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Incidence and Phenotypic Pattern of Antibiotic Resistance of *Vibrio* Species Isolated from Seafood Samples Caught from the Persian Gulf

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VIBRIO species, particularly *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. harveyi* are considered as an imperative foodborne pathogens associated with seafood consumption. An existing survey was carried out to determine the incidence and antibiotic resistance of *Vibrio* spp. isolated from diverse kinds of seafood samples. Seven-hundred and forty seafood samples including fish, shrimp, oyster, crab and shellfish were collected from the Boushehr port, Persian Gulf, Iran. Seafood samples were examined by culture method. Identification of *Vibrio* isolates was done by the PCR. Antibiotic resistance of bacteria was assessed by the disk diffusion. Seventy-nine out of 740 (10.67%) seafood samples were contaminated with *Vibrio* spp. Incidence of *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. harveyi* amongst the seafood samples was 18.98%, 41.77%, 13.92% and 10.12%, respectively. Incidence of other *Vibrio* species was 15.18%. The uppermost rate of contamination was found in shellfish (14.66%), shrimp (12%) and oyster (12%). The uppermost incidence of resistance was found toward tetracycline, penicillin, gentamicin, ampicillin, erythromycin and streptomycin. The lowermost rate of resistance was found toward vancomycin, nalidixic acid and azithromycin. Fish, shrimp, crab and oyster samples were considered as the main sources of transmission of *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. harveyi* bacteria. Proper cooking of seafoods before consumption and monitor the antibiotic prescription can reduce the risk of transmission of antibiotic resistant-*Vibrio* spp. through seafood consumption. Nevertheless, supplementary surveys are essential to originate more specifics about the impact of *Vibrio* spp. in seafood samples.

Keywords: *Vibrio* species. Incidence, Seafood, Antibiotic resistance.

Introduction

Seafoods, particularly fish, shrimp, crab, oyster and shellfish, are significant economic and nutrient seafoods. They are rich sources of lipids and indispensable fatty acids and also minerals including magnesium, sodium, calcium, potassium, copper zinc, iron and selenium [1]. They are so popular among people in most regions of the world [1]. Thus, they should have an acceptable level of hygiene and safety [1-3].

Vibrio species are associated with live seafood as they form part of the indigenous microflora of the sea environment [2]. Foodborne infections with *Vibrio* spp. are common all around the world and mainly associated with consumption of raw or undercooked seafoods [3]. Foodborne diseases caused by them are familiar with gastroenteritis, septicemia and even hospitalization and death [2, 3]. Only a few species, particularly *V. parahaemolyticus*, are frequently associated with human foodborne diseases caused by seafood's consumption, nevertheless there are sporadic outbreaks of foodborne diseases caused by other

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Vibrio spp. particularly *V. cholerae*, *V. vulnificus* and *V. harveyi* [2, 3].

V. cholerae, the causative agent of cholera, is a natural inhabitant of aquatic environments, but despite intensive efforts its ecology is still poorly understood [4]. Cholera is a life threatening disease associated with abdominal cramps, diarrhea, fever, vomiting and nausea and the appearance of blood and mucus in the stool of infected persons [4]. *V. parahaemolyticus* infections are considered with abdominal pain, vomiting, watery or bloody diarrhea and gastroenteritis [5]. 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 [5, 6]. *V. vulnificus* is an opportunistic human pathogen that may cause gastroenteritis, necrotizing soft-tissue infections and septicemia, with a boost lethality rate. Consumption of contaminated seafood and exposure of contaminated water are the main ways caused *V. vulnificus* infections [7, 8]. *V.* harveyi is found in the aquatic environment and distinct as nonpathogenic for humans; nevertheless, they are pathogenic for marine animals and they, although infrequently, have associated with infections in humans, particularly, wound infections [9].

Antibiotic therapy is one of the best choices for treatment of human vibriosis. However, *Vibrio* spp. are chiefly resistant toward numerous kinds of antibiotics including aminoglycosides, fluoroquinolone, tetracyclines, sulfonamides, and phenicols. Numerous investigations revealed that the incidence of resistance of *Vibrio* spp. toward commonly used antibiotic agents had a range between 10 to 100%. Thus, it is essential to assess the antibiotic resistance of *Vibrio* spp. recovered from seafood samples [10, 11].

Scarce data are available about the role of seafood samples in transmission of *Vibrio* spp. to human population in Iran. Thus, an existing survey was carried out to assess the incidence and antibiotic resistance of *Vibrio* spp. isolated from fish, shrimp, crab, oyster and shellfish samples.

Materials and Methods

Ethics

The current cross sectional survey was approved by the moral council of research of the Islamic Azad University, Shahrekord, Iran.

Samples

From October 2017 to October 2018, a total of 740 seafood samples including shrimp (n= 350), fish (n= 140), crab (n= 50), oyster (n= 50) 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 transferred to laboratory by ice box.

Isolation of Vibrio spp.

Twenty-five grams of seafood samples were homogenized 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, 13].

Polymerase Chain Reaction (PCR) detection of Vibrio spp.

Vibrio isolates were cultured on nutrient broth (Merck, Germany) and further incubated at 37 °C for 24 h. Principles of producing factory of DNA extraction kit (Thermo Fisher Scientific, Germany) were applied for DNA extraction. Extracted DNA samples were subjected to quantification by NanoDrop device (NanoDrop, Thermo Scientific, Waltham, USA), qualification (2% agarose gel) and purity checking (A260/A280). PCR detection of Vibrio spp. (V. cholerae, V. parahaemolyticus, V. vulnificus and V. harveyi) was conducted rendering beforehand documents (Table 1) [14, 15]. Thermo-cycler device (Flexrcycler, Germany) was used. 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. cholerae ATCC 9459, V. parahaemolyticus ATCC 17802, V. vulnificus ATCC 27562 and V. harveyi ATCC 14126).

