

Application of the Multiplex Polymerase Chain Reaction (M-PCR) for the Screening of *Vibrio* spp. from Rivers in Kuching, Sarawak

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ABSTRACT

The present study was conducted to investigate the occurrence of *Vibrio* spp. from selected rivers in Kuching, Sarawak (Malaysia) using Multiplex Polymerase Chain Reaction (m-PCR). During the six month study period, 19 samples were collected monthly from seven rivers, followed by simultaneous detection of three *Vibrio* spp., *Vibrio parahaemolyticus*, *Vibrio cholerae* and *Vibrio vulnificus*, in a single tube PCR reaction. Three sets of primers targeting the thermolabile (*tl*), outer membrane protein (*ompW*) and hemolysin/cytolysin genes (*vulCulsI*) of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*, respectively, were used. The results indicated that *V. parahaemolyticus* was the predominant species, occurring approximately 60.9% throughout the sampling period, followed by *V. cholerae* (23.1%) and *V. vulnificus* (16.0%). The months of July and December were found to be the months where all three *Vibrio* spp. were found to be at higher frequencies in the river samples. Results analyzed also indicated that the rivers with the highest prevalence of the three *Vibrio* spp. were Tambak Sejingkat, followed by Sungai Jernang and Sungai Tabuan. We conclude that m-PCR is a powerful and useful tool for the rapid and simultaneous detection of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* from the riverine environments without the need for isolation and culturing. Furthermore, this method is highly specific, and could be applied in diagnostic laboratories for larger scale epidemiological investigations of *Vibrio* spp.

Keywords: Multiplex PCR (m-PCR), *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Vibrio* spp., *Vibrio vulnificus*

INTRODUCTION

Vibrio spp. are Gram-negative, rod or curve shaped facultative halophilic anaerobes (Vincent *et al.*, 2014; Malcolm *et al.*, 2015). These bacteria are autochthonous microbial inhabitants of temperate estuarine ecosystems, brackish water, saltwater environments and coastal areas (Micky *et al.*, 2014; Shaw *et al.*, 2015). According to Lopez-Hernandez *et al.* (2015), vibrios can remain in a viable state in the marine environment for extended periods of time. *Vibrio* spp. are resistant to alkaline environments but do not tolerate acidic conditions and will die rapidly in solutions with pH lower than six. Different ecological parameters such as nutrient, temperature, salinity, zooplankton and phytoplankton concentration also have direct influence on the presence and abundance of *Vibrio* spp. in the environment (Alam, 2003; Lopez-Hernandez *et al.*, 2015). Warmer temperature quickly increases the concentration of *Vibrio* spp. in

both molluscan shellfish and seawater. It was also reported that fewer *Vibrio* spp. were cultured when temperature drop and low salinity are recorded (Alam, 2003). However, the degree of salt tolerance varies among the species of this genus.

Outbreaks of *Vibrio* associated diseases present public health problems in many countries, such as United States, India, Bangladesh, Canada, Africa, Latin America and Malaysia (Son *et al.*, 2002; Micky *et al.*, 2014; Lopez-Hernandez *et al.*, 2015). These diseases are usually associated with contaminated or improperly cooked seafood, especially raw seafood, such as raw oysters and also domestic use of highly polluted waters (Lesley *et al.*, 2011; Shaw *et al.*, 2015; Malcolm *et al.*, 2015). *Vibrio* spp. that are implicated in these cases are numerous. However, the three species that are of major concerns are *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*.

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