ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Sept. 2006, p. 3200–3202 Vol. 50, No. 9
0066-4804/06/$08.00+0 doi:10.1128/AAC.00293-06

Genetic Environment of Quinolone Resistance Gene *qnrB2* in a Complex *sul1*-Type Integron in the Newly Described *Salmonella enterica* Serovar Keurmassar

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Received 8 March 2006/Returned for modification 12 June 2006/Accepted 25 June 2006

A *qnrB2* determinant was described for a new complex *sul1*-type integron from *Salmonella enterica* serovar Keurmassar. The genetic structure contained two class 1 integrons surrounding two common regions (CRs) separated by a partial 3′ conserved segment. The *qnrB2* gene is adjacent to the first CR.

Quinolone resistance in gram-negative bacteria is mainly due to chromosomal mutations in genes encoding quinolone targets (DNA gyrase and topoisomerase IV) and/or in regulatory genes of outer membrane proteins or efflux pumps (7). The first plasmid-mediated quinolone resistance determinant, *qnrA*, was recently characterized (8), and several Qnr proteins (QnrA-like, QnrB, and QnrS) have since been described (6, 9; 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). Epidemiological studies of the distribution of *qnr* determinants show that *qnr*-positive strains frequently express extended-spectrum β-lactamases (ESBLs) (9). The genetic environment of *qnr* genes has been characterized for *qnrA* variants and *qnrS* (6) but not, to our knowledge, for *qnrB* determinants. The *qnrA* variants are located on plasmids and are often embedded in complex *sul1*-type integrons (9). These structures, first described for In6 and In7 (17), contain two partial copies of the 3′ conserved segment (3′-CS) of class 1 integrons, surrounding a common region (CR) which contains the *orf513* that encodes a putative recombinase (11, 18). Several antibiotic resistance genes have been described downstream of the CR, including $bla_{\text{DHA-1}}$ (20), *dfrA10* (10, 11), *cat* (16, 17), $bla_{\text{CTX-M}}$ genes (4), *dfrA19* (21), and $bla_{\text{CMY-9}}$ (3).

We have previously characterized two class-1 integrons in eight clonal strains of the newly described Keurmassar serovar of *Salmonella enterica* subsp. *enterica* (5). One integron contained the *aadA2* cassette and the other the *aac*(*6′*)-*IIc* and *ereA2* cassettes. The strain was resistant to amikacin, chloramphenicol, gentamicin, netilmicin, spectinomycin, streptomycin, sulfamethoxazole, tetracycline, tobramycin, and trimethoprim, and expressed the ESBL SHV-12; all the resistances were transferred en bloc to *Escherichia coli* by conjugation.

Here, we detected *orf513* by means of specific PCR method A (Fig. 1, Table 1). PCR A was positive with both the original strain and its transconjugants. Thus, to further investigate the genetic organization of the complex *sul1*-type integron, plasmid DNA from the transconjugants was extracted and amplified by PCR with primers located in known sequences (PCRs B and C) (Fig. 1, Table 1). Sequence analysis of fragment B showed, downstream of the CR, a 2,505-bp fragment containing (i) a 726-bp sequence with 90% identity to a fragment of the *Klebsiella pneumoniae* plasmid pRBDHA, including the first 535 bp of the *sapA* gene (AJ971343), and (ii) a 645-bp sequence with 100% identity to the *qnrB2* gene (DQ351242) (Fig. 1). In the plasmid pRBDHA, the *sapA* and *sapB* genes of the *sap* operon (ABC transporter family) were also adjacent to the CR and may have originated from *S. enterica* serovar Typhimurium (20). Sequence analysis of fragment C showed, upstream of the CR, the *aadA2*-containing class 1 integron (Fig. 1).

