Competing interests: None declared.
Ethical approval: Not required.

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14 December 2010

doi:10.1016/j.ijantimicag.2010.12.005

Presence of *qnrVC3* gene cassette in SXT and class 1 integrons of *Vibrio cholerae*

Sir,

The toxigenic strains of *Vibrio cholerae* serogroups O1 and O139 are responsible for epidemic cholera, however other serogroups, collectively referred to as 'non-O1/non-O139', are also clearly pathogenic and have caused outbreaks or sporadic cases of diarrhoea in humans [1]. Recently, three novel *qnr* genes, named *qnrVC1*, *qnrVC2* and *qnrVC3*, have been reported from *V. cholerae* O1 strains isolated from Brazil and Bangladesh, respectively [2,3]. However, no information is presently available regarding the prevalence of *qnr* genes in *V. cholerae* from India. In the current investigation, the presence of *qnrVC3* was analysed from O1 and non-O1/non-O139 strains isolated from Southern Kerala, India.

Antimicrobial susceptibility testing was performed using commercially available disks (Himedia, Mumbai, India). The minimum inhibitory concentration (MIC) of ciprofloxacin was determined by Etest (AB BIODISK, Solna, Sweden). Detection of class 1 integrons and the SXT element was carried out by polymerase chain reaction (PCR). For confirmation of the presence of *qnrVC3* in SXT, two pairs of primers were designed. PCR with rumB-F (GCAACCATTCTGCCGCATG) and qnr-R (CAAACCTCCGAGATACAC) yielded an amplicon of ca. 9 kb, and dfr-F (GGTACGTGTAATCAATATTTG) and tnp-R (CAGATCTTTATTCCCCACC) yielded an amplicon of ca. 2 kb. Sequencing of class 1 integrons and SXT (partial sequencing) was performed using an ABI PRISM® BigDye® Terminator Kit with an ABI PRISM® 3100 DNA Sequencer (Applied Biosystems, Foster City, CA). For dot–blot assay, plasmid and genomic DNA was spotted onto a nylon membrane (GE Healthcare, Piscataway, NJ), denatured and fixed by ultraviolet cross-linking, hybridised with biotin-labelled probe and detected using a Biotin Chromogenic Detection Kit (Fermentas, Burlington, Ontario, Canada) according to the manufacturer's instructions.

The non-O1/non-O139 strains showed the presence of class 1 integrons, whereas O1 strains possessed the SXT element. Strains DRV184 and DRV228 possessed two different class 1 integrons. Sequencing of the 1.2 kb amplicon revealed *dfrA1* and *orfC* (unknown function) gene cassettes, whereas sequencing of the 3.4 kb amplicon revealed the presence of *arr3*, *qnrVC3*, bla_{OXA-10} and *aadA1* gene cassettes (GenBank accession no. HM015626). Sequencing of the 2.7 kb amplicon from strains DRV181 and DRV229 revealed the presence of *qnrVC3*, bla_{OXA-10} and *aadA1* gene cassettes (GenBank accession no. HM015625). To our knowledge, these are novel *qnrVC3*-associated class 1 integrons reported from *V. cholerae* to date. All non-O1/non-O139 test strains showed the presence of 7 kb plasmids. Results of the dot–blot assay indicated the chromosomal location of class 1 integrons in all strains investigated. Partial sequencing of the 9 kb amplicon (rumB-F and qnr-R primers) and the 2 kb amplicon (dfr-F and tnp-R primers) confirmed the presence of *dfr6* and *qnrVC3* gene cassettes in SXT of *V. cholerae* O1 strains (GenBank accession no. HM015627). The ciprofloxacin MIC was 0.25 μg/mL in non-O1/non-O139 strains in contrast to MICs of 0.75 μg/mL and 2 μg/mL in MCV09 and A880, respectively (Table 1). Compared with the MIC of a susceptible strain (VC20), it could be inferred that strains possessing *qnrVC3* exhibit low-level resistance to ciprofloxacin. Moreover, mutations in *gyrA* (Ser83 → Ile) and *parC* (Ser85 → Leu) were detected, which may be responsible for the high MIC in O1 strains rather than the presence of *qnrVC3* alone.

