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Vibrio parahaemolyticus Chromosomal *qnr* Homologue VPA0095: Demonstration by Transformation with a Mutated Gene of Its Potential To Reduce Quinolone Susceptibility in *Escherichia coli*

The gene *qnr* (*qnrA* [G. A. Jacoby, K. Walsh, D. Mills, V. Walker, A. Robicsek, H. Oh, and D. C. Hooper, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., abstr. C2-1898a, 2004]; encoding QnrA) was first identified as a transferable quinolone resistance determinant on a multidrug-resistant plasmid in *Klebsiella pneumoniae* (6, 9) and has been detected in *K. pneumoniae*, *Escherichia coli*, *Enterobacter* spp., and *Citrobacter freundii* isolates in various countries (3, 4, 8, 10, 11). Furthermore, the *qnrA* homologues *qnrB* (encoding QnrB, 40% identity with QnrA [Jacoby et al., 44th ICAAC]) and *qnrS* (encoding QnrS, 59% identity with QnrA [2]) were discovered on plasmids in *K. pneumoniae* and *Enterobacter* spp. and in *Shigella flexneri*, respectively. Thus, several *qnr* genes have been found on enterobacterial plasmids, but their origin or ancestry is unknown.

Hata et al. recently reported that QnrS exhibited significant identity with a *Photobacterium profundum* protein (CAG22829; 64%) and a *Vibrio vulnificus* protein (AAO07889; 53%) (2), which also showed 66 and 60% identity with QnrA, respectively. In addition, by a homology search using the DNA Data Bank of Japan (http://www.ddbj.nig.ac.jp/search/blast-j.html), we had noticed that another *P. profundum* protein (CAG21998) and a *Vibrio parahaemolyticus* protein (BAC61438) both showed 58% identity with QnrA (52 and 56% with QnrS, respectively). Taken together, three species of the *Vibrionaceae* family were revealed to chromosomally possess putative *qnr* homologues, raising the hypothesis that *qnr* genes on enterobacterial plasmids had derived from one of them or their relatives. These chromosomal homologues are not associated with any integron-like structure. Meanwhile, *qnrA* is present in an integron (9, 11), but *qnrS* is not (2).

To gain a functional insight, we examined the *V. parahaemolyticus qnr* homologue, VPA0095, for *qnr*-like potential. A DNA fragment corresponding to the gene (part of the sequence, BA000032) was amplified from *V. parahaemolyticus* strain 8611 (isolated at Tohoku University Hospital, Sendai, Japan) by PCR with the primers 5′-TTAAAAACCGATCTC ATTTTTGAACGAG and 5′-ACTTCCTCGTCGAcGTTAT TCGGTAAGTC (the small character indicates an introduced artificial base exchange; the SalI site was newly established as underlined). The fragment, 701 base pairs from the second codon to 53 bases downstream of the stop codon, was digested with SalI after blunting and kination using a TaKaRa BKL kit (Takara Bio Inc., Otsu, Japan). It was next ligated into a plasmid vector, pTV118N (pUC118 derivative; Takara Bio Inc.), which was previously treated with NcoI, KOD DNA polymerase (TOYOBO, Osaka, Japan) for blunting, and then SalI, followed by introduction into *E. coli* strain MC1061 (5). Plasmids were extracted from some transformants of the strain, and their partial sequences encompassing the ligated fragment were determined. We consequently obtained a plasmid, pVPQNR8, carrying VPA0095 connected to a start codon derived from *lacZ* following the *lac* promoter and Shine-Dalgarno sequence, and a plasmid, pVPQNR2, carrying a gene with a single mutation (TGT to TAT) at the 115th codon of VPA0095, which probably occurred as a PCR error and was accompanied by an amino acid change from cysteine to tyrosine (C115Y). *E. coli* strain KL16 (1) was transformed with each plasmid and, as a control, with pTV118N and its derivative carrying *qnrA*, pKPQNR (constructed with *qnrA* from a *K. pneumoniae* isolate in Japan: unpublished). The transformants were then subjected to drug susceptibility tests, in which MICs of ciprofloxacin, levofloxacin, nalidixic acid (all synthesized at Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), and minocycline (Wyeth Japan, Tokyo, Japan) were determined by the agar dilution method (7). The results are shown in Table 1. pVPQNR8, compared to the negative control, pTV118N, did not result in significant changes of the MICs. Interestingly, on the other hand, 8- to 16-fold increases in quinolone MICs were provided by pVPQNR2 carrying a mutated gene, comparable to pKPQNR. The MIC of minocycline for strain KL16 was not changed by the plasmids, indicating the specific effect of introduced genes on quinolone susceptibility.

