Toxic genes and antibiotic resistance patterns in *Vibrio Parahaemolyticus* isolates from caught fish of the Caspian Sea

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Abstract

Vibrio parahaemolyticus (*V. parahaemolyticus*) is a marine bacterium that is widely acknowledged as a predominant causative agent responsible for bacterial foodborne outbreaks on a global scale. The objective of our study is to determine the prevalence of toxin-producing genes and antibiotic resistance patterns in *V. parahaemolyticus* isolates obtained from fish caught in the Caspian Sea. We conducted a descriptive cross-sectional study in which we collected 220 fish samples from the Caspian Sea, comprising four fish species (*Rutilus kutum, Mugilidae, Cyprinus carpio* and *Perca*). The samples underwent enriched and culture for bacteriological and biochemical examination. The isolates were confirmed using the 16S rRNA flagella-specific gene of *V.*

parahaemolyticus and then subjected to antimicrobial susceptibility testing using the disk-diffusion method. Additionally, PCR was employed to investigate the presence of three virulence genes (*toxR*, *tdh*, and *trh* genes). Out of a total of 220 fish samples, 40 (18.2%) were found to be contaminated with *V. parahaemolyticus*. All 40 confirmed isolates possessed the *toxR* gene and 29 (72.5%) of them harbored the *tdh* gene, while none of them contained the *trh* gene. The majority of the isolates exhibited susceptibility to ciprofloxacin (97.5%) and chloramphenicol (92.5%), but demonstrated resistance to amoxicillin (95%) and doxycycline (95%). The findings of this study yield valuable insights in to the microbial contamination of fish caught in the Caspian Sea. Appropriate control measures are suggested due to the high prevalence of *V. parahaemolyticus* in seafood and the subsequent presence of multi drug resistance (MDR) isolates.

Keywords: *Vibrio parahaemolyticus*, Marine environment, Foodborne disease, Virulence genes

1. Introduction

The existence of pathogenic bacteria in marine environments raises concerns regarding the safety of food, as these bacteria have the potential to cause foodborne diseases. *Vibrio parahaemolyticus* (*V. parahaemolyticus*) belongs to the Vibrionaceae family. It is a halophilic, gram-negative bacterium that has a rod-shaped morphology and is capable of motility (1). Being halophilic, it is able to survive and reproduce in environments with a sodium chloride (NaCl) concentration ranging from 1 to 9%. This bacterium naturally inhabits aquatic environment such as marine, estuarine and coastal environments. *V. parahaemolyticus* is commonly associated with various types of seafood, including fish, shrimp, lobster, and shellfish (2, 3).

The *toxR* gene, thermostable direct hemolysin (*tdh*) and *tdh*-related hemolysin (TRH) are some of the known virulence genes involved in Vibrio pathogenesis. Infections caused by *V. parahaemolyticus* occur due to the presence of different virulence factors, which include adhesins (Type I pilus), type III secretion systems (T3SS), and type VI secretion systems (T6SS). The *toxR* gene acts as a gene for *tdh* and *trh*. The *tdh* gene encodes a pore-forming protein that facilitates bacterial invasion in humans, while *trh* plays a similar role to *tdh* in causing disease (4, 5). The presence of *toxR*, *tdh*, and *trh* genes is typically used to differentiate potentially virulent strains of *V. parahaemolyticus* from non-virulent strains. The virulence genes associated with *V. parahaemolyticus*, particularly those related to in hemolysis and cytotoxicity, cause acute gastroenteritis in the host, leading to symptoms such as watery diarrhea, abdominal cramps, nausea, vomiting, fever, headache, and/or bloody diarrhea in humans who consume raw, undercooked, or mishandled seafood contaminated with *V. parahaemolyticus* (6).

Contact between open wounds and *V. parahaemolyticus* can also result in wound infections, and in rare cases, it can lead to life-threatening septicemia, particularly in individuals with pre-existing medical conditions. *V. parahaemolyticus* is responsible for numerous cases of food poisoning associated with seafood in many Asian countries including Japan, Taiwan and India (7, 8). Approximately 80% of the estimated 5.2 million cases of bacterial diarrhea that transpire annually in the United States can be attributed to foodborne illnesses (9). Given the global prevalence of *V. parahaemolyticus* gastroenteritis cases, it is crucial to investigate the prevalence of these bacteria, their virulence genes, and their impact on humans. According to a report from the Centers for Disease Control and Prevention (CDC), *V. parahaemolyticus* was identified as the most common foodborne pathogen, responsible for 39–51% of Vibrio infections compared to other *Vibrio* species such as *V. vulnificus*, *V. cholerae* (non-O1 and non-O139), *V. alginolyticus*, *V.fluvialis*, *V. mimicus*, and *V. hollisae* (10).

