ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 2009, p. 1665–1666 Vol. 53, No. 4
0066-4804/09/$08.00+0 doi:10.1128/AAC.01447-08

Temporal Appearance of Plasmid-Mediated Quinolone Resistance Genes▽

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Received 29 October 2008/Returned for modification 19 December 2008/Accepted 11 January 2009

One hundred fifty AAC(6′)-Ib-positive gram-negative isolates collected between 1981 and 1991 were examined by PCR for the presence of the *aac(6′)-Ib-cr* variant and other plasmid-mediated quinolone resistance (PMQR) genes. None had the *aac(6′)-Ib-cr* variant, *qnrA*, *qnrS*, *qnrC*, or *qepA*, but two strains collected in 1988 had *qnrB* alleles, making these the earliest known PMQR genes.

Plasmid-mediated aminoglycoside 6′-*N*-acetyltransferase, AAC(6′)-Ib, conferring resistance to amikacin, kanamycin, and tobramycin, was described in the 1980s (19, 20, 24) and found to be broadly distributed geographically and present in many clinically important gram-negative rods (3, 21). A variant, AAC(6′)-Ib-cr, with the surprising additional property of acetylating and inactivating fluoroquinolones with an accessible amino nitrogen on the piperazine ring, was described in 2006 (15), encoded by a plasmid isolated in 2000–2001 from Shanghai (23). AAC(6′)-Ib-cr differs from AAC(6′)-Ib by only two amino acid substitutions, both of which are required for the enhanced substrate recognition (9, 15, 22). The "cr" variant was subsequently found to be widely distributed around the world (1, 2, 4, 5, 10–12, 14, 17), suggesting an earlier origin to allow for such broad dissemination.

Fluoroquinolones modified by AAC(6′)-Ib-cr were approved for clinical use in the United States in 1986 (norfloxacin) and 1987 (ciprofloxacin). Hence, we hypothesized that if a historical collection of strains were available for examination, the cr variant would first be found after this time. Such a collection was begun at Schering-Plough in the 1980s and consists of more than 1,100 strains of gram-negative bacilli collected between 1981 and 1991, all of which were characterized as making AAC(6′)-Ib based on DNA hybridization probes (16). One hundred fifty-six of these strains were revived from storage, including 44 *Klebsiella pneumoniae* strains, 29 *Escherichia coli* strains, 20 *Enterobacter cloacae* strains, 19 *Acinetobacter calcoaceticus* strains, 8 *Enterobacter aerogenes* strains, 7 *Pseudomonas aeruginosa* strains, 5 *Proteus mirabilis* strains, 5 *Serratia marcescens* strains, 3 *Citrobacter freundii* strains, 3 *Klebsiella oxytoca* strains, 3 *Providencia stuartii* strains, 3 *Salmonella enterica* strains, 2 *Enterobacter sakazakii* strains, and 1 strain (each) of *Citrobacter diversus*, *Enterobacter agglomerans*, *Providencia rettgeri*, *Pseudomonas* sp., and *Stenotrophomonas maltophilia.* Fifty-seven strains came from Europe (Greece, 37; Belgium, 12; France, 3; Italy, 3; and Portugal, 2), 50 from South America (Argentina, 46; Chile, 2; Uruguay, 1; and Venezuela, 1), 47 from the United States, and 2 from the Far East (Japan and Taiwan). They were collected in 1981 (1 strain), 1982 (1 strain), 1983 (2 strains), 1984 (6 strains), 1985 (9 strains), 1986 (1 strain), 1987 (20 strains), 1988 (74 strains), 1989 (17 strains), 1990 (24 strains), and 1991 (1 strain).

The presence of *aac(6′)-Ib* was determined by PCR utilizing primers and conditions that amplify all known *aac(6′)-Ib* variants (11). The amplification products were digested with the restriction enzyme BtsCI (New England Biolabs, Ibswich, MA), which cleaves the wild-type gene but not the *cr* variant. Strains were also assayed by PCR for *qnrA*, *qnrB*, *qnrC*, *qnrS*, and *qepA*, the other currently known plasmid-mediated quinolone resistance genes, as described elsewhere (6), using the primers listed in Table 1.

One hundred fifty of the 156 strains were still positive by PCR for AAC(6′)-Ib, with 6 strains having lost the *aac(6′)-Ib* gene in storage. None contained the cr variant, confirming the expectation that more than a few years of exposure to potential quinolone substrates would be necessary for the enzyme to evolve to bifunctionality. None of the 156 strains was positive by PCR for *qnrA*, *qnrC*, or *qnrS*, genes that code for pentapeptide-repeat proteins that protect quinolone targets, or for *qepA*, a gene for a quinolone efflux pump. Two strains from 1988, however, were positive for *qnrB* alleles, including a *qnrB8*-like gene in a *C. freundii* isolate from Brooklyn, NY, and a *qnrB9*-like gene in a *K. pneumoniae* isolate from Cordoba, Argentina. Neither allele was transferred to azide-resistant *E. coli* J53 by mating and selection for nonquinolone resistances, suggesting possible chromosomal locations. It is interesting to note that strains from Argentina isolated in 2005 were the source of the QnrB10 variant, which differs from QnrB9 in five amino acids, and that *aac(6′)-Ib-cr* was found there in isolates from 2006 (14).

These 1988 strains are currently the earliest known to carry *qnr* alleles, the previous record holders having been the original *qnrA1* strain from the United States, isolated in 1994 (8), and an unspecified *qnr* allele from the same year found in Israel (18). Recent screening of *E. coli* bloodstream isolates collected between 1991 and 2005 in Israel found that the earliest strain positive for *aac(6′)-Ib-cr* came from 2000 (7). Detection of *qnr*-mediated resistance in gram-negative pathogens thus preceded detection of *aac(6′)-Ib-cr* by more than a decade. Faced with the lethal challenge of fluoroquinolones, mobilization of *qnr* alleles from commensal bacteria (13) ap-

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▽ Published ahead of print on 21 January 2009.

TABLE 1. PCR primers

Gene	Primer sequences (5′→3′)	Product size (bp)	Reference
qnrA	ATTTCTCACGCCAGGATTTG TGCCAGGCACAGATCTTGAC	468	This study
qnrB	CGACCTKAGCGGCACTGAAT GAGCAACGAYGCCTGGTAGYTG	513	This study
qnrC	GGGTTGTACATTTATTGAATCG CACCTACCCATTTATTTTCA	307	6
qnrS	ACTGCAAGTTCATTGAACAG GATCTAAACCGTCGAGTTCG	431	This study
aac(6′)-Ib	TTGCGATGCTCTATGAGTGGCTA CTCGAATGCCTGGCGTGTTT	482	11
qepA	AACTGCTTGAGCCCGTAGAT GTCTACGCCATGGACCTCAC	596	6

pears to have been accomplished more quickly than modification of an existing enzymatic mechanism.

This study was supported in part by grants AI43312 (to G.A.J.) and AI57576 (to D.C.H.) from the National Institutes of Health, U.S. Public Health Service.

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