TABLE 1. PCR circumstances applied for detection of Vibrio spp.

Target gene	Primer sequence (5'-3')	PCR product (bp)	PCR programs	PCR Volume (50µL)
V. cholerae	F: AAGACCTCAACTGGCGGTA R: GAAGTGTTAGTGATCGCCAGAGT	248	1 cycle: 93 ^{0C} ---------- 15 min. 35 cycle: 92 ^{0C} ---------- 40 s 57 ^{0C} ---------- 60 s 72 ^{0C} ---------- 90 s 1 cycle: 72 ^{0C} ---------- 7 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
V. parahaemolyticus	F: GCAGCTGATCAAAACGTTGAGT R: ATTATCGATCGTGCCACTCAC	897		
V. vulnificus	F: GTCTTAAAGCGGTTGCTGC R: CGCTTCAAGTGCTGGTAGAAG	410		
v. harveyi	F: GAAG CAGCACTCACCGAT R: GGTGAAGACTCATCAGCA	382	1 cycle: 95 ^{0C} ---------- 4 min. 30 cycle: 94^{0C} ---------- 60 s 55 ---------- 60 s 72 ^{0C} ---------- 60 S 1 cycle: 72 ^{0C} ---------- 10 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template
Vibrio spp.	F: CGGTGAAATGCGTAGAGAT R: TTACTAGCGATTCCGAGTTC	663	1 cycle: 93 ^{0C} ---------- 15 min. 35 cycle: 92 ^{0C} ---------- 40 s 57 ^{0C} ---------- 60 s 72 ^{0C} ---------- 90 s 1 cycle: 72 ^{0C} ---------- 7 min	5 µL PCR buffer 10X 2 mM $Mgcl_2$ 150 µM dNTP (Fermentas) 0.75 µM of each primers F & R 1.5 U Taq DNA polymerase (Fermentas) 3 µL DNA template

Antibiotic resistance pattern

Phenotypic profile of antibiotic resistance of Vibrio isolates were examined by disk diffusion test. Mueller–Hinton agar media (Merck, Germany) were applied for this goal. Protocols of the Clinical and Laboratory Standards Institute (CLSI) were applied for this goal [16]. Kirby-Bauer Disk Diffusion Susceptibility Test Protocol was applied for this goal [17]. A total of 0.5 McFarland concentration of bacteria were used for the antibiotic resistance analysis. Diverse antibiotic agents (Oxoid, UK) including ampicillin (10 µg/disk), penicillin G (10 units/disk), cefotaxime (30 µg/disk), cephalothin (30 µg/disk), gentamycin (10 µg/disk), streptomycin (10 µg/disk), erythromycin (15 µg/disk), azithromycin (15 µg/disk), tetracycline (30 µg/disk), ciprofloxacin (5 µg/disk), nalidixic acid (30 µg/disk), trimethoprim-sulfamethoxazole (25 µg/disk), and vancomycin (30 µg/disk) were examined in the antibiotic susceptibility testing. Media contained Vibrio spp. and also antibiotic disks were incubated at 37 °C for 24 h. After that, the diameter of growth inhibition zone were measured and interpreted according to CLSI. V. cholerae ATCC 9459, V. parahaemolyticus ATCC 17802, V. vulnificus ATCC 27562 and V. harveyi ATCC 14126 were applied as quality control microorganisms.

Statistical 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.

Results

Incidence of Vibrio spp. amongst examined seafood samples

Table 2 determines the incidence of *Vibrio* spp. isolated from diverse kinds of seafood samples. Seventy-nine out of 740 (10.67%) seafood samples were contaminated with *Vibrio* spp. Incidence of *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. harveyi* amongst the examined samples was 18.98%, 41.77%, 13.92% and 10.12%, respectively. Totally, 15.18% of examined samples were contaminated with other *Vibrio* spp., particularly *V. alginolyticus, V. mimicus*, *V. fluvialis*, and *V. anguillarum*. Shellfish (14.66%), shrimp (12%) and oyster (12%) were the most normally contaminated seafood samples with *Vibrio* spp. Fish samples (4.28%) had the lowest incidence of *Vibrio* spp. Fish was the most commonly contaminated sample with *V. cholerae* (50%). Shrimp was the most commonly contaminated sample with *V. parahaemolyticus* (45.23%). Crab was the most commonly contaminated sample with *V. vulnificus* (33.33%). Fish and oyster were the most commonly contaminated samples with *V. harveyi* (16.66%). Fish was the most commonly contaminated sample with other *Vibrio* spp. (21.42%). Statistically significant difference was found amid type of seafood samples and incidence of *Vibrio* spp. (P <0.05).

Antibiotic resistance of Vibrio spp.

Table 3 determines the antibiotic resistance pattern of *Vibrio* spp. isolated from diverse kinds of seafood samples. *V. cholerae* isolates displayed the uppermost incidence of resistance toward tetracycline (93.33%), penicillin (88%), gentamicin (86.66%), ampicillin (86.66%), erythromycin (60%) and streptomycin (53.33%) antibiotic agents. *V. parahaemolyticus* isolates displayed the uppermost incidence of resistance toward gentamicin (75.75%), tetracycline (57.57%%), penicillin (57.57%), and erythromycin (48.48%) antibiotic agents. *V. vulnificus* isolates displayed the uppermost incidence of resistance toward gentamicin (90.90%), tetracycline (90.90%), penicillin (90.90%), ampicillin (90.90%) and erythromycin (45.45%) antibiotic agents. *V. harveyi* isolates displayed the uppermost incidence of resistance toward ampicillin (100%), tetracycline (100%), gentamicin (87.50%), penicillin (75%), and erythromycin (62.50%) antibiotic agents. The lowermost incidence of resistance of *Vibrio* spp. was found toward vancomycin, nalidixic acid and azithromycin. Statistically significant difference was found amid type of seafood samples and incidence of antibiotic resistance (P <0.05).

