

## Plasmid-Mediated Quinolone Resistance in Isolates Obtained in German Intensive Care Units

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**Screening of 703 isolates of *Enterobacteriaceae*, obtained from 34 German intensive care units (ICUs), revealed *qnr*-positive, integron-containing isolates of *Enterobacter* sp. and *Citrobacter freundii* from four patients in 2 German ICUs. This is one of the first reports of *qnr*-positive strains obtained from patients in Europe.**

Integron-carrying isolates are statistically associated with multidrug resistance against various classes of antimicrobials (7). These episomal transmissible genetic elements transfer resistance to various antimicrobials, which is encoded by so-called “cassettes.” Despite the association of the integrons with a fluoroquinolone-resistant phenotype, no corresponding resistance cassette is known.

Usually, quinolone resistance results from mutational changes in the chromosomally encoded type II topoisomerases and the expression of efflux pumps or porins (2, 13). However, recent reports indicate that this resistance can also be mediated by plasmids (9). This first valid quinolone resistance (*qnr*) plasmid encodes a 218-amino-acid immune protein of the pentapeptide family, which directly protects the *Escherichia coli* gyrase from quinolone inhibition (16). Molecular characterizations of plasmids isolated from different clinical strains showed that the *qnr* gene was located together with other resistance determinants near class I integrons between a duplication of the 3' conserved sequence of integrons and *orf513* (16, 18, 19), a putative recombinase involved in site-specific acquisition of resistance genes (17).

The low level of resistance of *qnr* has been shown to contribute additively to the high level of resistance in *qnr*-positive strains (8). The presence of *qnr* facilitates the selection of chromosomal mutations causing high-level quinolone resistance (4, 9).

The occurrence of this novel plasmid-mediated quinolone resistance has been reported from few areas around the world. This transmissible quinolone resistance gene was detected in *Klebsiella pneumoniae* strains in different U.S. states, six *E. coli* strains from China, and one *Providencia stuartii* isolate from Egypt (4, 15, 18–20). The aim of this study was to determine the occurrence of the *qnr* gene in integron-positive strains in German intensive care units (ICUs).

Participants of the German surveillance system “Spread of Nosocomial Infections and Resistant Pathogens” were asked to collect fluoroquinolone- or cephalosporin-resistant *Enterobacteriaceae* (10). From February 2000 to December 2003, 703 strains were isolated from patients in 34 ICUs throughout

Germany. All of the strains were analyzed for the presence of integron cassettes and integrase by use of PCR (6). One hundred thirty-six strains contained both integron-defining structures. All of them were screened for the presence of the *qnr* gene.

PCR was performed as described previously with the primers QP1 and QP2, which results in a 543-bp amplicon (4). *E. coli* strain J53 containing the plasmid pMG252 (kindly provided by G. Jacoby, Lahey Clinic, Burlington, Mass.) served as a positive control (16). Six strains from four patients in two ICUs located in two different states were PCR positive. Sequence identities were confirmed by DNA sequencing of the six PCR products by use of the amplification primers and comparison with the published *qnr* coding sequence (16).

Biochemical species differentiation by the API 20E (bioMérieux, Marcy l'Etoile, France) identified one strain as *Enterobacter cloacae*, with just 67% identity. Subsequently, the 16S rRNA gene sequence of this isolate was determined with the universal 16S rRNA primers 806R, 8F, 13R, and 515F (3, 14). The sequence comparison by means of the BLASTN algorithm revealed closest similarity to one environmental isolate of *Enterobacter*, strain B901-2 (GenBank accession no. AB114268), with 99% sequence identity (1, 11). Therefore, this strain was designated as *Enterobacter* sp. The remaining five isolates originating from the second ICU were unambiguously identified as *Citrobacter freundii*. The suggested genotypic identity of these five strains isolated from three patients in a single ICU within 2 months was confirmed by XbaI macrorestriction analysis (data not shown) as described previously (5).

In all of the strains of *C. freundii* and *Enterobacter* sp., PCR detection of integron cassettes with primers hybridizing to the 5'- and 3'-conserved regions revealed PCR products 1,000 and 1,600 bp in size, respectively.

Antimicrobial susceptibility testing was performed according to the NCCLS (12). All of the strains were ciprofloxacin resistant, and none was susceptible to ceftazidime, ceftazidime, or ceftazidime (Table 1).

