

Complex Class 1 Integrons with Diverse Variable Regions, Including *aac(6′)-Ib-cr*, and a Novel Allele, *qnrB10*, Associated with *ISCR1* in Clinical Enterobacterial Isolates from Argentina[∇]

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Transferable quinolone resistance has not previously been reported in Argentina. Here we describe three complex class 1 integrons harboring the novel allele *qnrB10* in a unique region downstream of *orf513*, one of them also containing *aac(6′)-Ib-cr* within the variable region of integrons. The three arrays differed from *bla*_{CTX-M-2}-bearing integrons, which are broadly distributed in Argentina.

Three types of transferable (i.e., plasmid-encoded) quinolone resistance mechanisms have been reported so far, i.e., (i) a quinolone-protective mechanism encoded by the *qnr* genes (15), (ii) a double class antibiotic-modifying enzyme encoded by a two-point mutation allele of *aac(6′)-Ib* [named *aac(6′)-Ib-cr*] which acetylates ciprofloxacin and norfloxacin (14), and (iii) an efflux pump encoded by the *qepA* gene (12, 19). Different transferable *qnr* determinants have been described so far, i.e., *qnrA*, *qnrB*, and *qnrS* (15). The *qnrB* determinants were associated with either the *orf1005* gene, which encodes a putative transposase (7), or the *orf513* recombinase gene (5), the latter included in a region recently named *ISCR1* (16). The origin of *qnrB* is unknown, although QnrB-like proteins were recently found in members of the *Vibrionaceae* family (13). Here we describe a novel quinolone resistance determinant, *qnrB10*, downstream of *orf513* and embedded within different complex class 1 integrons.

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To investigate the occurrence of *qnr* genes in Argentina, we focused on clinical isolates resistant to ciprofloxacin and to at least two different families of antibiotics. Eighteen enterobacterial isolates from three hospitals from Buenos Aires were analyzed for the presence of the *qnrA*, *qnrB*, and *qnrS* genes by

PCR (Table 1). None of them contained *qnrA* or *qnrS*, but eight isolates were *qnrB* positive (Table 2). Sequence analysis (NCBI BLAST V2.0) confirmed the identification of a novel *qnrB* allele, designated *qnrB10* (accession number DQ631414), which showed maximal nucleotide identity (97%) with *qnrB5* (6). The deduced QnrB10 protein (226 amino acids) has 98% and 95% identity with QnrB5 and QnrB1, respectively. The *qnrB10* gene was cloned into the pCR2.1 vector (Invitrogen, Carlsbad, CA) by following the manufacturer's recommendations. The expression of this gene, under induction with 1 mM isopropyl-1-thio-β-D-galactopyranoside (IPTG), increased the ciprofloxacin MIC 32-fold compared to that for the host strain, *Escherichia coli* DH10B (Table 3).

Two *qnrB10*-bearing isolates showing different resistance profiles, *Enterobacter cloacae* E705 and *Klebsiella pneumoniae* M7943, were selected for further studies. Relevant susceptibility data (4) are shown in Table 3. To gain insights into the possible mechanisms of dissemination of *qnrB10*, we characterized its genetic environments in both isolates. For this, we used standard PCR long amplification conditions (fragments > 4 kb) implemented with the Elongase kit (Invitrogen) in accordance with the manufacturer's recommendations, followed by DNA sequencing (Table 1; Fig. 1A). Each isolate harbored a complex class 1 integron with a different variable region 1 (vr-1) composed of the 5′ conserved sequence (5′-CS) and the 3′-CS but exhibiting another unique variable region, vr-2, located between *ISCR1* and the second copy of the 3′-CS (Table 2; Fig. 1A). The E705 integron, named In131::*ISCR1*::*qnrB10* (see below for a discussion of the nomenclature), showed a new vr-1 comprising the *aac(6′)-IId* and *aadA1a* cassettes, while the In37::*ISCR1*::*qnrB10* integron harbored *aac(6′)-Ib-cr*, *bla*_{OXA-30}, *catB3*, and *arr-3*. This last array has been previously found in In37 from an *E. coli* isolate from China, which has *qnrA1* in vr-2 (accession number AY259086) (18), and in the integron from a *K. pneumoniae* strain isolated in France which harbors *qnrB4* in vr-2 [plasmid pRBDHA, accession number

