Detection and Molecular Characterization of *Vibrio Parahaemolyticus* in Shrimp Samples

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Abstract:

Background: Food safety has emerged as an important global issue with international trade and public health implications. Bacterial pathogens as *Vibrio parahaemolyticus* recognized as an important cause of foodborne diseases related to the consumption of raw, undercooked or mishandled seafood worldwide.

Methods: A total of 70 individual wild shrimp samples were collected from shrimp retail outlets in Zanjan, Iran and investigated for the presence of potentially pathogenic strains of *V. parahaemolyticus*. The shrimp samples were immediately homogenized and cultured on TCBS agar and subjected to confirmatory biochemical tests. Polymerase Chain Reaction (PCR) was performed for detection of total and pathogenic *V. parahaemolyticus* by amplification of *vp–toxR*, *tdh* and *trh* genes.

Results: The conventional method indicated that 16 (22.8%) of samples were positive for *V. parahaemolyticus*. However, PCR verified that only 12 (17.1%) shrimp samples were positive for *V. parahaemolyticus*. Of the 70 shrimp samples in our study, only 2 (2.8%) *tdh* and 1 (1.4%) *trh* positive strains were identified.

Conclusion: Detection of *tdh* and/or *trh* positive *V. parahaemolyticus* in shrimp marketed in Zanjan, Iran shows a probable risk for public health. Therefore, the reliable molecular methods for monitoring of potentially pathogenic *V. parahaemolyticus* are strongly recommended for the routine seafood examination.

Keywords: *Vibrio parahaemolyticus*, Shrimp, PCR, Molecular characterization, *vp–toxR*, *tdh*, *trh* genes.

1. INTRODUCTION

*Vibrio parahaemolyticus* is a halophilic marine bacterium and some strains can cause gastroenteritis in humans through the consumption of raw, undercooked or mishandled contaminated seafood [1, 2]. Although the gastroenteritis caused by *V. parahaemolyticus* is often self–limited and characterized by diarrhea, headache, vomiting, nausea, abdominal cramps and low fever, the infection may cause septicemia, a life-threatening infection, in immunocompromized patients [3 - 5]. The pathogenic strains of *V. parahaemolyticus* are characterized by the production of Thermo stable Direct Hemolysin (TDH) and/or TDH–Related Hemolysins in (TRH) that can lyse the red blood cells on Wagatsuma blood agar (referred to as the Kanagawa phenomenon) and encoded by *tdh* and *trh* genes,

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respectively [3, 6]. The ubiquitous nature of Vibrio spp. in marine and estuarine environments makes it impossible to obtain seafood completely free of these species. It has been implicated in several outbreaks of seafood poisoning worldwide [6, 7]. Previous epidemiological studies showed that V. parahaemolyticus is an important cause of foodborne disease in Asia, South America and the United States. The V. parahaemolyticus is frequently isolated from shellfish including oysters, clams, mussels, lobsters, crabs, shrimps and cockles, which provide an excellent substrate for the growth of these microorganisms in the aquatic habitats [8 - 13]. Many studies have been carried out on shellfish and findings concerning the distribution of V. parahaemolyticus in oysters and mussels are well documented. However, few data are available for crustaceans, despite the popularity of crabs and shrimps and their rising consumption worldwide [14]. Shrimp is one of the most important fishery products, and shrimp farming is an important economy characteristic of Iran [13]. The frequency of pathogenic V. parahaemolyticus infrozen ready-to-eat shrimps for human consumption was recently studied, and 7 to 8% of samples tested positive for tdh or trh virulence genes in countries such as Malaysia. Therefore, these shrimps might have the potential to cause V. parahaemolyticus-associated infections if consumed without further processing [14].

In recent years, V. parahaemolyticus has been recognized as the causative agent of 50–70% of all cases of gastroenteritis associated with consumption of seafood [5].

There are different methods for detection of V. parahaemolyticus in seafood samples. The most–probable–number (MPN) method is used for enumeration of V. parahaemolyticus from food and water, but this method is cumbersome and the recovery of the organism is low [15]. The culture-based approaches and PCR technique which is faster, easier and more sensitive can be used for identification of V. parahaemolyticus in seafood samples [10]. The V. parahaemolyticus strains possess a regulatory gene, toxR, which is present in all strains irrespective of their ability to produce tdh and/or trh. Therefore, the PCR targeted to the toxR gene can be used as a method for identification at the species level [16].

The objective of this study was to determine the frequency of pathogenic V. parahaemolyticus in wild shrimp samples using the culture and PCR methods based on detection of tdh and trh virulence genes in Zanan, Iran.

2. MATERIALS AND METHODS

2.1. Sampling

From March to June 2015, a total of 70 individual wild shrimp samples were collected from shrimp retail outlets in Zanjan, Iran. Shrimp samples were packed into a clean polyethylene bag then marked and transported to the laboratory of food microbiology in a cooler with ice packs for analysis within 1 h.

2.2. Reference Strain

The V. parahaemolyticus ATCC 17802 was kindly obtained from the Pasteur Institute of Iran and was grown on Thiosulphate Citrate Bile Salt Sucrose (TCBS) Agar (MERCK, Darmstadt, Germany).

