AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

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

Sachi Kanazawa, Takafumi Sato, Naoki Kohira, Tsukasa Ito-Horiyama, Masakatsu Tsuji, Yoshinori Yamano

Drug Discovery & Disease Research Laboratory, Shionogi & Co., Ltd., Osaka, Japan

KEYWORDS CPE, cefiderocol, IMP-6, ISMR

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 MIC_{90}s of cefepime, ceftolozane-tazobactam, ceftazidime-avibactam, and ciprofloxacin were ≥32 mg/liter, while the MIC_{90}s 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

Accepted manuscript posted online 24 April 2017

Citation Kanazawa S, Sato T, Kohira N, Ito-Horiyama T, Tsuji M, Yamano Y. 2017. Susceptibility of imipenem-susceptible but meropenem-resistant bla_{IMP-6}-carrying *Enterobacteriaceae* to various antibacterials, including the siderophore cephalosporin cefiderocol. Antimicrob Agents Chemother 61:e00576-17. https://doi.org/10.1128/AAC.00576-17.

Address correspondence to Takafumi Sato, takafumi.satou@shionogi.co.jp.

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

[a]Fixed concentration of 4 mg/liter.
[b]MIC, ≤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 MIC_{90}s 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.

ACKNOWLEDGMENTS

MIC data collection was supported by T. Hori and Y. Jinushi, Shionogi Techno-Advance Research Co., Ltd.

We thank A. Naito and T. Yamaguchi for useful advice.

All authors are employees of Shionogi & Co., Ltd., and we have no conflicts of interest to declare.

REFERENCES

1. Doi Y, Paterson DL. 2015. Carbapenemase-producing *Enterobacteriaceae*. Semin Respir Crit Care Med 36:74–84. https://doi.org/10.1055/s-0035-1544208.
2. Ohno Y, Nakamura A, Hashimoto E, Matsutani H, Abe N, Fukuda S, Hisashi K, Komatsu M, Nakamura F. 2017. Molecular epidemiology of carbapenemase-producing *Enterobacteriaceae* in a primary care hospital in Japan, 2010-2013. J Infect Chemother 23:224–229. https://doi.org/10.1016/j.jiac.2016.12.013.
3. Shigemoto N, Kuwahara R, Kayama S, Shimizu W, Onodera M, Yokozaki M, Hisatsune J, Kato F, Ohge H, Sugai M. 2012. Emergence in Japan of an imipenem-susceptible, meropenem-resistant *Klebsiella pneumoniae* carrying bla_{IMP-6}. Diagn Microbiol Infect Dis 72:109–112. https://doi.org/10.1016/j.diagmicrobio.2011.09.019.
4. Kayama S, Shigemoto N, Kuwahara R, Onodera M, Yokozaki M, Ohge H, Kato F, Hisatsune J, Sugai M. 2012. Rapid detection of bla_{IMP-6} by amplification refractory mutation system. J Microbiol Methods 88:182–184. https://doi.org/10.1016/j.mimet.2011.10.022.
5. Liu EM, Pegg KM, Oelschlaeger P. 2012. The sequence-activity relationship between metallo-β-lactamases IMP-1, IMP-6, and IMP-25 suggests an evolutionary adaptation to meropenem exposure. Antimicrob Agents Chemother 56:6403–6406. https://doi.org/10.1128/AAC.01440-12.
6. Sato T, Hara T, Horiyama T, Kanazawa S, Yamaguchi T, Maki H. 2015. Mechanism of resistance and antibacterial susceptibility in ESBL-phenotype *Klebsiella pneumoniae* and *Klebsiella oxytoca* isolated between 2000 and 2010 in Japan. J Med Microbiol 64:538–543. https://doi.org/10.1099/jmm.0.000057.
7. Clinical and Laboratory Standards Institute. 2012. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard—9th ed. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
8. Clinical and Laboratory Standards Institute. 2016. Performance standards for antimicrobial susceptibility testing, 26th ed. CLSI supplement M100S. Clinical and Laboratory Standards Institute, Wayne, PA.
9. Ito A, Nishikawa T, Matsumoto S, Yoshizawa H, Sato T, Nakamura R, Tsuji M, Yamano Y. 2016. Siderophore cephalosporin cefiderocol utilizes ferric iron transporter systems for antibacterial activity against *Pseudomonas aeruginosa*. Antimicrob Agents Chemother 60:7396–7401. https://doi.org/10.1128/AAC.01405-16.
10. Vasoo S, Cunningham SA, Cole NC, Kohner PC, Menon SR, Krause KM,

Harris KA, De PP, Koh TH, Patel R. 2015. *In vitro* activities of ceftazidime-avibactam, aztreonam-avibactam, and a panel of older and contemporary antimicrobial agents against carbapenemase-producing gram-negative bacilli. Antimicrob Agents Chemother 59:7842–7846. https://doi.org/10.1128/AAC.02019-15.

11. Osano E, Arakawa Y, Wacharotayankun R, Ohta M, Horii T, Ito H, Yoshimura F, Kato N. 1994. Molecular characterization of an enterobacterial metallo β-lactamase found in a clinical isolate of *Serratia marcescens* that shows imipenem resistance. Antimicrob Agents Chemother 38:71–78. https://doi.org/10.1128/AAC.38.1.71.
12. Ito-Horiyama T, Ishii Y, Ito A, Sato T, Nakamura R, Fukuhara N, Tsuji M, Yamano Y, Yamaguchi K, Tateda K. 2016. Stability of novel siderophore cephalosporin S-649266 against clinically relevant carbapenemases. Antimicrob Agents Chemother 60:4384–4386. https://doi.org/10.1128/AAC.03098-15.
13. Kelesidis T, Falagas ME. 2015. The safety of polymyxin antibiotics. Expert Opin Drug Saf 14:1687–1701. https://doi.org/10.1517/14740338.2015.1088520.
14. Boyer A, Gruson D, Bouchet S, Clouzeau B, Hoang-Nam B, Vargas F, Gilles H, Molimard M, Rogues A, Moore N. 2013. Aminoglycosides in septic shock. Drug Saf 36:217–230. https://doi.org/10.1007/s40264-013-0031-0.