

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

3
4 Yazdanpanah, E¹, Rajabi, Z^{2,3}, Maftoon, H⁴, Mirbagheri, SZ⁴, Soltan Dallal, MM^{3,4}

- 5 1. Division of Food Microbiology, Department of Pathobiology, School of Public Health,
6 Tehran University of Medical Sciences, Tehran, Iran
7 2. Zoonoses Research Center, Tehran University of Medical Sciences, Tehran, Iran
8 3. Food Microbiology Research Center, Tehran University of Medical Sciences, Tehran,
9 Iran
10 4. Department of pathobiology, School of Public Health, Tehran University of Medical
11 Sciences, Tehran, Iran
12 5.

13 Corresponding Author: Mohammad Mehdi Soltan Dallal, Food Microbiology Research Center,
14 Tehran University of Medical Sciences, Tehran, Iran.

15 E. mail: msoltandallal@gmail.com. Tel: 02188982971

16
17
18
19 **Abstract**

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

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

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

40 1. Introduction

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

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

71 Contact between open wounds and *V. parahaemolyticus* can also result in wound infections,
72 and in rare cases, it can lead to life-threatening septicemia, particularly in individuals with pre-
73 existing medical conditions. *V. parahaemolyticus* is responsible for numerous cases of food
74 poisoning associated with seafood in many Asian countries including Japan, Taiwan and India
75 (7, 8). Approximately 80% of the estimated 5.2 million cases of bacterial diarrhea that transpire
76 annually in the United States can be attributed to foodborne illnesses (9). Given the global
77 prevalence of *V. parahaemolyticus* gastroenteritis cases, it is crucial to investigate the
78 prevalence of these bacteria, their virulence genes, and their impact on humans. According to
79 a report from the Centers for Disease Control and Prevention (CDC), *V. parahaemolyticus* was
80 identified as the most common foodborne pathogen, responsible for 39–51% of *Vibrio*
81 infections compared to other *Vibrio* species such as *V. vulnificus*, *V. cholerae* (non-O1 and
82 non-O139), *V. alginolyticus*, *V. fluvialis*, *V. mimicus*, and *V. hollisae* (10).

83 In recent years, the rise of antibiotic-resistant infections has become a global health concern.
84 Therefore, timely surveillance of antibiotic-resistant bacteria and the dissemination of
85 surveillance data are essential to address these public health issues. A significant number of *V.*
86 *parahaemolyticus* strains isolated from clinical and environmental samples have shown high
87 resistance to multiple antibiotics such as amoxicillin, ampicillin, ceftazidime, and gentamicin
88 (11, 12). The extensive use and misuse of antibiotics for the treatment of seafood-related diseases
89 are likely the main contributors to the emergence of multiple drug resistance (MDR) in *V.*
90 *parahaemolyticus* isolates (13,14). This study aims to determine the prevalence, toxin-
91 producing genes and antimicrobial resistance patterns in *V. parahaemolyticus* isolates was
92 couth from the Caspian Sea.

83 2. Methods

84 2.1 Sample Collection and Isolation of *V. parahaemolyticus*

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

92 A 5-gram portion of each sample was enriched in 45 mL of alkaline peptone water containing
93 3% NaCl for 24 hours. A loopful of the enriched mixture was then cultured on thiosulphate
94 citrate bile salt sucrose (TCBS) agar (Merck, Germany). After incubation at 37°C for 24 hours,
95 the green colonies were selected and subjected to Gram staining, oxidase activity assessment,
96 ONPG, Triple-Sugar-Iron (TSI), Urease, Citrate, Lysin, and Arginine tests (15, 16).

97 **2.2 Antimicrobial Susceptibility Testing (AST)**

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

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

108 **2.3 DNA Extraction**

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

117 **2.4 PCR Confirmation (Detection of Virulence Genes: *tox R*, *tdh*, and *trh*)**

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

120 16S rRNA 16S rRNA-F: GCAGCTGATCAAAAACGTTGAGT, 16S rRNA-R:
121 ATTATCGATCGTGCCACTCAC. *toxR*-F: GTCTTCTGACGCAATCGTTG, *toxR*-R:
122 ATACGAGTGGTTGCTGTCATG, *tdhF*: GTAAAGGTCTCTGACTTTTGGGA, *tdh*-R:
123 TGG AATAGAACCTTCATCTTCACC, *trh*-F: TTGGCTTCGATATTTTCAGTATCT, and
124 *trh*-R: CATAACAAACATATGCCCATTTCCG (18,19).

