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Risk of *Vibrio* Transmission Linked to the Consumption of Crustacean in Coastal Towns of Côte d'Ivoire

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Abstract

The purpose of this study was to assess the risk of *Vibrio* spp. transmission from crustaceans to humans in two coastal towns of Côte d'Ivoire. Bacteriologic analysis was performed on 322 crustacean samples obtained from six markets in Abidjan and one in Dabou. Suspected colonies of *Vibrio* spp. were identified by morphological, cultural, biochemical and molecular tests, and matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). Polymerase chain reaction (PCR) was performed to further characterize *Vibrio* strains. Additionally, a survey on consumption of crustaceans was conducted among 120 randomly selected households in Abidjan. Overall, *Vibrio* spp. were isolated from 7.8% of the crustacean samples studied, at concentrations as high as 6.3 Log colony forming unit per gram. *Vibrio* strains identified were divided into 40% *Vibrio alginolyticus*, 36% *Vibrio parahaemolyticus*, and 24% non-toxicogenic *Vibrio cholerae* with the latter two species potentially causing mild to severe forms of seafood-associated gastroenteritis. Among interviewed households, 11.7% reported daily consumption of crustaceans, confirming the high probability of exposure of human population to *Vibrio* spp., and 7.5% reported symptoms of food poisoning after consumption of crustaceans. The absence of genes encoding major virulence factors in the studied strains, i.e., cholera toxin (*ctxA* and *ctxB*) for *V. cholerae* and thermostable direct hemolysin (*tdh*) and *tdh*-related haemolysin (*trh*) for *V. parahaemolyticus*, does not exclude the possibility of a potential exposure to pathogenic strains. However, food preparation practices prevent human infections, as most households boil crustaceans before consumption (96.7%), usually for at least 45 min (85.9%).

Keywords

Vibrio cholerae; *Vibrio parahaemolyticus*; crustaceans; household; food poisoning; PCR

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The consumption of fish and crustacean represents an important source of animal protein for humans worldwide. In 2006, an estimated 144 million tons of fish and crustaceans were obtained from fishery and aquaculture, the majority of which (110 million tons) were destined for human consumption (19). Due to the living environment of crustaceans (i.e., brackish lagoon waters and shallow sea waters), there is a considerable risk of contamination with environmental pollutants and infectious agents such as *Salmonella* spp., *Clostridium botulinum*, *Clostridium perfringens*, and bacteria naturally occurring in aquatic environments, such as those of the genus *Vibrio*, including species that can be pathogenic for humans (11, 32).

Vibrio are environmental bacteria widely distributed in aquatic environment, coastal waters, estuaries, and lakes, and their occurrence has been observed worldwide (29). Presence of *V. cholerae* in lake water is strongly suggested in the African Great Lakes Region where cases of cholera are reported nearly every year. Shallow shore waters are supposed to be the source of the disease due to persistence of cholera strains (4). *Vibrios* can survive and multiply in aquatic animals, with incomplete evidence linking vibrios with zoonoses. Some 90 species belong to the genus *Vibrio*, of which 12 are considered pathogenic to humans (31) and have been associated with different clinical syndromes, gastroenteritis being the most common presentation. Transmission may be through exposure of wounds to warm seawater, but primarily involves direct entry into the digestive tract through the consumption of seafood, mainly raw or undercooked crustaceans. The most common pathogenic vibrios primarily associated with food-borne gastrointestinal illness are *Vibrio cholerae* and *V. parahaemolyticus* (21). However, and even if this has not been extended today to other geographic areas of the world, it should be noted that in the United States, *V. vulnificus* has been associated with gastrointestinal infections and septicemia in individuals with chronic conditions following consumption of raw bivalves harvested and consumed locally. Indeed, *V. vulnificus* is the leading cause of death due to seafood consumption in this part of the world. The global incidence of food-borne diseases is difficult to estimate, but it has been reported by the World Health Organization (WHO) that, in 2005 alone, 1.8 million people died from diarrheal diseases. A considerable number of these cases can be attributed to contamination of food and drinking water (36). The Centers for Disease Control and Prevention (CDC) estimate that each year roughly 1 in 6 Americans (approximately 48 million people) acquire illness, 128,000 are hospitalized, and 3,000 die due to food-borne diseases (9). The incidence of infections with *V. parahaemolyticus* has increased in recent years (10, 15, 22). Between 1997 and 2006, 4,755 cases of *Vibrio* illness were reported by the Cholera and Other *Vibrio* Illness Surveillance System (COVIS) and 188 outbreaks of seafood-associated infections were reported to the Food-Borne Disease Outbreak Surveillance System in the United States (24). The most commonly reported *Vibrio* species causing food-borne infections were *V. parahaemolyticus* (1,931 [56.7%] food-borne *Vibrio* infections) and *V. vulnificus* (459 [13.5%] infections). Mollusks were implicated in 85 (45.2%) outbreaks, followed by fish in 73 outbreaks (38.8%), and crustaceans in 30 (16.0%) outbreaks (24). Indeed, *Vibrio* spp. are considered emerging pathogens due to the increased incidence in food-borne sporadic infections and outbreaks in recent years in many parts of the world (13).

