Incidence of Potentially Pathogenic *Vibrio* Spp. In Fresh Seafood from Itu Creek in Uyo, Akwa Ibom State, Nigeria


1Department of Microbiology, Faculty of Science, University of Ibadan, Ibadan, Nigeria
2Department of Microbiology, University of Port Harcourt, Choba, P.M.B. 5323, Port Harcourt, Rivers State, Nigeria
3Department of Microbiology, Faculty of Science, University of Uyo, Uyo, Akwa Ibom State, Nigeria
4Department of Pharmacology and Therapeutics, Igbinedion University, Okada, Edo State, Nigeria

Abstract: This study reported the incidence of potentially pathogenic *Vibrio* spp. in fresh seafood from Itu creek. Studies were carried out to isolate, identify and characterized different *Vibrio* species in fish, crayfish and periwinkle obtained from the Itu creeks. Different parts analyzed using standard microbiological techniques were the gills, intestine and the body surfaces. The *Vibrio* species isolated and identified were *Vibrio cholerae*, *Vibrio fluvialis*, *Vibrio mimicus*, *Vibrio parahaemolyticus* and *Vibrio vulnificus*. Among the species isolated, *Vibrio cholerae* was most predominant 24(30.4%). This was closely followed by *Vibrio mimicus* 22(27.8%), *Vibrio parahaemolyticus* 17(21.5%), *Vibrio fluvialis* 14(17.7%) and *Vibrio vulnificus* 2(2.5%). However, *Vibrio vulnificus* was only found in samples of crayfish and fish (gills). The presence of these organisms in the fresh seafood samples showed that seafoods are predisposed to contamination by bacteria. Thus, it is becomes advisable that sea foods be adequately subjected to proper boiling and cooking before consumption. Owing to the potential hazard of some pathogenic *Vibrio*, it is clearly necessary to put more emphasis on food hygiene. Therefore surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health.

Key words: *Vibrio cholerae* • *Vibrio fluvialis* • *Vibrio mimicus* • *Vibrio parahaemolyticus* • *Vibrio vulnificus* • Sea foods

INTRODUCTION

Fish and seafood constitute an important food component for a large section of world population. They come after meat and poultry as staple animal protein foods where fish forms a cheap source of protein [1]. Sea foods are prone to bacterial contamination, especially filter feeders such as mussels and oysters, which concentrate these bacteria in their filtration systems and, therefore, are ideally suited to trap all bacteria and viruses, pathogenic or otherwise, that live in the water [1, 2].

The importance of *Vibrio spp* as a contaminant of raw or under cooked seafood has been well established [3, 4]. Species such as *V. cholerae*, *V. parahaemolyticus*, *V. vulnificus*, *V. alginolyticus*, *V. mimicus*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii*, *V. hollisae* and *V. damsela* are human pathogens [5]. They account for a significant proportion of human infections such as gastroenteritis usually associated with consumption of raw or undercooked seafood, wound infections, septicemia and ear infections [5]. Most of these vibrios secrete enterotoxins in foods, water or in the gastrointestinal tract [6].

The occurrence of *Vibrio spp* in raw shellfish is common, especially shellfish from regions with temperate climates around the world from both natural and farm environments and all seafood types [7, 8].

The World Health Organization (WHO) defines food borne illness as a disease which is caused through the consumption of contaminated food [9]. Other than Vibrio pathogen such as *Campylobacter*, *Salmonella*, *Listeria monocytogenes* and *Escherichia coli* O157:H7 have been found to be responsible for major food borne outbreaks worldwide [9].
Pathogenic Vibrios have been a public health concern for seafood consumers and have been cause of import bans, detentions and rejections in international fish trade [1]. Vibrio parahaemolyticus and Vibrio vulnificus are part of the natural flora of estuarine and coastal marine environments worldwide and have been isolated from sea - and brackish water of both tropical and temperate regions, sediments and a variety of seafood especially shellfish and bivalve mollusks [10].

Although the vast majority of environmental V. parahaemolyticus isolates are non virulent, it is a leading cause of gastroenteritis linked to seafood consumption in the United States (U.S.) [11]. Vibrio vulnificus poses a significant health threat to humans who suffer from immune disorders, liver disease, or hemochromatosis [1]. In the Asian region, Vibrio spp. have been recognized as the leading cause of food borne outbreaks in many countries; Japan [12, 13], India [3, 14], China [4, 13-15], Taiwan [16], Korea [17], Thailand [18] and Iran [19]. Investigations have shown that many outbreaks were caused by consumption of contaminated seafood [20]. Cases of food borne outbreaks resulting from consumption of ready to eat seafood dishes especially those supplied by food catering food service establishments continually occur [21].

