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Detection of KPC-2 in a Clinical Isolate of *Proteus mirabilis* and First Reported Description of Carbapenemase Resistance Caused by a KPC β-Lactamase in *P. mirabilis*▽

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An isolate of *Proteus mirabilis* recovered from blood cultures of a diabetic patient was shown to be resistant to imipenem, meropenem, and ertapenem by disk diffusion susceptibility testing. Amplification of whole-cell and/or plasmid DNA recovered from the isolate with primers specific for the bla_{KPC} carbapenemase gene produced an amplicon of the expected size which was confirmed to be bla_{KPC-2} by sequence analysis. Transformation of a susceptible *Escherichia coli* host with plasmid preparations from the isolate generated a transformant for which the MICs of all of the carbapenems tested were increased three- to fourfold. We believe this to be the first report of carbapenem resistance in *P. mirabilis* caused by the acquisition of bla_{KPC}.

bla_{KPC}-mediated carbapenem resistance among gram-negative bacteria is a relatively recent and emerging concern among health care practitioners in the United States. Chiefly limited to isolates of *Klebsiella pneumoniae* in initial surveillance reports (3–5), this carbapenem-hydrolyzing molecular class A β-lactamase has since been identified in a variety of *Enterobacteriaceae* including *Escherichia coli* (12), *Enterobacter* spp. (3, 7), *Citrobacter* spp.(7), *Salmonella* spp. (11), and *Serratia marcescens* (7), as well as *Pseudomonas aeruginosa* (16). The continued transfer of bla_{KPC} between bacterial genera presents a serious challenge to infection prevention and control personnel because the enzyme mediates resistance not only to carbapenems but to all β-lactam antimicrobial agents, including cephalosporins, cephamycins, penicillins, and monobactams (13). Further, infection with bla_{KPC}-producing bacteria is associated with higher patient mortality rates (4). Like many members of the family *Enterobacteriaceae*, *Proteus* spp. can harbor numerous plasmid- and integron-mediated antimicrobial resistance determinants (8, 9). Carbapenem resistance among *Proteus* spp. has previously been shown to be mediated by the OXA-23 molecular class D β-lactamase (2) or through porin mutation with or without decreased expression of penicillin binding proteins (15). Recently, Tsakris et al. (14) reported on the first isolate of *P. mirabilis* producing a VIM-1 molecular class B metallo-β-lactamase resulting in carbapenem resistance. Herein, we give what is believed to be the first report of a carbapenem-resistant isolate of *P. mirabilis* possessing a transmissible plasmid-mediated bla_{KPC-2} gene.

(This work was presented in part at the European Conference of Clinical Microbiology and Infectious Disease, Munich, Germany, 2007.)

The isolate of interest was presumptively identified as *P. mirabilis* on the basis of Gram staining, swarming motility on blood agar plates, inability to ferment lactose, and production of hydrogen sulfide, oxidase, and indole reactions, as well as phenotypic identification with the Vitek-2 identification system (bioMérieux, Marcy l'Etoile, France). Confirmation of the identification was accomplished by extended microbiological and biochemical techniques performed by the Missouri Department of Health Laboratory (Jefferson City). Antimicrobial susceptibility testing was performed by disk diffusion according to CLSI guidelines (6). In addition, MICs of ertapenem, meropenem, imipenem, and doripenem were determined with the Etest (AB Biodisk, Solna, Sweden). Inhibition of bla_{KPC} activity was evaluated by disk approximation analysis as described previously (1).

Plasmid isolation, PCR, transformation, and restriction digestion. Total plasmid DNA was extracted from overnight cultures of the study isolate, the Top 10 *E. coli* host strain (TT), and selected transformants with the Qiagen plasmid mini extraction kit according to the manufacturer's procedures (Qiagen, Hilden, Germany). The presence of bla_{KPC} was determined by conventional PCR with primers specific for bla_{KPC} as described previously (5). Transformants were selected on nutrient agar plates containing 4 μg/ml imipenem. PCR-generated fragments were cloned into pCR-II and transformed into a chemically competent TT host according to the manufacturer's protocols (Qiagen). Transformants were selected on nutrient agar plates with 100 μg/ml ampicillin and screened by conventional PCR as described above.

