### AMENDED ABSTRACT

**Background:** Emerging resistance (R) in a wide variety of pathogens has seriously compromised infection treatment even for broad-spectrum carbapenem (CARB) agents. Doripenem (DOR), a novel parenteral CARB, was challenged with 415 R strains isolated from worldwide locations.

Methods: All susceptibility (S) tests were performed by NCCLS (M7-A6) methods for DOR, ertapenem (ERT), imipenem (IMP), meropenem (MER) and >20 other agents. Organisms included intrinsic CARB-R species (S. maltophilia [XM], E. faecium [EFM], methicillin-R staphylococci [MRS]; 115), ESBL- (74) or Amp C- (54) producing Enterobacteriaceae (6 species), and some strains of metallo-ß-lactamase-producing P. aeruginosa (MßL-PSA) and other R mechanisms (168).

Results: Seventeen &-lactamase-producing E. coli with TEM, SHV, CMY and CTX-M15 enzyme types were all DOR-S, BLNAR H. influenzae (5) had 16X higher DOR MICs than wild-types (WT), and nearly all IMP- or MER-R *Enterobacteriaceae* had DOR MIC values at  $\leq 4 \mu g/ml$ . Additional comparative results follow:

		MIC <sub>90</sub> (μg/ml)/% S						
Phenotype	Organism (no. tested)	DOR	ERT <sup>a</sup>	IMP	MER			
ESBL	E. coli (29)	0.03/100	0.25/100 <sup>a</sup>	0.5/100	≤0.06/100			
	Klebsiella (34)	0.06/100	0.25/100 <sup>a</sup>	0.25/100	0.12/100			
	P. mirabilis (11)	0.25/100	0.03/100 <sup>a</sup>	2/100	0.12/100			
AMP C	Citrobacter (11)	0.06/100	0.5/100	1/100	0.12/100			
	Enterobacter (33)	0.12/100	4/88 <sup>a</sup>	1/94	0.25/100			
	Serratia (10)	0.5/100	2/90 <sup>a</sup>	1/100	0.5/100			
CARB R	Acinetobacter (24)	>32/21	>32/0	>8/17	>8/4			
	PSA (49)	>32/22	>32/0	>8/0	>8/2			
PEN R	S. pneumoniae (23) <sup>b</sup>	1/100	2/70	2/44	8/-			
	vir. gr. strept (13)	4/100	8/8	4/-	>16/-			
a. Inoculum and hydrolysis effect detected (MIC ≥ 8X vs. WT).								

b. 11 strains were ceftriaxone-R.

DOR and other CARBs were not active against XM (0% at  $\leq 4 \mu g/ml$  [S]), EFM (MIC<sub>90</sub>, >32  $\mu g/ml$ ) or MRS (MIC<sub>90</sub>, 16  $\mu g/ml$ ). Also no CARB demonstrated significant potency versus *C. jeikium* (MIC<sub>90</sub>, >32 μg/ml) or MßL-PSA (IMP, VIM, SPM; 41% of DOR MICs at 8 μg/ml).

**Conclusions:** DOR retained activity at a MIC  $\leq 4$  or at 8  $\mu$ g/ml against many CARB-R strains and ESBL-producing species. Appropriate selection of DOR dosing may allow this more potent CARB to be applied to a larger number of contemporary Gram-positive and -negative R isolates

### INTRODUCTION

Infection therapy has been seriously compromised by emerging antimicrobial resistance in a wide variety of pathogens that have made current broad-spectrum carbapenem agents more attractive choices. The need for newer and more potent compounds has stimulated research in the field of carbapenems. This class of drugs most resembles penicillins, except that the 5-membered ring contains a double bond between carbons 2 and 3 and the sulphur atom is replaced by a carbon, which enhances their binding affinity to target PBPs and resistance to ßlactamase.

