ISSN: 2754-477X

Journal of Food Technology & Nutrition Sciences

SCIENTIFIC Research and Community

Research Article

Open Access

Vibrio Alginolyticus in Marine Foods of Animal Origin From Establishments in the Port of Chabihau, Yucatan, Mexico

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ABSTRACT

To determine if the marine foods of animal origin that are sold in establishments in the port of Chabihau, Yucatan, Mexico, represent potential risk factors for the Vibrio alginolyticus species for the development of acute gastroenteritis, wound infection, ear infection and primary septicemia. Three hundred and ninety seafood samples were studied: [298 (76.41%) raw, 8 (2.05%) marinated without heat, 77 (19.74%) partially cooked with heat and 7 (1.79%) completely cooked with heat]. For the homogenization and enrichment of the samples, as well as for the isolation and identification of the *Vibrio alginolyticus* species, we proceeded according to the methodology described in the eighth edition of the Bacteriological Analytical Manual (FDA). The criteria used in conducting the hypothesis tests for the difference between two percentages was based on the recommendations made by Cochran. The prevalences obtained in raw seafood, marinated without heat, partially cooked with heat and completely cooked with heat were, respectively, 7.72% (23/298), 0.00% (0/8), 7.79% (6/77) and 0.00% (0/7). In 29 (7.44%) samples an equal number of strains were isolated whose biochemical characteristics corresponded to the Vibrio alginolyticus species. It is concluded that raw seafood and partially cooked seafood represent potential risk factors for the *Vibrio alginolyticus* species for the development of acute gastroenteritis, wound infection, ear infection and primary septicemia.

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Received: June 03, 2021; **Accepted:** June 11, 2021; **Published:** June 13, 2021

Keywords: Vibrio Alginolyticus, Seafood, Establishments

Introduction

In the second edition of the Bergey Manual of Systematic Bacteriology the Family Vibrionaceae belongs to Order XI (*Vibrionales*) of Class III (*Gammaproteobacteria*) of the Phylum BXII (*Proteobacteria*) of the Domain *Bacteria*. In turn, the *Vibrionaceae* Family is made up of the *Vibrio*, *Allomonas*, *Catenococcus*, *Enterovibrio*, *Grimontia*, *Listonella*, *Photobacterium* and *Salinivibrio* genera. Twelve of the sixty–six species of the genus *Vibrio* are considered human pathogens and are *Vibrio alginolyticus*, *Vibrio carchariae*, *Vibrio cholerae*, *Vibrio cincinnatiensis*, *Vibrio damsela*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio parahaemolyticus*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* [1].

Vibrios are characteristically indigenous to marine, brackish, and estuarine habitats, and appear in large concentrations (*blooms*) when water temperatures rise (17–20°C). At low temperatures the vibrios remain in the sediment of the seabed and the counts are usually lower than those necessary to cause infection. In temperate countries, vibrios are present in seawater throughout the year, although their concentration experiences a notable increase in warm months due to favorable ecological conditions and plankton, increasing their accumulation by filter feeders and other marine animals [2].

Vibrio alginolyticus species, previously known as *Vibrio parahaemolyticus* biotype II, is the most halotolerant species; it supports a concentration of up to 10% of sodium chloride (NaCl) and is the most abundant in seawater; it is very common in the marine habitat of temperate countries [3,4]. It causes gastrointestinal infections in man and occasionally extra–intestinal. It was not considered pathogenic until 1973 but, since then, the number of infectious processes in which it has been implicated has been increasing [5]. It has been associated with enteritis, soft tissue infections abscesses, bacteremia, conjunctivitis, peritonitis, external conjunctivitis and external otitis [6-13]. In the case of otitis, there is often an underlying otic pathology [14]. Most of these infections are acquired by exposure to the marine environment or by contact with products derived from it. Vibrio alginolyticus is a ubiquitous organism in the sea and estuaries. Its distribution is worldwide; usually on the coasts of temperate countries; it is found in any marine organism such as fish, clams, oysters and corals, among others. It is a short, pleomorphic, large–negative bacillus, mobile by peritrichous and polar flagella. The signs and symptoms in humans are due to the ingestion of raw and/or insufficiently cooked marine products, or to damage to the skin when swimming in water where this microorganism is present. *Vibrio alginolyticus* is associated with acute gastroenteritis, wound infection, ear infection, and primary septicemia [15].

