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Similar Frequencies of *Pseudomonas aeruginosa* Isolates Producing KPC and VIM Carbapenemases in Diverse Genetic Clones at Tertiary-Care Hospitals in Medellín, Colombia

Johanna M. Vanegas,[a,b] Astrid V. Cienfuegos,[a,b] Ana M. Ocampo,[a,b] Lucelly López,[b] Helena del Corral,[b] Gustavo Roncancio,[c] Patricia Sierra,[d] Lina Echeverri-Toro,[e] Sigifredo Ospina,[e] Natalia Maldonado,[f] Carlos Robledo,[f] Andrea Restrepo,[g] J. Natalia Jiménez[a,b]

Línea de Epidemiología Molecular Bacteriana, Grupo de Microbiología Molecular, Universidad de Antioquia, Medellín, Colombia[a]; Grupo de Microbiología Básica y Aplicada, Universidad de Antioquia, Medellín, Colombia[b]; Clínica CardioVID, Medellín, Colombia[c]; IPS Universitaria Clínica León XIII, Medellín, Colombia[d]; Hospital Universitario San Vicente Fundación, Medellín, Colombia[e]; Clínica El Rosario, Medellín, Colombia[f]; Hospital Pablo Tobón Uribe, Medellín, Colombia[g]

Carbapenem-resistant *Pseudomonas aeruginosa* has become a serious health threat worldwide due to the limited options available for its treatment. Understanding its epidemiology contributes to the control of antibiotic resistance. The aim of this study was to describe the clinical and molecular characteristics of infections caused by carbapenem-resistant *P. aeruginosa* isolates in five tertiary-care hospitals in Medellín, Colombia. A cross-sectional study was conducted in five tertiary-care hospitals from June 2012 to March 2014. All hospitalized patients infected by carbapenem-resistant *P. aeruginosa* were included. Clinical information was obtained from medical records. Molecular analyses included PCR for detection of bla_{VIM}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}, and bla_{KPC} genes plus pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) for molecular typing. A total of 235 patients were enrolled: 91.1% of them were adults ($n = 214$), 88.1% ($n = 207$) had prior antibiotic use, and 14.9% ($n = 35$) had urinary tract infections. The bla_{VIM-2} and bla_{KPC-2} genes were detected in 13.6% ($n = 32$) and 11.5% ($n = 27$), respectively, of all isolates. Two isolates harbored both genes simultaneously. For KPC-producing isolates, PFGE revealed closely related strains within each hospital, and sequence types (STs) ST362 and ST235 and two new STs were found by MLST. With PFGE, VIM-producing isolates appeared highly diverse, and MLST revealed ST111 in four hospitals and five new STs. These results show that KPC-producing *P. aeruginosa* is currently disseminating rapidly and occurring at a frequency similar to that of VIM-producing *P. aeruginosa* isolates (approximately 1:1 ratio) in Medellín, Colombia. Diverse genetic backgrounds among resistant strains suggest an excessive antibiotic pressure resulting in the selection of resistant strains.

Pseudomonas aeruginosa is an opportunistic pathogen that is responsible for a wide variety of clinical infections, including bacteremia, pneumonia, urinary tract infection, and skin infections (1). This microorganism is intrinsically resistant to a variety of antimicrobials and is capable of developing resistance to almost any available antimicrobial compound (2). Carbapenems have been considered the last option for treating infections due to multidrug-resistant *P. aeruginosa*, because of their broad spectrum of antibacterial activity and their stability against hydrolysis by most β-lactamases. However, the emergence and spread of carbapenem resistance have limited their therapeutic efficacy (3–5). *Pseudomonas aeruginosa* bacteria possess several mechanisms that are involved in carbapenem resistance, such as overexpression of the MexAB-OprM efflux system and chromosomal AmpC, deficient expression of the outer membrane porin OprD, and acquired carbapenemases (6, 7). Ambler class B β-lactamases, such as VIM and IMP, are the most frequent carbapenemases involved in *P. aeruginosa* carbapenem resistance, while Ambler class A carbapenemases, such as KPC, frequently reported in *Enterobacteriaceae*, have started to be detected in *P. aeruginosa* isolates (8). In 2007, the presence of KPC was first reported in *P. aeruginosa* in Colombia, a country where KPC is endemic, and it has been reported subsequently in other countries from the Americas, such as Trinidad and Tobago, Argentina, and the United States, including Puerto Rico (8–12). Recently, an increasing frequency of KPC-producing *P. aeruginosa* isolates has been reported in hospitals from several Colombian cities, including Medellín (13, 14). To contribute to the understanding of the epidemiology of carbapenem-resistant *P. aeruginosa*, the aim of this study was to describe the clinical characteristics of patients infected by carbapenem-resistant *P. aeruginosa* and characterize the carbapenemases and the predominant resistant clones circulating in five tertiary-care hospitals within Medellín, Colombia.