To determine the outer boundaries of this complex *sul1*-type integron, plasmid DNA from the transconjugants was digested with BamHI. The fragments were then cloned into pUC18, and transformants were selected on brain heart infusion agar containing spectinomycin (25 μg/ml). A recombinant plasmid with a 12.9-kb BamHI insert was selected. PCRs A, B, and C were positive for this fragment, and sequence analysis of the boundaries with primers in pUC18 showed that the 5′-CS of class 1 integron was present at the left-hand boundary. The right-hand 700 bp showed 100% identity to the 3′ end of the complex *sul1*-type integron In-t1 containing the *dfrA19* gene, downstream of the CR. This integron has been described for plasmid IncFI/97 of *S. enterica* serovar Typhimurium (AJ310778) (21) (Fig. 1). We postulated that a second copy of the CR might be present on the 12.9-kb fragment and thus performed PCRs D and E (Fig. 1, Table 1). The PCR products were sequenced, confirming the presence (downstream of the sequence containing the first CR, the *sapA*-like, and *qnrB2*) of a fragment 100% identical to the CR and also the *dfrA19*-associated gene of plasmid IncFI/97 (Fig. 1). In the plasmid, this sequence was followed by the class 1 integron In-t2 containing the *oxa1* and *aadA1* cassettes (Fig. 1). By a combination of PCRs with primers located in the *orf513*, *oxa1*, *aadA1*, *dfrA19*, and *sul1* genes, we successfully amplified products F, G, and H from the transconjugant plasmid DNA (Fig. 1, Table 1). Sequencing of these fragments confirmed that the In-t2 integron was present downstream of the *dfrA19*-containing sequence.

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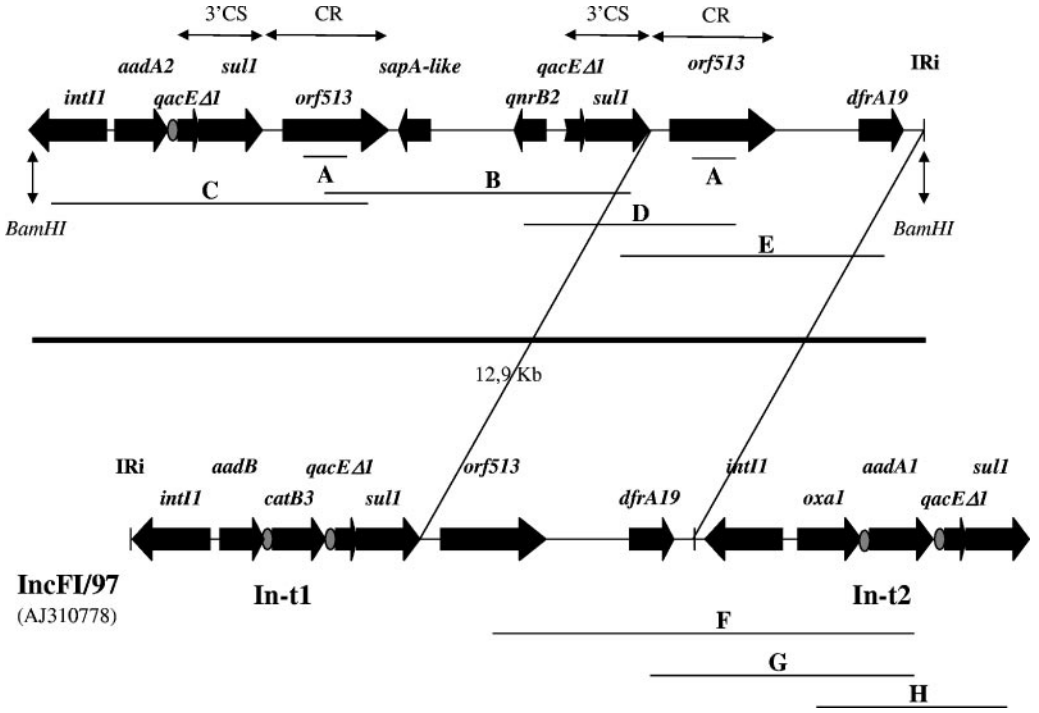

FIG. 1. Genetic organization of the *qnrB2*-containing complex *sul1*-type integron. Comparison with the organization of the plasmid IncFI/97 showed that the 12.9-kb BamHI fragment harbors the same orf513-*dfrA19*-containing fragment. Analysis by PCR mapping and sequencing of PCR products F, G, and H showed that integron In-t2 of IncFI/97 is located at the 3′ end of the 12.9-kb BamHI fragment. The horizontal bars correspond to PCR products, the gray ovals correspond to *attC* sites, and the thick arrows show the genes or ORFs and their direction of transcription.

This structure may have resulted from RecA-dependent homologous recombination between two copies of *sul1* or *orf513*.

