MOLECULAR CHARACTERISATION OF *VIBRIO VULNIFICUS* (AN EMERGING FOODBORNE PATHOGEN)

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Introduction

Of the vibrio pathogens, *Vibrio vulnificus* is a common bacterium in estuarine waters in temperate and tropical waters. *V. vulnificus* has been identified as the causative agents of a potentially fatal septicaemia (mortality >50%), which occurs following ingestion of raw shellfish, and nonfatal infections have resulted from handling of shellfish or from exposure of a pre-existing wounds to seawater. However, the relationship between the density of the ingested pathogen and the general public of the human host (with regards to the probability of disease) is unclear. To better determine the health risk associated with exposure to *V. vulnificus*, epidemiological tracking of strains is required. This may be achieved by the use of DNA fingerprinting which allows rapid and sensitive differentiation between *V. vulnificus* strains. In this study, *V. vulnificus* strains isolated from cockles (*Anadara granosa*) and sea water were characterised by antimicrobial resistance, plasmid profiles and random amplified polymorphic DNA (RAPD) analysis.

Materials and Methods

Cockles from wet markets were shucked with a sterile scalpel and the muscle and intravalvar fluids were collected in sterile stomacher bags for a cluster of individuals (~50g). Sea water samples were collected in sterile bottles from the resort are of Pulau Kapas, Terengganu. The cockles and seawater samples were enriched in peptone water overnight and were streaked onto thiosulfate-citrate-bile-salts agar. Identification of isolates followed the scheme of Tison et al. (1984). Disk diffusion tests were performed with antibiotic-containing disks as recommended by the manufacturer. Plasmid DNA was extracted followed by electrophoresis, essentially as described by Sambrook et al. (1989). Prior to amplification, chromosomal DNA of the *V. vulnificus* strains was extracted by the mini-preparation method by Wilson (1989). Reaction mixtures of 25 µl were made consisting of 2.5 µl 10x reaction buffer, 1 mM (final conc.) of each dNTP, 2 µM primer, 2.5 mM $MgCl_2$, 20-30 ng genomic DNA and 1 unit *Taq* polymerase. Amplifications were performed for 30 cycles (Perkin Elmer 2400) at 94°C for 2 min, 36°C for 1 min and 72°C for 2 min. A final elongation step at 72°C for 5 min was included.

Results and Discussion

V. vulnificus was isolated from 25 of the 100 cockles samples and all the 50 sea water samples analysed. All the 57 *V. vulnificus* strains from seawater were of biotype 1, whereas 26 and 10 strains from cockles were identified as biotype 1 and 2 respectively. Although biotype 2 strains have always been recovered from diseased eels and seem to be obligate fish pathogens, evidence of its survival in brackish water have been reported (Amaro et al. 1995). In the present study, we report the first observation on the isolation of *V. vulnificus* biotype 2 from cockles, found and harvested from shallow river estuaries along the coastal area of peninsular Malaysia (Son et al. 1998). Thirty one (86.1%) and 9 (15.8%) of strains from cockles and sea water demonstrated resistance to one or more antibiotics tested. About 23 (%) and 10 (17.5%) strains from cockles and sea water contained plasmid bands ranging in sizes from 1.4 to 9.7 MDa, which are in general agreement with previous reports on the plasmid carriage in *V. vulnificus*. Two 10-mer primers (GEN15003: 5'-AGGAT ACGTG-3' and GEN15009: 5'-AGAAGCGATG-3') generated polymorphism within the *V. vulnificus* strains. For the strains from cockles, six and five different RAPD types were apparent for primer GEN15003 and GEN15009, respectively (Son et al. 1998). Strains isolated from seawater demonstrated 27 and 37 RAPD types with primer GEN15003 and GEN15009. The results of the present study allowed us to recognise a high level of intraspecific diversity among the *V. vulnificus* strains. These laboratory observations, based on antibiotic susceptibility, plasmid profiles and RAPD types, suggest how complex the epidemiology of *V. vulnificus* in the study area, probably as a result of the concurrent action of several strains as opposed to the widespread transmission of a single type.

Conclusions

The presence of this pathogen may be a hazard to consumers of raw shellfish in the study area, as the cockle (*Anadara granosa*) is a popular ingredient in several types of local foods and as in many areas in Asia they are frequently consumed in a semi-cooked condition. The heterogeneity may have implication for our understanding of the distribution and evolution of this species in this geographic area.

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