Chapter

Survival Differences of *Vibrio vulnificus* and *Vibrio parahaemolyticus* Strains in Shellstock Oysters (*Crassostrea virginica*) from Harvest to Sale: A Risk Perspective

Violeta Pardío, Irma Wong, Leonardo Lizárraga, Karla López, Argel Flores, Guadalupe Barrera, Francisco Alarcón and Carlos Fernández

Abstract

As there is limited information on the risk for consuming market oysters contaminated with *V. vulnificus* and *V. parahaemolyticus*, the aim of this study was to estimate the risk associated with raw oyster consumption affected by contamination levels and temperature during postharvest and transportation. To evaluate the effect of the temperature during transportation from the Mandinga Lagoon to Mexico City on the growth of *V. vulnificus* and *V. parahaemolyticus*, a modified Gompertz model was fitted at ambient temperatures of 20.1, 25.6, and 24.4°C for 22 h in windy, dry, and rainy seasons, respectively. The risk was calculated using FDA/FAO/WHOv.2005 software. Results showed that the mean risk (cases per 100,000 servings) of a person acquiring *V. vulnificus vvha+/cvg*C infection by consuming raw oysters was 2.9×10^{-6}, 4.7×10^{-6}, and 4.3×10^{-6} during windy, dry, and rainy seasons, respectively. Risk for consuming oysters during windy season at-harvest contaminated with *V. parahaemolyticus tdh+* was 8×10^{-6} and 7.8×10^{-7} for consuming oysters at-market during rainy season contaminated with *V. parahaemolyticus tdh+* and *trh+*. These results suggest that maintaining temperatures above 20°C during oyster storage and transportation increases the risk of infections by pathogenic strains. The results provide a benchmark information to establish strategies to improve public health.

Keywords: *Crassostrea virginica*, *Vibrio*, harvest, market, season, survival, risk

1. Introduction

Vibrio vulnificus and *Vibrio parahaemolyticus* are the etiologic agents of seafood-associated fatalities worldwide. These Gram-negative, halophilic bacteria found naturally in marine and estuarine waters have the ability to cause lethal infections

including primary septicemia, wound infection, and gastroenteritis associated with the consumption of raw or undercooked seafood, particularly oysters, throughout the world [1–4]. *V. vulnificus* is more frequently associated with wound infections, with a case fatality rate as high as 50% [5], particularly in individuals with predisposing conditions, including patients with chronic liver disease, immunodeficiency, iron storage disorders, end-stage renal disease, and diabetes mellitus [6]. Similarly, *V. parahaemolyticus* infection can cause diarrhea and septicemia that may be life-threatening to people having underlying medical conditions such as liver disease, diabetes, or immune disorders [7, 8]. The *tlh* (thermolabile hemolysin) gene is a species-specific marker for *V. parahaemolyticus*, while the *tdh* (thermostable direct hemolysin) and *trh* (thermostable-related hemolysin) genes are pathogenicity markers for *V. parahaemolyticus* [9]. The occurrence of *orf8* genes has been considered an additional virulence factor for *V. parahaemolyticus* [10, 11]. *V. vulnificus* includes three biotypes of which Biotype 1 is capable of producing fatal disease to humans due to consumption of raw seafood. Biotype 1 has been further divided into two genotypes, C and E. The gene *vcg* (virulence-correlated gene) has two alleles, *vcgC* and *vcgE*, representing clinical and environmental strains, respectively [11].

Vibrio vulnificus and *V. parahaemolyticus* are commonly reported in many countries around the world with high mortality rates [12]. In Mexico, *V. vulnificus* was isolated in 27% (39/143) of oyster samples collected from Pueblo Viejo Lagoon, located on the North Gulf Coast of Veracruz state, Mexico. Isolation rates were significantly higher in June ($P < 0.0002$) and *V. vulnificus* was found to prefer salinity conditions above 18‰ and temperatures above 24°C ($P < 0.001$) [13]. Meanwhile, *V. parahaemolyticus tdh+* incidence has been reported in raw oysters (44.0%) sold in Guadalajara, México, during the warm months ($P = 0.0038$) [14], and in oyster samples (8.7%) from Pueblo Viejo Lagoon in Tamaulipas, México as well; likewise, in the coastal zone of Tamaulipas, México, a 19.9% prevalence of *V. parahaemolyticus* in oysters was reported, which increased 18.3 times during summer months (July, August, and September) [15]. Our studies [16] revealed the highest mean densities of *V. parahaemolyticus tlh+, tdh+/trh, tdh/trh+* and *tdh+/trh+* during spring season at 2.57, 1.74, 0.36, and 0.40 $\log_{10}$ MPN/g, respectively, and *tdh+/orf8+* during winter season (0.90 $\log_{10}$ MPN/g) in oysters harvested from Mandinga Lagoon System (MLS) located on the coast of Veracruz, Mexico. *V. parahaemolyticus tlh +* densities were associated to salinity ($R^2 = 0.372$, $P < 0.022$), *tdh+/trh+* to turbidity ($R^2 = 0.597$, $P < 0.035$), and *orf8+* to temperature, salinity, and pH ($R^2 = 0.964$, $P < 0.001$) [16]. In this context, the exposure to salinity and temperature conditions regulate the dynamics of *V. vulnificus* and *V. parahaemolyticus* harboring potentially pathogenic genotypes within the oyster. This adaptive response of *V. vulnificus* and *V. parahaemolyticus* to seasonal environmental changes may lead to an increase in survival and virulence, threatening the seafood safety and increasing the risk of illness [16].

The American oyster (*Crassostrea virginica*) is one of the most popular bivalve mollusks, widely consumed in large quantities. In Veracruz state, oysters are harvested extensively within the oyster-producing areas found along the Mexican Gulf coast. The state of Veracruz is the primary oyster producer, harvesting 26,713 tons annually, which accounts for 43% of the national average annual production (61,996 t) [17]. They are sold alive in whole shell, shucked in fresh form or packaged, and refrigerated in polyethylene bags. According to the Mexican Norm [18], which provides guidelines for the sanitary control and commerce of shellfish in Mexico, shellstock oysters should be kept alive and adequately refrigerated to an internal body temperature of 7°C for 7 days at most to ensure safe consumption. Nevertheless, during transport and storage of raw oysters, adverse conditions (low oxygen levels, accumulation of waste, feeding interruption, and temperature

abuse) favor recontamination and rapid deterioration [19]. *V. vulnificus* and *V. parahaemolyticus* can multiply in postharvest shellfish if they are held at temperatures >10°C [20, 21]. Although our previous studies have revealed a high prevalence of *V. parahaemolyticus* in oysters (*C. virginica*) in Veracruz, a relatively high proportion of oysters sold is not currently subjected to any postharvest process and is thus a health hazard. The MLS is an important area economically, where seafood production and consumption are common. It represents one of the most productive estuarine-lagoon systems in the Mexican Gulf of Mexico for year-round oyster harvesting with an oyster production of 306 t/y, resources that are supplying to seafood restaurants and oyster bars from nearby cities, mostly Veracruz—Boca del Río, and to Cancún and México City [22]. Because of the importance of raw oysters in gastronomy and economics, their microbial safety is of major interest. However, there is limited information on the loads of *V. vulnificus* and *V. parahaemolyticus* in oysters at market after long-distance transportation. Therefore, the aim of this study was to compare the seasonal survival ability of *V. vulnificus* and *V. parahaemolyticus* in shellstock oysters transported under ambient air and dry storage conditions from the MLS to a wholesale market in Mexico City, and to assess the risk as affected by storage and transportation conditions.

