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Wide Dissemination of *Pseudomonas aeruginosa* Producing β-Lactamase bla_{KPC-2} Gene in Colombia▽

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Ten bla_{KPC-2}-harboring *Pseudomonas aeruginosa* isolates from hospitals located in five different Colombian cities have been characterized. Isolates were multidrug resistant, belonged to five different pulsotypes, and possessed naturally chromosome-encoded bla_{AmpC} and bla_{OXA-50} genes and the acquired bla_{KPC-2} gene. In most cases, the bla_{KPC-2} genes were carried by plasmids of different sizes and were associated with Tn*4401b* or a new structure containing only part of the Tn*4401* sequence. This study revealed that several clones of *P. aeruginosa* producing bla_{KPC-2} are disseminating in Colombia.

Carbapenem resistance in *Pseudomonas aeruginosa* isolates represents a major threat to the management of nosocomial infections and involves several mechanisms, such as porin modifications, efflux pump overexpression, and acquired carbapenem-hydrolyzing β-lactamases (carbapenemases) (10, 14). Carbapenemases identified in *P. aeruginosa* are mostly metallo-ß-lactamases (14), but the Ambler class A β-lactamase KPC has also been reported (17). KPC carbapenemases were described initially in a report of a *Klebsiella pneumoniae* isolate collected in 2001 in North Carolina (20) and have since rapidly emerged and disseminated in the world, in enterobacterial species in particular (4, 12). KPC-producing *P. aeruginosa* isolates were reported first in 2006 from Colombia (17) and subsequently in Puerto Rico (19), in Trinidad and Tobago (1), in the southern part of the United States (13), and very recently in China (6). The rapid spread of KPC enzymes in *Enterobacteriaceae* has been linked to the genetic elements carrying the bla_{KPC-2} gene: plasmids of different sizes harboring a Tn*3*-like transposon, Tn*4401* (4, 11). Nevertheless, other genetic structures have been characterized with different insertion se-

TABLE 1. Origin, structure of Tn*4401*, other β-lactamases, plasmid analysis, pulsotype, and sequence type of the *P. aeruginosa* isolates

Isolate	Origin in Columbia	Yr of isolation	Site of isolation	Location	PCR and sequencing result (Tn*4401* homology)				bla_{KPC-2}-positive plasmid				Bacterial isolate characteristic	
									Name	Size (kb)	Transformant		Pulsotype	Sequence type
					bla_{KPC-2}	*TnpA*	IS*KPN6*	IS*KPN7*			EC[a]	PA[b]		
PA-1	Medellin	2006	Bronchial secretion	Tertiary care center	KPC-2	+	+	+	pCOL	45	+	+	A1	ST308
PA-2	Bogota	2006	Stool	Unknown	KPC-2	−	+	−	pPA-2	13	+	+	B	ST1006
PA-3	Barranquiila	2006	Bronchial secretion	Intensive care unit	KPC-2	+	+	+	pPA-3	60	−	−	C	ST1060
PA-4	Medellin	2006	Bone	General ward[c]	KPC-2	+	+	+	pPA-4	45	+	+	A2	ST308
PA-5	Medellin	2006	Surgical wound	General ward	KPC-2	+	+	+	pPA-5	45	+	+	A2	ST308
PA-6	Medellin	2006	Surgical wound	General ward	KPC-2	+	+	+	pPA-6	45	+	+	A1	ST308
PA-7	Medellin	2006	Urine	General ward	KPC-2	+	+	+	pPA-7	45	+	+	A1	ST308
PA-8	Medellin	2006	Tracheal aspirate	Intensive care unit	KPC-2	+	+	+	pPA-8	45	+	+	A1	ST308
PA-9	Cali	2007	Peritoneal fluid	General ward	KPC-2	+	+	+	−	−	−	−	D	ST235
PA-10	Pereira	2010	Urine	Intensive care unit	KPC-2	+	+	+	−	−	−	−	E	ST235

[a] Transformants into *E. coli* (EC) DH10B.
[b] Transformants into *P. aeruginosa* (PA) KG2505.
[c] General ward: patients hospitalized for surgical or medical disease.