Storage conditions, especially continuous refrigeration from harvest to consumption, are an important component of prevention of food-borne infections. In Côte d'Ivoire, however, seafood production is dominated by artisanal fishing in lagoons, with crustaceans sold on local markets without critical point inspections to ensure food safety. Fish and other aquatic products are often processed without additives or chemical preservatives and distributed without specific means of conservation such as refrigeration or freezing (23). Hence, there is a risk of microbiological contamination, which in turn might result in human infection. Among these potential risks, *Vibrio* spp. transmission is of particular concern. In 1985, an outbreak of diarrhea occurred in June and July (during the rainy season), which was linked to *V. parahaemolyticus* (34). Moreover, additional outbreaks of diarrhea occurred due to infections with other natural host of freshwater or saltwater, i.e., *Aeromonas caviae*, *A. hydrophila*, and *A. sobria* (16). In 2001, several outbreaks of diarrhea occurred, causing casualties in several parts of Côte d'Ivoire (33). According to Ekra et al. (17), between 2001 and 2005, 11,874 cases of cholera were notified with 564 deaths and a fatality rate of 4.7%.

The purpose of this study was to determine whether there are health risks associated with the consumption of crustaceans that are sold in markets in coastal towns of Côte d'Ivoire. Risk assessment was done by isolating and quantifying *Vibrio* spp. in shrimps and crabs obtained from local markets in Abidjan and Dabou. Molecular techniques were employed to characterize isolated strains. Additionally, a cross-sectional consumption survey was carried out in a random sample of households in Abidjan, using a pre-tested questionnaire.

MATERIALS AND METHODS

Study area and design

In August and September 2008, exploratory visits were made in markets of 10 municipalities of Abidjan (i.e., Abobo, Adjamé, Attécoubé, Cocody, Koumassi, Marcory, Plateau, Port Bouët, Treichville, and Yopougon), the economic capital of Côte d'Ivoire. Additionally, one market in Dabou, located 49 km west of Abidjan along the coast, was visited to investigate the main distribution channels of crabs and shrimps (Fig. 1A). Seven markets were retained for further in-depth studies; six in Abidjan (i.e., Adjamé, Koumassi, Marcory, Port Bouët, Treichville, and Yopougon) and the main market of Dabou. Between June 2009 and December 2010, cross-sectional surveys were conducted. Raw shrimps and crabs were purchased and subjected to laboratory investigation with an emphasis on *Vibrio* species identification. Additionally, in November and December 2009, a household cross-sectional survey pertaining to the consumption of crustaceans was carried out in 10 neighborhoods of Abidjan (Fig. 1B).