To our knowledge, this is the first characterization of the genetic organization of a *qnrB* determinant. The variant *qnrB2* gene has previously been detected on plasmid pMG301 in *Citrobacter koseri* (DQ351242) (Jacoby et al., Abstr. 44th Intersci. Conf. Antimicrob. Agents Chemother.), but its genetic environment has not yet been characterized. In the complex *sul1*-type integron described here, the *qnrB2* gene is in the opposite orientation (Fig. 1). To our knowledge, the only resistance gene so far found to be located downstream of the CR, in the opposite orientation, is $bla_{\text{DHA-1}}$ (20). The *qnrA* determinants previously described for complex *sul1*-type integrons were all inserted in the same orientation as *orf513* (9). Some antibiotic resistance genes located downstream of the CR have been shown to originate from bacterial chromosome segments (1, 2, 3, 15, 19). The reservoir of *qnrA*-like genes was recently shown to be *Shewanella* algae (14). The origin of *qnrB* is unknown, although QnrB-like proteins were recently found in members of the *Vibrionaceae* family (13).

TABLE 1. Primer sequences used for PCR mapping

Amplicon (target)	Primer	Sequence (5′–3′)	Size of amplicon (bp)	Reference
A (*orf513*)	341A	CGCCCACTCAAACAAACG	468	15
	341B	GAGGCTTTGGTGTAACCG		15
B (*orf513-sul1*)	341_{STOP}	ACATTAGTCGGCCAGCGG	4,262	15
	SulR	GATTGCGCTTCGCAGATCTCCAGG		12
C (*intI1-orf513*)	intI1L	ACATGTGATGGCGACGCACGA	3,943	12
	341B	GAGGCTTTGGTGTAACCG		15
D (*qnrB2-orf513*)	qnrL	TGAACCACTGAACGTCGC	2,696	This study
	341B	GAGGCTTTGGTGTAACCG		15
E (*sul1-dfrA19*)	SulL	CCTGGAGATCTGCGAAGCGCAATC	3,298	This study
	dfrR	CTCGCTGGCACTGGAAT		This study
F (*orf513-aadA1*)	341_{STOP}	ACATTAGTCGGCCAGCGG	5,333	15
	aadAR	AAGTATGACGGGCTGATACTG		This study
G (*dfrA19-aadA1*)	dfrL	ATTCCAGTGCCAGCGAG	3,531	This study
	aadAR	AAGTATGACGGGCTGATACTG		This study
H (*oxa1-qacEΔ1*)	oxaL	CTGAAATTGCTCAATTCAATAAAGC	2,431	This study
	SulR2	GTCGTTATAGCCCTATCTCGCGTC		This study

The *S. enterica* serovar Keurmassar strain expressed the ESBL SHV-12. Previous studies have shown an association between *qnr*-positive isolates and ESBLs, the two determinants sometimes being located on the same plasmid (9). In our *S. enterica* serovar Keurmassar strain, all the resistances, including the ESBL phenotype, were transferred en bloc. Thus, the SHV-12 determinant may be located on the same plasmid as the complex *sul1*-type integron described here.

We describe a new complex *sul1*-type integron in an *S. enterica* serovar Keurmassar strain containing two complete class-1 integrons surrounding two CRs separated by a partial 3′CS (Fig. 1). The resistance genes *qnrB2* and *dfrA19* were found adjacent to the two CRs.

Nucleotide sequence accession number. The nucleotide sequence of the 12.9-kb BamHI fragment has been deposited in the EMBL-GenBank databases under accession number AM234698.

This work was supported by grants from the French Ministère de la Recherche and from Conseil Régional du Limousin. A. Gassama was supported by a grant from EGIDE, and N. Raked was supported by a grant from Fondation pour la Recherche Médicale.

We thank Emmanuelle Charpentier for critical reading and Bertrand Luzier and Pierre Rivet for technical assistance and sequencing.