2. Materials and methods

2.1 Oyster collection and transportation

Six-specimen collections were performed from the same lot of oysters during dry, rainy, and windy seasons from January to December 2012 in two different sites: (1) at the oyster harvesting bank Mata Grande (Pescadores Unidos Union producer) in the MLS at 08:00 am by divers and (2) directly from the customer at the *Central de Abasto* in Mexico City at 08:00 am next morning, where oysters from this producer are sold. This is one of the most important wholesale seafood markets in Mexico City. Mata Grande oyster bank is located close to mangrove islands in Mandinga Grande lagoon (**Figure 1**). The MLS is located in southern state of Veracruz, Mexico, flows parallel to the northwestern coastline of the Gulf of Mexico, between 19°02′ N and 96°06′ W in Alvarado, Veracruz. MLS is formed by the confluence of the river Jamapa, and effluents of Huatusco, Cotaxtla, Totolapan rivers, ending in the Gulf of Mexico by the Boca del Rio, close to Veracruz City. It is

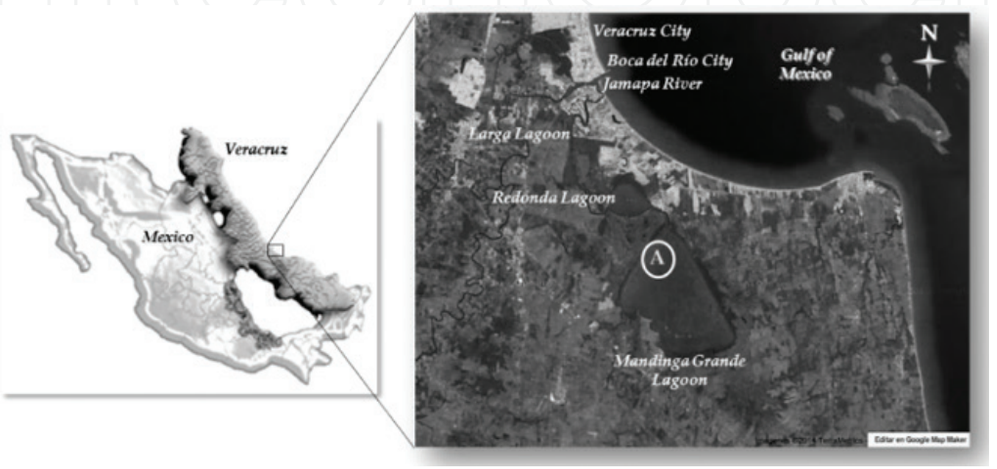

Figure 1.
Location of the study region and map of the MLS. Site of oyster samples collection monitored during dry, rainy, and windy seasons: bank A Mata Grande located close to mangrove islands 19° 01′ 53.8″N and 96° 04′ 23.1″W.

a shallow (1–3 m depth) tropical lagoon connected to the sea by a long and narrow deeper channel through the Jamapa River. This lagoon system consists of four lagoons (Conchal, Larga, Chica, and Grande) and flooded zones and cover an area of 3250 ha. The dry season occurs from March to June, the rainy season occurs from July to October, and the windy season from November to February when the MLS is affected by high-velocity northern winds (90–129 m/s) [16, 23].

Producers harvested the oysters at 08:00 am in the morning and stored at ambient temperature in a storage room until 18:00 pm when oysters were loaded in sacks for transport. Oyster sacks were transported stacked on a nonrefrigerated box truck overnight by road, arriving to the *Central de Abasto* market in Mexico City at 03:00 am. Product was delivered to customer at 6:00 am and samples were collected at 08:00 am. The average transit time was 22 h supply-chain from MLS to Mexico City. This producer transports oysters at ambient temperature and no records were available to document postharvest temperature exposure, which creates increased opportunity for temperature abuse. Therefore, specific practices and sampling points were selected based on those that are currently in use. A total of 80 legal-sized [24] live shellshock oysters were immediately transported to the laboratory according to Mexican Minister of Health approved method NOM-109-SSA1-1994 [25]. Dead animals were discarded, and the remaining oysters were scrubbed and rinsed under cold running tap water to remove debris and attached algae.

2.2 Bacteriological analysis

Within 2 h of collection, oysters were shucked, and meats and intravalvular liquids were pooled under aseptic conditions. Oyster samples were analyzed according to the protocol of Lizárraga-Partida et al. [26] modified. *V. vulnificus* and *V. parahaemolyticus* quantification was performed following the same most probable number-polymerase chain reaction (MPN-PCR) procedure described previously [16, 27], briefly: a 200 g of oyster sample (150 g of meat and 50 g of intravalvular fluid) were mixed with 200 mL phosphate-buffered saline (PBS) and blended for 120 s to make a 1:1 dilution. The shellfish homogenate was added to alkaline peptone water in a three-tube MPN dilution series prepared up to $1:10^4$ according to the standard three-tube MPN procedure. The tubes were incubated at 35°C for 24 h. After incubation, DNA was extracted from each positive APW tube showing growth and then purified. The densities of *V. vulnificus* and *V. parahaemolyticus* strains were calculated using positive results by PCR, employing the most probable number (MPN) tables. Simultaneously, one loopful from the top 1 cm of each positive broth tube from the MPN method categorized as positive for *V. vulnificus vvha* + and *V. parahaemolyticus tlh*+ based on the DNA amplification results was streaked onto CHROMagar™ Vibrio (CHROMagar Microbiology, Paris, France). Plates were incubated at 35°C for 24 h for the isolation of presumptive colonies. To confirm the presumptive *V. vulnificus vvha*+, *vcg* E, and *vcg* C, and *V. parahaemolyticus tlh*+, *tdh*+, *trh*+, and *orf*8, at least 15 blue-green and mauve well-grown colonies from each CHROMagar plates were selected and inoculated into APW tubes, incubated at 35°C for 18–24 h, and then subjected to DNA extraction, purification, and amplification. Presumptive strains that were confirmed with the direct PCR were scored as positive for the respective gene and stored in Trypticase soy agar (TSA; BIOXON Becton Dickinson S.A de C.V., Mexico) slants at −20°C. PCR assays were performed using specific primers (Sigma-Aldrich QUIMICA S.A. de C.V., Toluca, Mexico) for species and identification of pathogenic genes. Oligonucleotides targeting the *vvhA* (cytotoxin, cytolysin) and *tlh* (thermolabile hemolysin) genes were used for *V. vulnificus* and *V. parahaemolyticus*, respectively. Strains from the Collection of Aquatic Important Microorganisms (CAIM) of Centro de Investigaciones en Alimentación y Desarrollo A.C. Mazatlán, Sinaloa, México (www.ciad.mx/caim) were

used as positive controls. DNA of strain CAIM 610 was used as positive control for (*vvha*) gene [28], and strains CAIM 1860 and 1859 for *vcgC* and *vcgE* genes, respectively [29]. DNA of strain CAIM 1772 was used as positive control for *V. parahaemolyticus* nontoxigenic (*tlh*) and toxigenic (*tdh*, *trh*) genes [30], and strain CAIM 1400 for *orf*8 gene [31]. A 100-bp ladder (100–3000 bp; Axygen) was used as a DNA size marker. Densities of *V. vulnificus* and *V. parahaemolyticus* strains were expressed by the most probable number (MPN) as *V. vulnificus* or *V. parahaemolyticus* MPN/g of oyster [32]. *V. vulnificus* and *V. parahaemolyticus* presumptive isolates were identified by biochemical characteristics using Kligler iron agar slants (KIA), lysine iron agar (LIA), motility-indole-ornithine medium (MIO), Moeller decarboxylase broth media, and arginine dihydrolase test. All agar media were BD Bioxon (Becton Dickinson de México S.A. de C.V., México, México). The oxidase test (p-aminodimethylaniline; Becton Dickinson, NJ, USA) was performed on growth from presumptively positive isolates. Some *V. vulnificus* strains isolated from oysters collected at the *Central de Abasto* market in Mexico City were characterized for *vvhA* and *vcg* genotype, using PFGE, multilocus sequence typing (MLST), and *rtx*A1. Analyses included a comparison with *rtx*A1 reference sequences. Environmental *V. vulnificus* C genotype strains had high similarity to the virulent reference strain (CAIM 1860) [33].