TABLE 2. Incidence of Vibrio spp. isolated from diverse kinds of seafood samples.

Samples	N. samples collected	N. samples positive for Vibrio spp. (%)	N. samples positive for bacteria (%)				
			V. cholerae	V. parahaemolyticus	V. vulnificus	V. harveyi	Other species
Shrimp	350	42 (12)	8 (19.04)	19 (45.23)	3 (7.14)	3 (7.14)	9 (21.42)
Fish	140	6 (4.28)	3 (50)	1 (16.66)	1 (16.66)	1 (16.66)	-
Crab	50	3 (6)	1 (33.33)	1 (33.33)	1 (33.33)	-	-
Oyster	50	6 (12)	1 (16.66)	3 (50)	1 (16.66)	1 (16.66)	-
Shellfish	150	22 (14.66)	2 (9.09)	9 (40.90)	5 (22.72)	3 (13.63)	3 (13.63)
Total	740	79 (10.67)	15 (18.98)	33 (41.77)	11 (13.92)	8 (10.12)	12 (15.18)

TABLE 3. Antibiotic resistance pattern of Vibrio spp. isolated from diverse kinds of seafood samples.

Samples/Bacteria (N. positive) A10		N. isolates resist to each antibiotic agents (%)												
		P10	Cef	Cep	Gen	S10	Ert	Az	Tet	Cip	Nlx	Tri	Van	
Shrimp	*V. cholerae* (8)	6 (75)	5 (62.50)	3 (37.50)	3 (37.50)	7 (87.50)	5 (62.50)	4 (50)	2 (25)	7 (87.50)	2 (25)	1 (12.50)	3 (37.50)	2 (25)
	V. parahaemolyticus (19)	11 (57.89)	9 (47.36)	6 (31.57)	7 (36.84)	13 (68.42)	8 (42.10)	9 (47.36)	5 (26.31)	10 (52.63)	4 (21.05)	4 (21.05)	7 (36.84)	7 (36.84)
	V. vulnificus (3)	3 (100)	3 (100)	1 (33.33)	1 (33.33)	3 (100)	1 (33.33)	2 (66.66)	1 (33.33)	3 (100)	1 (33.33)	-	1 (33.33)	-
	V. harveyi (3)	3 (100)	2 (66.66)	1 (33.33)	1 (33.33)	3 (100)	1 (33.33)	2 (66.66)	-	3 (100)	1 (33.33)	-	1 (33.33)	-
	V. cholerae (3)	3 (100)	3 (100)	1 (33.33)	2 (66.66)	3 (100)	1 (33.33)	2 (66.66)	1 (33.33)	3 (100)	2 (66.66)	1 (33.33)	1 (33.33)	1 (33.33)
Fish	*V. parahaemolyticus* (1)	1 (100)	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	1 (100)	-	1 (100)	-
	V. vulnificus (1)	1 (100)	1 (100)	-	-	1 (100)	-	-	-	1 (100)	1 (100)	-	1 (100)	-
	V. harveyi (1)	1 (100)	1 (100)	-	-	1 (100)	-	-	-	1 (100)	-	-	-	-
	V. cholerae (1)	1 (100)	1 (100)	-	1 (100)	1 (100)	1 (100)	1 (100)	-	1 (100)	1 (100)	1 (100)	1 (100)	-
Crab	*V. parahaemolyticus* (1)	1 (100)	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	1 (100)	-	1 (100)	-
	V. vulnificus (1)	1 (100)	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	1 (100)	-	-	-
	V. harveyi (-)	-	-	-	-	-	-	-	-	-	-	-	-	-
Oyster	*V. cholerae* (1)	1 (100)	1 (100)	-	1 (100)	1 (100)	1 (100)	1 (100)	-	1 (100)	1 (100)	1 (100)	1 (100)	-
	V. parahaemolyticus (3)	3 (100)	1 (33.33)	1 (33.33)	1 (33.33)	3 (100)	1 (33.33)	2 (66.66)	-	3 (100)	1 (33.33)	-	1 (33.33)	-
	V. vulnificus (1)	1 (100)	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	1 (100)	-	-	-
	V. harveyi (1)	1 (100)	1 (100)	-	-	1 (100)	-	1 (100)	-	1 (100)	-	-	1 (100)	-
Shellfish	*V. cholerae* (2)	2 (100)	2 (100)	-	1 (50)	2 (100)	-	1 (50)	1 (50)	2 (100)	1 (50)	-	1 (50)	-
	V. parahaemolyticus (9)	8 (88.88)	7 (77.77)	2 (22.22)	4 (44.44)	7 (77.77)	2 (22.22)	3 (33.33)	3 (33.33)	4 (44.44)	2 (22.22)	1 (11.11)	2 (22.22)	2 (22.22)
	V. vulnificus (5)	4 (80)	4 (80)	1 (20)	3 (60)	4 (80)	-	1 (20)	1 (20)	4 (80)	1 (20)	-	-	1 (20)
	V. harveyi (3)	3 (100)	2 (66.66)	1 (33.33)	1 (33.33)	2 (66.66)	1 (33.33)	2 (66.66)	-	3 (100)	1 (33.33)	-	1 (33.33)	-
Total	*V. cholerae* (15)	13 (86.66)	12 (۸۸)	5 (۳۳/۳۳)	4 (۲۶/۶۶)	13 (۸۶/۶۶)	8 (53.33)	9 (60)	4 (26.66)	14 (93.33)	7 (46.66)	4 (26.66)	7 (46.66)	3 (20)
	V. parahaemolyticus (33)	24 (27.72)	19 (57.57)	9 (27.27)	12 (36.36)	25 (75.75)	11 (33.33)	16 (48.48)	8 (24.24)	19 (57.57)	9 (27.27)	5 (15.15)	12 (36.36)	9 (27.27)
	V. vulnificus (11)	10 (90.90)	10 (90.90)	2 (18.18)	4 (36.36)	10 (90.90)	1 (9.09)	5 (45.45)	2 (18.18)	10 (90.90)	5 (45.45)	-	2 (18.18)	1 (9.09)
	V. harveyi (8)	8 (100)	6 (75)	2 (25)	2 (25)	7 (87.50)	2 (25)	5 (62.50)	-	8 (100)	2 (25)	-	3 (37.50)	-