125 The total volume of the PCR reaction was adjusted to 20 µL, comprising 10 µL of Mastermix,
126 1.5 µL of DNA, 1 µL of primers and 7.5 µL of distilled water (D.W.).

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

135

136

137 3. Results

138 3.1 Prevalence of *V. parahaemolyticus* and virulence genes

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

144

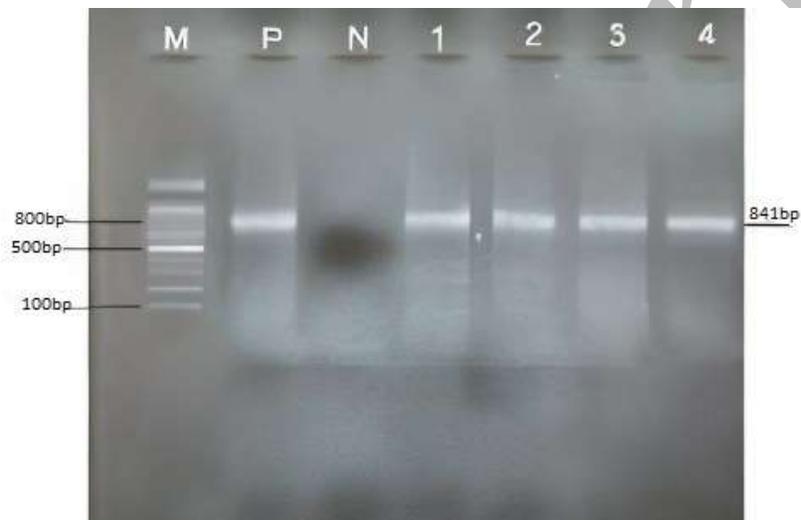
145 **Table1.** Prevalence of *Vibrio parahaemolyticus* in different species of fish samples

