Research Article
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Species of the genus *Vibrio* of clinical–epidemiological importance

Abstract

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 undergoes a notable increase in the warm months due to favorable ecological conditions and plankton, increasing their accumulation by filter feeders and other marine animals. The objective of the present study was to determine whether raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat represent potential risk factors for the species *Vibrio damsela*, *Vibrio fluvialis* and *Vibrio furnissii* for the development, respectively, of infection. wound; of acute gastroenteritis; and acute gastroenteritis. A list of establishments specialized in the sale of seafood for human consumption was obtained. The amount of seafood in these establishments was 390. For the homogenization and enrichment of each sample, as well as for the isolation and identification of the three species, we proceeded according to the methodology described in the eighth edition of the Bacteriological Analytical Manual (FDA). Estimation intervals at the 95% confidence level were constructed using the Cornfield method. In 32 (8.21%), 10 (2.56%) and 23 (5.90%) samples an equal number of strains were isolated whose biochemical characteristics corresponded, respectively, to *Vibrio damsela*, *Vibrio fluvialis* and *Vibrio furnissii*. The global prevalences obtained in raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat were, respectively, 19.46% (58/298), 0.00% (0/8), 9.09% (7/77) and 0.00% (0/7). It is concluded that raw seafood and partially cooked seafood represent potential risk factors for three of the twelve species of clinical–epidemiological importance.

Keywords: *Vibrio*, pathogenic species, seafood

Volume 9 Issue 3 - 2021

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Received: July 03, 2021 | **Published:** July 15, 2021

Introduction

The *Vibrio* genus is one of the eight genera that make up the *Vibrionaceae* family; its members are natural inhabitants of the waters and the marine environment. Until 1960, *Vibrio cholerae* was the only genus recognized as a human pathogen. It was first described by Pacini, in 1854, who found it in the intestine of a patient who died of cholera and at that time it was designated as *Vibrio cholerae*. In 1906, Gotschlich in El Tor, Sinai, isolated microorganisms very similar to *Vibrio cholerae*, but which differed from it in their hemolytic activity, which he called *Vibrio El Tor*, today this microorganism is considered a variant of *Vibrio cholerae*. Sixty–six species of the genus *Vibrio* are recognized, of which twelve are considered human pathogens, these are: *Vibrio alginolyticus*, *Vibrio carchariae*, *Vibrio cholerae*, *Vibrio cincinnatiensis*, *Vibrio damsela*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Some of these species are limited to intestinal disease, others cause extraintestinal disease, and others have both locations. Vibrios are short, curved rods that resemble a comma. Its size varies considerably; they range from 1 to 5 µm in length by 0.3 to 0.6 µm in width. Commas can appear elongated, thin, and delicate, or short and thick. They appear as simple S–shaped cells, in pairs or sometimes in very short chains. Spheroplast forms are frequently seen. In liquid media, spirillary forms are often seen. In old cultures, on the other hand, very small shapes are seen resembling granules and that do not fix the colorations well. In subcultures the vibrios lose their coma shape. These microorganisms are actively mobile by means of a flagella that is observed attached to the blepharoplast on electron microscopy. They are Gram–negative, but stain better when carbolfuchsin is used as a contrast instead of safranin. *Vibrio* can easily grow in culture media without high nutritional requirements. One of its main characteristics is its rapid growth in 1% alkaline peptone water with 0.5% sodium chloride (NaCl). The growth occurs mainly on the surface after 6–9 hours of incubation, so a film is observed on the surface. Vibrios are strongly aerobic, and an adequate source of oxygen is necessary for their isolation. Although some species grow very poorly or do not grow at 37°C, those associated with disease in man show that their optimum growth temperature ranges between 30–40°C. The presence of NaCl is essential in the culture media for the growth of vibrios; the concentration of NaCl required for each species of vibrios is different and this characteristic is one of the parameters used in their biochemical identification.

Physiological studies for species classification begin with the oxidase reaction; all species capable of causing disease in humans are oxidase positive with the exception of *Vibrio metschnikovii* which is oxidase negative. Most vibrios do not reduce nitrates (NO_3) to nitrites (NO_2), they do not ferment lactose ($C_{12}H_{22}O_{11}$) and glucose ($C_6H_{12}O_6$), maltose ($C_6H_{12}O_6$), mannitol ($C_6H_{12}O_6$) and sucrose ($C_{12}H_{22}O_{11}$). no gas formation. The hemolytic power of *Vibrio cholerae* strains has been one of the main elements in the differentiation of classical *Vibrio cholerae* and *Vibrio cholerae El Tor*; Classical *Vibrio cholerae* has been found not to produce true soluble hemolysin, whereas *Vibrio cholerae El Tor* does. At least two antigenic components have been detected in the different species of vibrios. The O (heat stable)

antigen and the H (heat labile) antigen and their agglutinability and immunogenicity are inactivated by heating at 100°C for 15 minutes and 2.5 hours, respectively. A characteristic of the species of the genus *Vibrio* is that all the strains of the same species possess the same H antigens, although they are divided into many serogroups by the O antigen. More than 80 serovars have been described in *Vibrio cholerae*.[1]

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 *Bacteria* Domain. In turn, the *Vibrionaceae* Family is made up of the *Vibrio*, *Allomonas*, *Catenococcus*, *Enterovibrio*, *Grimontia*, *Listonella*, *Photobacterium* and *Salinivibrio* genera. Of the sixty–six accepted species of *Vibrio* (*Vibrio cholerae*, *Vibrio aerogenes*, *Vibrio aestuarianus*, *Vibrio agarivorans*, *Vibrio albensis*, *Vibrio alginolyticus*, *Vibrio anguillarum*, *Vibrio brasiliensis*, *Vibrio calviensis*, *Vibrio campbellii*, *Vibrio carinnchariae*, *Vibrio chagasii*, *Vibrio cincinniensis*, *Vibrio cincinnarriens*, *Vibrio corallillyticus*, *Vibrio costicola*, *Vibrio cyclitrophicus*, *Vibrio damsela*, *Vibrio diabolicus*, *Vibrio diazotrophicus*, *Vibrio fischeri*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio gazogenes*, *Vibrio halioticoli*, *Vibrio harveyi*, *Vibrio hispanicus*, *Vibrio holichrioius*, *Vibrio ilaloioprioe*, *Vibrio lentus*, *Vibrio logei*, *Vibrio marinus*, *Vibrio mediterranei*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio myftili*, *Vibrio natriegens*, *Vibrio navarrensis*, *Vibrio neptunius*, *Vibrio nereis*, *Vibrio nigripulchritudo*, *Vibrio ordalus*, *Vibrio emolicida*, *Vibrio peticidaius*, *Vibrio emolicida*, *Vibrio penaeicida*, *Vibrio pomeroyi*, *Vibrio proteolyticus*, *Vibrio ruber*, *Vibrio rumoiensis*, *Vibrio salmonicida*, *Vibrio scophthalmi*, *Vibrio shilonii*, *Vibrio splendidus*, *Vibrio succinogenes*, *Vibrio superstes*, *Vibrio tapetis*, *Vibrio tasmaniensis*, *Vibrio trachuri*, *Vibrio tubiashii*, *Vibrio viscosus*, *Vibrio vulnificus*, *Vibrio woudanis*, and *Vibrio xuii*)[2] the following twelve are considered pathogenic: *Vibrio alginolyticus*, *Vibrio carchariae*, *Vibrio cholerae*, *Vibrio cincinnatiensis*, *Vibrio damsela*, *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus*.[3]