According to their method of preparation, seafood was classified into raw seafood, non–heat marinated seafood, partially cooked seafood with heat, and completely cooked seafood with heat. There were three varieties (crustaceans, mollusks and fish) and forty–two were the species studied (catfish, bosh or curucó; balá

or whip ray, sea bream, boquinete, squid, shrimp, crab, snail, carp, gut dogfish, cherna pinta, chopa, chucumite, cuddly, sea bass, cuberita, smedregal, blackfin mackerel, crab, black horse mackerel, smooth, longtail, largemouth bass, manta ray, mussel, grouper, white mojarra, carp mojarra, tilapia mojarra, oyster, string vine, snapper mulatto, pejelagarto, wahoo, octopus, ray, snook, villajaiba blonde, salmon, sierra, tilapia and white trout).

The objective of the present investigation was to determine the prevalences of the *Vibrio alginolyticus* species in raw marine foods, marine foods marinated without heat, marine foods partially cooked with heat and marine foods completely cooked with heat. In other words, determining whether raw seafood, non–heat marinated seafood, partially heat–cooked seafood, and completely heat–cooked seafood represent potential risk factors for *Vibrio alginolyticus* for the development of acute gastroenteritis, wound infection, ear infection and primary septicemia [15].

Null hypothesis (H_0). Raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat are not contaminated with Vibrio alginolyticus.Alternative hypothesis (H1). Raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat are contaminated with *Vibrio alginolyticus*.

Antecedents

The species *Vibrio alginolyticus* was isolated for the first time by Miyamoto et al. Reyes–Velázquez et al., carried out an investigation whose objective was to determine the concentrations of the species *Vibrio alginolyticus* and *Vibrio parahaemolyticus* in *Crassostrea virginica* of the lagoon system of Mandinga, Veracruz, Mexico, and their relationship with salinity and temperature of the water [3,16]. The times of greatest production and consumption of oysters were considered, that is, dry times and rainy seasons corresponding to 2008. Four sampling sites were selected and three samples were taken per site and per season, resulting in a total of twenty–four samples. Each sample consisted of thirty commercial–size microorganisms that were analyzed using the serial dilution technique. A biochemical characterization of the colonies found was made to determine the concentrations of Vibrio alginolyticus and *Vibrio parahaemolyticus* species in Most Probable Number per gram (MPN/g), whose values ranged from < 3 to 150 MPN/g. No correlations were found between the concentration of the *Vibrio alginolyticus* species and the temperature and salinity of the water in the rainy season. However, a positive correlation between the temperature and the concentration of the *Vibrio parahaemolyticus* species could be observed ($r= 0.69$; $p < 0.0500$), while the correlation of the concentration of the *Vibrio parahaemolyticus* species and salinity was negative ($r= –0.68$; $p < 0.0500$). The main contribution of this research work was the establishment of both safe zones and periods of oyster extraction. *Vibrio alginolyticus* is the most halotolerant species of the genus Vibrio since it supports concentrations of up to 10% of NaCl and is the most abundant species in seawater; it is very common in the oceanic habitat of temperate countries. The *Vibrio alginolyticus* species is a risk factor for the development of gastrointestinal infections in humans and, sporadically, for the development of extra–intestinal infections. It has low virulence and is frequently associated with other pathogenic microorganisms; its invasive power is low and the infections it causes are usually benign and self–limited. It was not considered a pathogenic species until 1973 but, since then, the number of infectious processes in which it has been implicated has been increasing. It has been associated with enteritis, soft tissue infections–especially in wounds and burns–and external otitis. Most of these infections are acquired by exposure to seawater or by contact with products derived from it. Clinically, the *Vibrio alginolyticus* species is associated with acute gastroenteritis, wound infection, ear infection, and primary septicemia.

