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Surveillance of Carbapenem-Resistant *Pseudomonas aeruginosa* Isolates from Puerto Rican Medical Center Hospitals: Dissemination of KPC and IMP-18 β-Lactamases▽

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During a 6-month period, 37/513 (7.2%) *Pseudomonas aeruginosa* isolates belonging to 13 pulsed-field gel electrophoresis (PFGE) groups from Puerto Rican hospitals were carbapenem nonsusceptible. Seven of 37 isolates from four PFGE groups carried bla_{IMP-18}, and 25/37 isolates from seven PFGE groups carried bla_{KPC}. The results indicated the clonal spread of bla_{KPC}-positive *P. aeruginosa* isolates into several Puerto Rican hospitals and the dissemination of bla_{IMP-18} and bla_{KPC} into genetically unrelated isolates.

Pseudomonas aeruginosa is a problematic nosocomial pathogen because it can cause complicated infections in immunocompromised and critically ill patients and can survive therapy through chromosomal mutations or acquisition of resistance-encoding genes. Carbapenems, including imipenem and meropenem, are β-lactam antibiotics used extensively for the treatment of *P. aeruginosa* infections. However, carbapenem resistance can emerge and involves several mechanisms, including porin downregulation, efflux pump overexpression, and the production of carbapenem-hydrolyzing enzymes (11, 12). In the absence of hydrolyzing enzymes, loss of the porin, OprD, can result in imipenem resistance and decreased meropenem susceptibility (10). Meropenem resistance requires OprD loss coupled with *mexAB-oprM* pump overexpression (7).

During a 2006 surveillance study of nosocomial pathogens from U.S. medical centers, 10.7 and 6.4% of the *P. aeruginosa* isolates were resistant to imipenem and meropenem, respectively (15). This report did not include hospitals from Puerto Rico, a U.S. commonwealth with close social and economic ties to the United States. In 1998, a study comprising seven different Puerto Rican hospitals reported that 11.3% of *P. aeruginosa* isolates were imipenem resistant (5). A retrospective surveillance study in 2000 at the Puerto Rico Medical Center (PRMC) found that 46.9% of *P. aeruginosa* isolates were imipenem resistant, but this high percentage may be an overestimation due to multiple isolations from individual patients (18).

The mechanism(s) associated with carbapenem resistance in *P. aeruginosa* isolates from Puerto Rico is unknown. The objectives of this study were to collect imipenem-resistant *P. aeruginosa* isolates from hospitals associated with the PRMC, determine the genetic relatedness of the isolates, and identify the mechanism(s) associated with the carbapenem-resistant phenotype.

The PRMC is located in the San Juan metropolitan area and includes six teaching hospitals, an emergency room, and a central laboratory. Between October 2006 and March 2007, 513 *P. aeruginosa* isolates were isolated at the PRMC laboratory. During this period, 37/513 (7.2%) unique, consecutive *P. aeruginosa* isolates were identified as being imipenem nonsusceptible, according to microdilution testing using the Vitek DCS-R5 system. MICs for piperacillin-tazobactam, ceftazidime, imipenem, and meropenem were obtained for the 37 isolates by using agar dilution (3). Thirty-one of 37 and 29/37 isolates had high MICs (≥32 μg/ml) to imipenem and meropenem, respectively (Table 1). The remaining isolates had MICs ranging from 8 to 16 μg/ml for both carbapenems, corresponding with an intermediate or resistant phenotype. Twenty-eight of 37 isolates were piperacillin-tazobactam resistant (MIC ≥ 128 μg/ml), while 31/37 isolates were ceftazidime resistant (MIC ≥ 32 μg/ml) (Table 1). Of the 37 carbapenem-nonsusceptible isolates, 70.2% (26/37) were resistant to all four β-lactams tested.