Vibrios are characteristically indigenous to marine, brackish and estuarine habitats, and appear in large concentrations (*blooms*) when the waters increase in temperature (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 undergoes a notable increase in the warm months due to favorable ecological conditions and plankton, increasing their accumulation by filter feeders and other marine animals.[3–4]

The marine environment occupies practically three–quarters of the earth's surface and in coastal areas it enters into relationship with man directly for work and/or sports reasons or indirectly through the handling and/or consumption of seafood. It is well known that the contamination of coastal waters from sewage discharges has been, and still is, a constant epidemiological source of salmonellosis, hepatitis, and other infections. A less publicized aspect is the infectious pathology caused by indigenous marine bacteria of the *Vibrionaceae* family.[5–12]

Table 1 shows the association of *Vibrio spp*., with different clinical syndromes. 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 belong to Group 5 (positive production of arginine dehydrolase).[13] The species *Vibrio damsela*[14] is a halophilic *Vibrio* belonging, together with *Vibrio fluvialis* and *Vibrio furnissii*, to the group of positive arginine dehydrolase vibrios provisionally called "group F vibrios and also "CDC group EF6".[15] The species *Vibrio fluvialis* is halodependent and has many similarities with *Vibrio damsela* and *Vibrio furnissii*.[16] It is much more common in the marine environment than the other species of the *Vibrio* genus of the "EF6 group" and has been implicated in sporadic cases of gastrointestinal infection with pictures of watery diarrhea, vomiting, abdominal pain, and severe dehydration.[17–20] The species *Vibrio furnissii* has been proposed to place the halophilic strains previously known as *Vibrio fluvialis* biovar II. It has been isolated from the feces of patients with diarrhea, but its pathogenicity remains uncertain.[21]

1. The causes that most frequently contribute to the appearance of cases and outbreaks in the population include: Consumption of raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat;
2. The defective or the absent refrigeration that favors the multiplication of the microorganism;
3. Inadequate handling of food in kitchens which leads to cross contamination;[22] and
4. Contamination of seafood by the handler through the anus–hand–food mechanism due to being an asymptomatic carrier.[23–25]

According to their method of preparation, seafood was classified as raw, marinated without heat, partially cooked with heat, and completely cooked with heat. There were three varieties (crustaceans, mollusks, and fish) and forty–two were the species studied (bagre, balá, besugo, bobo, calamar, camarón, cangrejo, caracol, carpa, cazón, cherna, chopa, chucumite, cojinuda, corvina, cuberita, esmedregal, guachinango, jaiba, jurel, lisa, liseta, lobina, mantarraya, mejillón, mero, mojarra, mojarra carpa, mojarra tilapia, ostión, pámpano, pargo, pejelagarto, peto, pulpo, raya, robalo, rubia, salmón, sierra, tilapia y trucha).

In Mexico, enteritis and other diarrheal diseases constitute one of the ten main causes of morbidity and mortality. In Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, the incidence of diarrheal syndromes of unknown etiology is high; its inhabitants habitually consume raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat. According to data from the Statistical Yearbooks of the state of Campeche (National Institute of Statistics, Geography, and Informatics) corresponding to the years 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006 and 2007, the second cause of morbidity is invariably represented by gastrointestinal infections with rates of 7.1%; 6.6%; 6.5%; 6.8%; 6.1%; 4.9%; 4.8%; 4.9%; 4.8% and 4.6%.

Vibrio damsela

The species *Vibrio damsela* was isolated for the first time by Love et al. [27] Vibrios are Gram–negative rod–shaped bacteria that have become widespread in both coastal and estuarine environments. Some species such as *Vibrio anguillarum* and *Vibrio tapetis* include serious pathogens of aquatic vertebrates and invertebrates. Other species such as *Vibrio fluvialis*, *Vibrio furnissii*, *Vibrio hollisae*, *Vibrio alginolyticus*, *Vibrio carchariae*, *Vibrio cholerae*, *Vibrio metschnikovii*, *Vibrio mimicus*, *Vibrio parahaemolyticus*, and *Vibrio vulnificus* can cause disease in aquatic animals and humans. Human

outbreaks, although low in number, usually involve wound infections and gastrointestinal illnesses, frequently with watery diarrhea. In a minority of cases there is sufficient evidence to effectively associate human infections with diseased animals. In other cases, the link is certainly feasible but the strong evidence is mostly lacking.[28]

As aquaculture production and consumption of aquaculture products increase the possibility of contracting zoonotic infections from any handling or ingestion of these products also increases. The main pathogens acquired topically from fish and/or shellfish through spinal puncture/forceps or open wounds are the species *Vibrio damsela*, *Aeromonas hydrophila*, *Edwardsiella tarda*, *Mycobacterium marinum*, *Streptococcus iniae* and *Vibrio vulnificus*. These pathogens, all indigenous to the aquatic environment, have also been associated with disease outbreaks in edible fish. Outbreaks are often related to management factors such as the quality and quantity of nutrients in the water and high population density that can increase the bacterial load on the outer surface of the fish. As a result, sick fish are more likely to transmit the infection to humans. This study offers a list of human cases of zoonosis around the world from the main zoonotic pathogens of fish and/or shellfish.[29]

The species *Vibrio damsela* is a halophilic species belonging together with the species *Vibrio fluvialis* and *Vibrio furnissii* to the group of vibrios positive arginine dehydrolase provisionally called "group F vibrios" and also "CDC group EF6".[30] The species *Vibrio damsela* has been recognized as a cause of disease in man and its cytolytic activity has also been demonstrated.[31] The species has been reported in soft tissue infections,[32] fatal necrotizing fasciitis[33] and sepsis.[34,35] Recently, the role of iron (Fe) in the experimental pathogenicity of *Vibrio damsela* for both fish and mammals has been studied.[36] The species *Vibrio damsela* it is related to wound infection [(26)].

