceftazidime alone and in combination with clavulanic acid.[8] Quality control strains utilized included *E. coli* ATCC 25922 and 35218, and *K. pneumoniae* ATCC 700603.

RESULTS

- In the tested collection of isolates, ESBLs were detected in 4%, 15%, and 3% of *E. coli*, *Klebsiella* spp., and *P. mirabilis*, respectively; stably de-repressed expression of AmpC was evident in 27%, 27%, 25%, and 4% of *E. aerogenes*, *E. cloacae*, *C. freundii*, and *S. marcescens*, respectively (ceftazidime resistance) (Table 1).
- Doripenem was the single most active agent against ESBL-confirmed *E. coli* and *Klebsiella* spp. (MIC_{90} values, ≤0.06 and 0.12 μg/mL, respectively) and was at least 4-fold more potent than either imipenem or ertapenem against both organisms (Table 1). One ESBL-confirmed *Klebsiella* spp. isolate (0.2%) displayed a reproducible doripenem MIC of 8 μg/mL.
- One year 2005 *P. mirabilis* isolate from a patient in Greece had a doripenem MIC of >8 μg/mL; PCR amplification and sequencing revealed a VIM metallo-β-lactamase (MBL; Tables 1 and 2).
- Among comparator antimicrobials, amikacin provided the broadest coverage of ESBL-producing *E. coli* (92.4% susceptible) and *Klebsiella* spp. (73.7%), whereas piperacillin/tazobactam (100%), ceftazidime (82.8%), and amikacin (79.3%) were most active against ESBL-producing *P. mirabilis* (Table 2).
- Doripenem inhibited >99% of all tested *Enterobacter* spp., *C. freundii*, and *S. marcescens* at ≤4 μg/mL (Table 1).
- Among those strains displaying constitutive AmpC production (ceftazidime resistance), >97% of strains were inhibited by doripenem; this agent was also the most potent agent tested, being 2- to 8-fold more active than either ertapenem or imipenem (Table 3).
- For those strains demonstrating doripenem MIC values >4 μg/mL, most (15 of 18) have been further characterized as expressing either Bush group 2f carbapenemases (4) or MBLs (9).
- In addition to the carbapenems, cefepime (64.0% to 91.4% susceptible) and amikacin (78.3% to 91.8%) were also effective in inhibiting a large majority of *Enterobacter*, *Citrobacter*, and *Serratia* spp. (Table 3).

Table 1. Antimicrobial activity of doripenem tested against a global collection of Enterobacteriaceae expressing confirmed ESBL or inferred AmpC cephalosporinases.

	MIC (μg/mL)		Cum. % inhibited at MIC (μg/mL)							
Organism (no. tested)	**50%**	**90%**	**≤0.06**	**0.12**	**0.25**	**0.5**	**1**	**2**	**4**	**8**
E. coli										
All (8,527)	≤0.06	≤0.06	99	>99	>99	>99	>99	>99	100	
ESBL-confirmed (329)	≤0.06	≤0.06	95	99	>99	>99	100			
Klebsiella spp.										
All (3,835)	≤0.06	≤0.06	90	96	97	98	98	98	99	>99
ESBL-confirmed (571)	≤0.06	0.12	78	92	95	98	99	>99	>99	100
P. mirabilis										
All (905)	0.12	0.25	26	70	96	>99	>99	>99	>99	>99
ESBL-confirmed (29)	0.12	0.25	14	62	96	100				
E. aerogenes										
All (423)	0.06	0.12	70	92	96	97	97	99	>99	>99
Ceftazidime-susceptible (307)	≤0.06	0.12	82	98	100					
Ceftazidime-resistant (116)	0.12	2	37	77	84	89	90	96	97	98
E. cloacae										
All (1,541)	≤0.06	0.12	77	92	96	98	99	>99	>99	>99
Ceftazidime-susceptible (1,120)	≤0.06	≤0.06	90	98	>99	>99	>99	100		
Ceftazidime-resistant (419)	0.12	0.5	40	76	88	93	96	96	97	98
C. freundii										
All (225)	≤0.06	≤0.06	92	96	97	98	>99	100		
Ceftazidime-susceptible (169)	≤0.06	≤0.06	96	97	98	98	100			
Ceftazidime-resistant (56)	≤0.06	0.12	77	93	94	96	98	100		
S. marcescens										
All (790)	0.12	0.25	26	84	97	99	>99	>99	>99	>99
Ceftazidime-susceptible (759)	0.12	0.25	26	84	98	>99	>99	>99	>99	>99
Ceftazidime-resistant (31)	0.12	0.5	1-	64	80	93	93	93	93	97