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TABLE 1. Primers used in PCR and DNA sequencing

Target and primer ^a	No. ^b	Sequence (5'-3')	Reference
<i>qnr</i> genes			
<i>qnrA</i> or - <i>C</i> (screening)			
<i>qnr</i> -F		TCAGCAAGAGGATTTCTCA	This study
<i>qnr</i> -R		GGCAGACTATTACTCCCA	
<i>qnrS</i> (screening)			
<i>qnrS</i> -F		ACGACATTCGTCAACTGCAA	6
<i>qnrS</i> -R		TAAATTGGCACCCCTGTAGGC	
<i>qnrB</i> (screening)			
<i>qnrB</i> -F	1	CCGACCTGAGCGGCACTGA	This study
<i>qnrB</i> -R	2	CGCTCCATGAGCAACGATGCCT	
<i>qnrB10</i> (complete gene) ^c			
<i>qnrB</i> -Met-F		ATGTTGTCATTACTGTATA	This study
<i>qnrB</i> -STOP-R		CTAACCAATCACAGCGATG	
Complex class 1 integrons			
<i>intI1</i>			
Inti1-R	3	TTCGAATGTCGTAACCGC	11
Inti1-F		CGAGGCATAGACTGTAC	
Sulpro3 (F)	4	GCCTGACGATGCGTGGA	8
vr-1			
5'CS (F)	5	GGCATCCAAGCAGCAAG	8
3'CS (R)	6	AAGCAGACTTGACCTGA	
<i>orf513</i> (3'-CS junction), <i>orf513</i> -R5'	7	CGTAACCGTTTGTGAGTG	This study
<i>orf513</i> (screening)			
<i>orf513</i> -F		ATGGTTTCATGCGGGTT	2
<i>orf513</i> -R		CTGAGGGTGTGAGCGAG	
vr-2			
<i>orf513</i> -F3'	8	GTTCGATCCATCACAGAG	This study
<i>sul1</i> -R	9	TTTGAAGGTTGACAGC	
<i>orf5</i> , <i>orf5</i> lower (R)		TGTCGACGTGGGTGAAT	This study
<i>orf6</i> , In21-R	10	CGGTCCGACTGCAAGTGA	This study
Resistance cassettes and other genes of the integrons characterized in this study			
<i>aadA1</i>			
<i>aadA1</i> -F		TTGCTGGCCGTACATTTG	This study
<i>aadA1</i> -R		TCATTGCGCTGCCATTC	
<i>aac(6')</i> -Ib (any variant) and <i>aac(6')</i> -IId			
<i>aacF7334</i>	11	CATCACAAAGTACAGCATCGTGACCAACAG	This study
<i>aacR7907</i>	12	TTAGGCAACTGCGTGTTCGCTCGAATGC	
<i>aac6IB</i> (R)		TGTGACGGAATCGTTGC	8
<i>aacA4R</i> (F)		AAACACGCCAGGCATTC	1
<i>bla</i> _{OXA-30}			
<i>oxaIII</i> -F		CATTATTTGAAGGAAGTGAAG	This study
<i>oxaIII</i> -R		CACCAGTTTCCCATACAGT	
<i>catB3</i>			
<i>catout3'-1</i> (F)	13	TTTCTGCTCTATCGGGAG	This study
<i>arr3out5'-1</i> (R)		TTGTTAGACGGCAAACCTC	
<i>arr-3</i> , <i>sapC-sapB</i>	14	GACTTGCTAACACAGGG	This study
<i>arr-3</i> , <i>arr3</i> -F			
<i>sapC1</i> -R		GAATGTGATTCAGTATCG	This study
<i>sapB4</i> -F		AACCCGTCCGCACAGGCCCG	
<i>arr-3</i> , <i>sapA</i>			
<i>sapA</i> -F	15	CAGTGGGTTTCGTCTATTGC	This study
<i>sapA</i> -R		CGTTACTGTCCGTCGCC	
<i>arr-3</i> , <i>sapA-qnrB10</i> Junction			
<i>sapA3</i> -R		G CAGCAGCCTGACCACTC	This study
<i>qnrB</i> -F3'		CTTGGCATCGCTGTGATTG	

^a Only relevant primers (F, forward; R, reverse) are shown. Other sequence-based primers used in primer walking were excluded for brevity.