2.3 Statistical analysis

2.3.1 Seasonal densities and survival

Most probable number (MPN) three-tube chart and formulas corresponding 95% confidence limits were used to identify MPN for each sample [32]. MPN values for *V. vulnificus* and *V. parahaemolyticus* counts were log-transformed to normalize the data and homoscedasticity requirements for appropriate analysis of variance. Significant differences in the seasonal distributions of $\log_{10}$ MPN/g *V. vulnificus* and *V. parahaemolyticus* densities were analyzed by analysis of variance ($P < 0.05$) and Tukey's test. All statistical analyses were carried out with *X*LSTAT > 2018 software (Addinsoft™) with the minimum level of significance set at $P < 0.05$. Nondetectable values of *V. vulnificus* and *V. parahaemolyticus* counts (<0.30 MPN/g) were replaced by half of the detection limit in oysters for statistical purposes.

To evaluate the effect of the transportation time on the growth ($\log_{10}$ NMP/g) of *V. vulnificus* and *V. parahaemolyticus*, a modified Gompertz model was fitted to the experimental data obtained at the ambient temperatures of 20.1, 25.6, and 24.4°C [34] for 22 h during supply-chain transportation in windy, dry, and rainy seasons, respectively, from MLS to Mexico City. Lag time and specific growth rate of strains were determined using Statistica 7.0 (Statistica Software, Palo Alto, CA, USA). This model has been used to describe *V. parahaemolyticus* growth (Eq. (1)) [35]:

$$Y_0 = N_0 + A\ x\ exp\ [-exp(2.718\frac{\mu_{max}}{A})\ x\ (\lambda - t) + 1] \quad (1)$$

where *Yt* is the log counts (CFU/g) at time *t*; N_0 is the initial level of bacteria ($\log_{10}$ CFU/g); $A = \log_{10}\ (N_{max}/N_0)$, where N_{max} represents the growth from the inoculum to stationary phase; and the parameters exp, μ_{max}, and λ represent *e* constant, maximum specific growth rate (h^{-1}), and lag time of the strain growth (h), respectively. The effect of temperature on *V. vulnificus* and *V. parahaemolyticus* growth was calculated with Eq. (2):

$$G = \ln 2/\mu_{max} \quad (2)$$

where G is the generation time (h) at 20.1, 25.6, and 24.4°C and μ_{max} is maximum specific growth rate (h^{-1}).

Goodness of fit of the modified model was evaluated using the coefficient of determination (R^2) and the standard deviation of the residuals (Syx), which were provided by Statistica software.

2.3.2 Risk assessment

The FDA/FAO/WHO v.2005 software in combination with Microsoft Excel was used to run the risk simulations using the model developed by the U.S. Food and Drug Administration and used by FAO/WHO to estimate the risk of illness associated to the consumption of raw oysters [37]. Results were expressed as number of cases per 100,000 servings (cocktails consumed). The consumption data considered *V. vulnificus* and *V. parahaemolyticus* levels in raw oysters at harvest and after transportation and serving size (a cocktail of 12 oysters).

3. Results and discussion

3.1 Seasonal densities and survival after transportation

As shown in **Table 1**, significant differences in *V. vulnificus vvha*+ densities between seasons were observed, with higher ($P < 0.05$) mean levels during windy (0.720 $\log_{10}$ MPN/g) and the lowest in rainy (−0.523 $\log_{10}$ MPN/g) seasons. During windy season, the average water temperature in the MLS-Mata Grande bank was 25.6°C, nevertheless mean *V. vulnificus vvha*+ densities decreased during rainy season when the average water temperature increased ($P > 0.05$) to 27.4°C. However, salinity was higher ($P < 0.05$) in windy (25.8‰) than in rainy (7.3‰) seasons (**Table 5**).

In contrast, *V. parahaemolyticus tlh*+ density levels were high ($P > 0.05$) in rainy (0.713 $\log_{10}$ MPN/g) and low in windy (0.477 $\log_{10}$ MPN/g) seasons. After 22 h of supply-chain transportation, *V. vulnificus vvha*+ and *V. parahaemolyticus tlh*+ densities increased ($P < 0.05$) in all seasons probably due to the high ambient temperatures observed during transportation (20.1, 25.6, and 24.4°C). **Table 2** shows that no *V. vulnificus vvha*+ *vgc*E densities were detected at-harvest and remain

Seasons	*Vibrio vulnificus vvha*+ ($\log_{10}$ MPN/g mean and range)		*V. parahaemolyticus tlh*+ ($\log_{10}$ MPN/g mean and range)	
	At-harvest	**At-market**	**At-harvest**	**At-market**
Windy	$0.720 \pm 0.344^{a,x}$ (0.477–0.964)	$3.351 \pm 0.041^{a,y}$ (3.322–3.380)	$0.477 \pm 0.001^{a,x}$ (0.477–0.0.477)	$3.041 \pm 0.001^{a,y}$ (3.041–3.041)
Dry	$-0.483 \pm 0.056^{b,x}$ (−0.523 to −0.444)	$1.055 \pm 0.129^{b,y}$ (0.964–1.146)	$0.686 \pm 0.0.149^{a,x}$ (0.580–0.792)	$3.210 \pm 0.239^{a,y}$ (3.041–3.380)
Rainy	$-0.523 \pm 0.001^{c,x}$ (<−0.523)*	$3.351 \pm 0.041^{a,y}$ (3.322–3.380)	$0.713 \pm 0.221^{a,x}$ (0.556–0.869)	$3.380 \pm 0.001^{a,y}$ (3.380–3.380)

Means with different letter (a, b, c) are significantly different ($P < 0.05$) between seasons.
Means with different letter (x, y) are significantly different ($P < 0.05$) between hours of transportation within each season.
**< −0.523 = not detected.*

Table 1.
Seasonal variations of V. vulnificus vvha *and* V. parahaemolyticus tlh *densities (log_{10} MPN/g) in* Crassostrea virginica *samples from the MLS during 22-h supply-chain transportation in windy, dry, and rainy seasons, respectively, from MLS to Mexico City.*

Seasons	*Vibrio vulnificus vgc*E (log_{10} MPN/g mean and range)		*Vibrio vulnificus vgc*C (log_{10} MPN/g mean and range)	
	At-harvest	**At-market**	**At-harvest**	**At-market**
Windy	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.483 \pm 0.056^{a,x}$ (−0.523 to −0.444)	$0.469 \pm 0.010^{a,x}$ (0.462–0. 477)	$0.781 \pm 0.005^{a,y}$ (0.778–0.785)
Dry	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.523 \pm 0.001^{b,x}$ (<−0.523)	$-0.523 \pm 0.001^{b,x}$ (<−0.523)
Rainy	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.483 \pm 0.056^{a,x}$ (−0.523 to −0.444)	$-0.523 \pm 0.001^{b,x}$ (<−0.523)	$0.469 \pm 0.010^{c,y}$ (0.462–0. 477)
	V. parahaemolyticus tlh+/tdh+		***V. parahaemolyticus tlh+/tdh−/trh+***	
Windy	$-0.020 \pm 0.707^{a,x}$ (<−0.523–0.477)	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.523 \pm 0.001^{a,x}$ (<−0.523)
Dry	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.523 \pm 0.001^{a,x}$ (<−0.523)
Rainy	$-0.523 \pm 0.001^{a,x}$ (<-0.523)*	$-0.484 \pm 0.056^{a,x}$ (−0.523 to −0.444)	$-0.523 \pm 0.001^{a,x}$ (<−0.523)	$-0.484 \pm 0.056^{a,x}$ (−0.523 to −0.444)

Means with different letter (a, b, c) are significantly different ($P < 0.05$) between seasons.
Means with different letter (x, y) are significantly different ($P < 0.05$) between hours of transportation within each season.
**<−0.523 = not detected.*