DNA sequencing and analysis. Sequencing of the bla_{KPC} PCR product cloned into pCR-II (TT/pPmir) was performed with primers F (5′-ATGTCACTGTATCGCCGTC-3′) and R (5′-CTCAGTGCTCTACAGAAAACC-3′) as described previously (16), with BigDye Terminator cycle sequencing kit v3.1 (Applied Biosystems, Inc., Foster City, CA) and an MJ Re-

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TABLE 1. MICs of the drugs tested in this study

Drug	MIC (μg/ml)			Fold change[d]
	P. mirabilis[a]	TT only[b]	TT/pPmir[c]	
Meropenem	>32	0.047	0.125	3
Ertapenem	>32	0.006	0.023	4
Imipenem	>32	0.38	1.5	4
Doripenem	>32	0.047	0.125	3

[a] KPC (pPmir)-positive *P. mirabilis*.
[b] Carbapenem-susceptible *E. coli* host strain.
[c] *E. coli* host strain transformed with pPmir from *P. mirabilis*.
[d] Fold difference between MICs for host strain and transformant.

search PTC-200 DNA Engine thermal cycler (Bio-Rad Laboratories, Waltham, MA). Sequencing reaction mixtures were purified by ethanol precipitation, separated, and analyzed with an ABI PRISM 3100 genetic analyzer (ABI, Foster City, CA) by following the manufacturer's protocols. Forward and reverse strands of two independent PCR products were each sequenced. Sequences were aligned and compared to published sequences of the bla_{KPC-2} gene with Vector NTI v10.3.0 software (Invitrogen, Carlsbad, CA).

The study isolate was recovered from one of three cultures of blood drawn from a 53-year-old patient with uncontrolled diabetes mellitus. The patient was noncompliant with diabetes management and had recently undergone bilateral amputation of the lower extremities for gangrene. Prior antimicrobial therapy included vancomycin, cefepime, metronidazole, ciprofloxacin, and clindamycin. However, the patient had not been previously treated with a carbapenem. Disk diffusion testing indicated that the organism was resistant to imipenem, ampicillin, and cefazolin. Additional analysis with the Vitek2 automated system indicated that this organism was resistant to ampicillin, piperacillin, cefazolin, cefuroxime, and ceftriaxone; intermediately resistant to cefepime; and susceptible to piperacillin-tazobactam and ciprofloxacin. Because our laboratory was currently performing a prospective analysis of all carbapenem-resistant *Enterobacteriaceae*, the isolate was further tested for the presence of the bla_{KPC} gene with conventional PCR assays. The patient was subsequently placed on piperacillin-tazobactam, followed by ciprofloxacin, and subsequent blood cultures were negative.

A single large plasmid (pPmir) was recovered from the study isolate (data not shown). Conventional and real-time (data not shown) PCR analyses of this plasmid, as well as total DNA, were positive for the presence of the bla_{KPC} gene. Additional antimicrobial susceptibility testing with E-strips demonstrated that the MICs of doripenem, meropenem, imipenem, and ertapenem were >32 μg/ml (Table 1). Following transformation of the TT host strain, a single colony resistant to 4 μg/ml imipenem was selected and subcultured. As indicated in Fig. 1, a fragment of the size expected was generated from the transformant but not from the TT host strain. MIC testing of the transformant indicated that there was a three- to fourfold increase in the MICs of doripenem, meropenem, imipenem, and ertapenem compared to those for the susceptible host strain (Table 1). In addition, disk approximation analysis indicated an inhibition of bla_{KPC} in the presence of clavulanate (data not shown).

Isolated pPmir and TT/pPmir were submitted for sequence analysis with primers specific for bla_{KPC}. A subsequent BLAST search showed that the amplified fragments were 100% homologous at the nucleic acid level (Fig. 2) and 100% identical at the amino acid level (Fig. 3) to the bla_{KPC-2} gene submitted to GenBank (accession no. AAK70220).

In this report, we describe what we believe to be the first reported case of infection caused by a strain of carbapenem-resistant *Proteus mirabilis* positive for the bla_{KPC-2} gene. While this finding is not totally unexpected, given the recent documented spread of bla_{KPC} carbapenemase to a number of genera within the family *Enterobacteriaceae* (3–5, 11, 12), it is a disturbing trend, given the relatively recent discovery of this family of β-lactamases. While extended-spectrum β-lactamase and carbapenemase activities have previously been documented in *Proteus* species (2, 10, 14, 15, 17), the addition of bla_{KPC} to the spectrum of resistance factors carried by an organism that traditionally has been placed in the low-level endogenous resistance category (similar to *Klebsiella* spp.) is equally troubling. It is possible that, as part of the normal gut flora, *Proteus* species may be unrecognized vectors for the dissemination of bla_{KPC} until, as in our case, they are identified as a clinically significant cause of infection. As a case in point, until recently, KPC-positive *K. pneumoniae* recovered from urine might have gone unrecognized in our laboratory as carbapenems were not routinely tested versus urinary tract isolates. However, a prospective study initiated at our institution following the identification of our first KPC-positive *K. pneumoniae* isolate showed that nearly 12% of the *K. pneumoniae* strains recovered in our laboratory from all sources were positive for bla_{KPC}. Most of these (64%) were urinary tract isolates with evidence of both clonal expansion and the introduction of unique, unrelated strains (data not shown). Currently, all of the carbapenem-resistant *Enterobacteriaceae* recovered in our laboratory are tested for the presence of bla_{KPC} and of these, 99.0% contain bla_{KPC}.

