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Evolution and Dissemination of OqxAB-Like Efflux Pumps, an Emerging Quinolone Resistance Determinant among Members of *Enterobacteriaceae*

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The OqxAB efflux pump, a plasmid-mediated quinolone resistance (PMQR) determinant, has become increasingly prevalent among members of *Enterobacteriaceae* over the past decade. To investigate the evolution and dissemination routes of the *oqxAB* operon, we assessed the prevalence of *oqxAB*-like elements among various Gram-negative bacterial species and analyzed the genotypic and phenotypic characteristics of organisms harboring such elements. With a comprehensive genotyping approach, a chromosome-based *oqxAB* operon was detectable in all *Klebsiella pneumoniae* strains tested, including organisms isolated before the year 1984. Sequence and phylogenetic analyses confirmed that the *oqxAB* operon in *K. pneumoniae* isolates was genetically closest to their plasmid-borne counterparts recoverable only from *Escherichia coli* and *Salmonella* isolates collected from the year 2003 onward. Chromosomal elements with much lower sequence homology were also found among the *Enterobacter* spp. but not other Gram-negative species. Contrary to the quinolone resistance phenotypes which were consistently observable among organisms with *oqxAB*-harboring plasmids, chromosomal *oqxAB* elements generally did not confer quinolone resistance, except for *K. pneumoniae* strains, which exhibited a typical *oqxAB*-mediated phenotype characterized by cross-resistance to olaquindox, chloramphenicol, and the quinolones. Gene expression analysis illustrated that such phenotypes were due to elevated expression of the chromosomal *oqxAB* operon. Furthermore, transposition of the *oqxAB* operon from the bacterial chromosome to plasmids was found to result in a >80-fold increase in the level of expression of the OqxAB pump, confirming its status as the first constitutively expressed efflux system located in bacterial mobile elements.

The mobile efflux pump OqxAB, first identified in *Escherichia coli* in 2003, belongs to the RND family and shares up to 40% homology with other RND-type efflux systems such as AcrAB in *Escherichia coli* and MexAB in *Pseudomonas aeruginosa* (1). At the time of its discovery, the gene encoding this pump was located in a conjugative plasmid designated pOLA52 and was found to contribute to phenotypic resistance toward nalidixic acid and chloramphenicol and reduced susceptibility to ciprofloxacin in *Escherichia coli* (2, 3). Since then, *oqxAB* has been frequently detected as a plasmid-mediated quinolone resistance (PMQR) determinant among members of *Enterobacteriaceae* (4–6). Sequencing analysis of pOLA52 initially showed that *oqxAB*, together with an open reading frame *orf68* of unknown function, was flanked by the insertion sequence IS*26* (2). A set of corresponding genes that shared 99% nucleotide homology with the *oqxAB* operon in pOLA52, including an *oqxR* gene that was genetically identical to the plasmid-borne *orf68* element, was subsequently detectable in the genome of *Klebsiella pneumoniae*, which did not exhibit phenotypic resistance to either nalidixic acid or chloramphenicol (7). More recently, Bialek-Davenet et al. (8) showed that mutations in *oqxR* induced overexpression of not only *oqxAB* but also *rarA*, which encoded the *oqxAB* transcriptional activator in *K. pneumoniae*. These findings infer that a mutated *oqxR* gene is required to elicit overexpression of *oqxAB* and cross-resistance to quinolone and chloramphenicol in *K. pneumoniae*. Despite these findings, however, the evolutionary origin of *oqxAB*-harboring plasmids and the molecular basis of the differential phenotypes observable in organisms harboring the chromosomal and plasmid-borne *oqxAB* genes remain ill defined. First, although *oqxAB* was detected frequently in *K. pneumoniae*, concrete evidence showing that *oqxAB* is intrinsic to this bacterial species is not available, as failure of *oqxAB* detection in *K. pneumoniae* is common (9, 10). In addition, *oqxAB* homologues in other members of *Enterobacteriaceae*, including *Enterobacter aerogenes* and *Enterobacter cloacae* and some other *Klebsiella* spp. (7), were also identified, prompting a need to perform cross-species analysis of the pattern of distribution for both chromosomal and plasmid-borne *oqxAB*-like elements and the respective roles of such elements in conferring phenotypic resistance. Second, whether translocation of the *oqxAB* genes from chromosome to plasmid results in overexpression of this efflux pump is not clear. Currently, data regarding the expression level of *oqxAB* in pOLA52 and the regulatory mechanisms concerned are not available.