Genetic relatedness of the carbapenem-resistant *P. aeruginosa* isolates from Puerto Rico was determined using pulsed-field gel electrophoresis (PFGE) (8). The 37 isolates were divided into 13 major PFGE groups (Fig. 1). Group 11 contained the largest number of isolates (25/37), was comprised of four highly related (i.e., ≥93% similarity) subgroups (11A to 11D), and was resistant to all β-lactams tested. Groups 1, 3, 4, 7, 9, and 10 consisted of at least two isolates cultured from different patients. Isolates in each respective group shared a common susceptibility pattern, except for isolates belonging to group 9. PS1 (group 9C) had a PFGE pattern that was 90% similar to those of PS4 (group 9B) and PS33 (group 9A) but had ceftazidime MICs that were at least 64-fold lower and carbapenem MICs fourfold lower than those of PS4 and PS33

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TABLE 1. Characteristics of the *P. aeruginosa* isolates from Puerto Rico

Strain	Sample site	Hospital[a]	Hospital ward[b]	PFGE group	MIC (μg/ml)[c]				PCR result[d]				pI	Sequence analysis[e]	Expression of[f]:		OprD production[g]
					P/T	CAZ	IPM	MEM	IMP	VIM	KPC	OXA			*mexA*	*oprD*	
PAO1					4	1	1	0.5							1.0	1.0	+
PS1	Sputum	1	General	9C	8	2	16	8	Neg	Neg	Neg				4.2	0.008	
PS2	Urine	1	ICU	10B	4	128	64	16	Pos	Neg	Neg	Pos	8.0	IMP-18, OXA-2			
PS3	Sputum	2	ICU	12	8	4	16	8	Neg	Neg	Neg				1.0	0.002	−
PS4	Sputum	3	General	9B	64	>128	>64	64	Pos	Neg	Neg	Neg	5.4, 7.5, ≥8.5	IMP-18			
PS5	Sputum	1	ICU	7A	>256	64	>64	>64	Neg	Neg	Pos	Neg	6.7, ≥8.5	KPC-2	1.8	0.005	−
PS6A	Sputum	4	ICU	11A	>256	32	>64	>64	Neg	Neg	Pos				1.8	0.0005	−
PS7A	Urine	4	ICU	11D	>256	64	>64	>64	Neg	Neg	Pos						
PS9	Sputum	1	General	3B	64	8	8	16	Neg	Neg	Neg						
PS10	Blood	1	General	1A	>256	64	>64	>64	Neg	Neg	Pos	Neg	6.7, ≥8.5	KPC-2	1.5	0.003	−
PS11A	Sputum	1	ICU	7A	>256	32	>64	>64	Neg	Neg	Pos						
PS12	Sputum	1	General	7B	>256	64	>64	32	Neg	Neg	Pos						
PS13	Sputum	1	ICU	11E	>256	64	>64	>64	Neg	Neg	Pos						
PS14	Sputum	1	ICU	10C	4	64	64	16	Pos	Neg	Neg						
PS15A	Blood	1	General	10A	8	128	32	16	Pos	Neg	Neg						
PS17B	Blood	1	ICU	11D	>256	64	>64	>64	Neg	Neg	Pos	Neg	6.7, ≥8.5	KPC-2	2.0	0.0004	−
PS18A	CSF	1	ICU	11D	>256	32	>64	>64	Neg	Neg	Pos						
PS19A	Urine	1	General	11C	>256	64	>64	>64	Neg	Neg	Pos						
PS20A	Skin	5	General	11D	>256	64	>64	>64	Neg	Neg	Pos				1.9	0.0003	−
PS21	Skin	4	General	11F	>256	64	>64	>64	Neg	Neg	Pos						
PS22	Urine	4	General	3A	256	16	8	16	Neg	Neg	Neg						
PS23	Blood	1	General	4A	>256	128	>64	>64	Neg	Neg	Pos		6.7, ≥8.5	KPC-2			
PS25	Sputum	1	General	1A	>256	64	>64	>64	Neg	Neg	Pos						
PS26	Sputum	3	ICU	3A	128	8	8	16	Neg	Neg	Neg	Pos	7.5, 8.0	OXA-2			
PS28	Skin	5	General	6	128	128	>64	>64	Neg	Neg	Pos		7.65, ≥8.5	KPC-5			
PS29	Sputum	4	General	11D	>256	64	>64	>64	Neg	Neg	Pos						
PS30	Sputum	4	General	11D	>256	64	>64	>64	Neg	Neg	Pos						
PS31	Skin	4	General	11B	>256	64	>64	>64	Neg	Neg	Pos						
PS33	Blood	1	General	9A	32	>128	64	32	Pos	Neg	Neg						
PS34	Blood	1	ICU	4A	>256	64	>64	>64	Neg	Neg	Pos						
PS35	Urine	4	General	11A	>256	64	>64	>64	Neg	Neg	Pos						
PS36	Urine	4	General	11B	>256	64	>64	>64	Neg	Neg	Pos						
PS37	Sputum	4	General	11D	>256	64	>64	>64	Neg	Neg	Pos						
PS39	Urine	1	General	8	128	>128	>64	64	Pos	Neg	Neg	Neg	5.4, 6.0, 7.5, ≥8.5	IMP-18	4.1	0.015	−
PS40	Urine	6	General	5	128	32	>64	>64	Neg	Neg	Pos	Pos	6.7, 8.0, ≥8.5	KPC-2, OXA-2			
PS41	Urine	4	General	11D	>256	64	>64	>64	Neg	Neg	Pos						
PS43	Skin	1	ICU	2	256	16	16	32	Neg	Neg	Pos	Pos	6.7, 8.0, ≥8.5	KPC-2, OXA-2	1.8	0.56	−
PS44	Blood	ER	NR	13	32	>128	64	32	Pos	Neg	Neg	Neg	5.4, 6.0, 7.5, ≥8.5	IMP-18	3.3	0.36	−