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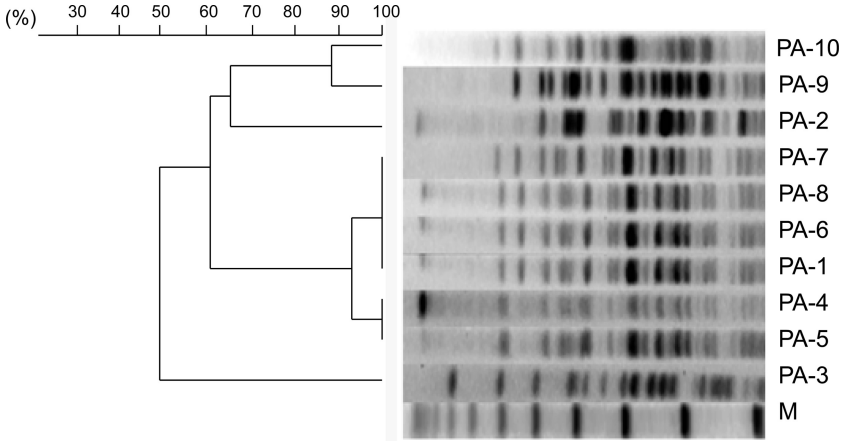

FIG. 1. Dendrogram of pulsed-field gel electrophoresis data (SpeI digests) showing genetic relatedness between the 10 KPC-producing reference isolates. M, molecular weight.

quences (IS) upstream of the bla_{KPC} gene, as seen in a *P. aeruginosa* isolate harboring bla_{KPC-5} (8, 19). Until now, little has been known about the clonal relationships and genetic background of KPC-producing *P. aeruginosa* strains. The aim of the present work was to characterize 10 bla_{KPC-2}-harboring *P. aeruginosa* isolates collected from Colombian hospitals located in different cities scattered throughout the country and sent to the International Center for Medical Research and Training (CIDEIM), Cali, Colombia, between 2006 and 2010.

Molecular typing by pulsed-field gel electrophoresis (PFGE) identified five pulsotypes (A to E) among the isolates (Table 1; Fig. 1). Two closely related patterns (A1 and A2), differing by only one band, were identified in isolates from three different wards in the same hospital in Medellin, Colombia. Four isolates belonged to clone A1, and two others belonged to clone A2. The other isolates were unrelated, differing by at least seven bands, thus suggesting heterogeneity among KPC-producing *P. aeruginosa* strains from Colombia. Four distinct allelic profiles were obtained by multilocus sequence type (MLST) analysis (Table 1) (3): ST308 (isolates PA-1 and PA-4 to -8), ST235 (isolates PA-9 and PA-10), ST1006 (isolate PA-2), and ST1060 (isolate PA-3). These results matched perfectly with the PFGE results. The four sequence types identified were not single- or double-locus variants of each other. Among them, only ST235 had already been identified as a single-locus variant of ST227 and ST230; all three correspond to clonal complex CC11 (7), which has been isolated in several countries and has been shown to be associated with various β-lactamases (5, 7). The dissemination of bla_{KPC} into genetically unrelated *P. aeruginosa* isolates has already been described in Puerto Rico (19), where seven different clones have been found, highlighting the rapid spread of the bla_{KPC} gene in this area.