Table 2.
Seasonal variations of pathogenic V. vulnificus *(genotypes E and C) and* V. parahaemolyticus *(*tlh/tdh, tlh/trh*) densities (log_{10} MPN/g) in* Crassostrea virginica *samples from the MLS during 22-h supply-chain transportation in windy, dry, and rainy seasons, respectively, from MLS to Mexico City.*

unculturable after 22-h transportation during dry season. A seasonal trend was observed, as higher ($P > 0.05$) *V. vulnificus vvha+ vgc*C density (0.469 log_{10} MPN/g) in oysters harvested from Mata Grande bank was found during windy season, and no densities were detected during dry and rainy seasons. Similarly, *V. parahaemolyticus tdh+* density in oysters increased ($P > 0.05$) in windy season (−0.020 Log_{10} MPN/g), but no densities were detected during dry and rainy seasons. In contrast, no *V. parahaemolyticus trh+* density was detected in all seasons. After 22 h of supply-chain transportation, a slight increase ($P > 0.05$) in *V. vulnificus vgc*E (−0.483 log_{10} MPN/g) in windy and rainy seasons was observed. *V. vulnificus vgc*C density in oysters increased ($P < 0.05$) in windy (0.781 log_{10} MPN/g) and rainy seasons (0.469 log_{10} MPN/g) as well. An increase in densities of *V. parahaemolyticus tdh+* and *trh+* (−0.484 log_{10} MPN/g) in oysters was observed in rainy season, probably due to the high ambient temperature observed (24.4°C) in rainy season. Our results were lower than those reported in oysters harvested from the U.S. Gulf of Mexico during dry season (3.36 log_{10} MPN/g), which were higher than those detected during windy season (1.0 log_{10} MPN/g) [37]. *V. vulnificus* proliferates in areas or during months where the water temperature exceeds 18°C as in MLS, and culturable concentrations of *V. vulnificus* are generally lower when water temperatures are cooler.

In other study, *V. vulnificus* was isolated from oyster samples collected from Pueblo Viejo Lagoon, Veracruz, and 27% (39/143) of the oyster samples were *vvha+*. Although positive samples were found during all seasons of a 1-year period, a seasonal fluctuation was observed. Isolation rates from oysters were significantly higher in June than in the period from November to February ($P < 0.0002$), indicating that water surface temperatures >24°C and salinity conditions >18‰ are more favorable for *V. vulnificus* [13]. In our study, we found higher ($P < 0.05$) *V. vulnificus vvha+* densities during windy (December to March) and dry seasons (April to July) when water temperature and salinity were 25.6°C/25.8‰ and 28.7°C/29.8‰,

respectively. However, a decrease was observed during rainy season when water temperature and salinity were 27.4°C and 7.3‰, respectively. Thus, *V. vulnificus* colonization of oysters in MLS may be influenced by water parameters such as temperature or salinity as previously reported [38]. An important finding in our study was the isolation of pathogenic *V. vulnificus vgc*C strains. This is the first study to report the presence of *V. vulnificus vgc*C in oysters from the Mexican coastline of the Gulf of Mexico. It is unclear if levels of the two *V. vulnificus* genotypes are unique to certain environmental conditions. As with previous studies of total *V. vulnificus* levels, a significant negative correlation with salinity was observed for the *vgc*C strains from oysters ($r = -0.35$, $P = 0.008$) [39]. In our study, there was a significant increase in the population of *V. vulnificus vgc*C in oysters in winter season when MLS water salinity levels were high. These results seem to indicate that *V. vulnificus vgc*C strains have evolved to cope with the stresses associated with changing environment. The fact that oysters have *vgc*C strains as the dominant strain type further suggests the possibility that those oysters harboring larger densities of this genotype would likely to be more infective to humans as *V. vulnificus vgc*C type is more infectious [29].

Regarding *V. parahaemolyticus*, our results demonstrated that *V. parahaemolyticus tlh*+ strains are present almost throughout the year as *V. parahaemolyticus* abundance in the Gulf of Mexico is almost constant because temperature is warmer (>11.6°C) [40]. The seasonal trends in *V. parahaemolyticus* densities observed in our study agree with previous studies since the seasonal cycle of the pathogen has been correlated with dry and rainy seasons in tropical waters, being salinity the major factor. *V. parahaemolyticus tlh*+ density was detected at 3.26 $\log_{10}$ MPN/g in oysters (*Crassostrea brasiliana*) harvested from Sao Paulo, Brazil during the dry season when mean water temperature was 29°C and salinity 29‰ [41]. Our previous studies have shown that there is a seasonal variation in the survival and virulence of *V. parahaemolyticus*, probably caused by a response of gene expression to stress. *V. parahaemolyticus tlh*+/*tdh*+ densities in oysters harvested from the MLS were observed during windy and dry seasons (0.97 and − 0.18 $\log_{10}$ MPN/g), respectively, and *V. parahaemolyticus tlh*+/*tdh*−/*trh*+ (−0.37 $\log_{10}$ MPN/g) was only detected during dry season. Meanwhile, during rainy season only, −0.509 $\log_{10}$ MPN/g was identified [42]. The presence of pathogenic *V. parahaemolyticus* strains raises important health issues and may be indicative of high risk in usual consumers of oyster from Mandinga lagoon during windy season where the maximum densities are found. These data suggest that *V. vulnificus* and *V. parahaemolyticus* populations in oysters are controlled by different factors. Moreover, the oyster, as a living host, may have contributed to the variation in these pathogen densities because of fluctuations in physiology resulting from reproductive status, diet, and health [11]. Densities above Mexican limits (absence in 50 g of sample) [19] for *V. parahaemolyticus tlh*+ and *V. vulnificus vvha*+ were detected in oyster samples at-harvest and at-market. In Mexico, these pathogens are not currently included in the microbiological surveillance programs of shellfish from harvesting areas and they are also excluded from the Mexican communicable disease surveillance plans. Thus, the presence of pathogenic strains is a public health concern, as these strains are not covered by current regulations.

The values for the kinetic growth parameters and performance statistics of the modified Gompertz model for *V. parahaemolyticus* (*tlh*+, *tlh*+/*tdh*+, and *tlh*+/*tdh*−/*trh*+) and *V. vulnificus vvha*+ and genotypes E and C, at ambient temperatures during 22 h transportation of oysters are shown in **Tables 3** and **4**, respectively. The average R^2 value of the model fitted to *V. parahaemolyticus* growth was 0.9999 for nonpathogenic *tlh*+ and for pathogenic *tdh*+ and *trh*+ strains. Similarly, R^2 value of the model fitted to *V. vulnificus* growth was 0.9999 for *vvha*+ and for *vcg*E and *vcg*c

strains, indicating that this model was able to describe both pathogens growth. As shown in **Table 3**, the predicted lag time values of the nonpathogenic *tlh* strains were 4.2909, 4.3582, and 4.2484 h in windy, dry, and rainy seasons, respectively; meanwhile, the predicted lag time values for both pathogenic *tdh*+ and *trh*+ strains were 6.3439 during rainy season, indicating faster growth and better adaptation of the nonpathogenic strain to ambient temperatures during transportation.

V. parahaemolyticus	μ_{max} (h^{-1})	λ (h)	*A*	*G* (h)	R^2	S_{yx}
Windy						
tlh+	1.0242	4.2909 (257.5 min)	2.564	0.6767 (40.6 min)	0.9999	0.00067
Dry						
tlh+	1.0117	4.3582 (261.5 min)	2.520	0.6851 (41.1 min)	0.9999	0.00073
Rainy						
tlh+	1.0736	4.2484 (254.9 min)	2.670	0.6456 (38.7 min)	0.9999	0.00073
tlh+/*tdh*+	0.0096	6.3439 (380.6 min)	0.039	72.0207 (4321.2 min)	0.9999	0.00018
tlh+/*tdh*−/*trh*+	0.0096	6.3439 (380.6 min)	0.039	72.0207 (4321.2 min)	0.9999	0.00018

Table 3.
Parameter values using the modified Gompertz model for V. parahaemolyticus *(*tlh+, tlh+/tdh + *and* tlh+/tdh−/trh+*) growth in oysters transported for 22 h at 20.1, 25.6, and 24.4°C (windy, dry, and rainy seasons) from MLS to Mexico City.*