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

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

۱۴۶

۱۴۷



۱۴۸

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

۱۵۱

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

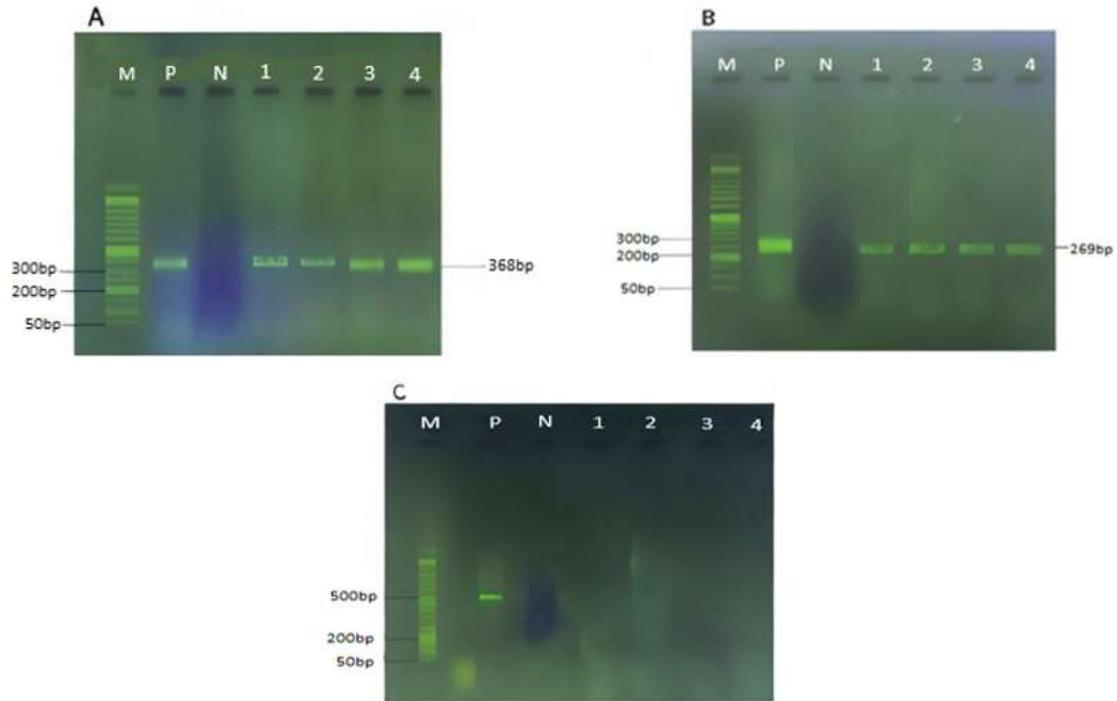


Fig 2. The presence of *V. parahaemolyticus* by detection of virulence factor genes. M: Ladder 50 bp; P: Positive control; N: Negative control. **A**, Numbers 1-4: Isolates of *V. parahaemolyticus* containing *toxR* gene (368 bp). **B**, Lines 1-4: Isolates of *V. parahaemolyticus* containing *tdh* gene (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(μ g)	<i>V. parahaemolyticus</i> (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

۱۷۱

۱۷۲

۱۷۳ 4. Discussion

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

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

۱۸۵ Our results are in line with previous studies conducted in Iran and other regions worldwide. For
 ۱۸۶ instance, Najafi (2006) and Jalali (2010) reported frequencies of *V. parahaemolyticus* in farmed
 ۱۸۷ and marine fish ranging from 5-10% and 3.9%, respectively (14,15). In comparison, our reported
 ۱۸۸ frequency is lower. These findings suggest a lower level of *V. parahaemolyticus* contamination in
 ۱۸۹ farmed fish and processed fish that have undergone proper cold storage procedures. Alipour *et al*,
 ۱۹۰ conducted a study on water and sediment samples from the Caspian Sea, revealing that 98 out of
 ۱۹۱ samples (20.3%) tested positive for *V. parahaemolyticus*, indicating a relatively high presence of
 ۱۹۲ the bacterium in the waters of the Caspian Sea and subsequent contamination of most fish and animals
 ۱۹۳ (16).

194 Rahimi *et al.*, conducted a study on 132 shrimp and crab samples, while Raisi *et al.*, examined
195 300 shrimp from the Persian Gulf. They observed contamination rates of 3.03% and 9.5% with *V.*
196 *parahaemolyticus*, respectively (18, 21). Another study by Safarpour *et al.*, reported a presence of
197 22% *V. parahaemolyticus* in fish from the Persian Gulf, which is higher compared to previous similar
198 studies. The majority of *V. parahaemolyticus* strains isolated from fish and lobster samples were found
199 to carry the *tdh* (23.45%) and *trh* (66.16%) virulence genes, confirming their high pathogenicity (19).

200 Zarei *et al.*, studied the infection rates of *V. parahaemolyticus* in shrimp caught during different
201 seasons. Their findings showed infection rates of 19% in summer, 13% in spring, 8% in autumn, and
202 4% in winter. The higher prevalence of *V. parahaemolyticus* in summer samples can be attributed to
203 increased salt concentration in the water resulting from evaporation caused by heat, creating favorable
204 conditions for bacterial growth and spread. Additionally, 0.6% of the *V. parahaemolyticus* strains
205 isolated from these samples carried the *toxR* virulence gene (22).

206 In a study conducted in Zanjan, Iran, shrimp samples were examined and found to have a 17.1%
207 positive rate for *V. parahaemolyticus* among the 70 samples tested. Among the *V. parahemolyticus*
208 positive samples, the *tdh* and *trh* genes were present in 2.8% and 1.4% of samples, respectively
209 (23).

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

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

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

۲۲۶ for the *tdh* and *trh* genes (27), indicating toxigenic potential. Mahmud *et al.*, isolated 192 strains
۲۲۷ of *V. parahaemolyticus* from seawater and seaweeds in the K channel in Japan, and 18 samples
۲۲۸ (9.3%) carried toxic or toxigenic genes (28).