Antibiotic susceptibility was determined by the disk diffusion

TABLE 2. Primers used in this study

Name of primer	Fig. 2 lane no.	Sequence (5′–3′)	Reference
KPC-A	1	CTGTCTTGTCTCTCATGGCC	11
KPC-B	2	CCTCGCTGTGCTTGTCATCC	11
3098U	3	TGACCCTGAGCGGCGAAAGC	11
4914L	4	GAAGATGCCAAGGTCAATGC	11
TnpA-178U	5	CTTGCAGATCGGCTACTTCA	This study
TnpA-1198U	6	CGCCATCTTCGTCCACTGTA	This study
TnpA-2537L	7	GCCCTGCGTCATTTCCTTCA	This study
ISKpn7-24U	8	CCCACCCCAGCGAAGTAAAAC	This study
ISKpn7-1916L	9	GACCCACTTTACCCCTGAAT	This study
ISKpn6-82U	10	CGTCGGCGCCAAGGATACCA	This study
ISKpn6-1429L	11	ATCTGCTGCCCCCTTCTCTG	This study
141R-6	12	TCACCGGCCCTCACCTTTGG	11
EcoRI-out	13	CACCCGACCTGGACGAACTA	11
Delta SHVa		AAGATCCACTATCGCCAGCAG	4
Delta SHVb		ATTCAGTTCCGTTTCCCAGCGG	4
Pre-TEM1		GTATCCGCTCATGAGACAATA	4
Pre-TEM2		TCTAAAGTATATATGAGTAAA CTTGGTCTG	4
CTX-MA		CGCTTTGCGATGTGCAG	4
CTX-MB		ACCGCGATATCGTTGGT	4
VIM-B		ATGGTGTTTGGTCGCATATC	11
VIM-F		TGGGCCATTCAGCCAGATC	1
IMP-2004A		ACAYGGYTTGGTDGTTCTTG	1
IMP-2004B		GGTTTAAYAAAACAACCACC	1
OXA-9A		TTCGTTTCCGCCACTCTCCC	4
OXA-9B		ACGAGAATATCCTCTCGTGC	4

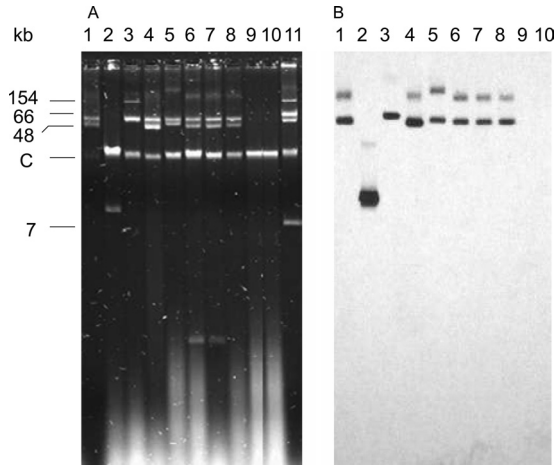

FIG. 2. (A) Plasmid extractions from cultures of the different isolates by the Kieser method. (B) Southern hybridization carried out with an internal probe for the bla_{KPC-2} gene. Lane 1, *P. aeruginosa* PA-1; lane 2, *P. aeruginosa* PA-2; lane 3, *P. aeruginosa* PA-3; lane 4, *P. aeruginosa* PA-4; lane 5, *P. aeruginosa* PA-5; lane 6, *P. aeruginosa* PA-6; lane 7, *P. aeruginosa* PA-7; lane 8, *P. aeruginosa* PA-8; lane 9, *P. aeruginosa* PA-9; lane 10, *P. aeruginosa* PA-10; lane 11, *E. coli* 50192 harboring four (7-, 48-, 66-, and 154-kb) plasmids. C, chromosome.