In recent years, the rise of antibiotic-resistant infections has become a global health concern. Therefore, timely surveillance of antibiotic-resistant bacteria and the dissemination of surveillance data are essential to address these public health issues. A significant number of *V. parahaemolyticus* strains isolated from clinical and environmental samples have shown high resistance to multiple antibiotics such as amoxicillin, ampicillin, ceftazidime, and gentamicin (11, 12). The extensive use and misuse of antibiotics for the treatment of seafood-related diseases are likely the main contributors to the emergence of multiple drug resistance (MDR) in *V. parahaemolyticus* isolates (13,14). This study aims to determine the prevalence, toxin-producing genes and antimicrobial resistance patterns in *V. parahaemolyticus* isolates was couth from the Caspian Sea.

2. Methods

2.1 Sample Collection and Isolation of V. parahaemolyticus

In a descriptive cross-sectional study, a total of 220 fish samples were collected from the Caspian Sea between August 2022 and August 2023. The samples consisted of four species of fish: *Rutilus kutum*, *Mugilidae*, *Cyprinus carpio* and *Perca*. To preserve their quality, the samples were placed in sealed containers with dry ice and transported frozen within approximately 24 h to the laboratory. The isolation of *V. parahaemolyticus* bacteria was carried out following the standard protocols established by the US Food and Drug (FDA). The protocols were summarized as follows:

A 5-gram portion of each sample was enriched in 45 mL of alkaline peptone water containing 3% NaCl for 24 hours. A loopful of the enriched mixture was then cultured on thiosulphate citrate bile salt sucrose (TCBS) agar (Merck, Germany). After incubation at 37°C for 24 hours, the green colonies were selected and subjected to Gram staining, oxidase activity assessment, ONPG, Triple-Sugar-Iron (TSI), Urease, Citrate, Lysin, and Arginine tests (15, 16).

2.2 Antimicrobial Susceptibility Testing (AST)

The antimicrobial susceptibility of the *V. parahaemolyticus* isolates was determined using the disk diffusion method following the guidelines set by the Clinical and Laboratory Standards Institute (CLSI) (17).

The susceptibility tests were performed using Nutrient Agar, Muller-Hinton agar, and a panel of 10 antibiotic disks (Mast, UK) was used for antibiotic susceptibility tests. The following 10 antimicrobial disks were used in the study: ampicillin (10 µg), ceftazidime (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), meropenem (10 µg), trimethoprim-sulfamethoxazole (25 µg), tetracycline (30 µg), amoxicillin (25 µg), and doxycycline (30 µg). *P. aeruginosa* ATCC 27853 and *V. parahaemolyticus* ATCC 17802 were used as quality control organisms.

2.3 DNA Extraction

The genomic DNA was extracted as described by the boiling method (12). Fresh colonies of the *V. parahaemolyticus* isolates were suspended in 400 µL of sterile deionized water and mixed well by using a vortex mixer. The mixture was then heated at 100°C for 15 minutes on a thermo block device. After heating, the samples were centrifuged at 11000 rpm for 10 minutes. The supernatants, which contained the genomic DNA, were transferred to microtubes and stored at -20° C until further molecular studies. The concentration of the extracted DNA was determined using a Nanodrop (Nano Drop™ One Microvolume UV-Vis Spectrophotometers).

2.4 PCR Confirmation (Detection of Virulence Genes: *tox R*, *tdh*, and *trh*)

PCR was performed, to detect the presence of 16S rRNA, *toxR*, *tdh*, and *trh* genes in the *V. parahaemolyticus* isolates (4). The primer sequences used for the genes were as follows:

16S rRNA 16S rRNA-F: GCAGCTGATCAAAACGTTGAGT, 16S rRNA-R: ATTATCGATCGTGCCACTCAC. *toxR*-F: GTCTTCTGACGCAATCGTTG, *toxR*-R: ATACGAGTGGTTGCTGTCATG, *tdhF*: GTAAAGGTCTCTGACTTTTGGA, *tdh*-R: TGGAATAGAACCTTCATCTTCACC, *trh*-F: TTGGCTTCGATATTTTCAGTATCT, and trh-R: CATAACAAACATATGCCCATTTCCG (18,19).

