# Molecular characterisation of Vibrio parahaemolyticus carrying tdh and trh genes using ERIC-, RAPD- and BOX-PCR on local Malaysia bloody clam and Lala

## ABSTRACT

Molecular typing methods have been widely applied for many purposes. In this study, such methods were adopted as DNA fingerprinting tools to determine the origin and divergence of virulent Vibrio parahaemolyticus strains found in local seafood. Although not all strain carry virulent tdh and trh gene, increasing prevalence demands an effective fingerprinting scheme which can constantly monitor and trace the sources of such emerging food pathogens. By using ERIC-, RAPD-, and BOX-PCR methods, 33 Vibrio parahaemolyticus isolates from local Malaysia bloody clam (Anadara granosa) and Lala (Orbicularia orbiculata) with confirmed presence of tdh and trh gene were characterised, followed by determination of clonal relatedness among virulent strains using cluster analysis and discriminatory index. This study also involved application of Immunomagnetic Separation (IMS) Method which significantly improved the specificity of strain isolation. Cluster analysis using Unweighted Pair Group Mathematical Averaging (UPGMA) and Dice Coefficient shown clustering according to isolation food source, IMS level and haemolysin gene possessed. Nevertheless, different DNA fingerprinting methods generated different clustering at different similarity cut-off percentage, regardless as individual or as composite dendrograms. ERIC- and RAPD-PCR composite fingerprinting relatively shown the highest discriminatory index at following similarity cut- off percentage: 0.68 at 50%; 0.83 at 65%; and 0.93 at 75%. Discriminatory power increased with similarity cut-off percentage. However, result also suggested that BOX-PCR might be an effective fingerprinting tool, as it generated three clusters with no single-colony isolate at 70% similarity cut-off. This study not only achieved its objective to determine clonal relatedness among virulent strains from local seafood via characterisation, but also speculated the best possible combination of molecular typing methods to effectively do so