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

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

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

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

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

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

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

۲۷۳ **Acknowledgment**

۲۷۴ We would like to extend our gratitude to the Vice Chancellor for Research of Tehran University
۲۷۵ of Medical Sciences for sponsor this research project. This article is the result of a research
۲۷۶ grant approved by the Zoonoses Research Center, Tehran University of Medical Sciences.

۲۷۷ **Authors' Contribution**

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

۲۸۱ **Ethics**

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

۲۸۴ **Conflict of Interest**

۲۸۵ The authors declare that they have no competing interests.

۲۸۶ **References**

- ۲۸۷ 1. Namadi P, Deng Zh. Optimum environmental conditions controlling prevalence of vibrio
۲۸۸ parahaemolyticus in marine environment. *Marine Env Res.* 2023;183:105828.
۲۸۹
- ۲۹۰ 2. Waidner L, Potdukhe T. Tools to Enumerate and Predict Distribution Patterns of
۲۹۱ Environmental *Vibrio vulnificus* and *Vibrio parahaemolyticus*. *Microorganisms.* 2023; 11(10):2502.
- ۲۹۲ 3. Masoumi H, Gouya M, Soltan-Dallal MM, Aghili N. Surveillance for foodborne disease outbreaks
۲۹۳ in Iran, 2006-2011. *Med J Islam Rep Iran.* 2015;29:285.
- ۲۹۴ 4. Kim YB, Okuda J, Matsumoto C, Takahashi N, Nishibuchi M. Identification of *Vibrio*
۲۹۵ *parahaemolyticus* strains at the species level by PCR targeted to the *toxR* gene. *J Clin Microbiol.*
۲۹۶ 1999;37(4):1173-7.
۲۹۷
- ۲۹۸ 5. Newton AE, Garrett N, Stroika SG, Halpin JL, Turnsek M, Rajal K M, Centers for Disease Control
۲۹۹ and Prevention (CDC). Increase in *Vibrio parahaemolyticus* infections associated with consumption of
۳۰۰ Atlantic Coast shellfish—2013. *MMWR.* 2014;63(15):335-6.
۳۰۱
- ۳۰۲ 6. Haibo Zh, Xinmei L, Zhaoxin L, Antuo H, Wenjie M, Changzheng S, et al. Quantitative detection
۳۰۳ of *Vibrio parahaemolyticus* in aquatic products by duplex droplet digital PCR combined with
۳۰۴ propidium monoazide. *Food Control.* 2023; 144:109353.
۳۰۵
- ۳۰۶ 7. Kunbawui P, Jong S M, Ryu AR, Ji YK, In Tae H, Kil B S. Occurrence and virulence of *Vibrio*
۳۰۷ *parahaemolyticus* isolated from seawater and bivalve shellfish of the Gyeongnam coast, Korea, in
۳۰۸ 2004–2016. *Marine Pollut Bullet.* 2018;137:382-387.
۳۰۹
- ۳۱۰ 8. Yutaka Y, Kaoru H, Masataka S, Isao T, Masatoshi B, Dusit A. Prevalence and antimicrobial
۳۱۱ susceptibility of *Vibrio* species related to food safety isolated from shrimp cultured at inland ponds in
۳۱۲ Thailand. *Food control.* 2014;38 (1):30-6.
۳۱۳
- ۳۱۴ 9. Soltan Dallal MM, Motalebi S, Masoumi H, Sharifi Y MK, Rahimi forushani A. Antimicrobial
۳۱۵ investigation on the multi-state outbreak of salmonellosis and shigellosis in Iran.
۳۱۶ *Med J Islam Rep Iran.* 2020; 34:49.
۳۱۷
- ۳۱۸ 10. Heba A A, Rasha M E B, Mohamed A H, Mariam H E K, Etab M A R, Ahmed M M E. Molecular
۳۱۹ characterization, antibiotic resistance pattern and biofilm formation of *Vibrio parahaemolyticus* and *V.*
۳۲۰ *cholerae* isolated from crustaceans and humans. *Int J Food Microbiol.* 2018;274:31-37.
۳۲۱
- ۳۲۲ 11. Chia W T, Yaya R, Hanan H, Tze Y T, Epeng L, Wendy D R, et al. Prevalence and antibiotic
۳۲۳ resistance patterns of *Vibrio parahaemolyticus* isolated from different types of seafood in Selangor,
۳۲۴ Malaysia. *Saudi J Biol Sci.* 2020; 27(6):1602-08.
۳۲۵
- ۳۲۶ 12. Reshma S, Deborah A, Mohamed A. Prevalence, antibiotic resistance, virulence and plasmid
۳۲۷ profiles of *Vibrio parahaemolyticus* from a tropical estuary and adjoining traditional prawn farm along
۳۲۸ the southwest coast of India. *Ann Microbiol.* 2015; 65(4):2141-49.
۳۲۹
- ۳۳۰ 13. Hyo-Won J, Jin-Ah K, Su-Jin J, Seong-Seon C, Min-Kyeong K, Hye-Jin Y, et al. Prevalence,
۳۳۱ antibiotic-resistance, and virulence characteristics of *Vibrio parahaemolyticus* in restaurant fish
۳۳۲ tanks in seoul, South Korea. *Foodborne Pathog Dis.* 2020; 17(3):209-14.
۳۳۳
- ۳۳۴
- ۳۳۵