MATERIALS AND METHODS

Study population. A cross-sectional study was conducted at five tertiary-care hospitals located in Medellín from June 2012 to March 2014. Hospitals A and B are large university hospitals of 662 and 700 beds, respectively. Hospitals C and D are medium-size tertiary-care centers of 286 and 300 beds, respectively, and hospital E is a 140-bed cardiology hospital. These five institutions are located in Medellín, Colombia's second-largest city. All patients infected by carbapenem-resistant *P. aeruginosa* during the study period were included, and molecular analyses were performed on the first bacterial isolate recovered during hospitalization. The study protocol was approved by the Bioethics Committee for Human Research at Universidad de Antioquia (CBEIH-SIU) (approval no. 11-35-415), as well

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Address correspondence to J. Natalia Jiménez, nataliajiudea@gmail.com.
J.M.V. and A.V.C. contributed equally to the work.

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as by the research ethics committees from each of the other participating institutions.

Clinical and epidemiological data. Clinical and epidemiological information was obtained from medical records for each patient. The information included sociodemographic characteristics, prior colonization, antimicrobial use, intensive care unit (ICU) stay, type of infection, comorbidities, treatment, and outcomes, including therapeutic failure, cure, and death. Infections were classified as either community or health care associated according to the standard epidemiological definitions established by the U.S. Centers for Disease Control and Prevention (CDC) (6).

Bacterial strains and antibiotic susceptibilities. *Pseudomonas aeruginosa* isolates intermediate or resistant to carbapenems according to CLSI 2012 cutoff points were selected (7). The identification of isolates and determination of their antibiotic susceptibilities were carried out with the automated Vitek 2 system (bioMérieux, Marcy l'Etoile, France). The antibiotics tested for *P. aeruginosa* were piperacillin-tazobactam, ceftazidime, cefepime, imipenem, meropenem, amikacin, gentamicin, ciprofloxacin, and colistin.

Detection of carbapenemases. The presence of carbapenemases was evaluated using a phenotypic screening assay that is a variation of the 3-dimensional test (15, 16) and PCR amplification of the bla_{KPC}, bla_{VIM}, bla_{IMP}, bla_{NDM}, and bla_{OXA-48} genes, using previously described primers and conditions (17, 18). After PCR amplification, forward and reverse sequencing were performed. The sequences were compared with those available at GenBank (www.ncbi.nlm.nih.gov/BLAST) and the Lahey database (http://www.lahey.org/Studies/).

A comparison of the clinical characteristics and resistance profiles between carbapenemase- and noncarbapenemase-producing (CP and NCP, respectively) *P. aeruginosa* isolates was performed.

Molecular typing. Pulsed-field gel electrophoresis (PFGE) was performed using 50 U of SpeI restriction enzyme (Thermo Scientific, United States). DNA fragment patterns were normalized using the bacteriophage lambda ladder PFGE marker (New England BioLabs, United Kingdom). Electrophoresis was performed on a CHEF DR III (Bio-Rad Laboratories, Hercules, CA) at 11°C for 21 h under the following conditions: initial switch time, 2.2 s; final switch time, 63.8 s; included angle, 120°; and voltage gradient, 6 V/cm. Cluster analysis was performed using the Dice coefficient with BioNumerics software version 6.0 (Applied Maths, Sint-Martens-Latem, Belgium). Dendrograms were generated by the unweighted-pair group method using average linkages (UPGMA), with 1% tolerance and 0.5% optimization settings. A similarity cutoff of ≥80% was used to define genetically related strains.

Multilocus sequence typing (MLST) was performed using the methodology described by Curran et al. (19) on a subset of 41 isolates representing the most frequent PFGE patterns (17.4% of all isolates). Allele numbers and sequence types (STs) were assigned using the database maintained at http://pubmlst.org/paeruginosa/.

Statistical analyses. Comparisons of clinical, epidemiological, and molecular characteristics were carried out between CP and NCP isolates. Categorical variables were described using absolute and relative frequencies and compared using the chi-square test or Fisher's exact test. *P* values of ≤0.05 were considered statistically significant. Statistical analyses were carried out using the SPSS version 20.0 software package (SPSS Inc., Chicago, IL).

RESULTS

Clinical and epidemiological characteristics. A total of 235 patients infected by carbapenem-resistant *P. aeruginosa* in five hospitals that participated in the study were enrolled. The patients' demographic and clinical characteristics are summarized in Table 1. The majority of patients with carbapenem-resistant *P. aeruginosa* infection were males (66.4%, $n = 156$), and most were adults (91.1%, $n = 214$). At the time of sample collection, 37.0% ($n = 87$) of patients were hospitalized in intensive care units (ICUs) and were frequently attended by personnel with surgical (26.4%, $n = 62$) and internal medicine (23.4%, $n = 55$) specialties.

