Advances in Biological Research 5 (6): 356-365, 2011
ISSN 1992-0067

Occurrence of Potentially Pathogenic *Vibrio* Species in Sea Foods Obtained from Oron Creek

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Abstract: This study reported the occurrence of potentially pathogenic *Vibrio* species in sea foods and water samples obtained from Oron creek. Studies were carried out to isolate, characterized, identify and to quantify the occurrence of different *Vibrio species* associated with sea food: crocker fish, crayfish, aquatic snail, periwinkle and crab obtained from the Oron creek and in samples of water harboring them. For fishes; samples of gills, intestines and body surfaces were analyzed microbiologically while only the body surfaces of other sea food were analyzed on thiosulphate citrate bile salt sucrose (TCBS) agar using standard microbiological techniques. A total of 53 isolates were recovered from samples of sea foods and water and were identified as *Vibrio cholerae*, *Vibrio alginolyticus, Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. The present study showed that out of the 120 seafoods and water samples examined, *Vibrio* was recovered from 44.2% of samples, with 90.0%, 45.0%, 30.0%, 50.0%, 25.0% and 25.0% of fish, crayfish, periwinkle, aquatic snail, crab and water respectively. The frequency of occurrence showed that among all the *Vibrio* species isolated; *Vibrio cholerae* was the most predominant 25(47.2%), this was followed by *Vibrio parahaemolyticus* 10(18.9%), *Vibrio mimicus* 8(15.1%), *Vibrio fluvialis* 7(13.2%) and *Vibrio alginolyticus* 2(3.8%) while *Vibrio vulnificus* was the least predominant 13(1.9%). *Vibrio vulnificus* and *Vibrio alginolyticus* was found only in aquatic snails and fish gills respectively. However, only *V. cholerae*, *V. alginolyticus, V. fluvialis* and *V. parahaemolyticus* was recovered from samples of water. *V. vulnificus* and *V.* mimicus weren't found in the water samples. The results of this study constitute an indicator of bacteriological contamination of a variety of seafood. The presence of these pathogenic organisms in these samples of sea food and water could pose a serious threat and hazard to susceptible people. Thus, sea foods should be adequately cooked before consumption. Good manufacturing practices should always be observed by the trade to minimize the risk of cholera and vibrio food poisoning associated with the consumption of seafood products. Hygienic quality of fish tank water in particular the source water for keeping live seafood is also important.

Key words: Seafoods · Pathogenic Organisms · *V. Parahaemolyticus* · *V. Cholerae* · *V. Alginolyticus* · *V. Fluvialis* · *V. Vulnificus* · *V.* Mimicus

INTRODUCTION

Seafood especially shellfish is a food substrate for some zoonotic vibrios of which these microorganisms, cause food poisoning and diarrhea in human [1]. Sea foods are prone to bacterial contamination and could cause health risk to consumers [2]. Vibrios are associated with live seafood as they form part of the indigenous microflora of the marine environment. Food-borne infections with *Vibrio* spp. are common in Africa. In Nigeria, *V. parahaemolyticus* is continuing to be the top causative agent among all the reported food poisoning outbreaks in recent years [3]. Also in Nigeria, there is a large number of public frozen seafood processing services distributed along the country, where a considerable number of people buy their frozen seafood products daily. Serious consequences relating to national productivity and development can arise from lack of hygiene and sanitation in such outlets [4-5].

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There have been several reports on the health risks associated with the consumption of processed seafood, ranging from allergic reactions, stomach and intestinal cancerous growths and a general degeneration of peripheral cellular tissues, to gradual breakdown of the digestive and excretive systems in a statistically high percentage of people examined. Few of these reports however, have looked at the likely risks from a microbiological food safety point of view [4, 5].

In the last 20 years, many halophilic *Vibrio* species such as *V. parahaemolyticus*, *V. alginolyticus*, *V. vulnificus*, *V. hollisae*, *V. fluvialis V. mimicus*, *V. furnissii and V. damsella*, have been implicated in human enteric infections, wound infections and septicemia due to consumption of shellfish and exposure to seawater [1, 6].