The total volume of the PCR reaction was adjusted to 20 µL, comprising 10 µL of Mastermix, 1.5 µL of DNA, 1 µL of primers and 7.5 µL of distilled water (D.W.).

The PCR reaction was performed using an amplification thermal cycler (Q lab, peckstar). The reaction consisted of pre-denaturation at 95 °C for 5 min; followed by 30 cycles of the main thermal program (denaturation at 95 °C for 45 seconds, annealing at 59 °C for 50 seconds, extension at 72 °C for 45 seconds), and a final extension at 72 °C for 5 min. The amplified PCR products were then subjected to gel electrophoresis (Bio-Rad), and the gel image was recorded using a Gel Doc device. *V. parahaemolyticus* strains ATCC33847 (*toxR*+, *tdh*+) and ATCC17802 (*toxR*+, trh+) were used as positive control templates, and sterile distilled water was used as the negative control.

3. Results

3.1 Prevalence of *V. parahaemolyticus* and virulence genes

Out of the 220 fish samples that were examined, it was discovered that 40 samples (18.2%) were contaminated with *V. parahaemolyticus* (Table 1). Molecular testing was conducted to confirm the presence of *V. parahaemolyticus*, as illustrated in Fig 1. All 40 isolates of *V parahaemolyticus*, which were confirmed through biochemical testing, were found to possess the 16S rRNA gene.

Table1. Prevalence of *Vibrio parahaemolyticus* in different species of fish samples

Fish Sample (Species)	No. of sample	Number of positive samples	(%) of positive samples

Rutilus kutum	55	9	16.4%
Mugilidae	55	18	32.7%
Perca	55	5	9.1%
Cyprinus carpio	55	8	14.5%
Total	220	40	18.2%

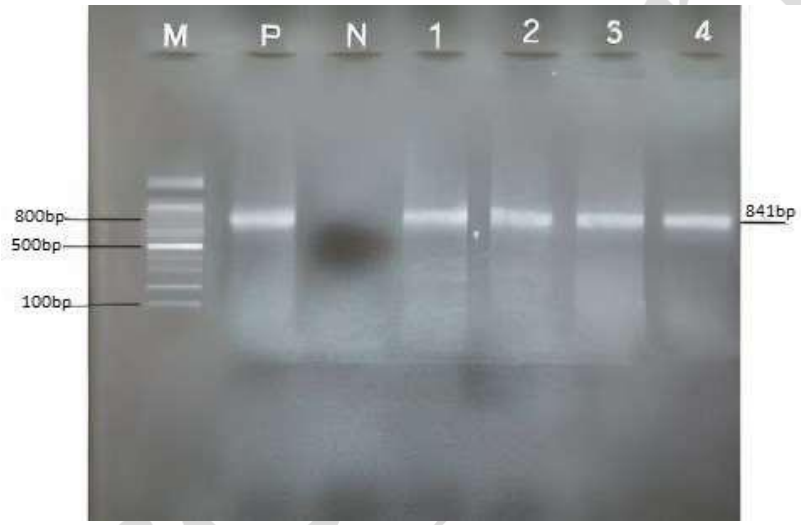

Fig 1. The presence of *V. parahaemolyticus* by PCR amplification of 16S rRNA gene. M Ladder: 100 bp; P: Positive control; N: Negative control; Numbers 1 to 4: Isolates containing 16S rRNA gene.

PCR assay was demonstrated that the *toxR* gene was detected in all 40 (100%) of the confirmed isolates. The presence of the thermostable direct hemolysin (*tdh*) gene was observed in 29 (72.5%) of the isolates. Notably, none of the *V. parahaemolyticus* isolates exhibited *tdh*-related hemolysin (*trh*) gene. The findings from agarose gel electrophoresis, employed for PCR amplification, are presented in Fig 2.