To address the above issues, we performed a comprehensive assessment of the prevalence of the *oqxAB* genes in various mem-

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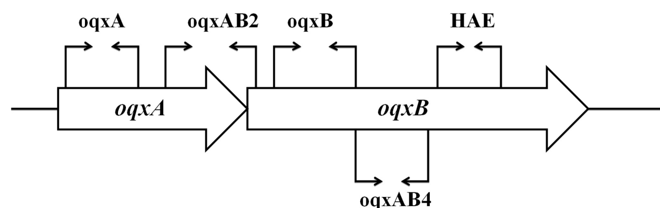

FIG 1 Target regions of the *oqxAB* operon in pOLA52 subjected to PCR genotyping with 5 primer sets.

bers of *Enterobacteriaceae* recovered from different time periods and regions to map the evolution and dissemination routes of this antibiotic resistance determinant. We then performed genetic analysis of *oqxAB*-like elements recoverable from the test strains to obtain evidence which suggests that the plasmid-borne *oqxAB* operon originated from the chromosome of *K. pneumoniae* and evolved to become even more functionally active than their chromosomal counterparts.

MATERIALS AND METHODS

Bacterial isolates. Eighty-five clinical *K. pneumoniae* isolates were collected from The Prince of Wales Hospital, Hong Kong, among which 15 were isolated in or before 1984. Another 8 *K. pneumoniae* isolates were obtained from the *Salmonella* Genetic Stock Centre (SGSC) at the University of Calgary, Canada (http://people.ucalgary.ca/~kesander/), including one *K. pneumoniae* type strain (MGH 78578). Fifty-seven isolates of other bacterial species collected from the SGSC, including *Klebsiella oxytoca* ($n = 8$), *E. cloacae* ($n = 27$), *E. aerogenes* ($n = 15$), *Serratia marcescens* ($n = 3$), *Serratia odorifera* ($n = 2$), and *Serratia liquefaciens* ($n = 2$), and 30 clinical isolates each of *Salmonella* spp., *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Vibrio parahaemolyticus*, other *Vibrio* spp., *Staphylococcus aureus*, and *Enterococcus* spp. were also included in this study. An *oqxAB*-positive *Salmonella enterica* serovar Typhimurium clinical isolate, ST07-37, isolated in 2007 at The Prince of Wales Hospital, Hong Kong, was used in gene expression analysis.

PCR and sequence analysis. The prevalence of *oqxAB* among different bacterial species was determined by utilizing multiple PCR primer sets (*oqxA*, *oqxB*, *hae*, *oqxAB2*, and *oqxAB4*) targeting different yet overlapping regions of the *oqxAB* operon in pOLA52 (Fig. 1). The presence of the IS*26* element upstream of *oqxA* was detected by the primer set IS*26*-*oqxA*. All PCR amplicons were subjected to nucleotide sequencing for confirmation. Primers used in this study are listed in Table 1. The full length of the *oqxAB* gene was amplified from the four oldest *K. pneumoniae* strains (QE137, QE319, QE321, and QE324) isolated in or before the year 1984, followed by nucleotide sequencing and comparison to known *oqxAB* homologues recovered from *K. pneumoniae*, *K. oxytoca*, *E. aerogenes*, and *E. cloacae*, as well as plasmid-carried elements recorded in GenBank. Nucleotide and protein BLAST analyses were performed by utilizing the NCBI BLAST services. Sequence alignment and maximum likelihood phylogenetic analysis were conducted by means of MEGA6.06 software (11).

Antimicrobial susceptibility testing. The MICS of five antimicrobials (ciprofloxacin, norfloxacin, nalidixic acid, chloramphenicol, and olaquindox) were determined for all test strains and interpreted according to the CLSI guidelines (12). *E. coli* ATCC 25922 and ATCC 35218 were used as quality controls.

Southern hybridization. S1-pulsed-field gel electrophoresis (PFGE) was performed to determine the location of *oqxAB* in selected strains. Briefly, agarose-embedded DNA was digested with S1 nuclease (New England Biolabs) at 37°C for 1 h. The restriction fragments were separated by electrophoresis in 0.5 M Tris-borate-EDTA buffer at 14°C for 18 h using a Chef Mapper electrophoresis system (Bio-Rad, Hercules, CA) with pulse times of 2.16 to 63.8 s. A phage lambda PFGE ladder (New England Biolabs) was used as a DNA size marker. The gels were stained with GelRed, and DNA bands were visualized with UV transillumination (Bio-Rad). Chromosomal and plasmid DNAs of *S.* Typhimurium strains were transferred and cross-linked onto nylon membranes and hybridized with DIG-labeled 16S rRNA and *oqxAB2* probes using the DIG high prime DNA labeling and detection starter kit I (Roche), following the manufacturer's instructions.

RNA extraction and qRT-PCR. Total RNA was extracted by the Qiagen Protect bacteria minikit, followed by DNase treatment. The quality and quantity of RNA were determined by using a NanoDrop spectrophotometer. One microgram of RNA was subjected to reverse transcription (RT) using Life Technologies SuperScript III reverse transcriptase; RT-quantitative PCR (qRT-PCR) was performed by using a Bio-Rad iQ5 iCycler and Life Technologies SYBR select master mix. *K. pneumoniae* strain MGH 78578 was used as a control, and expression levels of the test genes were normalized with that of 16S rRNA.

