Detection and characterisation of total and haemolysin-producing *Vibrio parahaemolyticus* in bivalve molluscan shellfish from U.K growing waters

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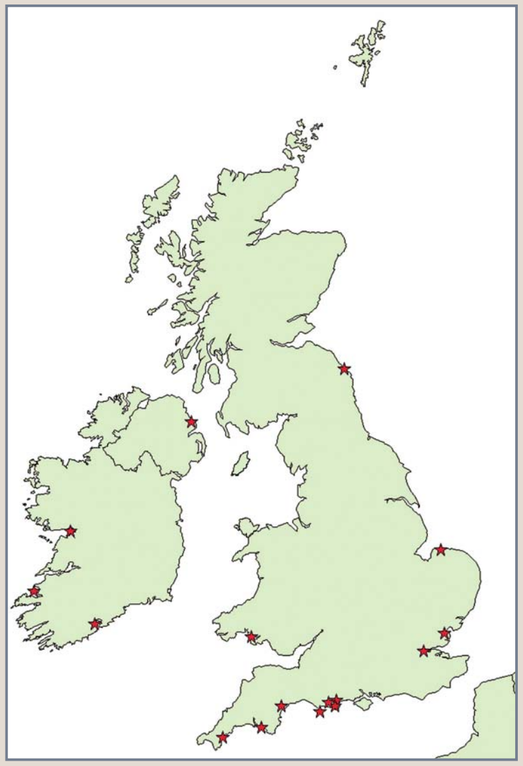
Introduction

The consumption of raw oysters contaminated with pathogenic *Vibrio* species such as *V. parahaemolyticus*, *V. vulnificus* and *V. cholerae* can lead to gastroenteritis, septicaemia and cholera respectively. In Europe, infection by this pathogen is mainly travel related and/or as a result of inadequate hygiene standards. However outbreaks in France, Spain and Italy have recently been reported. In 2004, an outbreak of O3:K6, *V. parahaemolyticus*-associated gastroenteritis was reported in Spain. Seventy-six cases were recorded following the consumption of edible crabs (*Cancer pagurus*) by wedding guests. The crabs had been harvested in the U.K. Pathogenicity is associated with the thermostable direct haemolysin (TDH) and the TDH-related haemolysin (TRH), encoded by the *tdh* and *trh* genes respectively, (Nishibuchi and Kaper, 1995). Up to 99% of clinical strains possess the tdh/trh gene however the presence of these genes in environmental strains is rare (1-5%). The prevalence of pathogenic *Vibrio* species in British waters appears to currently not be of major significance. Nevertheless the detection of pathogenic strains in shellfish harvesting areas could help prevent infections arising and improve shellfish safety.

Pulse Field Gel Electrophoresis (PFGE) is a molecular typing method used in epidemiological studies, in the differentiation of pathogenic strains and in the monitoring of their spread though the community. The objectives of this study were to detect total and potential pathogenic *V. parahaemolyticus* from samples received at Weymouth CEFAS laboratory on an *ad hoc* basis during the period 2001-2003. PFGE was used to evaluate the genetic diversity among *V. parahaemolyticus* strains obtained and assess the relatedness of strains with known pathogenic strains. PFGE has been used in a number of studies to illustrate genetic heterogeneity of non-toxigenic *V. parahaemolyticus* strains recovered from seafood.

Method



- Samples were analysed according to ISO/PDTS 21872. All biochemically confirmed *V. parahaemolyticus* strains, were tested by PCR amplification using the species target *toxR* described by Kim *et. al.,* 1999. The presence of *tdh* and *trh* genes was detected using primers previously described by Tada *et. al.,* 1992.
- PFGE was carried out based on the CDC's 'One-Day (24-48hrs) standardised laboratory protocol for molecular sub-typing of non-typhoidal *Salmonella*'. The running conditions were 18hrs, 6Volts/cm, switch time: 2-40 secs and pump speed 70.

Figure 1: A map of UK and Ireland showing sampling sites. During July-September of 2001- 2004, 71 samples were obtained from commercial shellfisheries in the UK and Eire. Samples comprised mainly Pacific oysters (Crassostrea gigas), Native oysters (Ostrea edulis) and mussels (Mytilus edulis).