Doripenem (formerly S-4661), is a novel parenteral carbapenem. Its chemical formula is (+)-(4R, 5S, 6S)-6-[(1R)-1-1hydroxyethyl]-4-methyl-7-oxo-3[3S,5S])-S-(sulfmoylaminomethyl) pyrrolidin-3-yl[thio-1-azabicyclo[3.2.0]hept-2-2ene-2-carboxylic acid monohydrate and it was developed by Shionogi & Co., Ltd. (Figure 1). This new carbapenem possesses ß-lactamase stability and resistance to inactivation by renal dehydropeptidases. Doripenem, however, remains unstable to the L1 enzyme produced by S. maltophilia and many metallo-ß-lactamases. Earlier in vitro studies show that doripenem's spectrum and potency resembles those of imipenem versus Gram-positive cocci and it has a Gram-negative activity most similar to that of meropenem (e.g. two- to four-fold greater than imipenem). The side chain located at position 2 provides greater activity among non-fermentative Gram-negative bacilli having multi-drug resistances. Preliminary reports have also demonstrated that the broad bacterial spectrum of doripenem and favorable pharmacokinetic properties allow elevated dosing. This study was conducted to evaluate the potency of doripenem versus other comparators tested against contemporary, 2001 - 2002 multi-drug-resistant (MDR) organisms.

### MATERIALS AND METHODS

A total of 415 organisms with well-characterized resistance mechanisms were selected from several worldwide surveillance programs and tested against more than 20 antimicrobial agents by the broth microdilution or agar dilution methods [NCCLS, 2003]. Seventy-one ESBLproducing *E. coli*, *K. pneumoniae* and *P. mirabilis* were selected based on NCCLS screening MIC criteria of  $\ge 2 \mu g/ml$  for aztreonam or ceftriaxone or ceftazidime with a confirmed MIC reduction of  $\geq$  eight-fold when combined with clavulanic acid. Fifty-four Amp C-producing *Citrobacter* spp., *Enterobacter* spp. and *S. marcescens* had MIC values of > 16 µg/ml for ceftazidime. Seventy-four carbapenem-resistant Acinetobacter spp., Enterobacter spp., S. marcescens, and P. aeruginosa were tested. Fifteen P. aeruginosa had proven MßL enzymes (IMP, VIM or SPM) by carbapenem hydrolysis assays, EDTA inhibition studies and gene sequencing. In addition, 17 clinical strains having multiple ß-lactamases were tested and these enzymes included TEM, SHV, CMY, OXA and CTX-M types. Five BLNAR H. influenzae, 23 penicillinresistant S. pneumoniae, 13 penicillin-resistant viridans group, 16 MRSA and 34 MR-CoNS were selected based on NCCLS resistance guidelines.

In the broth microdilution method, the organisms were tested against more than 20 antimicrobial agents using custom panels manufactured by TREK Diagnostics, Inc. (Cleveland, OH) according to NCCLS [2003] methods. The agar dilution method was utilized to test doripenem provided by Peninsula Pharmaceuticals Inc and ertapenem from Merck. Media were supplemented with defibrinated sheep blood for Streptococcus spp. testing and HTM with NAD supplement was used for *H. influenzae* testing. Fastidious organisms were incubated for 20 - 24 hours in 5% CO<sub>2</sub>. The appropriate ATCC QC strains (*E. coli* 25922, *P. aeruginosa* ATCC 27853, *S. pneumoniae* ATCC 49619, *E. faecalis* ATCC 29212 and S. aureus ATCC 25923) were utilized for all tests. An arbitrary susceptibility breakpoint for doripenem of  $\leq 4 \mu g/ml$  was used to compare carbapenem agents.

