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## Multiplex PCR for detection of antibiotic resistance genes and the SXT element: application in the characterization of *Vibrio cholerae*

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This study describes a multiplex PCR assay for the detection of antibiotic resistance genes and the SXT element in *Vibrio cholerae*. Conditions were optimized to amplify fragments of *sulII* (encoding sulfamethoxazole resistance), *dfrA1* (O1-specific trimethoprim resistance), *dfr18* (O139-specific trimethoprim resistance), *strB* (streptomycin B resistance) and the SXT element simultaneously in one PCR. This multiplex PCR was evaluated on 142 *V. cholerae* isolates and the results correlated with the phenotypic antibiotic data obtained using a disc diffusion assay and a colony blot assay. Thus this one-step PCR can be used as a simple, rapid and accurate method for identification of antibiotic resistance profiles and could be used for the surveillance of the spread of antibiotic resistance determinants in epidemiological and environmental studies.

### INTRODUCTION

*Vibrio cholerae* is the causative agent of cholera, a severe and sometimes lethal disease. Multiple drug resistance in *V. cholerae* has been reported frequently, usually after acquisition by strains of a conjugative plasmid (Falbo *et al.*, 1999; Glass *et al.*, 1983; Tabtieng *et al.*, 1989). Other genetic elements, such as the class 1 integron and SXT element, have also been reported to carry genetic determinants for antimicrobial resistance (Dalsgaard *et al.*, 2000, 2001; Hochhut *et al.*, 2000, 2001; Iwanaga *et al.*, 2004; Thungapathra *et al.*, 2002). Historically, only the O1 serogroup of *V. cholerae* has been the causative agent of epidemic cholera. However, in late 1992, in India and Bangladesh, a novel serogroup designated *V. cholerae* O139 emerged, replacing *V. cholerae* O1, and gave rise to major cholera outbreaks (Ramamurthy *et al.*, 1993). These strains were characteristically resistant to the antibiotics furazolidone (Fr), sulfamethoxazole (Sul), trimethoprim (Tmp), chloramphenicol (Cm) and low concentrations of streptomycin (Str). Genes encoding resistance to these antibiotics were found to be located on a novel transmissible genetic element, designated SXT (Waldor *et al.*, 1996).

After the extensive cholera outbreaks caused by *V. cholerae* O139, *V. cholerae* O1 El Tor strains re-emerged in 1994 as the predominant cause of cholera on the Indian continent. In contrast to El Tor strains isolated before the O139

outbreak, the re-emerged El Tor strains, like the initial O139 isolates, were resistant to Fr, Sul, Tmp, Cm and Str (Yamamoto *et al.*, 1994). The corresponding genes were found to be located in an integrating conjugative element that was closely related but not identical to the O139 SXT element (Hochhut & Waldor, 1999; Waldor *et al.*, 1996). Variations were noted in recent *V. cholerae* O139 isolates from India, i.e. they were generally no longer resistant to Sul and Tmp, and showed varying resistance to Str (Mitra *et al.*, 1996; Sinha *et al.*, 2002; Yam *et al.*, 1994).

As the SXT genetic element plays a role in the acquisition of antibiotic resistance, it is important to look for the presence of the *sulII* (encoding Sul resistance), *dfrA1* (O1-specific Tmp resistance), *dfr18* (O139-specific Tmp resistance) and *strB* (Str B resistance) genes and the SXT element (Bhanumathi *et al.*, 2003; Falbo *et al.*, 1999; Hochhut *et al.*, 2001) in *V. cholerae* strains. To trace the changes in antibiotic resistance, we developed a multiplex PCR for the detection of antibiotic resistance genes and the SXT element and used it to characterize *V. cholerae* strains.

### METHODS

**Bacterial strains.** A total of 142 strains of *V. cholerae* were included in this study. Of these, 72 were serogroup O1, 62 were serogroup O139 and eight were non-O1, non-O139 serogroup obtained from laboratory stocks identified previously using standard bacteriological methods (World Health Organization, 1987). All isolates were examined for their oxidase reaction, and the identities of the *V. cholerae* O1 strains were confirmed by serogrouping using growth from triple-sugar iron agar slants with polydonal O1 and monospecific Inaba and Ogawa antisera. *V. cholerae* strains that did not agglutinate with O1 antiserum were checked with O139

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Abbreviations: Cm, chloramphenicol; Fr, furazolidone; Str, streptomycin; Sul, sulfamethoxazole; Tmp, trimethoprim.

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