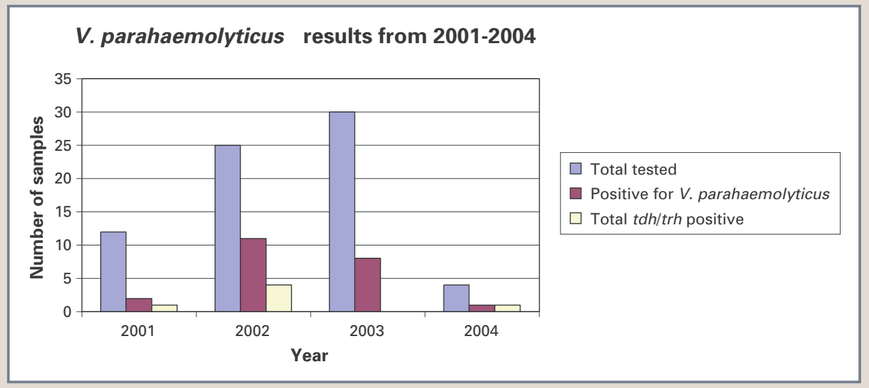


Figure 2: This bar graph shows the proportions of samples tested positive for V. parahaemolyticus. Further PCR testing for the presence of tdh and trh genes found 9 strains to be tdh positive while 0 were positive for trh.

- 22 (31%) samples tested positive for *V. parahaemolyticus* using conventional microbiological methods
- 15 (21%) samples tested positive using *toxR* targeted PCR for *V. parahaemolyticus* strains
- PFGE analyses showed a diverse heterogeneity among *V. parahaemolyticus* isolates.
- Three *V. parahaemolyticus* isolates originated from Portland and Limosa (Southwest of England) where found to be 100% similar.
- Two *V. parahaemolyticus* isolates originated from Arne and Holes Bay where also 100% similar.
- Dendrograms generated show little similarities between clinical *V. parahaemolyticus* O3:K6 (Samples V05/67and V05/68) and environmental strains of *V. parahaemolyticus*.

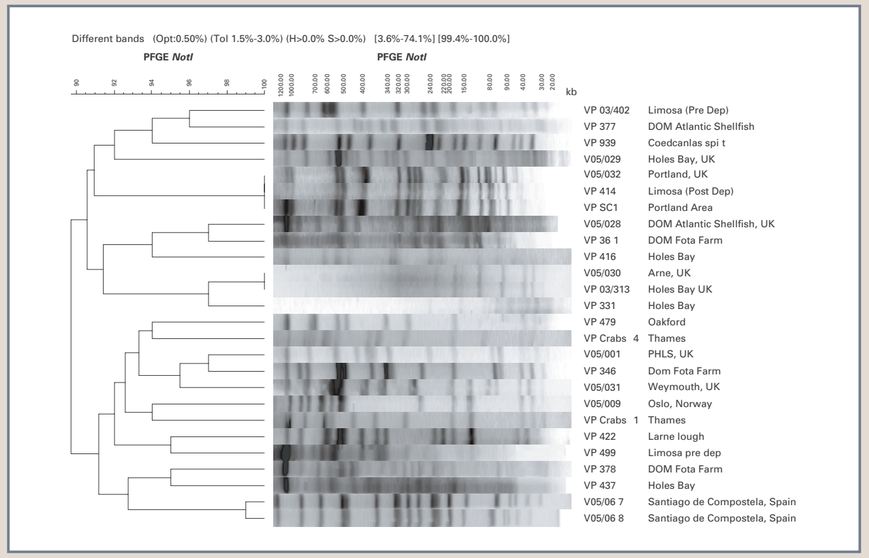


Figure 3: Dendrogram portraying the genetic diversity of various V. parahaemolyticus isolates. The dendrogram is based on PFGE isolates obtained with NotI-digested V. parahaemolyticus DNA.

Discussion

This study shows the presence of *V. parahaemolyticus* strains in 23 and 31% of samples tested dependent upon the method used. Classical microbiological methods consistently produced higher incidence of detection than molecular methods. PFGE patterns showed that strains isolated in U.K and Irish seafood were closely related to each other but showed little relationships to pandemic clones O3:K6. Further studies are underway to establish the homology between UK and Irish isolates with clinically significant European clones.

Future work

This data shows the need to develop standardised classical and molecular methods for the detection, enumeration and characterisation of potentially pathogenic *Vibrio* species in seafood's. The integration and harmonisation of these validated methods; on a Pan European level will advance the knowledge base within the community and provide important information to inform future decisions on controls. The outcomes will therefore reduce the potential human health hazards presented by the ingestion of *Vibrio* species thus promoting consumer confidence and have the economic and trade advantages of increased markets. It will also markedly reduce the occurrences of the inappropriate application of control measures with respect to *Vibrios*'. There will therefore be both advances in health and welfare benefits among European consumers through the increased consumption of premium fisheries products and support the seafood production industry.

References

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