The PCR-detectable *qnr* gene of *C. freundii* could be mobilized together with the antimicrobial-resistant phenotype into an *E. coli* J53Az<sup>R</sup> recipient by transconjugation and subsequent selection on 100- $\mu$ g/ml Na-azide in combination with either 10- $\mu$ g/ml tetracycline or 10- $\mu$ g/ml gentamicin after overnight filter mating (Table 2). Similar experiments with the *Enterobacter* sp. strain failed, even when 10  $\mu$ g of ceftazidime

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TABLE 1. Properties of the clinical isolates examined in this study

Isolate	Identity	Origin	ICU	Patient	Date of withdrawal (mo/day/yr)	MIC ( $\mu\text{g/ml}$ ) of <sup>a</sup> :												
						CIP	FOX	CAZ	CAZ + CLA	CTX	CTX + CLA	PIP	GEN	AMK	TET	SXT	TMP	SMZ
37	<i>C. freundii</i>	Tracheal	H1B	9096	8/27/01	4	>256	256	256	64	64	256	>64	128	>128	128	>128	>512
38	<i>C. freundii</i>	Tracheal	H1B	9702	9/3/01	2	>256	256	128	64	32	>256	>64	32	>128	128	>128	>512
39	<i>C. freundii</i>	Tracheal	H1B	9096	9/3/01	8	>256	256	256	64	64	>256	>64	32	>128	128	>128	>512
43	<i>C. freundii</i>	Surgical suture	H1B	9702	10/1/01	4	>256	128	128	64	32	>256	>64	32	>128	128	>128	>512
44	<i>C. freundii</i>	Tracheal	H1B	6485	9/27/01	2	>256	256	128	64	32	>256	>64	32	>128	128	>128	>512
458	<i>Enterobacter</i> sp.	Swab	IME1	7410	5/11/03	8	>256	128	128	64	128	256	4	4	16	128	>128	>512

<sup>a</sup> CIP, ciprofloxacin; FOX, cefoxitin; CAZ, ceftazidime; CLA, clavulanic acid; CTX, cefotaxime; PIP, piperacillin; GEN, gentamicin; AMK, amikacin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; TMP, trimethoprim; SMZ, sulfamethoxazole.

or piperacillin per ml or 0.25  $\mu\text{g}$  of ciprofloxacin per ml was employed for selection.

The plasmid preparation from *Enterobacter* sp. and *C. freundii* C39 as well as all three transconjugants revealed covalently closed circular DNA larger or equal to the *qnr*-positive plasmid pMG252, which is 180 kb in size (18). By use of a nonradioactively labeled *qnr* PCR fragment, these plasmids showed the presence of *qnr* DNA sequences in Southern hybridization experiments (data not shown).

The patients' records did not show that any of them had been abroad in the months preceding ICU admission. The patient carrying the *Enterobacter* sp. strain had been in a coma vigil state for 1 year prior to admission from a nursing home to the ICU because of cardiac arrhythmia; he stayed in the unit for 4 days. With regard to the small outbreak of *qnr*-positive *C. freundii*, the tracheal secretion of the presumptive index case patient was found to be colonized with *C. freundii* 10 days after admission because of craniocerebral injury. The second patient was admitted to the ICU the same day, and the identical strain was isolated 1 week later from the patient's tracheal secretion, from abdominal swabs taken during surgery two and a half weeks later, and finally from surgical sutures. Therefore, this strain was taken to be the infectious agent of a surgical site infection. The third patient was admitted to the ICU on the same day as the first patient. However, after just 1 month of stay in the unit, this patient's tracheal secretion and catheter urine turned out to be colonized with the outbreak strain. Only some of the strains were made available for investigation (Ta-

ble 1). As far as the patients' records showed, none of the three patients colonized or infected with *C. freundii* had been abroad in the months preceding admission.

The importance of the *qnr* transmissible resistance gene may be its additive effect in raising the quinolone MIC and subsequent greater ease in selecting high quinolone resistance from *qnr*-expressing strains (8, 9). It may present one possible explanation for a link between integron carriage and quinolone resistance. This is one of the first reports on *qnr*-positive strains obtained from patients in Europe. Considering recent reports on the detection of this plasmid-mediated resistance gene in isolates originating from the United States, China, and Egypt, the spread of this emerging resistance might be underscored in multidrug-resistant, integron-carrying *Enterobacteriaceae*.

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TABLE 2. Resistance profile of strains used in transconjugation experiments

Group and strain	MIC ( $\mu\text{g/ml}$ ) of <sup>a</sup> :										
	CIP	FOX	CAZ	CTX	PIP	GEN	AMK	TET	SXT	TMP	SMZ
Donor strain 39	8	>256	256	64	>256	>64	32	>128	128	>128	>512
Recipients strain J53Az <sup>R</sup>	0.03	8	2	0.125	1	0.25	4	1	0.5	0.5	16
Transconjugants											
39-1	4	>256	256	64	>256	>64	32	>128	128	>128	>512
39-2	4	>256	256	64	>256	>64	64	>128	128	>128	>512
39-5	0.5	1	64	32	>256	>64	16	>128	128	>128	>512

<sup>a</sup> CIP, ciprofloxacin; FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; PIP, piperacillin; GEN, gentamicin; AMK, amikacin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; TMP, trimethoprim; SMZ, sulfamethoxazole.

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