Table 2. Activity of doripenem and comparator antimicrobial agents tested against *E. coli*, *Klebsiella* spp., and *P. mirabilis*, including confirmed ESBL-producing strains.

	MIC (μg/mL)		% by Category[a]	
Organism (no. tested)/antimicrobial agent	**50%**	**90%**	**Susceptible**	**Resistant**
E. coli (8,527)				
Doripenem	≤0.06	≤0.06	-	-
Imipenem	≤0.12	0.25	>99.9	<0.1
Ertapenem	≤0.06	≤0.06	>99.9	0.0
Ampicillin	>16	>16	48.2	50.8
Amoxicillin/clavulanic acid	8	16	78.4	6.5
Piperacillin/tazobactam	2	4	95.6	2.1
Ceftriaxone	≤0.25	≤0.25	94.9	4.2
Ceftazidime	≤1	≤1	95.9	2.5
Cefepime	≤0.12	0.25	96.9	2.5
Ciprofloxacin	≤0.03	>4	81.4	18.5
Amikacin	2	4	99.3	0.2
Gentamicin	≤2	≤2	91.1	8.1
E. coli (ESBL-confirmed; 329)				
Doripenem	≤0.06	≤0.06	-	-
Imipenem	≤0.12	0.25	100.0	0.0
Ertapenem	≤0.06	0.25	99.7	0.0
Ampicillin	>16	>16	0.0	99.7
Amoxicillin/clavulanic acid	16	>16	23.4	22.2
Piperacillin/tazobactam	8	>64	69.3	12.5
Ceftriaxone	>32	>32	14.3	74.2
Ceftazidime	16	>16	38.9	39.2
Cefepime	16	>16	45.0	44.1
Ciprofloxacin	>4	>4	30.4	69.3
Amikacin	4	16	92.4	2.1
Gentamicin	≤2	>8	51.7	45.6
Klebsiella spp. (3,835)				
Doripenem	≤0.06	≤0.06	-	-
Imipenem	≤0.12	0.25	98.7	0.9
Ertapenem	≤0.06	0.12	97.7	1.8
Ampicillin	>16	>16	5.0	79.9
Amoxicillin/clavulanic acid	4	>16	74.8	11.4
Piperacillin/tazobactam	2	>64	83.9	12.3
Ceftriaxone	≤0.25	>32	81.3	13.3
Ceftazidime	≤1	>16	83.5	13.6
Cefepime	≤0.12	16	88.7	8.7
Ciprofloxacin	≤0.03	>4	84.1	14.2
Amikacin	1	16	92.5	4.1
Gentamicin	≤2	>8	81.9	16.0
Klebsiella spp. (ESBL-confirmed; 571)				
Doripenem	≤0.06	0.12	-	-
Imipenem	≤0.12	0.5	100.0	0.0
Ertapenem	≤0.06	0.5	97.0	1.8
Ampicillin	>16	>16	0.0	99.8
Amoxicillin/clavulanic acid	16	>16	27.3	25.7
Piperacillin/tazobactam	32	>64	44.8	42.6
Ceftriaxone	>32	>32	22.2	55.2
Ceftazidime	>16	>16	30.9	57.4
Cefepime	8	>16	54.5	35.4
Ciprofloxacin	1	>4	54.5	40.5
Amikacin	8	>32	73.7	15.6
Gentamicin	>8	>8	33.1	60.2
P. mirabilis (905 strains)				
Doripenem	0.12	0.25	-	-
Imipenem	1	2	99.8	0.1
Ertapenem	≤0.06	≤0.06	99.8	0.0
Ampicillin	2	>16	69.2	30.1
Amoxicillin/clavulanic acid	≤1	8	92.8	2.7
Piperacillin/tazobactam	≤0.5	1	99.2	0.0
Ceftriaxone	≤0.25	≤0.25	95.8	2.7
Ceftazidime	≤1	≤1	98.3	1.0
Cefepime	≤0.12	≤0.12	96.9	2.9
Ciprofloxacin	≤0.03	>4	78.6	16.4
Amikacin	2	4	98.2	1.5
Gentamicin	≤2	8	88.8	9.9
P. mirabilis (ESBL-confirmed; 29 strains)				
Doripenem	0.12	0.25	-	-
Imipenem	1	2	100.0	0.0
Ertapenem	≤0.06	≤0.06	100.0	0.0
Ampicillin	>16	>16	0.0	100.0
Amoxicillin/clavulanic acid	8	>16	62.1	10.3
Piperacillin/tazobactam	1	4	100.0	0.0
Ceftriaxone	>32	>32	17.2	62.1
Ceftazidime	2	>16	82.8	10.3
Cefepime	>16	>16	27.6	69.1
Ciprofloxacin	>4	>4	24.1	62.1
Amikacin	4	>32	79.3	20.7
Gentamicin	>8	>8	34.5	62.1