Nucleotide sequence accession numbers. Full-length *oqxAB* sequences from 4 *K. pneumoniae* isolates (QE137, QE319, QE321, and QE324) were deposited to GenBank (accession numbers KJ875814, KJ875815, KJ875816, and KJ875817, respectively).

Various nucleotide and genome sequences were retrieved from GenBank and used in assessment of the genetic relatedness of *oqxAB*-like elements: *Klebsiella pneumoniae* genome sequences MGH 78578 (CP000647.1), XH209 (CP009461.1), PMK1 (CP008929.1), PittNAM01 (CP006798.1), CG43 (CP006648.1), KPNIH31 (CP009876.1), blaNDM-1 (CP009114.1), ATCC BAA-2146 (CP006659.1), 342 (CP000964.1), 1084 (CP003785.1), JM45 (CP006656.1), and KCTC 2242 (CP002910.1); *Klebsiella variicola* strain At-22 (NC_013850.1); *Klebsiella oxytoca* strain

TABLE 1 Primers used in this study

Primer set	Forward: 5′ to 3′	Reverse: 5′ to 3′	Nucleotide position range in *oqxAB* operon[a]	Reference or study
16S rRNA	CTCCTACGGGAGGCAGCAG	GWATTACCGCGGCKGCTG		14
oqxB-RT	TATCTCATTGGCGGCGTGAA	CGCGATTTTGGCGTTGATCT		This study
rarA-RT	GCAGGTGCCACTTCGAATA	GCGCCATCATTCAGGATCT		15
oqxR-RT	TAACGAAGCCTGCTCTGCTT	AATGGTTCCGCTAACTCGTG		This study
IS*26*-oqxA	GCTGTTACGACGGGAGGAG	GGAGACGAGGTTGGTATGGA		6
OqxA	CTCGGCGCGATGATGCT	CCACTCTTCACGGGAGACGA	43−435	4
OqxB	TTCTCCCCCGGCGGGAAGTAC	CTCGGCCATTTTGGCGCGTA	1632−2144	4
HAE	GCCTGGTAAGTCGAGATCGG	CTCGAACGGCTATCAGGGAC	2792−3357	This study
OqxAB2[b]	ACGGTGTACGTCTACTTTGA	GTCTCGGCAATCACTTTCG	640−1384	16
OqxAB4	ATCGAGATGGGTTCCGGTAG	TAAACGGACGGAAAATCCAG	2010−2772	16

[a] The nucleotide position range was based on that of the *oqxAB* operon in plasmid pOLA52 (GenBank accession number NC_010378.1).
[b] Also used as a hybridization probe.

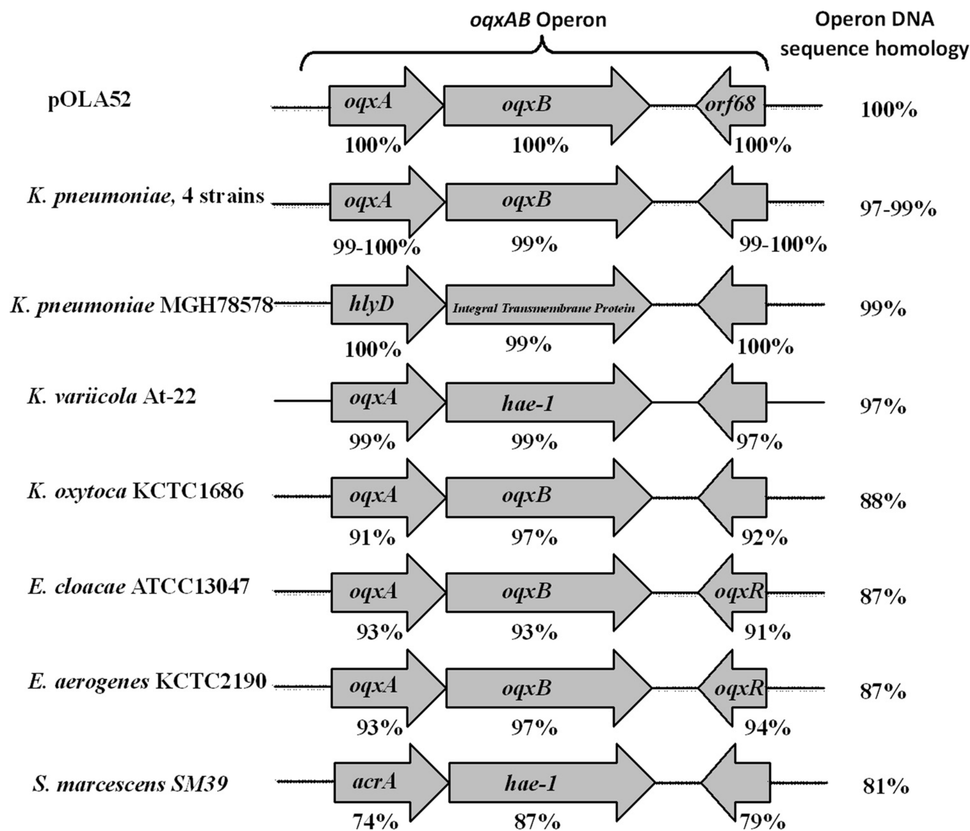

FIG 2 Amino acid sequence alignment of the *oqxAB* operon (or its synonyms) from genomes of various control strains and four *K. pneumoniae* strains recovered in Hong Kong in or before the year 1984, against *E. coli* plasmid pOLA52. The arrows depict open reading frames (ORF) and their respective orientations. The percentage below each ORF depicts the amino acid sequence homology to a specific gene in the *oqxAB* operon in pOLA52. The overall nucleotide homology with pOLA52 is shown on the right.

