ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2004, p. 4438–4440 Vol. 48, No. 11
0066-4804/04/$08.00+0 DOI: 10.1128/AAC.48.11.4438–4440.2004

Plasmid-Mediated Carbapenem-Hydrolyzing Enzyme KPC-2 in an *Enterobacter* sp.

Ashfaque Hossain,[1,2] M. J. Ferraro,[3] R. M. Pino,[3] R. B. Dew, III,[3] E. S. Moland,[1,2] T. J. Lockhart,[1,2] K. S. Thomson,[1,2] R. V. Goering,[2] and N. D. Hanson[1,2]*

Center for Research in Anti-Infectives and Biotechnology,[1] and Department of Medical Microbiology and Immunology,[2] Creighton University School of Medicine, Omaha, Nebraska, and Massachusetts General Hospital, Boston, Massachusetts[3]

Received 12 February 2004/Returned for modification 16 May 2004/Accepted 6 July 2004

A strain of an *Enterobacter* sp. with reduced susceptibility to imipenem, which produced a plasmid-mediated class A carbapenem-hydrolyzing enzyme, KPC-2 β-lactamase, was isolated from a patient with sepsis at a Boston hospital. This is the first report of the production of a plasmid-encoded KPC-2 β-lactamase by an *Enterobacter* sp.

Molecular class A carbapenem-hydrolyzing β-lactamases are clavulanate susceptible and may possess the ability to hydrolyze all anti-gram-negative β-lactam antibiotics, including carbapenems (3). Although rarely encountered in gram-negative clinical isolates, these enzymes are being reported with increasing frequency (5). The first of the KPC-type class A carbapenem-hydrolyzing enzymes, KPC-1, was named to indicate its occurrence in *Klebsiella pneumoniae* (11). To date, two related enzymes, KPC-2 and KPC-3, have been described (4, 9, 12; K. Young, P. Tierno, Jr., L. Tysall, M. Palepou, R. Painter, D. Suber, D. Shungu, L. Silver, K. Inglima, J. Kornblum, N. Woodford, and D. Livermore, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-50, 2003).

KPC-2 differs from KPC-1 by a single amino acid change of serine to glycine at position 175, while KPC-3 differs from both KPC-1 and KPC-2 by a single amino acid substitution of tyrosine for histidine at position 272 (9; Young et al., 43rd ICAAC). Genes encoding these enzymes can be of either chromosomal or plasmidic origin (5, 9).

In recent years, *Enterobacter* spp. have been recognized as increasingly important opportunistic pathogens particularly in debilitated and hospitalized patients (8). In this report, we describe an *Enterobacter* strain with reduced susceptibility to imipenem cultured on multiple occasions from a patient with sepsis. The reduced imipenem susceptibility was associated with the plasmid-encoded carbapenem-hydrolyzing enzyme, KPC-2. It was of epidemiologic relevance that the patient had received prior therapy with multiple antibiotics but not a carbapenem.

In 2001, four isolates of a strain of an *Enterobacter* sp. were obtained from blood (three isolates) and sputum (one isolate) from a patient admitted to a Boston hospital with sepsis after endoscopic retrograde cholangiopancreatography. Prior to the detection of these isolates, the patient had a very complicated hospital course, including initial sepsis with *Escherichia coli* which cleared after treatment with the combination of ampicillin, gentamicin, and metronidazole, a regimen which was later switched to levofloxacin and gentamicin. The *Enterobacter* sepsis was treated with amikacin, ceftazidime, metronidazole, and quinupristin-dalfopristin. The patient died, with the cause of death being attributed to myocardial infarction secondary to gram-negative septicemia secondary to a perforated duodenum.