[a] ER, emergency room.
[b] ICU, intensive care unit (includes medical, surgical, pediatric, and neurosurgical units); NR, not reported.
[c] P/T, piperacillin-tazobactam; CAZ, ceftazidime; IPM, imipenem; MEM, meropenem.
[d] Neg, negative for amplification; Pos, positive for amplification.
[e] The bla_{KPC} and bla_{IMP-18} sequence represents the entire structural gene. The bla_{OXA-2} sequence was obtained from 555 bp of the amplified fragment.
[f] Expression data are presented as fold differences compared to the wild-type strain PAO1.
[g] OprD production was assessed by outer membrane analysis.

(Table 1). Groups 2, 5, 6, 8, 12, and 13 were represented by single isolates.

A majority of isolates (19/37) were collected from hospital 1 and belonged to eight distinct PFGE groups (Table 1). Hospital 4 had the second highest number of isolates (11/37) but consisted only of two groups (groups 3 and 11), with group 11 isolates comprising the majority (10/11). The remaining hospitals had isolated ≤2 carbapenem-resistant *P. aeruginosa* isolates during the 6-month period. Group 11D isolates were from patients in three different hospitals, hospitals 1, 4, and 5 (Table 1), suggesting the clonal spread of this highly resistant isolate. Group 3 isolates were also present in three different hospitals, group 3A in hospitals 3 and 4 and group 3B in hospital 1.

All 37 isolates were evaluated for the presence of metallo-β-lactamases, using the MBL Etest strip (AB Biodisk, Solna, Sweden). Thirty-two of 37 isolates were positive for a metallo-β-lactamase (data not shown). The MBL Etest-negative isolates were PS1, PS3, PS9, PS22, and PS26. All 37 isolates were screened by PCR for bla_{IMP}, bla_{VIM}, and bla_{KPC} (17), using the following primers: IMP-1F (5′-GGAATAGAGTGGCTTAATTC-3′) and IMP-1R (5′-CAACCAGTTTTGCCTTACC-3′); VIM1F (5′-GGTGTTTGGTCGCATATCGC-3′) and VIM1R (5′-CCATTCAGCCAGATCGGCATC-3′); KPCF2 (5′-GTATCGCCGTCTAGTTCTGC-3′) and KPCR2 (5′-GGTCGTGTTTCCCTTTAGCC-3′). Seven of 37 isolates, representing four genetically distinct PFGE groups from patients in two different hospitals and the emergency room, were positive for bla_{IMP} (Table 1). Sequence analysis of bla_{IMP} from representatives of each group identified the gene as bla_{IMP-18} (Table 1). Twenty-five of 37 isolates were positive for bla_{KPC} (Table 1). The bla_{KPC} isolates were collected from four different locations and represented seven genetically distinct (<80% similarity) PFGE groups (Table 1). Sequence analysis of bla_{KPC} from representatives of each group revealed that six isolates contained bla_{KPC-2} while one isolate harbored a new variant, bla_{KPC-5} (GenBank accession number EU400222) (21). All of the isolates were PCR negative for bla_{VIM}. Interestingly, all IMP and KPC PCR-positive isolates were metallo-β-lactamase positive by the MBL Etest, as mentioned above. Upon retesting of several isolates from different PFGE groups by the MBL Etest, the IMP PCR-positive strains (PS2, PS4, PS39, and PS44) were positive, as previously determined. However, seven KPC PCR-positive strains (PS5, PS10, PS17B, PS23, PS28,