A10: ampicillin (10 µg/disk), P10: penicillin G (10 units/disk), Cef: cefotaxime (30 µg/disk), Cep: cephalothin (30 µg/disk), Gen: gentamycin (10 µg/disk), S10: streptomycin (10 µg/disk), Ert: erythromycin (15 µg/disk), Az: azithromycin (15 µg/disk), Tet: tetracycline (30 µg/disk), Cip: ciprofloxacin (5 µg/disk), Nlx: nalidixic acid (30 µg/disk), Tri: trimethoprim-sulfamethoxazole (25 µg/disk), Van: vancomycin (30 µg/disk).

Discussion

Seafoods are in close contact with the microbial flora of sea and ocean and also cross contamination in harbors and fishing centers. Thus, two potential source of microbial contamination are existed for contamination of seafoods. *Vibrio* spp. are extensively spread in sea and ocean water, globally. Furthermore, contaminated humans may be reservoir of some *Vibrio* spp. [18].

The incidence of *Vibrio* spp. in the current survey was 10.67% in which shellfish samples had the highest rate of contamination (14.66%). *V. parahaemolyticus* (41.77%) was the most routinely detected bacteria amongst the *Vibrio* spp. Total incidence of *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. harveyi* amongst the *Vibrio* spp. were 18.98%, 41.77%, 13.92% and 10.12%, respectively. The presence of *Vibrio* spp. in seafood samples, particularly 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 *Vibrio* spp. in assessed samples. Moreover, opportunity for occurrence of cross contamination with infected human and staffs of the hunting centers is a conceivable reason for the presence of *Vibrio* spp. in studied samples. Moreover, 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 *Vibrio* spp. in diverse samples. *V. cholerae* had the highest incidence in fish samples (50%). This may be owing to the occurrence of cross contamination by infected staffs and workers because *V. cholerae* is more prone to transmit from humans to seafood samples. *V. harveeyi*, *V. vulnificus* and *V. parahaemolyticus* were detected in low percent of examined fish and crab samples, it has been suggested that the possibility of transmission of these species through the consumption of fish and crab in Iran may be very low. Similarly, lower incidence of *V. cholerae* in crab and oyster, *V. vulnificus* in oyster and *V. harveyi* in oyster may express similar interpretation as above. However, role of fish and crab in transmission of *V. cholerae*, oyster, shrimp, shellfish and crab in transmission of *V. parahaemolyticus*, crab and shellfish in transmission of *V. vulnificus* and finally oyster and shellfish in transmission of *V. harveyi* have been approved in this survey. Moreover, roles of shrimp and shellfish samples have been approved for transmission of other *Vibrio* spp.

Some surveys have been conducted in this field in diverse parts of the world. Messelhäusser *et al*. [19] conveyed the boost incidence of *V. parahaemolyticus*, *V. cholerae* and *V. vulnificus* in seafood and fish samples. Likewise, roles of seafood samples as reservoirs of non-O1 or O139 strains of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* have been determined [3, 18]. Robert-Pillot *et al*. [20] determined that 34.70% of examined seafood 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%). They also revealed that *V. vulnificus* was perceived in crustaceans (16.51%) and fish (9.40%) samples. They also exhibited that only a frozen fish was positive for *V. cholerae*. Thongkao *et al*. [21] reported that the incidence of *V. harveyi*, *V. parahaemolyticus* and *V. vulnificus* amongst the marine shellfishes samples in Thailand was 9.33%, 5.33% and 0%, respectively. An Iranian survey [22] determined that the incidence of *V. vulnificus*, *V. parahaemolyticus*, *V. mimicus*, *V. alginolyticus* and *V. harveyi* in fish and shrimp samples caught from the Persian Gulf was 2.65%, 3.53%, 1.76%, 2.65% and 11.50%, respectively. Raissy *et al*. [23] reported that the incidence of *V. vulnificus*, *V. alginolyticus*, *V. mimicus*, *V. parahaemolyticus* and *V. harveyi* amongst the lobster and crab samples caught from the Persian Gulf, Iran was 13.63%, 9.09%, 4.54%, 3.03% and 3.03%, respectively. Considerable incidence of *Vibrio* spp. in diverse kinds of seafood samples from Iran was reported previously [24, 25]. *V. parahaemolyticus* was the most prevalent *Vibrio* spp. amongst the examined seafood samples. Similarly, *V. parahaemolyticus* was the most prevalent cause of seafood contamination in Vietnam [26], Malaysia [27], China [28] and India [29]. Total incidence of *V. parahaemolyticus* was 47.50% in surveys conducted on diverse kinds of seafood samples in recent years in which overall incidence of bacterium in oyster, clams, fish, shrimp, mussels, scallop and periwinkle was 63.40%, 52.90%, 51.00%, 48.30%, 28.00%, 28.00% and 28.00%, respectively [30]. Similarly, boost incidence of *V. cholerae* in fish samples has been reported from Israel [4], Bangladesh [31], Tanzania [32] and Czech Republic [33]. Similar to findings of the present research, both *V. vulnificus* and *V. harveyi* had lower incidences in

seafood samples examined previously [21, 34-36]. Accordingly, *V. harveyi* is more considered as a pathogen of marine fish, shrimp and invertebrates which caused boost economic burden into the aquacultures [37, 38]. The contamination rate of seafood samples with *Vibrio* spp. vary amid diverse researches. The difference in data advises that time, season, place of sampling, method of sampling, types of samples and even laboratory techniques applied in researches may affect the outcomes of surveys. Moreover, difference hygienic levels of fishing centers may affect the incidence of bacteria in diverse investigations.