a. Break point criteria those of CLSI M100-S16 [2006]; - = no break points established.

Table 3. Antimicrobial activity of doripenem against select Enterobacteriaceae with stably de-repressed AmpC production (ceftazidime-resistant).

	MIC (μg/mL)		% by Category[a]	
Organism (no. tested)/antimicrobial agent	**50%**	**90%**	**Susceptible**	**Resistant**
E. cloacae (419)				
Doripenem	0.12	0.5	-	-
Imipenem	≤0.5	1	98.3	0.5
Ertapenem	0.5	2	91.0	4.4
Piperacillin/tazobactam	64	>64	23.6	41.5
Cefepime	2	>16	81.1	12.7
Ciprofloxacin	0.25	>4	66.5	29.2
Amikacin	2	32	88.5	7.1
Gentamicin	≤2	>8	60.2	34.2
E. aerogenes (116)				
Doripenem	0.12	2	-	-
Imipenem	0.5	4	91.4	3.4
Ertapenem	0.25	>8	87.1	11.2
Piperacillin/tazobactam	32	>64	20.7	21.6
Cefepime	0.5	8	91.4	4.3
Ciprofloxacin	≤0.03	>4	67.1	30.6
Amikacin	2	16	91.8	8.2
Gentamicin	≤2	>8	85.9	12.9
C. freundii (56)				
Doripenem	≤0.06	0.12	-	-
Imipenem	0.5	1	100.0	0.0
Ertapenem	0.12	0.5	98.2	0.0
Piperacillin/tazobactam	64	>64	32.1	33.9
Cefepime	1	16	87.5	8.9
Ciprofloxacin	0.12	>4	62.8	34.9
Amikacin	2	16	90.7	4.7
Gentamicin	≤2	>8	69.8	25.6
S. marcescens (31)				
Doripenem	0.12	0.5	-	-
Imipenem	0.5	1	100.0	0.0
Ertapenem	≤0.06	1	93.3	6.7
Piperacillin/tazobactam	16	>64	51.6	25.8
Cefepime	4	>16	64.5	29.0
Ciprofloxacin	1	>4	56.5	39.1
Amikacin	4	32	78.3	8.7
Gentamicin	>8	>8	21.7	69.6

a. Break point criteria those of CLSI M100-S16 [2006]; - = no break points established.

CONCLUSIONS

- The dramatic increases in ESBL- and AmpC-producing enteric species are changing empiric therapy, with greater reliance upon carbapenems due to their inherent stability to the most commonly encountered β-lactamases.
- Overall, 99.7% and 97.7% of ESBL- and stably de-repressed AmpC-producing Enterobacteriaceae, respectively, were inhibited by ≤4 μg/mL of doripenem. Among non-susceptible isolates, sporadic Bush group 2f β-lactamases and MBLs were the responsible mechanisms.
- Doripenem may represent a significant new choice for broad-spectrum coverage, given its potent activity against prevalent Gram-positive and -negative pathogens, including those with resistant and problematic phenotypes, and a novel drug delivery option.

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