ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Aug. 2005, p. 3523–3525 Vol. 49, No. 8
0066-4804/05/$08.00+0 doi:10.1128/AAC.49.8.3523–3525.2005
Copyright © 2005, American Society for Microbiology. All Rights Reserved.

Origin of Plasmid-Mediated Quinolone Resistance Determinant QnrA

Laurent Poirel,[1] Jose-Manuel Rodriguez-Martinez,[1,2] Hedi Mammeri,[1] Alain Liard,[1] and Patrice Nordmann[1]*

Service de Bactériologie-Virologie, Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine Paris-Sud, Université Paris XI, 94275 K.-Bicêtre, France,[1] and University Hospital Virgen Macarena, University of Sevilla, Sevilla, Spain[2]

Received 15 March 2005/Returned for modification 19 April 2005/Accepted 21 May 2005

Plasmid-mediated resistance to quinolones is increasingly reported in studies of *Enterobacteriaceae*. Using a PCR-based strategy, a series of gram-negative species were screened for *qnrA*-like genes. *Shewanella algae*, an environmental species from marine and fresh water, was identified as its reservoir. This is a one of the very few examples of progenitor identification of an acquired antibiotic resistance gene.

Multidrug resistance in *Enterobacteriaceae*, including resistance to quinolones, is currently among the top antibiotic resistance problems in the United States and is rising worldwide (5, 13). Quinolone resistance in *Enterobacteriaceae* results mostly from chromosomal mutations in genes coding for DNA gyrase (topoisomerase II), for efflux and outer membrane proteins, or for their regulatory elements (11). However, Qnr (later termed QnrA), a plasmid-mediated quinolone resistance determinant (G. A. Jacoby, K. Walsh, D. Mills, V. Wolker, A. Robicsek, H. Oh, and D. C. Hooper, Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother., Abstr. C2-1898a, 2004), had been reported in 1998 from *Klebsiella pneumoniae* first from the United States (16). It has been reported since then in *Citrobacter freundii*, *Escherichia coli*, *Enterobacter cloacae*, *Enterobacter sakazakii*, *K. pneumoniae*, and *Klebsiella oxytoca* from Asia and Europe (8, 15, 18, 26, 32).

The 218-amino-acid protein QnrA, which belongs to the pentapeptide repeat family, protects DNA gyrase and topoisomerase IV from the inhibitory activity of quinolones (29, 30). QnrA confers resistance to nalidixic acid and increases MICs of fluoroquinolones up to 32-fold (7, 15, 16).

The recent emergence of plasmid-mediated quinolone resistance led us to search for its natural reservoir. Our working hypothesis was that this novel resistance determinant could derive from an environmental, human, or animal gram-negative species, taking into account our knowledge on horizontal gene transfer in bacteria. A total of 48 gram-negative bacterial species were screened that included clinically significant bacterial species such as representatives of the *Enterobacteriaceae*, *Aeromonadaceae*, *Pseudomonadaceae*, *Xanthomonadaceae*, *Moraxellaceae*, and *Shewanellaceae* families.

PCR-based experiments using specific primers for the *qnrA* gene (15) identified a positive signal for three *Shewanella algae* clinical isolates (KB-1 to KB-3) (10) and reference strain *S. algae* CIP106454T (Institut Pasteur strain collection, Paris, France). The locations of the *qnrA* genes were determined precisely by using the endonuclease I-Ceu-I technique (14). Pulsed-field gel electrophoresis (28) gave six DNA fragments from *Shewanella* sp. strains (Fig. 1). The DNA probe for rRNA consisting of a 1,504-bp PCR fragment for 16S and 23S rRNA genes (6) hybridized with all the fragments from the *Shewanella* strains. Hybridization with a DNA probe internal to *qnrA* gene (15) gave a single signal for the *S. algae* strains only (Fig. 1).