Sample size calculation

In a previous study in Morocco, Cohen et al. (14) isolated *Vibrio* spp. from 5.7% of the shrimps examined. Assuming a slightly lower prevalence estimate for our study (i.e., 5%), and allowing for a precision on the infection prevalence of 5% and a power of 80%, a minimum sample size of 76 crustaceans was required. In order to account for the uncertainty and likely variations within crustacean species, we opted for a considerably larger sample

size, and finally purchased 322 crustaceans on seven markets. The number of crustaceans collected from the individual markets was proportional to the number of vendors.

A preliminary household survey carried out in Abidjan revealed that most heads of households interviewed consumed shellfish (96%). Allowing an error on this prevalence of consumption of 5%, and a power of 80%, the estimated sample size was 60 households. We doubled this number to account for the uncertainty associated with the preliminary assessments in the household.

Ethical considerations

The study was approved by the Ministry of Higher Education and Scientific Research of Côte d'Ivoire (MESRS, decision no. 171) and the national ethics committee (CNE decision no. 1176 MSHP).

Collection of crustaceans on local markets

Crustacean samples were collected in the morning between 7 and 9 a.m. during a series of cross-sectional surveys carried out between June 2009 and December 2010. According to previous research, crabs mainly belonged to the genus *Callinectes* and *Cardisoma*, whereas shrimps primarily belonged to the genus *Penaeus* (35). The temperature in the containers where crustaceans were kept (e.g., baskets, bowls, and dishes), was measured. Since crabs are sold alive, they are not put on ice. Shrimps, in contrast, are put on ice as they are sold dead. However, ice quantities are often insufficient and, given the high ambient temperatures at open markets in tropical West Africa, shrimps are at times sold in the absence of ice. Vendors were interviewed about the origin of the crustaceans. Upon purchase, the specimens were placed individually into sterile plastic bags that were labeled with unique identifiers. Specimens were transferred on ice in a cool box to the bacteriologic laboratory of the Centre Suisse de Recherches Scientifiques en Côte d'Ivoire (CSRS) in Abidjan and processed within 1 h.

Laboratory procedures

We adhered to the following procedures. Crustaceans that were still alive upon arrival in the laboratory were killed using a sterile clamp inserted into the spinal cord. Next, specimens were milled including intact shells, using a stomacher. Then 25 g of homogenate was added to 225 ml of sterile alkaline peptone water at 2% of NaCl. This homogenized mixture served as the stock suspension. *Vibrio* were quantified by the method of most probable number (MPN) described in the FDA Bacteriological Analytical Manual (18). We generated serial 10-fold dilutions of the initial stock suspension in 2% APW (1% peptone, 2% NaCl, 0.3% yeast extract, pH 8.6) as the diluent, of which 1 ml were used to inoculate three tubes of MPN series in 9 ml of 2% APW. After 18 to 24 h of incubation at 37°C, culture from each MPN tube showing growth, was plated into selective agar (solid TCBS medium, Diagnostics Pasteur) and incubated at 37°C for 24 h. Typical colonies of *Vibrio* species (flat yellow colonies (2-3 mm in diameter) were considered *V. cholerae*, large yellow colonies *V. alginolyticus*, translucent or yellow colonies *V. fluvialis* and *V. vulnificus*, and green colonies *V. parahaemolyticus*) were isolated on GNA medium (alkaline nutrient agar) with 2% NaCl (Biorad) and subsequently incubated for 24 h at 37°C to obtain pure strains.