The isolates were identified as *Enterobacter cloacae* by using a Vitek Legacy system (bioMérieux, St Louis, Mo.) and as *Enterobacter asburiae* by using a Phoenix system (Becton Dickinson, Baltimore, Md.) and API 20E strips (bioMérieux). This ambiguity in identification by systems available to clinical microbiology laboratories was not resolved with the supplementary conventional test (methyl red test), by sequencing the chromosomal *ampC* β-lactamase gene, or by 16S rRNA gene sequencing (MIDI labs, Newark, Del.). The methyl red test was negative, indicating that the strain was *E. cloacae*. However, the sequence of the chromosomal *ampC* β-lactamase closely resembled that of the ACT-1 β-lactamase (97% similarity; GenBank accession no. U58495) and the chromosomal *ampC* gene of *E. asburiae* (94% similarity; GenBank accession no. AJ311172). The ACT-1 β-lactamase is considered to be derived from the chromosomal *amp*C β-lactamase of *E. asburiae* (7). Therefore, we finally decided to describe the strain as an *Enterobacter* sp. The closest match by 16S rRNA gene sequencing was *Enterobacter* sp. strain MS 412 (99% similarity, accession no. AY297788).

Antibiotic susceptibilities and β-lactamase phenotypes were determined by using TREK microdilution panels (Westlake, Ohio) (Table 1). Carbapenem-hydrolyzing β-lactamase activity and inhibition characteristics were determined by spectrophotometric hydrolysis assays using 100 μM imipenem as the substrate and 500 μM clavulanate and 250 μM EDTA as the inhibitors. Isoelectric focusing (IEF) was performed on polyacrylamide gels containing ampholines (pH range, 3.5 to 9.5) with crude β-lactamase extracts to determine isoelectric points (pIs) and general inhibitor characteristics. The genetic relatedness of the isolates was analyzed by pulse field gel electrophoresis with XbaI (10).

* Corresponding author. Mailing address: Department of Medical Microbiology and Immunology, Creighton University School of Medicine, 2500 California Plaza, Omaha, NE 68178. Phone: (402) 280-5837. Fax: (402) 280-1875. E-mail: ndhanson@creighton.edu.

TABLE 1. Characteristics of the strains used in this study

Strain	pI	KPC-2 PCR	Plasmid(s) (kb)	MIC (μg/ml) of[a]:									IMI hydrolysis[c]
				IMI	CPD	CPD/CV4	CAZ	CAZ/CV4	FEP	FEP/CV10	FOX	ATM	
Enterobacter sp. strain MGH 221	9.2, 6.9, 5.4	+	65, 9, 4.8, 4	8	>128	>32	>128	>32	32	8	>16	>128	37.59
E. coli J53AzR	ND[b]	−	None	0.25	2	0.5	0.12	0.25	≤0.06	≤0.03	≤4	<1	ND
E. coli J53AzR/KPC-2	6.9, 5.4	+	65	1	32	4	4	0.5	2	≤0.03	8	32	17.08

[a] IMI, imipenem; CPD, cefpodoxime; CPD/CV4, cefpodoxine-4 μg of clavulanate/ml; CAZ, ceftazidime; CAZ/CV4, ceftazidime-4 μg of clavulanate/ml; FEP, cefepime; FEP/CV10, cefepime-10 μg of clavulanate; FOX, cefoxitin; ATM, aztreonam.
[b] ND, not determined.
[c] Nanomoles of imipenem per minute per milligram of protein.

Plasmid DNA preparations were obtained by using an alkaline lysis technique (1). The conjugal transfer of plasmids was carried out in mixed broth cultures with the sodium azide-resistant *Escherichia coli* strain J53AzR as the recipient and Luria-Bertani agar plates containing 150 μg of sodium azide/ml and 2 μg of ertapenem/ml as selection media. The transconjugants were analyzed for plasmid content and by PCR for the presence of bla_{KPC-2}. Primers KPC1F (5′-GCTACACCTAGC TCCACCTTC-3′) and KPC3R (5′-TGGAGGGCCAATAGA TGATT-3′) designed from the bla_{KPC-1} gene (AF297554) were used for the detection and sequencing of the carbapenemase gene.