^b Numbers correspond to primer locations in the PCR chart shown in Fig. 1A.

^c Primers used for cloning *qnrB10* in the pCR2.1 vector.

TABLE 2. Relevant characteristics of clinical isolates and transconjugants used in this study

Isolate or strain	Yr of isolation	Hospital	Relevant resistance phenotype ^a	<i>qnrB10</i> ^b	vr-1 of <i>qnrB10</i> -bearing integrons
<i>C. freundii</i> CF701	2005	H2	AMP-AMC-CAZ-FEP-TZP-AMK-GEN-CHL-TET-SXT-NAL-CIP	+	<i>aac(6')-IId-aadA1a</i>
<i>C. freundii</i> CF702	2005	H3	AMP-AMC-CAZ-FEP-TZP-MEM-CHL-SXT-NAL-CIP	+	<i>aac(6')-Ib-aadA1a</i>
<i>E. aerogenes</i> E704	2005	H3	AMP-AMC-CAZ-TZP-GEN-CHL-SXT-NAL-CIP	+	<i>aac(6')-IId-aadA1a</i>
<i>E. cloacae</i> E701	2005	H3	AMP-AMC-CAZ-TZP-SFI-NAL-CIP	+	<i>aac(6')-Ib-aadA1a</i>
<i>E. cloacae</i> E702	2005	H2	AMP-AMC-CAZ-FEP-TZP-AMK-GEN-CHL-TET-SXT-NAL-CIP	+	<i>aac(6')-IId-aadA1a</i>
<i>E. cloacae</i> E703	2005	H2	AMP-AMC-CAZ-FEP-TZP-GEN-CHL-SXT-NAL-CIP	+	<i>aac(6')-IId-aadA1a</i>
<i>E. cloacae</i> E705	2005	H2	AMP-AMC-CAZ-TZP-GEN-CHL-TET-SXT-NAL-CIP	+	<i>aac(6')-IId-aadA1a</i>
<i>K. pneumoniae</i> M7943	2006	H1	AMP-AMC-CTX-CAZ-FEP-TZP-AMK-GEN-CHL-TET-SXT-NAL-CIP	+	<i>aac(6')-Ib-cr-bla_{OXA-30}-catB3-arr-3</i>
<i>E. coli</i> M7943-TC2			AMP-AMC-FEP-TZP-AMK-CHL-SFI-NAL-CIP	+	<i>aac(6')-Ib-cr-bla_{OXA-30}-catB3-arr-3</i>

^a Antimicrobial resistance and reduced susceptibility were tested by the disk diffusion method (4). Abbreviations: AMC, amoxicillin-clavulanic acid; AMK, amikacin; AMP, ampicillin; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin; CTX, cefotaxime; FEP, cefepime; GEN, gentamicin; MEM, meropenem; NAL, nalidixic acid; SFI, sulfisoxazole; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; TZP, piperacillin-tazobactam.

^b The presence of *qnrB10* was tested by PCR and sequence analysis (Table 1).

AJ971343; *aac(6')-Ib-cr* had not been recognized at the time it was published] (17). However, as shown in Fig. 1B, vr-1 of the In37::ISCR1::*qnrB10* integron differed from the other two since it lacks a 101-bp duplication of the 3'-CS located at the 5' end of the *aac(6')-Ib-cr* cassette. Also, the W→R mutation in this gene presented a silent change (CGG→AGG) with respect to the previously reported variant (14).