Vibrio parahaemolyticus has been frequently involved in outbreaks of food-borne diseases worldwide [7, 8]. *Vibrio parahaemolyticus* is often isolated from seawater, sediment and a variety of seafood including shrimp, crab, oyster and clam due to its halophilic characteristics [1]. This bacterium is one of the leading causes of food borne gastroenteritis associated with ingestion of undercooked shellfish throughout the world including the United States, China, Japan and Korea [1, 9-11]. Also, this microbial infection is characterized by diarrhea, vomiting, nausea, abdominal cramps and low grade fever [1, 12].

Vibrio cholerae also constitutes a very important risk. *Vibrio cholerae* (also *Kommabacillus*) is a gram negative, facultative, [13] comma-shaped bacterium with a polar flagellum that causes cholera in humans [14, 15]. *V. cholerae* and other species of the genus *Vibrio* belong to the gamma subdivision of the Proteobacteria. There are two major biotypes of *V. cholerae* identified by hemagglutination testing, classical and El Tor and numerous serogroups. *V. cholerae* was first isolated as the cause of cholera by Italian anatomist Filippo Pacini in 1854, but his discovery was not widely known until Robert Koch, working independently thirty years later, publicized the knowledge and the means of fighting the disease [16].

Vibrio vulnifus is another organism of great concern in seafood safety due to the severity of the disease and the high mortality rate it can cause [17]. In addition, *V. vulnificus* is a potentially lethal food borne pathogen and capable of causing primary septicemia and necrotizing wound infections in susceptible individuals [1, 18]. Regarding the public health hazard, vibrios have been implicated in food poisoning and gastroenteritis; Ballal *et al*. [19] and Horseman and Surani [20] reported *V. vulnificus* in their respective studies.

Other species that have been increasingly recognized as food pathogens in recent years are *V. mimicus* and *V. alginolyticus*. *V. mimicus* has genetic and many biochemical similarities to *V. cholerae* and its pathogenicity involves several toxins including that of *V. cholerae*. Many food-borne outbreak cases involving *V. mimicus* have been reported [21].

V. alginolyticus is one of the most common *Vibrio species* occurring in the marine environments and seafood [22-23]. This is an opportunistic pathogen and its pathogenicity is thought to be similar to that of *V. parahaemolyticus* [24]. Strains of the pandemic clone of *Vibrio parahaemolyticus* have been identified as dominant isolates from clinical cases of diarrhea reported in various Southeast Asian countries, including India, Japan, Thailand, Bangladesh, Taiwan and Vietnam, as well as from some cases in the United States [24] but not in the Southern Hemisphere. Most epidemic isolates initially identified were classified within serovar O3:K6, however, more recently, isolates classified in serovars other than O3:K6 have been identified as also forming part of the pandemic clone [24]. Hidalgo *et al*. [25] also found *V. alginolyticus* and *V. fluvialis* in mollscan shellfish in Spain.

Even though epidemiological evidence on outbreaks of food-borne disease is scarce, there are indications that foods could be contaminated to unsafe levels at the points of consumption with air flora and other microorganisms from handlers, equipments/utensils and the raw food materials [26]. Effective hygiene control through bacteriological testing is vital to ensure acceptable levels of contamination and avoid adverse human health consequences of food-borne illness [27-28]. The microbiological safety of food and water is achieved by as far as possible ensuring the absence of pathogenic microorganisms and by all means preventing their multiplication [29].

Thereby, monitoring of pathogenic *Vibrio species* in seafoods and water samples is crucial. Due to limited information available on vibriosis associated with shellfish in Uyo, Akwa Ibom, Nigeria the objectives of this study are to investigate the occurrence of zoonotic vibrios in sea foods products and water samples as well as to evaluate the local situation of *Vibrio* species in seafood products and to make recommendations to reduce risk associated with the consumption of seafood. Thus, this study reported the occurrence of potentially pathogenic *Vibrio* spp. in fresh sea food from oron creek in Uyo, Akwa Ibom State, Nigeria.

