

First Outbreak of KPC-3-Producing *Klebsiella pneumoniae* (ST258) Clinical Isolates in a Mexican Medical Center

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A KPC-producing *Klebsiella pneumoniae* isolate was first described in the United States (1), and strains with the carbapenemase enzyme have more recently been described as having a global spread (2). These KPC-type carbapenemase enzymes belong to molecular class A β -lactamases, and they are able to hydrolyze carbapenems and to confer resistance to broad-spectrum antibiotics (3). Risk factors associated with the acquisition of carbapenemase-producing *K. pneumoniae* bacteria include prolonged hospitalization, time in the intensive care unit (ICU), invasive devices, immunosuppression, and the use of multiple antibiotic agents before initial culture (4). In Mexico, no KPC carbapenemase has been described in recent studies of extended spectrum β -lactamase (ESBL)-producing *K. pneumoniae* (5, 6). We report the first outbreak of KPC-3-producing *K. pneumoniae* isolates of sequence type (ST) 258 in a hospital in Mexico City, along with their epidemiologic and molecular characteristics.

During a 9-month period (January to September 2010), we identified 22 patients (between 21 and 83 years of age) and 2 health workers with an imipenem-resistant *K. pneumoniae* isolate, identified by means of the MicroScan Walkaway 96 automated system (Siemens/Dade-Behring, Sacramento, CA, USA). ESBL production assays were performed using the disk diffusion method according to Clinical and Laboratory Standards Institute (CLSI) guidelines (1, 7), and 11/24 isolates (45.8%) were ESBL producers. However, all isolates were positive for carbapenemase production by the modified Hodge test (1, 7). Antibiotic susceptibility testing was carried out by the microdilution method, following CLSI recommendations (1, 8). All isolates were resistant to most of the antimicrobials tested (ceftazidime, cefotaxime, piperacillin, ciprofloxacin, imipenem, meropenem, and gentamicin), and they were susceptible to tigecycline and colistin, for which the susceptibility breakpoints proposed by the European Committee on Antimicrobial Susceptibility Testing (www.eucast.org) were used (2 μ g/ml for tigecycline and colistin) (Table 1). Typing for *bla*_{CTX-}, *bla*_{SHV-}, *bla*_{TEM-}, *bla*_{GES-}, and *bla*_{KPC-} type genes was carried out by PCR (6, 9) on a PerkinElmer/Applied Biosystems 3730 DNA sequencing system (Applied Biosystems, PerkinElmer, Foster City, CA, USA). For the 11 ESBL-producing *K. pneumoniae* isolates, the ESBL genes were not identified in the PCR screen, and the *K. pneumoniae* chromosomal and plasmidic β -lactamases corresponded to SHV-11 and TEM-1, respectively. Nevertheless, the KPC-3 gene was the carbapenemase enzyme identified in all isolates.

Genomic DNA was analyzed by pulsed-field gel electrophoresis (PFGE) (10, 11); by following the criteria of Tenover et al. (11)

and using GelCompar II software (Applied Math, Kortrijk, Belgium), three clonal groups (A, B, and C) and two unrelated isolates were revealed (see Fig. S1 in the supplemental material). Two of the isolates (6420 and 6219) were recovered from the hands of health care personnel and corresponded to isolates from clonal groups B and C (Table 1). Multilocus sequence typing (MLST) was carried out (12), and all KPC-3-producing *K. pneumoniae* isolates belonged to ST258 (Table 1), which has been described as a pandemic clone (13) and is part of clonal complex 292 (14). The identification of isolates with different phylogenetic relationships by PFGE but with the same MLST profile is consistent, according to Vimont et al. (15).

Plasmid analysis was carried out for all isolates (16); one to four high-molecular-weight plasmids were detected in the isolates (Table 1). According to the plasmid profiles, mating experiments were carried out in five isolates (17), and transfer of imipenem resistance was unsuccessful. In order to identify the replicon that contains the KPC-3 gene, a Southern hybridization experiment was carried out using a nonradioactive probe obtained by PCR amplification from the entire KPC-3 gene (ECL direct nucleic acid labeling and detection system; GE Healthcare, Piscataway, NJ, USA) (9). In most isolates, positive signals from the KPC-3 gene were obtained from both a 270-kb and a 70-kb plasmid (data not shown); however, the gene was identified in other plasmids (200, 160, 140, and 120 kb) as well (Table 1). Plasmid preparations obtained by means of ion exchange columns (Qiagen, Valencia, CA, USA) were transformed into *Escherichia coli* TOP10 by electroporation and, once separated in different cells, plasmids were digested with EcoRI enzyme. The restriction pattern revealed two 270- and 70-kb backbone plasmids with imipenem (1 μ g/ml), kanamycin (25 μ g/ml), gentamicin (16 μ g/ml), and ceftazidime (1 μ g/ml) resistance and imipenem (1 μ g/ml), tetracycline (15 μ g/ml), and ceftazidime (1 μ g/ml) resistance phenotypes, respectively. All plasmid incompatibility groups were screened using PCR-based replicon typing (18, 19), and the incompatibility

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TABLE 1 Characteristics of KPC-3 *K. pneumoniae* ST258 clinical isolates

