Detection and Isolation of *Vibrio* spp. in Seafood With Cultural and Molecular Method

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ABSTRACT

*Vibrio* spp. are gram-negative, facultative anaerobic, non-spore-forming bacilli which are oxidase positive and halophilic bacteria widely spread in sea- and brackwater worldwide. The genus *Vibrio* includes several foodborne pathogens which cause a spectrum of clinical conditions including septicaemia, cholera and milder forms of gastroenteritis. The species most commonly associated with foodborne transmission include *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus*. *V. parahaemolyticus* causes a much milder diarrhea than cholera.

In this study, totally 89 seafood samples were analysed for the detection of *Vibrio* spp. These samples are: calamary, mackerel, shrimp, red mullet, trout. The approach involved detecting *Vibrio* spp. by cultural and molecular method. For cultural method involves an enrichment phase with incubation at 37°C for 24 h in alkaline peptone water and an isolation phase on selective-differential medium thiosulphate citrate bile salts sucrose (TCBS), Modified cellobiose polymyxin B colistin agar (mCPC) plates incubating at 37°C for 24 h. For the detection and identification of *Vibrio* spp. with molecular method (*V. parahaemolyticus, V. cholerae and V. vulnificus*) three probe-based multiplex real-time PCR systems were used. Real Time PCR process were carried out in a Bax Q7 DUPONT.

The data demonstrated that the cultural methods allow an efficient recovery, isolation and identification of current species of *Vibrio* in seafood. Real Time PCR is the most quick analyses method. With cultural method, the results obtained within 2-5 days. With Real Time PCR, the results obtained within 1-5 days. Molecular protocols are needed for confirmation of the identity of the organism and are discussed in detail.

**Keywords**: Seafood, Vibrio spp., Real Time PCR

INTRODUCTION

In most countries there is no obligation to report on food borne diseases to public health authorities. It has been estimated that only a few of actual cases of food borne diseases are recorded, which could be due to lack of awareness of the etiological role of foods. Also, it is very often that the food responsible is not available for the analysis and therefore specific food vehicle is not identified. When identification of the etiological agent has been successful, pathogenic bacteria are the most frequent disease agent. Bacterial pathogens related to seafood can be categorized into three general groups. Bacteria which are normal components of the marine or estuarine environment (*Vibrio* spp., *Listeria monocytogenes*, *Clostridium botulinum* and *Aeromonas hydrophila*), enteric bacteria which are present due to faecal contamination and bacterial contamination during processing. *Vibrios* are frequently isolated from seafood (1).

*Vibrio* spp. are gram-negative, straight or curved rods are members of the family Vibrionaceae and facultative anaerobic, non-spore-forming bacilli which are oxidase positive and halophilic bacteria widely spread in sea and brackwater worldwide (1,2). The genus *Vibrio* includes at least 30 species. In recent years researchers have focused their attention on the halophilic noncholerae vibrios.
These organisms are not only natural inhabitants of aquatic environments, but are also more and more frequently involved in human gastroenteric episodes (3). Infections arise due to the consumption of raw, undercooked, or improperly processed fish or other seafood containing significant levels of bacteria (5).

The genus *Vibrio* includes several foodborne pathogens which cause a spectrum of clinical conditions including septicemia, cholera and milder forms of gastroenteritis. Especially people with underlying complications like HIV, hemochromatosis, diabetes, and liver disease are susceptible to systemic infections that produces fever, general discomfort, and secondary fluid-filled lesions on the extremities. The species most commonly associated with foodborne transmission include *V. cholerae*, *V. parahaemolyticus*, and *V. vulnificus* (2,4). It is important to point out that these pathogens have been isolated in larger number also from fish and shellfish. The danger of infections is higher because these pathogens are resistant to most antibiotics (4, 6).

There are lots of detection system for these bacteria like cultural method, PCR and Real Time PCR method, microarray method etc. In cultural method, detection of *Vibrio* spp. has consisted of enrichments steps and isolation steps on selective agar medium followed by biochemical and serological testing. In PCR and Real Time PCR method, target specific primers are used. Real Time PCR is more specific and quick method. In this study both cultural and molecular method were used for the detection of *Vibrio* spp in sea food.