Table 1 Association of *Vibrio spp*., with different clinical syndromes [26]

Species	Clínical sindromes					
	Gastroenteritis	Wound infection	Ear infection	Primary septicemia	Secondary septicemia	
Vibrio alginolyticus	(+)	++	++	+		
Vibrio carchariae		+				
Vibrio cholerae O1	+++	+				
Vibrio cholerae no O1	+++					
Vibrio cincinnatiensis		++	+	+	+	+
Vibrio damsela	++			+		
Vibrio fluvialis	(+)	++				
Vibrio furnissii	++					
Vibrio hollisae	(+)					
Vibrio metschnikovii	++			+		
Vibrio mimicus			+	(+)		
Vibrio parahaemolyticus	+++	+	+			+
Vibrio vulnificus	+	++		++		++

Source. Own elaboration

+++= Frequently reported; ++= Less common (6–100 reports); += Rare (1–5 reports); y (+)= The association is not clear.

Vibrio fluvialis

The species *Vibrio fluvialis* was isolated for the first time by Lee et al. [37]. Gracia–Valenzuela et al.[38] carried out a study that aimed to find an alternative to the use of commercial antibiotics using extracts of vegetable oils with non–specific antimicrobial activity. In this study, the minimum inhibitory concentration, and the plaque inhibition capacity of the essential oil of oregano (*Origanum vulgare*) were evaluated, with fractions high in thymol and carvacrol, compared with commercial antibiotics for the species *Vibrio fluvialis*, *Aeromonas hydrophila*, *Aeromonas salmonicida*, *Pseudomonas fluorescens*, *Pseudomonas putida*, *Vibrio mimicus* and *Vibrio vulnificus* isolated from whiteleg shrimp (*Litopenaeus vannamel*). The species *Vibrio fluvialis* is a pathogenic microorganism that has been found usually in coastal environments. Considering a current increase in the number of outbreaks of liquid stools and in occasional extraintestinal cases, the species *Vibrio fluvialis* has been considered an emerging pathogenic microorganism. Although the *Vibrio fluvialis* species can be simply isolated using existing culture methods, its characterization is still a difficult problem due to the close phenotypic similarity with either the *Vibrio cholerae* species or the *Aeromonas spp*. However, the use of molecular instruments has helped to facilitate the characterization of the *Vibrio fluvialis* species from both clinical and dissimilar environmental samples. Various virulence factors have been reported, but their pathogenicity mechanisms and their ability to survive in the environment have not yet been investigated. A work carried out by Ramamurthy *et al*., covers some of the great discoveries that have been carried out to understand the extent of the importance of the species *Vibrio fluvialis*.[39]

The species *Vibrio fluvialis* is halodependent and presents many similarities with the species *Vibrio damsela* and *Vibrio furnissii*.[40] In the oceanic environment it is much more frequent than the other species of the *Vibrio* genus of the "EF6 group". The *Vibrio fluvialis*

species has been responsible for occasional cases of gastrointestinal infection with liquid stools, vomiting, belly pain and severe dehydration.[41–44] It has been reported that the enterotoxin it possesses differs from the cholera toxin of the *Vibrio cholerae* species both in the recipient and in the mode of action and antigenicity.[45] An isolated case of otitis caused by the species *Vibrio fluvialis* associated with the species *Vibrio alginolyticus* has been recently reported.[46]

A case of liquid evacuation has also been reported in a patient with HIV/AIDS.[47] The species *Vibrio fluvialis* is related with acute gastroenteritis.[26] *Vibrio furnissii.* The species *Vibrio furnissii* was isolated for the first time by Brenner et al.[48] The species *Vibrio furnissii* in blood is occasionally reported, which may explain why the clinical characteristics of bloodstream infections by this species have not been reported. A study by Derber et al.[49] reports a patient who developed skin lesions and bacteremia due to the species *Vibrio furnissii* and was successfully treated with fluoroquinolones.

The species *Vibrio furnissii* can be a serious pathogen in patients with underlying comorbidities who are exposed to seafood. The species *Vibrio furnissii* has been formulated to locate the halophilic strains previously known as *Vibrio fluvialis* biovar II. The species *Vibrio furnissii* has been isolated from stool samples of patients with liquid stools, but its pathogenicity is still doubtful.[48]

The species *Vibrio furnissii* is found related to acute gastroenteritis.[26]

1. The causes that most frequently contribute to the appearance of cases and outbreaks in the population include: Consumption of raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat;
2. The defective or the absent refrigeration which favors the multiplication of the microorganism;
3. Inadequate handling of food in kitchens, which leads to cross contamination from raw seafood to cooked food;[50] and
4. Contamination of seafood by the handler through the anus–hand–food mechanism due to being an asymptomatic carrier.[51–53]

According to their method of preparation, seafood was classified into raw, marinated without heat, partially cooked with heat, and completely cooked with heat. There were three varieties (crustaceans, mollusks, and fish) and forty–two were the species studied (bagre, balá, besugo, bobo, calamar, camarón, cangrejo, caracol, carpa, cazón, cherna, chopa, chucumite, cojinuda, corvina, cuberita, esmedregal, guachinango, jaiba, jurel, lisa, liseta, lobina, mantarraya, mejillón, mero, mojarra, mojarra carpa, mojarra tilapia, ostión, pámpano, pargo, pejelagarto, peto, pulpo, raya, robalo, rubia, salmón, sierra, tilapia y trucha).

In Mexico, enteritis and other diarrheal diseases constitute one of the ten main causes of morbidity and mortality. In Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, the incidence of diarrheal syndromes of unknown etiology is high; its inhabitants habitually consume raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat. According to data from the Statistical Yearbooks of the state of Campeche (National Institute of Statistics, Geography, and Informatics) corresponding to the years 1998, 1999, 2000, 2001, 2002, 2003, 2004, 2005, 2006 and 2007, the second cause of morbidity is invariably represented by gastrointestinal infections with rates of 7.1%; 6.6%; 6.5%; 6.8%; 6.1%; 4.9%; 4.8%; 4.9%; 4.8% and 4.6%.

The objectives of this research were to determine the prevalences of *Vibrio damsela*, *Vibrio fluvialis* and *Vibrio furnissii* species in raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat. In other words, determine if raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat represent potential risk factors for *Vibrio damsela* species for the development of wound infection; by the species *Vibrio fluvialis* for the development of acute gastroenteritis; and by the species *Vibrio furnissii* for the development of acute gastroenteritis.[26]

Null hypothesis (Ho): Raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat are not contaminated with one or more of the three species studied of the twelve species of clinical–epidemiological importance of the genus *Vibrio*, and therefore are not factors potential risks for the fish and shellfish consuming population of Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico.