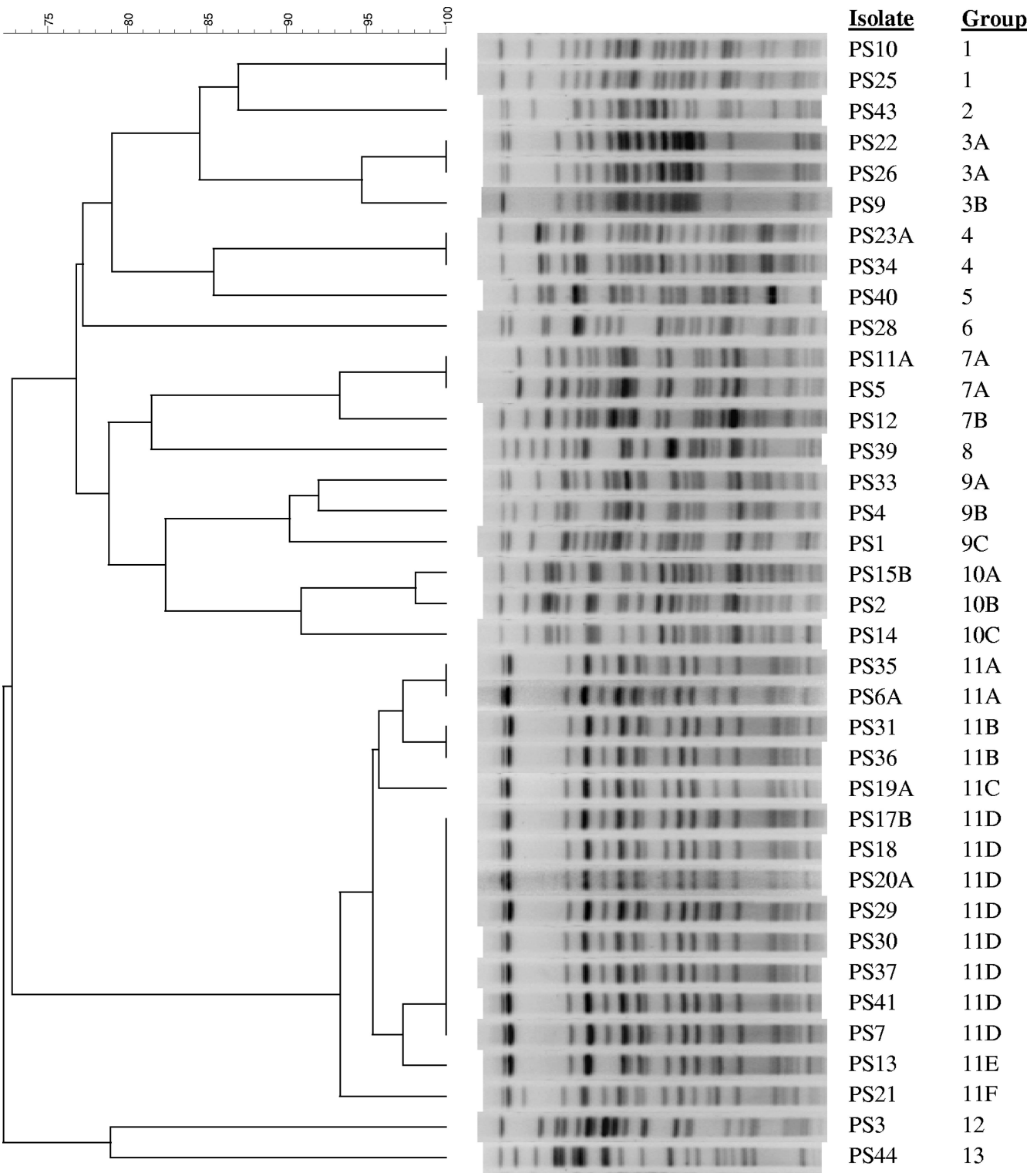

FIG. 1. PFGE of the 37 isolates analyzed. The names and PFGE types of the isolates are shown at the right of the figure. The dendrogram depicting genetic relatedness and generated using BioNumerics is shown at the left of the figure. The scale for the dendrogram represents percentage of relatedness. *Staphylococcus aureus* strain NCTC 8325 was used for the gel-to-gel normalization required to generate the dendrogram.

PS40, and PS43) were negative after repeat testing, indicating that the initial results were false positives.

Twelve isolates belonging to different PFGE groups were further evaluated by isoelectric focusing (17), including seven bla_{KPC}-positive isolates, four bla_{IMP}-positive isolates, and one isolate negative for both. Six KPC PCR-positive isolates produced a β-lactamase with a pI of 6.7, whereas no band was observed for KPC PCR-negative isolates at this pI (data not

shown). PS28, containing bla_{KPC-5}, produced a β-lactamase with a pI of 7.65, differing from the other KPC-positive isolates. All KPC enzymes were partially inhibited by clavulanate (data not shown). In addition to KPC, these isolates produced chromosomal AmpC (pI ≥ 8.5). PS40 and PS43 also produced an enzyme with a pI of 8.0, indicative of an OXA-2-like β-lactamase (Table 1). The presence of bla_{OXA-2} was verified by PCR, using OXA-2 family-specific primers (13), and sequencing the generated product. Of the IMP PCR-positive isolates, PS2 had a β-lactamase with a pI of 8.0, correlating with the presence of bla_{OXA-2} (Table 1). PS4, PS39, and PS44 produced at least three additional β-lactamases (Table 1). PS26 was negative for both bla_{KPC} and bla_{IMP} but produced an enzyme with a pI of 8.0, corresponding to OXA-2.

