

Plasmid-Mediated Quinolone Resistance Pump QepA2 in an *Escherichia coli* Isolate from France[∇]

Vincent Cattoir,^{1,2} Laurent Poirel,¹ and Patrice Nordmann^{1*}

INSERM U914 Emerging Resistance to Antibiotics, Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, K-Bicêtre,¹ and Service de Bactériologie-Virologie-Hygiène, Hôpital Henri Mondor, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine de Créteil, Université Paris XII, Créteil,² France

Received 15 May 2008/Returned for modification 22 June 2008/Accepted 12 July 2008

One hundred twenty-one extended-spectrum β -lactamase-producing enterobacterial clinical isolates were screened for the *qepA* gene. A single CTX-M-15-positive *Escherichia coli* isolate (0.8%) that produced the putative pump QepA2 was identified. This *qepA2* gene was located onto a 90-kb mobilizable plasmid that conferred reduced susceptibility to hydrophilic fluoroquinolones.

Although resistance to quinolones in *Enterobacteriaceae* is related mainly to chromosome-encoded mutations (16), emergence of plasmid-mediated quinolone resistance has been reported with three known mechanisms to date: the Qnr determinants (12, 15), aminoglycoside acetyltransferase AAC(6′)-Ib-cr (14), and QepA (13, 19). Whereas QnrA (10), QnrB (8), and QnrS (7) proteins protect target enzymes (DNA gyrase and type IV topoisomerases) from quinolone inhibition (12, 15), the AAC(6′)-Ib-cr determinant acetylates norfloxacin and ciprofloxacin (14).

The QepA determinant has been identified in two *Escherichia coli* clinical isolates from Japan and Belgium (13, 19). The *qepA* gene encodes a 14-transmembrane-segment efflux pump belonging to the major facilitator superfamily (13, 19). QepA confers decreased susceptibility to hydrophilic fluoroquinolones (e.g., norfloxacin, ciprofloxacin, and enrofloxacin) with a 32- to 64-fold increase of MICs (13, 19). To date, a single epidemiological survey has evaluated the prevalence of the QepA determinant among a collection of *E. coli* clinical isolates collected from 2002 to 2006 (cutoff of MICs of norfloxacin, ≥ 0.025 $\mu\text{g/ml}$), reporting its low prevalence (0.3%) in Japan (18).

In order to determine whether that determinant may have disseminated further worldwide, we have conducted an epidemiological survey to evaluate the prevalence of the *qepA* gene among extended-spectrum β -lactamase (ESBL)-producing enterobacterial isolates collected from a French university hospital, since ESBLs are often identified among quinolone-resistant isolates (12, 15).

One hundred twenty-one nonduplicate, ESBL-producing enterobacterial clinical isolates, collected at the Bicêtre Hospital, France, between January and October of 2007 have been studied. Screening was carried out by PCR using standard conditions and specific primers designed in this work: *qepA*-F (5′-CGTGTGCTGGAGTTCTTC-3′) and *qepA*-R (5′-CTG

CAGGTACTGCGTCATG-3′), giving a 403-bp product. The *qepA* gene was detected in a single *E. coli* isolate, indicating its low prevalence (0.8%) in that collection. Similarly, a low prevalence has also been reported (0.3%) in *E. coli* (18) from Japan. This work is the first epidemiological survey of the QepA determinant performed with European isolates. To date, only four *qepA*-positive clinical isolates have been reported from Japan and Belgium, all being *E. coli* isolates (13, 18, 19).

The *qepA* gene was identified in *E. coli* isolate BicA recovered in January 2007 from urine and blood samples of a 79-year-old female patient suffering from pyelonephritis. Antibiotic susceptibility testing by disk diffusion revealed that *E. coli* BicA was resistant to all β -lactams except carbapenems and cephamycins and exhibited an ESBL phenotype. PCR and sequencing (6) revealed that *E. coli* BicA harbored the *bla*_{TEM-1} and *bla*_{CTX-M-15} genes and was also resistant to chloramphenicol, tetracycline, sulfamethoxazole, trimethoprim, nalidixic acid, and fluoroquinolones but remained fully susceptible to aminoglycosides (Table 1), according to the CLSI standard (5). Sequence analysis of the chromosome-encoded quinolone resistance-determining regions (2) revealed that *E. coli* BicA had a *gyrA* gene with two mutations, leading to Ser83Leu and Asp87Asn substitutions, and one mutation in *parC*, leading to a Ser80Ile substitution, compared to the wild-type enzymes of *E. coli*. No mutation was found in *gyrB* and *parE* genes, and no *qnr*-like or *aac*(6′)-Ib-cr genes were detected by PCR (4, 11) in *E. coli* BicA.