Identification of *Vibrio* strains was performed based on morphological (Gram type and type of mobility), cultural (growth in alkaline peptone water containing from 0 to 10% NaCl), and biochemical (Oxydase test, API 20E galleries, BioMérieux) features, followed by analysis using matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF). We used a Microflex LT instrument (Bruker Daltonics GmbH; Leipzig, Germany) with FlexControl (version 3.0) software (Bruker Daltonics) for the automatic acquisition of mass spectra in the linear positive mode within a range of 2 to 29 kDa (2). As standards for the species *V. cholerae*, strains JF3514 (O1, 569B, classical Inaba) and JF3515 (O1, TCP-2, classical Ogawa) were used, in order to complement the present data for standards of the instrument, which comprised 77 different *Vibrio* species (including *V. parahaemolyticus* and *V. vulnificus*, but lacked standards for *V. cholerae*). Polymerase chain reaction (PCR) was done for confirmation of the species identification (7, 12, 28) and further characterization of strains. Specifically, *V. cholerae* and *V. parahaemolyticus* were characterized by PCR for the detection of the genes encoding virulence-associated factors, the cholera toxin genes (*ctxA* and *ctxB*), and the thermostable-direct and thermostable-related hemolysins (*tdh* and *trh*) (1, 20, 30). Biochemical, cultural and molecular identification and characterization of strains were performed at the Centre National de Référence des Vibrions et du Choléra (CNRVC) at Institut Pasteur in Paris, France. Reference strains from the French National Reference Centre for Vibrios and Cholera were used as positive control for species confirmation as well as for *V. cholerae* and *V. parahaemolyticus* characterization of virulence-associated factors. The primers used in this study and the target genes are summarized in Table 1.

Household survey

To investigate crustacean consumption patterns, we administered a questionnaire to 120 households. The questionnaire was pre-tested in two neighborhoods (Bingerville and Sideci) that were not otherwise involved in the study. The questionnaire consisted of three sections: (i) information pertaining to socioeconomic indicators (e.g., type of house and number of household members); (ii) food consumption habits and cooking techniques; and (iii) risk factors for infection with *Vibrio*, and potential signs and symptoms in relation to food poisoning in connection with the consumption of crustaceans. The number of households selected per municipality was proportional to the population size, based on estimates from the 1998 census. In each municipality, one neighborhood was randomly selected. Households were also collected at random. In brief, at a central place in the selected neighborhood, a pen was thrown and houses selected to the left of the pointing direction of the pen. The first household in this direction was chosen and the person responsible for the cooking in the household was asked whether or not crustaceans are consumed. If the answer was affirmative, the household was interviewed; in case of non consumption of crustaceans, the next household was selected. Only few households said that they do not consume crustaceans.

Statistical analysis

Data were double entered and analyzed using SPSS version 10 (IBM Corporation; Somers, NY, USA). Binomial generalized linear models were performed to associate presence of *Vibrio* detected in crustaceans with the genus of crustaceans (model 1) and market of selling

(model 2) by using R 2.10.1 software (R Foundation for Statistical Computing; Vienna, Austria).

RESULTS

Crustacean sample and characterization of *Vibrio*

Exploratory visits to local food markets in Abidjan revealed that crabs of the genus *Callinectes* and shrimps of the genus *Penaeus* were the most widely sold specimens. Crayfish were not found on local markets.

From 322 crustaceans subjected to detailed laboratory examinations, *Vibrio* spp. was isolated in 25 specimens, owing to an overall prevalence of 7.8%. As shown in Table 2, there was considerable spatial heterogeneity; at the unit of the market, *Vibrio* spp. in crustaceans ranged from 2.3% (Marcory) to 15.6% (Treichville). We found prevalences of 14.3% and 6.0% for *Penaeus* and *Callinectes*, respectively (Table 3). No *Vibrio* spp. was found in shrimps of the genus *Macrobrachium* spp. and crabs of the genus *Cardiosoma* spp. Moreover, our results revealed a presence of *Vibrio* in shrimps of the genus *Penaeus* significantly higher than in other crustaceans ($P < 0.05$) (Table 3).

Upon purchase of crustaceans in the early morning hours on local markets in Abidjan and Dabou, we measured average temperatures of 14.8°C and 26.8°C in the containers where shrimps and crabs were kept, respectively (Table 4). The average load of *Vibrio* in crustacean samples was 6.26 Log colony forming unit (CFU) per gram of crabs and 6.27 Log CFU per gram of shrimp (Table 4). These high levels of vibrios reflect their ability to grow rapidly in crustaceans when exposed to such temperatures. A previous study conducted on cooked crustaceans stored at a temperature of 37°C showed concentrations of *V. cholerae* O1 up to 10^{10} cells/g of product (25).