The genetic environments of *qnrB10* in the two integrons described here, Δ(3')*sapC-sapB-sapA* and Δ(5')*pspF*, mirrored a conserved architecture found in all of the *qnrB* contexts previously reported (accession numbers AM234698 and AJ971343) that includes the location of *qnrB* alleles in an orientation opposite to that of *orf513* (Fig. 1A) (3, 5, 17). The *sap* operon encodes a putative peptide transport system in gram-negative bacteria, while the *pspF* gene is a transcriptional activator of the stress-inducible *psp* operon. However, a deeper analysis of the *qnrB2*, *qnrB4*, and *qnrB10* environments showed relevant differences. The *sap* and *psp* operons were truncated at different outer points, and the intergenic regions between *sapA* and the *qnrB* alleles were different (Fig. 1A). Moreover, analysis of the inner boundary of the second 3'-CS showed deletions of different lengths in this element when comparing the complex class 1 integrons described so far (Fig. 1C). These data suggest that the *qnrB* alleles are located in similar genetic

contexts from probably different genomic sources, as has been described previously for *bla_{CTX-M-2}* alleles (1, 16).

To our knowledge, there is no real consensus on the naming of complex class 1 integrons. Therefore, we tried to follow the simplest criterion. We show here three class 1 integrons with essentially the same vr-1 [*aac(6')-Ib-cr*, *bla_{OXA-30}*, *catB3*, and *arr-3*] but different vr-2s. Since these three harbored the same array of cassettes with alleles that are supposed to confer the same antimicrobial resistance profile, we named the integron from *K. pneumoniae* M7943 In37, focusing on vr-1. In order to specify the genetic platform of the complex class 1 integrons, we added the genetic specification of vr-2, resulting In37::ISCR1::*qnrA1* and In37::ISCR1::*qnrB4-bla_{DHA-1}* for the integrons described by Wang et al. (18) and Verdet et al. (17), respectively, and In37::ISCR1::*qnrB10* for our finding in *K. pneumoniae* M7943.

Transfer of In131::ISCR1::*qnrB10* and In37::ISCR1::*qnrB10* was assayed by biparental conjugation as described before (10), with *E. coli* J53-AzR (azide resistant) as the recipient strain. Transconjugants were selected on Mueller-Hinton agar supplemented with sodium azide (100 μg/ml) and ampicillin (100 μg/ml), sulfamethoxazole (100 μg/ml), or kanamycin (50 μg/ml). Only In37::ISCR1::*qnrB10* was detected in a transconjugant, named M7943-TC2, that had the quinolone and amino-

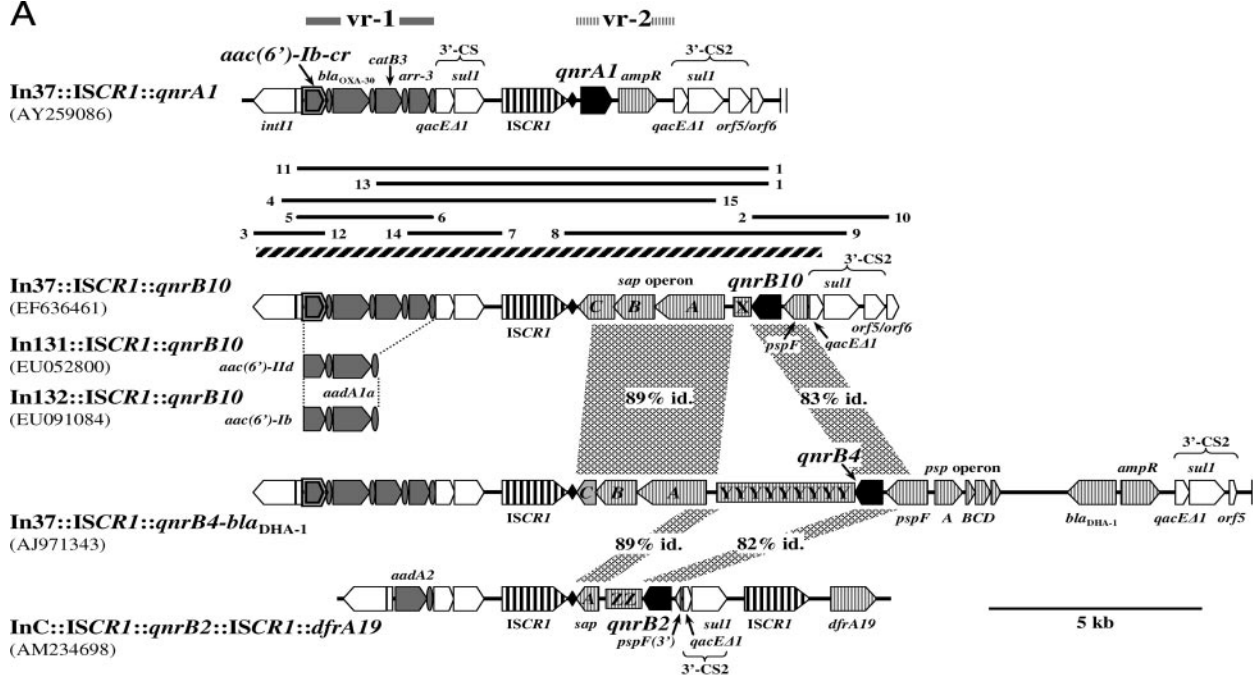
TABLE 3. Susceptibility profiles of relevant clinical isolates, bacterial recipient for conjugation experiments, transconjugants, clones, and strains used in this study