The QepA determinant was transferred as previously described (3) from *E. coli* BicA to the *E. coli* TOP10 recipient strain by electrotransformation after selection on Trypticase soy agar plates containing ciprofloxacin (0.01 $\mu\text{g/ml}$). Plasmid analysis performed by using the Kieser technique (9) identified a single ca. 90-kb plasmid (pQep) from the *E. coli* transformant. PCR-based replicon typing (1) indicated that pQep was a member of the IncFI incompatibility group. Once transferred in *E. coli* TOP10, pQep conferred increased MICs for hydrophilic fluoroquinolones (such as norfloxacin and ciprofloxacin) as previously described (13, 19) and also increased resistance to amoxicillin, ticarcillin, piperacillin, chloramphenicol, tetracycline, sulfamethoxazole, and trimethoprim (Table 1). In addition, plasmid pQep was confirmed by PCR (6) to harbor the *bla*_{TEM-1}

* Corresponding author. Mailing address: Service de Bactériologie-Virologie, Hôpital de Bicêtre, 78 rue du Général Leclerc, 94275 Le Kremlin-Bicêtre cedex, France. Phone: 33-1-45-21-36-32. Fax: 33-1-45-21-63-40. E-mail: nordmann.patrice@bct.aphp.fr.

[∇] Published ahead of print on 21 July 2008.

TABLE 1. MICs of antibiotics for the *E. coli* clinical isolate BicA, its J53 transconjugants harboring natural plasmids pCTX-M (*bla*_{CTX-M-15}⁺) or both pCTX-M and pQep (*qepA2*⁺), its TOP10 transformant harboring natural plasmid pQep, and reference strains J53 (azide resistant) and TOP10

Antibiotic	MIC (μg/ml) for ^a :					
	BicA	TC-1 (pCTX-M)	TC-2 (pCTX-M + pQep)	J53	TF (pQep)	TOP10
Nalidixic acid	>256	4	4	4	1.5	1
Norfloxacin	>256	0.06	1	0.06	0.5	0.03
Ofloxacin	>32	0.12	0.12	0.12	0.03	0.01
Pefloxacin	>32	0.06	0.06	0.06	0.03	0.03
Levofloxacin	>32	0.03	0.06	0.06	0.01	0.008
Ciprofloxacin	>32	0.01	0.12	0.01	0.03	0.003
Enrofloxacin	>32	0.06	0.06	0.03	0.008	0.003
Moxifloxacin	>32	0.06	0.12	0.06	0.01	0.004
Gatifloxacin	>32	0.06	0.12	0.01	0.01	0.003
Amoxicillin	>256	>256	>256	4	>256	4
Amoxicillin-CLA ^b	16	16	16	4	16	4
Ticarcillin	>256	>256	>256	4	>256	4
Piperacillin	>256	>256	>256	2	>256	2
Piperacillin-TZB ^c	4	2	2	1	2	1
Cephalothin	>256	>256	>256	8	8	4
Cefotaxime	>32	>32	>32	0.06	0.06	0.06
Ceftazidime	>32	16	16	0.12	0.12	0.06
Aztreonam	>32	16	16	0.06	0.12	0.06
Cefepime	>32	16	16	0.01	0.12	0.06
Imipenem	0.5	0.25	0.25	0.06	0.25	0.12
Gentamicin	0.5	0.12	0.12	0.12	0.25	0.25
Tobramycin	0.5	0.25	0.25	0.12	0.5	0.5
Amikacin	2	1	1	1	1	1
Netilmicin	0.5	0.12	0.12	0.12	0.25	0.25
Chloramphenicol	>256	8	>256	8	>256	4
Tetracycline	>256	2	2	2	>256	2
Sulfamethoxazole	>256	16	>256	16	>256	16
Trimethoprim	>256	0.25	>256	0.25	>256	0.12
Rifampin	16	8	8	8	16	16

^a TC-1 and TC-2, *E. coli* J53 transconjugants; TF, *E. coli* TOP10 transformant.

