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Detection of the KPC Gene in *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* during a PCR-Based Nosocomial Surveillance Study in Puerto Rico[▽]

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A 6-month, PCR-based, island-wide hospital surveillance study of beta-lactam resistance in *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* was conducted in Puerto Rico. Of 10,507 isolates, 1,239 (12%) unique, multi-beta-lactam-resistant isolates from all geographical regions were identified. The KPC gene was detected in 61 *E. coli*, 333 *K. pneumoniae*, 99 *P. aeruginosa*, and 41 *A. baumannii* isolates, indicating the widespread dissemination of the KPC gene in clinically significant nosocomial isolates.

During the past decades, the emergence of multi-beta-lactam-resistant (MβLR) Gram-negative bacilli has become an important clinical problem associated with increases in mortality rates and the length and cost of hospital stays. Acquired broad-spectrum beta lactamases have been identified with increasing frequency in clinical isolates of Gram-negative bacilli (2, 7). KPC (*Klebsiella pneumoniae* carbapenemase) belongs to the Ambler class A, Bush subgroup 2f, serine-based carbapenemases, which are active against all beta-lactams, including the carbapenems (9). Ten KPC variants (KPC-2 to -11) are currently known. The KPC enzyme has been detected worldwide in *Enterobacteriaceae* and recently in *Pseudomonas aeruginosa* isolates from Colombia, Puerto Rico, Trinidad and Tobago, and the United States and in *Acinetobacter baumannii* in Puerto Rico (1, 8, 11, 13). The KPC gene has been found associated with the plasmid-borne transposon Tn*4401*, which may be responsible for its rapid dissemination (4, 6).

Previous studies conducted in Puerto Rico have detected a significant number of KPC-positive Gram-negative bacilli in Puerto Rico Medical Center hospitals (10–12, 14). Pulsed-field gel electrophoresis of these isolates showed both clonally related and unrelated isolates. In September of 2008, the first outbreak caused by a KPC-positive *K. pneumoniae* strain was identified in a hospital located in the southern region of Puerto Rico (5). The aim of this study was to perform an island-wide surveillance study to identify and determine the geographical distribution of KPC-positive nosocomial Gram-negative bacilli.

A PCR-based surveillance study of beta-lactam resistance was conducted during a 6-month period (January to June 2009) in 17 hospitals across the island. Participating hospitals provided all unique, consecutive, MβLR *Escherichia coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* isolates, together with the corresponding susceptibility reports and basic epidemiologic information. Multi-beta-lactam resistance was defined as resistance to any of the carbapenems and/or two or more of the following antibiotics: ceftriaxone, cefotaxime, ceftazidime, cefepime, aztreonam, and piperacillin-tazobactam. The KPC PCR assay was performed utilizing primers and conditions as previously described (14). No attempts were made to evaluate patients' therapies or clinical outcomes. Statistical analysis was performed utilizing the two-tailed Fisher exact test. A P value of ≤ 0.05 was considered statistically significant.

Table 1 shows the total number of isolates together with the MβLR and KPC-positive organisms identified during the study period. Using the monthly bacteriology laboratory reports submitted by the participating hospitals, 10,507 *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* clinical isolates were identified. This, however, represents an overestimation due to the collection of multiple identical isolates from the same patients. A total of 1,239 unique, consecutive MβLR isolates were identified, representing 12% of the 10,507 isolates. The KPC gene was detected in 5% (534/10,507) of the total and 43% (534/1,239) of the MβLR isolates. The distribution of the KPC gene among the MβLR isolates was as follows: *E. coli*, 28% (61/219); *K. pneumoniae*, 73% (333/457); *P. aeruginosa*, 36% (99/272); *A. baumannii*, 14% (41/291). There was a statistically significantly higher number of KPC-positive *Klebsiella pneumoniae* isolates than of each of the other three organisms ($P \leq 0.05$) in all regions.