Antibiotic selection was done based on their availability, prescription rate (highly prescribed antibiotics were selected) and also principles of the CLSI. Unlawful and vague antibiotic prescription particularly in veterinary is may be the chief reason for the boost incidence of resistance in the *Vibrio* spp. *V. cholerae* isolates had the uppermost and most diverse incidence of resistance to antibiotic agents. These findings are may be owing to the transmission of bacteria from infected humans and staffs. Other *Vibrio* spp. had lower resistance toward examined antibiotic agents because they were often transmitted from the sea to seafood samples, which is not usually an antibiotic source. Boost incidence of resistance of *Vibrio* spp. toward tetracycline, penicillin, gentamicin, ampicillin, erythromycin and streptomycin antibiotic agents was also conveyed from Iran [39], Malaysia [40], Australia [41] and Brazil [42]. Amalina *et al*. [43] conveyed that the incidence of *Vibrio* spp. amongst the seafood samples was 72% in which the incidence of *V. parahaemolyticus*, *V. vulnificus*, *V. cholerae* and *V. harveyi* was 25%, 14%, 3% and 1%, respectively. They exhibited that incidence of resistance of *Vibrio* spp. toward ampicillin, penicillin g, bacitracin, erythromycin, streptomycin, tetracycline and vancomycin was 80%, 80%, 44%, 30%, 14%, 14%, and 54%, respectively. Kumar *et al*. [44] conveyed that *Vibrio* spp. isolated from seafood samples from India were resistant to erythromycin, penicillin and ampicillin antibiotic agents. Boost incidence of resistance of *V. parahaemolyticus* bacteria isolated from seafood samples toward ampicillin was also reported [28, 45, 46]. High incidence of resistance of *Vibrio* spp. against ampicillin and penicillin was also reported [47]. Ampicillin-, amoxicillin- and erythromycin-resistant *V. harveyi* was also reported in fish samples collected from Italy [48]. Oh *et al*. [49] determined that the incidence of resistance of *V. parahaemolyticus* bacteria isolated from seafood samples collected from Republic of Korea toward ampicillin, amoxicillin, cefepime, cefotaxime, streptomycin, gentamicin, amikacin, ciprofloxacin, nalidixic acid, trimethoprim-sulfamethoxazole, chloramphenicol, tetracycline, rifampin and erythromycin antibiotic agents was 57.80%, 0%, 1.40%, 0.9%, 8.70%, 1.80%, 2.80%, 0.50%, 2.80%, 1.40%, 3.70%, 3.70%, 11.90% and 0.90%, respectively. Unconditionally, occurrence of foodborne bacteria, particularly those with an emergence of antibiotic resistance, has been measured amongst other types of Iranian foodstuffs and in some cases veterinary samples [50-59]. Findings of the current investigation can use as a preliminary research about the epidemiology of *Vibrio* spp. in seafood samples to design some useful solutions to prevent outbreaks of foodborne diseases. Full cooking of seafood samples is recommended to decrease the risk of *Vibrio* spp. in seafood samples.

Conclusion

An existing survey is one of the most comprehensive research about the incidence and antibiotic resistance of *Vibrio* spp., particularly *V. parahaemolyticus* bacteria recovered from fish, shrimp, crab, oyster and shellfish samples in the Persian Gulf, Iran. Outcomes signifies boost incidence of *Vibrio* spp. amongst the examined samples. Furthermore, higher incidence of *Vibrio* spp. was found in shellfish, shrimp and oyster samples. The most routinely reservoirs and sources of *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus* and *V. harveyi* bacteria were fish, shrimp, crab and oyster, respectively. Most of isolates were resistant to tetracycline, penicillin, gentamicin, ampicillin, erythromycin and streptomycin antibiotic agents. *V. cholerae* strains had the highest and most diverse incidence of resistance toward antibiotic agents. 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 *Vibrio* spp. in seafood samples.

