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Epidemiology of *qnrVC* alleles and emergence out of the *Vibrionaceae* family

The quinolones are antibiotics effective in the treatment of several current nosocomial infections. Bacteria carrying *qnr* genes present with decreased susceptibility to fluoroquinolones. In addition to this low-level resistance, *qnr* genes are associated with reduced bactericidal activity of ciprofloxacin *in vitro* and *in vivo*, which represents a therapeutic threat (Allou *et al.*, 2009). Moreover, in association with mutations in *gyrA*/*parC* and with efflux pump regulatory systems, a full fluoroquinolone resistance phenotype can easily emerge, resulting in treatment failure (Rodríguez-Martínez *et al.*, 2011). Recently, due to increasing reports of *qnrVC* alleles in different genetic contexts, these genes were classified within a new transferable *qnr* family (Pons *et al.*, 2013). Here, using *in silico* analysis, we show that *qnrVC* has already emerged worldwide out of the *Vibrionaceae* family in bacterial species of public health relevance, and in association with mobile genetic elements.

The *qnrVC1* allele is a quinolone-resistance determinant that was first identified in a class 1 integron from a Brazilian *Vibrio cholerae* strain recovered in 1998, and was classified as a new and atypical member of the Qnr family (Fonseca *et al.*, 2008). Different from the plasmid-mediated *qnr* genes, which lack an *attC* site (Rodríguez-Martínez *et al.*, 2011), *qnrVC1* was associated with a *V. parahaemolyticus* repeat (VPR), the recombination site responsible for the cassette mobilization characteristic of chromosomal integrons (Fonseca *et al.*, 2008). Moreover, *qnrVC1* carries its own functional promoter (P_{qnrVC}) and its ability to confer decreased susceptibility to quinolones (MIC of ciprofloxacin, 0.25 μg ml^{-1}) was determined (Fonseca *et al.*, 2008; da Fonseca & Vicente, 2012). Subsequently, this *qnrVC1* cassette was identified in *V. cholerae* from Bangladesh and India in different genetic contexts, including SXT, which is a mobile element (Kim *et al.*, 2010; Kumar & Thomas, 2011) (Table 1). Concurrently with *qnrVC1* characterization the *qnrVC2* allele was identified, by *in silico* analysis, in a plasmid from *V. cholerae* isolated in Vietnam (Fonseca *et al.*, 2008). However, this gene is not functional because of the presence of several stop codons in its coding region and, consequently, does not contribute to the emergence of quinolone resistance.

Concomitantly to this *qnrVC1* spread, new alleles were identified (Table 1). The *qnrVC3* and *qnrVC4* genes were also found in class 1 integrons from *V. cholerae* and *Aeromonas punctata* strains from India and China, respectively (Kumar & Thomas, 2011; Xia *et al.*, 2010), and their deduced proteins differed by four and 41 amino acids, respectively, compared with QnrVC1. The *qnrVC4* gene was also found among environmental aquatic-borne species from other bacteria genera (Table 1). The P_{qnrVC} promoter and the VPR recombination site from *qnrVC1* are conserved and identical in *qnrVC3*. However, despite the presence of the canonical P_{qnrVC} in all *qnrVC4* cassettes already described, their recombination sites correspond to different *V. cholerae* repeats (VCRs) (Table 1).

The *qnrVC5* allele was recently identified in *V. parahaemolyticus*, *V. cholerae* and *V. fluvialis* from Haiti, China and India (Table 1). Some of these were erroneously annotated in GenBank as *qnrB1* or *qnrVC*-like, and here we have properly unified and named them as *qnrVC5*. The *qnrVC5* gene differs by one non-synonymous mutation from *qnrVC4* and by four indels from *qnrVC2* (Fonseca *et al.*, 2008). However, the *qnrVC5* recombination site has the highest identity with the *qnrVC2* VCR, and the plasmid from *V. fluvialis*, where *qnrVC5* was found, showed 99 % identity with pVN84 from *V. cholerae* O1 that harboured *qnrVC2* (Fonseca *et al.*, 2008; Rajpara *et al.*, 2009). These data indicate that these two plasmids are the same (Rajpara *et al.*, 2009) and, considering the high similarity between *qnrVC5* and *qnrVC2* cassettes, we could hypothesize that *qnrVC5* is in fact the *qnrVC2* functional form, and that *qnrVC2*, *qnrVC4* and *qnrVC5* are closely related.

Altogether, these findings show that the *qnrVC* family has amazing mobility and dispersion through different hosts and environments, which suggests that the distinct recombination sites they are associated with (VPRs and VCRs; Table 1) have been effectively recognized and mobilized during site-specific recombination events.