KCTC 1686 (NC_016612.1); *Serratia marcescens* genome sequences WW4 (CP003959.1) and SM39 (AP013063.1); *Enterobacter aerogenes* strain KCTC 2190 (CP002824.1); *Enterobacter cloacae* genome sequences ATCC 13047 (CP001918.1), EcWSU1 (CP002886.1), and ENHKU01 (CP003737.1); and plasmid sequences pOLA52 (NC_010378.1), pSDB58 (KF840373.1), pHXY (NG_041556.1), and E16 (GQ497565.1).

RESULTS

***K. pneumoniae* chromosome as origin of *oqxAB*.** To test the idea that *oqxAB* originated from *K. pneumoniae*, where it exists as a chromosomally encoded membrane transporter, five primer sets were used to determine the relative prevalence of *oqxAB* in different bacterial species. To obtain convincing evidence on the evolutionary origin of *oqxAB*, we included 15 *K. pneumoniae* clinical isolates collected in The Prince of Wales Hospital, Hong Kong, in or before the year 1984, which was 10 years earlier than the earliest date when *oqxAB* was first detected in a plasmid in *E. coli* (13). This *oqxAB* genotyping test was regarded as positive if one or more of the five primer sets resulted in successful amplification of *oqxAB*-like fragments. Based on this criterion, all *K. pneumoniae*, *K. oxytoca*, and *E. aerogenes* isolates, including the 30-year-old strains, were found to be positive, whereas 26 out of 27 *E. cloacae* strains were also found to contain *oqxAB*-related genes. The *oqxAB*-positive rate for *Salmonella* Typhimurium isolates was 29%. However, no *oqxAB*-like elements were detectable in *Serratia* spp., *Pseudomonas aeruginosa*, *Acinetobacter* spp., *Vibrio parahaemolyticus*, other *Vibrio* spp., *Enterococcus* spp., and *Staphylococcus aureus* isolates.

It should be noted that highly variable result patterns for the genotyping tests with five primer sets were observed among the test isolates; hence, only 14 *K. pneumoniae* strains were positive to all primer sets tested, including 10 collected after 2008. Alignment of primer sequences in known *K. pneumoniae* genomes suggested that the negative genotyping test results were due to sequence variations rather than to a lack of the priming regions in the respective genomes (results not shown). This idea is supported by our observation that, for each isolate which we defined as *oqxAB* positive by our *oqxAB* genotyping approach, at least one primer set targeting the *oqxA* gene and one targeting the *oqxB* gene produced a positive result. On the other hand, an association between IS*26* and *oqxA* was not observable in all isolates tested, suggesting that this gene was not introduced into the chromosome of *K. pneumoniae* by transposition events. S1-PFGE was performed on eight *K. pneumoniae* isolates for which the positive response rate to different primer sets varied. Only the chromosomal DNA band, but not the plasmid, was observed in these strains. Southern hybridization of the nylon membrane transferred with DNA from the same S1-PFGE gel showed that the *oqxAB* probe successfully hybridized to the chromosomal DNA band that also was hybridized by the 16S rRNA probe. However, only the 16S rRNA but not the *oqxAB* probe successfully hybridized to the chromosomal

