ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Feb. 2003, p. 755–758 Vol. 47, No. 2
0066-4804/03/$08.00+0 DOI: 10.1128/AAC.47.2.755–758.2003

Emergence in *Klebsiella pneumoniae* of a Chromosome-Encoded SHV β-Lactamase That Compromises the Efficacy of Imipenem

Laurent Poirel,[1] Claire Héritier,[1] Isabelle Podglajen,[2] Wladimir Sougakoff,[2] Laurent Gutmann,[2] and Patrice Nordmann[1]*

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Le Kremlin-Bicêtre,[1] and Laboratoire de Recherche Moléculaire sur les Antibiotiques, INSERM, Université Paris VI, Paris,[2] France

Received 19 June 2002/Returned for modification 8 September 2002/Accepted 10 November 2002

A *Klebsiella pneumoniae* isolate was identified that had reduced susceptibility to several expanded-spectrum cephalosporins and imipenem. That isolate produced a chromosome-encoded SHV-type β-lactamase, SHV-38, that had an alanine to valine substitution in position Ambler 146 compared to β-lactamase SHV-1. The kinetic parameters for purified β-lactamases SHV-38 and SHV-1 showed that the hydrolytic spectrum of SHV-38 included only ceftazidime and imipenem. This report is the first example of an SHV-type β-lactamase capable of hydrolyzing imipenem.

Extended-spectrum β-lactamase (ESBL) production is one of the main mechanisms of resistance to broad-spectrum β-lactam antibiotics among the *Enterobacteriaceae* (16). These enzymes are frequently encoded by conjugative plasmids and have evolved from the narrow-spectrum TEM-1 or TEM-2 and SHV-1 penicillinases through point mutations in regions critical for β-lactam binding and/or hydrolysis (16). They were reported recently as a plague of plasmids (7). ESBLs have spread worldwide, with prevalence and enterobacterial species distribution depending on the geographical situation (4, 16, 17). A surveillance study conducted across the United States indicated increased MICs (2 μg/ml or more) of ceftazidime for 15% of *Escherichia coli* and 24% of *Klebsiella pneumoniae* isolates, consistent with an ESBL phenotype (10).

Since the hydrolytic profile of ESBLs includes expanded-spectrum cephalosporins (cefotaxime, ceftazidime, cefepime, and cefpirome), the use of these cephalosporins for treating serious infections caused by an isolate with a confirmed ESBL phenotype should be avoided (12, 18). The mortality rate in patients who have such infections and who are treated with expanded-spectrum cephalosporins is high, and imipenem remains one of the very few β-lactams left for treating such infections (12, 18).

We report a novel point mutant derivative of the chromosome-encoded SHV-1 of *K. pneumoniae* that had a substrate profile extending not only to expanded-spectrum cephalosporins but also to imipenem.

MATERIALS AND METHODS

Bacterial strains. *K. pneumoniae* strain Lor-1 was a clinical isolate. *E. coli* DH10B (Life Technologies, Eragny, France) was used as a recipient strain in electroporation and for protein expression experiments. *E. coli* K-12 (a gift from P. Plésiat) and *K. pneumoniae* CIP53513 (Institut Pasteur Paris strain collection) were used as reference strains for endonuclease I-*Ceu*I digestion and susceptibility testing, respectively.

* Corresponding author. Mailing address: Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78, Rue du Général Leclerc, 94275 Le Kremlin-Bicêtre, France. Phone: 33 1 45 21 36 32. Fax: 33 1 45 21 63 40. E-mail: nordmann.patrice@bct.ap-hop-paris.fr.

Antimicrobial agents and resistance studies. The antimicrobial agents used and their sources were described previously (20). Antibiotic disks (Sanofi Diagnostics Pasteur, Marnes-la-Coquette, France) were used for routine antibiograms (http://www.sfm.asso.fr). MICs were determined by an agar dilution technique on Mueller-Hinton agar (Sanofi) with an inoculum of 10^4 CFU per spot (20).