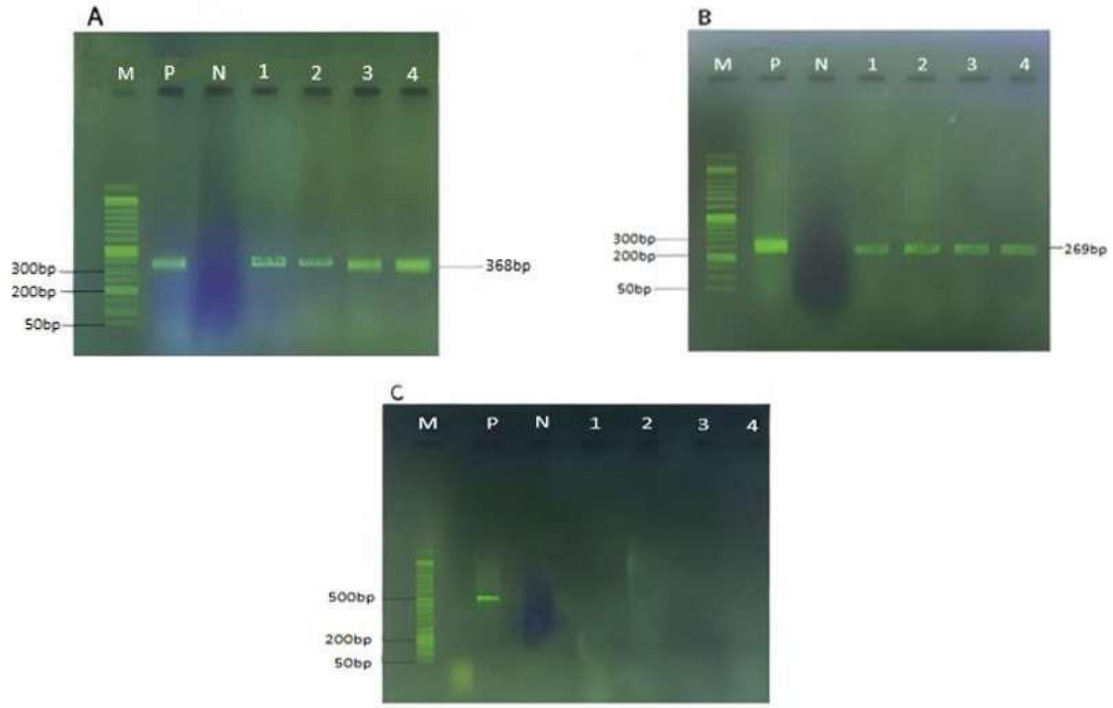

Fig 2. The presence of *V. parahaemolyticus* by detection of virulence factor genes. M: Ladder 50 bp; P: Positive control; N: Negative control. **A**, Numbers 1-4: Isolates of *V. parahaemolyticus* containing *toxR* gene (368 bp). **B**, Lines 1-4: Isolates of *V. parahaemolyticus* containing *tdh* gene (269 bp). **C**, Line 1-4: Isolates of *V. parahaemolyticus* containing *trh* gene (500 bp)

3.2 Antimicrobial susceptibility of the *V. parahaemolyticus* isolates

The antibiotic resistance profile of the *V. parahaemolyticus* isolates were assessed. The findings revealed high susceptibility to ciprofloxacin (97.5%), chloramphenicol (92.5%), and gentamycin (87.5%). However, resistance was noted for amoxicillin (95%), doxycycline (95%) and tetracycline (92.5%). Detailed results regarding antimicrobial resistance can be found in Table 2.

Table 2. Antimicrobial resistance profiles of *Vibrio parahaemolyticus* isolates

Antibiotics(µg)	***V. parahaemolyticus*(n=40)**			**Zone diameters (mm)**		
	No. (%) of Sensitive (S)	No. (%) of Intermediate (I)	No. (%) of Resistant (R)	Resistant	Intermediate	Sensitive
Ampicillin	7 (17.5)	8 (20)	25 (62.5)	≥13	14-16	≤17
Amoxicillin	(0)	2 (5)	38 (95)	≥13	14-17	≤18

