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First Isolation of $bla_{\text{IMI-2}}$ in an *Enterobacter cloacae* Clinical Isolate from China

Carbapenem resistance mediated by acquired carbapenemases is a growing concern worldwide. Acquired class A carbapenemases of the NMC/IMI, SME, and KPC types remain infrequent in *Enterobacteriaceae* (2, 6–8), but some of them have increasingly been reported recently (10). IMI-1 was first described in 1996 in clinical isolates of *Enterobacter cloacae* (9). Recently, IMI-2, a variant that differs from IMI-1 by 2 amino acid residues, was found in a strain of *Enterobacter asburiae* isolates in U.S. rivers (1). In this study, we report on the detection of IMI-2 in a clinical isolate of *Enterobacter cloacae* from China.

An imipenem-resistant *E. cloacae* isolate (isolate 8) was obtained from the blood of a patient in our hospital in August 2001. The patient had previously been treated with ampicillin-sulbactam and cefotaxime. *E. cloacae* isolate 8 was resistant to imipenem, meropenem, and ertapenem; had intermediate resistance to cefotaxime and ceftazidime; and was susceptible to cefepime (Table 1). Conjugation was carried out by a broth method as previously described (11). Transconjugant clones were selected on Mueller-Hinton agar containing rifampin (256 mg/liter) and imipenem (4 mg/liter). Imipenem resistance was transferred to a transconjugant along with a plasmid with a size of about 80 kb. The MIC for the transconjugant (*Escherichia coli* C600E8) showed that it has resistance to carbapenems but not to expanded-spectrum cephalosporins (Table 1). The β-lactamase activity was determined by UV spectrophotometry with imipenem as a substrate (1, 5). In *E. coli* C600E8, expression of carbapenemase activity increased from 643 to 1,352 U/mg of protein upon exposure to imipenem (4 mg/liter) as an inducer. These results demonstrated that the β-lactamase was produced at a high basal level and that expression was very poorly inducible. Isoelectric focusing was performed according to a published protocol (3). The result demonstrated that *E. cloacae* 8 had 5 pI bands at pI 5.1, 5.4, 6.9, 8.1, and 8.6, while *E. coli* C600E8 had only one band at pI 8.1, which was inhibited by clavulanic acid.

TABLE 1. MICs of selected antimicrobial agents for various strains determined by Etest

β-Lactam	MIC (mg/liter)		
	E. cloacae 8	*E. coli*	
		C600	C600E8
Imipenem	64	0.38	64
Meropenem	32	0.08	16
Ertapenem	32	0.08	16
Cefepime	2	0.064	0.064
Ceftazidime	16	0.5	0.38
Cefotaxime	12	0.05	0.125
Cefoperazone	≥256	0.5	1.5
Cefoperazone-sulbactam	128	0.25	1
Piperacillin	≥256	32	64
Piperacillin-tazobactam	≥256	2	4
Ampicillin	≥256	16	≥256
Ampicillin-sulbactam	≥256	8	64

FIG. 1. Schematic representation of the structure of the region containing the $bla_{\text{IMI-R2}}$-$bla_{\text{IMI-2}}$ gene complex from the *E. cloacae* 8 plasmid. The two sides of the $bla_{\text{IMI-R2}}$-$bla_{\text{IMI-2}}$ gene are inverted repeat sequences. "Tn*903*-like" downstream is an incomplete open reading frame that is part of "Tn*903*-like" upstream.

The imipenem resistance gene was cloned into pGEM-T Easy (Promega, Madison, Wis.) as a 10.6-kb EcoRI fragment. A 10,629-bp stretch of DNA sequence was obtained by nucleotide sequencing on both strands (GenBank accession no. AY780889). In the cloned fragment, $bla_{\text{IMI-2}}$ is preceded by a gene encoding a protein 97% identical to ImiR (9). The regions flanking the $bla_{\text{IMI-R2}}$-$bla_{\text{IMI-2}}$ gene contain sequences related to Tn*903* (4) and IS*2* (Fig. 1). Their structure apparently differs from that previously reported to be flanking the $bla_{\text{IMI-R2}}$-$bla_{\text{IMI-2}}$ gene complex from *E. asburiae* (1). The transposable elements flanking the $bla_{\text{IMI-R2}}$-$bla_{\text{IMI-2}}$ gene complex could be involved in mobilization of the carbapenemase gene to enterobacterial plasmids.

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REFERENCES

1. **Aubron, C., L. Poirel, R. J. Ash, and P. Nordmann.** 2005. Carbapenemase-producing Enterobacteriaceae, U.S. rivers. Emerg. Infect. Dis. **11:**260–264.
2. **Bush, K., G. A. Jacoby, and A. A. Medeiros.** 1995. A functional classification scheme for β-lactamases and its correlation with molecular structure. Antimicrob. Agents Chemother. **39:**1211–1233.
3. **Coudron, P. E., E. S. Moland, and K. S. Thomson.** 2000. Occurrence and detection of AmpC β-lactamases among *Escherichia coli*, *Klebsiella pneumoniae*, and *Proteus mirabilis* isolates at a veterans medical center. J. Clin. Microbiol. **38:**1791–1796.
4. **Grindley, N. D., and C. M. Joyce.** 1980. Genetic and DNA sequence analysis of the kanamycin resistance transposon Tn903. Proc. Natl. Acad. Sci. USA **77:**7176–7180.
5. **Laurent, P., T. Naas, M. Guibert, E. B. Chaibi, R. Labia, and P. Nordmann.** 1999. Molecular and biochemical characterization of VEB-1, a novel class A extended-spectrum β-lactamase encoded by an *Escherichia coli* integron gene. Antimicrob. Agents Chemother. **43:**573–581.
6. **Naas, T., and N. Patrice.** 1994. Analysis of a carbapenem-hydrolyzing class A β-lactamase from Enterobacter cloacae and of its LysR-type regulatory protein. Proc. Natl. Acad. Sci. USA **91:**7693–7697.
7. **Nordmann, P., and L. Poirel.** 2002. Emerging carbapenemases in Gram-negative aerobes. Clin. Microbiol. Infect. **8:**321–331.
8. **Nordmann, P., and L. Poirel.** 2002. Acquired carbapenem-hydrolyzing β-lactamases and their genetic support. Curr. Pharm. Biotechnol. **3:**117–127.
9. **Rasmussen, B. A., K. Bush, D. Keeney, Y. Yang, R. Hare, C. O'Gara, and A. A. Medeiros.** 1996. Characterization of IMI-1 β-lactamase, a class A carbapenem-hydrolyzing enzyme from *Enterobacter cloacae*. Antimicrob. Agents Chemother. **40:**2080–2086.
10. **Woodford, N., P. M. Tierno, Jr., K. Young, L. Tysall, M.-F. I. Palepou, E. Ward, R. E. Painter, D. F. Suber, D. Shungu, L. L. Silver, K. Inglima, J. Kornblum, and D. M. Livermore.** 2004. Outbreak of *Klebsiella pneumoniae* producing a new carbapenem-hydrolyzing class A β-lactamase, KPC-3, in a New York medical center. Antimicrob. Agents Chemother. **48:**4793–4799.

11. **Yan, J.-J., W. C. Ko, and J.-J. Wu.** 2001. Identification of a plasmid encoding SHV-12, TEM-1, and a variant of IMP-2 metallo-β-lactamase, IMP-8, from a clinical isolate of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45:**2368–2371.

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