Alternative hypothesis, working hypothesis or research hypothesis (H_1): Raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat are contaminated with one or more of the three species studied of the twelve species of clinical–epidemiological importance of the genus *Vibrio*, constituting, consequently, potential factors of risk for the fish and shellfish consuming population of Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico.[54]

a. The *Vibrio cholerae* and *Vibrio mimicus* species show positive growth both in nutrient broth with 0% NaCl and in nutrient broth with 1% NaCl (Group 1).
b. The species *Vibrio metschnikovii* has a negative test for oxidase and a negative test for the reduction of nitrates (NO_3) to nitrites (NO_2) (Group 2).
c. The species *Vibrio cincinnatiensis* shows positive fermentative metabolism of Myo–Inositol (Group 3).
d. The species *Vibrio hollisae* shows negative production of arginine dehydrolase, negative decarboxylation of lysine and negative decarboxylation of ornithine (Group 4).
e. The species *Vibrio damsela*, *Vibrio fluvialis* and *Vibrio furnissii* show positive arginine dehydrolase production (Group 5).

Finally, the species *Vibrio alginolyticus*, *Vibrio parahaemolyticus*, *Vibrio vulnificus* and *Vibrio carchariae* show negative production of arginine dehydrolase and positive decarboxylation of lysine (Group 6).

Material and methods

Epistemological approach

Quantitative approach, probabilistic approach, or positivist approach.[55]

Study design

Descriptive, cross–sectional epizootiological study without directionality (cause→effect, or effect→cause) and with prospective timing.[56]

Study universe

The study was carried out on the total samples of all establishments

specialized in the sale of seafood for human consumption in Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico (Figure 1), in the period from 1 June, 2009 to May 31, 2010. Samples were collected from August 1, 2009 to March 31, 2010.[57]

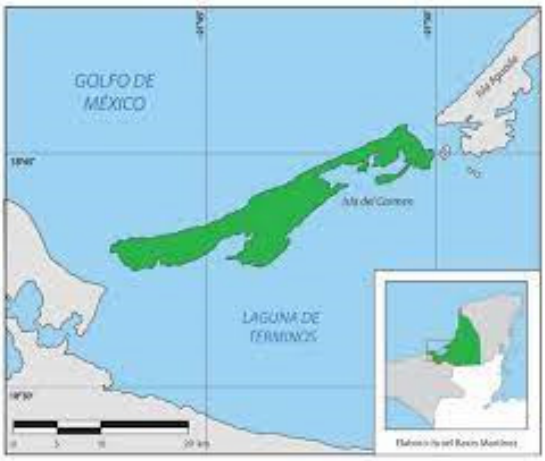

Figure 1 Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico[57]

The city takes its name from July 16, 1717, the day of the Virgen del Carmen, the date on which the pirates were defeated and expelled from the island by Alonso Felipe de Andrade at the San Felipe fort. The shield is an oval limited by a gray periphery that has the name of the city and the state to which it belongs. In the center of the oval is the Laguna de Terminos and above it Isla de Tris, Isla del Carmen or Perla del Golfo. The lion symbolizes the European siege of Mexico during the Spanish rule and the French intervention; he is on the island and on it an eagle wounds him with its beak and claws. The eagle symbolizes the people of the island preventing a foreign country from intervening both in its territory and in the nation (Figure 2). The original shield has the legend "La Laguna by Yucatán and both by the Mexican Republic". During the first years of Mexico as an independent country, the region of the island was known as La Laguna and even in the early years of the 20th century it was called that. The reason for the motto is that when it was created in 1828 the island was part of the Province of Yucatán.[58,59]

Isla del Carmen, Isla de Tris or Perla del Golfo is a Mexican island located in the state of Campeche between the Laguna de Terminos and the Gulf of Mexico. During the Spanish rule, the island was called the Island of Terms, but since "Terms" was abbreviated on maps as "Tris" it was more commonly known as Isla de Tris. It communicates in the North point through the "Puente de La Unidad" with the population of Isla Aguada and to the South by the Bridge "El Zacatal" and the Atasta peninsula. It is the most populated island in Mexico. It has an area of 153 kilometers², a length of 36 kilometers and is 7.5 kilometers in its widest part and is crossed throughout by Federal Highway 180 (Carmen–Champotón). In the westernmost part is Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, municipal seat of the municipality of the same name. The island has land, sea and air accesses. In the access by land, the island is communicated in the North point with the "Puente de La Unidad" that communicates with San Francisco de Campeche, the state capital, and in the southern part with the bridge "El Zacatal" (the longest of its kind in Latin America) that links the island with the Atasta peninsula. Access by sea is through the port "Laguna Azul".

Figure 2 Coat of arms of Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico[59]

Finally, access by air is achieved through an International Airport and a Heliport. According to the II Count of Population and Housing 2005 (INEGI), in the state of Campeche 1,867 schools of scholastic modality are listed; of these, 123 are located in Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, 51.2% are of primary education, 23.6% of secondary education, 11.4% of secondary education, 0.8% of higher technical education and 13% higher education. These school spaces house a school enrollment of 29,233 students; on average 238 students per school. The center for higher education studies with the greatest influence in Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, is the Autonomous University of Carmen (UNACAR) whose antecedents go back to the Liceo Carmelita.

This is one of the three most important universities in the state of Campeche; in addition, the Institute of Marine Sciences and Limnology of the National Autonomous University of Mexico (UNAM) has a monitoring and research station in Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico. Another of the most important universities in the state of Campeche is the Universidad Tecnologica de Campeche (UTC) located on the Atasta peninsula in San Antonio Cárdenas, which was the first university to be certified with ISO 9001–2000 for its quality management services and that in 2010 it was recognized as the best technological university in the country. In 2005, the population of Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, was 154,197 inhabitants, of which men and women have the same percentage –50% and 50% each gender. Compared to the year 2000, the City had a growth of 28,173 inhabitants which translates into a rate of 4.1%, annual average. The group of inhabitants that represents the highest percentage of the total population of Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, is the population of working ages: 30–59 years (18.2% men and 17.9% women). The 0–14 age group represents 29.4% in total (14.9% men and 14.5% women). The young population group, 15–29 years old, represents 29.1% of the population (14% men and 15.1% women). The inhabitants who are within the group of 60 years and + years are only 5.4% of the total (2.5% men and 2.9% women). The percentages of the population by age groups do not show significant variations with respect to the percentages registered

