OXA-163-Producing *Klebsiella pneumoniae* in Cairo, Egypt, in 2009 and 2010

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Two genetically unrelated OXA-163-carrying *Klebsiella pneumoniae* strains were identified from two infection cases in June 2009 and May 2010 in Cairo, Egypt. OXA-163-producing *Enterobacteriaceae* had been previously reported in Argentina only. Both patients had no history of travel abroad. The emergence of this newly recognized OXA-48-related β-lactamase able to hydrolyze cephalosporins and carbapenems is especially worrying in a geographic area where OXA-48 is endemic and effective surveillance for antibiotic resistance is largely unaffordable.

Carbapenems are first-line drugs for severe infections caused by extended-spectrum β-lactamase (ESBL)-producing *Enterobacteriaceae*. However, the emergence and rapid diffusion of carbapenemase-producing Gram negatives in the recent years is compromising the therapeutic efficacy of this class of antimicrobial drugs (2, 11, 16).

Recently, two bla*OXA-163* -producing isolates of *Klebsiella pneumoniae* and *Enterobacter cloacae* have been identified in Argentina (15). OXA-163 differs from OXA-48 by a single amino acid substitution and a 4-amino-acid deletion only, but it works as an extended-spectrum oxacillinase showing a weaker ability to hydrolyze carbapenems than OXA-48 but a concurrent significant ability to hydrolyze extended-spectrum cephalosporins (15). The bla*OXA-163* gene has been putatively proposed as the equivalent in South America of the bla*OXA-48* gene that is increasingly identified in the southern and eastern Mediterranean countries (1, 6, 8, 14).

Here, we report two autochthonous cases of infection caused by two genetically unrelated OXA-163-carrying *K. pneumoniae* strains in Cairo, Egypt, in 2009 and 2010.

Case 1 was a 31-year-old woman hospitalized in Cairo (Egypt) on June 5, 2009, due to acute myelocytic leukemia. She was on therapy by doxorubicin and cytarabine. After 16 days since admission, she was transferred to an intensive care unit because of heart and respiratory failure. Initial antibiotic therapy was started with vancomycin, azithromycin, and imipenem. Blood culture was positive for a carbapenem-resistant strain of *K. pneumoniae* (isolat e18). The patient died on June 23.

Case 2 was a 25-year-old man with an 18-month history of acute lymphocytic leukemia. He was in outpatient follow-up until May 2010, when he was admitted to the hospital due to a lower respiratory tract infection. He was started on cefepime and amikacin on May 14, but the clinical course was complicated. A sputum culture grew carbapenem-resistant *K. pneumoniae* (isolate 78). No further data about the treatment outcome were available for this patient.

Both patients had never traveled outside Egypt.

The two carbapenem-resistant *K. pneumoniae* strains were sent to the molecular epidemiology laboratory of the Department of Sciences for Health Promotion “G. D’Alessandro,” University of Palermo, Italy, for confirmation and typing. The two isolates were initially tested for antimicrobial susceptibility by disk diffusion according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (3). Susceptibility testing for extended-spectrum cephalosporins and carbapenems was performed with Etest strips (bioMérieux, Marnes-La-Coquette, France). Results were interpreted according to the CLSI interpretative criteria (3).

PCR screening for carbapenemase-encoding genes of classes A (bla*KPC*, bla*GES*), B (bla*VIM*, bla*IMP*), and D (bla*OXA*48 alleles was performed using a commercial kit. DNA fragment separation and dereplication were done using the BigDye Terminator version 1.1 cycle sequencing kit (Applied Biosystems, Warrington, United Kingdom) and the ABI Prism 310 Genetic Analyzer (Applied Biosystems, Foster City, CA). The nucleotide (nt) and deduced protein sequences were analyzed with the software available from the National Center for Biotechnology Information website (www.ncbi.nlm.nih.gov). Outer membrane protein (OMP) gene amplification was conducted using ompK35-F and -R and ompK36-F and -R, and PCR products were sequenced using OMP primers according to Kaczmarek et al. (8). The sequences were analyzed by comparison with reported nucleotide sequences in GenBank. Expression levels of the ompK35 and ompK36 genes were not investigated.

