

Detection and confirmation of toxigenic *Vibrio cholerae* O1 in environmental and clinical samples by a direct cell multiplex PCR

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ABSTRACT

Epidemic cholera caused by toxigenic *Vibrio cholerae* O1 is a major health problem in several developing countries. Traditional methods for identifying *V. cholerae* involve cultural, biochemical and immunological assays which are cumbersome and often take several days to complete. In the present study, a direct cell multiplex PCR was developed targeting the *ompW*, *ctxB* and *rfbO1* genes for confirmation of *V. cholerae*, its toxigenicity and serogroup O1, respectively from clinical and environmental samples. The detection sensitivity of the multiplex PCR was 1.9×10^3 *V. cholerae* per PCR reaction. A total of 31 environmental samples and 45 clinical *V. cholerae* isolates from different outbreaks were examined by the PCR. The assay was simple and specific, as there was no requirement for DNA extraction and no amplification was observed with other homologous bacteria used. The assay can be very useful for rapid surveillance of toxigenic *V. cholerae* O1 in environmental water samples, as well as for confirmation of clinical isolates.

Keywords: cholera, *Vibrio cholerae*, PCR, environmental samples

INTRODUCTION

Cholera is an epidemic diarrheal disease that continues to devastate many countries where primary health and sanitation is still a challenge. Several cholera episodes are reported from developing countries due to contamination of water supplies. In 2010, a total of 48 countries reported 317 534 cases of cholera to the WHO, of which 36% were reported from Africa and 56.6% from the Americas, where a large outbreak was reported in Haiti (WHO, 2011). A total of 5 155 cases including 9 deaths were reported from India (WHO, 2011). Globally, the true number of cholera cases may be much higher because many cholera cases remain unreported. Cholera is caused by *Vibrio cholerae*, which is primarily an inhabitant of aquatic environments (Faruque et al., 1998, Islam et al., 1994). Therefore, water plays a significant role in the transmission and epidemiology of cholera. Monitoring the presence of *V. cholerae* in drinking water sources prior to disease outbreak is important to enable effective resource management and public health protection (Choopun et al., 2002).

V. cholerae is classified into more than 200 serogroups, but only *V. cholerae* O1 and O139 that produce cholera toxin (CT) have been associated with epidemics and pandemics (Rivera et al., 2001). The other non-O1, non-O139 serogroups are usually associated with some cases of mild gastroenteritis (Kaper et al., 1995). The ability to produce CT has been used to distinguish between cholerae and non-cholerae strains. CT non-producer O1 and O139 (non-cholerae) strains do not cause epidemic cholera (Levine et al., 1983); and CT-producing non-O1/non-O139 strains, although pathogenic, lack epidemic potential. Hence, detection and monitoring of toxigenic *V. cholerae* O1 or O139 is important during surveillance.

Various conventional cultural-, biochemical- and immunological-based assays are used for detection of *V. cholerae*. However, many of these methods are time-consuming and laborious. Moreover, biochemical identification systems may not always be accurate as several *Vibrio* species display similar biochemical characteristics (Nishibuchi, 2006; O'Hara et al., 2003). Several investigators have employed a direct immunofluorescence technique using polyclonal or monoclonal antibodies for detection of various bacteria and spores (Goel et al., 2005a; Huq et al., 1990). In contrast to culturing and immunological techniques, molecular methods have several advantages: they are rapid, sensitive, highly selective and do not require extensive hands-on time. In this study, we have developed a simple, sensitive and specific multiplex PCR that confirms the presence of *V. cholerae* in environmental and clinical samples, and exhibits its epidemic potential.

MATERIALS AND METHODS

Bacterial strains

The reference strain of *Vibrio cholerae* 20 O1 Ogawa was procured from the National Institute of Cholera and Enteric Disease (NICED), Kolkata, India, and was maintained on Brain Heart Infusion (BHI) agar (Difco, USA) at 22 °C. The other bacterial strains used in this study were *V. cholerae* O1 El Tor N16961 (ATCC 39315), *V. cholerae* O1 El Tor (ATCC 14033), *V. cholerae* O1 Classical 569B (ATCC 25870), *V. cholerae* O1 Classical (ATCC 11623), *V. cholerae* O139 (ATCC 51394), *V. fischeri* (MTCC 1738), *V. parahaemolyticus* (MTCC 451), *Salmonella typhimurium* (MTCC 98), *S. paratyphi* A (MTCC 735), *S. virchow* (MTCC 1163), *S. infantis* (MTCC 1167), *S. bovis/morficans* (MTCC 1162), *Shigella sonnei* (MTCC 2957), *Shigella flexneri* (MTCC 1457), *Shigella dysenteriae* (NICED isolate), *Escherichia coli* (ATCC 11775) and *Bacillus cereus* (ATCC 10876).

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