The four corresponding QnrA-like proteins of *S. algae* reference strains and isolates KB-1 to KB-3 had only two to four amino acid substitutions compared to QnrA (termed now QnrA1) (Fig. 2). Note that the G+C content (52%) of the *qnrA*-like genes of *S. algae* matched exactly that of the genome

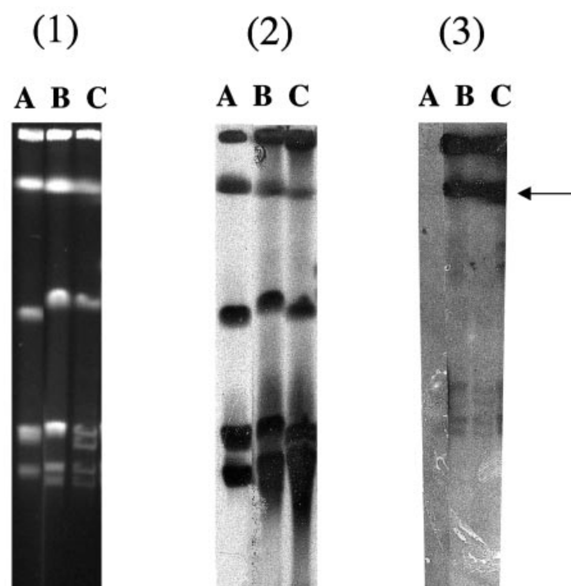

FIG. 1. (Panel 1) Pulsed-field gel electrophoresis profiles of I-Ceu-I digested whole-cell DNAs of *Shewanella* sp. strains. Lane A, *S. putrefaciens* reference strain CIP8040 (Institut Pasteur, Paris); lane B, *S. algae* clinical isolate KB-1; lane C, *S. algae* reference strain CIP106454T. (Panels 2 and 3) Southern hybridization was performed with a specific probe for the 16S-23S rRNA gene (6) and an internal probe for the *qnrA* gene (15). The horizontal arrow indicates hybridization position with the *qnrA* probe. Negative hybridization results with the *qnrA* probe were obtained for the *S. putrefaciens* reference strain.

