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qnrE1, a Member of a New Family of Plasmid-Located Quinolone Resistance Genes, Originated from the Chromosome of *Enterobacter* Species

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ABSTRACT *qnrE1*, found in a clinical *Klebsiella pneumoniae* isolate, was undetectable by PCR assays used for the six *qnr* families. *qnrE1* was located on a conjugative plasmid (ca. 185 kb) and differed from *qnrB* alleles by 25%. Phylogenetic reconstructions of *qnr* genes and proteins and analysis of the *qnrE1* surroundings showed that this gene belongs to a new *qnr* family and was likely mobilized by IS*Ecp1* from the chromosome of *Enterobacter* spp. to plasmids of *K. pneumoniae*.

KEYWORDS PMQR, quinolone, resistance, *qnr* detection, *qnrB*

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The epidemiology of quinolone resistance has dramatically changed since the discovery of the plasmid-mediated quinolone resistance (PMQR) genes in 1998 (1). Among them, the *qnr* genes encode pentapeptide repeat proteins that protect the quinolone targets, DNA gyrase and topoisomerase IV, from the action of these drugs (2). The *qnr* genes are grouped into six families: *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS*, all of which have been mainly found in *Enterobacteriaceae*, and *qnrVC*, which has been mostly found in *Vibrionaceae* (2–4). The six gene families differ in sequence by 30% or more from each other, and several alleles have been described for all families except *qnrC* (http://www.lahey.org/qnrStudies). Inside a given family, the allelic sequences differ on average by 9% or less (*qnrA*, *qnrB*, *qnrD*, and *qnrS*) or by 16% (*qnrVC*) (our unpublished data).

Recently, we reported a nationwide survey on PMQR genes based on a collection of 1,058 clinical enterobacteria from Argentina (5). In that study, *Klebsiella pneumoniae* Q1130, which was isolated in 2007 from the urine specimen of a 78-year-old female ambulatory patient, had a wild-type quinolone resistance-determining region of *gyrA* and showed low-level quinolone resistance. However, this isolate was negative in the PCR assays for *qnrA*, *qnrB*, *qnrC*, *qnrD*, and *qnrS* as well as for other kinds of PMQR genes, such as *aac(6′)-Ib-cr* and *qepA* (5). Herein, we investigated the possible presence of an unknown PMQR gene in this isolate.

Horizontal transference of low-level quinolone resistance. To know whether the low-level quinolone resistance observed in *K. pneumoniae* Q1130 may be horizontally transferred, a biparental conjugation assay was performed as described previously (6). Susceptibility to nalidixic acid (NAL), ciprofloxacin (CIP), and levofloxacin (LVX) was determined by agar dilution and disc diffusion according to the Clinical and Laboratory Standards Institute (7). The obtained transconjugant, *Escherichia coli* TC1130, showed MICs of NAL, CIP, and LVX that were 4, 16, and 16 times higher than those of the recipient strain *E. coli* J53-Az^r (Az^r indicates resistance to sodium azide), respectively (Table 1). By S1 nuclease assay (8), a unique plasmid band of the same size (ca. 185 kb) was observed for *K. pneumoniae* Q1130 and its transconjugant (data not shown). These

TABLE 1 Quinolone susceptibility profiles of the clinical isolate *K. pneumoniae* Q1130, the recipient strains *E. coli* J53-Az^r and TOP10, and their laboratory-derived strains

Strain	MIC (μg/ml)			Disc diffusion inhibition zone (mm)		
	NAL	CIP	LVX	NAL	CIP	LVX
K. pneumoniae Q1130	16	0.5	1	14	22	22
E. coli TC1130[a]	16	0.25	0.5	15	26	25
E. coli J53-Az^r	4	0.015	0.03	23	36	33
E. coli TOP10(pJET1.2-1130)[b]	8	0.125	0.125	22	32	36
E. coli TOP10(pJET1.2-C)[c]	1	0.002	0.004	36	48	43
E. coli TOP10	1	0.002	0.004	35	46	43

[a]Transconjugant selection was done with sodium azide (100 μg/ml) plus CIP (0.12 μg/ml).
[b]This is a *qnrE1*-harboring transformant selected with ampicillin (50 μg/ml).
[c]This is a *qnrE1*-negative transformant selected with ampicillin (50 μg/ml).

results indicated that *K. pneumoniae* Q1130 harbored a quinolone resistance mechanism encoded in a conjugative plasmid, which was named pKp1130.

The *qnrVC* genes had not been reported in clinical enterobacteria (2–4); therefore, they were the unique PMQR genes that were not previously analyzed in *K. pneumoniae* Q1130 (5). We confirmed this presumption by testing the presence of *qnrVC* genes in this clinical isolate and its transconjugant: the PCR assays used (see Table S1 in the supplemental material) rendered negative results in both cases.