V. vulnificus	μ_{max} (h^{-1})	λ (h)	*A*	*G* (h)	R^2	S_{yx}
Windy						
vvha+	1.0592	4.2838 (257.0 min)	2.630	0.6544 (39.3 min)	0.9999	0.00757
*vcg*E	0.0098	6.3022 (378.1 min)	0.040	70.7009 (4242.1 min)	0.9999	0.00021
*vcg*C	0.0836	5.9274 (355.6 min)	0.31	8.2914 (497.5 min)	0.9999	0.00013
Dry						
vvha+	0.5280	4.5347 (272.0 min)	1.540	1.3126 (78.8 min)	0.9999	0.00621
Rainy						
vvha+	1.7885	4.3926 (263.6 min)	3.870	0.3876 (23.3 min)	0.9999	0.00730
*vcg*E	0.0098	6.3022 (378.1 min)	0.04	70.7009 (4242.1 min)	0.9999	0.00021
*vcg*C	0.3063	4.9150 (294.9 min)	0.990	2.2633 (135.8 min)	0.9999	0.00005

Table 4.
Parameter values using the modified Gompertz model for V. vulnificus *(*vvha+, vcg*E, and* vcg*C) growth in oysters transported for 22 h at 20.1, 25.6, and 24.4°C (windy, dry, and rainy seasons) from MLS to Mexico City.*

Pathogenic strains were detected in oysters after 22 h of transportation only during rainy season. These results indicated that nonpathogenic *tlh*+ and pathogenic *tdh*+ and *trh*+ strains reached a maximum growth rate and the maximum density (6.670, 0.039, and 0.039 $\log_{10}$ MPN/g, respectively) after 22-h transportation at ambient temperature during rainy season. The values of lag time observed in this study were lower than those previously reported for nonpathogenic *tlh*+ (24.6 h) and pathogenic *trh*+ (38.7 h) strains of *V. parahaemolyticus* isolated from raw Korean oysters [35]. In the present study, the longer lag time of pathogenic strains may be due to the time required for colonization of the oyster tissue. It has been reported that *V. parahaemolyticus* colonized oyster tissues according to the change of time as it is digested by oysters [43]. The maximum specific growth rate (max) predicted for pathogenic *tdh*+ and *trh*+ strains (0.0096 h^{-1}) was lower than that for nonpathogenic *tlh*+ (1.0242, 1.0117, and 1.0736 h^{-1}) in windy, dry, and rainy seasons, respectively; generation times (*G*) of nonpathogenic (0.6767, 0.6851, 0.6456 h) in windy, dry, and rainy seasons, respectively, were shorter than that for pathogenic strains (72.0207 h). These results indicated that nonpathogenic *V. parahaemolyticus* strains reached a maximum growth rate faster by storage temperatures. However, both pathogenic and nonpathogenic *V. parahaemolyticus* grew during storage in rainy season, although it was not detected in at-harvest oysters. This finding suggests that the bacterium was most likely present in numbers below the limit of detection, or perhaps in a viable but nonculturable state. In addition, it has been also observed that *V. parahaemolyticus* multiplied rapidly in live oysters held at 26°C after harvest [20]. It has been reported that higher concentrations of *V. parahaemolyticus* are present in market oysters than in at-harvest oysters [44]. In our study, pathogenic *V. parahaemolyticus* strains had the ability to adapt and survive at 24.4°C during transportation in rainy season, prior to marketing. However, the growth characteristics of *V. parahaemolyticus* might vary by strain variation.

According to **Table 4**, the predicted lag time values of *V. vulnificus vvha*+ strains were 4.2838, 4.3547, and 4.3926 h in windy, dry, and rainy seasons, respectively. The predicted lag time values were 6.3022 for *vcg*E and 5.9274 and 4.9150 for *vcg*C during windy and rainy seasons, respectively, indicating faster growth and better adaptation to ambient temperatures during transportation of the *vvha*+ than *vcg*C strains. No *vcg*C and *vcg*E strains were detected in oysters after 22 h of transportation during dry season. *V. vulnificus vcg*E strains lag time values were similar to those of *V. parahaemolyticus tdh*+ and *trh*+ strains, but higher than those of *V. vulnificus vcg*C strains. The maximum specific growth rate (max) predicted for *vcg*E (0.0098 h^{-1}) and *vcg*C strains (0.0836 and 0.3063 h^{-1}) were lower than that for *vvha*+ (1.0592, 0.5280, and 1.7885 h^{-1}) in windy, dry, and rainy seasons, respectively. Generation times (*G*) of *vvha*+ (0.3876 h), *vcg*E (70.7009 h), and *vcg*C strains (2.2633 h) in rainy season were shorter than that observed in windy and dry seasons. These results indicated that *V. vulnificus vvha*+, *vcg*, and *vcg*C strains reached a maximum growth rate and the maximum density (3.870, 0.04, and 0.990 log10 MPN/g, respectively) after 22-h transportation at ambient temperature during rainy season. It has been reported a maximal growth rate of 0.175/h and a 1.3 log10 increase in *V. vulnificus* levels in oysters stored at 28°C [45]. Recently, a predictive growth model for *V. vulnificus* in Pacific oysters was developed [46], where growth rate and lag time of *V. vulnificus* in shucked oyster meat at 24°C were 0.0138 h^{-1} and 5.38 h, respectively. Overall, this growth rate is much lower than those observed in *V. vulnificus vvha*+ strains in our study. However, the lag time value is higher than our *V. vulnificus vvha*+ strains values. *V. vulnificus* and *V. parahaemolyticus* densities in shell oysters at the stage of distribution were greater than those observed in oysters at-harvest. Moreover, both *V. vulnificus* and *V. parahaemolyticus* grew during storage, although they were not detected at-harvest oysters. During transport and storage of raw oysters, adverse conditions (low oxygen

levels, accumulation of waste, and feeding interruption) are able to disrupt a variety of cellular processes and can promote the development of more stress-resistant cells, modulating the fitness and virulence of bacterial pathogens.

Studies have suggested that pathogenic strains have low levels of detection compared with nonpathogenic strains and are more sensitive to dystrophic conditions, rapidly becoming nonculturable [47]. Furthermore, differences in regulated genes between strains may more likely due to be a response against environmental stressors. Harvest and transport techniques used in this study were typical for the commercial oyster industry in the MLS and Alvarado Lagoon zones.

Therefore, these bacteriological findings in the commercial handling portion of the study are representative of the industry in Veracruz state and throughout perhaps the entire Mexican Gulf Coast oyster industry. These results indicate that the safety of raw oysters for consumption depends upon their initial degree of contamination, mainly due to the quality of seawater from which they are extracted or cultured, and to postharvest storage conditions. Because temperature abuse during postharvest handling and storage may increase the risk of illness due to the consumption of oysters, it is very important to predict the risk of *V. vulnificus* and *V. parahaemolyticus* to consumers. The infectious dose of *V. vulnificus* for the high-risk group is 2 log CFU/g [6]; therefore, for the protection of consumers, careful storage and consumption guidelines for oysters at retail markets and restaurants must be emphasized.

3.2 Risk assessment

According to **Table 5**, the results indicate that the risk of consuming a typical meal of 12 raw oysters contaminated with *V. vulnificus* would be higher in dry and rainy seasons. *V. vulnificus* levels in oysters and the corresponding consumer risk at the vending site are strongly influenced by climate, especially water and air temperatures. The findings indicate that the risk of oyster consumption from Veracruz, Mexico is slightly lower than those estimated by WHO/FAO [48] for *V. vulnificus* predicted to be associated with month- and year-specific water temperatures in the Gulf of Mexico, which were 3.37×10^{-5} and 4.28×10^{-5} during dry and rainy seasons, respectively. However, the risk of oyster consumption during windy season (2.9×10^{-6}) was similar to that reported by WHO/FAO (1.26×10^{-6}).

