ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2007, p. 3354–3360 Vol. 51, No. 9
0066-4804/07/$08.00+0 doi:10.1128/AAC.00339-07

New Plasmid-Mediated Fluoroquinolone Efflux Pump, QepA, Found in an *Escherichia coli* Clinical Isolate▽

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Received 13 March 2007/Returned for modification 6 May 2007/Accepted 24 May 2007

Plasmid-mediated Qnr and AAC(6′)-Ib-cr have been recognized as new molecular mechanisms affecting fluoroquinolone (FQ) resistance. C316, an *Escherichia coli* strain demonstrating resistance to various FQs, was isolated in Japan. Resistance to FQs was augmented in an *E. coli* CSH2 transconjugant, but PCR failed to detect *qnr* genes, suggesting the presence of novel plasmid-mediated FQ resistance mechanisms. Susceptibility tests, DNA manipulation, and analyses of the gene and its product were performed to characterize the genetic determinant. A novel FQ-resistant gene, *qepA*, was identified in a plasmid, pHPA, of *E. coli* C316, and both *qepA* and *rmtB* genes were mediated by a probable transposable element flanked by two copies of IS*26*. Levels of resistance to norfloxacin, ciprofloxacin, and enrofloxacin were significantly elevated in *E. coli* transformants harboring *qepA* under AcrB-TolC-deficient conditions. QepA showed considerable similarities to transporters belonging to the 14-transmembrane-segment family of environmental actinomycetes. The effect of carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) on accumulation of norfloxacin was assayed in a *qepA*-harboring *E. coli* transformant. The intracellular accumulation of norfloxacin was decreased in a *qepA*-expressing *E. coli* transformant, but this phenomenon was canceled by CCCP. The augmented FQ resistance level acquired by the probable intergeneric transfer of a gene encoding a major facilitator superfamily-type efflux pump from some environmental microbes to *E. coli* was first identified. Surveillance of the *qepA*-harboring clinical isolates should be encouraged to minimize further dissemination of the kind of plasmid-dependent FQ resistance determinants among pathogenic microbes.

Fluoroquinolones (FQs) are synthetic chemical agents and among the most commonly prescribed antimicrobials because of their broad-spectrum antimicrobial activity. Extensive clinical and agricultural use of FQs has led to high rates of resistance to these agents among pathogenic microbes (6, 9). The most common mechanism for resistance to FQs among pathogenic microbes is the mutation of chromosomal genes encoding DNA gyrase and/or topoisomerase IV (12, 13). Changes in the expression of efflux pumps and porin proteins are also a common FQ resistance mechanism in bacteria (1), but no plasmid-mediated FQ efflux pump has been documented to date. Four chromosome-dependent efflux systems responsible for FQ resistance have so far been reported, e.g., the resistance nodulation division family, AcrAB-TolC in *Escherichia coli* (7, 28) and MexAB-OprM in *Pseudomonas aeruginosa* (24); the major facilitator superfamily (MFS), NorA of *Staphylococcus aureus* (34); the multidrug and toxic compound extrusion family, NorM of *Vibrio parahaemolyticus* (19); and the ATP-binding cassette family, VcaM of non-O1 *Vibrio cholerae* (14).

Two molecular groups have been identified as plasmid-mediated FQ resistance mechanisms to date. Qnr peptides, QnrA (30), QnrB (15), and QnrS (11), were identified from *Klebsiella pneumoniae*, *Klebsiella oxytoca*, *E. coli*, *Citrobacter freundii*, *Citrobacter koseri*, *Enterobacter cloacae*, *Shigella flexneri*, and a *Salmonella* sp. (8, 16, 21, 31), and these peptides interfere with DNA gyrase by mimicking DNA (18). AAC(6′)-Ib-cr was also found to possess an N-acetylating activity of the piperazinyl substituent of ciprofloxacin and norfloxacin (26), and this enzyme has been reported to be geographically widespread (22, 25).

In the present study, we identified a novel FQ resistance mechanism, QepA, as a plasmid-mediated efflux pump found in an *E. coli* clinical isolate from Japan.

(These findings have been reported at the 46th Interscience Conference on Antimicrobial Agents and Chemotherapy, 2006, San Francisco, CA [33].)

MATERIALS AND METHODS

Bacterial strains, susceptibility testing, and DNA manipulation. *E. coli* strain C316, which displayed a multiple-resistance profile to aminoglycosides, FQs, and broad-spectrum β-lactams, except for ceftazidime and imipenem, was isolated from the urine of an inpatient at a medical facility in Hyogo Prefecture, Japan, in March 2002. The strains and plasmids used in this study are listed in Table 1. The bacteria were grown in Luria-Bertani (LB) broth supplemented with the appropriate antimicrobial agents.