Nucleic acid techniques. Whole-cell DNAs of *K. pneumoniae* Lor-1 and *E. coli* K-12 were extracted as described previously (20). Then, *Sau*3AI-digested DNA fragments of *K. pneumoniae* Lor-1 were ligated into the *Bam*HI site of phagemid pBK-CMV (20, 21). Recombinant plasmids were transformed by electroporation into *E. coli* DH10B (19). Antibiotic-resistant colonies were selected on Trypticase soy (TS) agar plates containing amoxicillin (100 μg/ml) and kanamycin (30 μg/ml). Recombinant plasmid DNAs were obtained from cultures of 100 ml of TS broth grown overnight in the presence of amoxicillin (100 μg/ml) at 37°C. Plasmid DNA was extracted and purified with a Qiagen (Courtaboeuf, France) plasmid DNA maxikit. Sequencing of the insert of one of the recombinant plasmids, pLP-1, which carried the novel β-lactamase gene bla_{SHV-38}, was performed and the results were analyzed as described previously (20). E xtraction of plasmid DNA from *K. pneumoniae* Lor-1 was attempted as previously reported (20).

A site-directed mutagenesis protocol was used as described by the manufacturer (Quick Change site-directed mutagenesis protocol; Stratagene, La Jolla, Calif.) for changing a T nucleotide to a C at position 425 of the gene to substitute a valine for an alanine residue at position Ambler 146 of the sequenced β-lactamase. Recombinant plasmid pLP-1 was used as a template with primer SHV-V146A1 (5′-CCACCGTCGGCGGCCCCGCAGGATTGACTGCC-3′) and primer SHV-V146A2 (5′-GGCAGTCAATCCTGCGGGGCCGACGGTGG-3′) to generate recombinant plasmid pLP-2. The insert of recombinant plasmid pLP-2 was also checked by sequencing.

To identify the chromosomal location of bla_{SHV-38} in *K. pneumoniae* Lor-1, endonuclease I-*Ceu*I (Ozyme; New England Biolabs, Saint-Quentin-en-Yvelines, France) was used to digest 26 bp of the *rrn* genes, coding for the 23S large-subunit rRNA (13). After digestion, separation of the resulting fragments by pulsed-field gel electrophoresis was performed with a CHEF-DRII apparatus (Bio-Rad) (24). The sizes of the endonuclease I-*Ceu*I-generated fragments were determined by comparison with those of *E. coli* K-12 (13). After transfer to a nylon membrane (Hybond N^+; Amersham Pharmacia Biotech, Orsay, France) according to the Southern technique (24), these fragments were UV cross-linked (Stratalinker; Stratagene Inc.) for 2 min. Then, they were hybridized with two different probes: a PCR-generated 1,502-bp probe for 16S rRNA genes of enterobacterial species (primers A and B) (2) and a PCR-generated 300-bp internal fragment of bla_{SHV-1} (20). Additionally, *Not*I- and *Hin*dIII-digested DNA of *K. pneumoniae* Lor-1 was subjected to gel electrophoresis as described previously (20), transferred by the Southern technique, and hybridized with the bla_{SHV-1} probe. A nonradioactive labeling and detection kit (enhanced chemiluminescence kit) was used (20).

The potential effect of the amino acid substitution found in SHV-38 on the protein structure was explored by using the Swiss PDB Viewer (8).

Biochemical analysis. Cultures of *E. coli* DH10B harboring recombinant plasmids pLP-1 (SHV-38) and pLP-2 (SHV-1) were grown overnight at 37°C in 4 liters of TS broth containing amoxicillin (100 μg/ml) and kanamycin (30 μg/ml). β-Lactamase extracts were obtained after sonication as described previously (20). They were dialyzed overnight against 20 mM Tris-HCl buffer (pH 7.9), loaded onto a Sepharose column preequilibrated with the same buffer, and eluted with a linear NaCl gradient (0 to 1 M). The fractions containing the highest β-lactamase activity were pooled and dialyzed overnight against 20 mM diethanolamine (pH 9.3) prior to a further purification step with a Q-Sepharose column. Final purification was obtained in two successive steps by using Centrisart (10 and 100 kDa; Sartorius, Göttingen, Germany). The protein contents and specific β-lactamase activities of the enzymes were determined as described previously (20). The purity of the enzymes was estimated by sodium dodecyl sulfate-polyacrylamide gel electrophoresis as described previously (24). Purified enzymes and β-lactamase extracts from cultures of *K. pneumoniae* Lor-1 were subjected to analytical isoelectric focusing (IEF) as previously described (20).