Citation: Monsreal JF, Serralta–Peraza LEDS, Abuxapqui JJF. Species of the genus *Vibrio* of clinical–epidemiological importance. *MOJ Biol Med.* 2021;6(3):116–125. DOI: 10.15406/mojbm.2021.06.00142

in 2000, except in the first ages [(0–14 years (32.1%) and 30–59 years (32.8%)], and the veneration of the Virgen del Carmen was born in a circumstantial way motivated by the order that Felipe V made to the Viceroy of New Spain, Marqués de Valeros, for which the Barlovento squad joined several vessels from Campeche manned by campechanos who were vividly interested in driving their terrible enemies off the Island, the expedition was entrusted to D. Alonso Felipe de Andrade who achieved a glorious victory over the pirates on July 16, 1717. In commemoration of that historic event every July 16 of each year the most important religious festival is celebrated in honor of Our Lady of Carmen, where religious and popular events are held in which the Virgin del Carmen walk by sea stands out. igiosa is called the International July Fair of Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, which takes place annually from July 15 to 31 with headquarters in the Tourist Complex.[60]

Operational definitions of the variables[61]

Cocktail bars, cooperative, fishmongers, restaurants and supermarkets: Establishments that sell marine foods of animal origin for human consumption and that have a sanitary license issued by the Secretary of Health of the state of Campeche.

Marine food: Any product of animal origin from the sea that provides the human body with micronutrients (vitamins and minerals) and macronutrients (carbohydrates, saccharides or sugars; lipids or glycerides; and proteins) for its nutrition.

Raw marine food: Any product of animal origin from the sea that provides the human body with micronutrients (vitamins and minerals) and macronutrients (carbohydrates, saccharides or sugars; lipids or glycerides; and proteins) for its nutrition and that in the time of sampling has been found in its natural state.

Marinated seafood without heat: Any product of animal origin from the sea that provides the human body with micronutrients (vitamins and minerals) and macronutrients (carbohydrates, saccharides or sugars, lipids or glycerides; and proteins) for its nutrition and that in the at the time of sampling, it was found cooked using the action of the acid of lemon juice, the acid of orange juice and vinegar, among others.

Sea food partially cooked with heat: Any product of animal origin from the sea that provides the human body with micronutrients (vitamins and minerals) and macronutrients (carbohydrates, saccharides or sugars; lipids or glycerides; and proteins) for its nutrition and that in the moment 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 five minutes; 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 cocktails and/or cebiches.

Sea food completely cooked with heat: Any product of animal origin from the sea that provides the human body with micronutrients (vitamins and minerals) and macronutrients (carbohydrates, saccharides or sugars; lipids or glycerides; and proteins) for its nutrition and that in the at the time of sampling, it was found cooked using the action of heat (grilled, fried and steam, among others).

Techniques and procedures: The Regional Coordination Nº. 3 of the Commission for the Protection against Sanitary Risks of the state of Campeche provided a list of establishments (cocktail bars, cooperative, fishmongers, restaurants and supermarkets) that specialize in the sale of seafood for human consumption. A first visit was made to each of the establishments and a list of 390 seafood samples was compiled that were classified, according to their method of preparation, into raw, marinated without heat, partially cooked with heat, and completely cooked with heat. The establishments received a second visit (in the period from August 1, 2009 to March 31, 2010) during which these samples were obtained. Each sample weighed approximately 50 grams; individually stored in sterile polyethylene bag (Ziploc); it was kept refrigerated; and it was sent for processing to the Food and Water Quality Control Laboratory of the UNACAR Faculty of Chemistry. The processing of the samples was carried out in the period from August 1, 2009 to March 31, 2010. For the homogenization and enrichment of each sample, as well as for the isolation and identification of the species *Vibrio damsela*, *Vibrio fluvialis* and *Vibrio furnissii*, we proceeded according to the methodology described in the eighth edition of the Bacteriological Analytical Manual (FDA).[62]

Homogenization: With the help of a sterile scalpel and a sterile anatomical forceps with a tooth, 25 grams were weighed into a sterile Petri dish; they were transferred to a sterile 200 ml blender glass; 125 ml of broth peptonized with 3% sodium chloride (NaCl) were added; and the contents were liquefied at low speed for 1 minute.

Enrichment: One ml of the resulting suspension was transferred to a culture tube containing 9 ml of broth peptonized with 3% NaCl; and incubated at 35–37 for 18–24 hours.

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 for 18–24 hours; from the pigmented colonies (yellow or green in color) that developed, smears were made to stain by the Gram method; when the colonies consisted of straight or slightly curved Gram–negative rods, the oxidase test was performed as a presumptive test.

Identification: Colonies that successfully passed the presumptive test (i.e., tested positive for oxidase) underwent the following complementary biochemical tests: arginine–dehydrolase production; 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–nitrophenyl–B–D–galactopyranoside (ONPG); Voges–Proskauer reaction; gelatin liquefaction; and Myo–Inositol fermentation.

Two x two contingency tables (also called tetrachoric tables or quadricellular tables) were constructed from which the proportions were calculated. As a hypothesis test or test of statistical significance, the Mantel and Haenszel Chi–Square statistic (x^2_{M-H}) was used. The Epi Info program for Windows, Version 3.4.3, was used to obtain

the values of the x^2_{M-H} statistic and the probability (*p*). The criterion applied in carrying out the hypothesis tests for the difference between two proportions was based on the following recommendations made by Cochran;[63] 1. When N > 40 use the statistic x^2_{M-H}; 2. When 20 ≤ N ≤ 40 use the statistic x^2_{M-H} if, and only if, all expected frequencies are > 5; if in any cell there is at least one expected frequency < 5, then use the Fisher's exact probability test (PPEF); and 3. When N < 20 use the PPEF.

x^2_{M-H}= [AD*BC / √(A+B) (C+D) (A+C) (B+D) (N–1)]²

PPEF= (A+B)! (C+D)! (A+C)! (B+D)! / N! A! B! C! D!

!= Factorial

The estimation intervals ($p - Z\sigma_p \leq P \leq p + Z\sigma_p$)[64] were constructed at the 95% confidence level (Z) for the percentages in the seafood populations with *Vibrio damsela*, *Vibrio fluvialis* and *Vibrio furnissii*.

Where:

p= Proportion of elements in the sample that have the characteristic of interest;

Z= Confidence level;

$Z\sigma_p$= Z times the Standard error; and

P= Proportion of elements in the population that have the characteristic of interest.