E. coli KAM32 (14), a highly susceptible antimicrobial laboratory strain, was transformed with *qepA*-carrying plasmids for a precise assay of the MICs of antimicrobials and chemical agents. Transformants were selected on LB agar plates containing chloramphenicol (20 μg/ml) and norfloxacin (0.025 μg/ml). The susceptibilities to antimicrobials, dyes, and other drugs were tested by the agar dilution method according to the procedure recommended by the NCCLS (presently CLSI) document M7-A6 (20).

Transconjugation analysis was performed with *E. coli* CSH2 as the recipient by the filter mating method. Transconjugants were selected on LB agar plates supplemented with rifampin (50 μg/ml) and kanamycin (25 μg/ml), because the R plasmid carried a kanamycin resistance determinant, *rmtB*. Plasmid DNA from a transconjugant was purified by the method of Kado and Liu (17). Transformation of *E. coli* DH10B with the plasmid DNA of the *E. coli* CSH2 transconjugant was performed by conventional electroporation techniques. Transformants were selected on LB agar containing kanamycin (25 μg/ml).

Basic recombinant DNA techniques described by Sambrook et al. (27) were

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▽ Published ahead of print on 4 June 2007.

TABLE 1. Bacterial strains and plasmids used in this study

E. coli strain	Plasmid	Characteristic(s)
C316	pHPA	Multidrug-resistant clinical isolate harboring an R plasmid, pHPA, from urine (Hyogo, Japan); GyrA mutations at Leu83 and Asn87
CSH2	None	Host strain of conjugation; resistant to rifampin and nalidixic acid
DH10B	None	Δ(*ara-leu*)*7696 araD139 galE15 galK16* Δ(*lac*)*X74 rpsL nupG* [ϕ80Δ(*lacZ*)M15]
KAM32	None	Highly susceptible antimicrobial laboratory strain (Δ*acrB ydhE hsd*) derived from *E. coli* K-12[a]
	pHPA	Transformant harboring the R plasmid, pHPA, derived from a wild strain *E. coli*, C316
	pSTV28	Transformant harboring a chloramphenicol-resistant cloning vector, pSTV28
	pSTVqepA	Transformant harboring a recombinant plasmid that carries a 3.2-kbp SacI-SalI fragment containing a *qepA* gene ligated to pSTV28
	pSTVΔqepA	Transformant harboring a recombinant plasmid carrying a disrupted *qepA* gene by EZ-Tn*5* <KAN-2> insertion

[a] See references 14 and 19.

employed in gene manipulations. The FQ resistance plasmid, pHPA, was prepared from *E. coli* DH10B and digested with SacI and SalI. The resultant fragments were ligated into a cloning vector, pSTV28 (Takara Bio, Inc., Otsu, Japan), that was restricted with the same enzymes, and the resultant recombinant plasmid that affects FQ resistance was named pSTVqepA. The nucleotide sequence of the insert of pSTVqepA was determined on both strands using BigDye Terminator cycle sequencing ready reaction kits and a DNA sequence analyzer, model 3100 (Applied Biosystems, Foster City, CA). The alignments of nucleotide and amino acid sequences were performed with Sequencher version 4.2.2 (Hitachi Software Engineering Co., Ltd., Yokohama, Japan) and GENETYX-MAC, version 12.2.3 (Software Development Co., Ltd., Tokyo, Japan).

Using the in vitro mutagenesis system of the EZ-Tn*5* <KAN-2> insertion kit (Epicenter, Madison, WI), a kanamycin-resistant transposon was inserted into the FQ resistance gene, *qepA*, for its inactivation, and the resultant plasmid was named pSTVΔqepA. Disruption of the *qepA* gene in pSTVΔqepA was checked by sequencing analysis using specific primers for the transposon ends.

Functional assay of gene product. The assay for norfloxacin accumulation in bacterial cells was performed according to Huda et al. (14) and Morita et al. (19). *E. coli* KAM32(pSTV28), *E. coli* KAM32(pSTVqepA), and *E. coli* KAM32 (pSTVΔqepA) cells were grown in the LB broth supplemented with 30 μg/ml of chloramphenicol. The cells were harvested at the late logarithmic phase and washed twice with 0.2 M MOPS (morpholinepropanesulfonic acid)-Tris buffer (pH 7.0), and the bacterial pellet was resuspended in the same buffer and adjusted to 50 μg of bacterial cells (wet weight) per ml for the assay. Norfloxacin (100 μM) was added to the assay mixture, and carbonyl cyanide *m*-chlorophenylhydrazone (CCCP) (100 μM) was added to the same mixture 10 min later. The first sample containing no CCCP was obtained 10 min after the addition of norfloxacin, and the second sample containing CCCP was taken 30 min after the addition of norfloxacin. Samples were centrifuged at 10,000 × *g* for 1 min at 4°C. The pellet was suspended in 1 ml of 100 mM glycine-HCl (pH 3.0), and the suspension was shaken overnight at room temperature and centrifuged at 10,000 × *g* for 5 min at room temperature. The supernatant was diluted twofold with 100 mM glycine-HCl (pH 3.0), and fluorescence was measured with excitation at 370 nm and emission at 450 nm with a Hitachi F2000 fluorescence spectrophotometer (Hitachi High-Technologies Co., Ltd., Tokyo, Japan).

