

Plasmid-Mediated *qepA* Gene among *Escherichia coli* Clinical Isolates from Japan[▽]

Kunikazu Yamane,* Jun-ichi Wachino, Satowa Suzuki, and Yoshichika Arakawa

Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, Tokyo, Japan

Received 29 August 2007/Returned for modification 9 September 2007/Accepted 30 January 2008

Seven hundred fifty-one *Escherichia coli* clinical isolates collected from 140 Japanese hospitals between 2002 and 2006 were screened for the *qepA* and *qnr* genes. Two *E. coli* isolates (0.3%) harbored *qepA*, but no *qnr* was identified. The results suggested a low prevalence of *E. coli* harboring *qepA* or *qnr* in Japan.

The most common chromosomal mechanism of resistance to fluoroquinolones (FQs) in pathogenic bacteria is amino acid substitutions in the quinolone resistance-determining regions of DNA gyrase (GyrA) and/or topoisomerase IV (ParC), which are the main target molecules of FQs (7, 8). Efflux pumps and alteration in the outer membrane proteins also contribute to chromosomal FQ resistance (6). Plasmid-mediated mechanisms of resistance to FQs such as Qnr and AAC(6′)-Ib-cr have also been described (13). We recently identified *qepA*, a new plasmid-mediated gene responsible for reduced FQ susceptibility from *Escherichia coli* C316, which was isolated in 2002 from the urine of an inpatient in Japan (21), and *qepA* was also reported from *E. coli* 1450, which was isolated in a Belgian hospital (12). *qepA* encodes an efflux pump belonging to the major facilitator subfamily (MSF). The MICs of norfloxacin, enrofloxacin, and ciprofloxacin were 32- to 64-fold higher for the experimental strains expressing QepA compared with the host strain (21). The MICs of ampicillin, erythromycin, kanamycin, tetracycline, and chemical substances such as carbonyl cyanide *m*-chlorophenylhydrazone, acriflavine, rhodamine 6G, crystal violet, and sodium dodecyl sulfate were not affected, however, indicating that FQs are the specific substrates of QepA. Moreover, a norfloxacin accumulation assay with or without carbonyl cyanide *m*-chlorophenylhydrazone, an efflux pump inhibitor, showed that QepA is an FQ-specific MSF-type efflux pump (21).

qnrA was the first plasmid-mediated gene that conferred resistance to quinolones such as nalidixic acid and increased MICs of FQs, originally reported in *Klebsiella pneumoniae* clinical isolates from the United States (11, 17). Subsequently, two other groups of *qnr* genes, *qnrB* (9) and *qnrS* (5), as well as their variants, have been reported. Qnrs belong to the pentapeptide repeat family and mimic DNA fragments bound to the DNA gyrase (17). The *qnr* genes have been identified in various bacterial species belonging to the family *Enterobacteriaceae* in many countries (13). In Japan, *qnrS* was first identified in *Shigella flexneri* (5) and *qnrA* was also identified recently (15, 16). Clinically, *E. coli* is the most frequent cause of urinary

tract infections and FQs are some of the preferred antimicrobial agents for treatment (19). In this study, we investigated the prevalence of *qepA*, as well as *qnrA*, *qnrB*, and *qnrS*, among *E. coli* clinical isolates collected from Japanese medical facilities.

