

Detection of Cholera Toxin-Producing *Vibrio cholerae* in Phytoplankton from Santubong and Samariang Estuaries

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ABSTRACT

Many cholera outbreaks worldwide were associated with cholera toxin-producing *Vibrio cholerae*. The bacteria are ubiquitous in aquatic environment, whilst phytoplankton is associated with adaptation of the *Vibrio* species. This study was conducted to detect cholera toxin-producing *Vibrio cholerae*, and to determine association of the selected water physicochemical parameters with the number of the bacteria. In this study, a total of ten phytoplankton samples were collected at Santubong and Samariang Estuaries in Kuching, Sarawak. Water physicochemical parameters (temperature, pH and salinity) were recorded. *Vibrio* bacteria were cultivated on thiosulfate citrate bile-salts sucrose selective agar and analysed for cholera toxin-producing *Vibrio cholerae* using polymerase chain reaction by targeting *ctxA* gene that encodes for virulence cholera enterotoxin subunit A. The result revealed that a range of $1.0 \times 10^7 - 8.0 \times 10^7$ CFU/ml of yellow colonies growing on the thiosulfate citrate bile-salts sucrose agars. Inversely, no samples were positive with cholera toxin-producing *Vibrio cholerae*. The physicochemical parameters at Samariang Estuary were more associated with the number of bacteria in the samples compared to Santubong Estuary.

Keywords: cholera toxin-producing, *ctxA* gene, *Vibrio cholerae*

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INTRODUCTION

Vibrio species is gram-negative bacteria, rod-curved shaped, halophile, non-spore forming, autochthonous living in marine and estuaries. The bacteria thrive in saline aquatic environments, that either be free in water or attached to animate surface like phytoplankton or zooplankton (Cavallo & Stabili, 2002). The presence of *Vibrio* bacteria in the environment is influenced by different ecological parameters such as temperature, salinity, nutrient, and concentration of zooplankton and phytoplankton (Alam *et al.*, 2003). *Vibrio cholerae* is one of the common species causing infection. In past record, cholera outbreaks occurred in Miri and Northern Division in Sarawak, Malaysia from November 1997 to April 1998 were due to the contaminated water supplies (Vincent *et al.*, 2015).

Phytoplankton is responsible as natural reservoirs for *Vibrio* bacteria especially *V. cholera*. Furthermore, phytoplankton blooms can promote the increase in *Vibrio* spp.

density (Huq *et al.*, 2012). Direct influence of phytoplankton with growth of *Vibrio* is explained as the number of different phytoplankton species that can contribute to the growth of *Vibrio* (Peterson *et al.*, 2010). Human risk from this event exists where the presence of *Vibrio* spp. in water and phytoplankton will lead to accumulation of *Vibrio* in the shellfish after filter feeding process (Huq *et al.*, 2012).

Cholera toxin (CT) is one of critical virulence factors involved in enteropathogenicity by certain strains of the species (Radu *et al.*, 2002). This toxin is strong heat-labile and encoded by *ctxA* gene. It was first discovered by Koch in 1884 (Broeck *et al.*, 2007). On the other hand, O1 and O139 serogroups are the only two *V. cholerae* serogroups critical for worldwide outbreaks. Other strains have been reported causing infections but being deemed as rare and trivial (Reidl & Klose, 2002). Pathogenicity of *V. cholerae* is influenced by several virulence factors including potent enterotoxin (CT) (Sharma & Chaturvedi, 2009), which is also carried by O1 and O139 groups (Dutta *et al.*, 2013). Nonetheless, other non-O1 and non-139 strains may also produce cholera toxin during infection. Clinical manifestations of