ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 1997, p. 2589–2590 Vol. 41, No. 11
0066-4804/97/$04.00+0

Letters to the Editor

Characterization of *bla*$_{TEM-3}$ and *bla*$_{SHV-4}$ β-Lactamase-Encoding Genes in *Citrobacter diversus*

Citrobacter diversus (also called *Citrobacter koseri*) is an opportunistic pathogen which causes various infections (4). *C. diversus* is intrinsically sensitive to all antibiotics active against gram-negative bacilli, except for penicillins (7). Additional resistances, to β-lactam antibiotics in particular (2), have rarely been reported. Between 1989 and 1991, we isolated three clinical strains of *C. diversus* (Cd2, Cd3, and Cd4) resistant to most β-lactam agents, including expanded-spectrum cephalosporins and aztreonam (Table 1); β-lactamase inhibitors were highly synergistic with these antibiotics against the isolates. These isolates were also resistant to most aminoglycosides, chloramphenicol, sulphonamides, and trimethoprim.

β-Lactam resistance of the three *C. diversus* strains was transferable to *Escherichia coli* by conjugation or transformation. Analytical isoelectric focusing on polyacrylamide gels indicated that the three *C. diversus* isolates produced two types of β-lactamases: one enzyme with a pI of ~5 (pI of 4.8 for Cd2 and Cd3 and pI of 5.4 for Cd4), likely to be the species-specific constitutive penicillinase (7), and one additional enzyme with a pI of 6.3 (Cd2 and Cd3) or 7.8 (Cd4), the single β-lactamase synthesized by the transformants or the transconjugants, expected to be an extended-spectrum β-lactamase (ESBL) (Table 1). Plasmid DNA extracted from the transformants in *E. coli* (pCd2, pCd3, and pCd4) demonstrated the presence of a single large plasmid. Plasmids pCd2 and pCd3 (~150 kb) gave the same patterns after restriction by *Bgl*II and *Ssp*I; an internal *bla*$_{TEM-1}$ probe hybridized with the same *Bgl*II fragment (~20 kb). Plasmid pCd4 (between 40 and 60 kb) gave a distinct restriction pattern with both enzymes; an internal *bla*$_{SHV-3}$ probe hybridized with an *Ssp*I fragment (2.7 kb).

It is highly probable that the ESBL produced by Cd4 was an SHV-4 enzyme, as SHV-4 is the only SHV-derived enzyme with a pI of 7.8 described at present. However, the identity of the ESBL produced by Cd2 and Cd3 was more uncertain, since several TEM-derived ESBLs have a pI of 6.3 (TEM-3, TEM-18, and TEM-22) (8). Consequently, the structural genes of the ESBLs synthesized by Cd2 and Cd4 were amplified by polymerase chain reaction by using a set of oligonucleotide primers complementary to both strands of the TEM-1 (10) and the SHV-1 (5) genes and directly sequenced. The nucleotide sequence of the *bla*$_{TEM}$-encoding gene of Cd2 differed from that of the *bla*$_{TEM-1}$ gene (10) at seven nucleotide positions: four of them were silent and the remaining three caused substitutions of amino acids (Gln 39→Lys, Glu 104→Lys, Gly 238→Ser) (Table 1) as previously described for TEM-3 (9). The nucleotide sequence of the *bla*$_{SHV}$-encoding gene of Cd4 differed from that of the *bla*$_{SHV-1}$ gene by three base changes, all of which resulted in amino acid substitutions: Arg 205→Leu, Gly 238→Ser, and Glu 240→Lys; the *bla*$_{SHVpCd4}$ sequence was identical to that of *bla*$_{SHV-4}$ except for one silent mutation (G→A, Lys-240) (6) (Table 1).

The present study is the first genetic characterization of ESBLs in *C. diversus* and the first report of an SHV-type ESBL in this species.