^b CLA, clavulanate.

^c TZB, tazobactam.

gene but not the *bla*_{CTX-M-15} gene. A second plasmid pCTX-M of ca. 90 kb and identified as an IncI1-type plasmid carried the *bla*_{TEM-1} and *bla*_{CTX-M-15} genes that were transferred by conjugation into *E. coli* J53 after selection with amoxicillin. Transcon-

jugants containing only pCTX-M (TC-1) exhibited an ESBL phenotype without any coresistance (Table 1). Noteworthy, pQep was cotransferred with pCTX-M but was not identified alone after selection with chloramphenicol, suggesting that pQep might be

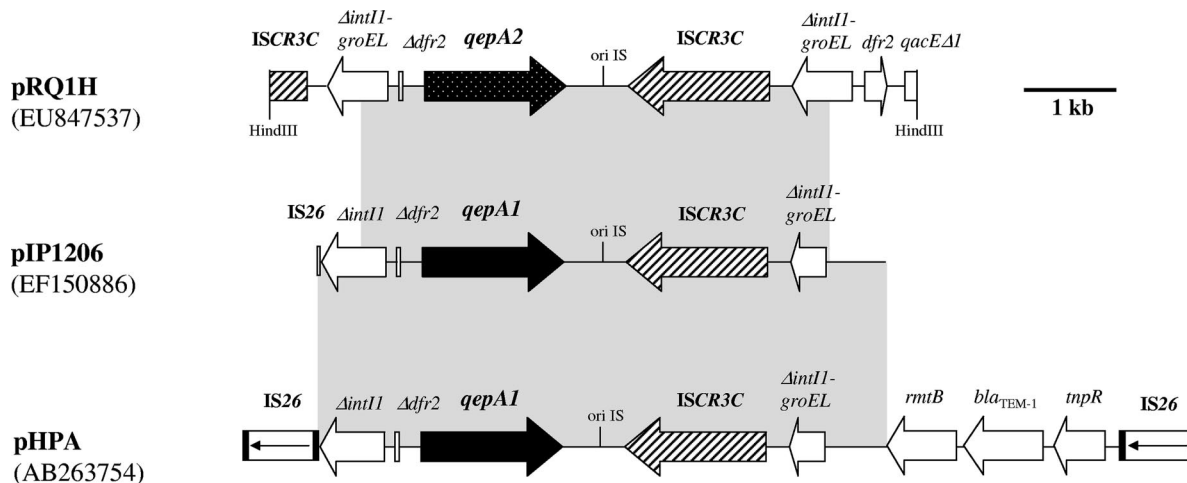


FIG. 1. Genetic environment of the *qepA2* gene in plasmid pQep from *E. coli* BicA and comparison with related QepA-positive plasmid structures. Plasmids pHPA and pIP1206 are from *E. coli* C316 from Japan (19) and *E. coli* 1540 from Belgium (13), respectively. Recombinant plasmid pRBicAH was obtained in the present study. Open reading frames are indicated by horizontal arrows. The HindIII restriction sites used for cloning experiments are indicated. Shaded areas indicate identities between sequences.