# Doripenem (S-4661), Antimicrobial Activity Tested Against **Drug-Resistant Pathogens**

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Table 1.	Antimicrobial activity of fo	our carbapenen	ns and 4 or 5 oth	her selected compa	rison agents teste	ed against 201
	Gram-negative organish			ce mechanisms.	0( by as	to com b
Organism (no. tested)	Antimicrobial	50%		Range	% Dy Ca Suscentible	Resistant
	Doripenem	<0.015	90%		100 0(<4)	
ESBL-producing (29) <sup>a</sup>	Ertapenem	0.03	0.25	<0.015-2	100.0	0.0
<u> </u>	Imipenem	0.12	0.5	≤0.06-0.5	100.0	0.0
	Meropenem	≤0.06	≤0.06	≤0.06-0.25	100.0	0.0
	Piperacillin/Tazobactam	2	>64	≤0.5->64	79.3	10.3
	Cefoxitin	4	16	2->32	79.3	6.8
	Cefepime	4	>16	0.25->16	72.4	24.1
		0.12	>2	≤0.03->2	58.6	41.4
K nneumoniae	Amikacin	2	>32	≤0.25->32	100.0(~4)	0.0(>16)
ESBL-producing (34) <sup>a</sup>	Ertapenem	0.06	0.25	<0.015-0.5	100.0(≤+)	0.0
<u>p.c.c.g.(c.)</u>	Imipenem	0.12	0.25	≤0.06-0.5	100.0	0.0
	Meropenem	≤0.06	0.12	≤0.06-1	100.0	0.0
	Piperacillin/Tazobactam	16	>64	2->64	67.6	26.5
	Cefoxitin	4	16	2->32	70.6	8.8
	Cefepime	4	>16	0.5->16	73.5	17.6
	Ciprofloxacin	≤0.03	>2	≤0.03->2	76.5	14.7
P mirabilis	Amikacin	0.12	>32	1->32	73.5	14.7
FSBL-producing (11) <sup>a</sup>	Ertapenem	<0.12	0.23	<0.00-0.23	100.0(≤4)	0.0(210)
		1	2	0.5-2	100.0	0.0
	Meropenem	≤0.06	0.12	≤0.06-0.12	100.0	0.0
	Piperacillin/Tazobactam	2	16	≤0.5->64	90.9	9.1
	Cefoxitin	8	16	2-16	81.8	0.0
	Cefepime	16	>16	0.25->16	36.4	45.5
	Ciprofloxacin	>2	>2	≤0.03->2	9.1	63.7
Citrabactarann	Amikacin	32	>32	2->32	45.5	45.5
Citrobacter spp.,	Ertapenem	0.03	0.06	0.03-0.12	100.0(≤4)	0.0(≥16)
	Imipenem	0.23	1	0.25-4	100.0	0.0
	Meropenem	≤0.06	0.12	≤0.06-0.12	100.0	0.0
	Piperacillin/Tazobactam	32	64	4->64	18.2	9.1
	Cefepime	1	2	0.5-4	100.0	0.0
	Ciprofloxacin	0.5	>2	≤0.03->2	72.7	18.2
	Amikacin	2	2	1-4	100.0	0.0
Enterobacter spp.,	Doripenem	0.06	0.12	≤0.015-4	100.0(≤4)	0.0(≥16)
<u>Cenazialme-R (33)</u>		0.5	4	≤0.015-8 0.12-8	87.9	9.1
	Meropenem	0.12	0.25	<0.06-4	100.0	0.0
	Piperacillin/Tazobactam	64	>64	≤0.5->64	15.2	36.4
	Cefepime	2	4	≤0.12-16	97.0	0.0
	Ciprofloxacin	1	>4	≤0.03->2	57.6	39.4
	Amikacin	2	>32	1->32	81.8	12.1
S. marcescens,	Doripenem	0.12	0.5	0.03-2	100.0(≤4)	0.0(≥16)
<u>Ceftazidime-R (10)</u>	Ertapenem	0.12	2	0.03-8	90.0	10.0
	Impenem	0.5	0.5	0.12-2	100.0	0.0
	Piperacillin/Tazobactam	<u>≤0.00</u>	>64	<u>≤0.00-2</u> 1->64	50.0	30.0
	Cefepime	4	>16	0.25->16	50.0	30.0
	Ciprofloxacin	>2	>2	0.03->2	30.0	60.0
	Amikacin	16	>32	2->32	60.0	30.0
Acinetobacter spp.,	Doripenem	8	>32	1->32	20.8(≤4)	50.0(≥16)
<u>Carbapenem-R (24)</u> c	Ertapenem	>32	>32	4->32	0.0	95.8
	Imipenem	>8	>8	2->8	16.7	83.3
	Riporacillin/Tazobactam	>8	>8	2->8	4.2	75.0
	Cefepime	>16	>16	8->16	4.2	79.2
	Ciprofloxacin	>4	>4	0.5->4	8.3	87.5
	Amikacin	>32	>32	2->32	25.0	62.5
P. aeruginosa,	Doripenem	8	>32	0.5->32	29.4(≤4)	29.4(≥16)
Carbapenem-R (34) <sup>c</sup>	Ertapenem	>32	>32	8->32	0.0	100.0
	Imipenem	>8	>8	8->8	0.0	91.2
		>8	>8	0.5->8	2.9	67.6
	Piperacillin/ lazobactam	>64	>64	4->64	44.1	55.9
	Ciprofloxacin	>2	>10	0 12->2	29.4	41.Z 82.4
	Amikacin	>32	>32	2->32	44 1	55.9
P. aeruginosa,	Doripenem	>32	>32	4->32	6.7(≤4)	86.7(≥16)
<u>MßL-R (15)</u> <sup>d</sup>	Ertapenem	>32	>32	>32	0.0	100.0
	Imipenem	>8	>8	8->8	0.0	93.3
	Meropenem	>8	>8	8->8	0.0	93.3
	Piperacillin/Tazobactam	64	>64	8->64	53.3	46.7
	Aztreonam	16	>16	4->16	46.7	33.3
<ul> <li>a. ESBL as defined by the</li> <li>b. Susceptibility criteria of t</li> </ul>	he NCCLS [2003], if available.		c. Resistant a d. MßL = met	at ≥ το μg/mi to imipener allo-β-lactamases (IMP.	VIM or SPM).	