Among the 25 *Vibrio* strains identified in our sample of 332 crustaceans, *V. alginolyticus*, *V. parahaemolyticus*, and non-toxigenic non-O1/non-O139 *V. cholerae* accounted for 40%, 36% and 24% of the isolates, respectively (Table 5). No major virulence factor, i.e., the genes encoding *ctxA* and *ctxB* in *V. cholera*, and *tdh* and *trh* in *V. parahaemolyticus*, were detected by PCR.

Household survey

Results obtained from the cross-sectional household survey are summarized in Table 6. Most respondents were females (116/120, 96.7%) and they primarily lived in small town houses. The mean number of people living in a household was 7.5, with a range from 1 to 24. Crabs of the genus *Callinectes* were consumed by 89.2% of households interviewed. Before consumption, crustaceans were boiled (96.7%) or fried (3.3%). None of the households interviewed reported raw consumption of crustaceans. Almost two-third of the households (64.2%) reported that they cooked crustaceans for more than 1 h, whereas one out of five (21.7%) households reported a cooking time of 45-60 min. The remaining 11.8% of the households reported shorter cooking times. Daily consumption of crustaceans was reported by 11.7% of the households, whereas 45.8% of the respondents reported occasional consumption (approximately once every month) and the remaining 42.5% consumed

crustaceans only rarely (once a year). Signs and symptoms potentially associated with food poisoning due to the consumption of crustaceans as soup were reported by 7.5% of the interviewees. Further probing and direct observations suggest that cross-contamination of vegetables and other foodstuff that were kept in the same bags as crustaceans while transferred from the market to the house might be the root cause of these food poisonings.

DISCUSSION

Analysis of 322 crustacean samples purchased on local markets in two coastal towns of Côte d'Ivoire between June 2009 and December 2010 revealed a prevalence of *Vibrio* spp. of 7.8%. This prevalence is somewhat higher than the one observed in shrimps in a recent study done in Casablanca, Morocco (5.7%) (14), but much lower than that reported in Mexico (80% in crustaceans) and in the People's Republic of China (70%) (6). However, the success of the methods for isolation of *Vibrio* from marine samples depends on the nature of the matrix, and the methods may therefore fail to detect very low levels of *Vibrio* in the background of bacteria present in natural samples.

The 25 strains of *Vibrio* identified were divided into *V. alginolyticus*, *V. parahaemolyticus*, and non-toxicogenic *V. cholerae*. Our study confirms the predominance of *V. alginolyticus* (40%) in crustaceans, as reported elsewhere (5, 13). The presence of *V. cholerae* in the crustacean samples analyzed here might be explained by the ability of this species to adhere to and colonize the shells of crustaceans, facilitating their survival in aquatic environment (8). Presence of multiple species of *Vibrio* in crustaceans suggests that they are also present in a free state in the Ebrié lagoon (spanning from Abidjan to Dabou in south Côte d'Ivoire). Indeed, several authors have reported the presence of *Vibrio* in marine and estuarine waters in coastal Côte d'Ivoire (26, 34).

Two of the three species identified, *V. cholerae* and *V. parahaemolyticus*, are responsible for most food-borne illnesses caused by *Vibrio* spp. *V. parahaemolyticus* is recognized as a major cause of acute seafood-borne gastroenteritis; non-O1/non-O139 *V. cholerae* strains cause sporadic cases of gastroenteritis, and have the ability to cause extraintestinal infection, with signs of invasive disease. *V. alginolyticus* is frequently isolated as a cause of *Vibrio* species-related illnesses, but is mainly associated with non-food-borne *Vibrio* infections, and cellulitis and otitis, resulting from saltwater exposure, are currently reported (31).