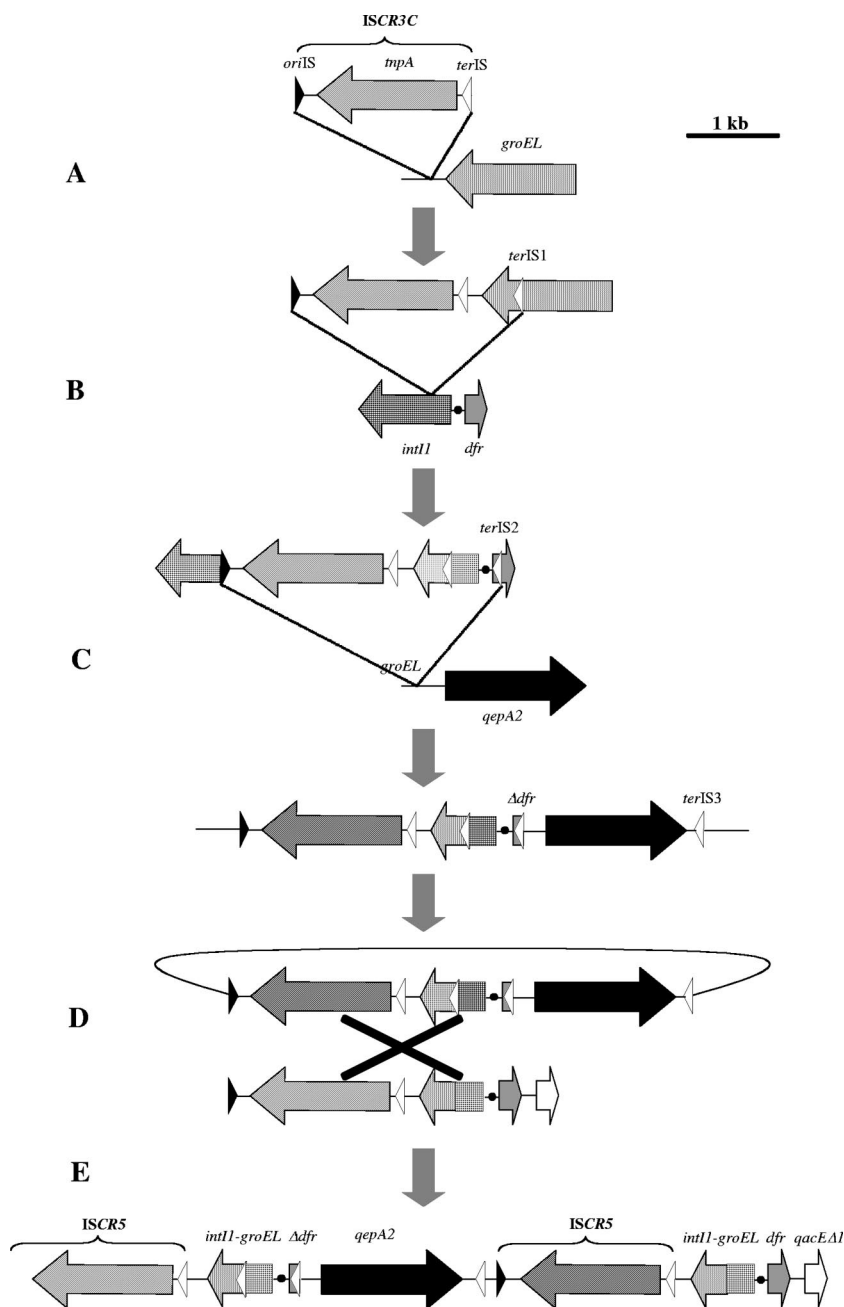


FIG. 2. Hypothesis of the ISCR3C-mediated mobilization of the *qepA2* gene according to the model proposed by Toleman et al. (17). The process may result from several steps. (A) Insertion of ISCR3C downstream of a *groEL* gene. Aberrant rolling circle (RC) replication of the ISCR3C element (fused to the *groEL* gene) generates a transposition intermediate starting at *oriIS* and ending at *terIS1*, located inside the *groEL* gene. (B) This intermediate thereafter transposes into a class 1 integrase gene. A second aberrant RC replication generates a transposition intermediate starting at *oriIS* and ending at *terIS2*, located inside a *dfr2* gene itself downstream of the *intI1* gene. (C) This second transposition intermediate transposes upstream of the *qepA2* gene. (D) A third aberrant RC replication produces circular intermediates which can then be rescued by recombination events between *mpA* or *intI1-groEL* genes, generating the *qepA2*-containing complex integron (E). Boxes represent the open reading frames of the various genes, with arrows indicating the direction of their transcription. The integrase-specific recombination sites are indicated as black dots, and *oriIS* and *terIS* are represented as black and white triangles, respectively.

mobilizable and not conjugative, as opposed to pIP1206, which is also classified as a broad-host-range IncFI-type plasmid (13).