To determine if OprD downregulation and/or increased *mexAB-oprM* pump expression contributed to the observed carbapenem MICs, *mexA* and *oprD* expression (20) and OprD production (20) were analyzed in 10 selected *P. aeruginosa* isolates. These isolates represented eight genetically distinct (<80% identity) PFGE groups from five different locations. Regardless of location or genetic relatedness, 8/10 isolates had a drastic decrease (between ~65- and 3,000-fold) in *oprD* transcript (Table 1), correlating with OprD protein loss, compared to wild-type strain PAO1. Two isolates, PS43 and PS44, had a small decrease (two- to threefold) in *oprD* expression; however, OprD protein was absent (Table 1). OprD loss in PS44 resulted from a mutation within the structural gene (G_{580}→T substitution) creating a premature stop codon. No mutations were observed in *oprD* of PS43. The *oprD* genes from PS5 and PS39 contained a nucleotide insertion (C_{73}) or a base substitution (G_{580}→T) resulting in a frameshift or premature stop codon, respectively. Sequence analysis revealed the insertion of IS element IS*1394* 1 bp upstream of the *oprD* translational start codon in PS17B. IS*1394* insertion between the promoter and start codon most likely contributed to the significant decrease (2,500-fold) in *oprD* expression.

Three isolates (PS1, PS39, and PS44) belonging to PFGE groups 9C, 8, and 13, respectively, produced *mexA* transcript levels three- to fourfold higher than that produced by the wild-type isolate, PAO1 (Table 1), which was comparable to the level produced by the *mexAB-oprM* overexpressing control strain, PS1454 (4.3-fold higher) (data not shown). PS1 had a meropenem MIC of 8 μg/ml, corresponding with OprD loss and *mexAB-oprM* overexpression. PS39 and PS44 had higher meropenem MICs (≥32 μg/ml), which were attributed to a combination of OprD loss, *mexAB-oprM* overexpression, and IMP-18 production.

Characteristics of the 37 patients with carbapenem-resistant *P. aeruginosa* isolates included a mean age of 50 years (range, 1 month to 99 years), with a statistically significant male gender distribution (70% male versus 30% female; $P \leq 0.05$) (Table 1). The most common anatomical site of infection was the respiratory tract (41%), followed by the urinary system (25%) and blood (19%).