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

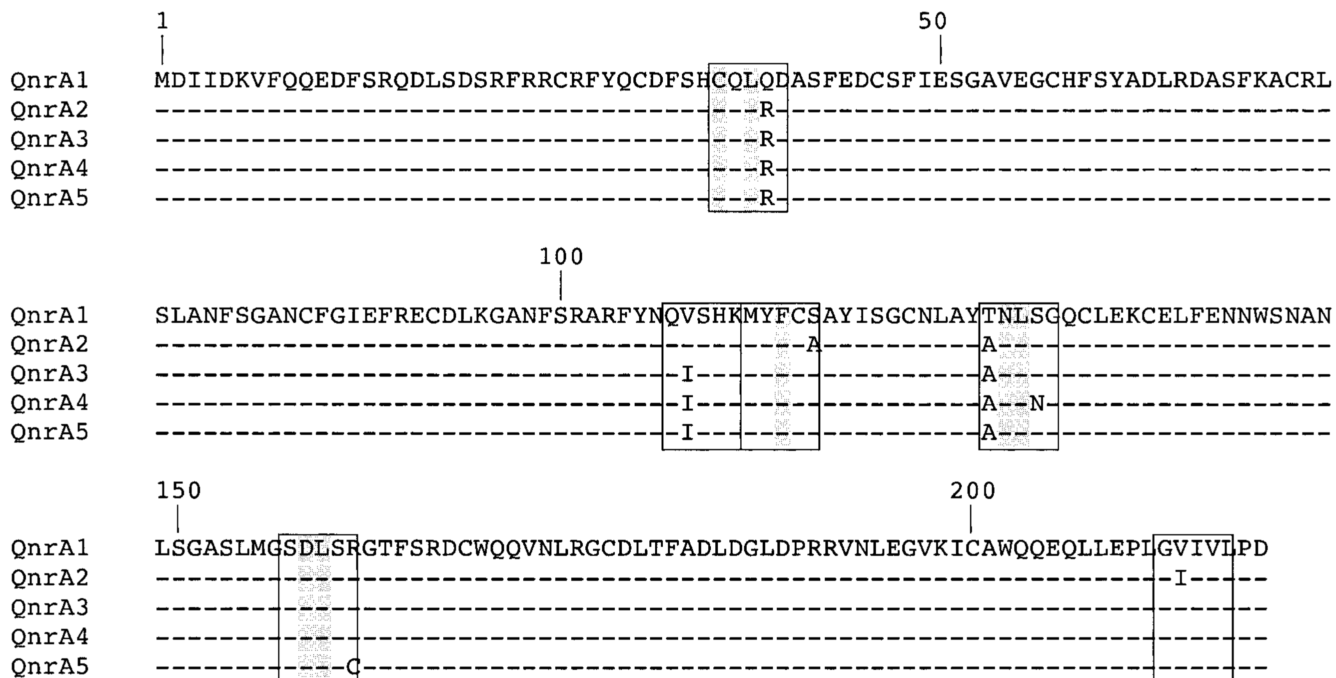

FIG. 2. Sequence comparison of plasmid-mediated QnrA-like determinants to those of *S. algae* strains. The plasmid-mediated QnrA1 and QnrA2 determinants are from *K. pneumoniae* from the United States (16) and from *Klebsiella oxytoca* from China (GenBank accession number AY675584), respectively. QnrA3 is from *S. algae* reference strain CIP106454T and *S. algae* clinical isolate KB-1, whereas QnrA4 and QnrA5 are from *S. algae* clinical isolates KB-2 and KB-3, respectively (10). DQ058661, DQ058662, and DQ058663 are GenBank accession numbers for QnrA3, QnrA4, and QnrA5, respectively. Dashes are for identical amino acid residues. Pentapeptide motifs in which amino acid substitutions have been identified compared to the QnrA1 sequence are boxed. In these motifs, the conserved residues identified by Tran and Jacoby (29) are shaded.

of *S. algae* (4). *S. algae* is a gram-negative species belonging to the *Shewanellaceae* family that is widely distributed in marine and freshwater environments (3, 19, 21). Whereas *S. putrefaciens* is also a human pathogen (2), it seems now that most of the *S. algae* isolates have been misidentified for *S. putrefaciens* (4) and most of the *S. algae* infections are related to seawater exposure (3). The MIC of nalidixic acid was 2 μg/ml, and those of fluoroquinolones ciprofloxacin, ofloxacin, sparfloxacin, and norfloxacin were 0.12, 0.5, 0.5, and 0.5 μg/ml, respectively, being identical for the four *S. algae* strains and remaining in the susceptibility range (17). Based on analysis of an antibiotic resistance phenotype, the presence of a QnrA-like determinant in *S. algae* could not be suspected. However, these MIC levels were four- to eightfold higher than those of the closely related species *Shewanella putrefaciens* that was *qnrA* negative.

Since the plasmid-encoded *qnrA* gene had been found in a *sul1*-type integron downstream of the open reading frame *orf513* coding for a recombinase (7, 15, 26, 29, 32), we also used a PCR-based strategy with primers ORF513D3 and ORF513D5 (15) for detecting this gene in *S. algae*. These experiments failed as well as those designed to amplify the *ampR* gene that has been identified in In*36* and In*37* just downstream of the *qnrA* gene (data not shown) (32). These results indicated that the CR1 element that provides promoter sequences for high-level expression of the plasmid-mediated QnrA gene in *Enterobacteriaceae* (15) was not associated with *qnrA* in *S. algae*.

Further work may identify in other psychrophilic species the reservoir of the two novel plasmid-mediated quinolone resistance determinants, i.e., QnrB identified from the United States and South India (Jacoby et al., abstr. C2-1898a, 2004) and QnrS identified from Japan (9), which share only 40 and 59% amino acid identity with QnrA, respectively. Notably, it has been shown recently that *Vibrio parahaemolyticus* possesses a QnrA homologue (58% identity) (27).

This report indicates that gram-negative bacterial species not only of the veterinary world but also of the environment may be a reservoir for emerging antibiotic resistance genes spreading in human pathogens, as suspected (33, 34). Our finding emphasizes the possible role of the aquatic environment as a reservoir of antibiotic resistance genes. In addition, we had determined recently that another *Shewanella* species, *Shewanella oneidensis*, is the natural reservoir of OXA-48, a plasmid-encoded carbapenem-hydrolyzing β-lactamase gene that was identified in *K. pneumoniae*, further indicating gene exchange between *Shewanella* spp. and *Enterobacteriaceae* (24). The present report adds knowledge on the origin of clinically significant antibiotic resistance genes that has been established without ambiguity in very few cases, such as for the SHV-, CTX-M-, and AmpC-type β-lactamase genes originating in enterobacterial species (12, 23, 25) and for tetracycline resistance determinants identified in mycobacteria and originating in *Streptomyces rimosus* (22).

The quinolones prescribed in human therapy are also extensively used in aquaculture (20) as synthetic molecules stable in

a water environment (31). Thus, it is tempting to speculate that subinhibitory concentrations of quinolones in water may select for waterborne *S. algae* strains and therefore enhance transfer of this naturally occurring quinolone resistance determinant to *Enterobacteriaceae*. The role of quinolones for inducing this antibiotic resistance gene transfer may be related to induction of the SOS bacterial repair system, as shown previously (1).

