ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Nov. 2009, p. 4959–4960 Vol. 53, No. 11
0066-4804/09/$12.00 doi:10.1128/AAC.00540-09


# Presence of *dfr6* Gene Cassette in Superintegron of Non-O1/Non-O139 Strain of *Vibrio cholerae*▽

Integrons, the DNA elements capable of capturing small mobile elements or gene cassettes, play a major role in the spread of antibiotic resistance in gram-negative bacteria. They are generally divided into two major groups: the multiresistance integrons (MRI), which carry mobile genetic elements, and the chromosomal superintegrons (SI). Gene cassettes carried by MRI encode resistance against antibiotics and are located in either chromosomes or plasmids. Based on the divergence of integrase genes, five classes of MRI have been reported (6). The SI identified in the genomes of the pathogenic *Vibrio* species sequenced to date have been found to contain a large number of gene cassettes, ranging from 72 to more than 200 (3, 7). The cassettes of the *Vibrio cholerae* SI were demonstrated to be substrates for the class 1 integrons of MRI (5, 8). Two class 1 integron gene cassettes, *CARB4* and *dfr6*, were found to contain *attC* sites similar to those of *V. cholerae* repeats (VCRs) (5). Hence, it was predicted that such genes were recruited from SI.

The strain identified (*V. cholerae* non-O1/non-O139, A444) was isolated from Vembanad Lake in the Allapuzha district of Kerala, India. The antimicrobial susceptibility test was done by using commercially available discs (Himedia). The presence of the SXT element and class 1 integrons was tested by PCR as described previously (1, 9). Shotgun cloning was performed by digesting genomic DNA with BfuC1 (NEB) and ligating the fragments with the BamHI (NEB)-digested pUC19 vector. The chemically competent *Escherichia coli* JM109 cells were transformed with a ligation mixture. The transformants were selected at 37°C on LB agar with ampicillin (100 mg/liter) and trimethoprim (TMP) (50 mg/liter). The plasmids from TMP-resistant clones were isolated and sequenced using a pUC/M13 universal pair of primers specific for the pUC series of vector. The sequencing was performed with an ABI Prism BigDye terminator kit using an ABI Prism 3100 DNA sequencer (Applied Biosystems). The nucleotide and deduced protein sequences were analyzed with BioEdit software (version 7.0.9.0; T. Hall, http://www.mbio.ncsu.edu/BioEdit/bioedit.html) and the BLAST search engine.

*V. cholerae* strain A444 was resistant to ampicillin, polymixin B, TMP, cotrimoxazole, streptomycin, and furazolidone. The SXT element and class 1 integrons were shown to carry several genes encoding resistance to TMP, sulfamethoxazole, streptomycin, and spectinomycin in clinical and environmental isolates of *V. cholerae* (2, 9), but neither the element nor the integrons could be detected in A444. The sequencing analysis revealed that the size of the insert in the pUC19 vector is 1,708 bp. The BLAST analysis of the sequence showed the presence of *intIA*, *dfr6*, hypothetical proteins, and VCR. The *dfr6* gene and one of the hypothetical proteins were flanked by VCR, and the arrangement resembled typical SI structure. The *dfr6* (474 bp) obtained showed 96% similarity to the *dfr6* gene reported from the class 1 integron in *V. cholerae* O1 (GenBank accession no. AB200915). Further, internal primers designed to amplify a 1,062-bp region from the cloned fragment in the pUC19 vector yielded a similar amplicon when PCR was done using genomic DNA as a template. The location of cassettes encoding hypothetical proteins was found within the SI of *V. cholerae* El Tor strain N16961 chromosome 2. Recently, novel *dfr* gene cassettes in the chromosomal integrons of environmental *Vibrio splendidus* were identified (4). Thus, the gene cassettes of SI in the *Vibrionaceae* family may constitute a reservoir of antibiotic resistance genes, such as *dfr6*, $bla_{\text{CARB-7}}$, and $bla_{\text{CARB-9}}$. To our knowledge, this is the first report of a demonstration of the *dfr6* gene in SI of *V. cholerae*. Further studies are required to identify other gene cassettes in SI from non-O1/non-O139 strains of *V. cholerae*. This study provides evidence to support the in vivo capture of VCR cassettes by class 1 integrons.

**Nucleotide sequence accession number.** The sequence described in this study has been deposited in GenBank under accession no. FJ905898.

We are grateful to M. Radhakrishna Pillai, Director, RGCB, for the facilities provided.

The research fellowship provided by the Council for Scientific and Industrial Research (CSIR) to Praveen Kumar is gratefully acknowledged.

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▽ Published ahead of print on 24 August 2009.