**MATERIALS & METHODS**

**Materials:** A total of 89 samples of sea food which included calamary, mackerel, shrimp, red mullet, trout, were collected from different markets in İzmir, Turkey. These samples were carried to the laboratory as soon as possible under refrigerated conditions (4°C) and analysed immediately upon receipt.

**Methods:** The approach involved detecting *Vibrio* spp. by cultural and molecular method.

**Cultural Method:** 25 g of fish products were homogenized with 225 ml of alkaline peptone water in a Stomacher for 2 min. It is incubated at 37 °C for 6 h ± 1 h for deep frozen products, or at 41,5 °C for 6 h ± 1 h for fresh products. For the second enrichment, enrichment medium (ASPW) is then inoculated with the first enrichment culture. It is incubated at 41,5 °C for 18 h ± 1 h. Followed by an isolation phase on selective-differential medium thiosulphate citrate bile salts sucrose (TCBS), Modified cellobiose polymyxin B colistin agar (mCPC) were inoculated with the cultures and plates were incubated at 37°C for 24 h (7).

**Molecular Method:** 25 g of fish products were homogenized with 225 ml of alkaline peptone water in a Stomacher for 2 min. It is incubated at 37°C for 24 h. For the detection of *Vibrio* spp. with molecular method (*V. parahaemolyticus*, *V. cholerae* and *V. vulnificus*) three probe-based multiplex real-time PCR systems were used. Internal control was also used each samples. Real Time PCR processes were carried out in a Bax Q7 DUPONT. If the reactions were positive for molecular method, the isolation step has continued like cultural method (8).

After the cultural and molecular method, green and yellow colonies were selected randomly. Totally 45 isolates were isolated from the TCBS and mCPC agar. These cultures were inoculated on the Nutrient Agar (2% total NaCl concentration) and were identified with “API 20E&API20NE”.
RESULTS & DISCUSSION

34 of the samples were positive according to Bax Q7 DUPONT and cultural method. Totally 45 isolates were identified. The most frequent isolated species were *V. parahaemolyticus*, *V. vulnificus*, *V. fulvialis*, and *V. alginolyticus*. *V. cholerae* and *V. metschikouri* was detected from only one sample. *V. parahaemolyticus* was isolated from ten samples. The data demonstrated that the cultural and molecular methods allow an efficient recovery, isolation and identification of current species of Vibrio in seafood. The similar results were obtained with the molecular and cultural methods. Real Time PCR is the most quick analyses method. With cultural method, the results obtained within 2-5 days. With Real Time PCR, the results obtained within 1-5 days. Molecular protocols are needed for confirmation of the identity of the organism, if the sample shows amplification in *V. parahaemolyticus*, *V. cholerae* and *V. Vulnificus* systems.

CONCLUSION

In this study fish and fish products were analysed and different *Vibrio* spp. cultures were isolated. The results of this study indicate that, *V. parahaemolyticus* and *V. cholerae* which are the most pathogen species, were detected our samples. The ubiquitous nature of *Vibrio* species in marine and estuarine environments makes it impossible to obtain seafood free of these bacteria (9). The most important means of controlling infection lay in simple hygienic measures to prevent multiplication of the organism in seafoods and cross contamination of cooked foods from raw seafood. Refrigeration or freezing is the most effective method for preventing multiplication. Pathogenic species of the genus Vibrio pose a considerable public health threat as the causative agents of both sporadic and epidemic human infections, for example *Vibrio parahaemolyticus* may cause septicemia that is life-threatening to especially people having underlying medical conditions such as liver disease or immune disorders. Illness is associated to the ingestion of raw or undercooked seafood and Vibrios are easily destroyed by heat, therefore proper cooking is sufficient to eliminate most vibrios. Because of these, people should avoid uncooked foods and these bacteria should be monitored and should be routinely analysed. Molecular and cultural method are the quick and suitable method for the routine analyses.

REFERENCES

[8] DUPONT Q7 BAX System Vibrio manuel