Vibrio alginolyticus causes soft tissue and bloodstream infection; little systematically collected clinical and epidemiological information is available. In the USA, *Vibrio alginolyticus* infections are reported to the Cholera and Other *Vibrio* Illness Surveillance system. Using data from 1988 to 2012, we categorized infections using specimen source and exposure history, analyzed case characteristics, and calculated incidence rates using US Census Bureau data. Most (96%) of the 1331 *Vibrio alginolyticus* infections were from coastal states. Infections of the skin and ear were most frequent (87%); ear infections occurred more commonly in children, lower extremity infections more commonly in older adults. Most (86%) infections involved water activity. Reported incidence of infections increased 12–fold over the study period, although the extent of diagnostic or surveillance bias is unclear. Prevention efforts should target waterborne transmission in coastal areas and provider education to promote more rapid diagnosis and prevent complications [17].

This study was conducted to determine the prevalence of *Vibrio spp*., in retail seafood in Berlin, Germany. A total of 160 raw seafood samples from supermarkets and seafood shops, consisting of shrimp (n= 80) and bivalves (n= 80), were investigated for the presence of Vibrio spp., using the International Organization for Standardization ISO/TS 21872 method and a multiplex PCR. The overall prevalence of *Vibrio spp*., in retail seafood was 55% (95% CI: 47.2 to 62.8%). The prevalence of *Vibrio spp*., in shrimp was slightly higher than in bivalves (57.5 *versus* 52.5%); however, the difference was not statistically significant. *Vibrio alginolyticus* was the most prevalent species (35.6%), followed by *Vibrio parahaemolyticus* (27.5%), Vibrio cholerae (6.3%), and Vibrio vulnificus (0.6%). None of the *Vibrio parahaemolyticus* (n= 110) isolates encoded tdh/trh genes, whereas all *Vibrio cholerae* isolates (n= 27) were lacking ctxA. Among the chilled samples (n= 105), the prevalence of *Vibrio spp*., in unpacked samples was significantly higher than in packed samples (p= 0.006). Among the packed samples (n= 55), no significant difference in the prevalence of *Vibrio spp*., was observed between chilled or frozen products. The results of this study indicated a high prevalence of *Vibrio spp*., in retail seafood in Germany; positive samples were detected in all types of seafood investigated. The detection of tdh/trh–negative *Vibrio parahaemolyticus* isolates should not be neglected because of previous findings on pathogenic strains lacking these virulence markers. Even though thorough cooking might limit the risk of foodborne illness caused by *Vibrio*, potential cross–contamination during preparation or consumption of raw and undercooked seafood might represent a risk of *Vibrio* infections [18].

Numerous prevalence studies of *Vibrio spp*., infection in fish have been extensively reported worldwide, including Malaysia. Unfortunately, information on the prevalence of *Vibrio spp*., in groupers (*Epinephelus spp*) is limited. In this study, groupers obtained from nine farms located at different geographical regions in Malaysia were sampled for the presence of pathogenic *Vibrio spp*., and their susceptibility profiles against seven antibiotics. Out of 270 grouper samples, 195 (72%) were detected with the presence of *Vibrio spp*., *Vibrio communis* showed highest prevalence in grouper (28%), followed by *Vibrio parahaemolyticus* (25%), *Vibrio alginolyticus* (19%), *Vibrio vulnificus* (14%), *Vibrio rotiferianus* (3%), *Vibrio spp*., (3%), *Vibrio campbellii* (2%), *Vibrio mytili* (2%), *Vibrio furnissii* (2%), *Vibrio harveyi* (1%), *Vibrio tubiashii* (1%), *Vibrio fluvialis* (0.3%) and *Vibrio diabolicus* (0.3%). Assessment on the antibiotic susceptibility profiles of the *Vibrio spp*., revealed