The potential of seafood to harbor microbial pathogens and causing subsequent illness is well documented for both developed and developing countries [22, 23]. Water-related diseases continue to be one of the major health problems globally. It is estimated that 80% of all illnesses are linked to use of food and water of poor microbiological quality [24]. Treatment of farm produce such as seafood with chlorinated water reduces populations of pathogenic and other microorganisms on fresh produce but cannot eliminate them. Reduction of risk for human illness associated with raw produce can be better achieved through controlling points of potential contamination in the field, during harvesting, during processing or distribution, or in retail markets, food service facilities, or the home [25, 26].

Little is known regarding the prevalence of pathogenic Vibrio in locally consumed seafood. Thus, this study investigate the occurrence and incidence of potentially pathogenic Vibrio spp. in fresh seafood from Itu creek in Uyo, Akwa Ibom State, Nigeria.

**MATERIALS AND METHODS**

**Sample Collection and Processing:** One hundred and fifty (150) samples of freshly harvested seafoods: crayfish (Parapandalus pritis), fishes (Parachonna Africana, Chrysichthys nigrodiaitatus and Hippoptamytus pictus) and periwinkle (Pugilina morio) were collected from Itu creeks in sterile plastic containers and taken to the laboratory for analysis. The samples were aseptically removed from the container. After the skin ranging, a sterile forceps and knife appropriate for opening the shell to remove the intestine and gills were used. For shrimps, the shell was peeled and separated from the freshly part and intestine. About 1g from each sample was weighed and dispensed into 9ml of sterile distilled water blank and shaken vigorously to mix properly. Ten-fold dilutions were made and dilutions (10⁻⁴ and 10⁻⁵) were selected for microbial enumeration using the standard pour plate method [27].

**Bacteriological Analysis**

**Enrichment Procedures:** Five grams of individual shellfish flesh were incised using a sterile scalpel after removal of the carpace. These 5 gm flesh samples were homogenized in 45 ml of 3% NaCl containing 1% alkaline peptone water (APW, pH: 8.6) using a sterile blender. The shellfish homogenates were incubated at 37°C for 18 hr [1, 29].

**Isolation Procedures:** Isolation of Vibrio spp. was made on thiosulphate citrate bile salt (TCBS) agar. Following incubation, the shellfish homogenates were inoculated on Thiosulphate Citrate bile salts sucrose agar media (TCBS, Hi Media, India) using an inoculating loop and kept at 37°C for 18 hrs [1, 29]. Bacteriological analysis of Vibrio spp. in seafood samples was carried out in duplicates. For each seafood sample, 25g were homogenized in 225ml sterile 0.1% peptone water in a Stomacher 400 Circulator Homogenizer at 120rev/min for 2 minutes. A 10 fold serial dilution in sterile 0.1% peptone water was prepared as described in the Bacteriological Analytical manual [30]. Spread plate method was carried out using 10⁻² and 10⁻³ dilutions on TCBS agar. The TCBS plates were incubated at 37°C for 18-24 hours and counts were made for each colony type. Discrete colonies were sub-cultured into fresh agar plates aseptically to obtain pure cultures of the isolates.
Identification of Bacterial Colonies: Colonies identifiable as discrete were carefully examined macroscopically for cultural characteristics. The isolated colonies were subjected to Gram staining and growing at various salt concentrations by transferring colonies into tubes containing peptone water and 0%, 3%, 6% and 10% NaCl and these tubes were incubated at 37°C for 24 hrs [31]. Also, all bacterial colonies from different samples; growing on TCBS plates were selected to be streaked onto the surface of Trypticase Soya agar slants (TSA; Oxoid, UK) supplemented with 2% NaCl, then incubated at 37°C for 24 hrs [32]. The further identification of Vibrio spp. were done using morphological, physiological and different biochemical tests. The isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt et al. [33], Cheesbrough [26], Farmer et al. [34] and Oyeleke and Manga [35].