Ninety-eight percent of infections were classified as health care associated according to CDC criteria after individual assessment of cases. The most common sites of infections were urinary tract and intra-abdominal (14.9% for each, $n = 35$), followed by skin and soft tissue (13.6%, $n = 32$). The medical histories of patients revealed frequent use of antibiotics within the past 6 months (88.1%, $n = 207$), mainly carbapenems (45.1%, $n = 106$), piperacillin-tazobactam (43.0%, $n = 101$), and glycopeptides (32.8%, $n = 77$). Targeted therapy was mainly fluoroquinolones, followed by colistin and aminoglycosides (30.2, 29.4, and 28.9%, respectively). The main outcomes in the patients studied were cure (46.2%, $n = 96$), and death (27.9%, $n = 58$). Therapeutic failures were reported in only 1.9% ($n = 4$) of cases. When comparing clinical characteristics among CP and NCP *P. aeruginosa* isolates, significant differences were only found in relation to empirical therapy using glycopeptides ($P = 0.029$) and targeted therapy using carbapenems ($P < 0.001$), aminoglycosides ($P = 0.002$), fluoroquinolones ($P < 0.001$), and colistin ($P < 0.001$) (Table 1).

Phenotypic and genotypic carbapenemase detection. The 3-dimensional test was positive in 23.8% ($n = 56$) of the *P. aeruginosa* isolates collected; among these, bla_{KPC} was detected by PCR in 48.2% ($n = 27$) and bla_{VIM} in 44.6% ($n = 25$) of the isolates. Remarkably, two (3.6%) isolates coharboring bla_{KPC} and bla_{VIM} were detected in two different hospitals and two isolates were negative for carbapenemase-encoding genes upon evaluation by PCR (3.6%). As for isolates with negative results by the 3-dimensional test (76.2%, $n = 179$), seven (3.9%) harbored bla_{VIM} and the remaining isolates were negative for the genes evaluated (96.1%, $n = 172$). In general, carbapenemases were detected by PCR in 26.0% ($n = 61$) of total isolates; bla_{KPC}, bla_{VIM}, and bla_{KPC} plus bla_{VIM} were detected in 11.5% ($n = 27$), 13.6% ($n = 32$), and 0.8% ($n = 2$), respectively, of total isolates. The bla_{NDM}, bla_{OXA-48}, and bla_{IMP} genes were not detected. On the other hand, 74.0% ($n = 174$) of isolates were negative for all carbapenemase-encoding genes evaluated.

Resistance profiles among carbapenem-resistant *P. aeruginosa* isolates. Of the total isolates, 86.1% ($n = 192$) and 80.3% ($n = 188$) were resistant to imipenem and meropenem, respectively. Almost half of the carbapenem-resistant *P. aeruginosa* isolates had resistance to ceftazidime (48.7%, $n = 114$), cefepime (45.5%, $n = 107$), and ciprofloxacin (47.7%, $n = 112$). Additionally, 70.8% ($n = 114$) were resistant to piperacillin-tazobactam, 67.9% ($n = 53$) to aztreonam, 40.1% ($n = 93$) to gentamicin, and 30.2% ($n = 71$) to amikacin. Resistance to colistin was found in 5.7% ($n = 12$) of isolates.

When comparing resistance patterns according to carbapenemases detected by PCR, resistance was higher in CP than in NCP *P. aeruginosa* isolates for all antimicrobials evaluated, with significant differences for most of them (Fig. 1A). Likewise, CP isolates were frequently multiresistant or resistant to three or more antibiotic groups, the most frequent profile being resistance to meropenem, imipenem, cefepime, ceftazidime, gentamicin, amikacin, and ciprofloxacin (53.8%). In contrast, for NPC isolates, the most usual profile was resistance to meropenem and imipenem (25.5%), followed by resistance to imipenem only (13.7%) (Fig. 1B).

Piperacillin-tazobactam, aztreonam, and colistin were excluded from resistance profile analyses due to missing data. However, separate analyses showed that isolates resistant to mero-

TABLE 1 Demographic and clinical characteristics of patients infected by carbapenem-resistant *P. aeruginosa*