Statistical analyses. The statistical analyses were done with SPSS 14.0J for Windows (SPSS Japan, Inc., Tokyo, Japan). Student's *t* test was used for analyses

TABLE 2. Results of susceptibility tests

Antimicrobial agent or chemical	MIC (μg/ml) for:						Fold increase in MIC for *E. coli* KAM32(pSTVqepA) vs. *E. coli* KAM32(pSTV28)
	E. coli C316	*E. coli* KAM32	*E. coli* KAM32(pHPA)	*E. coli* KAM32(pSTV28)	*E. coli* KAM32(pSTVqepA)	*E. coli* KAM32(pSTVΔqepA)	
Ampicillin	>128	2	>128	2	2	2	1
Erythromycin	>128	2	>128	1	2	1	2
Kanamycin	>128	1	>128	1	1	>128	1
Tetracycline	>128	0.25	0.25	0.25	0.25	0.125	1
Nalidixic acid	>128	1	1	1	2	1	2
Norfloxacin	>128	0.016	0.25	0.016	1	0.016	64
Enrofloxacin	>128	0.008	0.25	0.008	0.25	0.008	32
Tosufloxacin	>128	0.002	0.032	0.002	0.032	0.002	16
Levofloxacin	32	0.008	0.016	0.004	0.016	0.004	4
Ciprofloxacin	>128	0.004	0.064	0.004	0.125	0.004	32
Lomefloxacin	>128	0.032	0.064	0.032	0.064	0.032	2
Pazufloxacin	16	0.004	0.016	0.004	0.016	0.004	4
Sparfloxacin	16	0.001	0.002	0.001	0.002	0.001	2
Moxifloxacin	32	0.002	0.016	0.002	0.016	0.002	8
Gatifloxacin	32	0.004	0.016	0.002	0.016	0.004	8
CCCP	16	16	16	16	16	16	1
Acriflavine	>128	2	4	2	4	2	2
Rhodamine 6G	>128	4	4	4	4	4	1
Crystal violet	16	2	2	2	2	2	1
Sodium dodecyl sulfate	>128	128	128	128	128	128	1
Ethidium bromide	>128	4	8	4	8	4	2
Deoxycholate	>1,024	1,024	512	512	512	512	1
Benzalkonium	64	4	4	4	4	4	1

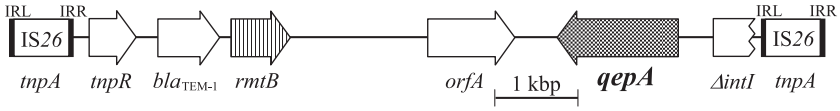

FIG. 1. Organization of the element mediating *qepA*. *qepA* is located in a 10-kb region flanked by two copies of IS*26*, and this probable transposable element also contains *rmtB*, together with *tnpR*, bla_{TEM-1}, and several probable open reading frames. IRL, 5′-GGCACTGTTGCA AA-3′; IRR, 5′-TTTGCAACAGTGCC-3′.

of norfloxacin accumulation in both *qepA*-expressing and -nonexpressing clones with or without CCCP.

Nucleotide sequence accession number. The nucleotide sequence of the *qepA* gene and its flanking regions appears in the EMBL/GenBank/DDBJ (E/G/D) databases under accession number AB263754.

RESULTS

Transfer of FQ resistance and antimicrobial susceptibility. FQ resistance was successfully transferred from *E. coli* C316 to *E. coli* CSH2 at a frequency of 10^{-5} to 10^{-6} cells per recipient cell by conjugation, and the MIC of norfloxacin for the CSH2 transconjugant was elevated above 0.125 μg/ml. Strain CSH2 shows resistance to nalidixic acid by *gyrA* mutation; therefore, the exact norfloxacin MIC was measured in *E. coli* DH10B. The norfloxacin MIC for DH10B was elevated from <0.008 μg/ml to 0.25 μg/ml by introduction of the plasmid pHPA, carrying *qepA*. For the *E. coli* KAM32 transformants that harbor pSTVqepA carrying a 3.2-kbp SacI-SalI fragment of pHPA, 32- to 64-fold-higher MICs of three FQs, norfloxacin, ciprofloxacin, and enrofloxacin, were observed and compared to those for both host strains of *E. coli* KAM32 with or without the vector plasmid (Table 2). The MICs of the other antimicrobial agents and various chemicals, including erythromycin, acriflavine, and ethidium bromide, were not changed when the 3.2-kbp SacI-SalI fragment of pHPA was introduced with pSTVqepA in *E. coli* KAM32. The *E. coli* KAM32 transcon-