MATERIALS AND METHODS

Sample Collection and Processing: One hundred and twenty sea foods and water samples including fish (20); crabs, (20), crayfish (20), periwinkle (20), aquatic snail (20) and water samples (20) were collected from Oron creek in Uyo, Akwa Ibom State, Nigeria then packed in sterile polyethylene bags placed in an ice box while samples of water were placed in sterile containers. For fishes, samples of gills, intestines and body surfaces were analyzed microbiologically while only the body surfaces of other sea food were analyzed on thiosulphate citrate bile salt sucrose (TCBS) agar using standard microbiological techniques. The samples were collected in sterile plastic containers and taken to the laboratory for analysis. The samples of *Penaeus* spp. (shrimp) and others were aseptically removed from the container. After the skin ranging, a sterile forceps and knife appropriate for opening the shell to remove the intestine and gills were used. For shrimps, the shell was peeled and separated from the freshly part and intestine. About 1g was weighed and dispensed into 9ml of sterile distilled water blank and shaken vigorously to mix properly. A ten-fold sterile dilutions were made and an appropriate dilutions (10^{-6} and 10^{-7}) were selected for microbial enumeration using standard pour plate method [30].

Bacteriological Analysis: Bacteriological analysis of seafood samples for *Vibrio* spp. was carried out in duplicates using 25g of crocker fish, crayfish, aquatic snail, periwinkle and crab meat. For each seafood sample, 25g were homogenized in 225ml sterile 0.1% peptone water in a Stomacher 400 Circulator Homogenizer at 120rev/min for 2 minutes. A 10 fold serial dilution in sterile 0.1% peptone water was prepared as described in the Bacteriological Analytical Manual [31]. Spread plate method was carried out using 10^{-2} and 10^{-3} dilutions on TCBS. Analysis of *Vibrio* spp. in the overlying water sample was carried out in duplicates, 1ml of the overlying water was dispensed into 9 ml of 0.1% peptone water. A ten-fold serial dilution of the water sample was done and 10^{-2} and 10^{-3} dilutions were spread plated on TCBS agar. Analysis of *Vibrio spp* in the mud flat sample was carried in duplicates using 25g of mud flat placed in 225ml of 0.1% peptone water and shacked. A ten-fold serial dilution of the mud flat sample was done and 10^{-2} and 10^{-3} dilutions were spread plated on TCBS agar. The TCBS plates were incubated at 37°C for18-24 hours and counts were made for each colony type. Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates. Colonies identifiable as discrete were carefully examined macroscopically for cultural characteristics. All isolates were Gram stained to determine their Gram reaction. The isolates were identified as described by Jolt *et al*. [32] and Oyeleke and Manga [33].

Purification and Conservation of Isolates: For *Vibrio* spp. identification for each sample, 10-20 representative colonies of each of green and yellow colony type were selected from TCBS plates containing 20-200 colonies. A total of 53 isolates was subjected to biochemical tests and sodium chloride tolerance leading to the species characteristics of human pathogenic *Vibrionaceae* commonly encountered in seafood listed in BAM [31].

RESULTS

The present study showed that out of the 120 seafoods and water samples examined, *Vibrio* was recovered from 44.2% of samples, with 90.0%, 45.0%, 30.0%, 50.0%, 25.0% and 25.0% of fish, crayfish, periwinkle, aquatic snail, crab and water respectively (Table 1).