Close examination of *qnrVC3* and *dfr6* gene cassettes in SXT as well as in class 1 integrons revealed the presence of *attC* or VCR (*Vibrio cholerae* repeat) sites similar to that of gene cassettes commonly found in superintegrons of the Vibrionaceae family. Recently, we have demonstrated the *dfr6* gene in the superintegron of a non-O1/non-O139 strain of *V. cholerae*. The gene cassettes of class 1 integrons are believed to be evolved from ancestral superintegrons [4]. Moreover, several gene cassettes have been shown to carry an *attC* site almost identical to the *attC* sites located in the superintegrons of *Xanthomonas* and *Vibrio* spp. [5]. Recently, Fonseca et al. [2] hypothesised superintegron ancestry of *qnrVC1* and *qnrVC2* from O1 strains. The data presented here are in agreement with previous reports hypothesising that the *qnr* gene in class 1 integrons has originated from superintegrons. The *qnrVC3* gene was first reported in SXT from O1 strains isolated from Bangladesh [3]. This study describes for the first time the presence of *qnr* genes in SXT of *V. cholerae* strains isolated from India. In SXT, the drug resistance genes *dfr18*, *floR*, *strB* and *sul2* are clustered within a composite transposon-like structure (rumB operon). Although *qnrVC3* and *dfr6* were located in the rumB operon in A880 and MCV09, further investigations are required to resolve how *qnrVC3* and *dfr6* genes were acquired by SXT. The involvement of integron-mediated gene capture cannot be ruled out. The presence of *qnr* genes in *V. cholerae* is clearly expanding and there is need for continuous and careful monitoring to control its dissemination.

Table 1
Characteristics of *qnrVC3*-positive *Vibrio cholerae* strains.

Strain	Serogroup (source)	Antibiogram	MIC of CIP (μg/mL)	SXT element	Class 1 integron(s)	
					No.	Size (kb)
MCV09	O1 (clinical)	TMP, NAL, TET, STR, SPT, AMP, PMB, CIP	0.75	+	–	–
A880	O1 (environmental)	TMP, NAL, TET, STR, SPT, AMP, PMB, CIP, NOR	2	+	–	–
DRV184	Non-O1/non-O139 (environmental)	TMP, STR, SPT, CTX, AMP, PMB	0.25	–	2	1.2 and 3.4
DRV228	Non-O1/non-O139 (environmental)	TMP, STR, SPT, CTX, AMP, PMB	0.25	–	2	1.2 and 3.4
DRV181	Non-O1/non-O139 (environmental)	STR, SPT, CTX, AMP, PMB, TET, GEN, NOR	0.25	–	1	2.7
DRV229	Non-O1/non-O139 (environmental)	SPT, CTX, AMP, PMB, GEN	0.25	–	1	2.7
VC20	O1 (clinical)	AMP	0.047	–	N/D	N/D

MIC, minimum inhibitory concentration; CIP, ciprofloxacin; TMP, trimethoprim; NAL, nalidixic acid; TET, tetracycline; STR, streptomycin; SPT, spectinomycin; AMP, ampicillin; PMB, polymyxin B; NOR, norfloxacin; CTX, cefotaxime; GEN, gentamicin; N/D, not done.

The sequences of the class 1 integron of DRV181 and DRV184 and of SXT of MCV09 are deposited in GenBank under accession nos. HM015625, HM015626 and HM015627, respectively.

Acknowledgment

The authors are grateful to Prof. M. Radhakrishna Pillai (Director, Rajiv Gandhi Centre for Biotechnology, Trivandrum, India) for the facilities provided.

Funding: Intramural support from Rajiv Gandhi Centre for Biotechnology (Trivandrum, India).

Competing interests: None declared.

Ethical approval: Not required.

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28 July 2010

doi:10.1016/j.ijantimicag.2010.12.006