Identification of a new *qnr* gene in pKp1130. pKp1130 was extracted from *E. coli* TC1130 with the Qiagen large-construct kit (Qiagen, Hilden, Germany) and sequenced using the MiSeq sequencer (Illumina, San Diego, CA) and CLC Genomics Workbench software v.5.5.1 (CLC bio, Qiagen) for read assembling. Open reading frames (ORFs) were annotated using the RAST Server (rast.nmpdr.org) (9) followed by manual comparative curation and determination of sequence similarity with BLAST (http://www.ncbi.nlm.nih.gov/BLAST/). Differences between nucleotide or amino acid sequences were calculated as *p*-distances with MEGA6 (http://www.megasoftware.net/) (10) and expressed as percentages. The sequencing of pKp1130 resulted in 14 contigs, giving a total length of 187,745 nucleotides, which is in agreement with the size estimated by the S1 nuclease assay. A BLAST analysis showed that the best hits (lowest BLAST E values) for the 14 contigs corresponded to sequences of enterobacterial plasmids (data not shown). Nine out of 14 contigs (total length of 154,020 nucleotides) shared 99.8% identity (query cover of 92%) with the plasmid p1 (199,497 bp) of *K. pneumoniae* NY9 (GenBank accession number CP015386) (see Fig. S1 in the supplemental material). The analysis of the longest contig of pKp1130 (56,313 nucleotides; Contig_5 in Fig. S1) revealed the presence of a 645-bp ORF that showed the highest identity with the *qnrB* family (average of 75% identity with all of the *qnrB* alleles previously described and a range of 73.2% [*qnrB31* and *qnrB53*] to 76.4% [*qnrB42*]) (see Fig. S2A in the supplemental material). This ORF showed 34 nucleotide changes in positions fully conserved among all of the *qnrB* alleles (Fig. S2B). The protein inferred from this ORF differed from all of the previously known QnrB proteins by an average of 15.8% (34 amino acids), with a range of 14.0% (QnrB1, QnrB17, QnrB66, and QnrB75; 30 amino acids) to 17.8% (QnrB73; 38 amino acids) (Fig. 1A). In addition, the sequence of this putative new protein displayed the typical pentapeptide repeat structure of the Qnr proteins and contained the loops A and B, which have proven to be essential for quinolone-protective activity (11–13) (Fig. 1B). Interestingly, an amino acid change was observed in each loop of the putative new Qnr protein regarding all of the previously described QnrB proteins: K52Q and S113C (QnrB amino acid numbering) for loops A and B, respectively. Only two reports analyzed the effect on quinolone susceptibility driven by substitutions in these positions of QnrB1: the CIP MIC was reduced 16 times in the presence of S113D or S113A (11, 13) while no effect was observed for K52A (13).

These results indicated that the ORF found in pKp1130 may correspond to a new *qnr* gene, named *qnrE1*, which is in agreement with the public database repository of *qnr* sequences (http://www.lahey.org/qnrStudies) (G. A. Jacoby, personal communication).

A

	QnrA	QnrC	QnrS	QnrVC	QnrD	QnrB
QnrC	36.1					
QnrS	40.0	39.4				
QnrVC	36.4	27.8	35.6			
QnrD	52.7	55.6	59.8	53.5		
QnrB	57.5	57.3	59.7	55.6	35.4	
QnrE1	56.7	57.5	62.6	56.7	34.3	15.8

B

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                10          20          30          40          50
QnrA1  MDIID KVFQQ EDFSR QDLSD SRFRR CRFYQ CDFSH CQLQD ASFED CSF IESGAVEG
QnrC1  MNYSH KTYDQ IDFSG QDLSS HHFSH CKFFG CNFNR VNLRD AKFMG CTF IESNDFEG
QnrS1  METYN HTYRH HNFSH KDLSD LTFTA CTFIR SDFRR ANLRD TTFVN CKF IEQGDIEG
QnrVC1 MEKSK QLYNQ VNFSH QDLQE HIFSN CTFIH CNFKR SNLRD TQFIN CTF IEQGALEG
QnrVC4 MDKTD QLYVQ ADFSH QDMSG QYFKN CKFFC CSFKR ANLRD TQFVD CSF IERGELEG
QnrD1  M---E KHFIN EKFSR DQFTG NRVKN IAFSN CDFSG VDLTD TEFVD CSF YDRNSLEG
QnrB1  M---A LALVG EKIDR NRFTG EKIEN STFFN CDFSG ADLSG TEFIG CQF YDRESQKG
QnrE1  M---A LIFEG EKIGR NRFTG EKIEN AIFRN CDFSG TDLTS SEFIG CQF YDRESQQG
                                                                  52

          61          71          81          91         101         111
QnrA1  CHFSY ADLRD ASFKA CRLSL ANFSG ANCFG IEFRE CDLKG ANFSR ARF YNQVSHKMYFCS
QnrC1  CNFIY ADLRD ASFMN CMLSM ANFQG ANCFG LELRE CDLKG ANFSQ ANF VNHVSNKMYFCS
QnrS1  CHFDV ADLRD ASFQQ CQLAM ANFSN ANCYG IEFRA CDLKG ANFSR TNF AHQVSNRMYFCS
QnrVC1 CDFSY ADLRD ASFKN CQLSM SHFKG ANCFG IELRD CDLKG ANFSQ VSF VNQVSNKMYFCS
QnrVC4 CDFSY SDLRD ASFKN CSLSM SYFKG ANCFG IEFRE CDLKG ANFAQ ASF MNQVSNRMYFCS
QnrD1  CDFNR AKLKN ASFKS CDLSM SNFKN ISALG LEISE CLAQG ADFRG ANF MNMITTRSWFCS
QnrB1  CNFSR AMLKD AIFKS CDLSM ADFRN SSALG IEIRH CRAQG ADFRG ASF MNMITTRTWFCS
QnrE1  GNFNR AQLKD AIFKS CDLSM ADFRH SNALG IEIRE CRAQG ADFRG ASF MNMITTRTWFCC
                                                                  113