A total of 53 *Vibrio* species was isolated and identified as *Vibrio cholerae*, *Vibrio alginolyticus, Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Table 2 shows the distribution and frequency of occurrence of *Vibrio* spp. isolates from sea foods samples. Among the *Vibrio* species isolated *Vibrio cholerae* was the most predominant 25/53(47.2%), this was followed by *Vibrio parahaemolyticus* 10/53(18.9%), *Vibrio mimicus* 8/53(15.1%), *Vibrio fluvialis* 7/53(13.2%) and *Vibrio alginolyticus* 2/53(3.8%) while *Vibrio vulnificus* was the least predominant 1/53(1.9%). *Vibrio vulnificus* and *Vibrio alginolyticus* were found only in aquatic snails and fish gills respectively (Table 2). However, only *V. cholerae*, *V. alginolyticus, V. fluvialis* and *V. parahaemolyticus* were recovered from samples of water.. *V. vulnificus* and *V.* mimicus weren't found in the water samples (Table 2).

Table 1: Distribution and Frequency of Occurrence of *Vibrio spp*. Isolates from Fresh SeaFood and Water Samples

Samples	No. Tested (%)	No. Positive for Vibrios (%)
Fish	20(16.7)	18(90.0)
Crayfish	20(16.7)	9(45.0)
Periwinkle	20(16.7)	6(30.0)
Aquatic snail	20(16.7)	10(50.0)
Crab	20(16.6)	5(25.0)
Water	20(16.6)	5(25.0)
Total	120(100.0)	53(44.2)

Table 1: Distribution and frequency of occurrence of *Vibrio* spp. isolates from sea foods and water

		Fish							
Vibrio Isolates	No. &(%)	Gills	Skin	Intestine	Aquatic snails	Crab	Crayfish	Periwinkle	Water
Vibrio cholerae	25(47.2)	4(16.0)	3(12.0)	2(8.0)	4(16.0)	3(12.0)	5(20.0)	2(8.0)	2(8.0)
V. parahaemolyticus	10(18.9)	1(10.0)	2(20.0)	2(20.0)	1(10.0)	0(0.0)	1(10.0)	2(20.0)	1(10.0)
Vibrio alginolyticus	2(3.8)	1(50.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(50.0)
Vibrio mimicus	8(15.1)	0(0.0)	1(12.5)	2(25.0)	3(37.5)	0(0.0)	1(12.5)	1(12.5)	0(0.0)
Vibrio fluvialis	7(13.2)	0(0.0)	0(0.0)	0(0.0)	1(14.3)	2(28.6)	2(28.6)	1(14.3)	1(14.3)
Vibrio vunificus	1(1.9)	0(0.0)	0(0.0)	0(0.0)	1(100.0)	0(0.0)	0(0.0)	0(0.0)	0(0.0)
Total	53 (100.0)	6(11.3)	6(11.3)	6(11.3)	10(18.9)	5(9.4)	9(17.0)	6(11.3)	5(9.4)

DISCUSSION

Sea foods are prone to bacterial contamination and could cause health risk to consumers [2]. The food poisoning associated with consumption of shellfish either raw or slightly cooked, contaminated with *Vibrio* spp. causes intestinal infection characterized by diarrhea, abdominal cramps, sickness, vomiting, fever and severe headache [1, 34]. The samples of sea foods and water samples analyzed bacteriologically showed varying degree of *Vibrio* contamination. The overall prevalence of zoonotic vibrios was 44.2% in all the samples, 90.0% in fishes (gills, skin and intestine), 45.0% in crayfish, 30.0% in periwinkle, 50.0% in aquatic snails, 25.0% in crab and 25.0% in water samples. The study also showed that skin, gill and intestine of fishes used in this study were heavily loaded with different species of *Vibrio*.

In the present study, *Vibrio parahaemolyticus, Vibrio mimicus, Vibrio vulnifus* and *Vibrio alginolyticus* occurred in the seafoods. This is in agreement with the studies of Gopal *et al*. [22] and Colakogu *et al.* [23]. *Vibrio cholerae* and Vibrio *parahaemolyticus* were present in the gills, skin, intestine of fish and overlying water. This corresponds to the studies of Amirmozafari *et al*. [35] and Ouseph *et al*. [36]. On the other hand, varying rates were demonstrated in some other studies. Multiple shrimp farm environments from the east and west coast of India were studied for abundance of *Vibrio* spp. [22]. The study by Gopal *et al*. [22] revealed the dominance of *V. alginolyticus*, followed by *V. parahaemolyticus* in east and west coast samples. While in Iran, a report by Hosseini *et al*. [37] showed that only 2.1% of studied shrimp samples harbored *Vibrio* spp. Yang *et al*. [38] revealed 19.0% prevalence of *V. parahaemolyticus* from seafood samples in China while Ji *et al*. [39] reported 58.6% prevalence of *V. vulnificus* in samples from different sources in 10 Chinese cities.