Strain	MIC (μg/ml) ^a									
	NAL	CIP	NOR	LVX	OFX	GAT	MXF	KAN	TOB	AMK
<i>E. cloacae</i> E705	≥256	≥64	≥64	≥64	≥64	32	≥64	≥256	32	8
<i>K. pneumoniae</i> M7943	128	32	32	4	8	4	8	128	16	16
<i>E. coli</i> M7943-TC2	16	2	2	0.5	0.5	0.5	1	64	16	4
<i>E. coli</i> J53-AzR	4	0.015	0.03	0.03	0.06	0.03	0.06	2	0.25	0.5
<i>E. coli</i> DH10B/pCRQB10 ^b	8	0.125	0.125	0.06	0.06	0.06	0.125	≥256	0.25	2
<i>E. coli</i> DH10B/pCR2.1	2	0.004	0.008	0.004	0.004	0.004	0.004	≥256	0.25	2
<i>E. coli</i> DH10B	2	0.004	0.008	0.004	0.004	0.004	0.004	1	0.25	1

^a MICs (4) were analyzed under induction by 1 mM IPTG. The antibiotics used for MIC determination were as follows: NAL, nalidixic acid; CIP, ciprofloxacin; NOR, norfloxacin; LVX, levofloxacin; OFX, ofloxacin; GAT, gatifloxacin; MXF, moxifloxacin; KAN, kanamycin; TOB, tobramycin; AMK, amikacin.

^b The complete *qnrB10* gene was cloned into pCR2.1 in the sense orientation with respect to the *lac* promoter.

A



B

In37::ISCR1::qnrA1 1 TTAGATGCACTAAGCACATAATTGCTCACAGCCAACTATCAGGTCAAGTCTGCTTTTATTATTTTAAAGCGTGCATAAT
 In37::ISCR1::qnrB10 -----TTAGGCATTAG---AACTTCACGGCGGATTAAAGA--AAAGGAGAAGACATCGTGAC

In37::ISCR1::qnrA1 81 AAGCCCTACACAAATTGGGAGTTAGACATCATGAGCAACGCACAAAACAAAGTTAGGCATCACAAAGTACAGCATCGTGAC
 In37::ISCR1::qnrB10 -----TTAGGCATTAG---AACTTCACGGCGGATTAAAGA--AAAGGAGAAGACATCGTGAC

In37::ISCR1::qnrA1 161 CAACAGCAACGATTCGGTACACTGCGCCMTGACTGAG...CGG...GCCTAACCCCTTC...TGTCAAACG 770
 In37::ISCR1::qnrB10 54 CAACAGCAACGATTCGGTACACTGCGCCMTGACTGAG...AGG...GCCTAACCCCTTC...TGTCAAACG 663