Cloning of HindIII-restricted DNA from whole-cell DNA (3) of *E. coli* BicA gave recombinant plasmid pRBicAH, containing a 7,020-bp insert including the *qepA* gene. Sequence

analysis revealed that the *qepA* gene was located inside a genetic structure in large part identical to that of previously described plasmids pHPA (19) and pIP1206 (13), identified from *qepA*-positive *E. coli* strains from Japan and Belgium, respectively (Fig. 1). However, detailed analysis of pRBicAH

showed that the *qepA* gene possessed two nucleotide substitutions, leading to Ala99Gly and Val134Ile changes in the deduced 511-amino-acid protein (13, 19). This variant (named QepA2) conferred a phenotype similar to that of the QepA determinant (renamed QepA1) (data not shown). Of note is the fact that the *qepA2* gene was not associated with the *rmtB* gene encoding an aminoglycoside ribosomal methylase, in contrast to what has been reported for plasmids pHPA and pIP1206 (13, 19). Interestingly, the *qepA2* gene was identified between two copies of an ISCR-like element and was not associated with IS26 elements, as opposed to what was described in pHPA (Fig. 1). The transposase gene of that ISCR element encoded a protein 100% identical to the putative transposase identified on plasmids pHPA and pIP1206 (13, 19). That transposase showed a single amino acid substitution, Leu331Arg or Leu477Pro, compared to transposases of the ISCR3-like elements ISCR3A (GenBank accession no. AAK02053, AF261825, BAE54320, and ABZ01838) and ISCR3B (GenBank accession no. ACA62823), respectively. This novel IS element was termed ISCR3C. Upstream of the *qepA2* gene, a truncated *dfr2* gene known to encode resistance to trimethoprim was identified and was located upstream of a truncated integrase gene (*ΔintI1*) of a class 1 integron (Fig. 1). This truncated integrase gene was identified close to a truncated chaperonin gene (*ΔgroEL*), thus encoding a fusion protein IntI1/GroEL previously described in association with ISCR-like elements (17).

In a study of *E. coli* C316 from Japan, the region comprising the *qepA* and *rmtB* genes was found to be flanked by two copies of IS26, and the authors suggested that those genes were likely part of a composite transposon (Fig. 1) (19). In pQep, the genetic environment of the *qepA2* gene was nearly identical to that of pHPA, but no IS26 element was found. The ISCR3-type element might be at the origin of the mobilization of the *qepA2* gene, and a possible hypothesis reconstituting the overall process of acquisition of that gene (involving transposition/recombination mechanisms) is proposed in Fig. 2, according to the model described by Toleman et al. (17).

By using a 5' rapid amplification of cDNA ends PCR system (Invitrogen, Cergy-Pontoise, France), the site of initiation of transcription of the *qepA2* gene was mapped in *E. coli* BicA and identified 24 bp upstream of the start codon of that gene. Upstream of this transcriptional start site, a putative -35 promoter sequence (ATGTCG) was found, separated by 17 bp from a putative -10 promoter sequence (TGTCGT). A putative ribosome binding site (GGAAG) was also identified 9 bp upstream of the *qepA2* start codon.

This is the first epidemiological survey evaluating the prevalence of the plasmid-mediated quinolone resistance determinant QepA in a European hospital; the prevalence was found to be low among ESBL-producing enterobacterial isolates. Identification of QepA with the widespread ESBL CTX-M-15 on a broad-host-range plasmid and in association with a peculiar ISCR-mediated genetic structure may indicate its further spread.

Nucleotide sequence accession number. The nucleotide sequence of the *qepA2*-positive strain *E. coli* BicA has been

submitted to the GenBank nucleotide sequence database (accession no. EU847537).

We thank Patrice Courvalin for the gift of the *qepA*-positive control, Thierry Naas for helpful discussion, and Mark Toleman and Timothy Walsh for their contribution to the ISCR nomenclature.

This work was funded by a grant from the Ministère de l'Éducation Nationale et de la Recherche (grant UPRES-EA3539), Université Paris XI, Paris, France, and mostly by a grant from the European Community (6th PCRD, LSHM-CT-2005-018705).