         121         131         141         151         161         171
QnrA1  AYISG CNLAY TNLSG QCLEK CELFE NNWSN ANLSG ASLMG SDLSR GTFSR DCWQQ VNLRG
QnrC1  AYITG CNLSY ANFDK QCLEK CDLFE NKWVG ASLQG ASFKE SDLSR GSFSD DFWEQ CRIQG
QnrS1  AFISG CNLSY ANMER VCLEK CELFE NRWIG TNLAG ASLKE SDLSR GVFSE DVWGQ FSLQG
QnrVC1 AYITG CNLSY ANFEQ QLIEK CDLFE NRWIG ANLRG ASFKE SDLSR GVFSE DCWEQ FRVQG
QnrVC4 AYITG CNLSY ANFER QCIEK CDLFE NRWIG ANLSG ASFKE SDLSR GVFSE GCWSQ CRLQG
QnrD1  AYITK TNLSY ANFSR VILEK CELWE NRWNG TVITG AVFRG SDLSC GEFSS FDWSL ADFTG
QnrB1  AYITN TNLSY ANFSK VVLEK CELWE NRWIG AQVLG ATFSG SDLSG GEFST FDWRA ANFTH
QnrE1  AYITK SNLSY ANFSK VVLEK CELWE NRWNG AQILG ATFSG SDLSG GEFSS FDWRA ANFTH

         181   186   191   196   201   206   211   216
QnrA1  CDLTF ADLDG LDPRR VNLEG VKICA WQQEQ LLEPL GVIVL PD          218
QnrC1  CDLTH SELNG LEPRK VDLTG VKICS WQQEQ LLEQL GVIVI PDKVF       221
QnrS1  ANLCH AELDG LDPRK VDTSG IKIAA WQQEL ILEAL GIVVY PD          218
QnrVC1 CDLSH SELYG LDPRK IDLTG VKICS WQQEQ LLEQL GVIIV PD          218
QnrVC4 CDLSH SELYG LDPRK VDLTG VKICS WQQEQ LLEQL GLIVV PD          218
QnrD1  CDLTG GALGE LDARR INLDG VKLDG EQALQ LVESL GVIVH R           214
QnrB1  CDLTN SELGD LDIRG VDLQG VKLDN YQASL LMERL GIAVI G           214
QnrE1  CDLTN SELGD LDVRG IDLQG VKLDS YQVSQ LMERL GIAVL G           214
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FIG 1 Comparison of Qnr proteins. (A) Paired comparisons of Qnr families. The averages of the percentage of amino acid differences between the indicated families are shown. (B) Pentapeptide repeat structures of Qnr proteins. Only representative variants for the six families are shown. Gaps introduced to maximize the alignment are indicated by hyphens. The amino acid numbering indicated above each block of sequences is based on the longest protein (QnrC1). Residues fully conserved among all of the Qnr variants shown are depicted with a gray-shaded background. The loops A and B (8 and 12 amino acids, respectively), essential for the quinolone-protective activity of the Qnr proteins, are indicated by boxes. The amino acid changes between QnrE1 and QnrB1 are marked with asterisks, and those located in positions for which no changes were found between QnrB1 and any of the remaining QnrB proteins previously described are highlighted with a black-shaded background. The locations in the loops A and B of the changes between QnrE1 and QnrB1 are indicated by bold numbers (QnrB amino acid numbering) below the block of sequences.

The genetic context analysis of *qnrE1* showed that the insertion sequence IS*Ecp1* was located 22 bp upstream of this gene while *araJ*, which encoded a transporter of the major facilitator superfamily, was located 121 bp downstream (see Fig. S3 in the supplemental material). Given that no putative promoter sequences were found in the