The kinetic parameters for purified β-lactamases SHV-38 and SHV-1 were determined by UV spectrophotometry as described previously (21). Briefly, k_{cat} and K_m values were determined by analyzing β-lactam hydrolysis under initial rate conditions by using the Eadie-Hofstee linearization of the Michaelis-Menten equation. In the presence of low K_m values, K_i values were determined by using amoxicillin as a β-lactam competitor. Various concentration of β-lactamase inhibitors were preincubated with the enzymes for 3 min at 30°C before the rate of benzylpenicillin (100 μmol) hydrolysis was tested. The 50% inhibitory concentrations of these inhibitors were determined as the concentrations of these inhibitors that inhibited hydrolytic activity by 50%. Results were expressed in micromolar units.

Nucleotide sequence accession number. The nucleotide sequence of bla_{SHV-38} has been assigned GenBank accession number AY079099.

TABLE 1. MICs of β-lactams for *K. pneumoniae* Lor-1, *E. coli* DH10B(pLP-1), *E. coli* DH10B(pLP-2), and *E. coli* DH10B (reference strain)

β-Lactam(s)[a]	MIC (μg/ml) for:			
	K. pneumoniae Lor-1 (SHV-38)	*E. coli* DH10B (pLP-1) (SHV-38)	*E. coli* DH10B (pLP-2) (SHV-1)	*E. coli* DH10B
SHV-38	SHV-1			
Amoxicillin	>512	>512	>512	4
Amoxicillin + CLA	>512	>512	64	4
Ticarcillin	>512	>512	>512	4
Ticarcillin + CLA	>512	>512	128	4
Piperacillin	>512	>512	>512	2
Piperacillin + TZB	>512	>512	256	2
Cephalothin	128	512	32	4
Cefuroxime	4	16	8	4
Cefoxitin	4	8	8	8
Ceftazidime	16	64	2	0.25
Ceftazidime + CLA	2	8	1	0.25
Cefotaxime	0.25	0.5	0.12	0.06
Ceftriaxone	0.12	0.25	0.06	0.06
Cefepime	2	4	0.25	0.03
Cefpirome	2	4	0.5	0.03
Cefpirome + CLA	0.12	0.5	0.06	0.03
Aztreonam	2	4	0.25	0.12
Moxalactam	1	2	0.25	0.06
Moxalactam + CLA	0.25	0.5	0.12	0.12
Imipenem	0.25	0.5	0.06	0.06
Meropenem	0.06	0.12	0.06	0.06

[a] CLA, clavulanic acid at a fixed concentration of 2 μg/ml; TZB, tazobactam at a fixed concentration of 4 μg/ml.

RESULTS

Clinical case. *K. pneumoniae* Lor-1 was isolated from a 61-year-old man hospitalized for pancreatitis in the surgical intensive care unit at the hospital Europeen Georges Pompidou (Paris, France) in February 2001. This isolate was from a blood culture and a digestive drain sample. The case history revealed previous treatment with amoxicillin-clavulanic acid, piperacillin-tazobactam, and gentamicin. Following the isolation of *K. pneumoniae* Lor-1, the patient was treated with cefotaxime and ciprofloxacin, to which the strain appeared susceptible in vitro, according to the results of disk diffusion testing. He has continued to do well after a 10-day antibiotic course.

Genetics. Recombinant plasmid pLP-1 was obtained by cloning of *Sau*3AI-restricted fragments of whole-cell DNA of *K. pneumoniae* Lor-1 into plasmid pBK-CMV. Sequencing of the 1,711-bp insert of pLP-1 identified an open reading frame that encoded an Ambler class A β-lactamase (1). The deduced protein, designated SHV-38, had one amino acid substitution (alanine to valine at position 146, according to the Ambler numbering) (1) compared to SHV-1 (3). Plasmid detection in *K. pneumoniae* Lor-1 failed. Hybridization of single *Hin*dIII and single *Not*I restriction fragments of whole-cell DNA of *K. pneumoniae* Lor-1 with a bla_{SHV-1} probe suggested that this isolate had a single copy of the bla_{SHV-38} gene (data not shown).

