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MOLECULAR ANALYSIS AND GENETIC STUDIES ON *VIBRIO PARAHAEMOLYTICUS* ISOLATED FROM SEAFOOD AND COSTAL WATER IN MALAYSIA

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ABSTRACT

Vibrio parahaemolyticus is a gram negative curved-rod bacterium that is widely distributed in the marine environment. This organism is frequently isolated from raw seafoods, particularly shellfish. Consumption of raw or undercooked seafood contaminated with *V. parahaemolyticus* may lead to development of acute gastroenteritis characterised by diarrhoea, headache, vomiting, nausea, and abdominal cramps. This pathogen is a common cause of food poisoning in many Asian countries, including China, Japan and Taiwan. This study was done to shed light on some molecular aspects of the pathogenic *V. parahaemolyticus* isolates found in seafood and seawaters of Malaysia. The bacterial isolates studied include 144 *V. parahaemolyticus* isolates collected from 2004 to 2007 plus 5 isolates as reference. All isolates were confirmed to be *V. parahaemolyticus* by culture on CHROMvibrio agar and biochemical tests (API 20NE). Antibiotic susceptibility of the isolates to a panel of antibiotics was determined using E-test. Polymerase chain reaction (PCR) was done to detect the *toxR* species-specific regulatory gene and *tlh* family-species gene, and the *tdh* and *trh* hemolysin genes; whilst the Enterobacterial repetitive intergenic consensus sequence (ERIC) PCR was performed to differentiate and to study the relatedness of the isolates and Pulsed-field gel electrophoresis (PFGE) was performed as well for epidemiological profile. PCR showed that the *toxR* and *tlh* genes were detected in all study isolates, and showed consistency with the API 20NE results, suggesting PCR as a potential diagnostic test for *V. parahaemolyticus*. The virulence gene *tdh+* was negative in all the isolates except one strain isolated from shrimp, whereas the *trh+* virulence gene was found in 8.5% shrimp and 10.7% cockle isolates respectively. The findings indicate a low prevalence of pandemic *tdh+ V. parahaemolyticus* (1/144; 0.69%) while a higher prevalence was found for *trh+* strains (12/144; 8.3%). The PFGE of *Not*I restriction patterns revealed that shrimp isolates from Perak are very similar in their genetic origin, while isolates from cockles and sea water have a more diverse PFGE profile. In contrast, ERIC-PCR patterns produced by strains isolated from the three sources (Perak, Penang and Selangor) were very diverse, a distinct pattern for any particular cluster was not observed. As a conclusion, *toxR* and *tlh* species-specific PCR is a reliable molecular approach for rapid detection of *V. parahaemolyticus,* while PFGE remains the gold standard in determining the genetic relatedness and epidemiology profile of the isolates. Most environmental isolates sampled in this study did not possess the virulence genes that are associated with acute gastroenteritis. This might be the reason behind the low incidence of *V. parahaemolyticus* associated seafood poisoning in Malaysia**.** The findings in this study indicate that the occurrence of pathogenic *V. parahaemolyticus* in seafood sell in the local retail market means the potential risk of *V. parahaemolyticus* outbreak or infection through seafood in Malaysia that should not be neglected.