First American Isolate of KPC-2 Producing Klebsiella pneumoniae Sequence Type 23

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Running title: KPC-2 producing Klebsiella pneumoniae ST23

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Abstract:

KPC-2 producing *Klebsiella pneumoniae* mainly correspond to the CC258, however we describe KPC-2 producing *K. pneumoniae* belonging to the invasive ST23. KPC-2 has been scarce reported in ST23 and this constitutes its first isolation in America. Acquisition of resistant markers in virulent clones could mark an evolutionary step towards their settling as major nosocomial pathogen.

Case report:

An elderly, 85-year-old, man was admitted at the Intensive Care Unit of a hospital at Buenos Aires, in March 19th, 2013. He presented poor general condition, sensory impairment, hypotension, poor peripheral perfusion, crackling rales and desaturation. He has history of acute myeloid leukemia in 2012, currently undergoing chemotherapy with methotrexate (20 mg/week) and prednisone (150 mg/day). Two days after his admission a meticillin susceptible *Staphylococcus aureus* was isolated from blood culture and tracheal aspirate (10⁴ UFC/ml) and the patient was treated with cefazolin. A week later, the patient developed a catheter associated bacteremia due to meticillin resistant *S. 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* (*K. pneumoniae* 3089) was recovered from a second tracheal aspirate culture (10⁵ UFC/ml). The general condition of the patient worsened and finally died on April 19th.

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

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

\textit{K. pneumoniae} belonging to ST23 correspond to a hypermucoviscous phenotype. Hypermucoviscous strains are associated to a highly invasive syndrome characterized by bacteremia, liver abscesses, metastatic infections, and even endophthalmitis, suppurative meningitis and brain abscess (10, 11). The invasive nature of \textit{K. pneumoniae} ST23 seems to correlate with the hypermucoviscosity that protects from phagocytosis and serum killing by complement. The plasmid-mediated \textit{regulator of mucoid phenotype A} (\textit{rmp}A) and the
mucoviscosity-associated gene A (magA) have been associated to this virulent phenotype (12-14). The last, renamed as wzyKpK1, is a chromosomal gene that is required for exopolysaccharide biosynthesis and is restricted to K. pneumoniae capsule serotype K1, considered to be the most virulent strains 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 KLAS are endemic in Taiwan, they have been reported 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 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-generation cephalosporins, monobactams, carbapenems and ciprofloxacin (17).

As K. pneumoniae 3089 exhibited an extreme colony stickiness and rendered a positive string test (18) (figure 2), the presence of magA and rpmA virulence genes was investigated using the following primers (5´-3´): wz y-F: CGCCGCAAATA CGAGAAGTG, wzy-R: GCAATCGAAGTG AGAGTGC and rmpA-F: ACTGGGCTACCTCTGCTTCA, rmpA-R: CTTGCATGAGCCATCTTTCA. Both hypermucoviscocity associated genes were detected in the studied isolate.

Although K. pneumoniae ST23 isolates usually display a susceptible profile, here we detected the presence of KPC-2 in an isolate belonging to this invasive sequencetype. The presence of KPC carbapenemases in K. pneumoniae ST23 has been only previously reported in isolates from China and Poland, in 2010 and 2011, respectively, displaying the same susceptibility profile as K. pneumoniae 3089 (17, 19, 20). However, no single mention to the virulence factors or hypermucoviscosity phenotype was included in those studies.
In the last few months three more hypermucoviscous \textit{K. pneumoniae} ST23 isolates have been referred to our laboratory, displaying antimicrobial susceptible phenotypes except for ampicillin. Considering the virulence factors associated to this phenotype and its highly invasive feature a 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. Antibiotics commonly used in \textit{K. pneumoniae} infections have been useful for the therapeutic treatment of ST23 clinical isolates, however the acquisition of resistance genes in this invasive strains could hinder their eradication, probably favoring the development of metastatic infections.

A rising number of cases of \textit{K. pneumoniae} ST23 from geographic regions other than Southeast Asia indicate that this is a globally emerging pathogen. According to Brisse \textit{et al.}, \textit{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 towards the settling of \textit{K. pneumoniae} ST23 as a major cause of nosocomial infections.

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Figure 1: Genetic context of \textit{bla}_{KPC-2}.

Legend figure 1: Primers (5'–3') used to perform the PCR mapping of \textit{bla}_{KPC-2}. KPC-F: ATGTCAC TGTATCGCCGTCT, KPC-R: TTTTCAGAGCCTTACTGCCC (2), 816U: CACCTACACCACGACGAACC, 3098U: TGACCCTGAGCGCGAAAGC and 4714: GAAGATGCCAAGGTCAATGC (3) and Is1B-RV:TTCCTGACCACCTCCCGCTTCC (this study).

Figure 2: Hypermucoviscous phenotype of \textit{K. pneumoniae} 3089

Legend figure 2: Hypermucoviscous phenotype is characterized by the formation of elongated (≥5 mm) mucoviscous strings when a loop is passed through a colony (positive string test).