A total of 751 nonduplicate *E. coli* isolates isolated from patients admitted to 140 medical facilities in Japan between 2002 and 2006 were submitted to our reference laboratory for characterization of the genetic determinants responsible for antimicrobial resistance, as well as their genetic relatedness. All of the isolates were suspended in Luria-Bertani (LB) broth supplemented with 25% glycerol and stored in a -80°C deep freezer until analysis. The isolates were initially screened by growth on LB agar plates containing 0.025 $\mu\text{g}/\text{ml}$ norfloxacin. PCR analyses for *qepA* and the three *qnr* genes were performed for all of the isolates that grew on the norfloxacin-containing plates. DNA templates for the PCR were prepared by the standard boiling method. The primer sets used for detection of *qnrA*, *qnrB*, and *qnrS* have been described by Cattoir et al. (1) and Robicsek et al. (14). The pairs of primers designed by Cattoir et al. (1) were able to amplify internal fragments with *qnrA1* to *qnrA6*, *qnrB1* to *qnrB8*, and *qnrS1* to *qnrS2*, respectively. A 199-bp fragment of *qepA* was amplified by PCR with primers QEPA-F (5′-GCA GGT CCA GCA GCG GGT AG-3′) and QEPA-R (5′-CTT CCT GCC CGA GTA TCG TG-3′). The pair of primers used for detection of *rmtB* have been described by Doi and Arakawa (4). *rmtB* is a 16S rRNA methylase gene that confers resistance to aminoglycosides and was located in close proximity to *qepA* on a transferable plasmid in *E. coli* C316 (21). Positive control strains for *qnrA*, *qnrB*, and *qnrS* were *E. coli* J53(pMG252) (11), *E. coli* J53(pMG298) (9), and *E. coli* DH10B(pBC-H2.6) (5), respectively, and that for *qepA* was *E. coli* KAM32(pSTVqepA) (21). The PCR conditions used for *qepA* were as follows: initial denaturation at 96°C for 1 min, followed by 30 cycles of amplification at 96°C for 1 min, annealing at 60°C for 1 min, and extension at 72°C for 1 min. The final extension step was at 72°C for 5 min. The multiplex PCR condition for the *qnr* genes has been described previously (1, 14).

Of the 751 *E. coli* isolates tested, 325 grew on LB agar plates supplemented with 0.025 $\mu\text{g}/\text{ml}$ norfloxacin. Two isolates (0.3%) were positive for *qepA* and *rmtB* (MRY04-1030 and MRY05-3283). The two isolates came from geographically distant hospitals. However, no *qnr* gene was detected among the *E. coli* isolates tested in this study.

* Corresponding author. Mailing address: Department of Bacterial Pathogenesis and Infection Control, National Institute of Infectious Diseases, 4-7-1 Gakuen, Musashi-Murayama, Tokyo 208-0011, Japan. Phone: 81-42-561-0771. Fax: 81-42-561-7173. E-mail: kazuwa@nih.gov.jp.

[▽] Published ahead of print on 19 February 2008.

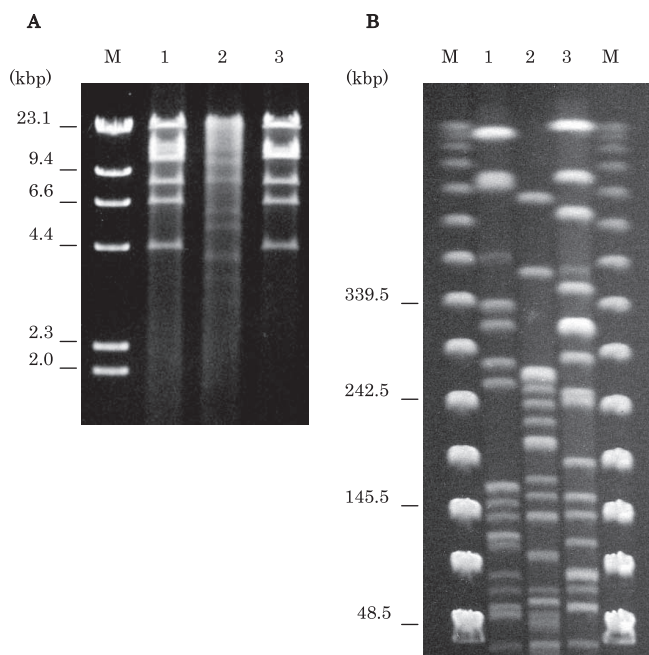


FIG. 1. (A) EcoRI restriction profiles of *qepA*-carrying plasmids from the transconjugants. Lane M, lambda HindIII marker; lane 1, pHPA from *E. coli* C316; lane 2, p041060 from *E. coli* MRY04-1060; lane 3, p05283 from *E. coli* MRY05-3283. (B) PFGE fingerprinting patterns of XbaI-digested total DNA preparations from three *E. coli* isolates. Lanes M, lambda ladder PFGE marker used as a molecular size marker. Lanes 1 to 3, *E. coli* C316, MRY04-1030, and MRY05-3283, respectively.

