First outbreak of KPC-3-producing *Klebsiella pneumoniae* (ST258) clinical isolate in a Mexican medical center

The KPC-producing *Klebsiella pneumoniae* isolate was first described in the USA (1), and it has been more recently described as a global spread carbapenemase enzyme (2). These carbapenemase KPC-type 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, intensive care unit (ICU) stay, invasive devices, immunosuppression and the use of multiple antibiotic agents before initial culture (4). In Mexico, no carbapenemase KPC has been described in recent studies with extended spectrum β-lactam (ESBL)-producing *K. pneumonia*, (5,6). We report the first outbreak of KPC-3 *K. pneumoniae* isolates of sequencing type (ST) 258 in a hospital in Mexico City, along with its 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 (Dade-Behring MicroScan; Sacramento, CA) automated system. ESBL production assays were performed using the disk diffusion method according to Clinical Laboratory Standard Institute (CLSI) (1,7), whereas 11/24 (45.8%) isolates were ESBL-producers. However, all isolates were positive for carbapenemase-production by...
the modified Hodge test (1,7). The antibiotic susceptibility testing was carried out by the microdilution method following CLSI recommendations (1,8). All isolates were resistant to 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 μg/ml for tigecycline and colistin) (Table 1). The typing for \( \text{bla}_{\text{CTX-}} \), \( \text{bla}_{\text{SHV-}}, \text{bla}_{\text{TEM-}}, \text{bla}_{\text{GES-}} \) and \( \text{bla}_{\text{KPC-types}} \) genes was carried out by PCR (6,9), and all were analyzed on a Perkin Elmer/Applied Biosystems 3730 (Applied Biosystems, PerkinElmer, Foster City, CA, USA), DNA sequencing system. On the eleven ESBL-producing \( \text{K. pneumoniae} \) isolates, the ESBL genes were not identified in the PCR screened and the chromosomal \( \text{K. pneumoniae} \) 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 Pulse Field Gel Electrophoresis (PFGE) (10,11), and following Tenover et al. criteria (11) and using the GelCompar II software (Applied Math, Kortrijk, Belgium), three clonal groups (A, B and C), and two unrelated isolates were revealed (S1). Two of the isolates (6420 and 6219) were recovered from the hands of health care personnel and corresponded to isolates from clones B and C (Table 1). The Multilocus Sequence Typing (MLST) was carried out (12) and all KPC-3-producing \( \text{K. pneumoniae} \) isolates belonged to sequence type (ST) 258 (Table 1). Which has been described as pandemic clone (13), and is part of clonal complex ST292 (14). The identification of isolates with
different phylogenetic relationship by PFGE with the same MLST is consistent according to Vimont et al (15).

Plasmid analysis was carried out in all isolates (16); one to four high-molecular-weight plasmids were detected in the isolates (Table1). According to plasmid profile, 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 non-radioactive probe obtained by PCR amplification from entire KPC-3 gene (9) (ECL Direct Nucleic Acid Labeling and Detection System, GE Healthcare, Piscataway, NJ). The result showed in most isolates, positive signals from the KPC-3 gene in both a 270-kb and a 70-kb plasmid (data not showed); however, it was identified in other plasmids (200-, 160- 140- and 120-kb) as well (Table 1).

The plasmid preparation obtained by means of ion exchange columns (Qiagen, Valencia, CA, USA) were transformed by electroporation in E. coli Top10 and once separated in different cells, these were digested with EcoRI enzyme. The restriction pattern revealed two 270- and 70-kb backbone plasmids, with imipenem (1 μg/ml), kanamycin (25 μg/ml), gentamicin (16 μg/ml) and ceftazidime (1 μg/ml); and imipenem (1 μg/ml), tetracycline (15 μg/ml) and ceftazidime (1 μg/ml) resistance phenotypes, respectively. All plasmid incompatibility groups were screened using the PCR-based replicon typing (18,19); and the incompatibility groups IncF (FIA, FIB, FII s), and repF were identified in both plasmids and confirmed by Southern hybridization (Data not showed), using the non-radioactive probe obtained by the PCR-based replicon typing.
This is the first outbreak report of hospital-acquired KPC-3-producing *K. pneumoniae* isolates in Mexico; in addition, two different plasmids encoding carbapenemase KPC-3 were identified. At the beginning of the outbreak, a misdiagnosis of KPC-producing *K. pneumoniae* isolates was identified. At the end of outbreak, a high prevalence of 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 this *K. pneumoniae* belonging to the international epidemic clone ST258 constitutes a high-risk factor for nosocomial infections.

**Competing interest**

All authors declare no financial or personal relationships with other people or organizations that could have inappropriately influenced their work.
Acknowledgements

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Running title: KPC-3 carbapenemase in Mexico
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References


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

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Abbreviations: CAZ = ceftazidime; CTX = cefotaxime; PIP = piperacillin; CIP = ciprofloxacin; IPM = imipenem; MEM = meropenem; GEN = gentamicin; TGC = ticarcillin-clavulanate.
a Values in bold indicate the plasmid-borne KPC-3 gene, identified by Southern hybridization.

b The minimal inhibitory concentration in all isolates for colistin was <1 μg/ml.

Abbreviations: NR, Not related; ICU, Intensive Care Unit; GS, General surgery; IM, Internal medicine.

Antibiotic abbreviations: ceftazidime, CAZ; cefotaxime, CTX; piperacillin, PIP; ciprofloxacin, CIP; imipenem, IPM; clavulanic acid, CLA, meropenem, MEM; gentamicin, GEN; and tigecycline, TGC.

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* Values in bold indicate the plasmid-borne KPC-3 gene, identified by Southern hybridization.

* The minimal inhibitory concentration in all isolates for colistin was <1 μg/ml.