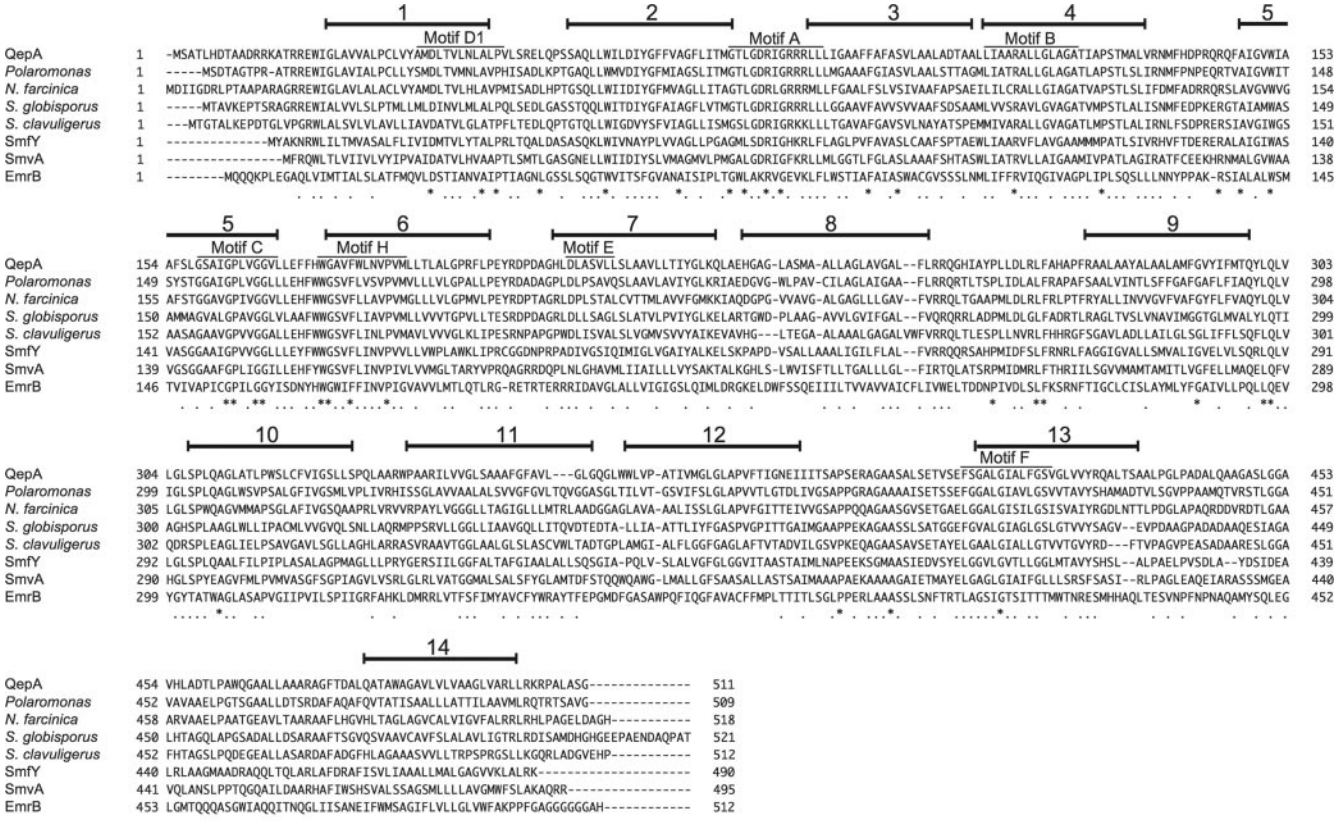

FIG. 2. Comparison of amino acid sequences of 14-TMS MFS-type efflux pumps with QepA. Fourteen portions of probable 14-TMS motifs and seven specific motifs, A, B, C, D1, E, F, and H, are shown by horizontal lines above the alignments. Asterisks and dots indicate identical and similar amino acid residues, respectively. Dashes represent gaps. *Polaromonas*, a putative efflux pump of the *Polaromonas* sp. strain JS666 chromosome (NCBI Protein Data Bank accession number ABE43318); *N. farcinica*, the putative arabinose efflux permease AraJ of the *Nocardia farcinica* chromosome (NCBI Protein Data Bank accession number BAD57397); *S. globisporus*, SgcB of *Streptomyces globisporus* (E/G/D accession number AY048670); *S. clavuligerus*, the putative efflux pump EpeA of the *Streptomyces clavuligerus* chromosome (E/G/D accession number AJ302083); SmfY, a *Serratia marcescens* chromosomal efflux pump protein (E/G/D accession number AB251607); SmvA, a *Salmonella enterica* chromosomal efflux pump protein (E/G/D accession number P37594); and EmrB, an *Escherichia coli* chromosomal efflux pump protein (E/G/D accession number AAC75733).