RESULTS

The present study showed that out of the 150 seafood samples examined, Vibrio was recovered from 52.7% of samples, with 70.0%, 36.7% and 16.7% of fish, crayfish and periwinkle respectively (Table 1). A total of 79 isolates of Vibrio spp. were obtained and identified as Vibrio cholerae, Vibrio fluvialis, Vibrio mimicus, Vibrio parahaemolyticus and Vibrio vulnificus. Table 2 shows the distribution and frequency of occurrence of Vibrio spp. isolates from fresh seafood. Among the species isolated, Vibrio cholerae was the most predominant 24(30.4%), this was followed by Vibrio mimicus 22(27.8%), Vibrio parahaemolyticus 17(21.5%), Vibrio fluvialis 14(17.7%) and Vibrio vulnificus 2(2.5%). Vibrio vulnificus was found only in samples of crayfish and fish (gills) as shown in Table 2.

DISCUSSION

Samples of sea foods analyzed microbiologically in this study showed varying degree of Vibrio contamination. The study showed that skin, gills and intestine of fish used in this study were heavily loaded with different species of Vibrio. This study showed that a total of 79 isolates of Vibrio spp. were obtained from 150 samples of fresh seafoods with Vibrio cholerae as the most predominant. This was followed by Vibrio mimicus, Vibrio parahaemolyticus, Vibrio fluvialis. It showed that Vibrio vulnificus was least predominant. This is comparable with the findings of some other authors in a similar study. Yang et al. [13] revealed 251 isolates of V. parahaemolyticus from 1293 seafood samples (19.0%) collected from the 25 sites in China, during July to October in 2007, while Ji et al. [36] reported 58.6% of 239 samples from different sources were positive for V. vulnificus in 10 Chinese cities from June to September 2009. Compared to other studies, nearly similar results were detected by a study in Turkey determining the presence of Vibrios in 66.0% of samples [29] and in Alexandria, North Coast of Egypt Mediterranean Seas determining the presence of Vibrios in 52.0% of samples. In a study by Merwad et al. [37], the overall prevalence of zoonotic vibrios was 57.3% in white shrimps, 48% in blue crabs and 54% in oysters. Pinto et al. [28] 2008 reported 32.6% for mussels in Italy; Blanco – Abad et al. [38] reported 11.2% for mussels in Spain and Kirs et al. [10] reported 94.8% for oysters in New Zealand.

The predominant presence of Vibrio cholerae in gills of fish could be due to the fact that gills serve as a filter that filters water that comes into the fish from their water ecosystem. The presence of other species of Vibrio (Vibrio parahaemolyticus, Vibrio fluvialis and Vibrio mimicus) agrees favourably with previous studies by Gopal et al. [3], Aberoumand [19] and Colakogu et al. [29] in a similar study on shellfishes. Vibrio spp. can occur naturally in an aquatic environment; the presence of these organisms in raw seafood may be expected. The presence of Vibrio spp. in samples of raw seafood suggests that food borne illness could arise if this seafood is consumed in the uncooked state.
The high prevalence of *Vibrio* spp. in fresh water seafood sample is of concern because it can cause illness in humans. The high incidence probably reflects the nature of *Vibrio* spp. which is known as halophilic water borne bacterium that commonly inhabits environmental water source worldwide. It has been found that fresh water as well as brackish water and marine environments may support the growth of these organisms which are pathogenic to humans [2].

Seafood products harvested from contaminated waters or which have been improperly preserved after harvesting are known to play an important role in infections by *Vibrio* spp. especially crustaceans [1]. This coincides with the results of the present study, where *Vibrio* were recovered from 52.7% of samples with fish showing significantly higher rate of contamination (70.0%). Shellfish poses a particular problem because they are filter feeders which could be a cause for the abundance of *Vibrio* [37].

*Vibrio cholerae* (30.4%) also happened to dominate over other *Vibrio* spp. because it is homogenous spp., comprising organisms that are similar. Biochemically, to each other they shared a common antigen and are closely related genetically. The percentage reported for *V. cholerae* in this study is comparable to what was reported by Aberoumand [19] in a similar study on raw sea foods. *V. cholerae* has been reported as the most predominant species in this study. This is contrary to Aberoumand [19] and Adeleye *et al.* [5] who reported *V. alginolyticus* as the most predominant, followed by *V. parahaemolyticus*. Popovic *et al.* [2] reported the absence of *V. cholerae* or *V. alginolyticus* in their study.