This work was funded by a grant from the Ministère de l'Education Nationale et de la Recherche (UPRES-EA3539), Université Paris XI, France, and by a grant from the European Community (6th PCRD, LSHM-CT-2003-503335). L.P. is a researcher from the INSERM (Paris, France), and J.-M.R.-M. was a recipient of a travel grant from the Spanish Society for Clinical Microbiology and Infectious Diseases in 2004. We also thank A. Pascual for constant support of J.-M.R.-M.

REFERENCES

1. **Beaber, J. W., B. Hochhut, and M. K. Waldor.** 2004. SOS response promotes horizontal dissemination of antibiotic resistance genes. Nature **427:**72–74.
2. **Brink, A. J., A. Van Straten, and A. J. Rensburg.** 1995. *Shewanella* (*Pseudomonas*) *putrefaciens* bacteremia. Clin. Infect. Dis. **20:**1327–1332.
3. **Fonnesbech-Vogel, B., H. M. Holt, P. Gerner-Smidt, A. Bundvad, P. Sogaard, and L. Gram.** 2000. Homogeneity of Danish environmental and clinical isolates of *Shewanella algae*. Appl. Environ. Microbiol. **66:**443–448.
4. **Fonnesbech-Vogel, B., K. Jorgensen, H. Christensen, J. Elmerdhahl-Olsen, and L. Gram.** 1997. Differentiation of *Shewanella putrefaciens* and *Shewanella alga* on the basis of whole-cell protein profiles, ribotyping, phenotypic characterization, and 16S rRNA gene sequence analysis. Appl. Environ. Microbiol. **63:**2189–2199.
5. **Garau, J., M. Xercavins, M. Rodriguez-Carballeira, J. R. Gomez-Vera, I. Coll, D. Vidal, T. Llovet, and A. Ruiz-Bremon.** 1999. Emergence and dissemination of quinolone-resistant *Escherichia coli* in the community. Antimicrob. Agents Chemother. **43:**2736–2741.
6. **Girlich, D., L. Poirel, A. Leelaporn, A. Karim, C. Tribuddharat, M. Fennewald, 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.
7. **Jacoby, G. A., N. Chow, and K. B. Waites.** 2003. Prevalence of plasmid-mediated quinolone resistance. Antimicrob. Agents Chemother. **47:**559–562.
8. **Jonas, D., K. Biehler, D. Hartung, B. Spitzmuller, and F. D. Daschner.** 2005. Plasmid-mediated quinolone resistance in isolates obtained in German intensive care units. Antimicrob. Agents Chemother. **49:**773–775.
9. **Hata, M., M. Suzuki, M. Matsumoto, M. Takahashi, K. Sato, S. Ibe, and K. Sakae.** 2005. Cloning of a novel quinolone resistance from a transferable plasmid in *Shigella flexneri* 2b. Antimicrob. Agents Chemother. **49:**801–803.
10. **Héritier, C., L. Poirel, and P. Nordmann.** 2004. Genetic and biochemical characterization of a chromosome-encoded carbapenem-hydrolyzing Ambler class D β-lactamase from *Shewanella algae*. Antimicrob. Agents Chemother. **48:**1670–1675.
11. **Hooper, D. C.** 2001. Emerging mechanisms of fluoroquinolone resistance. Emerg. Infect. Dis. **7:**337–341.
12. **Huemeniuk, C., G. Arlet, V. Gautier, P. Grimont, R. Labia, and A. Philippon.** 2002. β-Lactamases of *Kluyvera ascorbata*, probable progenitors of some plasmid-mediated CTX-M types. Antimicrob. Agents Chemother. **46:**3045–3049.
13. **Lautenbach, E., B. L. Strom, I. Nachamkin, W. B. Bilker, A. M. Marr, L. A. Larosa, and N. O. Fishman.** 2004. Longitudinal trends in fluoroquinolone resistance among *Enterobacteriaceae* isolates from inpatients and outpatients, 1989–2000; differences in the emergence and epidemiology of resistance across organisms. Clin. Infect. Dis. **38:**655–662.
14. **Liu, S. L., A. Hessel, and K. E. Sanderson.** 1993. Genomic mapping with I-*Ceu*-I, an intron-encoded endonuclease specific for genes for ribosomal RNA in *Salmonella* spp., *Escherichia coli*, and other bacteria. Proc. Natl. Acad. Sci. USA **90:**6874–6878.
15. **Mammeri, H., M. Van De Loo, L. Poirel, L. Martinez-Martinez, and P. Nordmann.** 2005. Emergence of plasmid-mediated quinolone resistance in *Escherichia coli* in Europe. Antimicrob. Agents Chemother. **49:**71–76.