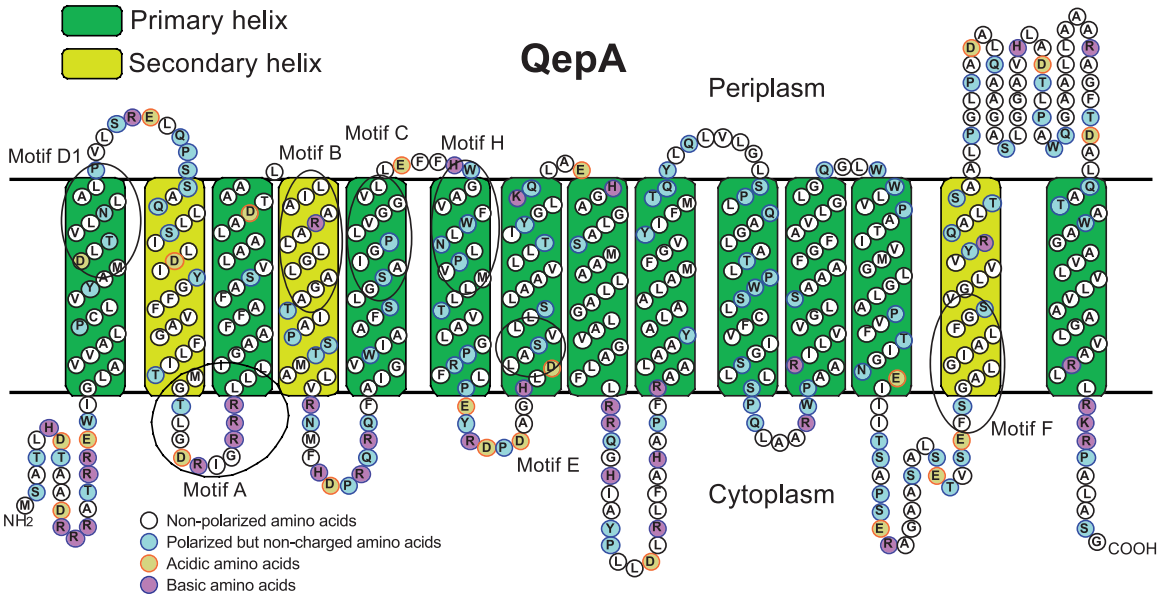

FIG. 3. Predicted secondary structure of QepA. The secondary transmembrane structure of QepA (511 amino acid residues) was calculated and illustrated by the SOSUI system (http://bp.nuap.nagoya-u.ac.jp/sosui/). Fourteen probable α-helix TMS and seven specific motifs are indicated. Classes of amino acid residues are differentiated by four colors.

jugant harboring pHPA showed high-level resistance to ampicillin, erythromycin, and kanamycin (Table 2). These resistance profiles were later found to depend on carriage by pHPA of a CTX-M-type β-lactamase gene ($bla_{CTX\text{-}M\text{-}12}$), a macrolide phosphotransferase gene (*mphA*), and a 16S rRNA methylase gene (*rmtB) (*data not shown). No positive band was observed in the strain harboring pHPA by a PCR analysis for detecting plasmid-mediated FQ resistance *qnr* genes (data not shown). Therefore, pHPA was considered to harbor a new plasmid-mediated FQ resistance determinant. The nucleotide sequence in the *ori* region of pHPA was very similar to that of R100, suggesting that it belongs to the group of IncFII plasmids.

Characteristics of FQ resistance determinant and its product. Sequence analysis of the 3.2-kbp SacI-SalI pHPA fragment cloned into pSTVqepA revealed a 1,536-bp open reading frame with a high G+C content (72%) that was named *qepA*. Considerable similarities between the codon usage patterns of *qepA* and genes for the MFS-type efflux pumps of *Nocardia farcinica*, *Streptomyces globisporus*, and *Streptomyces clavuligerus* were observed; however, no apparent similarities between the manners of codon usage of *qepA* and those of gram-negative bacteria, such as *rmrA* (E/G/D accession number AF233286), *smvA* (E/G/D accession number D26057), and *smfY* (E/G/D accession number AB251607) (data not shown) were seen. The *qepA*-harboring transferable plasmid, pHPA, also carried a 16S rRNA methylase gene, *rmtB* (5) (E/G/D accession number AB103506), which shows a considerable similarity to the 16S rRNA methylases essential for self-protection of actinomycetes from the hazardous effects of their own aminoglycosides (4, 29). The *qepA* gene is located in an ~10-kb region, flanked by two copies of IS*26* containing *tnpA*, and this region also contained several open reading frames, including *tnpR*, $bla_{TEM\text{-}1}$, and *rmtB*, as shown in Fig. 1.

The *qepA* gene encoded a putative protein, QepA, of 511 amino acids. The predicted amino acid sequence of QepA was subjected to a calculation with the SOSUI system (http://bp.nuap.nagoya-u.ac.jp/sosui/) and was found to have probably 14 transmembrane segments (TMS) (Fig. 2 and 3).