Vibrio chloreae was the most frequently isolated *Vibrio* spp. (47.2%) in the present study, followed by *V. parahaemolyticus* (18.9%), *V. mimcus* (15.1%), *V. fluvialis* (13.2%), *V. alginolyticus* (3.8%) and *V. vulnificus* (1.9%). These percentages are not similar to what was reported by Baffone *et al.* [40] in a similar study, recording *V. alginolyticus* (81.48%) and *V. parahaemolyticus* (14.8%) with greater isolation frequency from mussels. These percentages reported in this present study are not similar to what was reported by Wafaa *et al*. [2] in a similar study, recording that *V. alginolyticus* was the most frequently isolated *Vibrio* spp. (52.5%), followed by *V. parahaemolyticus* (14.1%) and *V. mimcus* (11.5%).

The predominant presence of *Vibrio cholerae* in gills of fish could be due to the fact that gills serve as a filter that filters water that comes into the fish from their water ecosystem.

Vibrio chloreae occurred in the investigated sea foods and water at a percentage of 47.2. This deviate from the findings of other researchers. Caldini *et al*. [41] reported that out of 150 *Vibrio* species isolated from the Arno River basin in Italy, *V. cholerae* non-O1 was the most prevalent species with 82.0% occurrence. This was attributed to the fact that the River basin is a freshwater. In contrast, Adeleye *et al*. [3] reported that out of 44 *Vibrio* species isolated from the sea foods in Lagos, *V. cholerae* was the least prevalent species with 6.8% occurrence.

In this study, the infection rate of *V. alginolyticus* in seafoods and water was 3.8%, with 50.0%% prevalence rate in fish and 50.0% water samples. This result was nearly close to the finding of Baffone *et al*. [40], who reported 8.16% in clams in Italy. Lower incidence of 6.3% from clams in Spain was recorded by Hidalgo *et al*. [25], respectively. The occurrence of *V. alginolyticus* in the analyzed sea foods is comparable to similar observations made by Elhadi *et al.* [42] in studies conducted in Malaysia and in Morocco by Bouchriti and El Marrakchi [43]. Elsewhere, high occurrence of *V. alginolyticus* has been reported in Indonesia by Molitoris *et al*. [44]. This high prevalence may be attributed to their high salt tolerance ability.

Vibrio alginolyticus is associated with white spot in shrimp in India and Taiwan while the zoonotic hazard of this pathogen has been implicated in ear, soft tissue and wound infections in human [1, 45]. Otherwise, previous studies cited higher infection rates for *V. alginolyticus*: Gopal *et al*. [22] reported 17.8% prevalence of *V. alginolyticus* in shrimps in India; Colakoglu *et al*. [23] reported 50.0% prevalence of *V. alginolyticus* in shrimps in Turkey and Hassanin [46] reported 40.0% prevalence of *V. alginolyticus* in shrimps in Egypt. Also, the infection rate of *V. alginolyticus* in water samples (50.0%) in this study contrasts other studies cited by Masini *et al*. [47] who reported 28.5% prevalence of *V. alginolyticus* and Hassanin [46] who reported 60.0% prevalence of *V. alginolyticus* in water.