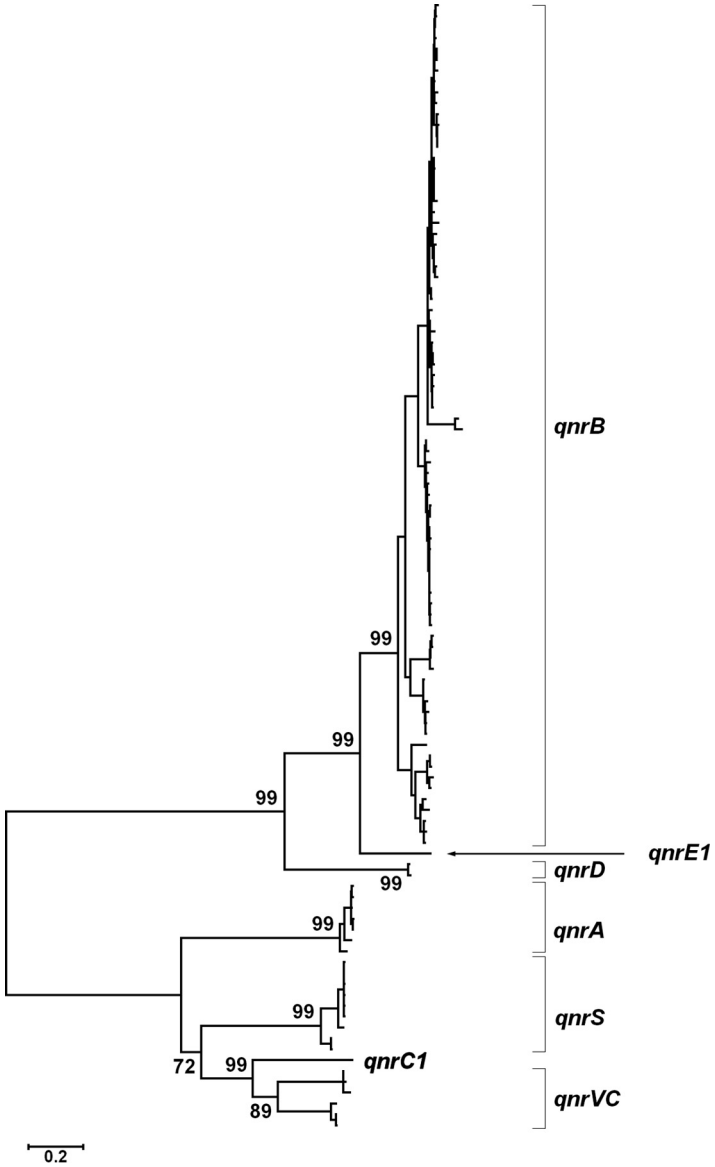

FIG 2 Phylogenetic relationships among *qnr* genes. The ML tree was generated from an alignment of *qnrE1* and all of the *qnr* genes currently known (accession numbers are indicated in Table S2 in the supplemental material). For simplicity, the taxon names of *qnr* alleles are excluded (see Table S3 in the supplemental material) and only the bootstrap percentages (over 1,000 replicates) for relevant nodes are shown. The clustering of the alleles for each *qnr* family is indicated with square brackets at the right. The branch lengths were drawn to the scale shown, which indicates the number of substitutions/site.

short stretch that separated the inverted repeat right (IRR) of IS*Ecp1* from *qnrE1*, it is very likely that the strong promoter located downstream of the transposase gene of IS*Ecp1* can drive the expression of *qnrE1* (14).

qnrE1 was completely amplified from *K. pneumoniae* Q1130 by PCR (see primers in Table S1). The sequence of the obtained amplicon (965 bp), based on MiSeq sequencing, was confirmed by direct Sanger sequencing (5). By using the CloneJet PCR cloning kit (Thermo Scientific/Thermo Fisher Scientific, Waltham, MA), this amplicon and an unspecific DNA fragment (976 bp), provided as a control in the cloning kit, were cloned into the vector pJET1.2/blunt, rendering plasmids pJET1.2-1130 and pJET1.2-C, respectively (Fig. S3). Both plasmids were introduced in *E. coli* TOP10 (Invitrogen/Thermo Fisher Scientific) by electroporation. The identity of the cloned fragment from *K. pneumoniae* Q1130 was also corroborated by Sanger sequencing (5). The MICs of NAL,