that majority of the isolates were susceptible to tetracycline, streptomycin, erythromycin and bacitracin, but resistance to ampicillin, penicillin G and vancomycin. The mean MAR index of the *Vibrio* isolates was 0.51, with 85% of the isolates showed MAR index value of higher than 0.2. Results indicate that the Vibrio spp., were continuously exposed to antibiotics. Furthermore, the plasmid profiles of *Vibrio spp*., showed that 38.7% of the isolates harbored plasmid with molecular weight of more than 10 kb, while 61.3% were without plasmid. During curing process, *Vibrio spp*., lost their plasmid, but remained resistant to ampicillin, penicillin G, bacitracin and vancomycin while a few isolates remained resistant to erythromycin, streptomycin and tetracycline. The results suggested that the resistance to antibiotics in isolated *Vibrio spp*., might be due to chromosomal and plasmid borne. This study demonstrates the prevalence of *Vibrio spp*., in groupers and the distribution of multidrug resistance strains that could be of concern to the farmers in Malaysia. In addition, data from this study can be further used in fish disease management plan [19].

Infection of seafood with pathogenic species of the genus *Vibrio* causes human food–borne illnesses. This study was executed to examine the antimicrobial resistance phenotypes, biofilm–forming capability and virulence–associated genes of *Vibrio* from fish and shellfishes. Three hundred fresh water and marine fish and shellfish samples were collected from wet markets and supermarkets in Mansoura, Egypt. Bacteriological examination and PCR amplification identified 92 *Vibrio spp*., including 42 *Vibrio parahaemolyticus* and 50 *Vibrio alginolyticus* isolates from the examined fish and shellfish (infection rate: 30.67%). However, *Vibrio vulnificus* was not found in this study. *Vibrio spp*., exhibited variable frequencies of antimicrobial resistance with higher percentages to ampicillin and penicillin. Multidrug resistance (MDR) was detected in 69.04 and 38% of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* respectively. PCR testing of virulence genes, tdh, trh and tlh revealed the presence of tlh and trh in 100 and 11.9% of *Vibrio parahaemolyticus* isolates respectively and none of *Vibrio alginolyticus* carried any of these genes. Biofilm–forming capability was displayed by 76% of *Vibrio parahaemolyticus* and 73.8% of *Vibrio alginolyticus* isolates. Both *Vibrio parahaemolyticus* and *Vibrio alginolyticus* showed no significant weak positive correlations ($r< 0.4$) between antimicrobial pairs belonging to different classes; however, a significant positive correlation ($p< 0.0500$) between trh and resistance to erythromycin ($r= 0.45$) and imipenem ($r= 0.38$) was only identified in *Vibrio parahaemolyticus*. This study reports the existence of MDR strains of *Vibrio parahaemolyticus* and *Vibrio alginolyticus* from the common types of fishes and shellfishes in Egypt. Furthermore, the presence of virulence genes in these isolates and the ability to produce a biofilm in *vitro* pose potential health hazards to consumers. Frequent monitoring of seafood for the presence of *Vibrio spp*., and their antimicrobial susceptibility, virulence determinants and biofilm–forming capability is important for assessing the risk posed by these organisms to the public and for improving food [20].