Transconjugation analysis was performed by the filter mating method with *E. coli* DH10B as the recipient (3). Transconjugants were selected on LB agar plates supplemented with streptomycin (50 µg/ml) and amikacin (50 µg/ml) because the plasmid carried *rmtB*, which confers resistance to amikacin. Plasmids were digested with EcoRI (New England BioLabs, Beverly, MA) and electrophoresed through a 1.0% agarose gel. FQ resistance was successfully transferred from the two *qepA*-positive *E. coli* isolates to *E. coli* DH10B at a frequency of 10⁻⁵ to 10⁻⁶ cells per recipient cell by conjugation. EcoRI restriction patterns for *qepA* carrying plasmids are shown in Fig. 1. The restriction patterns of pHPA from *E. coli* C316 and p05283 from *E. coli*

MRY05-3283 were very similar. However, those of p05283 and p041060 from *E. coli* MRY04-1060 were completely different from the other two. Neither of the *qepA*-positive plasmids conferred resistance to ceftazidime and cefotaxime.

Pulsed-field gel electrophoresis (PFGE) was performed with the CHEF-Mapper system (Bio-Rad Laboratories, Hercules, CA). Genomic DNA preparations from *E. coli* C316, MRY04-1060, and MRY05-3283 were digested with XbaI (New England BioLabs) (Fig. 1). The PFGE fingerprinting patterns of the three *qepA*-positive strains were apparently different from each other.

Antimicrobial susceptibility testing of the *qepA*-positive isolates and their transconjugants was performed by the agar dilution method according to the guidelines recommended by the Clinical and Laboratory Standards Institute (2) (Table 1). The MICs of norfloxacin for the transconjugants of each *qepA*-positive isolate were four- to fivefold higher than that for the recipient strain. The two *qepA*-positive isolates were also highly resistant to all of the aminoglycosides tested, including amikacin, tobramycin, and gentamicin, but susceptible to the expanded-spectrum cephalosporins and imipenem.

In our previous study, *qepA* and *rmtB* were found to be encoded on the same transferable plasmid, and the analysis of the genetic environment of *qepA* in *E. coli* showed that *qepA* and *rmtB* were likely mediated by a composite transposon flanked by two copies of IS26 (21). Interestingly, an *E. coli* strain positive for both *qepA* and *rmtB* has also been isolated in Belgium (12). The genetic organization of the region containing *qepA* and *rmtB* was very similar to that of *E. coli* C316, suggesting the *qepA*-harboring isolates demonstrating panresistance to aminoglycosides by production of RmtB may well have already spread worldwide.

Although *qnr* genes have been identified in *E. coli* and other members of the family *Enterobacteriaceae* isolated from other East Asian countries, such as China, Korea, and Taiwan (10, 18, 20), the results of our study indicate that *qnr*-harboring *E. coli* is still very rare in Japanese medical facilities.

We are grateful to George A. Jacoby for provision of the *qnrA*- and *qnrB*-positive control strains.

pBC-H2.6, deposited by Mami Hata, was provided by the DNA Bank, RIKEN BioResource Center (Tsukuba, Japan), with the support of the National Bio-Resources Project of the Ministry of Education, Culture, Sports, Science, and Technology, Japan (MEXT). Part

TABLE 1. Antimicrobial susceptibilities of the *qepA* donor, transconjugant, and recipient strains used in this study

Antimicrobial agent	MIC (µg/ml) for <i>E. coli</i> strain:				
	MRY04-1060	MRY05-3283	DH10B(p041060) ^a	DH10B(p05283) ^b	DH10B
Norfloxacin	>128	>128	0.25	0.25	≤0.008
Levofloxacin	64	128	0.008	0.015	≤0.008
Ciprofloxacin	>128	>128	0.015	0.015	≤0.008
Ceftazidime	0.5	0.5	0.5	0.5	0.25
Cefotaxime	0.13	0.13	0.06	0.06	0.06
Imipenem	0.13	0.13	0.25	0.25	0.25
Gentamicin	>128	>128	>128	>128	0.5
Amikacin	>128	>128	>128	>128	2
Tobramycin	>128	>128	>128	>128	0.5

^a Transconjugant of *E. coli* MRY04-1060.