REFERENCES

1. **Arduino, S. M., P. H. Roy, G. A. Jacoby, B. E. Orman, S. A. Pineiro, and D. Centron.** 2002. $bla_{CTX-M-2}$ is located in an unusual class 1 integron (In35) which includes Orf513. Antimicrob. Agents Chemother. **46:**2303–2306.
2. **Di Conza, J., J. A. Ayala, P. Power, M. Mollerach, and G. Gutkind.** 2002. Novel class 1 integron (InS21) carrying $bla_{CTX-M-2}$ in *Salmonella enterica* serovar Infantis. Antimicrob. Agents Chemother. **46:**2257–2261.
3. **Doi, Y., N. Shibata, K. Shibayama, K. Kamachi, H. Kurokawa, K. Yokoyama, T. Yagi, and Y. Arakawa.** 2002. Characterization of a novel plasmid-mediated cephalosporinase (CMY-9) and its genetic environment in an *Escherichia coli* clinical isolate. Antimicrob. Agents Chemother. **46:**2427–2434.
4. **Eckert, C., V. Gautier, and G. Arlet.** 2006. DNA sequence analysis of the genetic environment of various *blaCTX-M* genes. J. Antimicrob. Chemother. **57:**14–23.
5. **Gassama-Sow, A., A. Aïdara-Kane, N. Raked, F. Denis, and M. C. Ploy.** 2004. Integron-associated antibiotic resistance in the newly described *Salmonella enterica* serovar Keurmassar emerging in Senegal. Emerg. Infect. Dis. **10:** 1339–1341.
6. **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.
7. **Jacoby, G. A.** 2005. Mechanisms of resistance to quinolones. Clin. Infect. Dis. **15**(Suppl. 2)**:**S120–S126.
8. **Martinez-Martinez, L., A. Pascual, and G. A. Jacoby.** 1998. Quinolone resistance from a transferable plasmid. Lancet **351:**797–799.
9. **Nordmann, P., and L. Poirel.** 2005. Emergence of plasmid-mediated resistance to quinolones in *Enterobacteriaceae*. J. Antimicrob. Chemother. **56:** 463–469.
10. **Parsons, Y., R. M. Hall, and H. W. Stokes.** 1991. A new trimethoprim resistance gene, *dhfrX*, in the In7 integron of plasmid pDGO100. Antimicrob. Agents Chemother. **35:**2436–2439.
11. **Partridge, S. R., and R. M. Hall.** 2003. In34, a complex In5 family class 1 integron containing *orf513* and *dfrA10*. Antimicrob. Agents Chemother. **47:** 342–349.
12. **Ploy, M. C., F. Denis, P. Courvalin, and T. Lambert.** 2000. Molecular characterization of integrons in *Acinetobacter baumannii*. Description of a hybrid class 2 integron. Antimicrob. Agents Chemother. **44:**2684–2688.
13. **Poirel, L., A. Liard, J. M. Rodriguez-Martinez, and P. Nordmann.** 2005. Vibrionaceae as a possible source of Qnr-like quinolone resistance determinants. J. Antimicrob. Chemother. **56:**1118–1121.
14. **Poirel, L., J. M. Rodriguez-Martinez, H. Mammeri, A. Liard, and P. Nordmann.** 2005. Origin of plasmid-mediated quinolone resistance determinant QnrA. Antimicrob. Agents Chemother. **49:**3523–3525.
15. **Sabaté, M., F. Navarro, E. Miró, S. Campoy, B. Mirelis, J. Barbé, and G. Prats.** 2002. Novel complex *sul1*-type integron in *Escherichia coli* carrying $bla_{CTX-M-9}$. Antimicrob. Agents Chemother. **46:**2656–2661.
16. **Sørum, H., T. M. L'Abée-Lund, A. Solberg, and A. Wold.** 2003. Integron-containing IncU R plasmid Pras1 and pAr-32 from the fish pathogen *Aeromonas salmonicida*. Antimicrob. Agents Chemother. **47:**1285–1290.
17. **Stokes, H. W., C. Tomaras, Y. Parsons, and R. M. Hall.** 1993. The partial 3′-conserved segment duplications in the integrons In6 from pSa and In7 from pDGO100 have a common origin. Plasmid **30:**39–50.
18. **Valentine, C. R., R. M. Heinrich, S. L. Chissoe, and A. Roe.** 1994. DNA sequence of direct repeats of the *sulI* gene of plasmid pSa. Plasmid **32:**222–227.
19. **Verdet, C., G. Arlet, G. Barnaud, P. H. Lagrange, and A. Phillipon.** 2000. A novel integron in *Salmonella enterica* serovar Enteritidis, carrying the bla_{DHA-1} gene and its regulator *ampR*, originated from *Morganella morganii*. Antimicrob. Agents Chemother. **44:**222–225.
20. **Verdet, C., Y. Benzerara, V. Gautier, O. Adam, Z. Ould-Hocine, and G. Arlet.** 2006. Emergence of DHA-1-producing *Klebsiella* spp. in the Parisian region: genetic organization of the *ampC* and *ampR* genes originating from *Morganella morganii*. Antimicrob. Agents Chemother. **50:**607–617.
21. **Villa, L., P. Visca, F. Tosini, C. Pezzella, and A. Carattoli.** 2002. Composite integron array generated by insertion of an ORF341-type integron within a Tn21-like element. Microb. Drug Resist. **8:**1–8.