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ORIGINAL

Detection of virulence factors amongst the *Vibrio parahaemolyticus* strains isolated from marine food samples caught from the Persian Gulf

Detección de factores de virulencia entre las cepas de Vibrio parahaemolyticus aisladas de muestras de alimentos marinos capturados en el Golfo Pérsico

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Abstract

Background: Vibrio species, particularly *V. parahaemolyticus* are considered as an imperative foodborne pathogens occurred after marine food consumption. The present study was carried out to determine the incidence and virulence characters of V. parahaemolyticus isolated from shrimp and shellfish samples.
Methods: Five-hundred marine food samples including shrimp and shellfish were collected from the Boushehr port, Persian Gulf, Iran. Seafood samples were examined by culture and *V. parahaemolyticus* identification was done by the PCR. Virulence factors were also detected by the PCR.
Results: Twenty-two out of 500 (4.40%) marine food samples were contaminated with *V. parahaemolyticus*. Prevalence of *V. parahaemolyticus* amongst the examined shrimp and shellfish samples was 5.42% and 2%, respectively. Total distribution of *tdh, trh*, and *tlh* virulence factors amongst the examined samples was 18.18%, 9.09%, and 4.54%, respectively. *Trh* gene was only detected in shrimp samples (10.52%), while *tlh* gene was only detected in shellfish samples (33.33%).
Conclusions: Shrimp and shellfish samples were considered as the main sources of *V. parahaemolyticus*. Proper cooking of marine food samples before consumption can diminish the risk *V. parahaemolyticus*.

Keywords: *Vibrio parahaemolyticus*, Incidence, marine food, Virulence factors.

Resumen

Antecedentes: Las especies de Vibrio, en particular *V. parahaemolyticus*, se consideran patógenos de transmisión alimentaria imperativos tras el consumo de alimentos marinos. El presente estudio se llevó a cabo para determinar la incidencia y los caracteres de virulencia de *V. parahaemolyticus* aislado de muestras de camarones y mariscos.
Métodos: Se recogieron quinientas muestras de alimentos marinos, incluyendo camarones y mariscos, en el puerto de Boushehr, Golfo Pérsico, Irán. Las muestras de alimentos marinos se examinaron mediante cultivo y la identificación de *V. parahaemolyticus* se realizó mediante la PCR. También se detectaron los factores de virulencia mediante la PCR.
Resultados: Veintidós de 500 (4,40%) muestras de alimentos marinos estaban contaminadas con V. parahaemolyticus. La prevalencia de *V. parahaemolyticus* entre las muestras de camarones y mariscos examinadas fue del 5,42% y del 2%, respectivamente. La distribución total de los factores de virulencia *tdh, trh* y *tlh* entre las muestras examinadas fue del 18,18%, 9,09% y 4,54%, respectivamente. El gen *Trh* sólo se detectó en las muestras de camarones (10,52%), mientras que el gen *tlh* sólo se detectó en las muestras de mariscos (33,33%).
Conclusiones: Las muestras de camarones y mariscos fueron consideradas como las principales fuentes de *V. parahaemolyticus*. La cocción adecuada de las muestras de alimentos marinos antes de su consumo puede disminuir el riesgo *V. parahaemolyticus*.

Palabras clave: *Vibrio parahaemolyticus*, incidencia, alimentos marinos, factores de virulencia.

Introduction

Marine foods are rich sources of proteins, vitamin, and minerals[1]. Additionally, they are rich in essential fatty acids[1]. Their consumption rate among people all-around the world are so high. However, their raw or undercooked consumption may cause severe foodborne diseases[2]. Thus, strict monitoring is required to assess their quality and safety.

Vibrio species (spp.) are considered as one of the most important foodborne diseases regarding the consumption of raw or undercooked marine food samples, particularly shrimp and shellfish[3]. Among these species, Vibrio parahaemolyticus (*V. parahaemolyticus*) had a higher clinical standing and may cause more severe foodborne diseases for a longer period of time[4]. V. parahaemolyticus is a Gram-negative halophilic, non-spore forming, curved rod-shaped bacterium that naturally lives in estuarine and marine environments worldwide[5,6].

Potential virulent *V. parahaemolyticus* strains are usually differentiated from likely avirulent strains by the presence of some important virulence factors[7] responsible for adhesion and invasion into host cells and mainly supported reactions in inflammation[8]. Thermostable direct hemoylsin (*tdh*), TDH related hemolysin (*trh*), and thermolabile hemolysin (tlh) are the most important virulence factors of the V. parahaemolyticus[9].

V. parahaemolyticus infections are considered with abdominal pain, vomiting, watery or bloody diarrhea and gastroenteritis[10]. The bacterium harbored several kinds of virulence factors involved in the pathogenesis of disease. An open wound in skin comes in contact with *V. parahaemolyticus* is recommended as an infection pathway as well. Main syndromes caused by *V. parahaemolyticus* comprise gastroenteritis, wound infection, and septicemia[11]. Thus, it is essential to assess the epidemiological aspects of the *V. parahaemolyticus* in the cases of foodborne diseases. The present survey was done to assess the incidence and distribution of virulence fac tors in the *V. parahaemolyticus* strains isolated from marine food samples.