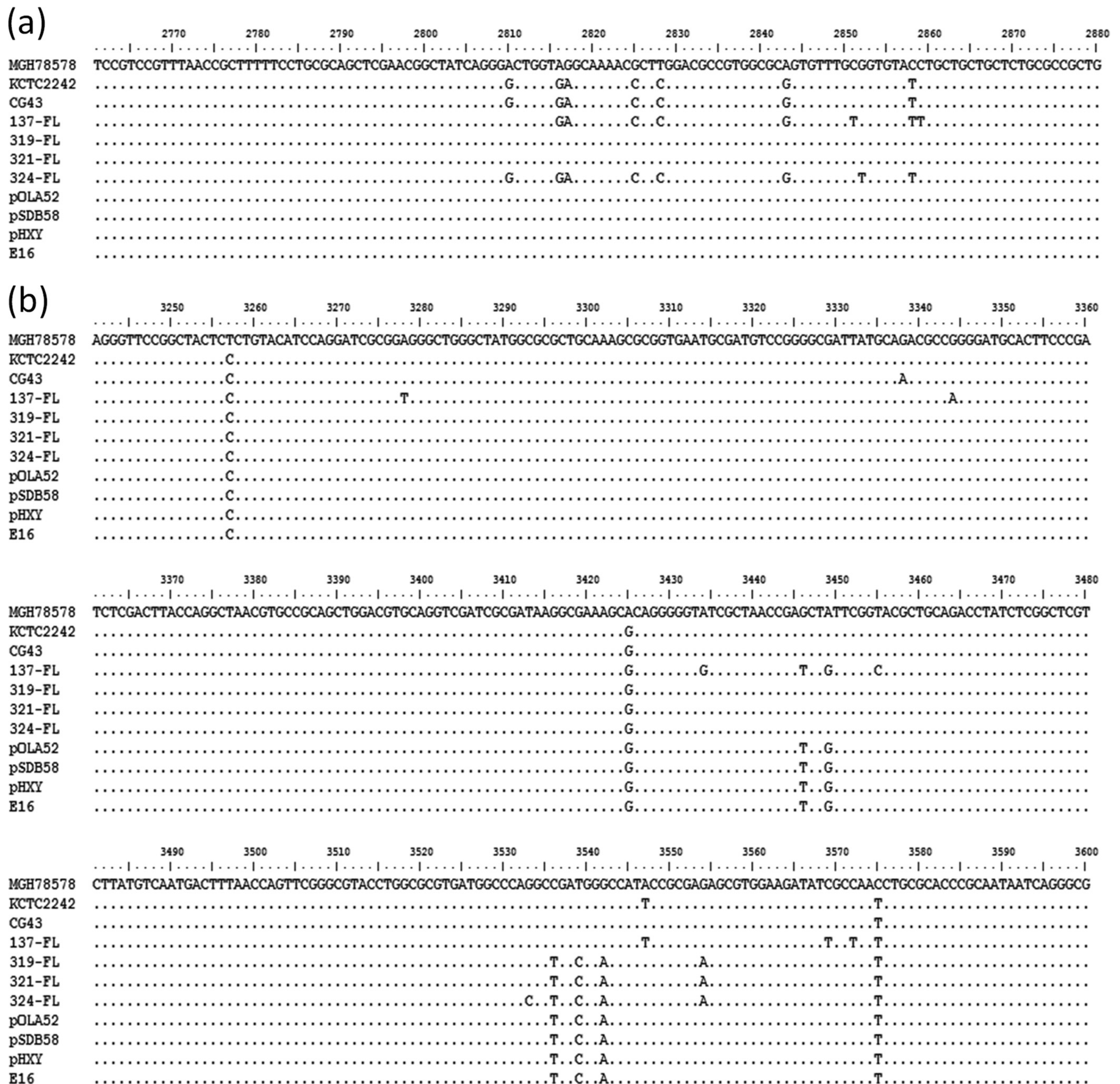

FIG 3 Nucleotide sequence alignment depicting identical sequence variations in two regions (a and b) of the chromosomal and plasmid-borne *oqxAB* operon recoverable from *K. pneumoniae* and *Salmonella/E. coli* strains, respectively. MGH 78578, KCTC 2242, and CG43: control *K. pneumoniae* strains. 137-FL, 319-FL, 321-FL, and 324-FL: clinical *K. pneumoniae* strains isolated in or before the year 1984. pOLA52, pSDB58, pHXY, and E16: plasmids harboring the *oqxAB* operon, recoverable from *Salmonella/E. coli* strains. The nucleotide sequence of strain MGH 78578 was used as the reference sequence. Sequence data of the four clinical *K. pneumoniae* strains were generated in this study. All other data are retrievable from GenBank.

DNA of two control *E. coli* strains. Taken together, the S1-PFGE and Southern hybridization data further confirm the chromosomal location of *oqxAB* (results not shown).

To assess the validity of the genotyping tests, the original *oqxAB* operon in pOLA52 (GenBank accession number NC_010378.1) was subjected to a BLASTN homology search in the NCBI database. All identical hits were plasmid-borne *oqxAB* operons in *E. coli* and *Salmonella* spp. Chromosomal high homology hits (≥97%) were also identified, but all such elements were membrane transporters (*oqxAB* homologues) in *K. pneumoniae* and *K. variicola*. The rest were intermediate homology hits (81 to 88%) involving *K. oxytoca*, *E. cloacae*, *E. aerogenes*, and *Serratia* spp. Nucleotide alignment data showed that the *oqxAB* operon in pOLA52 was 99% and 97% identical to the *K. pneumoniae* MGH 78578 strain and *K. variicola* At-22 strain, respectively, but exhibited only 88%, 87%, 87%, and 81% identity to *K. oxytoca*, *E. cloacae*, *E. aerogenes*, and