Ceftazidime	3 (7.5)	5 (12.5)	32 (80)	≥17	18-20	≤21
Meropenem	1 (2.5)	8 (20)	31 (77.5)	≥19	20-22	≤23
Chloramphenicol	37 (92.5)	2 (5)	1 (2.5)	≥12	13-17	≤18
Tetracycline	(0)	3 (7.5)	37 (92.5)	≥11	12-14	≤15
Doxycycline	(0)	2 (5)	38 (95)	≥11	12-14	≤15
Ciprofloxacin	39 (97.5)	1 (2.5)	(0)	≥15	16-20	≤21
Gentamicin	35 (87.5)	2 (5)	3 (7.5)	≥12	13-14	≤15
Trimethoprim-sulfamethoxazole	33 (82.5)	6 (15)	1 (2.5)	≥10	11-15	≤16

4. Discussion

The consumption of fish among Iranian households, coupled with the substantial statistics associated with Caspian Sea fisheries, underscores their significance in terms of public health (20). Consequently, this study on the prevalence of *V. parahaemolyticus* holds considerable importance in the promotion of public health.

The present study revealed that *V. parahaemolyticus* exhibited a prevalence rate of 18.1% in fish samples. This could be attributed to the species ability to tolerate high salt concentrations in seawater. These findings confirm the dominance of *V.parahaemolyticus* as the predominant microbial flora in the Caspian Sea. The majority of *V. parahaemolyticus* strains isolated from fish samples carried the *toxR* and *tdh* virulence genes, with rates of 100% and 72.5%, respectively, while the *trh* gene was not detected. The absence of the *trh* gene is noteworthy, as it is one of the causes of gastroenteritis resulting from the consumption of raw or undercooked fish and other related products.

Our results are in line with previous studies conducted in Iran and other regions worldwide. For instance, Najafi (2006) and Jalali (2010) reported frequencies of *V. parahaemolyticus* in farmed and marine fish ranging from 5-10% and 3.9%, respectively (14,15). In comparison, our reported frequency is lower. These findings suggest a lower level of *V. parahaemolyticus* contamination in farmed fish and processed fish that have undergone proper cold storage procedures. Alipour *et al*, conducted a study on water and sediment samples from the Caspian Sea, revealing that 98 out of samples (20.3%) tested positive for *V. parahaemolyticus*, indicating a relatively high presence of the bacterium in the waters of the Caspian Sea and subsequent contamination of most fish and animals (16).

Rahimi *et al*., conducted a study on 132 shrimp and crab samples, while Raisi *et al*., examined 300 shrimp from the Persian Gulf. They observed contamination rates of 3.03% and 9.5% with *V. parahaemolyticus*, respectively (18, 21). Another study by Safarpour *et al*., reported a presence of 22% *V. parahaemolyticus* in fish from the Persian Gulf, which is higher compared to previous similar studies. The majority of *V. parahaemolyticus* strains isolated from fish and lobster samples were found to carry the *tdh* (23.45%) and *trh* (66.16%) virulence genes, confirming their high pathogenicity (19).

Zarei *et al*., studied the infection rates of *V. parahaemolyticus* in shrimp caught during different seasons. Their findings showed infection rates of 19% in summer, 13% in spring, 8% in autumn, and 4% in winter. The higher prevalence of *V. parahaemolyticus* in summer samples can be attributed to increased salt concentration in the water resulting from evaporation caused by heat, creating favorable conditions for bacterial growth and spread. Additionally, 0.6% of the *V. parahaemolyticus* strains isolated from these samples carried the *toxR* virulence gene (22).

In a study conducted in Zanjan, Iran, shrimp samples were examined and found to have a 17.1% positive rate for *V. parahaemolyticus* among the 70 samples tested. Among the *V. parahemolyticus* positive samples, the *tdh* and *trh* genes were present in 2.8% and 1.4% of samples, respectively (23).

Numerous studies conducted in European countries and East Asia have shown the prevalence of Vibrio species, particularly *V. parahaemolyticus*, along the coasts of Asia and East Asia (24, 25, 26). Therefore, proper cooking of marine products is crucial in these countries to prevent gastroenteritis caused by *V. parahaemolyticus.*

In line with the aforementioned findings, Ottiviani *et al*., discovered that 11.6% of 559 oyster samples caught in the Adriatic Sea were infected with *V. parahaemolyticus*, with 7.7% of these strains carrying the *trh* gene. The *trh* gene was found to cause hemolysis of red blood cells and weakens the immune system (24). This report indicates that *V. parahaemolyticus* poses a higher pathogenic potential due to its virulence genes when consuming contaminated raw or uncooked products. Thus, the presence of these virulence genes in *V. parahaemolyticus* contributes significantly to its pathogenicity.