Materials and methods

Samples

A total of 500 marine food samples including shrimp (n= 350) and shellfish (n= 150) samples were randomly collected from the fishing centers in Bushehr Port, Iran. All samples were caught from the Persian Gulf, Iran. Samples (100 g from the dorsal muscle) were positioned in distinct sterile plastic bags to avoid from falling and cross contamination and were proximately elated to laboratory by icebox.

Isolation of V. parahaemolyticus

Twenty-five grams of seafood samples were hemogenized with 225 ml of Alkaline Peptone Water (Merck, Germany) supplemented with 2% w/v sodium chloride (NaCl) (pH 8.5) for 60 s using a stomacher (BagMixer 400W, Interscience, Saint-Nom-la-Bretèche, France) and then incubated at 37°C for 18 h. A loopful of enriched mixture was streaked on Thiosulphate Citrate Bile salt Sucrose agar (TCBSA, Merck, Germany) plates and incubated at 37°C for 24 h. Bacterial identification was performed according to the color of colonies and their morphology and some biochemical tests including Gram staining, triple sugar iron (TSI), sulfur reduction (cysteine desulfurase), indole production (tryptophanase), and motility (SIM), oxidase, catalase, O-nitrophenyl-beta-D-galactosifase (ONPG), lysine decarboxylase (LDC), Ornithine decarboxylase (ODC), Arginine dehydrolase (ADH) and Halotolerance tests[12].

PCE detection of V. parahaemolyticus

Vibrio isolates were cultured on nutrient broth (Merck, Germany) and further incubated at 37°C for 24h. Principles of producing factory of DNA extraction kit (Thermo Fisher Scientific, Germany) were applied for DNA extraction[13-15]. Extracted DNA samples were subjected to quantification by NanoDrop device (NanoDrop, Thermo Scientific, Waltham, USA), qualification (2% agarose gel) and purity checking (A260/A280)[16-18].

Table I shows the primers used for detection of the *toxR* gene of the *V. parahaemolyticus*[19]. Thermo-cycler device (Flexrcycler[2], Germany) was used.

PCR detection of virulence factors

Table II shows the primers used for detection of the virulence factors used for the detection of virulence factors in the *V. parahaemolyticus* strains[20]. Fifteen microliters of the PCR products were electrophoresed using 1.5% agarose gel. Runs were comprised a negative control (PCR grade water) and positive controls (*V. parahaemolyticus* ATCC 17802)[21-23].

Data examination

Data gotten from the experimentations were classified in the Excel software. SPSS/21.0 software was accompanied for statistical examination. Chi-square and Fisher's exact two-tailed tests were applied to measure any noteworthy association. Statistical signification was determined at a P value < 0.05[23,24].

Results

V. parahaemolyticus incidence

Table III shows the *V. parahaemolyticus* incidence amongst examined marine food samples. Twenty-two out of 500 (4.40%) marine food samples were contaminated with *V. parahaemolyticus*. Prevalence of *V. parahaemolyticus*

Table I: Primers used for detection of the toxR gene of the *V. parahaemolyticus*.

PCR Volume (50µL)	PCR programs	PCR product (bp)	Primer sequence (5'-3')	Target gene
5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template	1 cycle: 95^{0C} ---------- 4 min. 35 cycle: 94^{0C} ---------- 1 min 68^{0C} ---------- 1 min 72^{0C} ---------- 30 s 1 cycle: 72 0C ---------- 5 min	368	F: ATACGAGTGGTTGCTGTCATG R: GTCTTCTGACGCAATCGTTG	*V. parahaemolyticus*

Table II: Primers used for detection of the virulence factors used for the detection of virulence factors in the *V. parahaemolyticus* strains.

PCR Volume (50µL)	PCR programs	PCR product (bp)	Primer sequence (5'-3')	Target gene
5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase 3 µL DNA template	1 cycle: 94^{0C} ---------- 3 min. 30 cycle: 94^{0C} ---------- 60 s 58^{0C} ---------- 60 s 72^{0C} ---------- 60 s 1 cycle: 72^{0C} ---------- 5 min	245 410 450	F: GTAAAGGTCTCTGACTTTTGGAC R: TGGAATAGAACCTTCATCTTCACC F: TTGGCTTCGATATTTTCAGTATCT R: CATAACAAACATATGCCCATTTCCG F: AAAGCGGATTATGCAGAAGCACTG R: GCTACTTTCTAGCATTTTCTCTGC	TDH TRH TLH

amongst the examined shrimp and shellfish samples was 5.42% and 2%, respectively. Statistically significant difference was found amid type of seafood samples and incidence of *V. parahaemolyticus* (P <0.05).