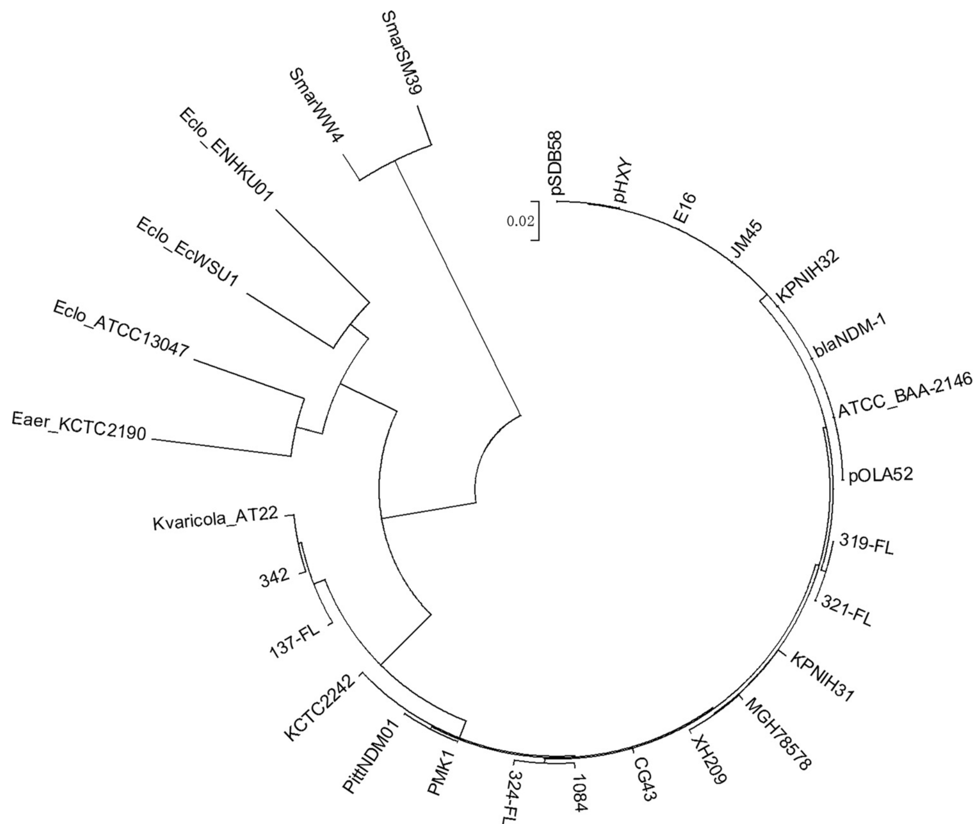

FIG 4 Phylogenetic tree depicting the genetic relatedness of *oqxAB* operons retrieved from various sources. OqxAB operons were extracted from the chromosomes of *K. pneumoniae* (JM45, CG43, MGH 78578, 1084, KPNIH31, KPNIH32, blaNDM-1, ATCC BAA-2146, PMK1, PittNDM01, KCTC 2242, 342, 137-FL, 319-FL, 321-FL, and 324-FL), *K. variicola* (At-22), *Enterobacter cloacae* (EcWSU1 and ENHKU01), *Enterobacter aerogenes* (KCTC 2190 and ATCC 13047), and *Serratia marcescens* (WW4 and SM39) strains and plasmids recoverable from *E. coli* and *Salmonella* (pSDB58, pHXY, E16, and pOLA52) strains.

Serratia marcescens, respectively; *oqxAB*-like elements were not found in organisms not belonging to the family *Enterobacteriaceae*. On the other hand, deduced amino acid sequences of the OqxA, OqxB, and transcriptional regulator Orf68 proteins encoded by genes located in pOLA52 were 99 to 100% identical to those of *K. pneumoniae* and *K. variicola*, but only 91 to 97% identical to those of *K. oxytoca*, *E. cloacae*, *E. aerogenes*, and *Serratia marcescens* (Fig. 2). Interestingly, the OqxB protein in different bacterial species shared 97 to 100% amino acid homology with pOLA52, indicating that it was more conserved than OqxA. Taken together, the genotyping and sequence alignment data suggest that *K. pneumoniae* is genetically most related to the plasmid-borne *oqxAB* genes detectable in *E. coli* and *Salmonella* clinical isolates in recent years.

To further investigate the genetic characteristics of the chromosomal *oqxAB*-like element in *K. pneumoniae*, the entire *oqxAB* operon in four of the oldest *K. pneumoniae* isolates, namely, QE137, QE319, QE321, and QE324, which were isolated in the year 1984 or before, was sequenced and compared to various plasmid-borne and chromosomal *oqxAB* operons. Consistent with the sequence alignment data, the *oqxAB* operons in these four isolates were found to share 97 to 99% homology at the nucleotide level and 99 to 100% identity at the amino acid level with pOLA52 (Fig. 2). Importantly, pockets of identical sequence variations or nucleotide polymorphism were observable among the chromosomal and plasmid-borne elements (Fig. 3). Among specific regions of genetic polymorphism, the plasmid-borne element was found to share a higher level of sequence identity with the 30-year-old *K. pneumoniae* isolates than with the more recent strains, suggesting that the plasmid-borne genes originated from the earlier *K. pneumoniae* strains. The results of phylogenetic analysis depicting the genetic relationship between various chromosomal and plasmid-borne *oqxAB* operons lend support to this idea (Fig. 4).