Characteristic	No. (%) of isolates			*P* value[a]
	Total no.	Noncarbapenemase producing	Carbapenemase producing	
Gender				0.430
Female	79 (33.6)	61 (35.1)	18 (29.5)	
Male	156 (66.4)	113 (64.9)	43 (70.5)	
Age (yrs)				0.116
<15	21 (8.9)	18 (10.3)	3 (4.9)	
15–30	26 (11.1)	18 (10.3)	8 (13.1)	
31–55	71 (30.2)	58 (33.3)	13 (21.3)	
>55	117 (49.8)	80 (46.0)	37 (60.7)	
Patient type				0.201
Adult	214 (91.1)	156 (89.7)	58 (95.1)	
Pediatric	21 (8.9)	18 (10.3)	3 (4.9)	
Hospital stay (days)				0.680
≤7	18 (8.6)	14 (9.1)	4 (7.3)	
>7	191 (91.4)	140 (90.9)	51 (92.7)	
History of surgery in past yr	153 (65.1)	110 (63.2)	43 (70.5)	0.389
History in past 6 mo				
Hospitalization	155 (66.0)	111 (63.8)	44 (72.1)	0.345
Dialysis	35 (15.0)	26 (15.0)	9 (14.8)	0.959
Stay in ICU	101 (43.0)	73 (42.0)	28 (45.9)	0.534
Antimicrobial use in past 6 mo	207 (88.1)	155 (89.1)	52 (85.2)	0.055
Carbapenems	106 (45.1)	80 (46.0)	26 (42.6)	0.651
Piperacillin-tazobactam	101 (43.0)	78 (44.8)	23 (37.7)	0.334
Glycopeptides	77 (32.8)	58 (33.3)	19 (31.1)	0.754
Fluoroquinolones	53 (22.6)	37 (21.3)	16 (26.2)	0.425
Infection type				0.232
Health care associated	231 (98.3)	170 (97.7)	61 (100)	
Community associated	4 (1.7)	4 (2.3)	0	
Hospitalization in ICU at time of isolate	87 (37.0)	62 (35.6)	25 (41.0)	0.456
Specialties				0.418
Surgery	62 (26.4)	46 (26.4)	16 (26.2)	
Internal medicine	55 (23.4)	33 (19.0)	22 (36.1)	
Intensive care	34 (14.5)	29 (16.7)	5 (8.2)	
Orthopedics	30 (12.8)	22 (12.6)	8 (13.1)	
Pediatrics	15 (6.4)	13 (7.5)	2 (3.3)	
Nephrology	11 (4.7)	9 (5.2)	2 (3.3)	
Other[b]	28 (11.9)	22 (12.6)	6 (9.8)	
Comorbidities	221 (94.0)	166 (95.4)	55 (90.2)	0.137
Trauma	46 (19.6)	35 (20.1)	11 (18.0)	0.724
Diabetes mellitus	49 (20.9)	35 (20.1)	14 (23.0)	0.639
Chronic renal disease	45 (19.1)	31 (17.8)	14 (23.0)	0.380
Cardiovascular disease	65 (27.7)	50 (28.7)	15 (24.6)	0.533
Cancer	26 (11.1)	22 (12.6)	4 (6.6)	0.192
Infection site				0.235
UTI[c]	35 (14.9)	25 (14.4)	10 (16.4)	
Catheter-associated UTI	24 (10.2)	14 (8.0)	10 (16.4)	
Intra-abdominal	35 (14.9)	27 (15.5)	8 (13.1)	
Skin and soft tissue	32 (13.6)	24 (13.8)	8 (13.1)	
Bloodstream	12 (5.1)	10 (5.7)	2 (3.3)	
Catheter-related bloodstream	14 (6.0)	10 (5.7)	4 (6.6)	

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TABLE 1 (Continued)

Characteristic	No. (%) of isolates			*P* value[a]
	Total no.	Noncarbapenemase producing	Carbapenemase producing	
Pneumonia	14 (6.0)	10 (5.7)	4 (6.6)	
Ventilator-associated pneumonia	17 (7.2)	13 (7.5)	4 (6.6)	
Osteomyelitis	15 (6.4)	8 (4.6)	7 (11.5)	
Surgical site	11 (4.7)	11 (6.3)	0	
Empirical therapy				
Piperacilin-tazobactam	52 (22.1)	37 (21.3)	15 (24.6)	0.590
Carbapenem	95 (40.4)	71 (40.8)	24 (39.3)	0.841
Glycopeptides	34 (14.5)	20 (11.5)	14 (23.0)	**0.029**
1st-generation cephalosporin	4 (1.7)	4 (2.3)	0	0.232
2nd-generation cephalosporin	0	0	0	
3rd-generation cephalosporin	4 (1.7)	4 (2.3)	0	0.232
4th-generation cephalosporin	15 8(6.4)	11 (6.3)	4 (6.6)	0.948
Targeted therapy				
Carbapenem	50 (21.3)	27 (15.5)	23 (37.7)	**<0.001**
4th-generation cephalosporin	37 (15.7)	1 (0.6)	0	0.141
Aminoglycosides	68 (28.9)	60 (34.6)	8 (13.1)	**0.002**
Fluoroquinolones	71 (30.3)	65 (37.8)	6 (9.8)	**<0.001**
Colistin	69 (29.4)	34 (19.5)	35 (57.4)	**<0.001**
Outcome				0.851
Cure	96 (46.2)	71 (46.4)	25 (45.5)	
Death	58 (27.9)	40 (26.1)	18 (32.7)	
Therapeutic failure	4 (1.9)	3 (2.0)	1 (1.8)	

[a] Values showing significantly different results are in boldface.
[b] Urology, transplant, hematology, neurology, pulmonology, vascular surgery, ophthalmology, and hepatobiliary.
[c] UTI, urinary tract infection.

penem plus imipenem and those resistant to imipenem only were frequently susceptible to those antibiotics (data not shown).

