OqxAB, a Quinolone and Olaquindox Efflux Pump, is Widely Distributed among Multidrug Resistant Klebsiella pneumoniae of Human Origin

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Running title: OqxAB in ESBL and KPC-producing Klebsiella pneumoniae

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Use of antibiotics among livestock contributes to the selection and dissemination of multidrug resistant (MDR) bacteria (1). Olaquindox and carbadox are quinoxiline derivatives with antibacterial properties that prevent dysentery and enhance weight gain in suckling pigs (2). Resistance to quinoxilines is mediated by the efflux pump OqxAB, which also extrudes antibiotics such as chloramphenicol and fluoroquinolones (3). The gene encoding for this efflux pump, oqxAB, was initially detected within plasmid pOLA52, which was found in Escherichia coli isolated from swine manure (4). Dissemination of oqxAB has been noted in Salmonella species, and the original genetic reservoir of oqxAB was traced to the chromosome of Klebsiella pneumoniae (5, 6). Surprisingly, OqxAB has only been reported in clinical isolates of K. pneumoniae from China, Korea and Spain (7-9).

Like other Enterobacteriaceae, K. pneumoniae is able to colonize and cause infection in animals and humans. As isolates harboring extended-spectrum β-lactamases (ESBLs) and Klebsiella pneumoniae-carbapenemases (KPCs) have become so prevalent, we hypothesized that OqxAB may be widely present among these clinically relevant types of K. pneumoniae. Isolates were screened by PCR for the oqxA gene (forward primer: 5’- GCGTCTCGGGATACATTGAT-3’; reverse primer: 5’-GGCGAGGTTTTGATAGTGGA-3’) and, if positive, were also screened for oqxB (forward primer: 5’- CTGGGCTTCTCGCTGAATAC-3’; reverse primer: 5’-CAGGTACACCGCAAACACTG-3’). Known positive strains were used as controls. Resistance to ciprofloxacin (as a representative fluoroquinolone) was recorded for each isolate, using a breakpoint of $\geq 4 \, \mu g/mL$ (10). Sequence type was established using the multilocus sequence typing (MLST) scheme developed at the Institut Pasteur (http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html).
The following sets of isolates were studied: 1) *K. pneumoniae* harboring *bla*KPC (n=12) collected in a pediatric long-term care facility in Northeast Ohio in 2004 (11); 2) *K. pneumoniae* harboring *bla*KPC (n=36) obtained from acute care hospitals in Northeast Ohio in 2012; 3) *K. pneumoniae* harboring *bla*KPC (n=43) obtained between 2004-2011 from the mid-Atlantic states of the United States (New Jersey, New York, Pennsylvania); and, 4) ESBL-producing *K. pneumoniae* (n=16) with a variety of *bla*SHV, *bla*TEM and *bla*CTX-M genes, collected in the 1990s from Taiwan, Australia, Argentina, Belgium, Turkey, South Africa and the United States (12).

The prevalence of *oqxAB* varied widely in the different sets of strains. Among KPC-producing *K. pneumoniae* from Northeast Ohio, *oqxAB* was present in 100% of the isolates. This included contemporary isolates from acute care facilities, which were also quinolone-resistant and belonged to ST 258, as well as isolates from a pediatric long term care facility that were quinolone-susceptible and belonged to ST 36. Among KPC-producing *K. pneumoniae* from the mid-Atlantic, prevalence was 71% (22/31) in ST 258 isolates, and 91.7% (11/12) among other non-ST 258 types; 96.8% and 58.3% of these isolates were ciprofloxacin-resistant, respectively. Also, *oqxAB* was present in 87.5% (14/16) of international ESBL-producing *K. pneumoniae*.

Our survey indicates that OqxAB, an efflux pump found in isolates of veterinary origin, is highly prevalent in diverse clinical MDR *K. pneumoniae* isolated from humans, including KPC-producing *K. pneumoniae* ST 258. Furthermore, its presence in *K. pneumoniae* isolated more than a decade ago suggests widespread dissemination. The association of OqxAB efflux pump with a diverse set of substrates including fluoroquinolones, commonly used in humans, may contribute to the MDR profile of *K. pneumoniae*. The occult dissemination of this efflux pump gene adds to the constellation of resistance determinants already circulating in *Enterobacteriaceae*. 
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REFERENCES


<table>
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<th>Location and year of isolation</th>
<th>Number of isolates</th>
<th>Source</th>
<th>Sequence type</th>
<th>ß-lactamase genes (bla)</th>
<th>Prevalence of oqxAB (MIC ≥ 4 μg/mL)</th>
<th>Ciprofloxacin resistance</th>
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<td>Pediatric long term care facility, Northeast Ohio, 2004</td>
<td>12</td>
<td>Stool</td>
<td>ST 36</td>
<td>KPC3</td>
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<tr>
<td>Acute care hospitals, Northeast Ohio, 2012</td>
<td>36</td>
<td>Blood, urine, sputum</td>
<td>ST 258</td>
<td>KPC-2, KPC-3</td>
<td>100 %</td>
<td>100 %</td>
</tr>
<tr>
<td>Acute care hospitals, New York, New Jersey, Pennsylvania, 2004-2011</td>
<td>31</td>
<td>Blood, urine, sputum</td>
<td>ST 258</td>
<td>KPC-2, KPC-3</td>
<td>71 %</td>
<td>96.8 %</td>
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<td>Acute care hospitals, New Jersey, Pennsylvania, 2006-2009</td>
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<td>Urine, sputum</td>
<td>ST 134; ST 234, ST 379, ST 429</td>
<td>KPC-2, KPC-3</td>
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<td>Hospitals in Taiwan, Australia, Argentina, Belgium, Turkey, South Africa and United States, 1996-1997</td>
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<td>Blood</td>
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<td>SHV-5, CTX-M-2, CTX-M-3</td>
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