P. aeruginosa can utilize numerous resistance mechanisms to counteract therapeutic selective pressures, as evident in the isolates examined from the PRMC hospitals. Although KPC β-lactamases have been predominantly encountered in the *Enterobacteriaceae* (1, 2, 4), the first report of KPC-producing *P. aeruginosa* isolates was described in three genetically related isolates from Colombia in 2007 (19). Therefore, we were surprised to find 25/37 bla_{KPC}-positive *P. aeruginosa* isolates representing seven genetically unrelated PFGE groups, collected from patients of various ages, from multiple sites of infection, and from four different PRMC hospitals. KPC enzymes have recently been identified in *Klebsiella pneumoniae* and *Escherichia coli* isolates from PRMC hospitals (16). Whether these organisms represent the source of bla_{KPC} transmission to *P. aeruginosa* in Puerto Rico remains unknown. However, the spread of bla_{KPC} into different genera is most likely associated with its residence within mobile genetic elements on plasmids of various sizes (14).

Little information is available regarding the metallo-β-lactamase, IMP-18. bla_{IMP-18}-positive single isolates of *P. aeruginosa* were identified in hospitalized patients from Mexico and the southwestern United States (6, 9). The spread of IMP-18 into multiple unrelated isolates from Puerto Rico indicates the movement of bla_{IMP-18} by mobile genetic elements.

The OprD porin was absent in all of the isolates examined. In the absence of acquired carbapenemases, carbapenem MICs were ≤16 μg/ml. The presence of KPC in OprD-deficient isolates often correlated with increased carbapenem MICs (>64 μg/ml). PS43, with imipenem and meropenem MICs of 16 μg/ml and 32 μg/ml, respectively, was the only exception. The presence of IMP-18 in OprD-deficient isolates PS39 and PS44 also corresponded with increased carbapenem MICs (32 to >64 μg/ml). In addition, these isolates overexpressed the *mexAB-oprM* pump, which may have further elevated the meropenem MICs. The absence of OprD coupled with KPC or IMP-18 appeared to have a synergistic effect on the carbapenem MICs. Since all KPC/IMP-18-positive isolates were also OprD deficient, the effect of these enzymes alone on carbapenem susceptibilities has yet to be determined.

In Puerto Rico, the carbapenem resistance rates are similar to those observed in the continental United States (15), but the high percentage of *P. aeruginosa* isolates producing a KPC is unique to this region. Our study has shown the dissemination of bla_{KPC} into several unrelated isolates of *P. aeruginosa* in addition to the spread of a clonal isolate into multiple PRMC hospitals. Therefore, the widespread carbapenem resistance observed in these *P. aeruginosa* isolates was not exclusively a hospital infection control issue.

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REFERENCES

1. **Bradford, P. A., S. Bratu, C. Urban, M. Visalli, N. Mariano, D. Landman, J. J. Rahal, S. Brooks, S. Cebular, and J. Quale.** 2004. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. Clin. Infect. Dis. **39:**55–60.
2. **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.
3. **Clinical and Laboratory Standards Institute.** 2008. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, 6th ed. Approved standard M7-A6. Clinical and Laboratory Standards Institute, Wayne, PA.
4. **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.
5. **Doern, G. V., R. N. Jones, M. A. Pfaller, M. Erwin, C. Ramirez-Rhonda, et al.** 1998. Multicenter evaluation of the in vitro activity of six broad-spectrum