The location of bla_{SHV-38} was assessed by using the endonuclease I-*Ceu*I technique. Five fragments were generated from restricted DNA of *K. pneumoniae* Lor-1 (2,200, 1,000, 600, 400, and 100 kb). The DNA probe for rRNA genes hybridized with all endonuclease I-*Ceu*I fragments, except the 2,200-kb fragment, whereas the bla_{SHV} probe hybridized only with the 600-kb fragment. A chromosomal location of bla_{SHV-38} was thus indicated. Additionally, the nucleotide sequence of a ca. 900-bp sequence downstream of bla_{SHV-38} was identical to that reported for bla_{SHV-1} in *K. pneumoniae* (6), further indicating the chromosomal origin of bla_{SHV-38}.

A site-directed mutagenesis experiment with pLP-1 as the template yielded plasmid pLP-2, which contained the same insert as pLP-1, except for a T-to-C change leading to a Val-to-Ala change at Ambler position 146. Plasmid pLP-2 encoded β-lactamase SHV-1.

IEF analysis and susceptibility testing. IEF analysis revealed for *K. pneumoniae* Lor-1 and *E. coli* DH10B harboring pLP-1 (SHV-38) or pLP-2 (SHV-1) a single and identical β-lactamase with a pI of 7.6 (data not shown).

K. pneumoniae Lor-1 was resistant to amino-, carboxy-, and ureidopenicillins with or without β-lactamase inhibitors and to cephalothin and showed reduced susceptibility to several expanded-spectrum cephalosporins, moxalactam, the monobactam aztreonam, and imipenem (Table 1). Although low, the MIC of imipenem for this isolate was still higher than that for reference strain *K. pneumoniae* CIP53513 (0.06 μg/ml). The isolate was fully susceptible to cefotaxime and ceftriaxone and showed intermediate susceptibility to ceftazidime (Table 1).

Once cloned into a multicopy plasmid and expressed in *E. coli* DH10B(pLP-1), bla_{SHV-38} conferred a β-lactam resistance pattern similar to that found for *K. pneumoniae* Lor-1, but at a higher resistance level. The MICs of β-lactams with or without β-lactamase inhibitors for *E. coli* DH10B(pLP-2) encoding SHV-1 mirrored those for *E. coli* DH10B(pLP-1) encoding SHV-38, but at a lower level (Table 1). No reduced susceptibility to most expanded-spectrum cephalosporins and imipenem was observed when SHV-1 was expressed, except for

TABLE 2. Kinetic parameters for purified β-lactamase SHV-38 and purified SHV-1

Substrate	Value for[a]:					
	SHV-38			SHV-1		
	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)	k_{cat} (s^{-1})	K_m (μM)	k_{cat}/K_m ($mM^{-1} \cdot s^{-1}$)
Benzylpenicillin	100	13	7,700	455	20	23,000
Amoxicillin	1,800	35	51,000	900	90	10,000
Ticarcillin	10	14[b]	700	60	22[b]	2,700
Piperacillin	100	80	1,300	570	60	10,000
Cephaloridine	40	150	270	170	110	1,500
Cephalothin	5	100	50	10	26	400
Cefuroxime	0.5	280	2	0.5	100	5
Cefotaxime	1	800	1	NH	ND	ND
Ceftriaxone	60	1,260	50	>15	>3,000	>5
Ceftazidime	110	3,800	30	NH	ND	ND
Cefepime	3	1,600	2	>100	>3,000	>35
Cefpirome	3	600	5	>4,000	>120	>30
Aztreonam	3	5,500	0.5	NH	ND	ND
Imipenem	0.01	24	0.5	NH	ND	ND

[a] Values are expressed as the mean of three independent measures (standard deviations were within 15%). NH, not hydrolyzed (the initial rate of hydrolysis was lower than 0.01 $\mu M^{-1} \cdot s^{-1}$); ND, not determined due to a K_m value that was too high or undetectable hydrolysis.