Molecular typing. The PFGE results revealed the presence of carbapenem-resistant *P. aeruginosa* strains with different genetic backgrounds circulating in hospitals from Medellín. Notably, the PFGE results for bla_{KPC}-harboring *P. aeruginosa* isolates showed a cluster in each hospital that included isolates that were indistinguishable or closely related (similarity index, 82 to 100%). Nevertheless, isolates from different hospitals were found to be unrelated (similarity index, <80%) (Fig. 2). MLST revealed bla_{KPC}-harboring *P. aeruginosa* isolates belonging to ST235 in hospitals A and B, ST362 in hospital C, and ST870 in hospital E, as well as two new STs, ST1801 in hospitals C and D and ST1803 in hospital C (Fig. 2; Table 2). In contrast, bla_{VIM}-harboring and NCP isolates showed high genotypic diversity by PFGE (Fig. 3A and B, respectively). The bla_{VIM}-harboring isolates belonged mainly to the ST111 clone, and the NCP isolates belonged to six different STs, including the novel ST1802 and ST1804 and ST227 that is part of clonal complex (CC) CC235 (Fig. 3; Table 2).

DISCUSSION

These results provide new evidence supporting the notion that the epidemiology of carbapenem-resistant *Pseudomonas aeruginosa* is very dynamic and highly context specific. The present study integrated the clinical and molecular data simultaneously in order to improve our understanding of the emergence of carbapenem resistance.

Overall, VIM is the most frequent carbapenemase reported in *P. aeruginosa* worldwide (20); however, in this study, isolates harboring KPC were similar in frequency to those harboring VIM. In fact, the detection of 27 isolates with bla_{KPC} in five tertiary-care hospitals within almost 2 years suggests a rapid dissemination of KPC-producing *P. aeruginosa* in Medellín. Previous studies conducted in six Colombian cities, including Medellín, revealed the presence of only 10 KPC-producing isolates circulating throughout the country during 2006 to 2010 (21). Other studies performed in five and seven Colombian cities during 2012 and 2013 showed the emergence of KPC-producing isolates, with frequencies of 5.1% ($n = 14$) and 16.4% ($n = 9$) of KPC-producing *P. aeruginosa* among the isolates in the two studies (13, 14). The latter result suggests an increasing frequency of this carbapenemase, possibly present throughout the country. Additionally, two isolates harboring both KPC-2 and VIM-2 were observed, which had been reported before in our country (14, 22). The presence of bla_{KPC} in *P. aeruginosa* and the increasing number of isolates harboring this enzyme throughout the country evidenced the capacity of dissemination of this gene outside the *Enterobacteriaceae* family.

Another significant finding was the close genetic relationship of strains within each hospital detected by PFGE in bla_{KPC}-harboring *P. aeruginosa* isolates. This result suggests mainly intrahospital transmission of these isolates, rather than dissemination from one hospital to another. The dissemination of KPC in *P. aeruginosa* was linked to four clones: two of them were ST235 and ST362, which have been reported previously in other countries, and the other two were novel clones assigned in this study, ST1801