Structural characteristics of QepA. The deduced amino acid sequence of QepA showed considerable similarity to probable membrane transporters of members of the order *Actinomycetales*, such as *Nocardia farcinica* (51%; NCBI protein database accession number BAD57397), *Streptomyces globisporus* (49%; E/G/D accession number AY048670), and *Streptomyces clavuligerus* (46%; E/G/D accession number AJ302083). A lower sequence homology (less than 38%) was seen with the major gram-negative bacterial 14-TMS family of MFS-type efflux pumps, such as EmrB (E/G/D accession number AAC75733), SmvA (E/G/D accession number P37594), and SmfY (E/G/D accession number AB251607), and with putative MFS-type efflux pumps of *Actinomycetales*. The phylogenetic tree in Fig. 4, which was calculated with the CLUSTAL W program (http://clustalw.ddbj.nig.ac.jp/top-e.html), also suggested that QepA belongs to the 14-TMS family transporters of gram-positive *Actinomycetales* but not those of gram-negative bacteria.

Efflux of norfloxacin by QepA. The accumulation of norfloxacin in *qepA*-harboring *E. coli* KAM32(pSTVqepA) was significantly lower ($P < 0.01$) than that in *qepA*-nonharboring *E. coli* KAM32(pSTV28) or *qepA*-disrupted *E. coli* KAM32 (pSTVqepA). The accumulation of norfloxacin in *E. coli* KAM32(pSTVqepA) increased significantly after the addition of CCCP (Fig. 5), suggesting the proton potential-dependent efflux of norfloxacin by QepA.

DISCUSSION

We first characterized a new quinolone efflux pump protein (QepA) responsible for FQ resistance. QepA showed a con-