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References

1. Venugopal, V. Gopakumar and K., Shellfish: Nutritive Value, Health Benefits, and Consumer Safety. *Comprehens.Rev.Food. Sci. Food. Safe,* **16**(6),1219-1242 (2017).
2. Traore, S., Bonfoh, B., Krabi, R., Odermatt, P., Utzinger, J., Rose, K.-N., Tanner, M., Frey, J., Quilici, M.-L. and Koussémon, M., Risk of Vibrio transmission linked to the consumption of crustaceans in coastal towns of Côte d'Ivoire. *J. Food. Protect.*, **75**(6),1004-1011 (2012).
3. Bonnin-Jusserand, M., Copin, S., Le Bris, C., Brauge, T., Gay, M., Brisabois, A., Grard, T. Midelet-Bourdin, G., Vibrio species involved in seafood-borne outbreaks (Vibrio cholerae, V. parahaemolyticus and V. vulnificus): Review of microbiological versus recent molecular detection methods in seafood products. *Critical. Rev. Food. Sci. Nutr.*, **59**(4),597-610 (2019).
4. Halpern, M. and Izhaki, I., Fish as hosts of Vibrio cholerae. *Frontiers in microbiology.* **8**:282 (2017).
5. Ghenem, L., Elhadi, N., Alzahrani, F. Nishibuchi, M., Vibrio parahaemolyticus: A review on distribution, pathogenesis, virulence determinants and epidemiology. *Saudi. J. Med. Medical. Sci.*, **5**(2),93 (2017).
6. Letchumanan, V., Chan, K.G. and Lee, L.H., Vibrio parahaemolyticus: a review on the pathogenesis, prevalence, and advance molecular identification techniques. *Front. Microbiol.*, **5**,Article 705, pp.1-13 (2014). https://doi.org/10.3389/fmicb.2014.00705
7. Heng, S.-P., Letchumanan, V., Deng, C.-Y., Ab Mutalib, N.-S., Khan, T.M., Chuah, L.-H., Chan, K.-G., Goh, B.-H., Pusparajah, P. and Lee, L.H., Vibrio vulnificus: an environmental and clinical burden. *Front. Microbiol.*, **8**, Article 997, pp-1-10(2017). 8:997. doi: ١٠,٣٣٨٩/fmicb.٢٠١٧,٠٠٩٩٧
8. Yun, N.R. and Kim, D.M., Vibrio vulnificus infection: a persistent threat to public health. *Korean. J. Internal. Med.*, **33**(6), Article 1070(2018).
9. Del Gigia-Aguirre, L., Sánchez-Yebra-Romera, W., García-Muñoz, S. and Rodríguez-Maresca, M., First description of wound infection with Vibrio harveyi in Spain. *New. Microb. New. Infect.,* **19**,15-16 (2017).
10. Lee, L.-H. and Raghunath, P., Vibrionaceae diversity, multidrug resistance and management. *Front. Microbiol.*, **9**, Aricl 563, p.1-3 (2018). doi. org/10.3389/fmicb.2018.00563
11. Lee, L.-H., Ab Mutalib, N.-S., Law, J.W.-F., Wong, S.H. and Letchumanan, V., Discovery on antibiotic resistance patterns of Vibrio parahaemolyticus in Selangor reveals carbapenemase producing Vibrio parahaemolyticus in marine and freshwater fish. *Front. Microbiol.*, **9**, Article 2513 (2018). doi: 10.3389/fmicb.2018.02513.
12. Jayasinghe, C., Ahmed, S. and Kariyawasam, M., The isolation and identification of Vibrio species in marine shrimps of Sri Lanka. *J. Food. Agri.*, **1**(1),36-44(2010).
13. ISO, International organization for standards. Specifies a horizontal method for detection of the enteropathogenic Vibrio species, causing illness in or via the intestinal tract, other than Vibrio parahaemolyticus and Vibrio cholerae Include Vibrio fluvialis, Vibrio mimicus and Vibrio vulnificus. (2007).
14. Tarr, C.L., Patel, J.S., Puhr, N.D., Sowers, E.G., Bopp, C.A. and Strockbine, N.A., Identification of Vibrio isolates by a multiplex PCR assay and rpoB sequence determination. *J. Clin. Microbiol.*, **45**(1),134-140 (2007).
15. Pang, L., Zhang, X.H., Zhong, Y., Chen, J., Li, Y. and Austin, B., Identification of Vibrio harveyi using PCR amplification of the toxR gene. *Letters. App. Microbiol.*, **43**(3),249-255 (2006).
16. CLSI, Methods for Antimicrobial Dilution and Disk Susceptibility Testing of Infrequently Isolated or Fastidious Bacteria, Approved. Guideline. (2010).
17. Bayer, A., Kirby, W., Sherris, J. and Turck, M., Antibiotic susceptibility testing by a standardized single disc method. *Am. J. Clin. Pathol.*, **45**(4),493-496 (1966).
18. Baker-Austin, C., Oliver, J.D., Alam, M., Ali, A., Waldor, M.K., Qadri, F. and Martinez-Urtaza, J., Vibrio spp. infections. *Nature. Rev. Dis. Primers.* **4**(1),1-19 (2018).
19. Messelhäusser, U., Colditz, J., Thärigen, D., Kleih, W., Höller, C. and Busch, U., Detection and differentiation of Vibrio spp. in seafood and fish samples with cultural and molecular methods. *Int. J. Food Microbiol.,* **142**(3),360-364 (2010).

20. Robert-Pillot, A., Copin, S., Himber, C., Gay, M. and Quilici, M.L., Occurrence of the three major Vibrio species pathogenic for human in seafood products consumed in France using real-time PCR. *Int. J. Food. Microbiol.*, **189**,75-81 (2014).

21. Thongkao, K. Sudjaroen, Y., Vibrio harveyi, V. parahaemolyticus, and V. vulnificus detection in Thai shellfishes by the triplex PCR method. *Annals of Tropical Medicine and Public Health.* **10** (2), **17,** 417-422 (2017).

22. Raissy, M., Rahimi, E., Azargun, R., Moumeni, M. and Sohrabi, H., Molecular detection of Vibrio spp. in fish and shrimp from the persian gulf. *J. Food. Biosci.Techno.*, **5**(2),49-52 (2015).

23. Raissy, M., Moumeni, M., Ansari, M. and Rahimi, E., Occurrence of Vibrio spp. in lobster and crab from the Persian Gulf. *Journal of Food Safety,* **32**(2),198-203 (2012).

24. Kiani, S., Naghavi, N.S. and Nazari, A., Detection of Vibrio species isolated from ornamental guppy fish in Kashan, Isfahan, Iran fish culturing pounds. BIOLOGICAL JOURNAL OF MICROORGANISM(16), 43-48 (2016).