beta-lactam antimicrobial agents in Puerto Rico. Diagn. Microbiol. Infect. Dis. **30:**113–119.
6. **Garza-Ramos, U., P. Tinoco, J. Silva-Sanchez, R. Morfin-Otero, E. Rodriguez-Noriega, G. Leon-Garnica, H. S. Sader, and R. N. Jones.** 2008. Metallo-beta-lactamase IMP-18 is located in a class 1 integron (In96) in a clinical isolate of Pseudomonas aeruginosa from Mexico. Int. J. Antimicrob. Agents **31:**78–80.
7. **Giske, C. G., C. Boren, B. Wretlind, and G. Kronvall.** 2005. Meropenem susceptibility breakpoint for *Pseudomonas aeruginosa* strains hyperproducing *mexB* mRNA. Clin. Microbiol. Infect. **11:**662–669.
8. **Goering, R. V.** 2004. Pulsed-field gel electrophoresis, p. 185–195. *In* D. H. Persing et al. (ed.), Molecular microbiology: diagnostic principles and practice. ASM Press, Washington, DC.
9. **Hanson, N. D., A. Hossain, L. Buck, E. S. Moland, and K. S. Thomson.** 2006. First occurrence of a *Pseudomonas aeruginosa* isolate in the United States producing an IMP metallo-β-lactamase, IMP-18. Antimicrob. Agents Chemother. **50:**2272–2273.
10. **Kohler, T., M. Michea-Hamzehpour, S. F. Epp, and J. C. Pechere.** 1999. Carbapenem activities against *Pseudomonas aeruginosa*: respective contributions of OprD and efflux systems. Antimicrob. Agents Chemother. **43:**424–427.
11. **Lister, P. D.** 2002. Chromosomally-encoded resistance mechanisms of *Pseudomonas aeruginosa*: therapeutic implications. Am. J. Pharmacogenomics **2:**235–243.
12. **Livermore, D. M.** 2002. Multiple mechanisms of antimicrobial resistance in *Pseudomonas aeruginosa*: our worst nightmare? Clin. Infect. Dis. **34:**634–640.
13. **Moland, E. S., N. D. Hanson, J. A. Black, A. Hossain, W. Song, and K. S. Thomson.** 2006. Prevalence of newer β-lactamases in gram-negative clinical isolates collected in the United States from 2001 to 2002. J. Clin. Microbiol. **44:**3318–3324.
14. **Naas, T., G. Cuzon, M. V. Villegas, M. F. Lartigue, J. P. Quinn, and P. Nordmann.** 2008. Genetic structures at the origin of acquisition of the β-lactamase bla_{KPC} gene. Antimicrob. Agents Chemother. **52:**1257–1263.
15. **Rhomberg, P. R., L. M. Deshpande, J. T. Kirby, and R. N. Jones.** 2007. Activity of meropenem as serine carbapenemases evolve in US medical centers: monitoring report from the MYSTIC program (2006). Diagn. Microbiol. Infect. Dis. **59:**425–432.
16. **Robledo, I. E., E. S. Moland, E. A. Aquino, G. J. Vázquez, M. I. Santé, J. Bertrán, and N. D. Hanson.** 2007. First report of a KPC-4 and CTX-M producing *K. pneumoniae* (Kp) isolated from Puerto Rico (PR), abstr. C2-1933. Abstr. 47th Intersci. Conf. Antimicrob. Agents Chemother., Chicago, IL.
17. **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.
18. **Vázquez, G. J., I. E. Robledo, A. Arroyo, E. Nadal, R. Rodríguez, and M. Bermúdez.** 2003. A comparison of the antimicrobial resistance patterns of gram-negative bacilli isolated from community-private and university-affiliated hospitals from Puerto Rico. P. R. Health Sci. J. **22:**265–271.
19. **Villegas, M. V., K. Lolans, A. Correa, J. N. Kattan, J. A. Lopez, and J. P. Quinn.** 2007. First identification of *Pseudomonas aeruginosa* isolates producing a KPC-type carbapenem-hydrolyzing β-lactamase. Antimicrob. Agents Chemother. **51:**1553–1555.
20. **Wolter, D. J., N. D. Hanson, and P. D. Lister.** 2005. AmpC and OprD are not involved in the mechanism of imipenem hypersusceptibility among *Pseudomonas aeruginosa* isolates overexpressing the *mexCD-oprJ* efflux pump. Antimicrob. Agents Chemother. **49:**4763–4766.
21. **Wolter, D. J., P. M. Kurpiel, N. Woodford, M. F. Palepou, R. V. Goering, and N. D. Hanson.** 2009. Phenotypic and enzymatic comparative analysis between the novel KPC variant, KPC-5, and its evolutionary variants, KPC-2 and KPC-4. Antimicrob. Agents Chemother. **53:**557–562.