[b] In the presence of a low K_m value, the K_i value was determined with amoxicillin as a competitor.

ceftazidime, as reported previously (18). In vitro resistance to inhibitors was observed with strains expressing SHV-1 and SHV-38, as reported for enterobacterial strains producing high levels of β-lactamase SHV-1 (18). The total activities of crude extracts of *E. coli* DH10B harboring plasmids pLP-1 (SHV-38) and pLP-2 (SHV-1) and of *K. pneumoniae* Lor-1 with 100 μM benzylpenicillin as a substrate were 150, 33, and 11 U per mg of protein, respectively. These results explain in part the differences in the MICs that have been observed.

Biochemical analysis. The specific activities of purified β-lactamases SHV-38 and SHV-1, measured with 100 μM benzylpenicillin as a substrate, were 20 and 100 U per mg of protein, respectively, with a 40-fold purification factor for each. The purity of both was estimated to be >90%. SHV-38 had strong activity against benzylpenicillin, amoxicillin, piperacillin, cephalothin, and cefuroxime (Table 2). Hydrolytic activity was also observed against expanded-spectrum cephalosporins and imipenem, whereas no activity was detected against cefoxitin, moxalactam, and meropenem. As expected (15, 25), SHV-1 had a narrow-spectrum hydrolytic profile, excluding expanded-spectrum cephalosporins (ceftazidime) and imipenem (Table 2).

The concentrations of β-lactamase inhibitors that reduced β-lactamase activity by 50% were similar for SHV-38 and SHV-1, i.e., 45 and 63 nM for clavulanic acid, respectively, 170 and 150 nM for tazobactam, respectively, and 5 μM for sulbactam for both. Based on its kinetic parameters, β-lactamase SHV-38 belongs to β-lactamase group 2be in the Bush-Jacoby-Medeiros classification (5).

DISCUSSION

We report here a novel β-lactamase that is structurally related to the narrow-spectrum penicillinase SHV-1. The bla_{SHV-38} gene, like bla_{SHV-1}, which is naturally present on the chromosome of *K. pneumoniae* (6), is also chromosome encoded.

The β-lactamase SHV-38 is, to the best of our knowledge, the first example of an SHV-1 derivative that has some carbapenem-hydrolyzing activity, considering that none of the plasmid-mediated extended-spectrum SHV and TEM derivatives that have spread worldwide has yet shown such an activity (4, 9, 16, 25). It is also the first example of a chromosome-encoded SHV-type enzyme with an extended hydrolysis profile that also includes, like the other ESBLs of the SHV type, some expanded-spectrum cephalosporins (9, 25).

Reduced susceptibility to carbapenems has been reported for *K. pneumoniae* and results from the acquisition of a plasmid-mediated class A carbapenem-hydrolyzing β-lactamase (KPC-1) (27); from metalloenzymes, which are distantly related to SHV-type β-lactamases (26); or from mechanisms combining decreased outer membrane permeability and the expression of plasmid-mediated AmpC-type β-lactamases and ESBLs (14). In none of these examples is the β-lactamase involved structurally related to the naturally expressed penicillinase of *K. pneumoniae*. Although quite low, the level of resistance to imipenem of SHV-38-producing *K. pneumoniae* Lor-1 was similar to that reported for several *K. pneumoniae* clinical isolates and *E. coli* reference strains that had metallocarbapenemase genes (22, 26). The penicillinase SHV-38 conferred some degree of resistance to moxalactam without detectable hydrolysis, a peculiar property for an ESBL that is shared by the ESBL TEM-52 (23). The kinetic parameters of SHV-38 and SHV-1 may explain in part the MICs obtained for SHV-38-producing strains. A difference in overall β-lactamase activity between SHV-38 and SHV-1 producers may explain the observed difference in the MICs of β-lactams as well as better in vivo stability of SHV-38.