At the same time: $\sigma_p = \sqrt{pq/n}$

Where:

σ_p= Standard error;

p= Proportion of elements in the sample that have the characteristic of interest;

q= Proportion of elements in the sample that do not have the characteristic of interest; and

n= Sample size.

Data processing

In the elaboration stage, the data were reviewed (information quality control); classified (qualitative scale), computerized [Statistical Package for the Social Sciences (SPSS) software for Windows, Version 22.0 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 6%.

Results

Three hundred ninety samples of seafood were studied that according to their preparation methods were classified as raw, marinated without heat, partially cooked with heat and completely cooked with heat. The absolute frequencies and relative frequencies of foods marine by prevalences of *Vibrio* according to preparation methods are presented in Table 4. The absolute and relative frequencies of seafood by prevalences of *Vibrio* according to preparation methods are presented in Table 3. One hundred and fifteen (38.59%) of the 298 samples labeled raw seafood, 2 (25.00%) of the 8 samples labeled non–heat marinated seafood, 24 (31.17%) of the 77 samples labeled partially heat–cooked seafood and 2 (28.57%) of the 7 samples labeled as completely cooked seafood with heat presented positive results. The global prevalence of the genus *Vibrio* in seafood was 36.92% (144/390).

Table 3 Absolute frequencies and relative frequencies of foods marine by prevalences of *Vibrio* according to preparation methods. Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico. I/VI/2009–31/V/2010

Preparation methods	Totals	Vibrio	
		Positives	Negatives
Raw	298	115 (38.59%)	183 (61.41%)
Marinated without heat	8	2 (25.00%)	6 (75.00%)
Partially cooked with heat	77	24 (31.17%)	53 (68.83%)
Completely cooked with heat	7	2 (28.57%)	5 (71.43%)
Totals	390	144 (36.92%)	246 (63.08%)

Source: Own elaboration

Discussion

In 32/390 (8.21%), 10/390 (2.56%) and 23/390 (5.90%) samples an equal number of strains were isolated whose biochemical characteristics corresponded, respectively, to *Vibrio damsela*, *Vibrio fluvialis* and *Vibrio furnissii*. The global prevalences obtained in raw seafood, marinated without heat, partially cooked with heat, and completely cooked with heat were, respectively, 19.46% (58/298), 0.00% (0/8), 9.09% (7/77) and 0.00% (0/7). The highest prevalence or positivity rate (19.46%; 58/298) was 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.

Second is the prevalence or positivity rate (9.09%; 7/77) observed in partially cooked seafood 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 five minutes. This result does not correspond to what was expected, 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 a[65] study in which the viability is reported. of microorganisms after having kept a crustacean in boiling water for five minutes after inoculation of 0.1 ml of culture broth–, or, because the food could have been contaminated by the handler after the "softening" process due to contamination crossed in kitchens, or, through the anus–hand–food mechanism because it is an asymptomatic carrier –supposition that is supported by studies[50–52] in which 0.72%, 0.68%–3.30% and 3.85% are reported, respectively, of seafood handlers who excrete the microorganism in their feces.

The following is the prevalence or positivity rate (0.00%) observed in marinated seafood without heat. Marine foods marinated without heat are those that at the time of sampling were cooked through the action of the acid of lemon juice, the acid of orange juice and vinegar, among others. This result does not correspond to the expected one because they are foods that have not been exposed to the action of heat. The explanation is probably based on the small number of samples studied.

Finally, there is the prevalence or positivity rate (0.00%) observed

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in seafood completely cooked with heat. Sea foods completely cooked with heat are those that at the time of sampling were cooked by the action of heat. Subsequently, this result corresponds to that expected since the probability of isolation is zero when the food has been prepared by means of adequate exposure to the action of heat.

When the observed prevalence or positivity rate in raw seafood (19.46%) was compared to the observed prevalence or positivity rate in partially cooked seafood (9.09%), statistically significant evidence was found at the level of significance or level of significance (α) of 5% to conclude that the prevalence observed in raw seafood (19.46%) is different from the prevalence observed in partially cooked seafood with heat (9.09%): $x^2_{M-H}(\alpha= 0.0500; gl= 1)= 4.5819; p= 0.0323$.

Conclusions

Raw seafood represents potential risk factors for the *Vibrio damsela* species for the development of wound infection. Raw seafood and seafood partially cooked with heat represent potential risk factors for *Vibrio fluvialis* species for the development of acute gastroenteritis; and raw seafood and partially heat–cooked seafood represent potential risk factors for *Vibrio furnissii* for the development of acute gastroenteritis. Finally, it is concluded that raw seafood and partially cooked seafood are contaminated with one or more of the three clinically important species of the genus *Vibrio*, thus constituting potential risk factors for the fish consuming population. and seafood from Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico. Finding partially heat–cooked seafood contaminated with clinically important species of the genus *Vibrio* 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 Isla del Carmen, Isla de Tris or Perla del Gulf, Campeche, Mexico.

Recommendations

Development of a health education program with the aim of promoting in the population of Isla del Carmen, Isla de Tris or Perla del Golfo, Campeche, Mexico, the development of attitudes and behaviors that allow them to participate in the prevention of individual diseases and collectives to protect themselves in this way from risks that endanger their health; Execution of a comprehensive sanitary control of establishments (cocktail bars, cooperative, fishmongers, restaurants, and supermarkets) that sell marine food of animal origin for human consumption; and Continuation of studies related to the twelve medically important species of the genus *Vibrio*. In summary, it is advisable to continue this research with a study that aims to search –both in the feces and in the sera of the handlers of seafood from cocktail bars, cooperatives, fishmongers, restaurants and supermarkets in Isla del Carmen, Isla de Tris or Pearl of the Gulf, Campeche, Mexico.

Acknowledgment

None.

Funding

None.

Conflict of interests

None.