It is important to point out that seasonal expansion of *V. vulnificus* illnesses associated with oysters harvested from the Gulf of Mexico corresponds with warmer water temperatures (>20°C). The evidence indicates that climate anomalies have already greatly expanded the risk for vibrio illnesses [49]. WHO/FAO [48] reported a risk assessment for primary septicemia cases associated with consumption of raw oysters from the Gulf Coast of USA with mean densities of 57,000 and 80 MPN/g during summer and winter harvest seasons, respectively. In this context, variation

Season	Air temperature (°C)	Water temperature (°C)	Salinity (‰)	Risk for at-risk population (cases per 100,000 servings; 95% confidence interval)
Windy	20.1	25.6	25.8	2.9×10^{-6} (2.0×10^{-6}–3.8×10^{-6})
Dry	25.6	28.7	29.8	4.7×10^{-6} (3.8×10^{-6}–5.8×10^{-6})
Rainy	24.4	27.4	7.3	4.3×10^{-6} (3.5×10^{-6}–5.4×10^{-6})

Table 5.
Estimated risk assessment to V. vulnificus *associated to consumption of raw oyster cocktail expended at-harvest at the MLS and at-market in Mexico City during windy, dry, and rainy seasons.*

in water and air temperatures and the characteristics and temperature of transportation and storage time have the effect of increasing the variation of *V. vulnificus* numbers at each point along the harvest-to-consumption continuum.

Table 6 summarized the results of risk to *V. parahaemolyticus*. Results indicated that the contamination rates of virulent *V. parahaemolyticus* (*tdh*+ and *trh*+) in raw oysters at-harvest and at-market, and the transportation temperatures significantly influence the probability of illness. The risk of recently harvested oysters during windy season in Veracruz-Boca del Río oyster bars and restaurants where oysters harvested at the MLS are sold was 1.1-fold higher than the mean risk of consuming oysters during the rainy season. These results indicate that the risk of raw oyster consumption in Veracruz, Mexico is lower than those of the U.S. which were 4.4×10^{-4} [50], similar to those reported in Australia (6×10^{-8}–6.1×10^{-6}), higher than those of Canada (7.5×10^{-10}–1.1×10^{-6}) and New Zealand (8.6×10^{-8}–3.2×10^{-7}), but lower to that in Japan during autumn (1.2×10^{-4}) [51] and Taiwan (8.56×10^{-5}) [52]. The estimated risk in our study is similar to that reported in Malaysia (1.76×10^{-6}) [53], but lower than the average risk associated with the consumption of raw oysters contaminated with pathogenic *V. parahaemolyticus* marketed at Sao Paulo, Brazil of 4.7×10^{-4}, 6.0×10^{-4}, 4.7×10^{-4}, and 3.1×10^{-4} for spring, summer, fall, and winter, respectively [36]. As the microbial risk assessment was conducted, several limitations were identified. The estimation did not include the growth model of *V. vulnificus* and *V. parahaemolyticus* during the time gap from markets to consumption.

However, the model's assumption can be referred for retail outlets that serve fresh raw oysters where there is minimal time for the growth of both pathogens to occur. There is a growing body of evidence to suggest that *V. vulnificus* and *V. parahaemolyticus* infections are increasing and tend to follow regional climatic trends, with outbreaks following episodes of unusually warm weather. Moreover, several epidemiological factors, such as growing consumption and international trade of seafood produce, may increase the incidence of these pathogens [12]. In Mexico, there is currently a lack of detailed surveillance information regarding *V. vulnificus* and *V. parahaemolyticus* infections, which probably disguises their real clinical burden. However, there have been some reports of outbreaks and deaths caused by consumption of oysters contaminated with these pathogens. Recently, a patient with hepatic cirrhosis and hepatic carcinoma

Season	*Vibrio parahaemolyticus* density (log_{10} NMP/g)	Pathogenic rate (%)		Risk for at-risk population (cases per-100,000 servings; 95% confidence interval)	
		tdh+	*trh*+	*tdh*+	*trh*+
Windy					
At-harvest	−0.020	10.0	ND	8×10^{-6} (6.4×10^{-7}–1.0×10^{-4})	ND
At-market	ND	ND	ND	ND	ND
Rainy					
At-harvest	ND	ND	ND	ND	ND
At-market	−0.484	0.2	0.2	7.8×10^{-7} (6.2×10^{-8}–9.9×10^{-6})	7.8×10^{-7} (6.2×10^{-8}–9.9×10^{-6})

Table 6.
Estimated risk assessment to V. parahaemolyticus *associated to consumption of raw oyster cocktail expended at-harvest at the MLS and at-market in Mexico City during windy, dry, and rainy seasons.*

suffered fulminant sepsis and necrohemorrhagic bullae secondary to a *V. vulnificus* infection. The patient had ingested oysters in Mexico City 36 h before [54]. Along Veracruz state in Mexican Gulf Coast, 18 *V. parahaemolyticus* infections were reported. Of 18 patients, 27.7% (5/18) consumed raw oysters at oyster bars and restaurants located in Boca del Río and Veracruz Port [55]. The information provided in this study is important for preventing public health problems as pathogenic genes such as *vcg*C, *tdh*+ and *trh*+ were detected. Moreover, the distribution and variation in numbers of virulent *V. vulnificus* and *V. parahaemolyticus* in oysters may need to be determined before harvest as these data should be valuable for assessment of the human health risk due to consumption of raw oysters which represents a significant threat to human health and seafood safety.

4. Conclusions

The evidence indicates that there are significant differences in *V. vulnificus vvha*+ densities between seasons, with higher mean levels during windy and the lowest in rainy seasons. In contrast, *V. parahaemolyticus tlh*+ density levels were high in rainy and low in windy seasons. After 22 h of supply-chain transportation, *V. vulnificus vvha*+ and *V. parahaemolyticus tlh*+ densities increased due to the high ambient temperatures observed during transportation in all seasons. Pathogenic *V. vulnificus vvha*+ *vgc*C and *V. parahaemolyticus tdh*+ densities in oysters increased in windy season as well. After 22 h of supply-chain transportation, *V. parahaemolyticus tdh*+ and *trh*+ densities increased in rainy season, and *V. vulnificus vgc*C density in oysters increased in windy and rainy seasons. This is the first study to report the presence of *V. vulnificus vgc*C in oysters from the Mexican coastline of the Gulf of Mexico.

Densities above Mexican limits for *V. parahaemolyticus tlh*+ and *V. vulnificus vvha*+ were detected in oyster samples at-harvest and at-market. The presence of pathogenic strains is a public health concern, as these strains are not covered by current regulations. After 22-h transportation at ambient temperature during rainy seasons, nonpathogenic *V. parahaemolyticus tlh*+ and pathogenic *tdh*+ and *trh*+ strains and *V. vulnificus vvha*+, *vcg*E, and *vcg*C strains reached a maximum growth rate and the maximum densities. The risk of consuming a typical meal of 12 raw oysters contaminated with *V. vulnificus* would be higher in dry and rainy seasons, and during windy season for *V. parahaemolyticus*. Although the risk of recently harvested oysters from MLS during the windy, dry, and rainy seasons in Veracruz-Boca del Río oyster bars and restaurants is low, results indicated that the contamination rates of virulent *V. vulnificus* and *V. parahaemolyticus* in raw oysters at-harvest and at-market and the transportation temperatures significantly influence the probability of illness.

Adjustments in industry practices and regulatory policy should be considered, especially for seafood that is consumed raw, such as oysters. The results of this study could help Mexican regulatory agencies to develop sanitary norms to protect the population against health risks caused by consumption of raw oysters contaminated with pathogenic strains, and oyster processors to implement control measures. To reduce the risk of *V. vulnificus* and *V. parahaemolyticus* infection from consuming raw oysters, more rigorous postharvest time-temperature controls and surveillance during transportation and marketing of raw oysters must be implemented for immediate detection of these pathogens, especially if oysters are exported to other countries. In this context, the public should be educated by the local government that foodborne illness must never be measured as a minor illness. If measures for mitigating *V. vulnificus* and *V. parahaemolyticus*

could not lead to a reduction of predicted risk associated with these pathogens and the global climate scenario worsens, the predicted risk will increase.

Acknowledgements

This work was supported by Mexican National Council of Science and Technology CONACYT and the Mexican Ministry of Health project research grant [114024] under the technical responsibility of Dr. Leonardo Lizárraga-Partida. We thank Centro de Investigaciones en Alimentación y Desarrollo A.C. (CIAD) of Vibriomex Group for supplying the control strains of *V. vulnificus* and *V. parahaemolyticus* from their Collection of Aquatic Important Microorganisms (CAIM, www.ciad.mx/caim).