Relative antimicrobial susceptibility and *oqxAB* expression profiles of *K. pneumoniae* and organisms harboring the pOLA52-like plasmid. With *oqxAB* being consistently detectable in *K. pneumoniae*, drug susceptibility phenotypes were checked for the 85 clinical isolates tested in this study, with results being consistent with previous findings that *K. pneumoniae* clinical isolates were generally susceptible to quinolones (Table 2). Among these 85 *K. pneumoniae* clinical isolates, only 20 were resistant to chloramphenicol and 28 were resistant to nalidixic acid (MIC of $\geq$32 μg/ml), 14 of which were also resistant to ciprofloxacin (MIC of $\geq$4 μg/ml). The majority of the isolates (51 out of 85) had a nalidixic acid MIC of $<$4 μg/ml, whereas 49 strains had a ciprofloxacin MIC of $<$0.006 μg/ml. However, high-level resistance to olaquindox, chloramphenicol, and nalidixic acid and reduced susceptibility to ciprofloxacin, a typical resistance phenotype conferred by the *oqxAB*-harboring plasmid in *Salmonella*, were observable in only two *K. pneumoniae* strains isolated during or after the 1990s (strains 94-3 and GN53) (Table 2). We confirmed, by S1-PFGE, that such a phenotype was not caused by extrachromosomal

TABLE 2 Summary of the genotypic and phenotypic characteristics of organisms harboring *oqxAB*-like elements

Bacterial species and test population/specific strain[a]	Place/yr(s) of isolation	Location of *oqxAB*[b]	Nucleotide sequence homology with pOLA52 (%)	*oqxAB* genotyping with 5 primer sets (no. [%] or present [+] or absent [−])						MIC (mg/liter)[c]				
				oqxA	oqxB	HAE	oqxAB2	oqxAB4	Overall	CIP	NAL	NOR	CHL	OLA
K. pneumoniae														
HK ($n = 85$)	HK/1984–2011			67	35	32	83	73	85 (100)					
SGSC ($n = 8$)	SGSC/1996			8	4	8	8	8	8 (100)					
MGH 78578		Chromosome	99	+	−	+	+	+	+	1	≥128	4	≥128	16
QE319	HK/1984	Chromosome	99	−	−	+	+	−	+	<0.006	<4	<2	<4	<16
94-3[d]	HK/1994	Chromosome	ND[e]	+	−	−	+	−	+	0.25	≥128	≤2	64	512
GN53[d]	HK/2006	Chromosome	ND	+	−	+	+	+	+	4	≥128	≥64	≥128	512
06-2	HK/2006	Chromosome	ND	+	−	−	+	+	+	≤0.006	≤4	≤2	≤4	≤16
K. oxytoca (SGSC, $n = 8$)	SGSC/1996		88[f]	0	0	4	8	8	8 (100)	<0.012–0.05	<4–>128	<4	<4	8–32
E. aerogenes (SGSC, $n = 15$)	SGSC/1996–1999		87[f]	0	10	15	15	4	15 (100)	<0.012	<4–8	<4	<4	8–64
E. cloacae (SGSC, $n = 27$)	SGSC/1996–2000		87[f]	3	15	9	14	15	26 (96)	<0.012–0.05	<4–16	<4	<4–16	16–128
S. Typhimurium ($n = 17$)	HK/2007			5	5	3	4	4	5 (29)					
1792	HK/2007			+	+	−	−	+	+	2	≥128	ND	≥128	≥512
2005	HK/2007	Plasmid	100	+	+	−	−	+	+	2	≥128	ND	≥128	≥512
Other species[g]	HK/2006–2010	Plasmid	100											

[a] HK, Hong Kong; SGSC, *Salmonella* Genetic Stock Centre, Calgary, Canada.
[b] Based on the results of S1-PFGE and Southern hybridization studies.
[c] CIP, ciprofloxacin; NAL, nalidixic acid; NOR, norfloxacin; CHL, chloramphenicol; OLA, olaquindox.
[d] *K. pneumoniae* strains 94-3 and GN53 exhibited the typical *oqxAB*-mediated antibiotic resistance profile (resistance to olaquindox, chloramphenicol, and the quinolones) without an *oqxAB*-harboring plasmid.
[e] ND, not determined.
[f] Based on the nucleotide sequences of the standard strains KCTC 1686, KCTC 2190, and ATCC 13047 for *K. oxytoca*, *E. aerogenes*, and *E. cloacae*, respectively.
[g] All other bacterial species tested, including *Serratia* spp. ($n = 7$), *Pseudomonas aeruginosa* ($n = 30$), *Acinetobacter* spp. ($n = 30$), *Vibrio parahaemolyticus* ($n = 30$), other *Vibrio* spp. ($n = 30$), *Staphylococcus aureus* ($n = 30$), and *Enterococcus* spp. ($n = 30$), were *oqxAB* negative.

