ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2007, p. 1553–1555 Vol. 51, No. 4
0066-4804/07/$08.00+0 doi:10.1128/AAC.01405-06

First Identification of *Pseudomonas aeruginosa* Isolates Producing a KPC-Type Carbapenem-Hydrolyzing β-Lactamase▽

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Received 9 November 2006/Returned for modification 11 December 2006/Accepted 21 January 2007

In Medellin, Colombia, three *Pseudomonas aeruginosa* isolates with high-level carbapenem resistance (MIC ≥ 256 μg/ml) and an isolate of *Citrobacter freundii* with reduced susceptibility to imipenem produced the plasmid-mediated class A carbapenemase KPC-2. This is the first report of a KPC-type β-lactamase identified outside of the family *Enterobacteriaceae*.

The molecular class A carbapenemases of the KPC family (KPC-1 to -4) are a potent group of carbapenemases documented in numerous pathogens. While well identified in *Klebsiella pneumoniae*, these enzymes have been acknowledged throughout many members of the family *Enterobacteriaceae*, with reports in *Klebsiella oxytoca* (2, 27), *Salmonella enterica* (12), *Escherichia coli* (6, 14), *Citrobacter freundii* (6), *Enterobacter* spp. (3, 6, 7), and *Serratia marcescens* (6).

The KPC enzymes were once limited to sporadic occurrences in the eastern United States. They are now widespread in some facilities in New York and show an expanding geographic range (6). Sporadic identifications have also occurred on a global level, with reports of KPC-2 in four *E. coli* strains in Israel (14) and single isolates of *K. pneumoniae* from France (13) and China (24). We recently reported KPC-2 in two *K. pneumoniae* strains isolated from separate centers in Medellin, Colombia (22).

We now report isolates of *P. aeruginosa* and *C. freundii* producing KPC enzymes which were recovered from one of those same centers in Medellin, Colombia, where KPC-2 was identified in *K. pneumoniae* (22). Their identification in *Pseudomonas* represents the first report of KPC enzymes outside of the family *Enterobacteriaceae*.

(This work was presented in part at the 46th Annual Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., 2006, late breaker abstr. C2-421a.)

In January 2006, a tertiary care center in Medellin, Colombia, identified a *C. freundii* isolate resistant to imipenem by disk. The isolate was recovered from an aspirate of an intra-abdominal abscess. The patient responded clinically to drainage. In April, in the same facility, three imipenem-resistant isolates of *P. aeruginosa* were recovered. Two of the isolates were from patients with ventilator-associated pneumonia. They received colistin and both subsequently died. The third *P. aeruginosa* isolate was from a surgical wound and appeared to reflect only colonization. All four patients had prolonged lengths of hospital stay, extensive antibiotic exposure, and were hospitalized concurrently between January and April 2006. No apparent association with the United States could be established in these patients.

The submitting hospital participates as part of a bacterial resistance surveillance network in Colombia. Therefore, the isolates were sent to the research facility Centro Internacional de Entrenamiento e Investigaciones Medicas (CIDEIM) for determination of the responsible resistance mechanism. Susceptibility testing by broth microdilution (4) confirmed the clinical lab findings. All three *P. aeruginosa* isolates showed unusually high-level β-lactam and carbapenem resistance (MICs ≥ 256 μg/ml) (Table 1). The isolates remained susceptible only to amikacin and colistin. In the *C. freundii* isolate, frank resistance occurred to ertapenem (MIC, 16 μg/ml), while reduced susceptibility was noted for imipenem and meropenem (MIC, 8 μg/ml). A two- to fourfold decrease in carbapenem MICs was seen with the addition of clavulanic acid for the *Citrobacter* isolate. No change was noted with clavulanic acid in *P. aeruginosa*, suggesting contributions to the carbapenem resistance by additional resistance mechanisms. Absence of OprD in all three isolates (data not shown) was confirmed by Western blot analysis (15) of outer membrane proteins (16), using an OprD-specific mouse monoclonal antibody (YD12; gift of Naomasa Gotoh, Kyoto Pharmaceutical University, Kyoto, Japan). Alterations in OprD expression affect imipenem and meropenem susceptibilities (8). Additionally, meropenem is affected by efflux overexpression; however, contributions by this mechanism were not examined.

Chromosomal DNA was fingerprinted by pulsed-field gel electrophoresis with XbaI digestion (Invitrogen, Carlsbad, CA) (11). The *P. aeruginosa* isolates revealed identical banding patterns, indicating clonal spread (data not shown). The high-level β-lactam resistance in *Pseudomonas* suggested a metallo-β-lactamase, a group which is commonly responsible for high-level resistance and has been found to occur in Colombian *P. aeruginosa* (5, 23). However, a PCR analysis for bla_{VIM} and bla_{IMP} (20) using genomic DNA was negative.