Our surveillance data indicate that bla_{KPC} disseminates rapidly within a health care environment—primarily among

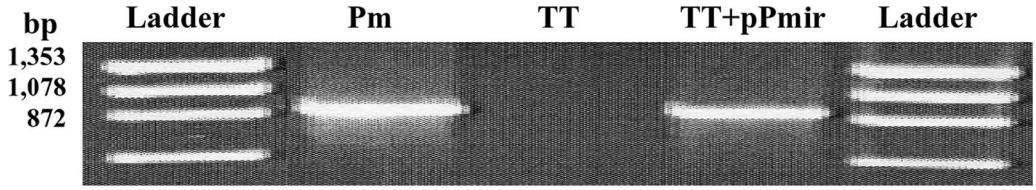

FIG. 1. KPC-specific PCR results generated from plasmid preparations of wild-type *P. mirabilis* (Pm), the TT host strain, and TT/pPmir. A fragment of the size expected (892 bp) was generated from wild-type *P. mirabilis* and the host strain transformed with the *P. mirabilis* KPC plasmid (pPmir) but not from the host strain.

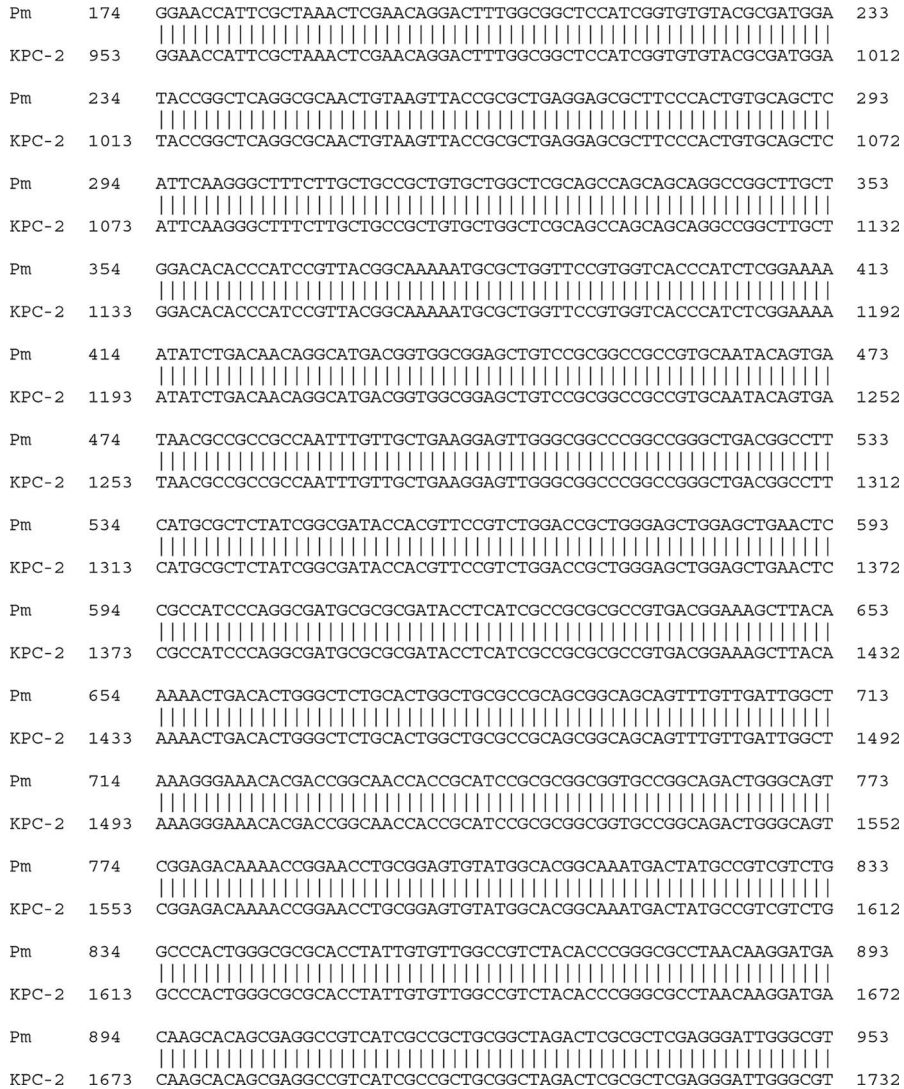

FIG. 2. PCR and sequencing of pPmir and a subsequent BLAST search determined that the PCR fragment generated showed 100% homology with the KPC-2 gene sequence submitted to GenBank.

strains of *K. pneumoniae*. However, we have also identified KPC-positive isolates of *Enterobacter cloacae*, *Citrobacter freundii*, *Klebsiella oxytoca*, and most recently, *P. mirabilis*. In an effort to curb this trend, we have begun routine screening of all gram-negative, carbapenem-resistant organisms for bla_{KPC} with a real-time PCR assay. If positive, results are conveyed to infection prevention personnel for appropriate precautions.

Although the carbapenem MICs for transformed *E. coli*

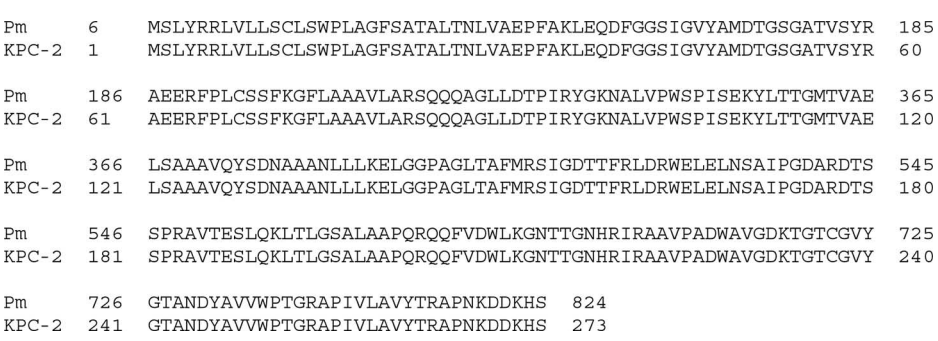

FIG. 3. PCR and sequencing of pPmir and a subsequent BLAST search determined that the PCR fragment generated shared 100% identity with the KPC-2 protein sequence submitted to GenBank.

were considerably lower than those for the donor strain of *P. mirabilis*, all were three- to fourfold higher than those for the TT host recipient strain. While this finding was somewhat perplexing, it was not extraordinary since similar results have been reported by others (12, 18). Several possibilities exist to explain this observation: (i) the promoter associated with pPmir has low affinity for *E. coli* RNA polymerase, (ii) pPmir has a much lower copy number in the *E. coli* host, and/or (iii) the *P. mirabilis* donor strain has multiple mechanisms of carbapenem resistance, i.e., porin mutation or genetic determinants that the *E. coli* host does not have. In terms of the latter, we were unable to demonstrate the presence of a metallo-β-lactamase with an EDTA disk approximation test (data not shown).

In conclusion, we give here what we believe to be the first description of a KPC-positive strain of *P. mirabilis*. Based on this finding, it would be prudent to routinely screen all clinically relevant isolates of *Enterobacteriaceae* for carbapenem resistance—even under circumstances where the use of this class of drug for the treatment of infection would be less likely, i.e., uncomplicated urinary tract infection.

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The mention of trade names or commercial products in this report is solely for the purpose of providing specific information and does not imply recommendation or endorsement by the U.S. Department of Agriculture.