Conflict of interest

The authors have no conflict of interest to declare.

Author details

Violeta Pardío[1*], Irma Wong[2], Leonardo Lizárraga[3], Karla López[1], Argel Flores[1], Guadalupe Barrera[4], Francisco Alarcón[1] and Carlos Fernández[4]

1 Faculty of Veterinary Medicine and Zootechnics, University of Veracruz, Veracruz, Mexico

2 National Autonomous University of Mexico, Institute of Ocean Science and Limnology, Mexico City, Mexico

3 Center of Scientific Research and Superior Education of Ensenada Baja California, Marine Biotechnology, Ensenada, Baja California, Mexico

4 Biological and Health Sciences Division, Metropolitan Autonomous University, Mexico City, Mexico

*Address all correspondence to: vpardio@uv.mx

References

[1] DePaola A, Hopkins L, Peeler J, Wentz B, McPhearson R. Incidence of *Vibrio parahaemolyticus* in US coastal waters and oysters. Applied and Environmental Microbiology. 1990;**56**:2299-2302

[2] Dileep V, Kumar H, Kumar Y, Nishibuchi M, Karunasaga I. Application of polymerase chain reaction for detection of *Vibrio parahaemolyticus* associated with tropical seafoods and coastal environment. Letters of Applied Microbiology. 2003;**36**:423-427

[3] Wang D, Yu W, Chen D, Zhang D, Shi X. Enumeration of *Vibrio parahaemolyticus* in oyster tissues following artificial contamination and depuration. Letters in Applied Microbiology. 2010;**51**:104-108

[4] Song E, Lee S, Lim H, Kim J, Jang K, Choi S, et al. *Vibrio vulnificus vvhA* induces autophagy-related cell death through the lipid raft-dependent c-Src/NOX signaling pathway. Scientific Reports. 2016;**6**:27080

[5] Johnson C, Bowers J, Griffitt K, Molina V, Clostio R, Pei S, et al. Ecology of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in the coastal and estuarine waters of Louisiana, Maryland, Mississippi, and Washington (United States). Applied and Environmental Microbiology. 2012;**78**:7249-7257

[6] Horseman M, Surani S. A comprehensive review of *Vibrio vulnificus*: An important cause of severe sepsis and skin and soft-tissue infection. International Journal of Infectious Diseases. 2011;**15**:e157-e166

[7] Igbinosa E, Okoh O. Emerging Vibrio species: An unending threat to public health in developing countries. Research in Microbiology. 2008;**159**:495-506

[8] Su Y, Liu C. *Vibrio parahaemolyticus*: A concern of seafood safety. Food Microbiology. 2007;**24**:549-558

[9] Nordstrom J, Vickery M, Blackstone G, Murray S, DePaola A. Development of a multiplex real-time PCR assay with an internal amplification control for the detection of total and pathogenic *Vibrio parahaemolyticus* bacteria in oysters. Applied and Environmental Microbiology. 2007;**73**:5840-5847

[10] Chao G, Jiao X, Zhou X, Yang Z, Huang J, Pan Z, et al. Serodiversity, pandemic O3:K6 clone, molecular typing and antibiotic susceptibility of foodborne and clinical *Vibrio parahaemolyticus* isolates in Jiangsu, China. Foodborne Pathogens and Disease. 2009;**6**:1021-1028

[11] Froelich B, Oliver J. The Interactions of *Vibrio vulnificus* and the oyster *Crassostrea virginica*. Microbial Ecology. 2013;**65**:807-816

[12] Baker-Austin C, Stockley L, Rangdale R, Martinez-Urtaza J. Environmental occurrence and clinical impact of *Vibrio vulnificus* and *Vibrio parahaemolyticus*: A European perspective. Environmental Microbiology. 2010;**2**:7-18. DOI: 10.1111/j.1758-2229.2009.00096.x

[13] Quiñones-Ramirez E, Natividad I, Betancourt-Rule M, Ramirez-Vives F, Vazquez-Salinas C. Putative virulence factors identified in *Vibrio vulnificus* strains isolated from oysters and seawater in Mexico. International Journal of Environmental Health Research. 2010;**20**:395-405. DOI: 10.1080/09603123.2010.491856

[14] Torres M, Fernández E. Incidence of *Vibrio parahaemolyticus* in raw fish, oysters, and shrimp. Revista Latinoamericana de Microbiología. 1993;**35**:267-272

[15] Charles-Hernández G, Cifuentes E, Rothenberg J. Environmental factors associated with the presence of *Vibrio parahaemolyticus* in sea products and the risk of food poisoning in communities bordering the Gulf of Mexico. Journal of Environmental Health Research. 2006;**2**:1-7

[16] López K, Pardío V, Lizárraga L, Williams J, Martínez D, Flores A, et al. Environmental parameters influence on the dynamics of total and pathogenic *Vibrio parahaemolyticus* densities in *Crassostrea virginica* harvested from Mexico's Gulf coast. Marine Pollution Bulletin. 2015;**91**:317-329

[17] [CONAPESCA] Comisión Nacional de Acuacultura y Pesca. Anuario Estadístico de Acuacultura y Pesca. 2017. Available from: http://www.conapesca.sagarpa.gob.mx/ [Accessed: 08-01-2018]

[18] [SSA] Secretaría de Salud, Gobierno de México. NORMA Oficial Mexicana NOM-242-SSA1-2009. Productos y servicios. Productos de la pesca frescos, refrigerados, congelados y procesados. Especificaciones sanitarias y métodos de prueba. 2009. Available from: http://portal.salud.gob.mx/ [Accessed: 08-08-2018]

[19] Kinsey T, Lydon K, Bowers J, Jones J. Effects of dry storage and resubmersion of oysters on total *Vibrio vulnificus* and total and pathogenic (*tdh+/trh+*) *Vibrio parahaemolyticus* levels. Journal of Food Protection. 2015;**78**:1574-1580. DOI: 10.4315/0362-028X.JFP-15-017

[20] Gooch J, Depaola A, Owers J, Marshall D. Growth and survival of *Vibrio paraemolyticus* in postharvest American oysters. Journal of Food Protection. 2002;**65**:970-974

[21] Cook D. Refrigeration of oyster shellstock: Conditions which minimize the outgrowth of *Vibrio vulnificus*. Journal of Food Protection. 1997;**60**:349-352

[22] [SIPESCA] Sistema de Información de Pesca y Acuacultura. 2018. Available from: https://sipesca.conapesca.gob.mx/loginfiel.php [Accessed: 08-08-2018]

[23] Contreras Espinosa F, Castañeda O. Las lagunas costeras y estuarios del Golfo de México: Hacia el establecimiento de índices ecológicos. In: Caso M, Pisanty I, Ezcurra E, editors. Diagnóstico Ambiental del Golfo de México, México. SEMARNAT/INE/INECOL/HARP-Institute; 2004. p. 628

[24] [SE] Secretaría de Economía, Gobierno de México. NMX-FF-001-SCFI-2011 Productos de la pesca-ostion en concha vivo, y pulpa envasada-refrigerada (litoral Atlántico)-especificaciones. 2011. Available from: http://www.economia.gob.mx [Accessed: 03-01-2018]

[25] [SSA] Secretaría de Salud, Gobierno de México. NOM-109-SSA1-1994. Procedimiento para la toma, manejo y transporte de muestras de alimentos para su análisis microbiológico. 1994. Available from: http://portal.salud.gob.mx/ [Accessed: 03-01-2018]

[26] Lizárraga-Partida M, Gómez-Gil B, Méndez-Gómez E, Wong-Chang I, Pardío-Sedas V, Cabanillas-Beltrán H. Molecular detection of *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus* in oyster from Mexico by the vibriomex group. In: Proceedings of the International Conference on Vibrios in the Environment; 7-12 November 2010; Biloxi, Mississippi, USA