REFERENCES

1. **Ambler, R. P., A. F. W. Coulson, J. M. Frère, J. M. Ghuysen, B. Joris, M. Forsman, R. C. Levesque, G. Tiraby, and S. G. Waley.** 1991. A standard numbering scheme for the class A β-lactamases. Biochem. J. **276:**269–272.
2. **Amicosante, G., A. Oratore, B. Joris, M. Galleni, J. M. Frère, and J. Van Beeumen.** 1988. Chromosome-encoded β-lactamases of *Citrobacter diversus*. Interaction with β-iodopenicillinate and labelling of the active site. Biochem. J. **254:**891–893.
3. **Goussard, S., and P. Courvalin.** 1991. Sequences of the genes *bla*T-1B and *bla*T-2. Gene **102:**71–73.
4. **Lipsky, A., E. W. Hook, A. A. Smith, and J. J. Plorde.** 1980. Citrobacter infections in humans: experience at the Seattle Veterans Administration Medical Center and a review of the literature. Rev. Infect. Dis. **2:**746–760.
5. **Mercier, J., and R. C. Levesque.** 1990. Cloning of SHV-2, OHIO-1, and

TABLE 1. MIC, pI, and nucleotide and amino acid substitutions in *bla*$_{TEM}$ and *bla*$_{SHV}$ genes

β-Lactamase	MIC[a] (mg/liter)			pI	Amino acid and substitution at position[b]:					Reference
	CTX	CAZ	ATM		39	104	205	238	240	
TEM-1	0.125	0.25	0.06	5.4	Gln GAG	Glu GAG	Gln CAA	Gly GGT	Glu GAG	10
TEM-2	0.125	0.25	0.125	5.6	Lys AAG					3
TEM-3	32	64	32	6.3	Lys AAG	Lys AAG		Ser AGT		9
TEM pCd2[c]	8	16	2	6.3	Lys **A**AG	Lys **A**AG		Ser **A**GT		This study
SHV-1	0.125	0.125	0.06	7.6	Gln CAG	Asp GAC	Arg CGG	Gly GGC	Glu GAG	5
SHV-4	32	128	256	7.8			Leu CTG	Ser AGC	Lys AAG	6
SHV pCd4[d]	4	32	32	7.8			Leu G**T**G	Ser **A**GC	Lys **AAA**	This study

[a] CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam.
[b] The number refers to the position of the amino acid according to Ambler et al. (1).
[c] From *C. diversus* Cd2.
[d] From *C. diversus* Cd4.

OXA-6 β-lactamases and cloning and sequencing of SHV-1 β-lactamase. Antimicrob. Agents Chemother. **34:**1577–1583.
6. **Péduzzi, J., M. Barthélémy, K. Tiwari, D. Mattioni, and R. Labia.** 1989. Structural features related to hydrolytic activity against ceftazidime of plasmid-mediated SHV-type CAZ-5 β-lactamase. Antimicrob. Agents Chemother. **33:**2160–2163.
7. **Philippon, A., G. Paul, M. Barthélémy, R. Labia, and P. Névot.** 1980. Properties of the beta-lactamase (penicillinase) produced by *Levinea malonatica*. FEMS Microbiol. Lett. **8:**191–194.
8. **Sirot, D.** 1995. Extended-spectrum plasmid-mediated β-lactamases. J. Antimicrob. Chemother. **36(**Suppl. A**):**19–34.
9. **Sougakoff, W., S. Goussard, and P. Courvalin.** 1988. The TEM-3 β-lactamase, which hydrolyzes broad-spectrum cephalosporins, is derived from the TEM-2 penicillinase by two amino acid substitutions. FEMS Microbiol. Lett. **56:**343–348.
10. **Sutcliffe, J. G.** 1978. Nucleotide sequence of the ampicillin resistance gene of *Escherichia coli* plasmid pBR322. Proc. Natl. Acad. Sci. USA **75:**3737–3741.

Z. El Harrif-Heraud
Laboratoire de Microbiologie
Hôpital Robert Boulin
112 cours de la Marne, BP 119
33550 Libourne, France
C. Arpin
C. Quentin
Laboratoire de Microbiologie
Université de Bordeaux 2
146 rue Léo Saignat
33076 Bordeaux Cedex, France