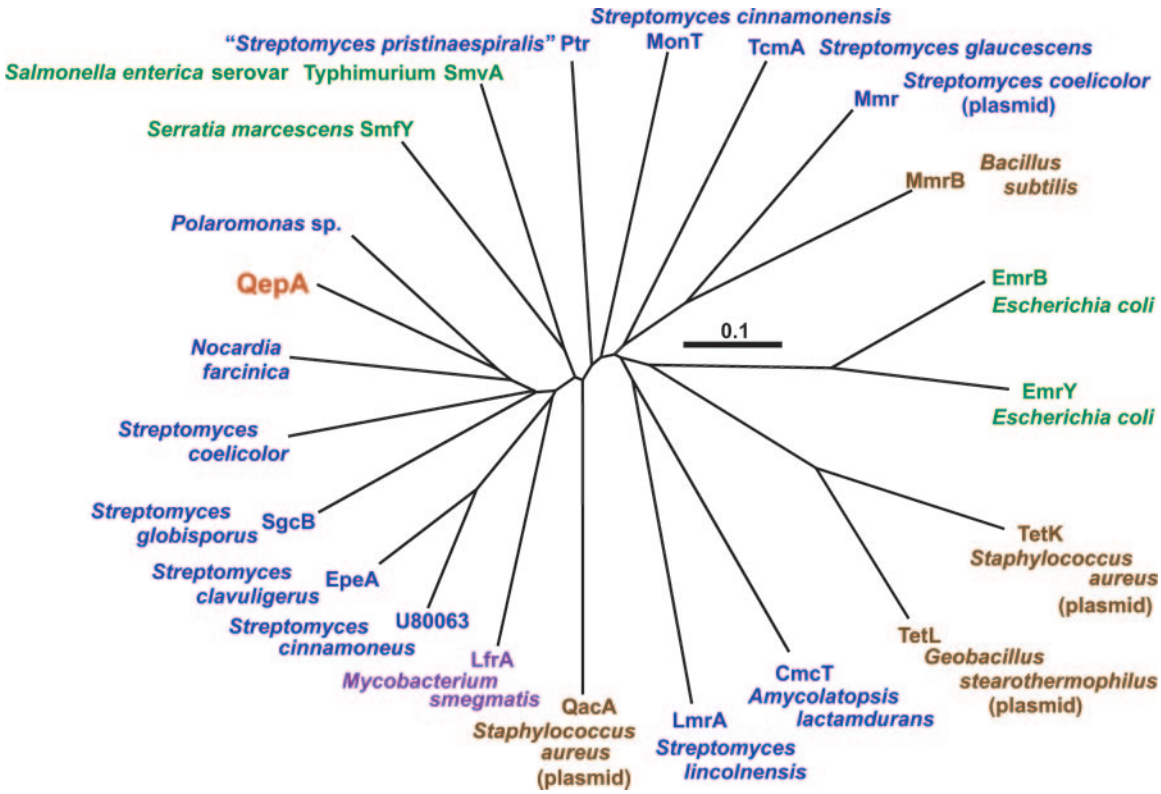

FIG. 4. Phylogenetic tree of 14-TMS efflux pumps belonging to MFS transporters. Each amino acid sequence was subjected to analysis with reference to the following sources: LfrA, a *Mycobacterium smegmatis* proton antiporter efflux pump (E/G/D accession number U40487); Ptr, a "*Streptomyces pristinaespiralis*" pristinamycin compound and rifampin resistance protein (E/G/D accession number X84072); TcmA, a *Streptomyces glaucescens* tetracenomycin C resistance and export protein (E/G/D accession number M80674); EmrB, an *E. coli* multidrug efflux system protein (E/G/D accession number AAC75733); EmrY, an *E. coli* multidrug resistance protein (E/G/D accession number D78168); TetK, a *Staphylococcus aureus* tetracycline efflux protein (E/G/D accession number M16217); TetL, a *Geobacillus stearothermophilus* tetracycline efflux protein (NCBI Protein Data Bank accession number P07561); Mmr, a *Streptomyces coelicolor* methylenomycin A resistance protein (E/G/D accession number M18263); MmrB, a *Bacillus subtilis* methylenomycin A resistance protein (E/G/D accession number X66121); CmcT, an "*Amycolatopsis lactamdurans*" cephamycin export protein (NCBI Protein Data Bank accession number Q04733); LmrA, a *Streptomyces lincolnensis* lincomycin resistance protein (E/G/D accession number X59926); QacA, an *S. aureus* antiseptic resistance protein (E/G/D accession number X56628); SmfY, a *Serratia marcescens* multidrug efflux pump (E/G/D accession number AB251607); SmvA, a *Salmonella enterica* serovar Typhimurium methyl-viologen resistance protein (NCBI Protein Data Bank accession number P37594); U80063, a *Streptomyces cinnamoneus* proton-dependent transport protein (E/G/D accession number U80063); EpeA, a *Streptomyces clavuligerus* DHA2 subfamily multidrug transporter (E/G/D accession number AJ302083); *Nocardia farcinica*, *N. farcinica* strain IFM 10152, probable arabinose efflux permease AraJ (NCBI Protein Data Bank accession number BAD57397); *Polaromonas* sp., *Polaromonas* sp. strain JS666, a probable MFS efflux pump (NCBI Protein Data Bank accession number ABE43318); QepA, an *E. coli* plasmid-mediated FQ efflux pump (E/G/D accession number AB263754); *Streptomyces coelicolor*, a probable membrane transporter of *S. coelicolor* (NCBI Protein Data Bank accession number CAC37879); SgcB, a *Streptomyces globisporus* transmembrane efflux protein (E/G/D accession number AAL06672); and MonT, a *Streptomyces cinnamonensis* putative monensin transporter (NCBI Protein Data Bank accession number AAO65793). Blue, actimomycetes; brown, gram-positive cocci; green, gram-negative rods; purple, *Mycobacterium smegmatis*. The horizontal bar (0.1) indicates a 10% change in the amino acid residues calculated by the CLUSTAL W program.

siderable similarity to the MFS-type efflux pumps belonging to the 14-TMS family of environmental microorganisms, including actinomycetes (Fig. 2). Norfloxacin accumulation in the *qepA*-expressing *E. coli* strain was significantly lower than that in the *qepA*-nonexpressing strains. Moreover, the addition of CCCP readily augmented the accumulation of norfloxacin even in the *qepA*-expressing strain (Fig. 5). These findings clearly revealed that the QepA protein is involved in the excretion of norfloxacin from the cytoplasm to the exterior of bacterial cells. As with FQs, the MICs of erythromycin, acriflavine, and ethidium bromide for the *qepA*-expressing clinical isolate and transconjugant were higher than those for the *qepA*-nonexpressing strains. However, the chemical substances tested, except FQs, showed at most twofold elevations in the MICs for a strain carrying only the *qepA* gene (Table 2). Therefore, it was strongly suggested that the QepA protein is involved mainly with the excretion of FQs as a proton antiporter efflux pump system.

Interestingly, the *qepA* gene had a high G+C content (72%), and the deduced amino acid sequence of QepA showed considerable homology to the probable efflux pumps belonging to the MFS-type membrane transporters found in *Polaromonas* spp. and members of the order *Actinomycetales* (Fig. 4). Moreover, considerable similarities between the codon usage patterns of *qepA* and genes for the MFS-type efflux pumps of *Nocardia farcinica*, *Streptomyces globisporus*, and *Streptomyces clavuligerus* were observed, and both *qepA* and *rmtB* genes were mediated by a probable transposable element flanked by