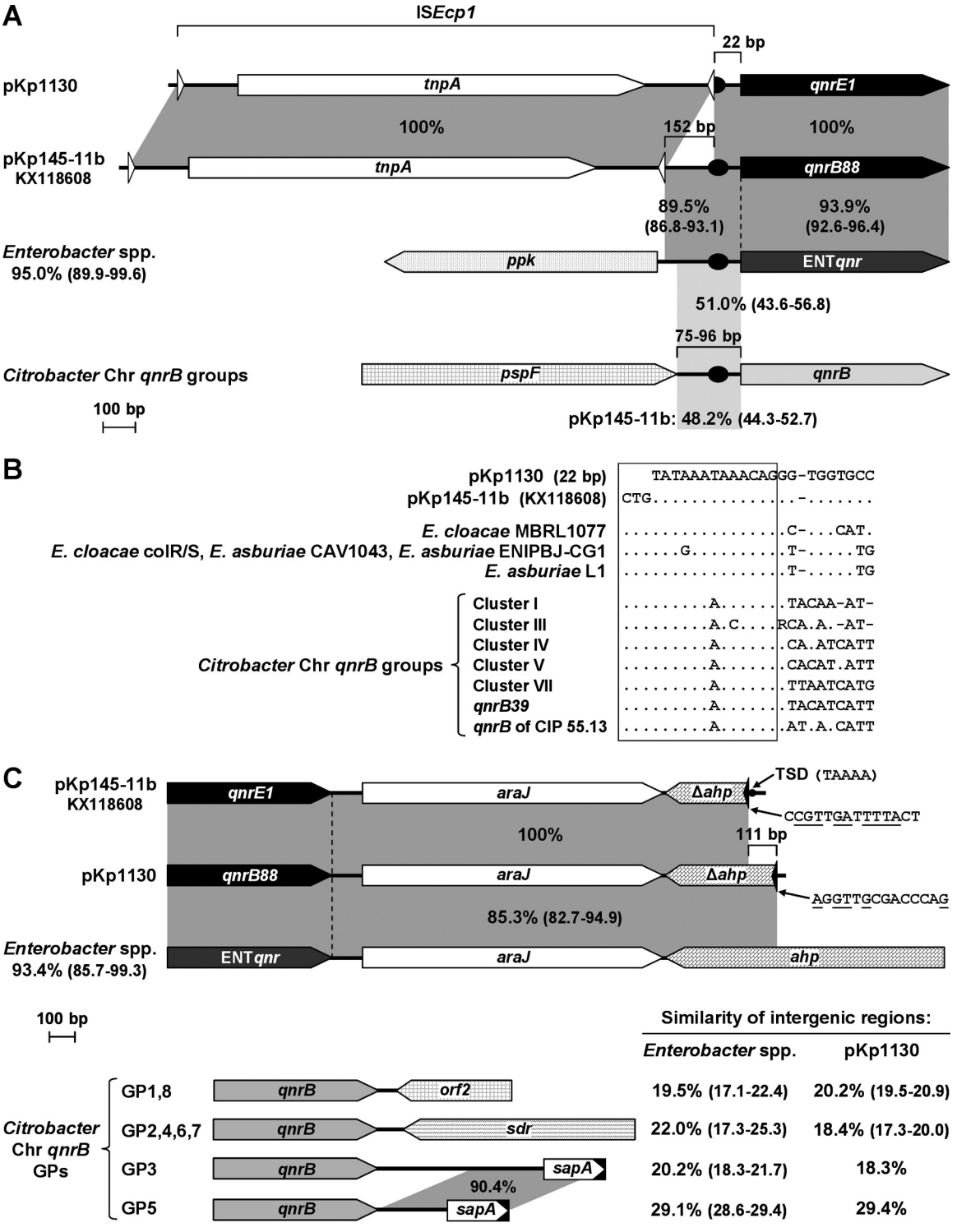

FIG 3 Comparison of genetic surroundings of *qnrE1*, chromosomal *qnr* genes of *Enterobacter* spp. (ENT*qnr*), and the *qnrB* alleles. Only relevant regions are shown. A 4,062-bp fragment of the Contig_5 of pKp1130 (see Fig. S1 in the supplemental material) is depicted. In pKp145-11b, *qnrE1* was named as *qnrB88* according to GenBank report KX118608. The ENT*qnr*-containing chromosomal regions of *E. cloacae* MBRL1077, *E. cloacae* colR/S, *E. asburiae* CAV1043, *E. asburiae* ENIPBJ-CG1, and *E. asburiae* L1 (GenBank accession numbers CP014280, CP010512, CP011591, CP014993, and CP007546, respectively) are represented altogether and indicated as "*Enterobacter* spp." (the identity among them is shown below this name); *ppk* and *ahp* encode polyphosphate kinase and alkyl hydroperoxidase, respectively. Genes/ORFs are represented by arrow-shaped boxes. Short horizontal square brackets are used to indicate the length of relevant DNA fragments. Percentages show the percentage of identity between sequences and, when multiple paired comparisons were done, the average percentages of identity and range (between brackets) are given. Dark and light gray-shaded areas indicate regions of high and low identity, respectively. (A) *qnrE1* upstream region. The *qnrB*-containing chromosomal regions of *Citrobacter* spp., which have been associated with seven phylogenetic groups of *qnrB* genes (26), are represented altogether and indicated as "*Citrobacter* Chr *qnrB* groups"

(Continued on next page)

CIP, and LVX of *E. coli* TOP10(pJET1.2-1130) were 8, 64, and 32 times higher, respectively, than those of the *qnrE1*-negative isogenic strain *E. coli* TOP10(pJET1.2-C) (Table 1). The levels of resistance conferred by *qnrE1* agree well with those observed for other *qnr* genes (2). These results strongly suggest that the substitutions K52Q and S113C found in loops A and B of QnrE1 do not impair its quinolone-protective activity.

***qnrE1* is a member of a new *qnr* family.** According to the current nomenclature for *qnr* genes, *qnrE1* should be considered to belong to the *qnrB* family because the genetic differences with the *qnrB* alleles were lower than 30%, the cutoff proposed for differentiating *qnr* families (15). However, the previously known *qnrB* alleles differed in sequence by an average of 8.6% (range, 0.2% to 19.8%) while *qnrE1* differed from the former by an average of 25.0% (range, 23.6% to 26.8%). In addition, the forward and reverse primers previously used for the screening of *qnrB* genes (5) showed a large proportion of nucleotide mismatches (8/19 and 7/22, respectively) regarding the *qnrE1* sequence. This fact can explain the lack of amplification observed for *K. pneumoniae* Q1130 in the PCR for *qnrB* genes (5). Similarly, we found relevant proportions of mismatches when the sequences of other primers used for *qnrB* screening in several surveys on PMQR genes (16–18), including degenerate primers (19), were compared with *qnrE1*.

Therefore, to further analyze the relationship between *qnrE1* and the *qnrB* alleles, we performed phylogenetic reconstructions using two different methods, neighbor-joining (NJ) and maximum likelihood (ML). Phylogenetic trees for DNA or amino acid sequences were constructed with MEGA6 using the general time reversible model, with gamma distribution of the substitution rate heterogeneity over sites (G) and a proportion of invariant sites, or the Jones-Taylor-Thornton model, with G, as the DNA and amino acid substitution models, respectively, that best fitted the data. The reliability of the tree topology was assessed by bootstrapping (20) using 1,000 replicates.