Seafood samples obtained in seafood markets and supermarkets at 11 sites selected from four states in Malaysia were examined for the presence of nine potentially pathogenic species from the genus Vibrio between July 1998 and June 1999. We examined 768 sample sets that included shrimp, squid, crab, cockles, and mussels. We extensively examined shrimp samples from Selangor State to determine seasonal variation of *Vibrio* populations. Eight potentially pathogenic *Vibrio* species were detected, with overall incidence in the samples at 4.6% for *Vibrio cholerae*, 4.7% for *Vibrio parahaemolyticus*, 6.0% for *Vibrio vulnificus*, 11% for *Vibrio alginolyticus*, 9.9% for *Vibrio metschnikovii*, 1.3% for *Vibrio mimicus*, 13% for *Vibrio damsela*, 7.6% for *Vibrio fluvialis*, and 52% for a combined population of all of the above. As many as eight *Vibrio* species were detected in shrimp and only four in squid and peel mussels. The overall percent incidence of any of the eight *vibrios* was highest (82%) in cockles (Anadara granosa) among the seafoods examined and was highest (100%) in Kuching, Sarawak State, and lowest (25%) in Penang, Pulau Penang State, among the sampling sites. Of 97 strains of *Vibrio cholerae* isolated, one strain belonged to the 01 serotype and 14 to the 0139 serotype. The results indicate that the various seafood markets in Malaysia are contaminated with potentially pathogenic *Vibrio* species regardless of the season and suggest that there is a need for adequate consumer protection measures [21].

Material and methods

Epistemological approach

Quantitative approach, probabilistic approach or positivist approach [22].

Study design

Descriptive, cross–sectional observational study with no directionality and prospective temporality [23].

Study universe

The study was carried out in the total of samples of the twenty one establishments specialized in the sale of seafood for human consumption in the port of Chabihau, Yucatan, Mexico, in the period from June 1, 2019 to May 31, 2020.

Chabihau, Yucatan, Mexico (Figure 1). Chabihau is a Yucatan fishing port in Mexico belonging to the municipality of Yobain and located on the northern coast of the Yucatan peninsula. It is located on the northern coast of the state of Yucatan, 58 km east of the city and port of Progreso de Castro, between the town of San Crisanto (Sinanche municipality) to the East and Santa Clara (Dzidzantun municipality) to the West and directly to the North connected with a paved highway with Yobain, the municipal seat of the homonymous municipality. The term Chabihau in the Mayan language means "where the anteater" (Chab) "opens the way" (haw). It is a patronymic and toponymic. There are no recorded data on the foundation of the current population of Chabihau, although it is known that there was a Mayan occupation before the conquest of Yucatan in the region, which belonged to the chiefdom or jurisdiction of Ah Kin Chel. Towards 1825 the town belonged to the Partido de la Costa whose head was Izamal. Later, Chabihau became part of the jurisdiction of Dzilam Gonzalez. Finally, as of 1921, the port is part of the municipality of Yobain. Ecotourism has become one of the main activities in the region. The entire area of beaches on the north coast of the peninsula is a natural attraction that attracts a large number of local and foreign tourists. There is a small archaeological site of the pre–Hispanic Mayan civilization called Chabihau near the town [24].

Figure 1: Chabihau, Yucatan, Mexico

Operational definitions of variables

Establishments

Establishments that sell seafood of animal origin for human consumption and that have a health license issued by the Health Services of the state of Yucatán [25].

Marine food

Any product of animal origin from the sea that provides the human body with elements for its nutrition [25].

Raw marine food

Any product of animal origin from the sea that provides the human body with elements for its nutrition and that at the time of sampling has been found in its natural state [25].

Marinated seafood without heat

Any product of animal origin from the sea that provides the human body with elements for its nutrition and that at the time of sampling has been found cooked using the action of the acid of lemon juice, the acid of orange juice and vinegar, among others. [25].

Sea food partially cooked with heat

Any product of animal origin from the sea that provides the human body with elements for its nutrition and that at the time of sampling has been found prepared in the following way: a) heat water to boiling; b) turn off the heat source and add the marine food; c) let the seafood "soften" in the hot water for at least 5 min; and d) transferring the marine food to a container allowing it to stand until cool. This food is ready to be used in the preparation of ceviche and / or cocktails [25].