Genes encoding major virulence factors, the cholera toxin of *V. cholerae* (i.e., *ctxA* and *ctxB*) and the thermostable direct and thermostable-related hemolysins of *V. parahaemolyticus* (i.e., *tdh* and *trh*) were not detected in the strains isolated during our study. These results are consistent with those obtained in most previous studies, showing that environmental and seafood isolates rarely contained the major virulence factors found in clinical isolates and involved in human pathology. Indeed, in a previous investigation, Cohen et al. (14) also failed to detect genes encoding *ctxA* and *ctxB* of *V. cholerae*, and *tdh* and *trh* of *V. parahaemolyticus*. However, these species possess numerous virulence factors which potentially contribute to disease and have not been investigated in this study. For example, Tiékoura et al. (34) characterized 30 strains of non-O1/non-O139 *V. cholerae* from the Grand Lahou lagoon waters by PCR, and found they were negative for *ctxA* gene, zonula

occludens toxin gene (*zot*), accessory cholera enterotoxin gene (*ace*), and toxin-coregulated pilus gene (*tcpA*), but positive for the hemolysin gene *hlyA* (all strains), for the outer membrane protein gene *ompU* (1 strain) and for the regulatory gene *toxR* (21 strains). For all these reasons and, although the genes encoding the major and best-characterized virulence factors were not detected in our samples, we cannot conclude that these species are not potentially pathogenic and do not represent a risk to human health. Furthermore, even though no toxigenic strains were found in this study, it is useful to obtain baseline data and to maintain vigilance by regularly monitoring potentially pathogenic microbial agents. Our cross-sectional household survey revealed that crabs and shrimps were consumed by most of the contacted households, and that symptoms of food poisoning, following the consumption of crustaceans, were sometimes observed. Although symptoms such as diarrhea, stomach ache, and abdominal pain are unspecific, and hence can be caused by a number of pathogens, *V. parahaemolyticus* and *V. cholerae*, which have been specifically associated with gastroenteritis following ingestion of seafood, are to be considered.

Different steps have been identified as particularly important with regard to the exposure of human to contamination by *Vibrio*, namely (i) the post-harvest stage (storage and transportation conditions); (ii) the food preparation practices at home, in relation to cross-contamination; and (iii) the frequency of consumption and the quantity of crustaceans consumed. With respect to the first step, we observed that temperature and the method of selling crustaceans on local markets were conducive to the growth of *Vibrio*. Shrimp, sold dead, should be continuously maintained on ice during sale, which, however, was not the case on the markets studied here in coastal Côte d'Ivoire. In the early morning hours (before 9 a.m.) when our cross-sectional surveys were done, we measured an average temperature of 14.8°C in the buckets from which shrimps were sold. It is conceivable that, during the day, temperatures rose, promoting the growth of organisms already present in crustaceans and amplified the risk of microbial contamination. In a survey pertaining to the quality of dish crabs (*Callinectes amnicola*), Koussémon et al. (27) noted that the temperature of selling seafood on local markets (between 25.5 and 26.5°C) promotes the proliferation of alteration germs in crustaceans. Concerning food preparation practices, the low percentage of households (7.5%) who reported to have had signs or symptoms after consumption of crustaceans is likely to be explained by the fact that most households (96.7%) consume shellfish in soup, which require a long cooking time at a high temperature, and hence destroys *Vibrio* bacteria (13), while *Vibrio* are generally acquired through consumption of contaminated raw or undercooked shellfish such as oysters, clams, mussels, or crabs. The reported illness due to food consumption in households who regularly consume crustaceans in soup might be explained by cross-contamination of crustaceans after cooking. The cooking time, however, is a critical point in the prevention of *Vibrio* infections, as it has indeed been shown that *Vibrio* can survive several min at boiling temperature (3). It should also be noted that the frequency of consumption of crustaceans is a potential risk factor for infection with *Vibrio*. This is important, as 11.7% of households reported daily consumption of crabs and/or shrimp.