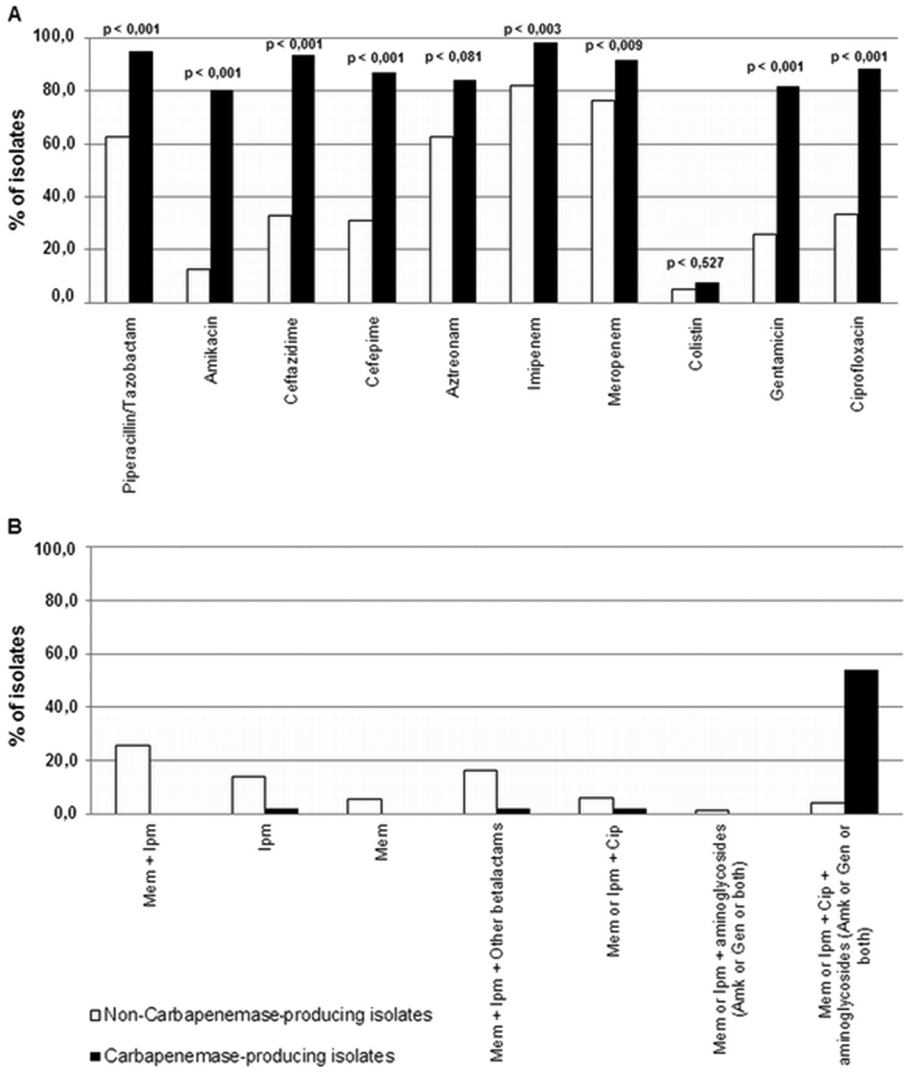

FIG 1 Rates of resistance among noncarbapenemase-producing and carbapenemase-producing *P. aeruginosa* isolates. (A) Percentages of isolates resistant to individual antibiotics. (B) Resistance profiles of carbapenem-resistant isolates. Mem, meropenem; Ipm, imipenem; Cip, ciprofloxacin; Amk, amikacin; Gen, gentamicin.

and ST1803. ST235 is a major *P. aeruginosa* multidrug-resistant clone that is involved in extended-spectrum β-lactamase (ESBL) (such as BEL, GES, and PER) and metallo-β-lactamase (such as VIM, IMP, and NDM) dissemination in Europe and Asia (3, 23–25) and has also been involved in KPC-2 dissemination in two cities of Colombia, Cali and Pereira (21). Conversely, there is only one report on the ST362 clone (26). Although KPC-2 harboring *P. aeruginosa* has also been detected in Trinidad and Tobago, the United States, Puerto Rico, Brazil, Argentina, and China, the global spread of epidemic strains has been difficult to assess due to MLST not having been used in the majority of countries (8–12). Contrary to the situation in Colombia, where the emergence of KPC-2 producing isolates has been linked to several clones, mainly of ST235 but also of ST308, ST1006, and ST1060, in Argentina it seems that the spread of KPC-2 in five provinces occurred through the dissemination of one successful clone, ST654 (8, 27).

In addition, high genetic diversity according to PFGE and MLST was found in bla_{VIM} and noncarbapenemase-producing isolates. The bla_{VIM}-harboring isolates belonged mainly to ST111, a successful clone carrying metallo-β-lactamase-encoding genes and the major *P. aeruginosa* epidemic strain throughout Europe (28). In Colombia, this ST has also been reported in an isolate harboring bla_{KPC-2} and bla_{VIM-2}, similar to one of the isolates harboring both genes found in this study (22). Previous studies in China and Spain have shown that clones of carbapenem-resistant *P. aeruginosa* can emerge from diverse genetic backgrounds and that MLST diversity could actually be higher than detected (29, 30). In accordance with this, it appears that the population structure of carbapenem-resistant *P. aeruginosa* isolates in Colombia is also diverse, with several distinctive clones currently able to harbor KPC and VIM carbapenemases, mainly ST235 and ST111, respectively, but with the parallel emergence of other clones. These results suggest a high antibiotic selection pressure favoring the emergence of multiple clones and point to appropriate antibiotic use as one of the main strategies to halt the emergence of carbapenem resistance in *P. aeruginosa*.