Sea food fully cooked with heat

Any product of animal origin from the sea that provides the human body with elements for its nutrition and that at the time of sampling has been found cooked using the action of heat (grilled, fried and steam, among others) [25].

Techniques and procedures

A first visit was made to each of the establishments specialized in the sale of seafood for human consumption and a list of one hundred ninety six samples was compiled which, according to their method of preparation, were classified into raw seafood, non–heat marinated seafood, partially cooked seafood with heat and completely cooked seafood with heat. The establishments received a second visit (in the period from June 1, 2019 to May 31, 2020) during which the samples were obtained.

Each sample weighed approximately 50 g; individually stored in sterile polyethylene bag (Ziploc); it was kept refrigerated; and it was sent for processing to Laboratorios Micro–Clin, S.A. de C.V., from the city of Merida. The processing of the samples was carried out in the period from June 1, 2019 to May 31, 2020.

For the homogenization and enrichment of each sample, as well as for the isolation and identification of the *Vibrio alginolyticus* species, we proceeded according to the methodology described in the eighth edition of the Bacteriological Analytical Manual (FDA) [26].

Homogenization

With the help of a sterile scalpel and a sterile anatomical forceps with a tooth, 25 g were weighed into a sterile Petri dish; transferred to a sterile 200 ml blender glass; 125 ml of broth peptonized with 3% NaCl were added; and the contents were liquefied at low speed for 1 min;

Enrichment

1 ml of the resulting suspension was transferred to a culture tube containing 9 ml of broth peptonized with 3% NaCl; and it was incubated at 35–37 ° C for 18–24 h;

Isolation

The growth on the surface was reseeded by streaks on a Thiosulfate – Citrate – Bile salts – Sucrose agar plate (TCBS agar) and on a modified Cellobiose – Polymyxin B – Colistin agar plate (mCPC agar); incubated at 35–37 ° C for 18–24 h; From the green pigmented colonies that developed, smears were made to stain by the Gram method; when the colonies consisted of curved or slightly curved gram-negative bacilli, the oxidase test was performed as a presumptive test;

Identificación

Colonies that successfully passed the presumptive test, that is, that were positive to the oxidase test, underwent the following complementary biochemical tests: production of arginine dehydrolase; ornithine decarboxylation; decarboxylation of lysine; growth on nutrient agar with 0% NaCl; growth on nutrient agar with 3% NaCl; growth on nutrient agar with 6% NaCl; growth on nutrient agar with 8% NaCl; growth on nutrient agar with 10% NaCl; growth at 42 °C (107.6 ° F); fermentative metabolism of sucrose; fermentative metabolism of D-cellobiose; fermentative metabolism of lactose; fermentative metabolism of arabinose; fermentative metabolism of D-mannose; fermentative metabolism of D-mannitol; hydrolysis of o-nitro-D-galactopyranoside (ONPG); Voges – Proskauer reaction; and gelatin liquefaction.

Table 1 shows the differential characteristics or biochemical properties of the *Vibrio alginolyticus* species associated with clinical syndromes related to the consumption of fish and shellfish.

Table 1: *Vibrio alginolyticus* according to differential characteristics or biochemical properties

Differential characteristics o biochemical properties	Vibrio alginolyticus
1. TCBS agar	A
2. mCPC agar	NG
3. Oxidase	+
4. Production of arginine dehydrolase	–
5. Decarboxylation of ornithine	+
6. Decarboxylation of lysine	+
7. Growth in nutrient agar with 0% NaCl	–
8. Growth in nutrient agar with 3% NaCl	+
9. Growth in nutrient agar with 6% NaCl	+
10. Growth in nutrient agar with 8% NaCl	+
11. Growth in nutrient agar with 10% NaCl	+
12. Growth at 42°C (107.6°F)	+
13. Fermentative metabolism of sucrose	+
14. Fermentative metabolism of D–cellobiose	–
15. Fermentative metabolism of lactose	–
16. Fermentative metabolism of arabinose	–
17. Fermentative metabolism of D–mannose	+
18. Fermentative metabolism of D–mannitol	+
19. ONPG	–
20. Voges–Proskauer reaction	+

