



Detection of cholera toxin (*ctxA* and *ctxAB*) genes in *Vibrio cholerae* isolated from clinical and environmental samples in Limbang Sarawak by multiplex polymerase chain reaction (PCR)

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ABSTRACT

Aims: Cholera epidemics have been occurred in Malaysia since 1991 till 2003 which can be proved from the records by the Infectious Diseases Division of the Ministry of Health. Moreover, there were also course of cholera epidemics from the year 1994 to 2003 which had been happened in Sarawak. Cholera outbreaks in Malaysia mostly caused by the El Tor O1 *Vibrio cholerae* serogroup. The aims of this study were to detect the presence of *V. cholerae* in clinical and environmental samples (n=28) from Limbang, Sarawak by collaboration with Sarawak Government Hospital and to detect the toxin genes from the isolates.

Methodology and results: All the isolates were sub-cultured in alkaline peptone water (APW). The boiled-cell method was used for DNA extraction. The total DNA extracted was amplified by polymerase chain reaction (PCR). Two types of PCR were used in this study which are 16S rRNA PCR and multiplex PCR. The results obtained from the study found out that 16 out of 28 (57.14%) samples were confirmed to be *V. cholerae* species. Four primers specific for *V. cholerae* were used in multiplex PCR (O1 type, O139 type, *ctxA* and *ctxAB*) to confirm the species type and the toxin genes. All samples shown positive for *V. cholerae* O1 serotype and 100% positive to all genes for the identification of *ctxA* and *ctxAB* genes.

Conclusion, significance and impact of study: From this study, it showed that multiplex PCR can be used for research purposes in molecular genetics field involving cholera outbreak.

Keywords: *Vibrio cholerae*, O1 serogroup, *ctxAB* genes, polymerase chain reaction

INTRODUCTION

Vibrio cholerae is a Gram-negative bacterium with non-spore rods that caused cholera disease. *Vibrio* species which found in marine environment most likely to prefer conditions such as salty, warm and alkaline. There are more than 200 serogroups of *V. cholerae* that successfully identified, but two biotypes of *V. cholerae* serogroup O1 are mostly pathogenic which are the "classical" and the El Tor (Mala *et al.*, 2014). Each O1 biotype has three serotypes which are Ogawa, Inaba and Hikojima. *V. cholerae* O1 strains are capable of interchanging serotypes between Ogawa and Inaba. The most prevalent causative agents of cholera epidemics are serogroups O1 and O139 (Hajia *et al.*, 2016). *V. cholerae* O139 Bengal is a new serogroup which has appeared in Bangladesh in year 1992. This type of serogroup has been isolated in Pakistan and India, while it is originally

limited to areas of Southeast Asia. Some people believed that the O139 serogroup could derive the eighth pandemic. However, only small proportion of the total cases of cholera has been caused by this type of serogroup. Furthermore, other types of *V. cholerae* serogroup possibly causes human illnesses, yet they do not develop into an epidemic form (Zuckerman *et al.*, 2007).

According to Teh *et al.* (2012), cholera outbreaks in Malaysia mostly caused by the El Tor O1 *V. cholerae* serogroup. The O139 serogroup occasionally causes most cases compare to the non-O1/non-O139 because the non-O1/non-O139 *V. cholerae* serogroup has not been incriminated in major outbreak. The vehicles of transmission of cholera outbreak are mainly from contaminated drinking water, unwashed contaminated food and undercooked seafood that presence from *V. cholerae* endemic estuaries (Teh *et al.*, 2012; Griffith *et*

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