

First Isolation of *bla*_{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)		
	<i>E. cloacae</i> 8	<i>E. coli</i>	
		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

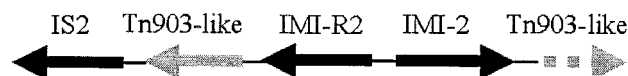


FIG. 1. Schematic representation of the structure of the region containing the *bla*_{IMI-R2}-*bla*_{IMI-2} gene complex from the *E. cloacae* 8 plasmid. The two sides of the *bla*_{IMI-R2}-*bla*_{IMI-2} gene are inverted repeat sequences. “Tn903-like” downstream is an incomplete open reading frame that is part of “Tn903-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*_{IMI-2} is preceded by a gene encoding a protein 97% identical to ImiR (9). The regions flanking the *bla*_{IMI-R2}-*bla*_{IMI-2} gene contain sequences related to Tn903 (4) and IS2 (Fig. 1). Their structure apparently differs from that previously reported to be flanking the *bla*_{IMI-R2}-*bla*_{IMI-2} gene complex from *E. asburiae* (1). The transposable elements flanking the *bla*_{IMI-R2}-*bla*_{IMI-2} gene complex could be involved in mobilization of the carbapenemase gene to enterobacterial plasmids.

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Yun-Song Yu*
Xiao-Xing Du
Zhi-Hui Zhou
Ya-Gang Chen
Lan-Juan Li

*The Key Lab of Infectious Diseases of
Public Health Ministry
The 1st Affiliated Hospital
Medical School
Zhejiang University
Zhejiang
Hangzhou, China*

*Phone: 86 571 8723 6756
Fax: 86 571 8723 6994
E-mail: yys119@163.com