In conclusion, we found a prevalence of *Vibrio* in randomly selected crustaceans in coastal towns of Côte d'Ivoire of 7.8%. The identification of 25 strains of *Vibrio* isolated from crustaceans revealed that they belong to three species of *Vibrio*, two of which, *V. cholerae*

and *V. parahaemolyticus* are associated with gastrointestinal diseases. The genes encoding major virulence factors were not detected by PCR. Nevertheless, our findings revealed that there is considerable environmental contamination with *Vibrio* spp. and this might represent a risk for human health. As a precautionary measure, it is recommended to avoid cross-contamination between ready to eat foods and fresh crustaceans through food separation (different containers) when purchasing, transferring, and preparing crustaceans at home, and to properly cook the food for at least 8 min at a temperature of 100°C.

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Fig 1.

Map of Côte d'Ivoire, indicating the two coastal towns where the current study was carried out (A) and detailed map of the economic capital Abidjan, showing the municipalities where household were interviewed (highlighted in pink) (B).

TABLE 1.

List of primers and target genes for PCR-based identification and characterization of *Vibrio* spp. recovered from crustaceans in the current study.

Target gene	Primers	Sequence (5-3)	Interpretation	Ref.
<i>ctxA</i>	CTX2	CGG GCA GAT TCT AGA CCT CCT G	Vc virulence factor (CT A subunit)	20
	CTX3	CGA TGA TCT TGG AGC ATT CCC AC		
<i>ctxB</i>	CTX7	GGT TGC TTC TCA TCA TCG AAC CAC	Vc virulence factor (CT B subunit)	30
	CTX9B	GAT ACA CAT AAT TAG AAT TAA GGA TG		
tdh	L.tdh	GTA AAG GTC TCT GAC TTT TGG AC	Vp virulence factor (Hemolysin TDH)	1
	R.tdh	TGG AAT AGA ACC TTC ATC TTC ACC		
trh	L.trh	TTG GCT TCG ATA TTT TCA GTA TCT	Vp virulence factor (Hemolysin TRH)	1
	R.trh	CAT AAC AAA CAT ATG CCC ATT TCC G		
16S/23S	prVC-F	TTAAGCSTTTTCRCTGAGAATG	Species identification(Vc)	12
ISR	prVCM-R	AGTCACTTAACCATACAACCCG-		
hly	V _{v-1}	CGC CGC TCA CTG GGG CAG TGG CTG	Species identification (Vv)	7
	V _{v-3}	CCA GCC GTT AAC CGA AAC ACC CGC		
pR72H	VP32	CGA ATC CTT GAA CAT ACG CAG C	Species identification (Vp)	28
	VP33	TGC GAA TTC GAT AGG GTG TTA ACC		

CT, cholera toxin; Vc, *Vibrio cholerae*; Vp, *Vibrio parahaemolyticus*; Vv, *Vibrio vulnificus*

TABLE 2.

Prevalence of crustaceans contaminated with *Vibrio* spp. purchased on seven markets of Abidjan, Côte d'Ivoire between June 2009 and December 2010 (n=322)

Market	No. of crustaceans examined	No. of crustaceans contaminated	% crustaceans contaminated (95% CI)
Treichville	45	7	15.6 (6.5-29.5)
Port Bouët	29	3	10.3 (2.2-27.4)
Koumassi	50	5	10.0 (3.3-21.8)
Dabou	45	4	8.9 (2.5-21.2)
Adjamé	34	3	8.8 (1.8-23.7)
Siporex	75	2	2.7 (0.3-9.3)
Marcory	44	1	2.3 (0.1-12.0)
TOTAL	322	25	7.8 (5.1-11.2)

CI, confidence interval

TABLE 3.