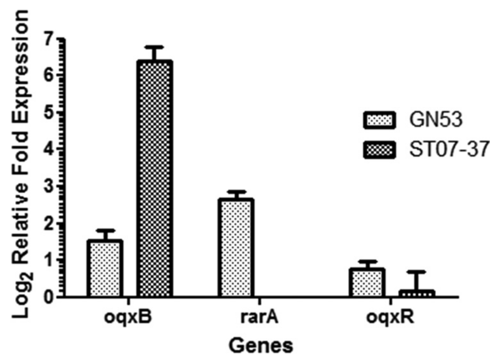

FIG 5 Relative expression levels of the *oqxB*, *rarA*, and *oqxR* genes in the olaquindox-resistant *K. pneumoniae* strain GN53 and a *Salmonella* Typhimurium strain ST07-37 harboring a pOLA52-like plasmid. The *K. pneumoniae* strain MGH 78578 was used as a control.

oqxAB elements. On the other hand, bacterial species harboring chromosomal *oqxAB* homologues such as *E. cloacae* and *E. aerogenes* were mostly susceptible to the test agents, suggesting that these homologues did not contribute to drug resistance under the test conditions.

To probe the molecular basis of the discrepancy in susceptibility phenotypes observable among various *oqxAB*-borne organisms, *K. pneumoniae* strain GN53 and a *Salmonella* strain harboring the pOLA52 plasmid (ST07-37), which were both olaquindox resistant, were subjected to RT-quantitative PCR analysis, with results showing that the expression levels of both *rarA* and *oqxB* in strain GN53 were significantly higher than those of the wild-type strain MGH 78578 (Fig. 5). This finding suggests that the drug resistance phenotypes of this strain were at least partially due to up-regulated expression of *oqxAB*. Interestingly, expression of *oqxR* was also moderately elevated, inferring that the effect of its gene product as an *oqxAB* repressor might have been counteracted by the extraordinarily large amount of the RarA protein produced in this strain. On the other hand, the expression level of *oqxB* in the *Salmonella* strain ST07-37 was elevated as much as 85-fold compared to that in the wild-type, drug-sensitive *K. pneumoniae* strain. Whether such high expression levels of the plasmid-borne *oqxAB* genes were due to derepression of the *oqxAB* operon as a result of lack of an OqxR binding site remains to be elucidated.

DISCUSSION

This study highlighted several important issues regarding the evolutionary origin and dissemination features of the PMQR determinant *oqxAB*. First, although this resistance determinant has become increasingly prevalent among Gram-negative pathogens, our study showed that it is mainly confined to members of *Enterobacteriaceae*. Second, *oqxAB* was most prevalent among the *Enterobacter* spp. and *Klebsiella* spp. In particular, both the detection rate and level of sequence homology with the *oqxAB* operon in pOLA52, the original plasmid in which *oqxAB* was first recovered, approached 100% even in *K. pneumoniae* strains recovered 10 years earlier than the time of discovery of pOLA52. This finding has important implications for the origin of mobile *oqxAB* elements. Third, organisms containing chromosome-based and plasmid-borne elements exhibited drastically different levels of gene expression and susceptibility to the quinolones; in particular, the expression level of the plasmid-borne *oqxAB* operon was >80-fold higher than that of the chromosomal genes.

We postulate that the *oqxAB* operon, together with a transcriptional regulator (*orf68*) in the chromosome of *K. pneumoniae*, was captured by IS*26* transposase and transferred to foreign plasmids, which were subsequently disseminated to other bacterial species that do not harbor *oqxAB*-like elements in the chromosome. It should be noted that, although the *oqxAB* operon is also prevalent among other species such as *Enterobacter* spp., a significantly lower degree of sequence homology with the plasmid-borne element was observed, suggesting that these *oqxAB* homologues were not as readily captured by transposition activities as the *K. pneumoniae* genes. The underlying principle by which structural differences between various *oqxAB* homologues affect transposition efficiency remains to be elucidated. In addition, although factors limiting horizontal transfer of existing *oqxAB*-carrying plasmids to non-*Enterobacteriaceae* species are not understood, the possibility that the *oqxAB* operon may be captured and transferred to other types of plasmids that can be taken up by other Gram-negative pathogens should be investigated.

Taken together, our findings indicate that *oqxAB* or its homologues represent one of the many endogenous efflux systems in *K. pneumoniae* and *Enterobacter* spp., a role which is functionally similar to that of *acrAB* in other members of *Enterobacteriaceae*. Yet a major difference between *oqxAB* and other chromosomal efflux genes, as illustrated by findings in this work, is that the *oqxAB* operon can become plasmid borne via transposition events, during which the *oqxAB* genes become overexpressed, presumably as a result of loss of the OqxR repressor function. The dissemination patterns of mobile elements harboring overexpressed efflux pumps should be closely monitored.

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