Distribution of V. parahaemolyticus virulence factors

Table IV shows the distribution of *V. parahaemolyticus* virulence factors amongst examined samples. Total distribution of *tdh, trh*, and *tlh* virulence factors amongst the examined samples was 18.18%, 9.09%, and 4.54%, respectively. *Trh* gene was only detected in shrimp samples (10.52%), while *tlh* gene was only detected in shellfish samples (33.33%). Statistically significant difference was found amid type of seafood samples and distribution of virulence factors (P <0.05).

Table IV: *V. parahaemolyticus* incidence amongst examined marine food samples.

Samples	N. collected samples	N. samples positive for *V. parahaemolyticus* (%)
Shrimp	350	19 (5.42)
Shellfish	150	3 (2)
Total	500	22 (4.40)

Table V: Distribution of *V. parahaemolyticus* virulence factors amongst examined samples.

Samples (N. positive)		N. isolates positive for virulence factors (%)		
		tdh	trh	tlh
Shrimp	19	3 (15.78)	2 (10.52)	-
Shellfish	3	1 (33.33)	-	1 (33.33)
Total	22	4 (18.18)	2 (9.09)	1 (4.54)

Discussion

According to the filter feeding food intake manner of shrimp and shellfish, they are more prone to microbial contamination from the aquatic environment[25]. In this regard, marine food contamination with Vibrio spp. can originate from both the aquatic environment (sea or ocean water) and also through the cross contamination by staffs of the fishing centers.

The present survey showed the high prevalence of *V. parahaemolyticus* in keeping with the high distribution of virulence factors in bacterial isolates. The *V. parahaemolyticus* presence in marine food samples, particularly shrimp and shellfish, could be linked to their filter-feeding activity. Water particle-associated and water free-living pathogenic microorganisms may be filtered throughout seafood's feeding and can gather in gastral tract or gills. Feeding of contaminated zooplanktons is supplementary imperative likely hazard issue for the boost incidence of *V. parahaemolyticus* in samples. Furthermore, opportunity for occurrence of cross contamination with infected human and staffs of the hunting centers is a conceivable reason for the presence of *V. parahaemolyticus* in studied samples. Furthermore, using contaminated ice for cooling of seafood samples is another important factor. Differences in diet of studied samples, distance of living from the beach, depth of their lives and finally their route of maintenance are probable factors affecting differences in the incidence of different *V. parahaemolyticus* in diverse samples.

Several surveys have been conducted in this field. In similar surveys, Messelhäusser et al. (2010)[26] and Baker-Austin et al. (2018)[27] reported the high prevalence of *V. parahaemolyticus* in marine food samples. Robert-Pillot et al. (2014)[28] signified that 34.70% of marine food samples were contaminated with Vibrio spp. in which 89.60% were positive for V. parahaemolyticus with higher incidence in crustaceans (79.30%), fish (8.60%) and shellfish (1.70%). Similarly, Thongkao et al. (2017)[29] stated that the distribution of *V. parahaemolyticus* amongst the marine shellfishes samples in Thailand was

5.33%. Previously[30] authors described that the incidence of *V. parahaemolyticus* in fish and shrimp samples caught from the Persian Gulf was 3.53%. Raissy et al. (2012)[31] stated that the incidence of V. parahaemolyticus amongst the lobster and crab samples caught from the Persian Gulf, Iran was 3.03%. *V. parahaemolyticus* was the most prevalent specie amongst the seafood samples. of surveys conducted in India[32], Vietnam[33], China[34] and Malaysia[35].

High distribution of *tdh, trh*, and *tlh* virulence factors amongst the *V. parahaemolyticus* strains isolated from seafood samples was reported previously[36,37]. However, not all *V. parahaemolyticus* strains are pathogenic. TDH and TR) encoded by *tdh* and *trh* genes, respectively, are considered major virulence factors in *V. parahaemolyticus*. However, about 10% of clinical strains do not contain *tdh* and/or *trh*. Environmental isolates of *V. parahaemolyticus* lacking *tdh* and *trh* are also highly cytotoxic to human gastrointestinal cells. Additionally, some of the tlh-associated *V. parahaemolyticus* strains may cause severe gastrointestinal diseases[38]. Even in the absence of these hemolysins, *V. parahaemolyticus* remains pathogenic indicating other virulence factors exist. This mini review aims at discussing the possible roles of tdh and trh genes in clinical and environmental isolates of *V. parahaemolyticus*[39].

Conclusion

High prevalence of *V. parahaemolyticus* with putative virulence factors were reported in this survey. Presence of *tdh, trh,* and *tlh* virulence factors amongst the *V. parahaemolyticus* strains isolated from shrimp and shellfish samples may pose an important public health issue regarding their consumption in raw or undercooked condition. Thus, full cooking of seafood samples before consumption and monitor the prescription of antibiotic can diminish the occurrence of antibiotic resistant-Vibrio foodborne diseases. However, further surveys are essential to found more details about the impact of *V. parahaemolyticus* in marine food samples.

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Interests conflict

The researchers declare that they have no conflict of interest.

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