The Ala146Val amino acid substitution has not been reported in any TEM- or SHV-type β-lactamases (http://www.lahey.org/studies/webt.htm). According to an analysis of the crystal structure of SHV-1 (11), this change does not occur in either the β-lactam binding site or the catalytic site of this enzyme. It is located at the N terminus of an alpha helix lying parallel to the region from positions 161 to 164 of the omega loop structure known to play a key role in β-lactam hydrolysis (11, 25). The way in which the Ala146Val substitution alters the behavior of the mutant enzyme remains unclear. Indeed, the side chain of residue 146 is oriented toward the solvent, so that it does not establish direct contacts with other residues in the protein. However, because it occupies a position highly exposed to the solvent, the side chain of Val-146, a hydrophobic residue preferentially found in the inside of proteins, may significantly disturb the conformation of the N-terminal part of the helix, with possible consequences for the neighboring omega loop located only 3.1 Å away.

Since the SHV-type enzymes, apart from being naturally chromosome encoded, may also be plasmid encoded (9, 25) and since *K. pneumoniae* is known to be an efficient vehicle for transmitting ESBL genes (4, 7, 16), it may also support the dispersion of this novel type of β-lactamase. Indeed, another patient hospitalized concomitantly in the same intensive care unit was found to be infected by the same SHV-38 producer. Finally, the emergence of SHV-38 may constitute a first step in the selection of higher-level carbapenem-resistant enterobac-

terial strains. Treatment of infections due to SHV-38-expressing strains with imipenem should probably be done with caution, since high inocula may be present at the infection site and may raise further the MIC of imipenem.

ACKNOWLEDGMENTS

We thank E. Collatz for critical reading of the manuscript.

This work was funded by a grant from the Ministères de l'Education Nationale et de la Recherche (UPRES-EA 3539), Université Paris XI, Paris, France.