The family Vibrionaceae is autochthonous to aquatic environments including estuarine, coastal waters and sediments worldwide and some species are well- known pathogens of marine organisms including fish and shellfish [1, 47]. The overall prevalence of *Vibrio* spp. in water samples from Oron creek in Uyo, Akwa Ibom State, Nigeria was 25.0% and 9.8% in this study. This comparable to what was reported by Merwad *et al*. [1], who also reported overall prevalence of 25.0% for *Vibrio* spp. in water samples from Suez Canal in Ismailia province belonging to Egypt. In Table 2, it was clearly that *V. mimicus* and *V. vulnificus* were not detected in water samples collected from Oron creek in Uyo, Akwa Ibom State, Nigeria. On the contrary, higher reports of *V. vulnificus* in water were noticed in other studies: Merwad *et al*. [1] detected *V. vulnificus* in water samples with infection rate of 5.0%.

In the current study, the infection rate of seafoods with *V. parahaemolyticus* was 18.9%; 20.0% in periwinkle, fish skin and intestine and 10.0% in crayfish, aquatic snail and water samples. *Vibrio parahaemolyticus* are regularly linked to human food borne infections caused by consumption of undercooked or recontaminated shellfish, but there are also occasional reports of food-borne or waterborne infections caused by environmental *Vibrio* e.g. *V. mimicus* [1, 48] and *V. alginolyticus* [1, 49]. From zoonotic point of view, *Vibrio parahaemolyticus* may spread into humans orally via contaminated mollscan shellfish particularly oysters leading to development of gastroenteritis with diarrhea [1, 50-51] accounting for 60-80% of cases, wound infections in 34% and 5% have septicemia [1].

The percentage reported for *V. parahaemolyticus* (18.9%) in this study was not comparable to some other studies. Some studies reported higher infection rates of *V. parahaemolyticus* in shrimps [22, 46]. Hassanin [46] reported infection rate of 27.6% in Abu-Kir fishing ground, Egypt; and Gopal *et al*. [22] reported infection rate of 12.2% in West coast farm, India. However, some studies reported lower infection rates of *V. parahaemolyticus* in seafoods. A prevalence of 2.8% in tropical shrimp culture in East coast farm in India was reported by Gopal *et al*. [22]. The percentage of *V. parahaemolyticus* in shrimps harvested from Dardanelles Market in Turkey was zero [1, 23]. In line with the assertions of Parveen *et al*. [52] and Merwad *et al*. [1], the differences between studies concerned percentages of *V. parahaemolyticus* in different seafoods could be attributed to the differences in the range of variation of salinity level and the sample sizes of studies.

Vibrio parahaemolyticus were not detected from crabs as shown in Table 2. This compared favourable with the findings of Merwad *et al*. [1] who reported zero infection rate of *V. parahaemolyticus* in crabs. However, the infection rate of aquatic snails with *V. parahaemolyticus* was 10.0%. Oysters pose high risk of *V. parahaemolyicus* food-borne illness due to their ability to concentrate pathogenic vibrios and toxins during the filter feeding process [1, 53-54]. The 10.0% reported for *V. parahaemolyicus* in aquatic snails deviates from what was reported in other studies elsewhere. Lower values were reported by Merwad *et al*. [1] in Egypt, Baffone *et al*. [40] in Italy, Colakoglu *et al*. [23] in Italy and Turkey, respectively. On the other hand, higher percentages were recorded in previous studies by Pinto *et al*. [12] in Italy who reported 32.6% for mussels; Blanco – Abad *et al*. [55] in Spain who reported 11.2% for mussels and Kirs *et al*. [56] in New Zealand who reported 94.8% for oysters. The higher reports of *V. parahaemolyticus* in previous studies may be associated with the growing of bivalve mollusks in uncontrolled waters subjected to contamination and their peculiar characteristic of filtering large amounts of water [1, 40].