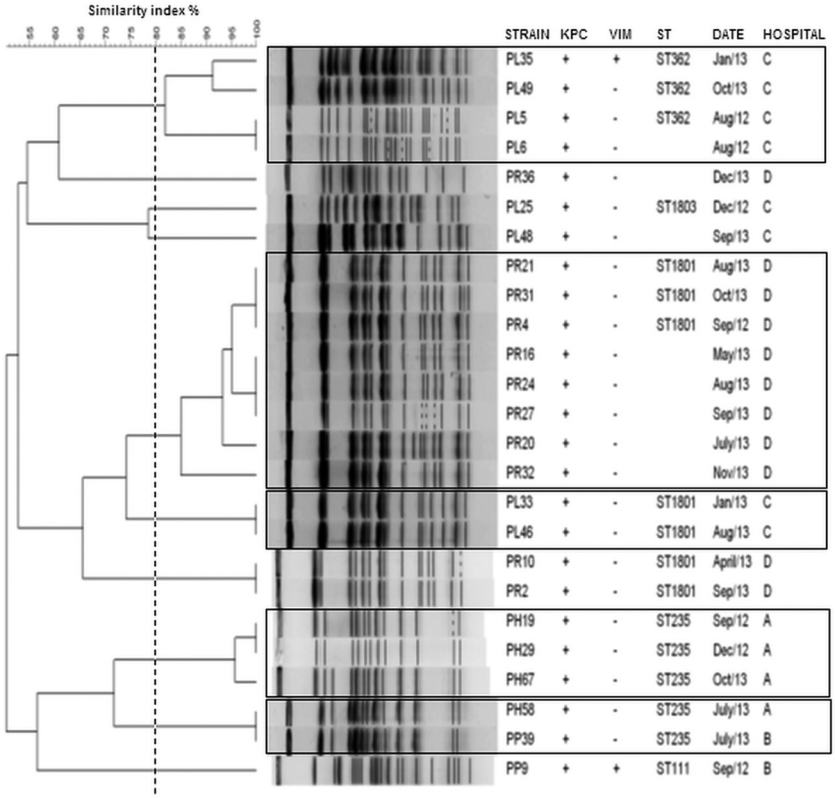

FIG 2 Genetic relatedness of KPC-producing *P. aeruginosa* isolates. The broken line corresponds to the cutoff level (80%) used to define PFGE clones as related. Boxes indicate the main clusters found at each hospital.

Regarding the clinical and epidemiological characteristics of the study population, the patients infected with carbapenem-resistant *P. aeruginosa* were mostly adults (above 55 years old), with various underlying diseases, long hospital stays, and histories of prior antibiotic use, which have been reported as risk factors for infection by carbapenem-resistant *P. aeruginosa* in health care settings (31–33). In this study, less than half of the patients were hospitalized in ICUs at the time the isolate was obtained; this highlights the importance of strengthening surveillance and preventive measures in these and other hospital wards. Although a modest percentage of mortality was found, this could not be attributed to carbapenem-resistant *P. aeruginosa* since the patients presented various underlying conditions.

TABLE 2 STs obtained by MLST of carbapenem-resistant *P. aeruginosa* isolates

Type of isolate	Hospital	ST	No. of isolates
bla_{KPC} harboring	A	235	4
	B	235	1
	C	362, 1801,[a] 1803[a]	2, 2, 1
	D	1801[a]	6
	E	870	1
bla_{VIM} harboring	A	111	3
	B	111, 856	1, 2
	C	111, 1800,[a] 1801[a]	1, 1, 1
	D	1249, 1799[a]	1, 1
	E	111	1
bla_{VIM} and bla_{KPC} harboring	B	111	1
	C	362	1
Noncarbapenemase producing	A	1798,[a] 1212	1, 1
	B	1802,[a] 1804[a]	1, 1
	C	882, 1724	1, 1
	D	170, 155, 1123	1, 1, 1
	E	267, 260	1, 1

[a] Novel ST.

Interestingly, high percentages of prior antibiotic use, mainly carbapenems, were observed. Previous studies have shown that the use of agents with anti-*Pseudomonas* activity, such as meropenem and imipenem, is an independent risk factor associated with carbapenem- and multidrug-resistant *P. aeruginosa* (31, 34, 35). Meropenem and imipenem are used as empirical therapy for infections due to aerobic Gram-negative bacteria where coverage for *P. aeruginosa* is not necessary, and the lack of de-escalation of empirical therapy could lead to the elimination of susceptible colonizing microbiota, thus favoring the multiplication of carbapenem-resistant strains (36). Another antibiotic frequently used as empirical therapy in suspected *P. aeruginosa* infections is piperacillin-tazobactam; this antibiotic has also been associated with the emergence of carbapenem resistance in this pathogen (37, 38).