Prevalence of crustaceans contaminated with *Vibrio* spp. obtained from seven markets in Abidjan, Côte d'Ivoire between June 2009 and December 2010, stratified by genus (n=322)

Crustacean	No. of specimens examined	No. of specimens contaminated	% contaminated (95% CI)
Shrimps of the genus <i>Penaeus</i>	112	16	14.3 (8.4-22.2)
Crabs of the genus <i>Callinectes</i>	150	9	6.0 (2.8-11.1)
Shrimps of the genus <i>Macrobrachium</i>	40	0	0
Crabs of the genus <i>Cardisoma</i>	20	0	0
TOTAL	322	25	7.8 (5.1-11.2)

CI, confidence interval

TABLE 4.

Mean temperature and mean number of *Vibrio* spp. in crustaceans obtained from markets in coastal towns in Côte d'Ivoire between June 2009 and December 2010

Type of crustacean	Mean (SD) temperature in buckets from which crustacean were sold (°C)	Mean (SD) number of <i>Vibrio</i> spp. (log CFU/g)
Crabs <i>Callinectes</i>	26.8 (1.6)	6.26 (0.50)
Crabs <i>Cardiosoma</i>	26.4 (0.7)	0
Shrimps	14.8 (6.8)	6.27 (0.98)

SD, standard deviation

TABLE 5.

Species of *Vibrio* identified from crustaceans purchased on local markets in coastal towns of Côte d'Ivoire between June 2009 and December 2010 (n=25)

Species of <i>Vibrio</i> identified	No. of species identified from crustaceans				
	Crabs <i>Callinectes</i>	Crabs <i>Cardiosoma</i>	Shrimps	Total	% of all
<i>V. alginolyticus</i>	6	0	4	10	40
<i>V. parahaemolyticus</i>	2	0	7	9	36
Non-toxigenic <i>V. cholerae</i>	1	0	5	6	24
Total	9	0	16	25	100

TABLE 6.

Food consumption and cooking habits among 120 households in Abidjan, Côte d'Ivoire

Characteristics	No. (%) of household
Sex of respondent	
Female	116 (96.7)
Male	4 (3.3)
Educational level of respondent	
No schooling	31 (25.8)
Primary school	30 (25.0)
Secondary school	46 (38.3)
University	13 (10.8)
Number of person in the household	
Mean	7.5
Range	1-24
Risk factors	
Consumption of crustacean	
Yes	120 (100)
Boiled	116 (96.7)
Fried	4 (3.3)
Condition of crustacean when purchased	
Alive crabs	106 (88.3)
Dead crabs	14 (11.7)
Type of crustacean	
Crab <i>Callinectes</i>	107 (89.2)
Crab <i>Cardiosoma</i>	7 (5.8)
Shrimp	6 (5.0)
Frequency of crustacean consumption	
Daily	14 (11.7)
Occasionally (~once every month)	55 (45.8)
Rarely (~once every year)	51 (42.5)
Quantity of crustacean consumed per person	
Half a crab <i>Callinectes</i>	52 (43.3)
One crab <i>Callinectes</i>	7 (5.8)
Two crab <i>Callinectes</i>	6 (5.0)
Three crab <i>Callinectes</i>	4 (3.3)
Approximately 300 g of shrimp	3 (2.5)
Crab <i>Cardiosoma</i>	1 (0.8)
Five shrimps	1 (0.8)
Time of cooking of crustacean	
5-15 min	8 (6.8)
15-30 min	3 (2.5)
30-45min	3 (2.5)

Characteristics	No. (%) of household
45-60 min	26 (21.7)
>1 h	77 (64.2)
Unknown	3 (2.5)
Signs and symptoms reported to be linked to crustacean consumption	
Total	9 (7.5)
Diarrhea	3 (2.5)
Stomach ache	3 (2.5)
Tonsillitis	1 (0.8)
Allergy	1 (0.8)
Ear ache	1 (0.8)
Source of drinking water	
Tap water	119 (99.2)
Water supplied by street vendors	1 (0.8)
Respondent report past cough	
No	96 (80.0)
Yes	24 (20.0)