References

1. Llop–Hernández A. Microbiología y Parasitología Médicas. Tomo I. 2001.
2. Garrity GM, JA Bell, TG Lilburn. Taxonomic Outline of the Prokaryotes Release 5.0. Bergey's Manual of Systematic Bacteriology 2nd edition. 2004:12–113.
3. West PA. The human pathogenic vibrios. A public health update with environmental perspectives. *Epidem Infec*. 1989;103(1):1–34.
4. Robertson WJ, RS Tobin. The relationship between three potential pathogens and population indicator organisms in Nova Scotian coastal waters. *Can J*. 1983;29(10):1261–1269.
5. Pérez–Trallero E, M Urbieta–Egaña. Aislamiento de *Vibrio vulnificus* (*Beneckea vulnifica*) en la costa de Guipúzcoa. *Laboratorio*. 1982;74:347–55.
6. Pérez–Trallero E, M Urbieta Egaña, et al. *Vibrio alginolyticus*. Estudio comparativo entre cepas de procedencia humana y aisladas del medio ambiente. *Clin*.1983;(1):102–106.
7. Lantero M, Perales L, Michans, et al. Septicemia por Non O1 *Vibrio cholerae*. *Clin*. 1984;2:62–64.
8. López Brea M, Jiménez, C de las Cuevas, et al. Non–01 *Vibrio cholerae* septicemia. *Trans Roy Soc Trop Med Hyg*. 1985;79: 878–9.
9. Pérez JL, M Cabré, L Riera, et al. Gastroenteritis por *Vibrio parahaemolyticus* asociada a consumo de ostras. *Clin*. 1987;5:1603,
10. Revillo MJ, B Moles, E Lomba, et al. Aislamiento de *Vibrio mimicus* en muestras clínicas. *Clin*. 1988;6:189–202.
11. Pérez JL, J Ayats, P López, et al. Infección de herida por *Vibrio alginolyticus*. *Rev Esp Clin*. 1989;4:314–315.
12. García–Martos P, M Benjumeda, D Delgado. Otitis externa por *Vibrio alginolyticus*: descripción de cuatro casos. *Acta Otorrinolaring Esp*. 1999;44:557.
13. Kelly MT, FW Hickman–Brenner, JJ Farmer. *Vibrio*. In: Balows A, Hausler WJ, Herrmann KL, Isenberg HD, Shadomy HJ, ed. Manual of Clinical Microbiology (5% ed.) American Society for Microbiology; Washington. 1991:389.
14. Love M, D Teebken–Fisher, JE Hose, et al. *Vibrio damsela* a marine bacterium, causes skin ulcers on the damselfish Chromis punctipinnis. *Science*. 1981;214(4525):1139–1140.
15. Lee JV, P Shread, AL Furniss, et al. Taxonomy and description of *Vibrio fluvialis* sp. nov. (synonym group F vibrios, group EF6). *J Appl Bacteriol*. 1981;50(1):73–95.
16. Lee JV, Shread P, Furniss AL, et al. Taxonomy and description of *Vibrio fluvialis* sp. nov (synonym group F vibrios, group EF6). *Journal of Applied Bacteriology*. 1981;50(1):73–94.
17. Lee JV, Shread P, Fumiss AL, et al. Taxonomy and description of V*ibrio fluvialis* sp. nov (synonym group F vibrios, group EF6). *Journal of Applied Bacteriology*. 1981;50(1):73–94.
18. Hug MI, AKMJ Alam, DJ Brenner, et al. Isolation of *Vibrio*–like group EF–6 from patients with diarrhea. *J Clin*. 1980;11(6):621–624.
19. Tacket CO, F Hickman, GV Pierce, et al. Diarrhea associated with *Vibrio fluvialis* in the United States. *J Clin*. 1982;16(5):991–992.
20. Bellet J, B Klein, M Altieri, et al. *Vibrio fluvialis*, an unusual pediatric

enteric pathogen. *Pediatr Emerg Care*. 1989;5(1):27–28.

21. Brenner DJ, FW Hickman–Brenner, JV Lee, et al. *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new «species isolated from human feces and the environment. *J Clin Microbiol*. 1983;18(4):816–824.

22. Bryan FL. Factors that contribute to outbreaks of food–borne disease. *J Food Prot*. 1978;41(10):816–827.

23. Franco Monsreal J, JJ Flores Abuxapqui. Prevalencia de *Vibrio parahaemolyticus* en productos marinos y en heces de manipuladores de alimentos. *Rev Lat–amer Microbiol*. 1988;30: 223–227,

24. Fujino TT. Report of the food hygiene sub–committee on *Vibrio parahaemolyticus*. In: Fujino T, Fukumi H, ed. Vibrio parahaemolyticus. Nayashoten. 196:673–725.

25. Pérez–Memije E, ML Vélez–González, F Galván–Rodríguez. Búsqueda de *Vibrio parahaemolyticus* en heces de manejadores de alimentos en el puerto de Acapulco, Guerrero. *Rev Lat–amer Microbiol*. 1980;22:18.

26. Pavia AT, Bryan JA, Maher KL, *Vibrio carchariae* infection after a shark bite. *Annals of Internal Medicine*. 1989;11(1):85–86.

27. Love M, Teebken–Fisher D, Hose JE, et al. *Vibrio damsela*, a marine bacterium, causes skin ulcers on the damselfish Chromis punctipinnis. *Science*. 1981;214(4525):1139–40.

28. Revista del Comité Científico de la Agencia Española de Seguridad Alimentaria y Nutrición. Gobierno de España. Ministerio de Sanidad y Política Social. 2010:1–134.

29. Haenen OLM, Evans JJ, Berthe F. Bacterial infections from aquatic species: potential for and prevention of contact zoonoses. *Revue Scientifique et Technique de L Office International des Epizooties*. 2013;32(2):497–507.

30. Lee JV, Shread P, Fumiss AL, et al. Taxonomy and description of *Vibrio fluvialis* sp. nov (synonym group F vibrios, group EF6). *Journal of Applied Bacteriology*. 1981;50(1):73–94.

31. Kreger AS. Cytolytic activity and virulence of *Vibrio damsela*. *Infection and Immunity*. 1984;44:326–31.

32. Coffey JA, Harris RL, Rutledge ML, et al. *Vibrio damsela* another potentially virulent marine Vibrio. *Japanese Journal of Infectious Diseases*. 1986;153:800–802.

33. Yuen KY, Ma L, Wong SS, et al. Fatal necrotizing fasciitis due to *Vibrio damsela*. Scandinavian *Journal of Infectious Diseases*. 1993;25(5):659–661.

34. Love M, Teebken–Fisher D, Hose JE, et al. *Vibrio damsela* a marine bacterium, causes skin lesions on the damselfish Cromis punctipinnis. *Science*. 1981;214(4525):1.139–1.140.

35. Morris JG, Miller HG, Wilson R. et al. Illness caused by *Vibrio damsela* and *Vibrio hollisae*. *Lancet*. 1982;319(8284);1.294–1.296.

36. Fouz B, Toranzo AE, Biosca EG, et al. Role of iron in the pathogenicity of *Vibrio damsela* for fish and mammals. *FEMS Microbiology Letters*. 1994;121(2):181–188.

37. Lee JV, Shread P, Furniss AL, Bryant TN. Taxonomy and description of *Vibrio fluvialis* sp. nov. (Synonym Group F Vibrios, Group EF6). Journal of Applied Microbiology. 1981;50(1):73–94.