Figure 1:

type and CTX-type ß-lactamases.								
Enzyme genotype						MIC (μg		
TEM-1	SHV-1	CTX-M15	OXA-type <sup>a</sup>	CMY-type	No. tested	Doripenem	Ertapenem	
+	+	+	1	-	3	0.03-0.06	0.03-0.25	
+	-	+	1	-	5	≤0.015-0.03	0.03-0.5	
+	-	+	2	-	3	0.03	0.03-0.25	
+	-	+	-	-	2	0.03	0.03-0.06	
+	-	+	-	CMY-6	1	0.03	1	
-	+	+	2	-	1	≤0.015	0.06	
-	-	+	1	-	1	≤0.015	0.25	
NEW	-	+	1	-	1	0.03	0.03	
<ul> <li>Number indicates how many enzymes of this type were present and NEW = novel TEM ESBL enzyme (characterization pending).</li> </ul>								

Table 3.	Direct comparison of the doripenem potency and three other carbapenems tested against selected Gram-negative pathogen wild-type and resistant subsets.								
		Doripenem		Ertapenem		Imipenem		Meropenem	
Organism	(no. tested) <sup>a</sup>	MIC <sub>90</sub>	% ≤4	MIC <sub>90</sub>	% S	MIC <sub>90</sub>	% S	MIC <sub>90</sub>	% S
E. coli	WT (31)	≤0.015	100	≤0.015	100	0.25	100	≤0.06	100
	ESBL (29)	0.03	100	0.25	100	0.5	100	≤0.06	100
K. pneumoniae	WT (26)	0.03	100	0.03	100	0.25	100	≤0.06	100
	ESBL (34)	0.06	100	0.25	100	0.25	100	0.12	100
Enterobacter spp.	WT (35)	0.06	100	0.25	100	0.5	100	≤0.06	100
	AMP-C (33)	0.12	100	4	88	1	94	0.25	100
Serratia spp.	WT (24)	0.12	100	0.12	100	2	100	0.12	100
	AMP-C (10)	0.5	100	2	90	1	100	0.5	100
a. WT = wild-type, ESBL = extended spectrum ß-lactamase, and AMP-C = amp C cephalosporinase (hyper-expression with ceftazidime resistance).									