REFERENCES

1. **Ambler, R. P., A. F. 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:**269–270.
2. **Avidor, B., Y. Kletter, S. Abulafia, Y. Golan, M. Ephros, and M. Giladi.** 1997. Molecular diagnosis of cat scratch disease: a two step approach. J. Clin. Microbiol. **35:**1924–1930.
3. **Barthélémy, M., J. Péduzzi, and R. Labia.** 1988. Complete amino acid sequence of p453-plasmid-mediated PIT-2 β-lactamase (SHV-1). Biochem. J. **251:**73–79.
4. **Bush, K.** 2001. New β-lactamases in gram-negative bacteria: diversity and impact on the selection of antimicrobial therapy. Clin. Infect. Dis. **32:**1085–1089.
5. **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.
6. **Chaves, J., M. G. Ladona, C. Segura, A. Coira, R. Reig, and C. Ampurdanes.** 2001. SHV-1 β-lactamase is mainly a chromosomally encoded species-specific enzyme in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45:** 2856–2861.
7. **Fierer, J., and D. Guiney.** 1999. Extended-spectrum β-lactamases: a plague of plasmids. JAMA **281:**563–564.
8. **Guex, N., and M. C. Peitsch.** 1997. Swiss-model and the Swiss-Pdb viewer: an environment for comparative protein modeling. Electrophoresis **18:**2714–2723.
9. **Heritage, J., F. H. M'Zali, D. Gascoyne-Binzi, and P. M. Hawkey.** 1999. Evolution and spread of SHV extended-spectrum β-lactamases in gram-negative bacteria. J. Antimicrob. Chemother. **44:**309–318.
10. **Jones, R. N., M. A. Pfaller, G. V. Doern, M. E. Erwin, R. J. Hollis, et al.** 1998. Antimicrobial activity and spectrum investigation of eight broad-spectrum β-lactam drugs: a 1997 surveillance trial in 102 medical centers in the United States. Diagn. Microbiol. Infect. Dis. **30:**215–228.
11. **Kuzin, A. P., M. Nukaga, Y. Nukaga, A. M. Hujer, R. A. Bonomo, and J. R. Knox.** 1999. Structure of the SHV-1 β-lactamase. Biochemistry **38:**5720–5727.
12. **Lautenbach, E., J. B. Patel, W. B. Bilker, P. H. Edelstein, and N. O. Fishman.** 2001. Extended-spectrum β-lactamase-producing *Escherichia coli* and *Klebsiella pneumoniae*: risk factors for infection and impact of resistance on outcomes. Clin. Infect. Dis. **32:**1162–1171.
13. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-*Ceu*-I, an intron-encoded endonuclease specific for genes for ribosomal RNA, in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. USA **90:**6874–6878.
14. **Martinez-Martinez, L., A. Pascual, S. Hernandez-Alles, D. Alvarez-Diaz, A. I. Suarez, J. Tran, V. J. Benedi, and G. A. Jacoby.** 1999. Roles of β-lactamases and porins in activities of carbapenems and cephalosporins against *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **43:**1669–1673.
15. **Matagne, A., J. Lamotte-Brasseur, and J. M. Frère.** 1998. Catalytic properties of class A β-lactamases: efficiency and diversity. Biochem. J. **330:**581–598.
16. **Medeiros, A. A.** 1997. Evolution and dissemination of β-lactamases accelerated by generation of β-lactam antibiotics. Clin. Infect. Dis. **24**(Suppl. 1)**:** S19-S45.
17. **Nordmann, P.** 1998. Trends in β-lactam resistance among *Enterobacteriaceae*. Clin. Infect. Dis. **27**(Suppl. 1)**:**S100-S106.
18. **Paterson, D. L., W. C. Ko, A. Von Gottberg, J. M. Casellas, L. Mulazimoglu, K. P. Klugman, R. A. Bonomo, L. B. Rice, J. G. McCormack, and V. L. Yu.** 2001. Outcome of cephalosporin treatment for serious infections due to apparently susceptible organisms producing extended-spectrum β-lactamases: implications for the clinical microbiology laboratory. J. Clin. Microbiol. **39:**2206–2212.
19. **Petit, A., H. Ben Yaghlane-Bouslama, L. Sofer, and R. Labia.** 1992. Does high level production of SHV-type penicillinase confer resistance to ceftazidime in *Enterobacteriaceae*? FEMS Microbiol. Lett. **71:**89–94.
20. **Poirel, L., M. Guibert, S. Bellais, T. Naas, and P. Nordmann.** 1999. Integron- and carbenicillinase-mediated reduced susceptibility to amoxicillin-clavulanic acid in isolates of multidrug-resistant *Salmonella enterica* serotype Typhimurium DT104 from French patients. Antimicrob. Agents Chemother. **43:**1098–1104.
21. **Poirel, L., I. Le Thomas, T. Naas, A. Karim, and P. Nordmann.** 2000. Biochemical sequence analyses of GES-1, a novel class A extended-spectrum β-lactamase, and the class 1 integron In52 from *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **44:**622–632.
22. **Poirel, L., T. Naas, D. Nicolas, L. Collet, S. Bellais, J. D. Cavallo, and P. Nordmann.** 2000. Characterization of VIM-2, a carbapenem-hydrolyzing metallo-β-lactamase and its plasmid- and integron-borne gene from a *Pseudomonas aeruginosa* clinical isolate in France. Antimicrob. Agents Chemother. **44:**891–897.
23. **Poyart, C., P. Mugnier, G. Quesne, P. Berche, and P. Trieu-Cuot.** 1998. A novel extended-spectrum TEM-type β-lactamase (TEM-52) associated with decreased susceptibility to moxalactam in *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **42:**108–113.
24. **Sambrook, J., E. F. Fritsch, and T. Maniatis.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
25. **Tzouvelekis, L. S., and R. A Bonomo.** 1999. SHV-type β-lactamases. Curr. Pharm. Des. **5:**847–864.
26. **Yan, J. J., W. C. Ko, S. H. Tsai, H. M. Wu, and J. J. Wu.** 2001. Oubreak of infection with multidrug-resistant *Klebsiella pneumoniae* carrying bla_{IMP-8} in a university medical center in Taiwan. J. Clin. Microbiol. **39:**4433–4439.
27. **Yigit, H., A. M. Queenan, G. J. Anderson, A. Domenech-Sanchez, J. W. Biddle, C. D. Steward, S. Alberti, K. Bush, and F. C. Tenover.** 2001. Novel carbapenem-hydrolyzing β-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45:**1151–1161.