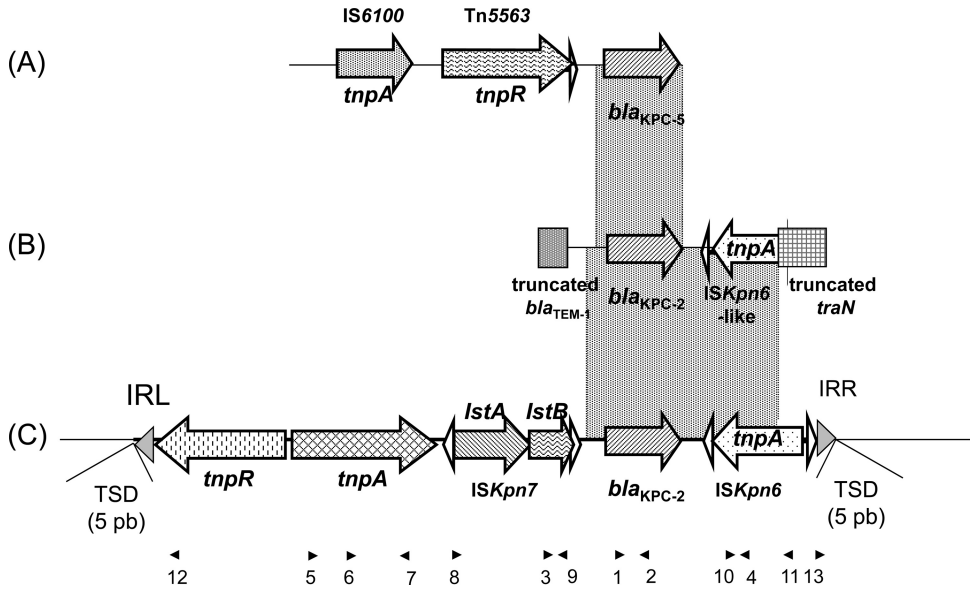

FIG. 3. Schematic representations of genetic structures surrounding the bla_{KPC} gene in different *P. aeruginosa* isolates. (A) *P. aeruginosa* PR280 harboring bla_{KPC-5} from Puerto Rico (19); upstream of the bla_{KPC-5} gene, a portion of the transposable element Tn*5563*, previously described in plasmid pRA2 from *P. alcaligenes*, was found. (B) Novel structure characterized in PA-2 from Colombia. (C) Structure of Tn*4401b*. The dotted rectangles indicate the common regions between the different structures. Horizontal arrows indicate genes and their corresponding transcription orientations. Gray triangles represent the left inverted repeat (IRL) and right inverted repeat (IRR) of Tn*4401*. Small and empty triangles represent the inverted repeats of IS*Kpn6* and IS*Kpn7*. Target site duplications (TSDs) are indicated above the sequence. Small black triangles above the sequence represent the primers listed in Table 2.

method, and MICs of carbapenems were determined using Etest strips (bioMérieux, Marcy l'Etoile, France) and interpreted according to Clinical and Laboratory Standards Institute guidelines (2). The 10 isolates were resistant to all penicillins and expanded-spectrum cephalosporins tested and showed carbapenem MIC values above 32 mg/ml (Table 1). The use of cloxacillin (200 mg/liter)-containing Mueller-Hinton (MH) agar plates did not alter the antibiogram results. All isolates remained susceptible to colistin. Eight isolates (PA-1 and PA-4 to -10) were also fully resistant to all tested aminoglycosides (amikacin, gentamicin, netilmicin, and tobramycin) and to ciprofloxacin; one isolate (PA-2) was susceptible to all tested aminoglycosides and resistant to ciprofloxacin; and a single isolate (PA-3) was susceptible to ciprofloxacin, amikacin, and fosfomycin, according to guidelines from CA-SFM (http://www.sfm-microbiologie.org).

PCR and sequencing (Table 2) (11) revealed that all isolates possessed bla_{KPC-2} genes and naturally and chromosomally encoded bla_{AmpC} and bla_{OXA-50} genes, but bla_{SHV}, bla_{TEM}, bla_{CTX-M}, bla_{VIM}, bla_{IMP}, and bla_{OXA-9} genes could not be evidenced.

By the Kieser extraction method (9), the six isolates from Medellin (PA-1 and PA-4 to -8) and Barranquilla (PA-3) showed several plasmids of different sizes (45 kb to ≥150 kb) (Fig. 2A). One isolate from Bogota (PA-2) showed only one plasmid (13 kb), and no plasmid could be evidenced for strains from Cali (PA-9) and Pereira (PA-10), suggesting a chromosomal location of the bla_{KPC-2} gene, as has already been described in *P. aeruginosa* isolates (17) (Fig. 2B). Only one plasmid hybridized with an internal probe for a bla_{KPC} gene in each isolate, with sizes ranging from 13 to 60 kb (Fig. 2A; Table 1). Transformants into *Escherichia coli* DH10B could be obtained with all the natural plasmids except with the 60-kb pPA-3 plasmid. No transformants could be obtained with Kieser extracts from PA-9 and PA-10. No other antibiotic resistance marker was cotransferred. The natural plasmids pPA-1, pPA-2, and pPA-4 to -8 were also electroporated into the AmpC-deficient *P. aeruginosa* KG2505 strain (16) and conferred a high level of resistance to all ß-lactams tested, including carbapenems. Previous studies have reported that OprD porin was absent in numerous KPC-producing *P. aeruginosa* isolates (17, 18) and is associated both with overexpression of the *mexAB-oprM* efflux pump and with increases in carbapenem MICs. However, in the present study, electroporation of plasmids harboring the bla_{KPC-2} gene was sufficient to confer a high level of resistance to the naturally susceptible *P. aeruginosa* KG2505 isolate.