To date, *qnrVC* alleles have been found in the *Vibrionaceae* family and among environmental aquatic-borne species. Moreover, *qnrVC1* had until recently been identified only in *V. cholerae*. However, our GenBank search (March 2013) revealed the occurrence of the entire *qnrVC1* gene cassette (including the 5′UTR and the VPR site) in different class 1 integrons from carbapenem-resistant *Pseudomonas aeruginosa* strains found in Tunisia (Table 1) and a *qnrVC*-like allele in a class 1 integron array from *Acinetobacter baumannii* in China, two opportunistic pathogens responsible for nosocomial outbreaks worldwide. The QnrVC-like deduced protein had only one amino acid substitution (N71D) in comparison with QnrVC1. The putative P_{qnrVC} presented one mismatch in the −35 hexamer compared with the canonical *qnrVC1* promoter (da Fonseca & Vicente, 2012), and the recombination site is also a VPR, although different from that of *qnrVC1*. Considering the polymorphism in the amino acid sequence and that a gene cassette is characterized by its *attC* site (Stokes *et al.*, 1997), we can assume that this *qnrVC*-like is a new allele, named here *qnrVC6* (Table 1).

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Table 1. Alleles of the *qnrVC* family

qnr allele	GenBank accession no.†	P_{qnrVC} sequence‡	*attC* site§	Genetic context	Organism	Country/year of identification
qnrVC1	EU436855.2	Canonical	VPR1	Class 1 integron	*Vibrio cholerae*	Brazil/1998
	FJ968160	Canonical	VPR1	SXT	*V. cholerae*	Bangladesh/2004–2008
	HM015627	Canonical	VPR1	SXT	*V. cholerae*	India/–
	HM015625‖	−35 TTAAGA (20 bp) −10 GGGTCT	VPR2	Class 1 integron	*V. cholerae*	India/–
	AFOP01000199	Canonical	VPR1	–	*V. cholerae*	–
	JX861889	Canonical	VPR1	Class 1 integron	*Pseudomonas aeruginosa*	Tunisia/–
	KC000001	Canonical	VPR1	Class 1 integron	*P. aeruginosa*	Tunisia/–
*qnrVC2**	AB200915	Canonical	VCR1	Plasmid	*V. cholerae*	Vietnam/2004
qnrVC3	HM15626	Canonical	VPR1	Class 1 integron	*V. cholerae*	India/–
qnrVC4	GQ891757	Canonical	VCR2	Class 1 integron	*Aeromonas punctata*	China/2008
	JQ837999	Canonical	VCR2	Class 1 integron	*E. coli*	Portugal/–
	JQ838001	Canonical	VCR2	Class 1 integron	*A. hydrophila*	Portugal/–
	JQ838003	–	–	–	*A. hydrophila*	Portugal/–
	JQ838004	–	–	–	*Aeromonas* sp.	Portugal/–
	JQ838005	–	–	–	*Pseudomonas* sp.	Portugal/–
	JQ838006	–	–	–	*Pseudomonas* sp.	Portugal/–
	JQ838007	–	–	–	*A. hydrophila*	Portugal/–
	JQ838008	–	–	–	*Pseudomonas* sp.	Portugal/–
	JQ838009	–	–	–	*Pseudomonas* sp.	Portugal/–
	ALED01000008	Canonical	VCR3	–	*V. cholerae*	Haiti/2010
	AFOQ01000016#	Canonical	VCR3	–	*V. cholerae*	–
qnrVC5	JX826517	Canonical	VCR1	Class 1 integron	*V. parahaemolyticus*	China/–
	ALEB01000307	Canonical	VCR1	–	*V. cholerae*	Haiti/2010
	JN408080	–	–	Plasmid	*V. fluvialis*	India/1998–2002
	JN571549	–	–	Plasmid	*V. fluvialis*	India/2006
	JN571550	–	–	Plasmid	*V. fluvialis*	India/2006
qnrVC6	GU944730	−35 TTGACA (16 bp) −10 TAGTCT	VPR3	Class 1 integron	*Acinetobacter baumannii*	China/–

–, No sequence information provided; VCR, *V. cholerae* repeat; VPR, *V. parahaemolyticus* repeat.

GenBank accession nos: FJ968160, HM015627, HM015625 (erroneously annotated as *qnrVC3*); AFOP01000199, ALED01000008, AFOQ01000016, ALEB01000307 (erroneously annotated as *qnrB1*); JN408080, JN571549, JN571550 (published as *qnrVC*-like without allelic definition; properly named here as *qnrVC5*).

**qnrVC2* is a non-functional allele because of the presence of premature stop codons.

†The GenBank accession nos AFOP01000199, FJ968160, HM015627, ALED01000008, ALEB01000307, JN571549 and JN571550 presented total identity with their alleles only at the amino acid level.

‡Point mutations in P_{qnrVC} hexamers are underlined.

§Numbers are included in order to distinguish different recombination sites.

‖GenBank accession no. HM015625 presents one silent mutation in the *qnr*VC1 coding region.

#AFOQ01000016 presents one substitution relative to *qnrVC4*, but considering the conservation observed in the 5′ UTR and VCR, it remains assigned as a *qnrVC4* allele.

infections. Therefore, the presence of functional *qnrVC* genes in these species, contextualized in a clinic environment, is worrisome, since their expression and, consequently, the emergence of quinolone resistance in these strains becomes imminent.

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