

Genomic Diversity of Cholera Outbreak Strains in East Malaysia

Lesley Maurice Bilung,¹ Yong Sy Fuh,¹ Velnetti Linang,¹ Adom Benjamin,¹
Micky Vincent,¹ Kasing Apun,¹ Samuel Lihan,¹ Chien Su Lin²

¹Department of Molecular Biology, Faculty of Resource Science and Technology,
Universiti Malaysia Sarawak, 94300 Kota Samarahan, Sarawak, Malaysia

²Department of Pathology, Hospital Bintulu, 97000 Bintulu, Sarawak, Malaysia

ABSTRACT

Thirty one *Vibrio cholera* isolates recovered from cholera outbreak in Bintulu, Sarawak (Malaysia) were detected with the presence of *ctx* gene by using specific PCR. These isolates were further characterized and differentiated by using the Enterobacterial Repetitive Intergenic Consensus PCR (ERIC-PCR) and BOX-PCR to determine their genomic fingerprints. The specific PCR result confirmed the identities of 27 isolates out of 31 as pathogenic *V. cholerae*. The ERIC-PCR generated several genetic profiles consisting of 4-6 bands with sizes in the range of 100 to 600 bp, while the BOX-PCR produced profiles numbering 2-7 bands in the sizes between 200 to 1000 bp. Based on the dendrogram generated from the DNA fingerprinting profiles (ERIC-PCR and BOX-PCR), all of the isolates can be divided into 2 main clusters that is further divided into 2 sub-clusters. The low genetic diversity of the isolates indicated the outbreak of *V. cholerae* in the study area was due to the contamination from a single or few sources of *V. cholerae*.

Keywords: *Vibrio cholerae*, *Ctx* gene, ERIC-PCR, BOX-PCR, Genetic fingerprinting

INTRODUCTION

Cholera is a severe infectious diarrheal disease caused by toxigenic *Vibrio cholerae*. The disease is characterized by stools of rice water diarrhea that rapidly leads to dehydration. The pathogen lives freely in aquatic environments and cholera is always associated with poor sanitation.¹ In Malaysia, cholera outbreaks caused by the El Tor O1 *V. cholerae* serogroup occur periodically, cases from the O139 serogroup occur sporadically, and the non-O1/non-O139 *V. cholerae* serogroup has not been implicated in any major outbreak.²⁻⁴ Contaminated drinking water, cooked food, and raw or undercooked seafood served as vehicles of transmission in Malaysia.⁵ In Sarawak (East Malaysia), the socio-economic activities practiced by the various ethnic groups, the natural phenomena (the La Nina in 1997), the dry seasons (drought) and other contributing factors such as lack of proper treated water supply and the poor sanitary system encountered by the toxigenic *V. cholerae* in the rural area had facilitated the spread of the diseases in Sarawak.⁶

Studies on genomic variation and molecular epidemiology of O1 and O139 *V. cholerae* are often carried out to track sources and spread of the pathogen.⁷ It has been reported that molecular typing methods such as Enterobacterial Repetitive Intergenic Consensus (ERIC) PCR and BOX-PCR were the discriminatory typing methods for *Vibrio* spp., and were very useful for tracing the temporal and geographic relatedness of epidemic strains of *V. cholerae*.⁸ In this study, *V. cholerae* strains were recovered from the cholera outbreaks in Bintulu, Sarawak during August 2012. Preliminary analysis of the isolates was carried out by culturing the isolates on CHROMagar *Vibrio* and followed by serotyping. Molecular characterization was done by screening the isolates for the presence of virulent gene, *ctx* genes. DNA fingerprinting by repetitive sequence based molecular markers like ERIC and BOX PCRs were carried out to study the strain level differences.

*Corresponding author: Dr. Lesley Maurice Bilung
mblesley@frst.unimas.my