C

In0 1 TTTAAGCGTG CATAATAAGC CCTACACAAA TTGGGAGATA TATCATGAAA GGCTGGCCTT TTCTTGTTAT
 In131::ISCR1::qnrB10 GGCAAGCGTG CATAATAAGC CCTACACAAA TTGGGAGATA TATCATGAAA GGCTGGCCTT TTCTTGTTAT
 In37::ISCR1::qnrB10 GGCAAGCGTG CATAATAAGC CCTACACAAA TTGGGAGATA TATCATGAAA GGCTGGCCTT TTCTTGTTAT
 In35::ISCR1::bla_{CTX-M-2} ATACGCCGTG CATAATAAGC CCTACACAAA TTGGGAGATA TATCATGAAA GGCTGGCCTT TTCTTGTTAT
 In7::ISCR1::dfrA10 ATACGTGATT ACTAATAAGC CCTACACAAA TTGGGAGATA TATCATGAAA GGCTGGCCTT TTCTTGTTAT
 In37::ISCR1::qnrA1 CAGAAATGCA GTGCACCTCC CTCACACATCC GCCAGTGC GCAGTCCAG CCAGACATCC GTTACCCGCG
 In6::ISCR1::cat2 TGTCTTTT TTTGTCCGCG GGGCGCGGAT AATGGATCAG ATTATGCAGT GTCACAATGG CCTTACCCGG

In0 71 CGCAATAGTT GCGGAAGTAA TCGCAACATC CGCATTAAAA TCTAGCGAGG GCTTTACTAA GCTTGCCCTT
 In131::ISCR1::qnrB10 CGCAATAGTT GCGGAAGTAA TCGCAACATC CGCATTAAAA TCTAGCGAGG GCTTTACTAA GCTTGCCCTT
 In37::ISCR1::qnrB10 CGCAATAGTT GCGGAAGTAA TCGCAACATC CGCATTAAAA TCTAGCGAGG GCTTTACTAA GCTTGCCCTT
 In35::ISCR1::bla_{CTX-M-2} CGCAATAGTT GCGGAAGTAA TCGCAACATC CGCATTAAAA TCTAGCGAGG GCTTTACTAA GCTTGCCCTT
 In7::ISCR1::dfrA10 CGCAATAGTT GCGGAAGTAA TCGCAACATC CGCATTAAAA TCTAGCGAGG GCTTTACTAA GCTTGCCCTT
 In37::ISCR1::qnrA1 CAATGCCGTG CTGTTACGCC TGTTCGCGGA TAATGTCCGC CGCACCGAGG GCTTTACTAA GCTTGCCCTT
 In6::ISCR1::cat2 ATTGGCGTAA GCGTCCGCGA TATCCGATG GAAGCCGAGG GATTCCCGCG CAGAAACGGT GTGCCACTCA

In0 141 TCCGCCGTTG TCATAATCGG TTATGGCATC GCATTTTATT TTCTTTCTCT
 In131::ISCR1::qnrB10 TCCGCCGTTG TCATAATCGG TTATGGCATC GCATTTTATT TTCTTTCTCT
 In37::ISCR1::qnrB10 TCCGCCGTTG TCATAATCGG TTATGGCATC GCATTTTATT TTCTTTCTCT
 In35::ISCR1::bla_{CTX-M-2} TCCGCCGTTG TCATAATCGG TTATGGCATC GCATTTTATT TTCTTTCTCT
 In7::ISCR1::dfrA10 TCCGCCGTTG TCATAATCGG TTATGGCATC GCATTTTATT TTCTTTCTCT
 In37::ISCR1::qnrA1 TCCGCCGTTG TCATAATCGG TTATGGCATC GCATTTTATT TTCTTTCTCT
 In6::ISCR1::cat2 TCCGCCGTTG TCATAATCGG TTATGGCATC GCATTTTATT TTCTTTCTCT