The infection rate of 10.0% was reported for *V. parahaemolyicus* in water samples in this study. On the contrary, higher infection rates of *V. parahaemolyticus* in water were noticed in other previous studies. Mahmoud *et al*. [57] reported 20.8% in Japan and Hassanin [46] reported 40.0% in Abu-Kir fishing farm, Egypt. However, lower infection rates were also reported in some previous studies. Blanco-Abad *et al*. [55] reported 5.6% in Spain, Masini *et al*. [58] reported 6.5% in Italy and Merwad *et al*. [1] reported 0.0% in Egypt. The value reported for *V. parahaemolyticus* in water may be associated with sample size and water turbidity. This finding was supported by Merwad *et al*. [1]

and Zimmerman *et al*. [59], who observed that *V. parahaemolyticus* levels in water are strongly correlated with sample size, water clearness and turbidity.

The total infection rate of *V. fluvialis* in seafoods in this study was 13.2%. *V. fluvialis* was not detected in any parts of the fishes used in this study. This result was higher compared to the finding of Merwad *et al*. [1], who recorded 8.0% in Egypt. Regarding the public health hazard, *V. fluvialis* was reported in a human case of severe watery diarrhea and bacteremia in Taiwan [60]. On the other side, *V. fluvialis* was detected in water samples with a similar infection rate (14.3%) with periwinkle and aquatic snails. Otherwise, low incidence of *V. fluvialis* (0.6%) was reported by Gopal *et al*. [22]. With respects to *V. fluvialis*, crayfish and crabs were more infected (28.6%). Otherwise, lower infection rates were detected in other studies. Gopal *et al*. [22] recorded 4.6% in shrimps and Hidalgo *et al*. [25] obtained 3.7% in clams. The variations in the incidence of *V. fluvialis* in seafoods and water may be accounted for the differences in water contamination levels in many geographic areas [1].

The total infection rate of *V. mimcus* in seafoods in this study was 15.1%. *V. mimcus* was not detected in fish gills, crabs and water samples used in this study. On the other side, *V. mimcus* was detected in higher proportion in aquatic snails (37.5%), followed by fish intestine (25.0%) and similar infection rates of 12.5% in periwinkle, crayfish, crabs and fish skin. This is higher than what was reported by Wafaa *et al*. [2], who detected in 11.5% of the sea food in a similar study.

Vibrio vulnificus is one of the emerging food and waterborne zoonotic bacteria that represents a human health hazard [1, 61]. This pathogen causes gastroenteritis and primary septicemia due to consumption of contaminated oysters, while skin and soft tissue infection results from handling contaminated shellfish or from exposure of open wounds to sea water [1, 20]. The total infection rate of *V. vulnificus* in seafoods in this study was 1.9% and was detected in aquatic snails only. This result was nearly close to the finding of Merwad *et al*. [1], who reported infection rate of *V. vulnificus* to be 2.0% for crabs and oysters in Egypt. On the contrary, higher infection rate of 6.6% and 5.0% was also reported by Merwad *et al*. [1] for shrimps and water samples respectively. Compared with prevalence of *V. vulnificus* in water in the present work, higher percentages of 2.0, 3.7, 5.0 and 32.0 were detected by Masini *et al*. [58], Gugliandolo *et al*. [62], Merwad *et al*. [1] and and Canigral *et al*. [61], respectively. This result also contrasts the findings of Colakoglu *et al*. [23]; who reported higher percentages of 16.6. Also, lower infection rate of 2.2% in East coast shrimp was reported [22]. However, in some other previous studies higher infection rates were reported: Canigral *et al*. [61] reported 10.0% in oysters and Kirs *et al*. [56] reported 17.2% in oysters.

Vibrio vulnificus is an opportunistic human pathogen that is highly lethal and is responsible for the overwhelming majority of reported seafood-related deaths in the United States [63-64]. This bacterium is a part of the natural flora of coastal marine environments worldwide and has been isolated from water, sediments and a variety of seafood, including shrimp, fish, oysters and clams [64-65]. Overall, *V. vulnificus* is a complex microorganism with physiological characteristics that contribute to its survival in the marine environment and in the human host [64]. On average, 34 cases of *V. vulnificus* infection are reported annually by the U.S. Food and Drug Administration [63- 65]. However, in recent years this number has risen dramatically. The CDC reported *Vibrio* infections increase by 78% between 1996 and 2006 and in 2005, 121 cases of *V. vulnificus* disease were confirmed [66]. While reasons for these increases have yet to be determined, it has been noted that outbreaks of *V. vulnificus* disease in Israel are associated with high temperature records [67]. These data suggest that global climate change resulting in higher water temperatures may increase the frequency of *V. vulnificus* disease and influence the global distribution of this pathogen [67]. Despite increases in the number of cases, the rate of infection remains relatively low [64].