16. **Martinez-Martinez, L., A. Pascual, and G. A. Jacoby.** 1998. Quinolone resistance from a transferable plasmid. Lancet **351:**797–799.
17. **National Committee for Clinical Laboratory Standards.** 2004. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically: approved standard M100–S14. NCCLS, Wayne, Pa.
18. **Nazik, A., L. Poirel, and P. Nordmann.** 2005. Further identification of plasmid-mediated quinolone resistance determinant in *Enterobacteriaceae*, in Turkey. Antimicrob. Agents Chemother. **49:**4050–4053.
19. **Nedoluha, P. C., S. Owens, E. Russek-Cohen, and D. C. Westhoff.** 2001. Effect of sampling method on the representative recovery of microorganisms from the surfaces of aquaculture finfish. J. Food Prot. **10:**1515–1520.
20. **NORM/NORM-VET2002.** 2003. Consumption of antimicrobial agents and occurrence of antimicrobial resistance in Norway. Tromso/Oslo 2003. ISSN: 1502–2307, p. 13.
21. **Nozue, H., T. Hayashi, Y. Hashimoto, T. Ezaki, K. Hamasaki, K. Ohwada, Y., and Y. Terawaki.** 1992. Isolation and characterization of *Shewanella alga* from human clinical specimens and emendation of the description of *S. alga* Simidu et al., 1990, 335. Int. J. Syst. Bacteriol. **42:**628–634.
22. **Pang, Y., B. A. Brown, V. A. Steingrube, R. J. Wallace, Jr., and M. C. Roberts.** 1994. Tetracycline resistance determinants in *Mycobacterium* and *Streptomyces*. Antimicrob. Agents Chemother. **38:**1408–1412.
23. **Philippon, A., G. Arlet, and G. A. Jacoby.** 2002. Plasmid-determined AmpC-type β-lactamases. Antimicrob. Agents Chemother. **46:**1–11.
24. **Poirel, L., C. Héritier, and P. Nordmann.** 2004. Chromosome-encoded Ambler class D β-lactamase of *Shewanella oneidensis* as a progenitor of carbapenem-hydrolyzing oxacillinase. Antimicrob. Agents Chemother. **48:**348–351.
25. **Poirel, L., P. Kämpfer, and P. Nordmann.** 2002. Chromosome-encoded Ambler class A β-lactamase of *Kluyvera georgiana* as a probable progenitor of a subgroup of CTX-M extended-spectrum β-lactamases. Antimicrob. Agents Chemother. **46:**4038–4040.
26. **Poirel, L., M. Van De Loo, H. Mammeri, and P. Nordmann.** 2005. Association of plasmid-mediated resistance with extended-spectrum β-lactamase VEB-1. Antimicrob. Agents Chemother. **49:**3091–3094.
27. **Saga, T., M. Kaku, Y. Onodera, S. Yamachika, K. Sato, and H. Takase.** 2005. *Vibrio parahaemolyticus* chromosomal *qnr* homologue VPA0095: demonstration by transformation with a mutated gene of its potential to reduce quinolone susceptibility in *Escherichia coli*. Antimicrob. Agents Chemother. **49:**2144–2145.
28. **Sambrook, J., and D. W. Russell.** 2001. Molecular cloning: a laboratory manual, 3rd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, N.Y.
29. **Tran, J. H., and G. A. Jacoby.** 2002. Mechanism of plasmid-mediated quinolone resistance. Proc. Natl. Acad. Sci. USA **99:**5638–5642.
30. **Tran, J. H., G. A. Jacoby, and D. C. Hooper.** 2005. Interaction of plasmid-encoded quinolone resistance protein Qnr with *Escherichia coli* DNA gyrase. Antimicrob. Agents Chemother. **49:**118–125.
31. **Turiel, E., A. Martin-Esteban, G. Bordin, and A. R. Rodriguez.** 2004. Stability of fluoroquinolones antibibiotics in river water samples and in octadecyl silica solid-phase extraction cartridges. Anal. Bioanal. Chem. **380:**123–128.
32. **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.
33. **Witte, W.** 1998. Medical consequences of antibiotic use in agriculture. Science **279:**996–997.
34. **Young, H. K.** 1993. Antimicrobial resistance spread in aquatic environments. J. Antimicrob. Chemother. **31:**627–635.