The topologies of the inferred NJ and ML trees for DNA sequences were very similar, and both showed, with high percentages of bootstrap support, that *qnrE1* was separated by the same evolutionary distance (0.4 nucleotide substitutions per site) from a cluster that comprised all of the *qnrB* alleles previously described (Fig. 2; see also Fig. S4 in the supplemental material). The analysis of the NJ and ML trees for amino acid sequences showed equivalent results, with the exception that, as expected, a lower distance (0.2 amino acid substitutions per site in both trees) was observed between QnrE1 and the cluster that comprised all of the QnrB proteins previously described (data not shown).

Source and mobilization of *qnrE1*. To see if there were sequences in the public databases more closely related to *qnrE1* than the *qnrB* alleles, we performed a BLAST search using the complete *qnrE1* sequence as the query. Six genes showing more than 90% identity were found.

First, the same *qnrE1* gene (named *qnrB88* in the GenBank report) was described in the plasmid pKp145-11b of a *K. pneumoniae* isolate from Brazil (GenBank accession number KX118608), which indicates that the distribution of this gene is not limited to Argentina. Second, 5 fluoroquinolone resistance genes (named here as ENT*qnr*; 96.4% to 92.6% identity with *qnrE1*) were found in the chromosomes of *Enterobacter cloacae*

FIG 3 Legend (Continued)
(*pspF* encodes a transcriptional activator of the phage shock protein operon; the YheO-like-encoding gene only found upstream of *qnrB* in *Citrobacter pasteurii* CIP 55.13 [26] was omitted for simplicity). Empty triangles indicate inverted repeats of IS*Ecp1*, and black ovals indicate the LexA binding site (truncated in pKp1130). The fragments upstream of all of the *qnr* genes that contain the LexA boxes (see details in B) are not drawn to scale. (B) Sequence comparison of the 24- to 26-bp fragments upstream of the *qnr* genes (22 bp in pKp1130) that contain the LexA binding site (boxed). The sequences of the seven *Citrobacter* Chr *qnrB* groups (26) are indicated (*qnrB* of CIP 55.13 and new chromosomal *qnrB* allele of *C. pasteurii* CIP 55.13 [26]). Dots indicate nucleotide identity to pKp1130 or pKp145-11b sequences. (C) *qnrE1* downstream region. The black triangles indicate the possible alternative IRRs of IS*Ecp1*, and their corresponding sequences are shown (nucleotides identical to IRR are underlined); TSD, target site duplication. The eight genetic platforms (GP1 to GP8) found for the chromosomal *qnrB* genes of *Citrobacter* spp. (26) are grouped by sequence similarity of the region depicted in the figure (*orf2*, putative gene of unknown function; *sdr* and *sapA* [truncated at the 3′ end] encode a short-chain dehydrogenase/reductase and a protein involved in antimicrobial peptide resistance, respectively). The identity among the intergenic regions of the GPs in each group and those of *Enterobacter* spp. or pKp1130 is shown at the right.

MBRL1077, *E. cloacae* colR/S (21), *Enterobacter asburiae* CAV1043, *E. asburiae* ENIPBJ-CG1 (22), and *E. asburiae* L1 (GenBank accession numbers CP014280, CP010512, CP011591, CP014993, and CP007546, respectively). The 100% identity between pKp1130 and pKp145-11b extended beyond *qnrE1* along a 3,951-bp fragment from IS*Ecp1* to a truncated alkyl hydroperoxidase-encoding gene (Δ*ahp*; Fig. 3A and C). The sequence identity in the vicinities of the *ahp* interruption point found in pKp1130 was confirmed by PCR and Sanger sequencing (5) using the primers araJ-F and R2 (Table S1) and both *K. pneumoniae* Q1130 and its transconjugant. To gain insights into the possible source of *qnrE1* and its occurrence in the plasmids of *K. pneumoniae* pKp1130 and pKp145-11b, the genetic surroundings of this gene were compared with those of the 5 ENT*qnr* genes. Both the upstream (excluding IS*Ecp1*) and the downstream regions of *qnrE1* showed high identity to those of ENT*qnr* (Fig. 3A and C), with *E. cloacae* MBRL1077 showing maximal identities (93.1% and 94.9%, respectively). The results of these comparisons were also consistent with the notion that IS*Ecp1* was responsible for the mobilization of *qnrE1* from the *Enterobacter* spp. chromosome to pKp1130 and pKp145-11b, truncating the *ahp* gene. Indeed, we found possible alternative IRRs (23, 24) at the 5′ edges of Δ*ahp* in pKp1130 and pKp145-11b, and a 5-bp duplication of the target site, which flanked IS*Ecp1-qnrE1-araJ-*Δ*ahp* in pKp145-11b (Fig. 3C). The *qnrE1* acquisitions in pKp1130 and pKp145-11b should have occurred through different IS*Ecp1* insertions in the chromosome of *Enterobacter* spp. because the region mobilized to pKp1130 begins 152 bp closer to and ends 111 bp farther from *qnrE1* than the region mobilized to pKp145-11b (Fig. 3A and C).