REFERENCES

- Carattoli, A., A. Bertini, L. Villa, V. Falbo, K. L. Hopkins, and E. J. Threlfall. 2005. Identification of plasmids by PCR-based replicon typing. *J. Microbiol. Methods* **63**:219–228.
- Cattoir, V., P. Lesprit, C. Lascols, E. Denamur, P. Legrand, C. J. Soussy, and E. Cambau. 2006. In vivo selection during ofloxacin therapy of *Escherichia coli* with combined topoisomerase mutations that confer high resistance to ofloxacin but susceptibility to nalidixic acid. *J. Antimicrob. Chemother.* **58**:1054–1057.
- Cattoir, V., L. Poirel, C. Aubert, C.-J. Soussy, and P. Nordmann. 2008. Unexpected occurrence of plasmid-mediated quinolone resistance determinants in environmental *Aeromonas* spp. *Emerg. Infect. Dis.* **14**:231–237.
- Cattoir, V., L. Poirel, V. Rotimi, C.-J. Soussy, and P. Nordmann. 2007. Multiplex PCR for detection of plasmid-mediated quinolone resistance *qnr* genes in ESBL-producing enterobacterial isolates. *J. Antimicrob. Chemother.* **60**:394–397.
- Clinical and Laboratory Standards Institute. 2008. Performance standards for antimicrobial susceptibility testing; 18th informational supplement M100–S18. Clinical and Laboratory Standards Institute, Wayne, PA.
- Girlich, D., L. Poirel, A. Leelaporn, A. Karim, C. Tribuddharat, M. Fennwald, and P. Nordmann. 2001. Molecular epidemiology of the integron-located VEB-1 extended-spectrum β -lactamase in nosocomial enterobacterial isolates in Bangkok, Thailand. *J. Clin. Microbiol.* **39**:175–182.
- Hata, M., M. Suzuki, M. Matsumoto, M. Takahashi, K. Sato, S. Ibe, and K. Sakae. 2005. Cloning of a novel gene for quinolone resistance from a transferable plasmid in *Shigella flexneri* 2b. *Antimicrob. Agents Chemother.* **49**:801–803.
- Jacoby, G. A., K. E. Walsh, D. M. Mills, V. J. Walker, H. Oh, A. Robicsek, and D. C. Hooper. 2006. *qnrB*, another plasmid-mediated gene for quinolone resistance. *Antimicrob. Agents Chemother.* **50**:1178–1182.
- Kieser, T. 1984. Factors affecting the isolation of CCC DNA from *Streptomyces lividans* and *Escherichia coli*. *Plasmid* **12**:19–36.
- Martinez-Martinez, L., A. Pascual, and G. A. Jacoby. 1998. Quinolone resistance from a transferable plasmid. *Lancet* **351**:797–799.
- Minarini, L. A., L. Poirel, V. Cattoir, A. L. Darini, and P. Nordmann. Plasmid-mediated quinolone resistance determinants among enterobacterial isolates from outpatients in Brazil. *J. Antimicrob. Chemother.*, in press.
- Nordmann, P., and L. Poirel. 2005. Emergence of plasmid-mediated resistance to quinolones in *Enterobacteriaceae*. *J. Antimicrob. Chemother.* **56**:463–469.
- Périchon, B., P. Courvalin, and M. Galimand. 2007. Transferable resistance to aminoglycosides by methylation of G1405 in 16S rRNA and to hydrophilic fluoroquinolones by QepA-mediated efflux in *Escherichia coli*. *Antimicrob. Agents Chemother.* **51**:2464–2469.
- Robicsek, A., J. Strahilevitz, G. A. Jacoby, M. Macielag, D. Abbanat, C. H. Park, K. Bush, and D. C. Hooper. 2006. Fluoroquinolone-modifying enzyme: a new adaptation of a common aminoglycoside acetyltransferase. *Nat. Med.* **12**:83–88.
- Robicsek, A., G. A. Jacoby, and D. C. Hooper. 2006. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect. Dis.* **6**:629–640.
- Ruiz, J. 2003. Mechanisms of resistance to quinolones: target alterations, decreased accumulation and DNA gyrase protection. *J. Antimicrob. Chemother.* **51**:1109–1117.
- Toleman, M. A., P. M. Bennett, and T. R. Walsh. 2006. ISCR elements: novel gene-capturing systems of the 21st century? *Microbiol. Mol. Biol. Rev.* **70**:296–316.
- Yamane, K., J. I. Wachino, S. Suzuki, and Y. Arakawa. 2008. Plasmid-mediated *qepA* gene among *Escherichia coli* clinical isolates from Japan. *Antimicrob. Agents Chemother.* **52**:1564–1566.
- Yamane, K., J. Wachino, S. Suzuki, K. Kimura, N. Shibata, H. Kato, K. Shibayama, T. Konda, and Y. Arakawa. 2007. New plasmid-mediated fluoroquinolone efflux pump, QepA, found in an *Escherichia coli* clinical isolate. *Antimicrob. Agents Chemother.* **51**:3354–3360.