Susceptibility of Imipenem-Susceptible but Meropenem-Resistant *bla*_{IMP-6}-Carrying *Enterobacteriaceae* to Various Antibacterials, Including the Siderophore Cephalosporin Cefiderocol

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Carbapenemase-producing *Enterobacteriaceae* (CPE) have been spreading worldwide and are a great concern among health care settings (1). Intriguingly, IMP-6 (encoded by the *bla*_{IMP-6} gene), one of the IMP-type metallo-carbapenemases, has been reported to confer the paradoxical imipenem-susceptible but meropenem-resistant (ISMR) phenotype to *Enterobacteriaceae* strains, and strains carrying *bla*_{IMP-6} have recently been isolated throughout Japan (2, 3). This phenotype might lead to misdiagnosis of CPE infections when imipenem is used for antimicrobial susceptibility testing, resulting in inappropriate antimicrobial use and failure to prevent nosocomial transmissions caused by this pathogen. The *bla*_{IMP-6} gene differs from the *bla*_{IMP-1} gene by one point mutation: adenine at nucleotide 640 in *bla*_{IMP-1} is replaced by guanine, corresponding to the amino acid substitution of glycine for serine (4). Although IMP-6 has weaker hydrolysis activity to imipenem as well as some kinds of β -lactams, such as benzylpenicillin and ceftazidime, than IMP-1 (5), CPE-carrying *bla*_{IMP-6} shows resistance to almost all the β -lactams except for imipenem, due to the concomitant production of CTX-M-2, an extended-spectrum β -lactamase (ESBL) (2, 3). Here, we investigated the antimicrobial activity of various antibacterials, including compounds recently marketed or currently under development, against the problematic ISMR strains carrying *bla*_{IMP-6}.

Eighty-two ISMR phenotype *Enterobacteriaceae* strains (29 *Escherichia coli*, 22 *Klebsiella pneumoniae*, 20 *Klebsiella oxytoca*, 9 *Enterobacter cloacae*, and 2 *Citrobacter freundii* strains) isolated in a tertiary hospital in Japan from 2010 to 2014 were used. The existence of *bla*_{IMP-6} (guanine at nucleotide position 640) was confirmed by allele-specific PCR in all test strains (4), and *bla*_{CTX-M-2-group} was detected by PCR in 80 of 82 strains (6). MIC was determined by the CLSI broth microdilution method (7), and the ISMR phenotype was defined as an imipenem MIC of ≤ 1 mg/liter and a meropenem MIC of ≥ 4 mg/liter (8). The MIC medium, iron-depleted cation-adjusted Mueller-Hinton broth prepared by treatment with Chelex (Bio-Rad, Hercules, CA), was used for cefiderocol (siderophore cephalosporin) (9).

The MIC₉₀s of cefepime, ceftolozane-tazobactam, ceftazidime-avibactam, and ciprofloxacin were ≥ 32 mg/liter, while the MIC₉₀s of colistin and amikacin were 0.5 and 8 mg/liter, respectively (Table 1). The two strains with resistance to colistin were *K. pneumoniae* and *E. cloacae* strains (MIC data by species are shown in the supplemental file). Recently marketed β -lactam- β -lactamase inhibitor combinations such as ceftolozane-tazobactam and ceftazidime-avibactam have been reported to be active against ESBL producers but inactive against metallo- β -lactamase producers (1, 10), and the results of

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TABLE 1 Susceptibility distribution of 82 imipenem-susceptible but meropenem-resistant *Enterobacteriaceae* strains carrying *bla*_{IMP-6}

Antibacterial(s)	No. of strains with indicated MIC (mg/liter)												MIC ₅₀ (mg/liter)	MIC ₉₀ (mg/liter)
	≤0.031	0.063	0.125	0.25	0.5	1	2	4	8	16	32	>32		
Imipenem			2	4	41	35							0.5	1
Meropenem								3	19	33	24	3	16	32
Cefepime						1	1	2	1		1	76	>32	>32
Cefiderocol	25	17	4	6	15	13	2						0.063	1
Ceftolozane-tazobactam ^a								1	4	7	25	45	>32	>32
Ceftazidime-avibactam ^a									6	13	28	35	32	>32
Aztreonam-avibactam ^a		30 ^b	26	18	8								0.125	0.25
Ciprofloxacin	27	4		2	4	1	2	15	3	7	10	7	4	32
Amikacin				2	24	25	22	8	1				2	8
Colistin		1	16	46	13	4				1		1	0.25	0.5

^aFixed concentration of 4 mg/liter.^bMIC, ≤0.063 mg/liter.

the present study were consistent with those reports, although the IMP-6 enzyme has remarkable substrate specificity changes. However, the antibacterials in the compound under development, cefiderocol and aztreonam-avibactam, showed MIC₉₀s of 1 and 0.25 mg/liter, respectively, against these ISMR strains, and there were no strains for which the MICs were ≥4 mg/liter. The reports that cefiderocol and aztreonam have quite low k_{cat}/K_m or relative V_{max}/K_m values for IMP-1 suggest that one reason for the good antimicrobial activities of both compounds is the low level of hydrolysis by IMP-6 as well as IMP-1 (5, 11, 12). Although colistin and amikacin showed potent activity against ISMR *Enterobacteriaceae* in this study, safety issues such as nephrotoxicity remain concerns in clinical settings (13, 14). Alternative treatment options for CPE infections, including those caused by IMP-6-producing strains, are needed.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at <https://doi.org/10.1128/AAC.00576-17>.

SUPPLEMENTAL FILE 1, XLSX file, 0.1 MB.

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