25. Aghaee, B.L., Shirazi, M.H., Pourmand, M.R., Hosseini, M., Afshar, D., Hajikhani, S. and Shobeiri, S., Phenotypic and Molecular Detection of Pathogenic Vibrio Species in Two Different Regions of the Caspian Sea in Mazandaran, Iran. *J. Med. Bacteriol.*, **4**(3-4),30-34 (2015).

26. Tran, T.H.T., Yanagawa, H., Nguyen, K.T., Hara-Kudo, Y., Taniguchi, T. and Hayashidani, H., Prevalence of Vibrio parahaemolyticus in seafood and water environment in the Mekong Delta, Vietnam. *J. Vet. Med. Sci.*,80(11):1737-1742(2018). doi: 10.1292/jvms..

27. Tan, C.W., Malcolm, T.T., Kuan, C.H., Thung, T.Y., Chang, W.S., Loo, Y.Y., Premarathne, J.M., Ramzi, O.B., Norshafawatie, M.F. and Yusralimuna, N., Prevalence and antimicrobial susceptibility of Vibrio parahaemolyticus isolated from short mackerels (Rastrelliger brachysoma) in Malaysia. *Front. Microbiol.*, **8**, Article 1087 (2017). doi: 10.3389/fmicb.2017.01087. eCollection 2017

28. Yang, Y., Xie, J., Li, H., Tan, S., Chen, Y. and Yu, H., Prevalence, antibiotic susceptibility and diversity of Vibrio parahaemolyticus isolates in seafood from South China. *Fron. Microbiol.*, **8**, Article 2566, pp.1-9(2017). doi: 10.3389/fmicb.2017.02566

29. Reyhanath, P.V. and Kutty, R., Incidence of multidrug resistant Vibrio parahaemolyticus isolated from Ponnani, South India. *Iranian. J. Microbiol.*, **6**(2), 60-67(2014).

30. Odeyemi, O.A., Incidence and prevalence of Vibrio parahaemolyticus in seafood: a systematic review and meta-analysis. *Springerplus.* **5**(1): Article 464 (2016). DOI: 10.1186/s40064-016-2115-7

31. Hossain, Z.Z., Farhana, I., Tulsiani, S.M., Begum, A. and Jensen, P.K., Transmission and toxigenic potential of Vibrio cholerae in hilsha fish (Tenualosa ilisha) for human consumption in Bangladesh. *Front. Microbiol.*, **9**, Article 222(2018). doi: 10.3389/fmicb.2018.00222

32. Hounmanou, Y.M., Mdegela, R.H., Dougnon, T.V., Mhongole, O.J., Mayila, E.S., Malakalinga, J., Makingi, G. and Dalsgaard, A., Toxigenic Vibrio cholerae O1 in vegetables and fish raised in wastewater irrigated fields and stabilization ponds during a non-cholera outbreak period in Morogoro, Tanzania: an environmental health study. *BMC. Res. Notes,* **9**(1), Article 466(2016). doi: 10.1186/s13104-016-2283-0.

33. Rehulka, J., Petras, P., Marejkova, M. and Aldova, E., Vibrio cholerae non-O1/non-O139 infection in fish in the Czech Republic. *Vet. Med. Czech.* **60**,16-22 (2015).

34. Changchai, N. and Saunjit, S., Occurrence of Vibrio parahaemolyticus and Vibrio vulnificus in retail raw oysters from the eastern coast of Thailand. *Southeast. Asian. J. Tropic. Med. Public. Health.*, **45**(3),662-669(2014). PMID: 24974651

35. Cañigral, I., Moreno, Y., Alonso, J.L., González, A. and Ferrús, M.A., Detection of Vibrio vulnificus in seafood, seawater and wastewater samples from a Mediterranean coastal area. *Microbiol. Res.*, **165**(8),657-664 (2010).

36. Givens, C., Bowers, J., DePaola, A., Hollibaugh, J. and Jones, J., Occurrence and distribution of V ibrio vulnificus and V ibrio parahaemolyticus–potential roles for fish, oyster, sediment and water. Letter. App. Microbiol., 58(6),503-510 (2014).

37. Zhou, J., Fang, W., Yang, X., Zhou, S., Hu, L., Li, X., Qi, X., Su, H. and Xie, L., A nonluminescent and highly virulent Vibrio harveyi strain is associated with "bacterial white tail disease" of Litopenaeus vannamei shrimp. PLoS. One, 7(2), e29961(2012). doi: 10.1371/journal.pone.029961.

38. Zhu, Z., Dong, C., Weng, S. and He, J., The high prevalence of pathogenic Vibrio harveyi with multiple antibiotic resistance in scale drop and muscle necrosis disease of the hybrid grouper, Epinephelus fuscoguttatus (♀)× E. lanceolatus (♂), in China. *J. Fish. Dis.*, **41**(4),589-601 (2018).

39. Raissy, M., Moumeni, M., Ansari, M. and Rahimi, E., Antibiotic resistance pattern of some Vibrio strains isolated from seafood. *Iranian Journal of Fisheries Sciences,* **11**(3),618-626 (2012).

40. Tan, C.W., Rukayadi, Y., Hasan, H., Thung, T.Y., Lee, E., Rollon, W.D., Hara, H., Kayal, A.Y., Nishibuchi, M. and Radu, S., Prevalence and antibiotic resistance patterns of Vibrio parahaemolyticus isolated from different types of seafood in Selangor, Malaysia. *Saudi. J. Biol. Sci.*, pages 7, (in press) (2020). 10.1016/j.sjbs.2020.01.002

41. Akinbowale, O.L., Peng, H. and Barton, M., Antimicrobial resistance in bacteria isolated from aquaculture sources in Australia. *J. App. Microbiol.*, **100**(5),1103-1113 (2006).

42. Albuquerque Costa, R., Araújo, R.L., Souza, O.V. and Vieira, R.H.S., Antibiotic-resistant Vibrios in farmed shrimp. *Bio.Med. Res. Int.*, **2015** , Article 505914 (2015).: doi: 10.1155/**2015**/505914.

