**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'GATCCATGGTG-3' and 5'-GCCAGTAATAAAACGAGAAAGAAACATGGTG-3'. The fragment, 701 base pairs from the second codon to 53 bases downstream of the stop codon, was digested underlined). The fragment, 701 base pairs from the second codon to 53 bases downstream of the stop codon, was digested with *Sal*I after blunting and kination using a TaKaRa BKL kit (TaKaRa BCL, Japan). The fragment containing the putative gene was cloned into the unique *Sal*I site of a plasmid vector, pTV118N (pUC118 derivative; Takara Bio Inc.), which was previously treated with Ncol, KOD DNA polymerase (TOYOBO, Osaka, Japan) for blunting, and then Sall, 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, pVPQN8, carrying VPA0095 connected to a start codon derived from lacZ following the lac promoter and Shine-Dalgarno sequence, and a plasmid, pVPQN2, 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 a plasmid 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. pVPQN8R, 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 pVPQN8R 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 introduced using a QuikChange site-directed mutagenesis kit (Stratagene, La Jolla, Calif.) and primers 5'-CACCAGTTTCTCATTGCTTTATGAGTCTGC-3' and 5'GCCAGTAATAAAACGAGAAAGAAACATGGTG-3' for the exchange in pVPQN8 (TGT to TAT) [resulting in pVPQN8R(TGT)115Y, the mutant type]) or 5'-CACCAGTTTCTCATTGCTTTATGAGTCTGC-3' and 5'GCCAGTAATAAAACGAGAAAGAAACATGGTG-3' for the exchange in pVPQN8R (TAT to TGT) [resulting in pVPQN8R(TAT)115C, 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, pVPQN8R(TGT)115Y clearly increased the quinolone MICs for the strain to the same level as pVPQN8R, while pVPQN8R(TAT)115C was basically identical to pVPQN8R, 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 reduce quinolone susceptibility. Although the in vivo activity of VPA0095 remains to be determined, if VPA0095 is present in *V. parahaemolyticus* outside Japan, the plasmid-borne *qnr* homologue of *V. parahaemolyticus* could be involved in the spread of *qnr*-like determinants in *V. parahaemolyticus*.
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