The *Enterobacter* sp. isolates exhibited elevated imipenem MICs of 8 μg/ml, which is intermediate by NCCLS interpretive criteria (Table 1). Crude enzyme preparations demonstrated carbapenem hydrolyzing activity in spectrophotometric hydrolysis assays. IEF showed three bands, focusing at 9.2, 6.9, and 5.4. The band with a pI value of 6.9 was not inhibited well by either cloxacillin or clavulanate and hydrolyzed 1 μg of imipenem/ml on IEF gel overlay (data not shown). KPC-specific PCR and sequencing demonstrated the presence of bla_{KPC-2} in the *Enterobacter* isolates. Each *Enterobacter* isolate had an identical plasmid profile, consisting of four plasmids of approximately 65, 9, 4.2, and 4.0 kb in size (Fig. 1). Pulse field gel electrophoresis profiles of the isolates were identical, suggesting that the isolates were probably of the same strain. Only the high-molecular-weight plasmid was transferred by conjugation. PCR and antibiotic susceptibility assays of the transconjugant indicated that bla_{KPC-2} was carried by the ~65-kb plasmid (Fig. 1). The transconjugant was susceptible to imipenem (MIC, 1 μg/ml). The specific activities of crude cell lysates from the *Enterobacter* strain and the *Escherichia coli* transconjugants with imipenem as substrate were 37.59 and 17.08 nmol/min/mg of protein, respectively (Table 1). These data indicated that production of the carbapenem-hydrolyzing enzyme KPC-2 contributed to the reduced imipenem susceptibility of the *Enterobacter* sp.

It is intriguing that the strain exhibited reduced susceptibility to imipenem even though the patient from whom the strain was isolated was not treated with a carbapenem. Certain class B carbapenemase enzymes are often physically associated with aminoglycoside resistance genes, and evolution of carbapenem resistance following treatment with aminoglycosides has been predicted (6; R. E. Mendes, J. Riberio, M. Castanheira, M. Toleman, H. S. Sader, R. N. Jones, and T. R. Walsh, Abstr. 43rd Intersci. Conf. Antimicrob. Agents Chemother., abstr. C1-661, 2003). However, it is not known whether such an association exists for class A carbapenemases, such as KPC-type enzymes. Another possible explanation is that therapy with other β-lactam agents was responsible. The occurrence of class A carbapenem-hydrolyzing enzymes in clinical isolates that predated the use of carbapenems suggested that other β-lactam antibiotics may also select for their spread in clinical environments (6).

A recent preliminary report indicated bla_{KPC-2} to be chromosomal in *E. cloacae* (S. Petrell, M. Renard, R. Bismuth, V. Jarlier, and W. Sougakoff, Abstr. 13th Eur. Congr. Clin. Microbiol. Infect. Dis., abstr. 0288, 2003). However, this is the first report of detection of the plasmid-encoded carbapenem-hydrolyzing enzyme KPC-2 in an *Enterobacter* sp. The detection of KPC-2 in *Klebsiella oxytoca*, *Escherichia coli*, *Salmonella enterica* serovar Typhimurium, and an *Enterobacter* sp. in a short period of time after the first detection of this enzyme in *K. pneumoniae* (4, 9, 12) indicates the potential of bla_{KPC-2} to spread among clinical enterobacteria. The detection of this resistance mechanism in an *Enterobacter* strain is particularly disturbing, because carbapenems are one of the few remaining therapies for infections caused by high-level AmpC- or

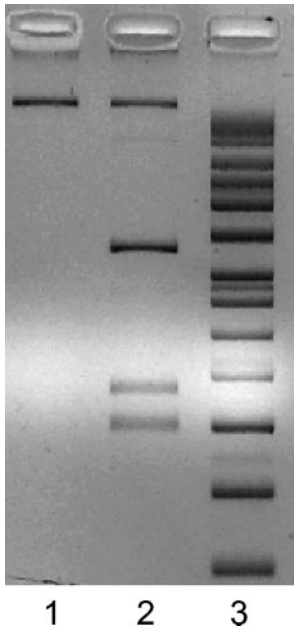

FIG. 1. Plasmid profiles. Lane 1, *Escherichia coli* transconjugant; lane 2, *Enterobacter* sp.; lane 3, supercoiled plasmid ladder (Invitrogen).

extended-spectrum β-lactamase-producing *Enterobacter* spp. (2). Loss of the carbapenems would jeopardize the therapy for serious infections caused by a major nosocomial pathogen. Studies to determine the prevalence, sources, and selection pressures responsible for the occurrence of this and other plasmid-encoded carbapenem-hydrolyzing β-lactamases among clinically relevant gram-negative organisms are urgently needed due to the importance of this drug class in the treatment of serious infections.