2.3. Isolation and Identification of V. Parahaemolyticus

The isolation and biochemical identification of V. parahaemolyticus were carried out as recommended in the FDA’s Bacteriological Analytical Manual. Twenty five gram of the samples were homogenized for 60 s in a stomacher (Heidolph, Schwabach, Germany) with 225 mL of alkaline peptone water (APW) containing 3% NaCl and then incubated for enrichment at 37°C for 24 h. After primary enrichment, a loopful (without shaking the flask) from each of the enriched homogenate was streaked onto the surface of Thiosulfate Citrate Bile Sucrose (TCBS) agar plates (MERCK, Darmstadt, Germany) and incubated at 37°C for 24 h. On TCBS plates, sucrose negative colonies (green or blue-green colonies with 2–3 mm in diameter), were picked up and inoculated into tryptonesoya broth with 3% NaCl, incubated at 37°C for 24 h, then purified onto nutrient agar slants with 3% NaCl and subjected for confirmatory biochemical tests using different media contained 2.5% NaCl. Every single colony was screened for Gram staining, motility, oxidase and urease activity, NaCl requirement, citrate utilization, triple sugar iron agar, arginine dehydrolase, lysine and ornithine decarboxylase, O/129 sensitivity, voges proskauer, indole and acid production from lactose, arabinose, cellobiose, mannitol and mannose.

2.4. Genomic DNA Extraction

A colony of V. parahaemolyticus (one colony per sample) was picked from nutrient agar and inoculated into 5 ml of
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strains indicated that 22.8% (16/70) of shrimp samples were positive for V. parahaemolyticus. In our study was lower than some previous studies. According to Abd–Elghany & Sallam [10], the frequency of V. parahaemolyticus in shrimp samples was 33.3%, and 36.8%, respectively. However, lower incidence of this pathogen in fish samples [1, 18, 19].

This variation in V. parahaemolyticus frequency among seafood samples may be due to the difference of the geographical region, type of shellfish sample, watersalinity, seasons of sampling, post-harvest practices and hygienic standards applied during the handling, transport and storage of seafood products, as well as the methods used for isolation and identification of the organism [10].

Fast and accurate diagnosis of food–borne pathogens is very important for a positive outcome of eradication programs. PCR based methods which target the conserved region of V. parahaemolyticus such as toxR gene is more efficient, reliable and faster compare to the conventional techniques [20]. In our study, the biochemically identified isolates were further verified using PCR targeting the vp–toxR gene. It has been indicated that 12 (17.1%) samples out of a total 70 shrimp samples were positive for vp–toxR gene. In the present study, the frequency of V. parahaemolyticus positive samples based on vp–toxR gene, was approximately similar to those reported by Abd–Elghany & Sallam in Egypt [10], who found that 16.7% of shellfish samples were positive for vp–toxR gene and also by Hassan et al. [18] in the Netherlands, who detected that 19% (38/200) of retailed shellfish samples were positive for toxR gene. Only a few reports on the frequency of V. parahaemolyticus in seafood samples from Iran have been previously published. According to the previous reports from Iran, 9.3% and 11% of the shrimp samples [13, 21] and 21.4% of the fish samples [22] were positive for the presence of this pathogen.

As the presence of V. Parahaemolyticus strains carrying tdh and/or trh genes in seafood represents a public health risk, their detection would be of paramount importance. It is well known that only 1–2% of the environmental strains
possess the \textit{tdh} gene. Of the 70 shrimp samples in our study, only 2 (2.8\%) \textit{tdh} and 1 (1.4\%) \textit{trh} positive strains were identified. In the previous report from Iran, the prevalence of \textit{tdh}–positive and \textit{trh}–positive \textit{V. parahaemolyticus} was 1.7\% and 0.7\%, respectively [21]. Similar to our results, in a study conducted in Malaysia, 5 (3.9\%) and 1 (0.78\%) strains isolated from live and frozen shrimp, respectively were positive for \textit{tdh} gene, whilst 2 (1.56\%) and 1 (0.78\%) strains were positive for \textit{trh} gene [23]. However, higher incidence of virulence \textit{V. parahaemolyticus} was identified in several studies such as the study conducted in Turkey by Terzi \textit{et al.} [24] who found that 24 (75\%) out of the 32 strains isolated from mussel were potentially pathogenic depending on \textit{tdh} and \textit{trh} genes.

**CONCLUSION**

In conclusion, the detection of \textit{tdh} and/or \textit{trh} positive \textit{V. parahaemolyticus} in shrimp marketed in Zanjan, Iran shows a probable risk for public health. Therefore, intensive and continuous monitoring of potentially pathogenic \textit{V. parahaemolyticus} are strongly recommended in order to evaluate the human health risk arising from seafood consumption.

**ETHICS APPROVAL AND CONSENT TO PARTICIPATE**

Not applicable.

**HUMAN AND ANIMAL RIGHTS**

No Animals/Humans were used for studies that are base of this research.

**CONSENT FOR PUBLICATION**

Not applicable.

**CONFLICT OF INTEREST**

The authors declare no conflict of interest, financial or otherwise.

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