To confirm the expression of *qnr*-like potential by a single mutation, we next performed site-directed mutagenesis experiments. Single base exchanges at the 115th codon of the gene on plasmids were induced using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, Calif.) and primers 5′-CACCATGTTTTTCTaTTCTGCTTTTATTACTGGC and 5′-GCCAGTAATAAAAGCAGAAtAGAAAAAACATGGTG for the exchange in pVPQNR8 (TGT to TAT [resulting in pVPQNR8C115Y, the mutant type]) or 5′-CACCATGTTTT TCTgTTCTGCTTTTATTACTGGC and 5′-GCCAGTAATA AAAGCAGAAcAGAAAAACATGGTG for the exchange in pVPQNR2 (TAT to TGT [resulting in pVPQNR2Y115C, the native type]). Lowercase letters in the primer sequences indicate an introduced artificial base exchange. Acquired plasmids were introduced into *E. coli* strain KL16. As shown in Table 1, pVPQNR8C115Y clearly increased the quinolone MICs for the strain to the same level as pVPQNR2, while pVPQNR2Y115C was basically identical to pVPQNR8, indicating the verified effect of the mutation conferring C115Y in VPA0095.

Thus, one of the *Vibrionaceae* chromosomal *qnr* homologues was experimentally confirmed to possess the potential to

TABLE 1. Influence of *qnrA* and its homologue, VPA0095 from *V. parahaemolyticus*, on the drug susceptibilities of *E. coli*

Plasmid	Gene carried[b]	Change in MIC (*n*-fold) for *E. coli* strain KL16[a]			
		Ciprofloxacin	Levofloxacin	Nalidixic acid	Minocycline
pTV118N (vector)		1 (0.015)	1 (0.03)	1 (4)	1 (1)
pKPQNR	*qnrA*	16	16	4	1
pVPQNR8	VPA0095(TGT)	1–2	1	1–2	1
pVPQNR2	VPA0095(TAT)	16	16	8–16	1
pVPQNR8C115Y[c]	VPA0095(TAT)	16	16	8–16	1
pVPQNR2Y115C[d]	VPA0095(TGT)	2	1–2	1–2	1

[a] The change is the ratio of the MIC for the strain with the indicated plasmid to the MIC, shown in parentheses, for the strain with pTV118N.
[b] The 115th codon is shown in parentheses.
[c] Acquired from pVPQNR8 by site-directed mutagenesis.
[d] Acquired from pVPQNR2 by site-directed mutagenesis.

reduce quinolone susceptibility in *E. coli*, suggesting that the homologues should relate closely to the origin or ancestry of *qnr* genes carried on enterobacterial plasmids. In addition, the potential enhancement of VPA0095 by a single mutation suggests, although its mechanism remains to be elucidated, that *qnr* genes and/or its homologues could be comparatively easily converted to or selected as a higher quinolone resistance determinant.

REFERENCES

1. **Bachmann, B. J.** 1996. Derivations and genotypes of some mutant derivatives of *Escherichia coli* K-12, p. 2460–2488. *In* F. C. Neidhardt (ed.), *Escherichia coli* and *Salmonella*: cellular and molecular biology, 2nd ed. American Society for Microbiology, Washington, D.C.
2. **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.
3. **Jacoby, G. A., N. Chow, and K. B. Waites.** 2003. Prevalence of plasmid-mediated quinolone resistance. Antimicrob. Agents Chemother. **47:**559–562.
4. **Jonas, D., K. Biehler, D. Hertung, B. Spitzmüller, and F. Daschner.** 2005. Plasmid-mediated quinolone resistance in isolates obtained in German intensive care units. Antimicrob. Agents Chemother. **49:**773–775.
5. **Maniatis, T., E. F. Fritsch, and J. Sambrook.** 1989. Molecular cloning: a laboratory manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
6. **Martinez-Martinez, L., A. Pascual, and G. A. Jacoby.** 1998. Quinolone resistance from a transferable plasmid. Lancet **351:**797–799.
7. **National Committee for Clinical Laboratory Standards.** 2003. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A6. National Committee for Clinical Laboratory Standards, Wayne, Pa.
8. **Rodriguez-Martinez, J. M., A. Pascual, I. Garcia, and L. Martinez-Martinez.** 2003. Detection of the plasmid-mediated quinolone resistance determinant *qnr* among clinical isolates of *Klebsiella pneumoniae* producing AmpC-type beta-lactamase. J. Antimicrob. Chemother. **52:**703–706.
9. **Tran, J. H., and G. A. Jacoby.** 2002. Mechanism of plasmid-mediated quinolone resistance. Proc. Natl. Acad. Sci. USA **99:**5638–5642.
10. **Wang, M., D. F. Sahm, G. A. Jacoby, and D. C. Hooper.** 2004. Emerging plasmid-mediated quinolone resistance associated with the *qnr* gene in *Klebsiella pneumoniae* clinical isolates in the United States. Antimicrob. Agents Chemother. **48:**1295–1299.
11. **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.

Tomoo Saga
Mitsuo Kaku
Department of Infection Control and Laboratory Diagnostics
Internal Medicine
Tohoku University Graduate School of Medicine
Sendai, Japan

Yoshikuni Onodera
Shinichiro Yamachika
Kenichi Sato
Hiroyuki Takase*
New Product Research Laboratories I
Daiichi Pharmaceutical Co., Ltd.
Tokyo, Japan

*Phone: 81-3-5696-8182
Fax: 81-3-5696-4264
E-mail: takas4px@daiichipharm.co.jp