- ۳۳۶ 14. Najafi A, Sadeghifar NKH, Bagheri T Y, Salari MH, Shirazi MH, Ranjbar R. Isolation of *Vibrio*
 ۳۳۷ *parahaemolyticus* from fish in Tehran and their antimicrobial resistance. *Iran J Infect Dis Trop Med.*
 ۳۳۸ 2006;11(35): 65-8.
 ۳۳۹
- ۳۴۰ 15. Jalali M MM, Mahdavi M, Javadi A, Khorvash F. Prevalence of *Vibrio parahaemolyticus* in
 ۳۴۱ seafoods in Isfahan. *Iran J Infect Dis Trop Med.* 2010; 14(46):33-6.
 ۳۴۲
- ۳۴۳ 16. Alipour M, Issazadeh K, Soleimani J. Isolation and identification of *Vibrio parahaemolyticus* from
 ۳۴۴ seawater and sediment samples in the southern coast of the Caspian Sea. *Comp Clin Path.* 2012;
 ۳۴۵ 23(1):129-33.
 ۳۴۶
- ۳۴۷ 17. Hindler J, Humphries R, Richter S, Jorgensen J, Bernard K, Killian S, et al. Methods for
 ۳۴۸ antimicrobial dilution and disk susceptibility testing of infrequently isolated or fastidious bacteria. M45,
 ۳۴۹ 3rd edn.
 ۳۵۰
- ۳۵۱ 18. Rahimi E, Ameri M, Doosti A, AR Gholampour. Occurrence of toxigenic *Vibrio parahaemolyticus*
 ۳۵۲ strains in shrimp in Iran. *Foodborne Pathog Dis.* 2010; 7(9):1107-11.
 ۳۵۳ 7(9):1107-11.
 ۳۵۴
- ۳۵۵ 19. Safarpourdehkordi F, Hosseini S, Rahimi E, Momeni M, Yahaghi E, Khodaverdi E. Investigate the
 ۳۵۶ frequency of virulence genes *Vibrio parahaemolyticus* isolated from fish, lobsters and crabs caught
 ۳۵۷ from Persian Gulf. *Iran J Med Microbiol.* 2018; 8(2):1-7.
 ۳۵۸
- ۳۵۹ 20. Hashempour-Baltork F, Jannet B, Tajdar-Oranj B, Aminzare M, Sahebi H, Alizadeh A, et al. A
 ۳۶۰ comprehensive systematic review and health risk assessment of potentially toxic element intakes via
 ۳۶۱ fish consumption in Iran. *Ecotoxicol Env Safety.* 2023;249:114349.
 ۳۶۲
- ۳۶۳ 21. Raissy M, Moumeni M, Ansari M, Rahimi E. Antibiotic resistance pattern of some *Vibrio* strains
 ۳۶۴ isolated from seafood. *Iran J Fisheries Sci.* 2012;11(3):618-26.
 ۳۶۵
- ۳۶۶ 22. Zarei M, Pourmahdi B M, Jamnejad A, Khezzadeh M. Seasonal prevalence of *Vibrio* species in
 ۳۶۷ retail shrimps with an emphasis on *Vibrio parahaemolyticus*. *Food Control.* 2012;25(1):107-9.
 ۳۶۸
 ۳۶۹
- ۳۷۰ 23. Asgarpour D, Haghi F, Zeighami H. Detection and Molecular Characterization of *Vibrio*
 ۳۷۱ *Parahaemolyticus* in Shrimp samples. *Open Biotech J.* 2018; 12: 46-50.
 ۳۷۲
- ۳۷۳ 24. Ottaviani D, Santarelli S, Bacchiocchi S, Masini L, Ghittino C, Bacchiocchi I. Presence of
 ۳۷۴ pathogenic *Vibrio parahaemolyticus* strains in mussels from the Adriatic Sea, Italy. *Food microbiol.*
 ۳۷۵ 2005; 22(6):585-90.
 ۳۷۶
- ۳۷۷ 25. Vengadesh L, Wai-Fong Y, Learn-Han L, Kok-Gan C. Prevalence and antimicrobial susceptibility
 ۳۷۸ of *Vibrio parahaemolyticus* isolated from retail shrimps in Malaysia. *Front Microbiol.* 2015;6:33.
 ۳۷۹
- ۳۷۹ 26. Chang-Ho K, YuJin S, WooRi K, YongGyeong K, KiCheol S, Eun-Gyoung O, et al. Prevalence
 ۳۸۰ and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from oysters in Korea. *Env Sci*
 ۳۸۱ *Pollut Res.* 2016; 23 (1):918-26.
 ۳۸۲
- ۳۸۳ 27. Ying Y, Jiafang X, Hua L, Shuwen T, Yanfeng C, Hui Y. Prevalence, antibiotic susceptibility and
 ۳۸۴ diversity of *Vibrio parahaemolyticus* isolates in seafood from South China. *Front Microbiol.* 2017;
 ۳۸۵ 8:2566.
 ۳۸۶