A PCR mapping approach revealed that the bla_{KPC-2} gene was associated with Tn*4401b* isoform in PA-1 and PA-3 to -10 (Table 2, Fig. 3). The natural plasmids pPA-1 and pPA-4 to -8 were extracted from electroporants and directly sequenced using outward-directed primers located next to the inverted repeats (IRs). For these six plasmids, the flanking sequences of Tn*4401b* were identical; it was inserted into an open reading frame (ORF) of 297 bp initially described as being carried on plasmid pRSB105, which was extracted from uncultured bacteria of a sewage plant in Germany (15). Upon insertion, Tn*4401b* generated identical 5-bp GCGCT target site duplications. For plasmid pPA-2, PCR mapping gave negative results, suggesting a different genetic organization, which was confirmed by direct sequencing of the plasmid. Only a 2,072-bp region, including the bla_{KPC-2} gene and an IS*Kpn6*-like ORF previously described in a study of *K. pneumoniae* isolates collected in China, was identical to Tn*4401* (8). This IS*Kpn6*-like

element shared a 1,039-bp fragment with IS*Kpn6*. The target site duplication and the left inverted repeat (IRL) as described in Tn*4401* were present, but the ORF encoding the putative transposase (439 amino acids in IS*Kpn6*) was truncated in its N terminus by a 117-bp fragment encoding the C-terminal region of a protein sharing 100% amino acid identity with the *traN* protein from plasmid pFBAOT6 previously described in a study of *Aeromonas punctata* (GenBank accession no. NC_006143) (14a). Upstream of the bla_{KPC-2} gene, only a 73-bp segment, which is interrupted by a truncated bla_{TEM} gene, is identical to Tn*4401*.

Unlike *Enterobacteriaceae*, *P. aeruginosa* isolates expressing KPC carbapenemase seem to be geographically limited. The presence of bla_{KPC} in *P. aeruginosa* isolates is worrying in a species that is known to be prone to becoming carbapenem resistant by multiple mechanisms. Our analysis of several KPC-producing *P. aeruginosa* isolates from different Colombian hospitals revealed the spread of different clones that harbor different plasmids and different genetic structures, including the Tn*4401b* isoform associated with the bla_{KPC-2} gene. The emergence of bla_{KPC-2} in different species of *Enterobactriaceae* and the spread of this gene to *P. aeruginosa* isolates in different countries emphasizes the potential for dissemination worldwide. Since phenotypic detection is still difficult, it remains to be determined to what extent KPC-producing *P. aeruginosa* have spread worldwide, particularly in South America.

Nucleotide sequence accession number. The nucleotide sequence reported in this paper has been assigned to the GenBank nucleotide database (accession number JN545009).