[27] Barrera-Escorcia G, Wong-Chang I, Fernandez-Rendon C, Vazquez-Botello A, Gomez-Gil B, Lizarraga-Partida M. Quantification of Vibrio species in oysters from the Gulf of Mexico with two procedures based on MPN and PCR. Environmental Monitoring

and Assessment. 2016;**188**:602. DOI: 10.1007/s10661-016-5620-9

[28] Brauns L, Hudson M, Oliver J. Use of polymerase chain-reaction in the detection of culturable and nonculturable *Vibrio vulnificus* cells. Applied and Environmental Microbiology. 1991;**57**:2651-2655

[29] Rosche T, Yano Y, Oliver J. A rapid and simple PCR analysis indicates there are two subgroups of *Vibrio vulnificus* which correlate with clinical or environmental isolation. Microbiology and Immunology. 2005;**49**:381-389

[30] Bej A, Patterson D, Brasher C, Vickery M, Jones D, Kaysner C. Detection of total and hemolysin producing *Vibrio parahaemolyticus* in shellfish using multiplex PCR amplification of *tlh*, *tdh* and *trh*. Journal of Microbiological Methods. 1999;**36**:215-225

[31] Myers M, Panicker G, Bej A. PCR detection of a newly emerged pandemic *Vibrio parahaemolyticus* O3:K6 pathogen in pure cultures and seeded waters from the Gulf of Mexico. Applied and Environmental Microbiology. 2003;**69**:2194-2200

[32] [USDA] United States Department of Agriculture. [LQAD] Laboratory Quality Assurance Division. Most Probable Number Procedure and Tables. MLG Appendix 2.03. United State. 2008

[33] Guerrero A, Gómez Gil B, Wong Chang I, Lizárraga Partida M. Genetic characterization of *Vibrio vulnificus* strains isolated from oyster samples in Mexico. International Journal of Environmental Health Research. 2015;**25**:614-627

[34] [CONAGUA] Comisión Nacional del Agua. 2018. Available from: https://smn.cna.gob.mx/tools/DATA/Climatolog%C3%ADa/Pron%C3%B3stico%20clim%C3%A1tico/Temperatura%20y%20Lluvia/TMED/2012.pdf [Accessed: 03-01-2018]

[35] Yoon K, Min K, Jung Y, Kwon K, Lee J, Oh S. A model of the effect of temperature on the growth of pathogenic and nonpathogenic *Vibrio parahaemolyticus* isolated from oysters in Korea. Food Microbiology. 2008;**25**:635-641

[36] Costa Sobrinho P, Destro M, Franco D, Landgraf M. A quantitative risk assessment model for *Vibrio parahaemolyticus* in raw oysters in Sao Paulo State, Brazil. International Journal of Food Microbiology. 2014;**180**:69-77

[37] Johnson C, Flowers A, Noriea N, Zimmerman A, Bowers J, DePaol A, et al. Relationships between environmental factors and pathogenic vibrios in the northern Gulf of Mexico. Applied and Environmental Microbiology. 2010;**76**:7076-7084

[38] Pfeffer C, Hite M, Oliver J. Ecology of *Vibrio vulnificus* in estuarine waters of eastern North Carolina. Applied and Environmental Microbiology. 2003;**69**:3526-3531

[39] Warner E, Oliver J. Population structures of two genotypes of *Vibrio vulnificus* in oysters (*Crassostrea virginica*) and seawater. Applied and Environmental Microbiology. 2008;**74**:80-85

[40] Cabrera-García E, Vázquez-Salinas C, Quiñones-Ramírez E. Serologic and molecular characterization of *Vibrio parahaemolyticus* strains isolated from seawater and fish products of the Gulf of México. Applied and Environmental Microbiology. 2004;**7**:6401-6406

[41] Costa Sobrinho P, Destro M, Franco D, Landgraf M. Correlation between environmental factors and prevalence of *Vibrio parahaemolyticus* in oysters harvested in the southern coastal área

of Sao Paulo State, Brazil. Applied and Environmental Microbiology. 2010;**76**:1290-1293. DOI: 10.1128/AEM.00861-09

[42] Flores A, Pardío VT, Lizárraga L, López K, Uscanga R, Flores R. Seasonal abundance of total and pathogenic *V. parahaemolyticus* isolated from American oyster harvested in the Mandinga Lagoon System, Veracruz, Mexico: Implication for food safety. Journal of Food Protection. 2014;**77**:1069-1077

[43] Cabello A, Espejo R, Romero J. Tracing *Vibrio parahaemolyticus* in oysters (*Tiostrea chilensis*) using a green fluorescent protein tag. Journal of Experimental Marine Biology and Ecology. 2005;**327**:57-166

[44] Ellison R, Malnati E, DePaola A, Bowers J, Rodrick G. Populations of *Vibrio parahaemolyticus* in retail oysters from Florida using two methods. Journal of Food Protection. 2001;**64**:682-686

[45] Cook D. Effect of time and temperature on multiplication of *Vibrio vulnificus* in postharvest Gulf coast shellstock oysters. Applied and Environmental Microbiology. 1994;**60**:3483-3484

[46] Kim Y, Lee S, Hwang I, Yoon K. Effect of temperature on growth of *Vibrio parahaemolyticus* and *Vibrio vulnificus* in flounder, salmon sashimi and oyster meat. International Journal of Environmental Research and Public Health. 2012;**9**:4662-4675. DOI: 10.3390/ijerph9124662

[47] Raghunath P. Roles of thermostable direct hemolysin (TDH) and TDH-related hemolysin (TRH) in *Vibrio parahaemolyticus*. Frontiers in Microbiology. 2015;**5**:1-4

[48] [WHO/FAO] World Health Organization, Food and Agriculture Organization of the United Nations. Risk assessment of *Vibrio vulnificus* in raw oysters. Microbiological Risk Assessments 8. 2005. Available from: http://www.fao.org/docrep/008/a0252e/a0252e00.htm [Accessed: 08-01-2018]

[49] Martínez-Urtaza J, Bowers J, Trinanes J, DePaola A. Climate anomalies and the increasing risk of *Vibrio parahaemolyticus* and *Vibrio vulnificus* illnesses. Food Research International. 2010;**43**:1780-1790. DOI: 10.1016/j.foodres.2010.04.001

[50] [U.S FDA] U.S. Food and Drug Administration. Quantitative risk assessment on the public health impact of pathogenic *Vibrio parahaemolyticus* in raw oysters. 2005. Available from: http://www.fda.gov/food/scienceresearch/researchareas/riskassessmentsafetyassessment/uc%m050421.htm [Accessed: 03-01-2018]

[51] [WHO/FAO] World Health Organization/Food and Agriculture Organization of the United Nations. Risk Assessment of *Vibrio parahaemolyticus* in Raw and Under-Cooked Seafood: Interpretative Summary and Technical Report. Microbiological Risk Assessment Series No. 16. Rome: FAO; 2011

[52] Huang Y, Hwang C, Huang L, Wu V, Hsiao H. The risk of *Vibrio parahaemolyticus* infections associated with consumption of raw oysters as affected by processing and distribution conditions in Taiwan. Food Control. 2018;**86**:101-109. DOI: DOI.org/10.1016/j.foodcont.2017.10.022

[53] New C, Kantilal H, Tan M, Nakaguchi Y, Nishibuchi M, Son R. Consumption of raw oysters: A risk factor for *Vibrio parahaemolyticus* infection. International Food Research Journal. 2014;**21**:2459-2472

[54] Cervera-Gaviria J, Armenta-Alvarez A, Mendez-Ceballos A, Bolaños-Aguilar M, Martinez-Hernandez L, Virgen-Cuevas M, et al. Choque séptico secundario an infección por *Vibrio vulnificus*. Reporte de un caso. In: Proceedings of the XLI National Congress on Infectology and Clinical Microbiology; 25-28 May 2016; Monterrey, Nuevo León, México. p. S99

[55] [INAI] Instituto Nacional de Transparencia y Acceso a la Información. Secretaría de Salud: Listado nominal de resultados. México: Veracruz; 2016. Available from: https://www.infomex.org.mx/gobiernofederal/home.action [Accessed: 03-01-2018]