^b Transconjugant of *E. coli* MRY05-3283.

of this work was funded by a grant from the Kurozumi Medical Foundation and Grant-in-Aid for Young Scientists (B) 19790400 from the Ministry of Education, Culture, Sports, Science, and Technology, Japan.

REFERENCES

1. 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.
2. Clinical and Laboratory Standards Institute. 2005. Performance standards for antimicrobial susceptibility testing; 15th informational supplement. CLSI M100-S15. Clinical and Laboratory Standards Institute, Wayne, PA.
3. de Lorenzo, V., and K. N. Timmis. 1994. Analysis and construction of stable phenotypes in gram-negative bacteria with Tn5- and Tn10-derived minitransposons. *Methods Enzymol.* **235**:386–405.
4. Doi, Y., and Y. Arakawa. 2007. 16S rRNA methylation: emerging resistance mechanism against aminoglycosides. *Clin. Infect. Dis.* **45**:88–94.
5. 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.
6. Hooper, D. C. 1999. Mechanisms of fluoroquinolone resistance. *Drug Resist. Updates* **2**:38–55.
7. Hooper, D. C. 2001. Emerging mechanisms of fluoroquinolone resistance. *Emerg. Infect. Dis.* **7**:337–341.
8. Hopkins, K. L., R. H. Davies, and E. J. Threlfall. 2005. Mechanisms of quinolone resistance in *Escherichia coli* and *Salmonella*: recent developments. *Int. J. Antimicrob. Agents* **25**:358–373.
9. 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.
10. Jeong, J. Y., H. J. Yoon, E. S. Kim, Y. Lee, S. H. Choi, N. J. Kim, J. H. Woo, and Y. S. Kim. 2005. Detection of *qnr* in clinical isolates of *Escherichia coli* from Korea. *Antimicrob. Agents Chemother.* **49**:2522–2524.
11. Martínez-Martínez, L., A. Pascual, and G. A. Jacoby. 1998. Quinolone resistance from a transferable plasmid. *Lancet* **351**:797–799.
12. 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.
13. Robicsek, A., G. A. Jacoby, and D. C. Hooper. 2006. The worldwide emergence of plasmid-mediated quinolone resistance. *Lancet Infect. Dis.* **6**:629–640.
14. Robicsek, A., J. Strahilevitz, D. F. Sahn, G. A. Jacoby, and D. C. Hooper. 2006. *qnr* prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. *Antimicrob. Agents Chemother.* **50**:2872–2874.
15. Saga, T., T. Akasaka, H. Takase, M. Tanaka, K. Sato, and M. Kaku. 2007. First detection of the plasmid-mediated quinolone resistance determinant *qnrA* in *Enterobacteriaceae* clinical isolates in Japan. *Int. J. Antimicrob. Agents* **29**:738–739.
16. Saito, R., W. Kumita, K. Sato, T. Chida, N. Okamura, K. Moriya, and K. Koike. 2007. Detection of plasmid-mediated quinolone resistance associated with *qnrA* in an *Escherichia coli* clinical isolate producing CTX-M-9 β -lactamase in Japan. *Int. J. Antimicrob. Agents* **29**:600–602.
17. Tran, J. H., and G. A. Jacoby. 2002. Mechanism of plasmid-mediated quinolone resistance. *Proc. Natl. Acad. Sci. USA* **99**:5638–5642.
18. Wang, M., J. H. Tran, G. A. Jacoby, Y. Zhang, F. Wang, and D. C. Hooper. 2003. Plasmid-mediated quinolone resistance in clinical isolates of *Escherichia coli* from Shanghai, China. *Antimicrob. Agents Chemother.* **47**:2242–2248.
19. Warren, J. W., E. Abrutyn, J. R. Hebel, J. R. Johnsin, A. J. Schaeffer, and W. E. Stamm. 1999. Guidelines for antimicrobial treatment of uncomplicated acute bacterial cystitis and acute pyelonephritis in woman. *Clin. Infect. Dis.* **29**:745–758.
20. Wu, J. J., W. C. Ko, S. H. Tsai, and J. J. Yan. 2007. Prevalence of plasmid-mediated quinolone resistance determinants QnrA, QnrB, and QnrS among clinical isolates of *Enterobacter cloacae* in a Taiwanese hospital. *Antimicrob. Agents Chemother.* **51**:1223–1227.
21. 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.