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REFERENCES

1. **Akpaka, P. E., et al.** 2009. Emergence of KPC-producing *Pseudomonas aeruginosa* in Trinidad and Tobago. J. Clin. Microbiol. **47:**2670–2671.
2. **Clinical and Laboratory Standards Institute.** 2011. Performance standards for antimicrobial susceptibility testing; 21st informational supplement M100-S21. Clinical and Laboratory Standards Institute, Wayne, PA.
3. **Curran, B., D. Jonas, H. Grundmann, T. Pitt, and C. G. Dowson.** 2004. Development of a multilocus sequence typing scheme for the opportunistic pathogen *Pseudomonas aeruginosa*. J. Clin. Microbiol. **42:**5644–5649.
4. **Cuzon, G., et al.** 2010. Worldwide diversity of *Klebsiella pneumoniae* that produce ß-lactamase bla_{KPC-2} gene. Emerg. Infect. Dis. **16:**1349–1356.
5. **Empel, J., et al.** 2007. Outbreak of *Pseudomonas aeruginosa* infections with PER-1 extended-spectrum ß-lactamase in Warsaw, Poland: further evidence for an international clonal complex. J. Clin. Microbiol. **45:**2829–2834.
6. **Ge, C., et al.** 2011. Identification of KPC-2-producing *Pseudomonas aeruginosa* isolates in China. J. Antimicrob. Chemother. **66:**1184–1186.
7. **Giske, C. G., et al.** 2006. Establishing clonal relationships between VIM-1-like metallo-ß-lactamase-producing *Pseudomonas aeruginosa* strains from four European countries by multilocus sequence typing. J. Clin. Microbiol. **44:**4309–4315.
8. **Jiang, Y., et al.** 2010. Complete nucleotide sequence of *Klebsiella pneumoniae* multidrug resistance plasmid pKP048, carrying bla_{KPC-2}, bla_{DHA-1}, *qnrB4*, and *armA*. Antimicrob. Agents Chemother. **54:**3967–3969.

8a. **Jolley, K. A., M. S. Chan, and M. C. Maiden.** 2004. mlstdbNet—distributed multi-locus sequence typing (MLST) databases. BMC Bioinformatics **5:**86.

9. **Kieser, T.** 1984. Factors affecting the isolation of cccDNA from *Streptomyces lividans* and *Escherichia coli*. Plasmid **12:**19–36.
10. **Livermore, D. M.** 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa:* our worst nightmare? Clin. Infect. Dis. **34:**634–640.
11. **Naas, T., et al.** 2008. Genetic structures at the origin of acquisition of the beta-lactamase bla_{KPC} gene. Antimicrob. Agents Chemother. **52:**1257–1263.
12. **Nordmann, P., G. Cuzon, and T. Naas.** 2009. The real threat of KPC carbapenemase-producing bacteria. Lancet Infect. Dis. **9:**228–236.
13. **Poirel, L., E. Lagrutta, T. Cleary, L. S. Munoz-Price, and P. Nordmann.** 2010. Emergence of KPC-producing *Pseudomonas aeruginosa*, United States. Antimicrob. Agents Chemother. **54:**3072.
14. **Queenan, A. M., and K. Bush.** 2007. Carbapenemases: the versatile ß-lactamases. Clin. Microbiol. Rev. **20:**440–458.

14a. **Rhodes, G., et al.** 2004. Complete nucleotide sequence of the conjugative tetracycline resistance plasmid pFBAOT6, a member of a group of IncU plasmids with global ubiquity. Appl. Environ. Microbiol. **70:**7497–7510.

15. **Schlüter, A., et al.** 2007. Erythromycin resistance-conferring plasmid pRSB105, isolated from a sewage treatment plant, harbors a new macrolide resistance determinant, an integron-containing Tn402-like element, and a large region of unknown function. Appl. Environ. Microbiol. **73:**1952–1960.
16. **Smith, A. W., and B. H. Iglewski.** 1989. Transformation of *Pseudomonas aeruginosa* by electroporation. Nucleic Acids Res. **17:**10509.
17. **Villegas, M. V., et al.** 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing ß-lactamase. Antimicrob. Agents Chemother. **51:**1553–1555.
18. **Wolter, D. J., et al.** 2009. Surveillance of carbapenem-resistant *Pseudomonas aeruginosa* isolates from Puerto Rican medical center hospitals: dissemination of KPC and IMP-18 ß-lactamases. Antimicrob. Agents Chemother. **53:**1660–1664.
19. **Wolter, D. J., et al.** 2009. Phenotypic and enzymatic comparative analysis of the novel KPC variant KPC-5 and its evolutionary variants, KPC-2 and KPC-4. Antimicrob. Agents Chemother. **53:**557–562.
20. **Yigit, H., et al.** 2001. Novel carbapenem-hydrolyzing ß-lactamase KPC-1 from a carbapenem-resistant strain of *Klebsiella pneumoniae*. Antimicrob. Agents Chemother. **45:**1151–1161.