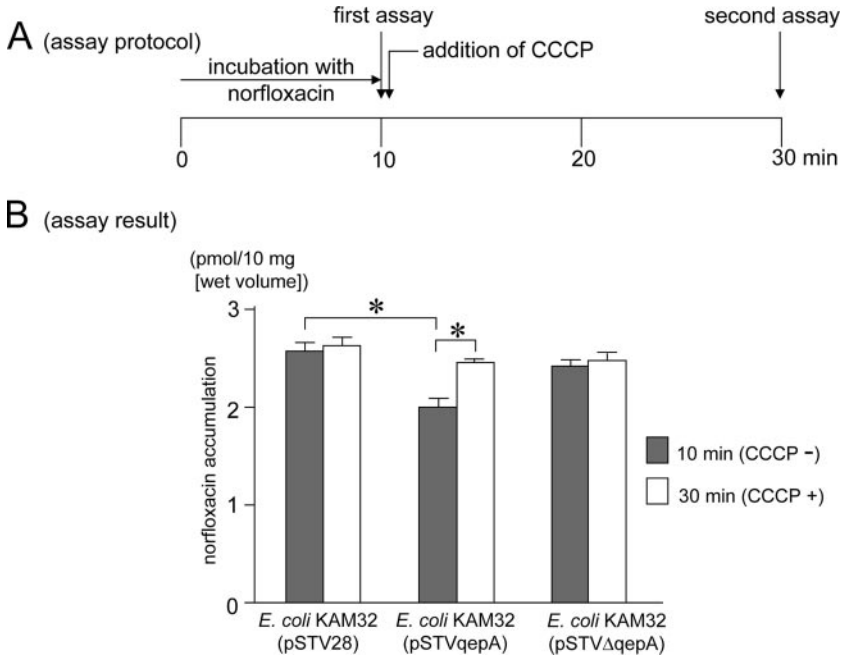

FIG. 5. Assay protocol and accumulation of norfloxacin in *E. coli* cells. The accumulations of norfloxacin in transformants that harbor a vacant plasmid (pSTV28), a *qepA*-carrying plasmid (pSTVqepA), and a disrupted *qepA*-carrying plasmid (pSTVΔqepA) are shown. *, statistical significance ($P < 0.01$) in the reduction of intracellular accumulation of norfloxacin that was seen in *E. coli* strains that produce QepA compared to QepA-nonproducing *E. coli* strains. The effect of CCCP on the accumulation of norfloxacin was also statistically significant in the QepA-producing strain. However, no statistical significance was seen in the norfloxacin levels of *E. coli* KAM32(pSTVqepA) cells between a sample taken at 10 min and a sample taken at 30 min in the absence of CCCP.

two copies of IS*26* on a transferable plasmid, pHPA. We therefore speculated that the chromosomal fragment of some actinomycetes with high G+C contents carrying both *qepA* and *rmtB* genes might be introduced into an IncFII plasmid by transposition and that pHPA could have been subsequently transferred to *E. coli*.

Antibiotic-producing actinomycetes have their own intrinsic genes for antibiotic efflux transporters for excretion of their metabolic products. FQs, however, are fully synthetic chemical agents, so no innate FQ transporter is expected to exist in the microbial world. It is possible, however, that some bacterial transporters that excrete antibiotics or bioactive agents possessing structural similarities to FQs might become transporters that can excrete FQs. Thus, we speculate that the QepA protein may well have originated from environmental microbes that produce bioactive metabolites, including antibiotics with structural similarity to FQs. A similar finding has been reported for OqxAB, a resistance nodulation division-type efflux pump that gives resistance to an artificial growth promoter, olaquindox (10).

Since resistance to broad-spectrum β-lactams has already developed among gram-negative bacteria, the emergence of multidrug-resistant gram-negative bacteria that harbor plasmids bearing *qepA* and/or *qnr*, as well as *rmtB* and genes for CTX-M-type extended-spectrum β-lactams and/or metallo-β-lactamases, could become a serious clinical concern. Although the MICs of FQs for QepA-producing strains are not high at present and the potential impact of *qepA*-harboring strains on FQ therapy has not been fully understood, the additive effect of QepA production on FQ resistance caused by mutations in genes for DNA gyrase and/or topoisomerase IV might promote further spread of FQ-resistant strains in clinical settings.

Quite recently, the *qepA* gene was also found in an RmtB-producing *E. coli* strain isolated in a Belgian hospital (23), suggesting a probable worldwide dissemination of *qepA* accompanied by *rmtB* that has already been identified in various gram-negative bacteria isolated in many countries of Asia (3, 5, 32) and Europe (2). QepA production confers resistance to enrofloxacin, a veterinary FQ, and *rmtB*-producing *E. coli* strains have frequently been isolated from pigs (3), for which aminoglycosides have tended to be used as a growth promoter in some countries or geographical areas. Thus, coproduction of QepA and RmtB may well give an advantage to bacteria to survive in livestock breeding environments as well as in human clinical settings. Hence, active surveillance of *qepA*-harboring gram-negative bacteria in animals might reveal a greater prevalence of such kinds of multidrug-resistant microbes.

In conclusion, we have newly identified a plasmid-mediated novel efflux pump, QepA, that is responsible for the elevation of levels of resistance to several clinically important FQs, such as ciprofloxacin, norfloxacin, and veterinary enrofloxacin.

ACKNOWLEDGMENTS

We thank Tomofusa Tsuchiya, a professor of the faculty of Pharmaceutical Sciences, Okayama University, for donating *E. coli* KAM32.

This work was funded by grants from the Ministry of Health, Labor and Welfare, Japan (H15-Shinkou-9, H15-Shinkou-10), and in part by a grant from the Kurozumi Medical Foundation.

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