38. Gracia–Valenzuela MH, Orozco–Medina C, Molina Maldonado C. Efecto antibacteriano del aceite esencial de orégano (*Lippia benrlandieri*) en bacterias patógenas de camarón *Litopenaeus vannamel*. *Hidrobiológica*. 2012;22(3):201–206.

39. Ramamurthy T, Chowdhury G, Pazhani GP, et al. *Vibrio fluvialis*: an emerging human pathogen. *Frontlers in Microbiology*. 2014;5:91.

40. Lee JV, Shread P, Furniss AL, Bryant T. Taxonomy and description of *Vibrio fluvialis* sp. nov (synonym group F vibrios, group EF6). *Journal of Applied Bacteriology*. 1981;50(1):73–94.

41. Lee JV, Shread P, Furniss AL, Bryant T. Taxonomy and description of *Vibrio fluvialis* sp. nov (synonym group F vibrios, group EF6). *Journal of Applied Bacteriology*. 1981;50(1):73–94.

42. Huq MI, Alam AKMJ, Brenner DJ, et al. Isolation of *Vibrio*–like group EF–6 from patients with diarrhea. *Journal of Clinical Microbiology*. 1980;11(6):621–624.

43. Tacket CO, Hickman F, Pierce GV, et al. Diarrhea associated with *Vibrio fluvialis* in the United States. *Journal of Clinical Microbiology*. 1982;16(5):991–992.

44. Bellet J, Klein B, Altieri M, et al. *Vibrio fluvialis*, an unusual pediatric enteric pathogen. *Pediatric Emergency Care*. 1989;5(1):27–28.

45. Ahsan CR, Sanyal SC, Zamman A, et al. Immunobiological relationships between *Vibrio fluvialis* and *Vibrio cholerae* enterotoxins. *Immunology and Cell Biology*. 1988;66(3):251–252.

46. Puy H, Canarelli B, Denamur E, et al. Otitis caused by *Vibrio alginolyticus*. *Presse Medicale*. 1989;18(19):985.

47. Hodge TW, Levy CS, Smith MA. Diarrhea associated with *Vibrio fluvialis* infection in a patient with AIDS. *Clinical Infectious Diseases*. 1995;21(1):237–238.

48. Brenner DJ, Hickman–Brenner FW, Lee JV, et al. *Vibrio furnissii* (formerly aerogenic biogroup of *Vibrio fluvialis*), a new species isolated from human feces and the environment. *Journal of Clinical Microbiology*. 1983;18(4):816–824.

49. Derber C, Coudron P, Tarr C, et al. *Vibrio furnissii*. an unusual cause of bacteremia and skin lesions after ingestion of seafood. *Journal of Clinical Microbiology*. 2011;49(6):2348–2349.

50. Bryan FL. Factors that contribute to outbreaks of foodborne disease. *Journal of Food Protection*. 1978;41(10):816–827.

51. Fujino TT. Report of the food hygiene subcommittee on *Vibrio parahaemolyticus*. En: Fujino T, Fukumi H, editores. *Vibrio parahaemolyticus*. Nayashoten. 1967:673–725.

52. Pérez–Memije E, Vélez–González ML, Galván–Rodríguez F. Búsqueda de *Vibrio parahaemolyticus* en heces de manejadores de alimentos en el puerto de Acapulco, Guerrero. *Revista Latinoamericana de Microbiología*. 1980;22:18.

53. Franco–Monsreal J, Flores–Abuxapqui JJ. Prevalencia de *Vibrio parahaemolyticus* en productos marinos y en heces de manipuladores de alimentos. *Revista Latinoamericana de Microbiología*. 1980;30:2237.

54. Kelly MT, Hickman Brenner FW, Farmer JJ. Vibrio. En: Versalovic J, Carroll KC, Funke G, Jorgensen JH, Landry ML, Warnock DW (editors). Manual of Clinical Microbiology (10. edition). Washington, D.C. *American Society for Microbiology*. 2011:389.

55. Van Belkum A. Bergey's Manual of Systematic Bacteriology (Volume 2, Parts A–C, 2nd Edition). *FEMS Immunology and Medical Microbiology*. 2006;46(3):476.

56. Lilienfeld AM, Lilienfeld DE. Foundations of Epidemiology, Second Edition. Oxford University Press, Inc. Nueva York, EUA. 1983:172–202.

57. https://www.google.com.mx/search?hl=esésite=imghpé8tbm=ischésource=hpg8biw=19208bih=9168q=isla+del+carmengog=isla+del+carmenégs_l=img.12..35i39k1 j019.2029.6746.0.9272.16.16.0.0.0.0.560.2435.0j2j6j5–1.9.0....0...1ac.1.64.img..7.9.2425.0.eEAU9GF–SO

58. https://es.wikipedia.org/wiki/Ciudad_del_Carmen.

59. https://www.google.com.mx/search?hl=esésite=imghpétbm=ischésource=hpg8biw=19208bih=9168q=isla+del+carmengog=isla+del+carmenégs_l=img.12..35i39k1 j019.2029.6746.0.9272.16.16.0.0.0.0.560.2435.0j2j6j5–1.9.0....0...1ac.1.64.img..7.9.2425.0.eE8AU9GFSOHimgrc=ee8gjEBD6h3yPM:

60. https://es.wikipedia.org/wiki/Isla_del_Carmen_(Campeche).

61. Franco–Monsreal J, Flores–Abuxapqui JJ. *Prevalencia de Vibrio parahaemolyticus* en productos marinos y en heces de manipuladores de alimentos. *Revista Latinoamericana de Microbiología*. 1988;30:2237.

62. Kaysner CA, DePaola A Jr. *Vibrio* En: Bacteriological Analytical Manual. 8th edition. *Sustancialmente reescrita y revisada*. 2004.

63. Cochran WG. Some methods for strengthening the common x² tests. *Biometrics*. 1954;10(4):417–51.

64. Daniel WW. Bioestadística: Base para el Análisis de las Ciencias de la Salud. México, D.F. Editorial Limusa Wiley. 176–7. Cuarta edición. 2014;978–968–18–6164–3.

65. Peffers ASR, Bayley J, Barrow Gl, et al. *Vibrio parahaemolyticus* gastroenteritis and international air travel. *The Lancet*. 1973;301(7795):143–145.

Citation: Monsreal JF, Serralta–Peraza LEDS, Abuxapqui JJF. Species of the genus *Vibrio* of clinical–epidemiological importance. *MOJ Biol Med.* 2021;6(3):116–125. DOI: 10.15406/mojbm.2021.06.00142