FIG. 1. Genetic organization of *qnrB10*-containing complex class 1 integrons. (A) Genes are represented by arrowed boxes (*qnr* genes are in black), *attC* is represented by vertical ovals, and the putative origin of replication of *ISCR1* (16) is represented by a black diamond. The 3'-CS and the second copy of this element (3'-CS2) are shown by white arrowed boxes. The hatched horizontal bar indicates the sequenced regions (13,281 bp in In37::ISCR1::*qnrB10* and 10,762 bp in In131::ISCR1::*qnrB10*). Thick horizontal lines indicate the principal amplicons obtained by PCR cartography of the *qnrB10*-bearing integrons (numbers indicate the corresponding primers in Table 1). The integrons In37::ISCR1::*qnrA1* (18) and In37::ISCR1::*qnrB4-bla_{DHA-1}* (17), having essentially the same *vr-1* as In37::ISCR1::*qnrB10*, were included for comparison (sequences were truncated at the two vertical thin lines). Shaded areas with percentages depict identities (id.) between *vr-1* and *vr-2*. The X, Y, and Z boxes indicate regions of no homology with GenBank sequences. (B) Comparison of the *aac(6')*-*Ib-cr* cassettes of In37::ISCR1::*qnrA1* and In37::ISCR1::*qnrB10* (nucleotides 1492 to 2261 and 1040 to 1702 in the sequences with accession numbers AY259086 and EF636461, respectively). Sequences are numbered from the first nucleotide following the junction with the 5'-CS, and dashes represent gaps introduced to maximize alignment (ClustalX software, available at ftp://ftp-igbmc.u-strasbg.fr/pub/). Identical nucleotides in the two sequences are in bold. The 101-bp duplication of the 3'-CS is underlined. The start (boxed) and stop (*) codons of each *aac(6')*-*Ib-cr* cassette are indicated, and the amino acid sequence of the deduced protein is shown below each nucleotide sequence (14). For brevity, other regions showing 100% nucleotide (or amino acid) identity are represented by dots. The silent CGG-to-AGG change (see the text for details) is also shown. (C) Deletions at the inner boundary of 3'-CS2. Nucleotide sequences correspond to In0 (normal 3'-CS, M73819), In131::ISCR1::*qnrB10* (EU052800), In37::ISCR1::*qnrB10* (EF636461), In35::ISCR1::*bla_{CTX-M-2}* (AY079169), In7::ISCR1::*dfrA10* (L06418), In37::ISCR1::*qnrA1* (AY259086), and In6::ISCR1::*cat2* (U04277). The deletion endpoint in 3'-CS2 is defined by the first base appearing in bold. Base 1 of In0 corresponds to the nucleotide at position 65 from the normal 3'-CS.

glycoside resistance profiles for *aac(6′)-Ib-cr* and *qnrB10* genes (Table 2). The presence of In37::ISCR1::*qnrB10* was also confirmed by PCR cartography.

The other six *qnrB10*-producing isolates studied here showed the same *vr-2* rearrangement and either *vr-1* of In131::ISCR1::*qnrB10* or a different one composed of the *aac(6′)-Ib* and *aadA1a* cassettes (Table 2; Fig. 1A), which was named In132. None of the *qnrB10*-bearing integrons studied here showed the In35 rearrangement [*aac(6′)-Ib-bla_{OXA-2}-orfD*], which is the most widespread complex class 1 integron involved in the dispersion of *bla_{CTX-M-2}* in the bacterial population from Argentina (2).

Recently, Toleman et al. proposed that ISCR1 is involved in the spread of class 1 integrons through a mechanism that mobilizes adjacent DNA sequences (16). This is in agreement with the finding that cassettes are more often transferred as part of the entire integron structure than as individual gene cassettes (9). The finding of In37 derivatives from different geographical regions with diverse *vr-2*s is consistent with the speculations of Toleman et al. On the basis of this hypothesis, we would also expect to find In35 associated with *qnrB10* in our nosocomial isolates.

In conclusion, the particular epidemiology of complex class 1 integrons found in isolates harboring a novel *qnrB* allele, as well as the emergence of *aac(6′)-Ib-cr* cassettes in our bacterial population, evidences the need for national surveillance in order to estimate the prevalence of these mechanisms.

Nucleotide sequence accession numbers. The nucleotide sequences determined in this work have been submitted to the GenBank database and assigned accession no. DQ631414, EF636461, EU052800, and EU091084.

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