Regarding the zoonotic significance, *V. vulnificus* are usually acquired through ingestion of shellfish or through contaminating open wounds during swimming, crabbing, shellfish cleaning and other marine activities as was previously sustained and are implicated in epidemic human gastroenteritis [1, 19]. Outbreaks of *Vibrio vulnificus* wound infections in Israel were previously attributed to tilapia aquaculture [68]. In a study by Zahid *et al*. [68], *V. vulnificus* was frequently isolated from coastal but not freshwater aquaculture in Bangladesh. Tilapia in Bangladesh is cultured in conjunction with shrimp and other local fish species [68]. *Vibrio vulnificus* is a leading cause of seafood-related deaths in the United States [69]. This bacterium is an uncommon but serious cause of human illness due to the consumption of raw or undercooked seafood, especially oysters [70]. As a matter of fact, *V. vulnificus* has been regarded as the predominant cause (95%) of seafood-related deaths in the United States, responsible for approximately 30 deaths annually [70]. Additionally, the CDC's recent FoodNet report suggested a continued increase in *Vibrio* incidences since 2001, pointing to a need for improved prevention measures [70]. In this regard, rapid and reliable detection methods are particularly needed to facilitate better control of potential *V. vulnificus* risks in raw oysters [69].

From zoonotic point of view, detection of *V. parahaemolyticus* in other seafoods suggests a probable risk for health of people consuming raw seafood. Therefore, it is recommended to pay attention to postharvest handling and adequate cooking to safeguard public health.

The finding of this study with regards to the high contamination of *V. parahaemolyticus* in these sea foods and in the overlying water is in concurrence with studies of Thararat *et al*. [71]; and Aberoumand [72], since *Vibrio spp* are widely distributed in marine environment and has been studied extensively by various researchers [71-73].

Vibrio parahaemolyticus, a Gram-negative halophilic bacterium, is responsible for human gastroenteritis worldwide and sporadic cases and outbreaks occur regularly in Asia as well as in other countries [74]. *Vibrio parahaemolyticus* is a major cause of gastroenteritis worldwide [74]. A total of 95 *V. parahaemolyticus* isolates belonging to 23 different serovars were identified in a case–control study of expatriates and Thai adults from 2001 to 2002 in Thailand [74]. Three well-known serovars of pandemic *V. parahaemolyticus* isolates were identified as a major cause of diarrhea in Thailand and a new *V. parahaemolyticus* isolate, serovar O3:K46, with pandemic traits was detected in a study by Serichantalergs *et al*. [74]. Subsequently, O3:K6 clones have disseminated throughout South America [24].

This study revealed eight Vibrio spp. from shellfish and all of them have a zoonotic importance. Therefore, the surveillance of contaminant *Vibrio* in shellfish is crucial for sustenance of public health. In this study the presence of contaminating bacteria in seafoods could be attributed to cross-contamination from environment, source and handling by the sellers [5]. Most microorganisms found on the samples indicate that the samples might have been contaminated from handles and environment processing due to unsanitary practices and processing condition. Some authors had earlier stated that *Vibrio* species are basically used as test organism for recent contamination of stream water. The presence of this organism cause gastroenteritis and it has great implication on human health. The results of this study constitute an indicator of bacteriological contamination of a variety of seafood. However, sea foods should be properly cooked before consumption and good quality control measures should be adopted in culturing, processing, harvesting and consumption of sea foods.

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