

# OqxAB, a Quinolone and Olaquinox Efflux Pump, Is Widely Distributed among Multidrug-Resistant *Klebsiella pneumoniae* Isolates of Human Origin

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Use of antibiotics among livestock contributes to the selection and dissemination of multidrug resistant (MDR) bacteria (1). Olaquinox and carbadox are quinoxaline derivatives with antibacterial properties that prevent dysentery and enhance weight gain in suckling pigs (2). Resistance to quinoxalines is mediated by the efflux pump OqxAB, which also extrudes antibiotics such as chloramphenicol and fluoroquinolones (3). The gene encoding 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 been reported only in clinical isolates of *K. pneumoniae* from China, South 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  $\beta$ -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'-GGCGAGGTTTTGATAGTGGGA-3') and, if positive, were also screened for *oqxB* (forward primer, 5'-CTGGGCTTCTCGCTGATAC-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  $\geq 4$   $\mu\text{g/ml}$  (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 (Table 1): (i) *K. pneumoniae* isolates harboring *bla*<sub>KPC</sub> ( $n = 12$ ) collected in a pediatric long-term-care facility in northeast Ohio in 2004 (11), (ii) *K. pneumoniae* isolates harboring *bla*<sub>KPC</sub> ( $n = 36$ ) obtained from acute-care hospitals in northeast Ohio in 2012, (iii) *K. pneumoniae* isolates harboring *bla*<sub>KPC</sub> ( $n = 43$ ) obtained between 2004 and 2011 from the mid-Atlantic states of the United States (New Jersey, New York, Pennsylvania), and (iv) ESBL-producing *K. pneumoniae* isolates ( $n = 16$ ) with a variety of *bla*<sub>SHV</sub>, *bla*<sub>TEM</sub>, and *bla*<sub>CTX-M</sub> 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 (Table 1). Among KPC-producing *K. pneumoniae* isolates from northeast Ohio, *oqxAB* was present in 100%. This included contemporary isolates from acute-care facilities that were also quinolone resistant and belonged to sequence type (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* isolates from the mid-Atlantic, the genes

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TABLE 1 Presence of *oqxAB* in diverse clinical isolates of *Klebsiella pneumoniae*

Location, yr of isolation	No. of isolates	Source(s)	Sequence type(s)	Acquired $\beta$ -lactamase ( <i>bla</i> ) gene type(s)	Prevalence of <i>oqxAB</i> (%)	% with ciprofloxacin resistance (MIC $\geq 4$ $\mu\text{g/ml}$ )
Pediatric long-term-care facility, northeast Ohio, 2004	12	Stool	ST 36	KPC-3	100	0
Acute-care hospitals, northeast Ohio, 2012	36	Blood, urine, sputum	ST 258	KPC-2, KPC-3	100	100
Acute-care hospitals, New York, New Jersey, Pennsylvania, 2004–2011	31	Blood, urine, sputum	ST 258	KPC-2, KPC-3	71	96.8
Acute-care hospitals, New York, New Jersey, Pennsylvania, 2006–2009	6	Urine, sputum	ST 134, ST 234, ST 379, ST 429	KPC-2, KPC-3	83.3	83.3
Acute-care hospitals, New York, New Jersey, Pennsylvania, 2006–2009	6	Urine	Not determined	KPC-3, KPC-4, KPC-5	100	33.3
Hospitals in Taiwan, Australia, Argentina, Belgium, Turkey, South Africa, and the United States, 1996–1997	16	Blood	Not determined	TEM-10, SHV-2, SHV-5, CTX-M-2, CTX-M-3	87.5	Not determined

were present in 71% (22/31) of ST 258 isolates and 91.7% (11/12) of 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* isolates.

Our survey indicates that OqxAB, an efflux pump found in isolates of veterinary origin, is highly prevalent in diverse clinical MDR *K. pneumoniae* strains isolated from humans, including KPC-producing *K. pneumoniae* isolates of ST 258. Furthermore, its presence in *K. pneumoniae* isolated more than a decade ago suggests widespread dissemination. The association of the 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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