Citation: José Franco-Monsreal, Lidia Esther del Socorro Serralta-Peraza, Javier Jesús Flores-Abuxapqui (2021) Vibrio Alginolyticus in Marine Foods of Animal Origin From Establishments in the Port of Chabihau, Yucatan, Mexico. Journal of Food Technology & Nutrition Sciences. SRC/JFTNS/115.
DOI: doi.org/10.47363/JFTNS/2021(3)118

21. Gelatin liquefaction	+
22. Fermentation of myo–inositol	–

A= Yellow pigmented colonies; and NG= No growth [26,27].

Two x two contingency tables were constructed from which prevalences were calculated. As a hypothesis test or test of statistical significance, the Mantel and Haenszel Chi–Square statistic ($x^2M–H$) was used. The Epi Info program for Windows, Version 7.1.5.2, was used to obtain the values of the $x^2M–H$ statistic and the probabilities (p). The criterion applied in carrying out the hypothesis tests for the difference between two percentages was based on the recommendations made by Cochran: 1. When $N > 40$ use the $x^2M–H$ test; 2. When $20 \leq N \leq 40$ use the $x^2M–H$ test if, and only if, all expected frequencies are ≥ 5; if in any cell there is at least an expected frequency < 5, then use the Fisher's exact probability test (PPEF); and 3. When $N < 20$ use the PPEF. [28]

$x^2M–H= \Sigma(|O – E| – ½)^2 / E$
PPEF= (A+B)! (C+D)! (A+C)! (B+D)! / N! A! B! C! D!

The Cornfield estimation interval was constructed at the 95% confidence level for the percentage in the population of seafood with the species *Vibrio alginolyticus* [29].
$p – Z\sigma p \leq P \leq p + Z\sigma p$

Data processing

In the elaboration stage, the data were reviewed (information quality control); classified (on a qualitative scale); computerized (the IBM SPSS Statistics software for Windows, Version 22) was used; presented (in Tables and in Graphs); and summarized (the corresponding summary measures were used for data classified on a qualitative scale). In the analysis and interpretation stages, the data were analyzed and interpreted, respectively.

Results

One hundred ninety six seafood samples were studied and classified according to their preparation methods into raw seafood, non–heat marinated seafood, partially heat–cooked seafood, and completely heat–cooked seafood.

The eight differential key tests to divide the twelve clinically significant species of the genus *Vibrio* into six groups are presented in Table 2. The species investigated in the present work belongs to Group 6 (negative production of arginine dehydrolase and positive decarboxylation of lysine).

Table 2: Eight differential key tests to divide the twelve clinically significant Vibrio species into six groups [30].

Differential key tests	Group 1		Group 2	Group 3	Group 4	Group 5			Group 6			
	Vibrio cholerae	Vibrio mimicus	Vibrio metschnikovii	Vibrio cincinnatiensis	Vibrio hollisae	Vibrio damsela	Vibrio fluvialis	Vibrio furnissii	Vibrio alginolyticus	Vibrio parahaemolyticus	Vibrio vulnificus	Vibrio carchariae
1. Growth in nutrient agar with 0% NaCl	+	+										
2. Growth in nutrient agar with 1% NaCl	+	+										
3. Oxidase test			–									
4. Reduction of nitrates (NO_3) a nitrites (NO_2)			–									
5. Fermentation of myo–inositol				+								
6. Production of arginine dehydrolase					–	+	+	+	–	–	–	–
7. Decarboxylation of lysine					–				+	+	+	+
8. Decarboxylation of ornithine					–							

Table 3 shows the absolute and relative frequencies of the *Vibrio alginolyticus* species in seafood according to preparation methods. Five (3.36%) of the 149 samples labeled raw seafood, 0 (0.00%) of the 4 samples labeled non–heat marinated seafood, 2 (5.13%) of the 39 samples labeled partially heat–cooked seafood, and 0 (0.00%) of the 4 samples labeled as completely heat cooked seafood had positive results. The global prevalence of the *Vibrio alginolyticus* species was 3.57% (7/196).