The finding of Letchumanan *et al*., in Malaysia and Kang *et al*., on the coast of Korea indicated a high prevalence of *V. parahaemolyticus* species in seafood, with rates of 100% and 37.6%, respectively (25, 26). Similarly, Yang *et al*., conducted a study on 504 samples of shrimp, fish, and oysters from the southern coast of China and found that 64% of the samples were infected with *V. parahaemolyticus.* Among these samples, 8.1% and 12.2% of the strains were positive

for the *tdh* and *trh* genes (27), indicating toxigenic potential. Mahmud *et al*., isolated 192 strains of *V. parahaemolyticus* from seawater and seaweeds in the K channel in Japan, and 18 samples (9.3%) carried toxic or toxigenic genes (28).

Haque *et al*., and Xiaoke *et al*., in studies conducted on fish, oysters, and shrimp in Bangladesh and China respectively, reported frequencies of 95% and 37.7% for *V. parahemolyticus* (29, 30). Although the results of these studies were higher than those of our current study, none of the *V. parahaemolyticus* isolates in our study were positive for the *tdh* or *trh* genes. Additionally, Kshirsagar *et al*., in a study on fish and shrimp samples in Gujarat, India, reported an infection rate of 11.61% for *V. parahaemolyticus* species. The *tdh* gene was found in 11.11% of the samples, but the *trh* gene was absent in all isolates, which was consistent with our results (31).

The occurrence rates of contamination by various Vibrio species in marine products exhibit regional variation within Iran. This variation can be ascribed to multiple factors, such as sample types, collection seasons, ecological circumstances, environmental pollution, species discrepancies, and substantial disparities in sanitary conditions from the point of fish capture to its delivery. It is noteworthy to mention that, in addition to the primary contamination stemming from fish caught in the Caspian Sea, secondary contamination can also contribute to the heightened prevalence of *V. parahaemolyticus* in fish. The lack of proper hygiene standards in fishing and processing platforms, as well as in centers for selling and distributing marine products, likely plays a role. Contact between the caught marine products and contaminated surfaces are likely one of the key factors leading to secondary contamination. Additionally, inadequate cooling processes for these products can further contribute to contamination.

Our study found that the isolated *V. parahaemolyticus* strains demonstrated the highest resistance pattern to amoxicillin (95%) and doxycycline (95%). Other studies conducted in Iran showed that this bacterium is sensitive to chloramphenicol and cephalothin and resistance to streptomycin, ampicillin, and nalidixic acid (14), these findings are consistent with global studies that have reported sensitivity to chloramphenicol and ciprofloxacin, and resistance to streptomycin, nalidixic acid, and ampicillin (26, 27).

The variations in antibiotic resistance patterns and the unique spectrum of resistance highlight the presence of diverse antibiotic patterns among different strains of *V. parahaemolyticus* in different regions. This underscores the significance of this species in fish contamination and the subsequent development of gastroenteritis from consuming contaminated seafood.

The findings of this study underscore the relatively high microbial contamination with *V. parahaemolyticus* in fish samples caught from the Caspian Sea. Consequently, consuming these marine products raw or partially cooked can pose a problem. Therefore, it is crucial to determine the antibiotic resistance pattern in these isolates to identify the most effective antibiotic and treatment approach.

In conclusion, this study provides valuable and information regarding the microbial contamination of fish caught from the Caspian Sea. The high prevalence of *V. parahaemolyticus* in seafood, along with the identification of multidrug-resistant isolates, presents a potential risk to human health. Hence, appropriate control measures should be implemented to minimize the risk of contamination. Consuming raw or undercooked fish can result in gastrointestinal issues such as heartache, diarrhea, and gastroenteritis.

This research highlights the importance of adequately cooking marine products as the principal preventive measure against vibriosis caused by *V. parahaemolyticus*. Implementing effective health monitoring practices in fishing and distribution centers for marine products can help reduce pollution levels in these products. Furthermore, providing up-to-date information on antibiotic-resistant *V. parahaemolyticus* strains is crucial for ensuring the effective treatment of human and aquatic product infections.

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Authors' Contribution

Conception and design: MMSD. Methodology: MMSD, ZR and EY. Investigation: ZR and EY. Acquisition and analysis of data: MMSD and EY. Writing the original draft: MMSD and EY. Critical revision of the manuscript for important intellectual content: HM.