387
388 28. Zahid H M, Afework K, Alizadeh M, Masayuki Y, Bhuiyan N, Balakrish N. Isolation and molecular
389 characterization of toxigenic *Vibrio parahaemolyticus* from the Kii Channel, Japan. *Microbiol Res.*
390 2006;161(1):25-37.
391
392 29. Manirul H, Anwar H, Shankar C M, Mohammad R, Zahid M. Prevalence, characterization and
393 antibiotic susceptibility of *Vibrio parahaemolyticus* isolated from fishes and shellfishes of coastal
394 regions of Bangladesh. *Dhaka Uni J Biol Sci.* 2015;24(2):121-9.
395
396 30. X Xu, Qingping W, Jumei Z, Jianheng C, Shuhong Z, Kui W. Prevalence, pathogenicity, and
397 serotypes of *Vibrio parahaemolyticus* in shrimp from Chinese retail markets. *Food Control.*
398 2014;46:81-5.
399
400 31. Kshirsagar D, Brahmhatt M, Chatur Y, Sinha N. Studies on occurrence and distribution of virulent
401 strains of *Vibrio Parahaemolyticus* in finfishes and prawns from different ecosystem of Gujarat (India).
402 *J Vet Health Sci.* 2013;11(1):07-10.
403
404
405
406
407
408
409
410
411
412
413
414
415
416
417
418
419
420

٤٢١
٤٢٢
٤٢٣
٤٢٤
٤٢٥
٤٢٦
٤٢٧
٤٢٨
٤٢٩
٤٣٠
٤٣١
٤٣٢
٤٣٣
٤٣٤
٤٣٥
٤٣٦
٤٣٧
٤٣٨
٤٣٩
٤٤٠
٤٤١
٤٤٢
٤٤٣
٤٤٤
٤٤٥
٤٤٦
٤٤٧
٤٤٨
٤٤٩
٤٥٠

Preprint

ε01

ε02

ε03

ε04

ε00

preprint