The Cornfield estimation interval at the 95% confidence level for the percentage in the population of seafood with the species *Vibrio alginolyticus* was $–10.18\% \leq P \leq 17.32\%$. In this estimation interval is zero (0), which agrees with the hypothesis test in the sense that there is no statistically significant difference between the prevalence of raw seafood (3.36%) and the prevalence of raw seafood. partially cooked seafood with heat (5.13%).

Table 3: Absolute frequencies and relative frequencies of the *Vibrio alginolyticus* species in seafood according to preparation methods. Chabihau, Yucatan, Mexico. June/1/2019–May/31/2020

Preparation methods	*Vibrio alginolyticus*		Totals
	Positive samples	Negative samples	
1. Raw	23 (7.72%)	275 (92.28%)	298 (100.00%)
2. Marinated without heat	0 (0.00%)	8 (100.00%)	8 (100.00%)
3. Partially cooked with heat	6 (7.79%)	71 (92.21%)	77 (100.00%)
4. Completely cooked with heat	0 (0.00%)	7 (100.00%)	7 (100.00%)
Totals	29 (7.44%)	361 (92.56%)	390 (100.00%)

Source. Own elaboration

Discussion

The highest prevalence (7.79%; 6/77) was observed in seafood partially cooked with heat. Sea foods partially cooked with heat are those that at the time of sampling were cooked through a "softening" process in which hot water is used for at least five minutes. This result does not correspond to the expected one and the observed prevalence can be explained either because the procedure used to "soften" the food is not enough to destroy the microorganism –an assumption that is supported by the study of Peffers et al., in which the viability of the microorganism is reported after having kept a crustacean in boiling water for five min prior inoculation of 0.1 ml of culture broth, or, because the food could have been contaminated by the handler after the process of "softening" due to cross contamination in the kitchens, or, through the anus–hand–food mechanism due to being an asymptomatic carrier–supposition that is supported by the study by Franco & Flores in which 3.85% is reported of seafood handlers who excrete the microorganism in their feces [25, 31].

Second is the prevalence (7.72%; 23/298) observed in raw seafood. Raw marine foods are those that were in their natural state at the time of sampling. Consequently, this result corresponds to that expected because the probability of insulation is high when the food has not been exposed to the action of heat.

No statistically significant difference was found when comparing the relative frequency or rate of positivity observed in partially cooked seafood with heat (7.79%) versus the relative frequency or rate of positivity observed in raw seafood (7.72%): $x^2M–H(\alpha= 0.0500; gl= 1) < 3.8416; p > 0.0500$.

Conclusions

Sea foods partially cooked with heat and raw seafood represent potential risk factors for *Vibrio alginolyticus* species for the development of acute gastroenteritis, wound infection, ear infection, and primary septicemia.

Finding partially heat–cooked seafood contaminated by the species *Vibrio* alginolyticus suggests the existence of asymptomatic carrier seafood handlers. The foregoing should occupy the attention of the corresponding authorities in order to continue carrying out related studies in this regard.Likewise, if to date it has not been considered a public health problem, it is convenient to keep it in mind in order to prevent health problems that could at some point affect the health of the population of the port of Chabihau, Yucatan, Mexico.

The results obtained in the present work are consistent with the results obtained in studies carried out by other authors [17-21].

Acknowledgements

Thanks our gratitude to the Universidad Intercultural Maya de Quintana Roo and the Servicios de Salud de Yucatán for each and every one of the facilities provided to carry out this work.

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