43. Amalina, N.Z., Santha, S., Zulperi, D., Amal, M.N.A., Yusof, M.T., Zamri-Saad, M. and Ina-Salwany, M.Y., Prevalence, antimicrobial susceptibility and plasmid profiling of Vibrio spp. isolated from cultured groupers in Peninsular Malaysia. *BMC. Microbiol.*, **19**(1),251 (2019). doi: 10.1186/s12866-019-1624-2.

44. Kumar, P.A., Patterson, J. and Karpagam, P., Multiple antibiotic resistance profiles of Vibrio cholerae non-O1 and non-O139. *Jpn. J. Infect. Dis.*, **62**(3),230-232 (2009).

45. Han, F., Walker, R.D., Janes, M.E., Prinyawiwatkul, W. and Ge, B., Antimicrobial susceptibilities of Vibrio parahaemolyticus and Vibrio vulnificus isolates from Louisiana Gulf and retail raw oysters. *Appl. Environ. Microbiol.*, **73**(21),7096-7098 (2007).

46. Oramadike, C. and Ogunbanwo, S.T., Prevalence and antimicrobial susceptibility of Vibrio parahaemolyticus isolated from seafoods in Lagos Lagoon Nigeria. *Cogent. Food. Agri.*, **1**(1),1-10 (2015) . doi.org/10.1080/23311932.2015.1041349

47. Vaseeharan, B., Ramasamy, P., Murugan, T. and Chen, J., In vitro susceptibility of antibiotics against Vibrio spp. and Aeromonas spp. isolated from Penaeus monodon hatcheries and ponds. *Int. Journal Antimicrob. Agents.*, **26**(4),285-291 (2005).

48. Scarano, C., Spanu, C., Ziino, G., Pedonese, F., Dalmasso, A., Spanu, V., Virdis, S. and De Santis, E., Antibiotic resistance of Vibrio species isolated from Sparus aurata reared in Italian mariculture. *New. Microbiol.*, **37**(3),329-337 (2014).

49. Oh, E.-G., Son, K.-T., Yu, H., Lee, T.-S., Lee, H.-J., Shin, S., Kwon, J.-Y., Park, K. and Kim, J., Antimicrobial resistance of Vibrio parahaemolyticus and Vibrio alginolyticus strains isolated from farmed fish in Korea from 2005 through 2007. *J. Food Protect.*, **74**(3),380-386 (2011).

50. Momtaz, H., Davood Rahimian, M. and Safarpoor Dehkordi, F., Identification and characterization of Yersinia enterocolitica isolated from raw chicken meat based on molecular and biological techniques. *J. App. Poultry. Res.*, **22**(1),137-145 (2013).

51. Madahi, H., Rostami, F., Rahimi, E. and Dehkordi, F.S., Prevalence of enterotoxigenic Staphylococcus aureus isolated from chicken nugget in Iran. *Jundishapur. J. Microbiol.* **7**(8),1-6 (2014).

52. Rahimi, E., Yazdanpour, S. and Dehkordi, F., Detection of Toxoplasma gondii antibodies in various poultry meat samples using enzyme linked immuno sorbent assay and its confirmation by polymerase chain reaction. *J. Pure. Appl. Microbio.*, **8**(1),421-427 (2014).

53. Dehkordi, F., Parsaei, P., Saberian, S., Moshkelani, S., Hajshafiei, P., Hosseini, S., Babaei, M. and Ghorbani, M., Prevalence study of Theileria annulata by comparison of four diagnostic techniques in southwest Iran. *Bulgar. J. Vet. Med.*, **15**,123-130 (2012).

54. Ghorbani, F., Gheisari, E. and Dehkordi, F.S., Genotyping of vacA alleles of Helicobacter pylori strains recovered from some Iranian food items. *Tropical Journal of Pharmaceutical Research,* **15**(8),1631-1636 (2016).

55. Rahimi, E., Sepehri, S., Dehkordi, F.S., Shaygan, S. and Momtaz, H., Prevalence of Yersinia species in traditional and commercial dairy products in Isfahan Province, Iran. *Jundishapur. J. Microb.*, **7**(4),1-6 (2014).

56. Safarpoor Dehkordi, F., Khamesipour, F. and Momeni, M., Brucella abortus and Brucella melitensis in Iranian bovine and buffalo semen samples: The first clinical trial on seasonal, Senile and geographical distribution using culture, Conventional and real-time polymerase chain reaction assays. *Kafkas. Üni. Vet. Fakült. Derg.*, **20**(6),821-828 (2014).

57. Safarpoor Dehkordi, F., Haghighi, N., Momtaz, H., Rafsanjani, M.S. and Momeni, M., Conventional vs real-time PCR for detection of bovine herpes virus type 1 in aborted bovine, buffalo and camel foetuses. *Bulgarian. J. Vet. Med.*, **16**(2),102−111 (2013).

58. Safarpoor Dehkordi, F., Barati, S., Momtaz, H., Hosseini Ahari, S.N. and Nejat Dehkordi, S., Comparison of shedding, and antibiotic resistance properties of listeria monocytogenes isolated from milk, feces, urine, and vaginal secretion of bovine, ovine, caprine, buffalo, and camel species in Iran. *Jundishapur. J. Microbiol.*, **6**(3),284-294 (2013).

59. Safarpoor Dehkordi, F., Valizadeh, Y., Birgani, T. and Dehkordi, K., Prevalence study of Brucella melitensis and Brucella abortus in cow's milk using dot enzyme linked immuno sorbent assay and duplex polymerase chain reaction. *J. Pure. Appl. Microbiol.*, **8**,1065-1069 (2014).