β-Lactamases in the cell extract from each isolate were evaluated by isoelectric focusing (10), with multiple and varied

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▽ Published ahead of print on 29 January 2007.

TABLE 1. Antimicrobial susceptibility patterns of *P. aeruginosa* and *C. freundii* KPC-possessing clinical isolates

Antimicrobial agent(s)	MIC (μg/ml)			
	P. aeruginosa PA2404	*P. aeruginosa* PA4012	*P. aeruginosa* PA4036	*C. freundii* CF6010
Imipenem	256	>256	>256	8
Imipenem-CLA[a]	256	>256	>256	4
Meropenem	>256	>256	>256	8
Meropenem-CLA	>256	>256	>256	2
Ertapenem	>256	256	>256	16
Ampicillin	>256	>256	>256	>256
Ampicillin-SUL[b]	>256	>256	>256	>256
Piperacillin	>256	>256	>256	>256
Piperacillin-TZB[c]	>256	>256	>256	>256
Ceftazidime	256	256	256	32
Cefotaxime	>256	>256	>256	32
Ceftriaxone	>256	>256	>256	256
Cefepime	>256	>256	>256	8
Aztreonam	>256	>256	>256	128
Aztreonam-CLA	>256	>256	>256	128
Amikacin	16	16	16	2
Gentamicin	>128	>128	>128	>128
Tobramycin	>128	>128	>128	64
Tigecycline[d]	16	16	16	0.5
Colistin	1	2	1	0.5

[a] CLA, clavulanic acid; inhibitor tested at a fixed concentration of 2 μg/ml.
[b] SUL, sulbactam. Penicillin/inhibitor ratio, 2:1.
[c] TZB, tazobactam; inhibitor tested at a fixed concentration of 4 μg/ml.
[d] Tested in CA-MHB medium <12 h old.

β-lactamases (pI range, 5.0 to 8.5) identified. One common pI ~6.8 β-lactamase was identified in all isolates. A number of carbapenemases, including KPC-2 (22) and OXA-23 (J. N. Kattan, A. M. Guzman, A. Correa, S. Reyes, K. Lolans, N. Woodford, M. V. Villegas, J. P. Quinn and D. M. Livermore, Abstr. 46th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-598, 2006) have been identified within the institution providing the isolates; both β-lactamases have isoelectric points in the pI 6.8 range. Thus, PCR analysis was performed with KPC (26) and OXA (25) primers. Surprisingly, the three *Pseudomonas* isolates as well as the *C. freundii* isolate were positive for bla_{KPC}. Subsequent sequencing of the pCR-XL-TOPO (Invitrogen, Carlsbad, CA)-cloned amplicon from representative isolates (PA4036 and CF6010) confirmed bla_{KPC-2}.

Attempts to transfer β-lactam resistance from both the *P. aeruginosa* and *C. freundii* representative isolates to *E. coli* J53 Az^r by a mixed broth mating procedure (17) were unsuccessful. The genetic location for the bla_{KPC} gene was investigated. Southern transfer of EcoR1- or BamH1-digested plasmids to a positively charged nylon membrane (Roche, Indianapolis, IN) (19) was performed. In every isolate, the bla_{KPC-2} probe (22) hybridized to a single band under high-stringency conditions (Fig. 1B). The BamHI digestion indicated that while similar bla_{KPC}-containing plasmid fragments were identified among the *P. aeruginosa* isolates, the fragment differed in size from that in *C. freundii*. This difference in the plasmid profiles argues against conjugal transfer of a single plasmid between the species as facilitating the horizontal spread of this gene. A chromosomal location was investigated using the I-CeuI endonuclease technique (9). The KPC probe cohybridized with a single rRNA-hybridizing band from the representative *P. aeruginosa*, indicating a chromosomal location for this gene as well (Fig. 2, panel C). No similar hybridization of the KPC probe was identified for the *C. freundii* isolate. This is the first identification of a chromosomally encoded bla_{KPC}. Further analysis of these plasmids and the genetic context associated with bla_{KPC} is planned to further elucidate the mechanisms behind this gene dissemination.

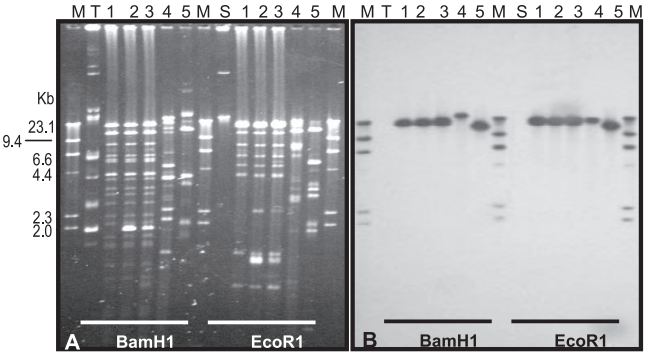

FIG. 1. Localization of the bla_{KPC-2} gene. (A) Electrophoretic profiles of plasmids digested with the indicated enzyme. (B) Hybridization with a bla_{KPC-2}-specific probe. Lanes: 1 to 3, *P. aeruginosa* PA2404, PA4012, and PA4036, respectively; 4, *C. freundii* CF6010; 5, KPN633 (positive control) (22); M, digoxygenin-labeled marker; T and S, TEM-10- and SHV-12-containing plasmids, respectively (negative controls).