Ethics

This research has obtained approval from the Ethics Committee of Tehran University of Medical Sciences under the code IR.TUMS.VCR.REC.1398.1069.

Conflict of Interest

The authors declare that they have no competing interests.

References

1. Namadi P, Deng Zh. Optimum environmental conditions controlling prevalence of vibrio parahaemolyticus in marine environment. Marine Env Res. 2023;183:105828.

2. Waidner L, Potdukhe T. Tools to Enumerate and Predict Distribution Patterns of Environmental Vibrio vulnificus and Vibrio parahaemolyticus. Microorganisms. 2023; 11(10):2502.

3. Masoumi H, Gouya M, Soltan-Dallal MM, Aghili N. Surveillance for foodborne disease outbreaks in Iran, 2006-2011. Med J Islam Rep Iran. 2015:29:285.

4. Kim YB, Okuda J, Matsumoto C, Takahashi N, Nishibuchi M. Identification of Vibrio parahaemolyticus strains at the species level by PCR targeted to the toxR gene. J Clin Microbiol. 1999;37(4):1173-7.

5. Newton AE, Garrett N, Stroika SG, Halpin JL, Turnsek M, Rajal K M, Centers for Disease Control and Prevention (CDC). Increase in Vibrio parahaemolyticus infections associated with consumption of Atlantic Coast shellfish—2013. MMWR. 2014;63(15):335-6.

6. Haibo Zh, Xinmei L, Zhaoxin L, Antuo H, Wenjie M, Changzheng S, et al. Quantitative detection of Vibrio parahaemolyticus in aquatic products by duplex droplet digital PCR combined with propidium monoazide. Food Control. 2023; 144:109353.

7. Kunbawui P, Jong S M , Ryu AR, Ji YK, In Tae H, Kil B S. Occurrence and virulence of Vibrio parahaemolyticus isolated from seawater and bivalve shellfish of the Gyeongnam coast, Korea, in 2004–2016. Marine Pollut Bullet. 2018;137:382-387.

8. Yutaka Y, Kaoru H, Masataka S, Isao T, Masatoshi B, Dusit A. Prevalence and antimicrobial susceptibility of Vibrio species related to food safety isolated from shrimp cultured at inland ponds in Thailand. Food control. 2014;38 (1):30-6.

9. Soltan Dallal MM , Motalebi S, Masoumi H, Sharifi Y MK , Rahimi forushani A. Antimicrobial investigation on the multi-state outbreak of salmonellosis and shigellosis in Iran. Med J Islam Rep Iran. 2020; 34:49.

10. Heba A A, Rasha M E B, Mohamed A H, Mariam H E K, Etab M A R, Ahmed M M E. Molecular characterization, antibiotic resistance pattern and biofilm formation of Vibrio parahaemolyticus and V. cholerae isolated from crustaceans and humans. Int J Food Microbiol. 2018;274:31-37.

11. Chia W T, Yaya R, Hanan H, Tze Y T, Epeng L, Wendy D R, et al. Prevalence and antibiotic resistance patterns of Vibrio parahaemolyticus isolated from different types of seafood in Selangor, Malaysia. Saudi J Biol Sci. 2020; 27(6):1602-08.

12. Reshma S, Deborah A, Mohamed A. Prevalence, antibiotic resistance, virulence and plasmid profiles of Vibrio parahaemolyticus from a tropical estuary and adjoining traditional prawn farm along the southwest coast of India. Ann Microbiol. 2015; 65(4):2141-49.

13. Hyo-Won J, Jin-Ah K, Su-Jin J, Seong-Seon C, Min-Kyeong K, Hye-Jin Y, et al. Prevalence, antibiotic-resistance, and virulence characteristics of Vibrio parahaemolyticus in restaurant fish tanks in seoul, South Korea. Foodborne Pathog Dis. 2020; 17(3):209-14.

14. Najafi A, Sadeghifar NKH, Bagheri T Y, Salari MH, Shirazi MH, Ranjbar R. Isolation of Vibrio paraheamoliticus from fish in Tehran and their antimicrobial resistance. Iran J Infect Dis Trop Med. 2006;11(35): 65-8.