Table 4.       Activity of doripenem tested against other antimicrobial-resistant organism (193 strains).								
			MIC (µg/r	nl)	% by ca	tegory <sup>b</sup>		
Organism (no. tested)	Resistance phenotype <sup>a</sup>	50%	90%	Range	Susceptible	Resistant		
Corynebacterium spp. (10)	MDR	32	>32	0.03->32	40.0(≤4)	60.0(≥16)		
E. faecium (29)	MDR	>32	>32	0.06->32	3.4	86.2		
S. aureus (16)	MR	16	16	0.25-32	0.0	100.0		
CoNS (34)	MR	0.5	16	≤0.015->32	0.0	100.0		
S. pneumoniae (11)	CTX-R	0.5	1	0.5-2	100.0	0.0		
S. pneumoniae (23)	PEN-R	0.5	1	0.25-2	100.0	0.0		
viridans group strept (13)	PEN-R	2	4	0.25-4	100.0	0.0		
H. influenzae (5)	BLNAR	2	-	2-4	100.0	0.0		
H. influenzae (10)	IMP-R	0.5	0.5	0.12-1	100.0	0.0		
Enterobacter spp. (4)	CARB-R	4	-	2-16	75.0	25.0		
S. marcescens (2)	CARB-R	0.25	-	0.25-4	100.0	0.0		
S. maltophilia (36)	MDR	>32	>32	32->32	0.0	100.0		
<ul> <li>a. MDR = multi-drug resistant; MR = methicillin-resistant; CTX-R = ceftriaxone-resistant; PEN-R = penicillin MIC at ≥ 2 μg/ml; BLNAR = β-lactamase-negative ampicillin-resistant; IMP-R = imipenem MIC at ≥ 2 μg/ml; and CARB-R = carbapenem-resistant (MIC, ≥ 16 μg/ml).</li> <li>b. NCCLS interpretive criteria for other carbapenems with activity against <i>P. aeruginosa</i>.</li> </ul>								



of doripenem tested against othe	r antimicrobial-resistant	t organism (	(193 strains)
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### RESULTS

- for doripenem when directly compared to ertapenem (Table 2).
- piperacillin/tazobactam, cefepime, fluoroquinolones and aminoglycosides (Table 1).
- e.g. eight- to 16-fold MIC elevations for ß-lactamase-producing isolates.
- (86.2%), methicillin-resistant staphylococci, and *S. maltophilia* (100.0%), see Table 4.

### CONCLUSIONS

- adjusted doses)
- clinical trial evaluations.

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• Among 74 documented ESBL-producing *E. coli*, *K. pneumoniae* and *P. mirabilis* strains, the doripenem MIC<sub>90</sub> values ranged from 0.03 to 0.25 μg/ml. The highest MIC for doripenem was only 0.25 μg/ml, four- to eight-fold less than MICs for imipenem or meropenem (Table 1).

• Similarly, clinical strains with multiple ß-lactamases (TEM-1, SHV-1, CTX-M15, OXA-types or CMY-6) consistently had lower MIC values

• Amp C-producing species (*Citrobacter, Enterobacter, S. marcescens*) with resistant level MIC results for ceftazidime were most susceptible (MIC<sub>ao</sub>s, 0.06 - 0.5 µg/ml) to doripenem among the carbapenems, and markedly superior in total spectrum when compared to

• Enzyme stability of doripenem, imipenem and meropenem was demonstrated by comparing ESBL- and Amp C-producing strains to wildtype (WT) isolates of the same species (Table 3; ≤ four-fold MIC increase). This contrasts to apparent enzyme instability for ertapenem,

• Carbapenem-resistant Acinetobacter spp. and P. aeruginosa had decreased susceptibility to doripenem (29.4 - 50.0% resistant), but these resistance rates were lowest among all carbapenems (Table 1). As seen with other carbapenems, nearly all (86.7%) metallo-ß-lactamase (IMP, VIM, SPM)-producing *P. aeruginosa* strains were resistant to doripenem as were the tested *Corynebacterium* spp. (60.0%), *E. faecium* 

• Various resistant populations of *S. pneumoniae* and viridans group streptococci (CTX-R or PEN-R; Table 4) remained susceptible to doripenem (MICs,  $\leq 4 \mu g/ml$ ), as did the *H. influenzae* strains having BLNAR and "imipenem resistance" phenotypes (Table 4).

• Overall, doripenem had the widest spectrum of activity versus the tested organisms with various resistance phenotypes when directly compared to other available carbapenems, broad-spectrum ß-lactams, fluoroquinolones or aminoglycosides (Tables 1-4).

• Doripenem exhibited ß-lactamase stability against commonly occurring ESBL and Amp C strains, but remained inactive against S. maltophilia (L1 enzyme) and P. aeruginosa isolates producing a metallo-ß-lactamase.

• Doripenem consistently had more multi-drug-resistant isolates with MIC values at  $\leq$  4 or  $\leq$  8 µg/ml compared to other carbapenems (potential therapy at appropriate

• Doripenem demonstrated class resistance features for *E. faecium* and methicillinresistant staphylococci, but continues to show characteristics that warrant wider