Resistance rates for gram-negative bacilli tend to be much higher in Latin America than in the United States and Europe (18, 21). The carbapenems, retaining the broadest spectrum of activity of the β-lactams, are commonly considered the treatment of choice for infections resulting from multiresistant gram-negative organisms. *P. aeruginosa* possesses a larger array of resistance mechanisms than other organisms, which allows it to circumvent the activities of antimicrobial agents (1). The occurrence of the KPC enzyme in *Klebsiella* and *Citrobacter* and the emergence of an additional weapon in the pseudomonads' arsenal are serious concerns, especially as the future development of new antibiotics for gram-negatives looks bleak. The high-level carbapenem resistance seen in these *Pseudomonas* isolates, likely a result of both OprD loss and KPC production, may have facilitated the identification of the KPC enzyme. Accurate identification of carbapenem resis-

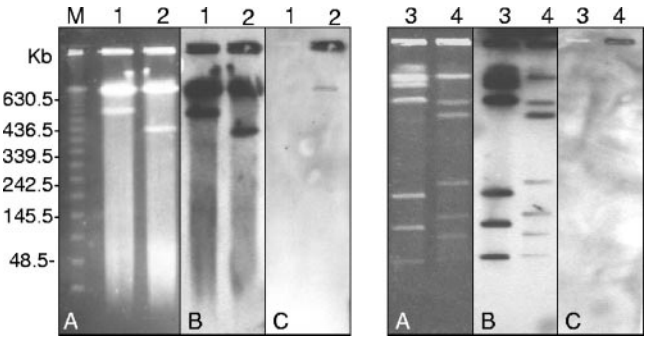

FIG. 2. Localization of the bla_{KPC} gene in I-CeuI-generated, pulsed-field gel electrophoresis-separated chromosome fragments of *P. aeruginosa* and *C. freundii*. (A) I-CeuI fragment restriction pattern. (B) Hybridization with a probe specific to 16S rRNA genes. (C) Hybridization with a probe specific to bla_{KPC}. Lanes: 1, *P. aeruginosa* ATCC 27853; 2, *P. aeruginosa* PA4036; 3, *C. freundii* CH32 (imipenem-sensitive isolate); 4, *C. freundii* CF6010; M, lambda DNA ladder standard with sizes (in kilobases) indicated on the left.

tance mechanisms will help determine the epidemiology, risk factors, and appropriate therapeutic options for such strains.

The conformation of the network of institutions that form the Colombian Nosocomial Resistance Study Group and funding for this study were made possible thanks in part to the support of the following: Bristol Myers Squibb, Colombia; Pfizer Inc., Groton, Conn.; Merck Research Laboratories, Whitehouse Station, N.J.; Baxter, Colombia; the Chicago Infectious Disease Research Institute, Chicago, Ill.

We thank Naomasa Gotoh for the kind gift of the OprD antibody and the participating institutions from the Colombian Nosocomial Resistance Study Group, whose members are as follows: CIDEIM, Ana María Guzmán and Sandra Lorena Reyes; Cali, Ernesto Martínez, Lena Barrera, Luz Marina Gallardo, Alba Lucía Bohorquez, and Nancy Villamarín; Bogotá, Carlos Alquichire, Aura Lucía Leal, Martha Ruiz, Mariluz Paez, Pilar Hurtado, Andrés Torres, Adela Cubides, Henry Mendoza, Alba Lucía Sanín, Nancy Botía Claudia Rodríguez, Sandra Reina, Martha Patricia Melendez, Sonia Cuervo, Jorge Cortés, María Cristina Paredes, and Patricia Arroyo; Medellín, Carlos Ignacio Goméz, Jaime López, Mónica Cuartas, Ana Lucía Correa, Jorge Donado, Julián Betancourth, Juan David Villa, Ana Cristina Quiroga, Jorge Nagales, Luz Teresita Correa, Eugenia Loaiza, Luz María Melguiza, Martha Vallejo, Rubén Darío Trejos, Victoria García, and Dora Rivas; Barranquilla, Ezequiel Guijarro, Rubén Darío Camargo, Adriana Marín, Angela Mendoza, and Ivan Zuluaga; Pereira, Carmen Elisa LLano, Araceli Cano, Martha Lucía Gómez, and Liliana Villa; Bucaramanga, Claudia Bárcenas, Adriana Pinto, and Luis Angel Villar; Ibague, Claudia Echeverry, Amparo Ovalle, and Araceli Vargas.

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