Isolate	Isolation date (day/mo/yr)	Ward ^a	Sample origin	PFGE ^b	MLST (ST)	Plasmid pattern (kb) ^c	MIC (μg/ml) ^d								
							CAZ	CTX	PIP	CIP	IPM	IPM-CLA	MEM	GEN	TGC
6406	03/08/2010	ICU	Respiratory tract	A	258	120 , 70	>256	>256	>256	>64	16	8	64	16	0.5
6407	25/08/2010	ICU	Respiratory tract	A	258	270 , 70	>256	>256	>256	>64	8	1	16	16	0.5
6408	16/04/2010	GS	Respiratory tract	A	258	270 , 70	>256	>256	>256	>64	4	1	16	16	0.5
6409	04/08/2010	GS	Surgical wound	A	258	270	>256	>256	>256	>64	16	4	32	16	0.5
6411	27/07/2010	ICU	Biological fluids	A	258	270	>256	>256	>256	>64	16	2	32	16	0.5
6412	20/09/2010	GS	Surgical wound	A	258	270 , 200	>256	>256	>256	>64	16	4	32	16	0.5
6413	16/03/2010	ICU	Biological fluid	A	258	270 , 70	>256	>256	>256	>64	4	1	16	16	0.5
6421	30/01/2010	IM	Urine	A	258	270 , 140	>256	>256	>256	>64	>256	>256	>256	16	0.5
6414	15/06/2010	ICU	Biological fluid	B	258	270 , 70	>256	>256	>256	>64	16	2	16	16	0.5
6415	15/02/2010	ER	Respiratory tract	B	258	70	>256	>256	>256	>64	4	1	16	32	0.5
6416	20/02/2010	ER	Respiratory tract	B	258	270 , 70	>256	>256	>256	>64	4	1	16	16	0.5
6417	18/02/2010	IM	Respiratory tract	B	258	200 , 100 , 70	>256	>256	>256	>64	4	1	8	16	0.5
6418	17/02/2010	ICU	Respiratory tract	B	258	270 , 110 , 70	>256	>256	>256	>64	4	1	16	16	0.5
6420	16/02/2010	ICU	Hands of health worker	B	258	270 , 230 , 70	>256	>256	>256	>64	4	1	8	16	0.5
6423	29/01/2010	IM	Urine	B	258	270 , 200 , 100 , 70	>256	>256	>256	>64	8	1	16	16	0.5
6424	26/04/2010	ICU	Respiratory tract	B	258	270 , 160 , 70	>256	>256	>256	>64	32	4	16	16	0.5
6422	31/01/2010	IM	Respiratory tract	B	258	270 , 120 , 70	>256	>256	>256	>64	32	4	32	16	0.5
6440	02/04/2010	ICU	Respiratory tract	C	258	270 , 160	>256	>256	>256	>64	4	1	16	16	0.5
6419	16/02/2010	GS	Hands of health worker	C	258	270 , 70	>256	>256	>256	>64	4	1	16	16	0.5
6439	15/04/2010	ICU	Respiratory tract	C	258	270	>256	>256	>256	>64	32	32	64	32	0.5
6490	28/06/2010	GS	Biological fluid	C	258	270 , 100 , 70	>256	>256	>256	>64	16	4	128	8	0.5
9900	12/02/2010	ICU	Surgical wound	C	258	270 , 100 , 70	>256	>256	>256	>64	4	1	8	16	0.5
6410	11/04/2010	IM	Blood	NR	258	270 , 70	>256	>256	>256	>64	4	1	8	16	0.5
9901	12/06/2010	ICU	Biological fluid	NR	258	270 , 160 , 70	>256	>256	>256	>64	8	2	16	16	0.5

^a ICU, intensive care unit; GS, general surgery; IM, internal medicine; ER, emergency room.

^b NR, not related.

^c Values in boldface indicate the presence of the plasmid-borne KPC-3 gene, identified by Southern hybridization.

^d The colistin MIC was <1 μg/ml for all isolates. CAZ, ceftazidime; CTX, cefotaxime; PIP, piperacillin; CIP, ciprofloxacin; IPM, imipenem; CLA, clavulanic acid; MEM, meropenem; GEN, gentamicin; TGC, tigecycline.

groups IncF (FIA, FIB, and FIIs) and repF were identified in both plasmids and confirmed by Southern hybridization (data not shown), using a nonradioactive probe obtained by PCR-based replicon typing.

This is the first report of an outbreak of hospital-acquired KPC-3-producing *K. pneumoniae* isolates in Mexico; in addition, two different plasmids encoding the KPC-3 carbapenemase were identified. At the beginning of the outbreak, a misdiagnosis of KPC-producing *K. pneumoniae* isolates was identified. At the end of the outbreak, a high crude mortality rate (55%) was determined. Moreover, this mortality corresponded to patients with a KPC-producing *K. pneumoniae* isolate culture, a respiratory tract infection, and mechanical ventilation, constituting recognized risk factors (4). Unfortunately, mortality due to this outbreak was higher than the previously reported figures (20). Our considerations on such mortality include the following: before identifying the first isolate of KPC-producing *K. pneumoniae*, we did not use phenotypic methods for carbapenemase screening, which caused a delay in terms of diagnosis and appropriate treatment; also, the use of a tigecycline-colistin combination at the beginning of the outbreak could have been helpful, but colistin was not available in our institution. The spread of *K. pneumoniae* with KPC carbapenemases in Mexican hospitals should be evaluated by multicenter epidemiological studies, since these *K. pneumoniae* isolates belonging to the international epidemic clone ST258 constitute a high-risk factor for nosocomial infections.

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