The chromosome of *Citrobacter* spp. has been proposed as the source of *qnrB* alleles (25, 26). Moreover, the genetic platforms of all of the chromosomal *qnrB* alleles of *Citrobacter* spp. available in public databases have been deeply analyzed and classified into eight groups (GP1 to GP8), which are strongly associated with seven phylogenetic groups of *qnrB* alleles (26). By comparative analyses, we found that the genetic surroundings of the chromosomal *qnrB* alleles of *Citrobacter* spp. were quite different from those of *qnrE1* or the ENT*qnr* genes, in both gene content and similarity of intergenic regions (Fig. 3A and C). Interestingly, as observed for the *qnrB* alleles and *qnrD1* (26–29), a LexA binding site was found a few nucleotides upstream of both *qnrE1* and ENT*qnr* genes, although, in pKp1130, it was truncated at its 5′ end by the IS*Ecp1* insertion (Fig. 3B).

The fact that the source of *qnrE1* is different from that of the *qnrB* alleles is in agreement with the results of the phylogenetic analysis, further supporting the notion that *qnrE1* belongs to a new *qnr* family.

Conclusions. The new plasmid-located *qnrE1* gene described here is undetectable by the PCR detection assays commonly used for the six *qnr* families currently known. This fact is highly relevant from the point of view of both the epidemiology of PMQR mechanisms and its detection in the clinical setting. Unlike the *qnrB* alleles, our results strongly suggest that the origin of *qnrE1* is the chromosome of *Enterobacter* spp. and that this gene would have been mobilized from there to plasmids of *K. pneumoniae* by IS*Ecp1*. These notions may imply that a new source of *qnr* genes is supplying the reservoir of plasmid-located *qnr* genes in clinical settings.

Accession number(s). The *qnrE1* sequence depicted in Fig. 3 has been assigned GenBank accession number KY073238.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.02555-16.

SUPPLEMENTAL FILE 1, PDF file, 0.2 MB.