This work was supported by a grant from Merck Co., Inc.

REFERENCES

1. **Hanson, N. D., E. S. Moland, A. Hossain, S. A. Neville, I. B. Gosbell, and K. S. Thomson.** 2002. Unusual *Salmonella enterica* serotype Typhimurium isolate producing CMY-7, SHV-9 and OXA-30 β-lactamases. J. Antimicrob. Chemother. **49:**1011–1014.
2. **Jones, R. N., D. J. Biedenbach, and A. C. Gales.** 2003. Sustained activity and spectrum of selected extended-spectrum β-lactams (carbapenems and cefepime) against *Enterobacter* spp. and ESBL-producing *Klebsiella* spp.: report from the SENTRY antimicrobial surveillance program (USA, 1997–2000). Int. J. Antimicrob. Agents **21:**1–7.
3. **Livermore, D. M.** 2002. The impact of carbapenemases on antimicrobial development and therapy. Curr. Opin. Investig. Drugs **3:**218–224.
4. **Miriagou, V., L. S. Tzouvelekis, S. Rossiter, E. Tzelepi, F. J. Angulo, and J. M. Whichard.** 2003. Imipenem resistance in a *Salmonella* clinical strain due to plasmid-mediated class A carbapenemase KPC-2. Antimicrob. Agents Chemother. **47:**1297–1300.
5. **Poirel, L., and P. Nordmann.** 2002. Acquired carbapenem-hydrolyzing β-lactamases and their genetic support. Curr. Pharm. Biotechnol. **3:**117–127.
6. **Queenan, A. M., C. Torres-Viera, H. S. Gold, Y. Carmeli, G. M. Eliopoulos, R. C. Moellering, Jr., J. P. Quinn, J. Hindler, A. A. Medeiros, and K. Bush.** 2000. SME-type carbapenem-hydrolyzing class A β-lactamases from geographically diverse *Serratia marcescens* strains. Antimicrob. Agents Chemother. **44:**3035–3039.
7. **Rottman, M., Y. Benzerara, B. Hanau-Bercot, C. Bizet, A. Philippon, and G. Arlet.** 2002. Chromosomal *ampC* genes in *Enterobacter* species other than *Enterobacter cloacae*, and ancestral association of the ACT-1 plasmid-encoded cephalosporinase to *Enterobacter asburiae*. FEMS Microbiol. Lett. **210:**87–92.
8. **Sanders, W. E., Jr., and C. C. Sanders.** 1997. *Enterobacter* spp.: pathogens poised to flourish at the turn of the century. Clin. Microbiol. Rev. **10:**220–241.
9. **Smith Moland, E., N. D. Hanson, V. L. Herrera, J. A. Black, T. J. Lockhart, A. Hossain, J. A. Johnson, R. V. Goering, and K. S. Thomson.** 2003. Plasmid-mediated, carbapenem-hydrolysing β-lactamase, KPC-2, in *Klebsiella pneumoniae* isolates. J. Antimicrob. Chemother. **51:**711–714.
10. **Wiener, J., J. P. Quinn, P. A. Bradford, R. V. Goering, C. Nathan, K. Bush, and R. A. Weinstein.** 1999. Multiple antibiotic-resistant *Klebsiella* and *Escherichia coli* in nursing homes. JAMA **281:**517–523.
11. **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.
12. **Yigit, H., A. M. Queenan, J. K. Rasheed, J. W. Biddle, A. Domenech-Sanchez, S. Alberti, K. Bush, and F. C. Tenover.** 2003. Carbapenem-resistant strain of *Klebsiella oxytoca* harboring carbapenem-hydrolyzing β-lactamase KPC-2. Antimicrob. Agents Chemother. **47:**3881–3889.