Table 2 shows the total number of hospitals, beds, and KPC-

TABLE 1. Numbers of KPC-positive *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* isolates among the total number of isolates and the multi-beta-lactam-resistant isolates

Organism	No. of isolates			No. of KPC producers/ total (%)	
	Total	MβLR	KPC producers	All isolates	MβLR isolates
E. coli	4,329	219	61	61/4,329 (1.4)	61/219 (33)
K. pneumoniae[a]	2,805	457	333	333/2,805 (12)	333/457 (73)
P. aeruginosa	2,415	272	99	99/2,415 (4.1)	99/272 (44)
A. baumannii	958	291	41	41/958 (4.3)	41/291 (14)
Total	10,507	1,239	534	534/10,507 (5)	534/1,239 (43)

[a] $P \leq 0.05$.

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TABLE 2. Total numbers of hospitals, beds, and KPC-positive isolates by geographic region

Geographic region	Total no. (%) of:					No. of KPC-positive isolates/total (%)				
	Hospitals	Beds	Isolates	MβLR isolates	KPC producers	*E. coli*	*K. pneumoniae*[a]	*P. aeruginosa*	*A. baumannii*	Overall
Puerto Rico Medical Center	6	858	2,472	354	180	19/32 (59)[a]	101/122 (83)	41/67 (61)[a]	19/133 (14)	180/354 (51)
Metropolitan	3	906	2,371	196	72	8/52 (15)	39/59 (66)	18/46 (39)	7/39 (18)	72/196 (37)
North	2	439	1,706	259	87	15/73 (21)	65/94 (69)	3/56 (5)	4/36 (11)	87/259 (34)
West	2	324	1,316	95	43	1/13 (8)	26/38 (68)	15/27 (56)[a]	1/17 (6)	43/95 (45)
South	2	593	1,299	176	86	15/35 (43)[a]	55/75 (73)	13/48 (27)	3/18 (17)	86/176 (49)
Central/East	2	497	1,343	159	66	3/14 (21)	47/69 (68)	9/28 (32)	7/48 (15)	66/159 (42)
Total	17	3,617	10,507	1,239/10,507 (12)	534/1,239 (43)	61/219 (28)	333/457(73)	99/272 (36)	41/291 (14)	534/1239 (43)

[a] $P \leq 0.05$.

positive isolates by geographic region. The metropolitan region had the highest number of hospital beds. Comparison of the numbers of KPC-positive isolates of these organisms by geographic region showed that the number of *E. coli* isolates was significantly higher in the southern and Puerto Rico Medical Center regions ($P \leq 0.05$) and that of *P. aeruginosa* was significantly higher in the Puerto Rico Medical Center and western regions ($P \leq 0.05$), while *K. pneumoniae* and *A. baumannii* were equally distributed among all areas.

Table 3 shows the baseline epidemiological information of the KPC-positive isolates. KPC-positive isolates were similarly distributed between cultures obtained from male and female patients. Samples obtained from the respiratory and urinary tracts yielded significantly higher numbers of such organisms than those obtained from other anatomical sites ($P \leq 0.05$). The distribution of the KPC-positive isolates by hospital area revealed that 167 were identified in the intensive care unit (ICU) and 349 in the general wards. The reason(s) for this difference is not clear from our data; it could suggest simply a higher number of specimens obtained from the general wards, the transfer of infected or colonized patients from the ICU to the general wards, hospitalization of patients already colonized with KPC-positive isolates, and/or that the organisms are not confined to a specific hospital area. Table 4 shows the antibiotic susceptibilities of the KPC-positive isolates to selected agents. The imipenem susceptibility breakpoints were reported as ≤4 μg/ml since the samples were collected prior to the June 2010 Clinical and Laboratory Standards Institute carbapenem susceptibility breakpoint changes. With the exception of the susceptibility of *E. coli* to imipenem (83%), the antimicrobial activity of the beta- and non-beta-lactam antibiotics was marginal to very poor. Unfortunately, susceptibility to polymyxins and tigecycline was not reported. Phenotypic detection of extended-spectrum beta-lactamases (ESBLs) was observed in 24% and 62% of the KPC-positive *K. pneumoniae* and *E. coli* isolates, respectively. These results are in agreement with the recent literature reports and suggest the presence of multiple different mechanisms of antibiotic resistance in these isolates (2, 3, 7).