15. Jalali M MM, Mahdavi M, Javadi A, Khorvash F. Prevalence of Vibrio parahaemolyticus in seefoods in isfahan. Iran J Infect Dis Trop Med. 2010; 14(46):33-6.

16. Alipour M, Issazadeh K, Soleimani J. Isolation and identification of Vibrio parahaemolyticus from seawater and sediment samples in the southern coast of the Caspian Sea. Comp Clin Path. 2012; 23(1):129-33.

17. Hindler J, Humphries R, Richter S, Jorgensen J, Bernard K, Killian S, et al. Methods for antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. M45, 3rd edn.

18. Rahimi E, Ameri M, Doosti A, AR Gholampour. Occurrence of toxigenic Vibrio parahaemolyticus strains in shrimp in Iran. Foodborne Pathog Dis. 2010; 7(9):1107-11.
7(9):1107-11.

19. Safarpourdehkordi F, Hosseini S, Rahimi E, Momeni M, Yahaghi E, Khodaverdi E. Investigate the frequency of virulence genes Vibrio parahaemolyticus isolated from fish, lobsters and crabs caught from Persian Gulf. Iran J Med Microbiol. 2018; 8(2):1-7.

20. Hashempour-Baltork F, Jannet B, Tajdar-Oranj B, Aminzare M, Sahebi H, Alizadeh A, et al. A comprehensive systematic review and health risk assessment of potentially toxic element intakes via fish consumption in Iran. Ecotoxicol Env Safety. 2023;249:114349.

21. Raissy M, Moumeni M, Ansari M, Rahimi E. Antibiotic resistance pattern of some Vibrio strains isolated from seafood. Iran J Fisheries Sci. 2012;11(3):618-26.

22. Zarei M, Pourmahdi B M, Jamnejad A, Khezrzadeh M. Seasonal prevalence of Vibrio species in retail shrimps with an emphasis on Vibrio parahaemolyticus. Food Control. 2012;25(1):107-9.

23. Asgarpoor D, Haghi F, Zeighami H. Detection and Molecular Characterization of Vibrio

Parahaemolyticus in Shrimp samples. Open Biotech J. 2018; 12: 46-50.

24. Ottaviani D, Santarelli S, Bacchiocchi S, Masini L, Ghittino C, Bacchiocchi I. Presence of pathogenic Vibrio parahaemolyticus strains in mussels from the Adriatic Sea, Italy. Food microbiol. 2005; 22(6):585-90.

25. Vengadesh L, Wai-Fong Y, Learn-Han L, Kok-Gan C. Prevalence and antimicrobial susceptibility of Vibrio parahaemolyticu s isolated from retail shrimps in Malaysia. Front Microbiol. 2015;6:33.

26. Chang-Ho K, YuJin S, WooRi K, YongGyeong K, KiCheol S, Eun-Gyoung O, et al. Prevalence and antimicrobial susceptibility of Vibrio parahaemolyticus isolated from oysters in Korea. Env Sci Pollut Res. 2016; 23 (1):918-26.

27. Ying Y, Jiafang X, Hua L, Shuwen T, Yanfeng C, Hui Y. Prevalence, antibiotic susceptibility and diversity of Vibrio parahaemolyticus isolates in seafood from South China. Front Microbiol. 2017; 8:2566.

28. Zahid H M, Afework K, Alizadeh M, Masayuki Y, Bhuiyan N, Balakrish N. Isolation and molecular characterization of toxigenic Vibrio parahaemolyticus from the Kii Channel, Japan. Microbiol Res. 2006;161(1):25-37.

29. Manirul H, Anwar H, Shankar C M, Mohammad R, Zahid M. Prevalence, characterization and antibiotic susceptibility of Vibrio parahaemolyticus isolated from fishes and shellfishes of coastal regions of Bangladesh. Dhaka Uni J Biol Sci. 2015;24(2):121-9.

30. X Xu, Qingping W, Jumei Z, Jianheng C, Shuhong Z, Kui W. Prevalence, pathogenicity, and serotypes of Vibrio parahaemolyticus in shrimp from Chinese retail markets. Food Control. 2014;46:81-5.

31. Kshirsagar D, Brahmhhatt M, Chatur Y, Sinha N. Studies on occurrence and distribution of virulent strains of Vibrio Parahaemolyticus in finfishes and prawns from different ecosystem of Gujarat (India). J Vet Health Sci. 2013;11(1):07-10.

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