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REFERENCES

1. Martínez-Martínez L, Pascual A, Jacoby GA. 1998. Quinolone resistance from a transferable plasmid. Lancet 351:797–799. https://doi.org/10.1016/S0140-6736(97)07322-4.
2. Jacoby GA, Strahilevitz J, Hooper DC. 2014. Plasmid-mediated quinolone resistance. Microbiol Spectr 2(5):PLAS-0006-2013.
3. Pons MJ, Gomes C, Ruiz J. 2013. QnrVC, a new transferable Qnr-like family. Enferm Infecc Microbiol Clin 31:191–192. https://doi.org/10.1016/j.eimc.2012.09.008.
4. Fonseca EL, Vicente AC. 2013. Epidemiology of *qnrVC* alleles and emergence out of the *Vibrionaceae* family. J Med Microbiol 62:1628–1630. https://doi.org/10.1099/jmm.0.062661-0.
5. Albornoz E, Lucero C, Romero G, Quiroga MP, Rapoport M, Guerriero L, Andres P, Rodriguez C, Galas M, Centrón D, Corso A, Petroni A. 2017. Prevalence of plasmid-mediated quinolone resistance genes in clinical enterobacteria from Argentina. Microb Drug Resist 23:177–187. https://doi.org/10.1089/mdr.2016.0033.
6. Melano R, Corso A, Petroni A, Centrón D, Orman B, Pereyra A, Moreno N, Galas M. 2003. Multiple antibiotic-resistance mechanisms including a novel combination of extended-spectrum β-lactamases in a *Klebsiella pneumoniae* clinical strain isolated in Argentina. J Antimicrob Chemother 52:36–42. https://doi.org/10.1093/jac/dkg281.
7. Clinical and Laboratory Standards Institute. 2016. Performance standards for antimicrobial susceptibility testing; approved standard—26th ed. CLSI document M100S. Clinical and Laboratory Standards Institute, Wayne, PA.
8. Barton BM, Harding GP, Zuccarelli AJ. 1995. A general method for detecting and sizing large plasmids. Anal Biochem 226:235–240. https://doi.org/10.1006/abio.1995.1220.
9. Aziz RK, Bartels D, Best AA, DeJongh M, Disz T, Edwards RA, Formsma K, Gerdes S, Glass EM, Kubal M, Meyer F, Olsen GJ, Olson R, Osterman AL, Overbeek RA, McNeil LK, Paarmann D, Paczian T, Parrello B, Pusch GD, Reich C, Stevens R, Vassieva O, Vonstein V, Wilke A, Zagnitko O. 2008. The RAST server: rapid annotations using subsystems technology. BMC Genomics 9:75. https://doi.org/10.1186/1471-2164-9-75.
10. Tamura K, Stecher G, Peterson D, Filipski A, Kumar S. 2013. MEGA6: molecular evolutionary genetics analysis version 6.0. Mol Biol Evol 30:2725–2729. https://doi.org/10.1093/molbev/mst197.
11. Rodríguez-Martínez JM, Briales A, Velasco C, Conejo MC, Martínez-Martínez L, Pascual A. 2009. Mutational analysis of quinolone resistance in the plasmid-encoded pentapeptide repeat proteins QnrA, QnrB, and QnrS. J Antimicrob Chemother 63:1128–1134. https://doi.org/10.1093/jac/dkp111.
12. Vetting MW, Hegde SS, Wang M, Jacoby GA, Hooper DC, Blanchard JS. 2011. Structure of QnrB1, a plasmid-mediated fluoroquinolone resistance factor. J Biol Chem 286:25265–25273. https://doi.org/10.1074/jbc.M111.226936.
13. Jacoby GA, Corcoran MA, Mills DM, Griffin CM, Hooper DC. 2013. Mutational analysis of quinolone resistance protein QnrB1. Antimicrob Agents Chemother 57:5733–5736. https://doi.org/10.1128/AAC.01533-13.
14. Nakano R, Okamoto R, Nagano N, Inoue M. 2007. Resistance to gram-negative organisms due to high-level expression of plasmid-encoded *ampC* β-lactamase bla_{CMY-4} promoted by insertion sequence IS*Ecp1*. J Infect Chemother 13:18–23. https://doi.org/10.1007/s10156-006-0483-6.
15. Jacoby G, Cattoir V, Hooper D, Martínez-Martínez L, Nordmann P, Pascual A, Poirel L, Wang M. 2008. *qnr* gene nomenclature. Antimicrob Agents Chemother 52:2297–2299. https://doi.org/10.1128/AAC.00147-08.
16. Kim HB, Park CH, Kim CJ, Kim EC, Jacoby GA, Hooper DC. 2009. Prevalence of plasmid-mediated quinolone resistance determinants over a 9-year period. Antimicrob Agents Chemother 53:639–645. https://doi.org/10.1128/AAC.01051-08.
17. Ciesielczuk H, Hornsey M, Choi V, Woodford N, Wareham DW. 2013. Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. J Med Microbiol 62:1823–1827. https://doi.org/10.1099/jmm.0.064428-0.
18. Zhao L, Zhang J, Zheng B, Wei Z, Shen P, Li S, Li L, Xiao Y. 2015. Molecular epidemiology and genetic diversity of fluoroquinolone-resistant *Escherichia coli* isolates from patients with community-onset infections in 30 Chinese county hospitals. J Clin Microbiol 53:766–770. https://doi.org/10.1128/JCM.02594-14.
19. Briales A, Rodríguez-Martínez JM, Velasco C, de Alba PD, Rodríguez-Baño J, Martínez-Martínez L, Pascual A. 2012. Prevalence of plasmid-mediated quinolone resistance determinants *qnr* and *aac(6′)-Ib-cr* in *Escherichia coli* and *Klebsiella pneumoniae* producing extended-spectrum β-lactamases in Spain. Int J Antimicrob Agents 39:431–434. https://doi.org/10.1016/j.ijantimicag.2011.12.009.
20. Felsenstein J. 1985. Confidence limits on phylogenies: an approach using the bootstrap. Evolution 39:783–791. https://doi.org/10.2307/2408678.
21. Napier BA, Band V, Burd EM, Weiss DS. 2014. Colistin heteroresistance in *Enterobacter cloacae* is associated with cross-resistance to the host antimicrobial lysozyme. Antimicrob Agents Chemother 58:5594–5597. https://doi.org/10.1128/AAC.02432-14.
22. Liu F, Yang J, Xiao Y, Li L, Yang F, Jin Q. 2016. Complete genome sequence of a clinical isolate of *Enterobacter asburiae*. Genome Announc 4:e00523-16. https://doi.org/10.1128/genomeA.00523-16.
23. Zong Z, Partridge SR, Iredell JR. 2010. IS*Ecp1*-mediated transposition and homologous recombination can explain the context of $bla_{CTX-M-62}$ linked to *qnrB2*. Antimicrob Agents Chemother 54:3039–3042. https://doi.org/10.1128/AAC.00041-10.
24. Partridge SR. 2007. Genetic environment of IS*Ecp1* and bla_{ACC-1}. Antimicrob Agents Chemother 51:2658–2659. https://doi.org/10.1128/AAC.00364-07.
25. Jacoby GA, Griffin CM, Hooper C. 2011. *Citrobacter* spp. as a source of *qnrB* alleles. Antimicrob Agents Chemother 55:4979–4984. https://doi.org/10.1128/AAC.05187-11.
26. Ribeiro TG, Novais Â, Branquinho R, Machado E, Peixe L. 2015. Phylogeny and comparative genomics unveil independent diversification trajectories of *qnrB* and genetic platforms within particular *Citrobacter* species. Antimicrob Agents Chemother 59:5951–5958. https://doi.org/10.1128/AAC.00027-15.
27. Wang M, Jacoby GA, Mills DM, Hooper DC. 2009. SOS regulation of *qnrB* expression. Antimicrob Agents Chemother 53:821–823. https://doi.org/10.1128/AAC.00132-08.
28. Briales A, Rodriguez-Martinez JM, Velasco C, Machuca J, Díaz de Alba P, Blazquez J, Pascual A. 2012. Exposure to diverse antimicrobials induces the expression of *qnrB1*, *qnrD*, and *smaqnr* genes by SOS-dependent regulation. J Antimicrob Chemother 67:2854–2859. https://doi.org/10.1093/jac/dks326.
29. Albornoz E, Lucero C, Romero G, Rapoport M, Guerriero L, Andres P, WHONET-Argentina Group, Galas M, Corso A, Petroni A. 2014. Analysis of plasmid-mediated quinolone resistance genes in clinical isolates of the tribe *Proteeae* from Argentina: first report of *qnrD* in the Americas. J Glob Antimicrob Resist 2:322–326. https://doi.org/10.1016/j.jgar.2014.05.005.