This surveillance study clearly demonstrated that the KPC gene has readily spread among important nosocomial pathogens in Puerto Rico. The reasons for this dissemination are not clear from our results; however, it can be speculated that multiple social and microbiological factors may be at play, such as: the small size of the island (3,435 square miles) with a high population density of 1,158.5 inhabitants per square mile (2009 estimate); the ease of ground transportation that facilitates the movement of patients to different hospitals; constant air travel between Puerto Rico and the continental United States and other countries; the extensive use of broad-spectrum antibiotics due to the high number of ESBL-positive isolates; antibiotic misuse; lax infection control practices; and/or the horizontal

TABLE 3. Baseline clinical information on KPC-positive *E. coli*, *K. pneumoniae K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* isolates

Organism	Total no. of KPC-positive isolates	No. of isolates from patients of following gender:			No. of isolates from following hospital unit:			No. of isolates from following anatomical site:					
		F[a]	M[b]	NR[c]	ICU	General ward	NR	RT[d,h]	UT[e,h]	SST[f]	Blood	Misc.[g]	NR
E. coli	61	26	35	0	13	44	4	9	23	12	10	7	0
K. pneumoniae	333	162	167	4	108	212	13	90	93	57	49	42	2
P. aeruginosa	99	46	50	3	28	71	0	35	22	18	10	13	1
A. baumannii	41	18	22	1	18	22	1	16	4	7	8	5	1
Total	534	252	274	8	167	349	18	150	142	94	77	67	4

[a] F, female.
[b] M, male.
[c] NR, not reported.
[d] RT, respiratory tract.
[e] UT, urinary tract.
[f] SST, skin and soft tissue.
[g] Misc., miscellaneous.
[h] $P \leq 0.05$.

TABLE 4. Total numbers and percentages of isolates susceptible to selected antibiotics[a]

Organism	Total no. of KPC producers	No. of isolates susceptible/total (%)						
		Ceftriaxone	Cefepime	Imipenem	Piperacillin-tazobactam	Amikacin	Ciprofloxacin	ESBL
K. pneumoniae	333	21/288 (7)	31/300 (10)	89/316 (28)	7/299 (2)	114/330 (35)	46/332 (14)	80/332 (24)
E. coli	61	10/52 (19)	10/51 (20)	44/53 (83)	24/52 (46)	39/60 (65)	10/60 (17)	37/60 (62)
P. aeruginosa	99	2/92 (2)	6/98 (6)	6/95 (6)	16/98 (16)	62/95 (59)	7/98 (7)	ND[b]
A. baumannii	41	0/40 (0)	0/40 (0)	11/33 (33)	2/34 (6)	4/41 (10)	0/41 (0)	ND

[a] Data, including those on ESBL detection, are from the participating hospitals' antimicrobial susceptibility reports. The susceptibility breakpoints (μg/ml) for all isolates are as follows: ≤8, cefepime; ≤4, imipenem; ≤16, amikacin; ≤1, ciprofloxacin. The susceptibility breakpoints of ceftriaxone are ≤1 μg/ml for *Enterobacteriaceae* and ≤8 μg/ml for *P. aeruginosa* and *A. baumannii.* The susceptibility breakpoints (μg/ml) of ceftriaxone are ≤1 and ≤8 for the *Enterobacteriaceae P. aeruginosa* and *A. baumannii*. The susceptibility breakpoints (μg/ml) of piperacillin-tazobactam are ≤64 and 4 for *P. aeruginosa* and ≤16 and 4 for *A. baumannii*.
[b] ND, not done.

transmission of the KPC and other antibiotic resistance genes. This study clearly emphasizes the importance of prompt recognition of these isolates and the establishment of proper therapeutic and infection control measures to reduce the spread of these organisms among patients and within hospitals.

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