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CASE REPORT

First Isolate of KPC-2-Producing *Klebsiella pneumonaie* Sequence Type 23 from the Americas

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KPC-2-producing *Klebsiella pneumoniae* isolates mainly correspond to clonal complex 258 (CC258); however, we describe KPC-2-producing *K. pneumoniae* isolates belonging to invasive sequence type 23 (ST23). KPC-2 has scarcely been reported to occur in ST23, and this report describes the first isolation of this pathogen in the Americas. Acquisition of resistant markers in virulent clones could mark an evolutionary step toward the establishment of these clones as major nosocomial pathogens.

CASE REPORT

An 85-year-old man was admitted at the intensive care unit of a hospital in Buenos Aires, Argentina, on 19 March 2013. He presented with poor general condition, sensory impairment, hypotension, poor peripheral perfusion, crackling rales, and desaturation. He had a history of acute myeloid leukemia in 2012 and was currently undergoing chemotherapy with methotrexate (20 mg/week) and prednisone (150 mg/day). Two days after his admission, a methicillin-susceptible *Staphylococcus aureus* isolate was obtained from a blood culture and a tracheal aspirate (10^4 CFU/ml), and the patient was treated with cefazolin. A week later, the patient developed catheter-associated bacteremia due to methicillin-resistant *Staphylococcus epidermidis*, and he received linezolid. He presented intercurrent hypovolemic shock and hypotension, requiring transfusion of 2 units of red blood cells. Concurrently, a hypermucoviscous *Klebsiella pneumoniae* strain, 3089, was recovered from a second tracheal aspirate culture (10^5 CFU/ml). The general condition of the patient worsened, and the patient died on 19 April.

Antimicrobial susceptibility tests were conducted on *K. pneumoniae* 3089 according to CLSI guidelines (1). The isolate was resistant to all beta-lactams, including carbapenems, but remained susceptible to aminoglycosides, fluoroquinolones, trimethoprim sulfamethoxazole, doxycycline, fosfomycin, colistin, and tigecycline. A positive result for a test of synergy between imipenem (30 μg) and phenyl boronic acid (300 μg) containing disks indicated the possible presence of KPC beta-lactamases. The presence of bla_{KPC} was confirmed by PCR amplification, and its genetic context was investigated by PCR mapping and sequencing, using plasmid DNA as the template (Fig. 1) (2). As expected, bla_{KPC-2} was located in Tn*4401* as previously reported by Naas et al. (3). Replicon typing, determined according to the method of Carattoli et al. (4), indicated that the bla_{KPC-2}-containing plasmid corresponded to the FIA incompatibility group, which had previously been reported to occur in *Escherichia coli* in Argentina by Gomez et al. (5). Conjugation assays, using both *Escherichia coli* HB101 and *Escherichia coli* CAG 12177 as receptor strains, did not yield transconjugants, according to a previously mentioned study (5).

KPC-producing *K. pneumoniae* isolates are, nowadays, endemic in different countries. The successful dissemination of *K. pneumoniae* isolates belonging to clonal complex 258 was a critical factor resulting in their pandemic expansion (6). In our country, a substantial increase of KPC-2-producing *K. pneumoniae* was observed in 2010, due to the huge dissemination of the hyperendemic sequence type 258 (ST258) clone, which displayed a multidrug-resistant phenotype (5, 7, 8). A multilocus sequence typing (MLST) scheme was conducted on *K. pneumoniae* 3089 (9). Unexpectedly, this strain displayed the following allelic profile: *gapA*, 2; *infB*, 1; *mdh*, 1; *pgi*, 1; *phoE*, 9; *rpoB*, 4; *tonB*, 12. This profile corresponded to ST23.

K. pneumoniae strains belonging to ST23 correspond to a hypermucoviscous phenotype. Hypermucoviscous strains are associated with a highly invasive syndrome characterized by bacteremia, liver abscesses, metastatic infections, and even endophthalmitis, supurative meningitis, and brain abscess (10, 11). The invasive nature of *K. pneumoniae* ST23 seems to correlate with the hypermucoviscosity that protects from phagocytosis and serum killing by complement. The plasmid-mediated *rmpA* (*r*egulator of *m*ucoid *p*henotype *A*) and *magA* (*m*ucoviscosity-*a*ssociated *g*ene *A*) genes have been associated with this virulent phenotype (12–14). The latter gene, renamed wzy_{KpK1}, is a chromosomal gene that is required for exopolysaccharide biosynthesis and is restricted to *K. pneumoniae* capsule serotype K1, whose strains are considered the most virulent of *K. pneumoniae* (13). Most of the isolates from patients with *K. pneumoniae* liver abscess syndrome (KLAS) belong to the K1 serotype and correspond to ST23 (14, 15). Although KLASs are endemic in Taiwan, they have been reported to occur with increasing frequency in other countries in Southeast Asia. They constitute an emerging infectious disease in the United States and Europe; moreover, they were recently reported to occur in Argentina (11, 13, 16). Hypermucoviscous *K. pneumoniae* isolates, including ST23 clinical strains, have been found to be susceptible to most antibiotics, including third- and fourth-gen-