The multidrug resistance phenotype observed among carbapenemase-producing *P. aeruginosa* isolates may be explained by the presence of several resistance genes in the same genetic elements harboring the carbapenemase genes, which limits the effective antimicrobial options (20). Conversely, resistance only to imipenem

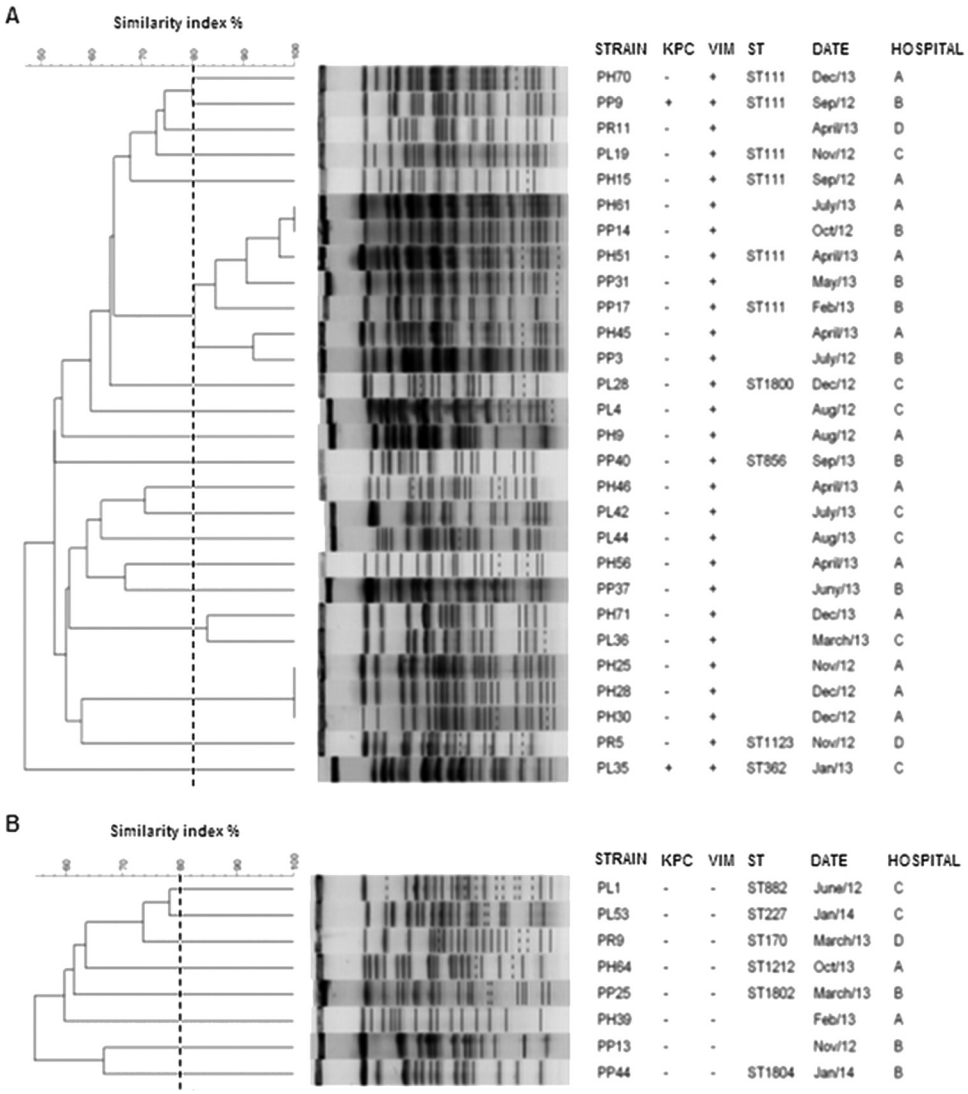

FIG 3 Genetic relatedness of VIM- and noncarbapenemase-producing *P. aeruginosa* isolates. The broken line corresponds to the cutoff level (80%) used to define PFGE clones as related. (A) VIM-producing isolates. (B) Noncarbapenemase-producing isolates.

or to meropenem plus imipenem occurred mainly in noncarbapenemase-producing *P. aeruginosa* isolates, thus opening up the possibility for more therapeutic options; this is in agreement with the differences found in targeted therapy for infections caused by CP and NCP isolates.

The high frequency of isolates negative by the 3-dimensional test and PCR suggests that other mechanisms are responsible for carbapenem resistance in this population. Alternative mechanisms could be overexpression of the MexAB-OprM efflux system and chromosomal AmpC or deficient expression of the outer membrane porin OprD, which have been previously reported (39, 40). Further studies comparing the clinical characteristics of patients infected with isolates with susceptible and resistant phenotypes could help to identify the main risk factors associated with carbapenem resistance in *P. aeruginosa* in hospital settings in this middle-income country.

In conclusion, the increasing number of isolates harboring KPC found in a much shorter period of time than in previous reports for the same country shows the importance of evaluating the presence of this carbapenemase in *P. aeruginosa* as well as in *Enterobacteriaceae*. In addition, the emergence of several clones of carbapenem-resistant *P. aeruginosa* suggests that excessive drug pressure is likely to be giving rise to selection of resistant phenotypes. This underscores the need to design and implement control strategies based on rational antibiotic use.

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