To evaluate their clonal relationship, the two *K. pneumoniae* isolates were submitted to pulsed-field gel electrophoresis (PFGE) after XbaI DNA digestion. Strain differentiation by PFGE was based on the criteria of Tenover et al. (17). The rep-PCR DiversiLab Microbial Typing System (bioMérieux, Marcy l’Étoile, France) was also used with the DiversiLab *Klebsiella* kit. DNA fragment separation and detection were done using the Agilent 2100 Bioanalyzer (Agilent Technologies, Santa Clara, CA), and results were analyzed and interpreted using the Kullback-Leibler method, as previously reported (13). Moreover, multilocus sequence typing (MLST) was performed on both isolates according to the protocol described on the *K. pneumoniae* MLST website (http://www.pasteur.fr/recherche/genopole/PF0034/macro/MLST.html)

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The findings of this study are of concern for a number of reasons. First, the detection of OXA-163 outside the continent where it has been firstly identified was unexpected. This finding seems to stand against the hypothesis of OXA-163 as the equivalent in South America of OXA-48 in the Mediterranean area (15). The two patients had no history of travel abroad. Moreover, possible contacts with source patients from Argentina, though it was not possible to investigate them in detail, appear to be unlikely due also to the 1-year-long interval of time between the two cases. An autochthonous emergence in Egypt of the OXA-163 resistance determinant appears to be the more plausible explanation. Second, the two *K. pneumoniae* isolates were genetically unrelated based upon PFGE and MLST. This rules out the clonal expansion of an OXA-163-producing strain in Egypt and, alternatively, supports the hypothesis of horizontal transmission of this genetic determinant via plasmids, according with the previous report (14). Notably, strains belonging to ST20 and ST37 have been previously identified in different countries as ESBL or carbapenemase producers (11, 12). Moreover, it is of special interest that OXA-163 has been identified in Egypt, a country of North Africa, a geographical area where OXA-48-producing Gram negatives are considered endemic (6, 9, 10). Third, both strains had much higher MICs for carbapenems than those reported by Poirel et al. (15). Studies suggest that outer membrane permeability defects coupled with ESBL production can confer resistance to carbapenems (5, 7, 9). This is likely to account for the higher level of carbapenem resistance detected in our strains. It is also noteworthy that the presence of similar mutations altering the properties of the OmpK35 and OmpK36 porins have been previously described in an ST37 *K. pneumoniae* clone carrying bla<sub>CTX-M-15</sub> and exhibiting reduced susceptibility to carbapenems (5). However, our study did not definitively attribute the carbapenem resistance phenotype to OXA-163, because transfer of antimicrobial agent resistance experiments and plasmid analysis were not performed. This is a substantial limitation of our report.

As previously reported about OXA-163-producing strains in Argentina, because of its unique hydrolysis ability mimicking a class A ESBL, OXA-163 could be able to spread unrecognized as a variant of ESBL, which may be an important factor in the epidemiology of carbapenem resistance (16).

### Table 1: Main characteristics of two OXA-163-producing *K. pneumoniae* isolates detected in Egypt, June 2009 and May 2010

<table>
<thead>
<tr>
<th><em>K. pneumoniae</em> strain (patient)</th>
<th>MIC (μg/ml)&lt;sup&gt;a&lt;/sup&gt;</th>
<th>bla genes</th>
<th>Sequence type</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Cefotaxime</td>
<td>Ceftriaxone</td>
<td>Ceftazidime</td>
</tr>
<tr>
<td>18 (case 1)</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
<tr>
<td>78 (case 2)</td>
<td>&gt;256</td>
<td>&gt;256</td>
<td>&gt;256</td>
</tr>
</tbody>
</table>

<sup>a</sup> MICs assessed by Etest.
carbapenemase (15). Surveillance of emergence and dissemination of enterobacteria with complex carbapenem resistance mechanisms is a serious public health problem where the costs can be unaffordable, such as low-resource countries. Further studies are urgently needed to assess prevalence of the bla\textsubscript{OXA-163} genetic determinant worldwide.

Nucleotide sequence accession numbers. The \textit{ompK35} of isolate 78 was assigned accession number JQ660370, and the \textit{ompK36} sequences of isolates 18 and 78 were assigned accession numbers JQ660371 and JQ660372, respectively, in the GenBank database.

REFERENCES