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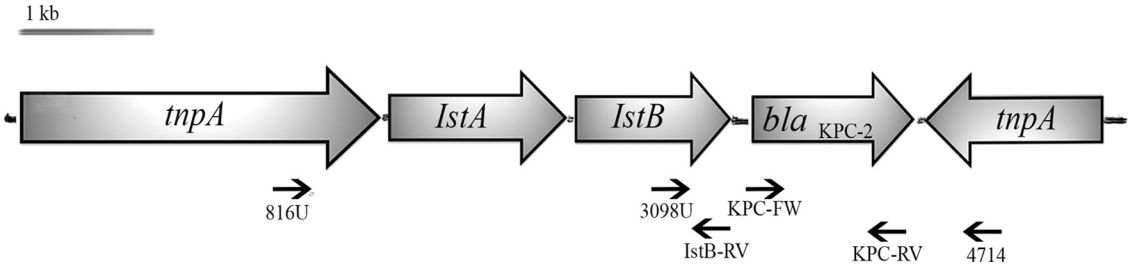

FIG 1 Genetic context of bla_{KPC-2}. The primers (5′ to 3′) used to perform the PCR mapping of bla_{KPC-2} were as follows: KPC-F, ATGTCACTGTATCGCCGTCT; KPC-R, TTTTCAGAGCCTTACTGCCC (2); 816U, CACCTACACCACGACGAACC; 3098U, TGACCCTGAGCGGCGAAAGC; 4714, GAAGATGCCAAGGT CAATGC (3); and IstB-RV, TTCCTGACCACTCCCGCCTTCC (this study).

eration cephalosporins, monobactams, carbapenems, and ciprofloxacin.

As *K. pneumoniae* 3089 exhibited an extreme colony stickiness and rendered a positive string test result (17) (Fig. 2), the presence of *magA* and *rpmA* virulence genes was investigated using the following primers (5′ to 3′): wzy-F, CGCCGCAAATACGAGAA GTG; wzy-R, GCAATCGAAGTGAAGAGTGC; rmpA-F, ACTGG GCTACCTCTGCTTCA; and rmpA-R, CTTGCATGAGCCATCT TTCA. Both hypermucoviscocity-associated genes were detected in the studied isolate.

Although *K. pneumoniae* ST23 isolates can be characterized as susceptible to most antibiotics, here we detected the presence of KPC-2 in an isolate belonging to this invasive sequence type. The presence of KPC carbapenemases in *K. pneumoniae* ST23 has previously been reported to occur only in isolates from China and Poland, in 2010 and 2011, respectively, displaying the same susceptibility profile as *K. pneumoniae* 3089 (18, 19, 20). However, no single mention of the virulence factors or hypermucoviscosity phenotype was included in those studies.

In the last few months, three more hypermucoviscous *K. pneumoniae* ST23 isolates have been referred to our laboratory, displaying phenotypes of susceptibility to all antimicrobials except ampicillin. Considering the virulence factors associated with this phenotype and its highly invasive nature, prompt identification and accurate treatment should be mandatory. These strains can be readily detected by the string test, MLST, and molecular characterization of the hypermucoviscous-phenotype-associated genes.

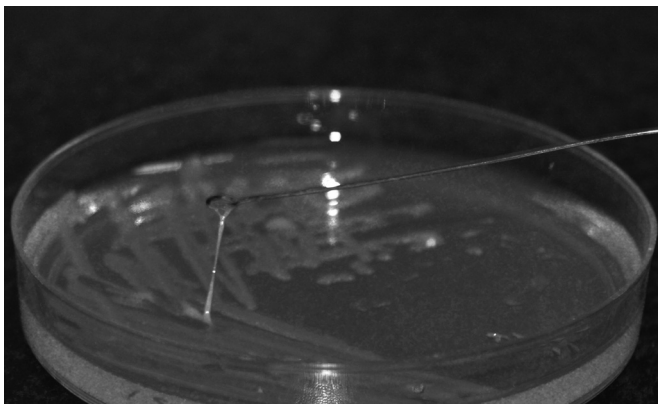

FIG 2 Hypermucoviscous phenotype of *K. pneumoniae* 3089. The hypermucoviscous phenotype is characterized by the formation of elongated (≥5-mm) mucoviscous strings when a loop is passed through a colony (positive string test result).

Antibiotics commonly used in *K. pneumoniae* infections have been useful for the therapeutic treatment of ST23 clinical isolates; however, the acquisition of resistance genes by these invasive strains could hinder the eradication of these strains, probably making the development of metastatic infections favorable.

A rising number of cases of *K. pneumoniae* ST23 infection in geographic regions other than Southeast Asia indicate that ST23 is a globally emerging pathogen. According to Brisse et al., *K. pneumoniae* ST23 constitutes an emerging highly virulent and metabolically versatile clone (14), so the acquisition of an important mechanism of antibiotic resistance such as KPC-2 could mark an evolutionary step toward the establishment of *K. pneumoniae* ST23 as a major cause of nosocomial infections.

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