REFERENCES

1. **Anderson, K. F., D. R. Lonsway, J. K. Rasheed, J. Biddle, B. Jensen, L. K. Mcdougal, R. B. Carey, A. Thompson, S. Stocker, B. Limbago, and J. B. Patel.** 2007. Evaluation of methods to identify the *Klebsiella pneumoniae* carbapenemase in *Enterobacteriaceae*. J. Clin. Microbiol. **45:**2723–2725.
2. **Bonnet, R., H. Marchandin, C. Chanal, D. Sirot, R. Labia, C. De Champs, E. Jumas-Bilak, and J. Sirot.** 2002. Chromosome-encoded class D β-lactamase OXA-23 in *Proteus mirabilis*. Antimicrob. Agents Chemother. **46:**2004–2006.
3. **Bratu, S., D. Landman, M. Alam, E. Tolentino, and J. Quale.** 2005. Detection of KPC carbapenem-hydrolyzing enzymes in *Enterobacter* spp. from Brooklyn, New York. Antimicrob. Agents Chemother. **49:**776–778.
4. **Bratu, S., M. Mooty, S. Nichani, D. Landman, C. Gullans, B. Pettinato, U. Karumudi, P. Tolaney, and J. Quale.** 2005. Emergence of KPC-possessing *Klebsiella pneumoniae* in Brooklyn, New York: epidemiology and recommendations for detection. Antimicrob. Agents Chemother. **49:**3018–3020.
5. **Bratu, S., P. Tolaney, U. Karumudi, J. Quale, M. Mooty, S. Nichani, and D. Landman.** 2005. Carbapenemase-producing *Klebsiella pneumoniae* in Brooklyn, N.Y.: molecular epidemiology and in vitro activity of polymyxin B and other agents. J. Antimicrob. Chemother. **56:**128–132.
6. **Clinical and Laboratory Standards Institute.** 2006. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically; approved standard. CLSI document M7-A7. Clinical and Laboratory Standards Institute, Wayne, PA
7. **Deshpande, L. M., P. R. Rhomberg, H. S. Sader, and R. N. Jones.** 2006. Emergence of serine carbapenemases (KPC and SME) among clinical strains of Enterobacteriaceae isolated in the United States medical centers: report from the MYSTIC program (1999-2005). Diagn. Microbiol. Infect. Dis. **56:**367–372.
8. **Giske, C. G., D. L. Monnet, O. Cars, and Y. Carmeli on Behalf of ReAct—Action on Antibiotic Resistance.** 2008. Clinical and economic impact of common multidrug-resistant gram-negative bacilli. Antimicrob. Agents Chemother. **52:**813–821.
9. **Hall, R. M., and C. M. Collis.** 1998. Antibiotic resistance in gram-negative bacteria: the role of gene cassettes and integrons. Drug Resist. Updates **1:**109–119.
10. **Luzzaro, F., M. Perilli, G. Amicosante, G. Lombardi, R. Belloni, A. Zollo, C. Bianchi, and A. Toniolo.** 2001. Properties of multidrug-resistant, ESBL-producing *Proteus mirabilis* isolates and possible role of β-lactam/β-lactamase inhibitor combinations. Int. J. Antimicrob. Agents **17:**131–135.
11. **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.
12. **Navon-Venezia, S., I. Chmelnitsky, A. Leavitt, M. J. Schwaber, D. Schwartz, and Y. Carmeli.** 2006. Plasmid-mediated imipenem-hydrolyzing enzyme KPC-2 among multiple carbapenem-resistant *Escherichia coli* clones in Israel. Antimicrob. Agents Chemother. **50:**3098–3101.
13. **Queenan, A. M., and K. Bush.** 2007. Carbapenemases: the versatile β-lactamases. Clin. Microbiol. Rev. **20:**440–458.
14. **Tsakris, A., A. Ikonomidis, A. Poulou, N. Spanakis, S. Pournaras, and F. Markou.** 2007. Transmission in the community of clonal *Proteus mirabilis* carrying VIM-1 metallo-β-lactamase. J. Antimicrob. Chemother. **60:**136–139.
15. **Villar, H., F. Danel, and D. Livermore.** 1997. Permeability to carbapenems of *Proteus mirabilis* mutants selected for resistance to imipenem or other beta-lactams. J. Antimicrob. Chemother. **40:**365–370.
16. **Villegas, M. V., K. Lolans, A. Correa, J. N. Kattan, J. A. Lopez, J. P. Quinn, and the Colombian Nosocomial Resistance Study Group.** 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing β-lactamase. Antimicrob. Agents Chemother. **51:**1553–1555.
17. **Vourli, S., H. Tsorlini, H. Katsifa, M. Polemis, L. S. Tzouvelekis, A. Kontodimou, and A. C. Vatopoulos.** 2006. Emergence of *Proteus mirabilis* carrying the bla_{VIM-1} metallo-β-lactamase gene. Clin. Microbiol. Infect. **12:**691–694.
18. **Zhang, R., H. W. Zhou, J. C. Cai, and G.-X. Chen.** 2007. Plasmid-mediated carbapenem-hydrolysing β-lactamase KPC-2 in carbapenem-resistant *Serratia marcescens* isolates from Hangzhou, China. J. Antimicrob. Chemother. **59:**574–576.