In this study, *Vibrio mimicus* (27.8%) was the second most predominant *Vibrio* species isolated from fresh seafoods. This is comparable with the findings of Adeleye *et al.* [5], who reported *Vibrio mimicus* predominance over *V. cholera* and *V. paraahaemolyticus*. *V. paraahaemolyticus* (21.5%) was the third most predominant *Vibrio* species isolated from fresh seafoods. This deviate from what was reported by Aberoumand [19], who reported *V. paraahaemolyticus* as the second most predominant *Vibrio* species in his study. The 21.5% prevalence value reported for *V. paraahaemolyticus* in this study is lower than what has been previously reported by some other authors [19, 2]. Aberoumand [19] reported 43.6% in his study. However, it was higher than the 5.0% reported by Popovic *et al.* [2] in a similar study.

The seafoods showed an infection rate of 17.7% for *Vibrio fluvialis* in this study. With respects to *V. fluvialis*, fish gills was more infected, 50.0%, followed by fish skin and intestine, 28.6% and crayfish, 21.4%. None was recovered from periwinkle. Otherwise, lower infection rates were detected in other studies. Gopal *et al.* [3] recorded 4.6% in shrimps, Hidalgo *et al.* [39] obtained 3.7% in clams and Merwad *et al.* [37](2011) recorded 16.0% in oysters, 14.0% in crabs and 12.0% in shrimps. The variations in the incidence of *V. fluvialis* in shellfish may be accounted for the differences in water contamination levels in many geographic areas. In addition, higher reports of *V. fluvialis* in this study may be associated with presence of planktons which are a tool for survival and distribution of these bacteria in aquatic environments as was advocated by Gugliandolo *et al*. [40], 2005 and Merwad *et al.* [37]. Regarding the public health hazard, *V. fluvialis* was reported in a human case of severe watery diarrhea and bacteremia in Taiwan [41].

The seafoods showed an infection rate of 2.5% for *V. vulnificus*. This result contrasts the findings of Colakoglu *et al.* [29] and Merwad *et al.* [37]; who reported higher percentages of 16.6 and 6.6%, respectively. Also, lower infection rate of 2.2% in East coast shrimp was cited [3]. Moreover, crabs and oysters showed a similar infection rate of 2.0% for *V. vulnificus*. However, previous studies cited irrelevant higher reports. Canigral *et al.* [42] reported 10.0% in oysters and Kirs *et al.* [10] reported 17.2% in oysters. The prevalence reported for *V. vulnificus* correlated with Aberoumand [19] who reported similar value for *Vibrio vulnificus* in their study. Warner and Oliver [43] also reported the isolation of population structures of two genotypes of *Vibrio vulnificus* from Oysters (*Crassostrea virginica*) and seawater.

The finding of this study with regards to the high contamination of seafoods with *V. cholerae*, *Vibrio mimicus* and *V. paraahaemolyticus*, could be explained by the fact that *Vibrio* spp are widely distributed in marine environment and has been studied extensively by various researcher [29, 44]. Colakoglu *et al.* [29] also reported the occurrence of *Vibrio spp* and *Aeromonas spp* in shellfish harvested off Dardanelles coast of Turkey.

*Vibrio paraahaemolyticus* is often isolated from seawater, sediment and a variety of seafood including shrimp, crab, oyster and clam due to its halophilic characteristics [37]. This bacterium is one of the leading causes of food borne gastroenteritis associated with ingestion of undercooked shellfish throughout the world including the United States, China, Japan and Korea [45-47]. Also, this microbial infection is characterized by diarrhea, vomiting, nausea, abdominal cramps and low grade fever [28].
Previous studies determined the occurrence of *V. parahaemolyticus* in shellfish in different geographic areas over the world: in shrimp in India [3], in Egypt [48] and in oyster in New Zealand [10], whereas in mussels in Turkey [29] and in Spain [38].

In Nigeria, *V. parahaemolyticus* associated gastroenteritis due to consumption of contaminated seafood has been reported as well as sporadic cases of cholera [5]. Infection with this organism occurs during handling of seafood and it does not grow under refrigeration. The organism is salt tolerant but very sensitive to heat and is destroyed by cooking. *Vibrio parahaemolyticus* diseases are usually associated with the ingestion of raw or insufficiently cooked seafood, improper post harvest storage conditions or poor handling of seafood during preparation [49].

*Vibrio vulnificus* is one of the emerging food and waterborne zoonotic bacteria that represents a human health hazard [42]. This pathogen causes gastroenteritis and primary septicemia due to consumption of contaminated oysters, while skin and soft tissue infection results from handling contaminated shellfish or from exposure of open wounds to sea water [50]. *Vibrio vulnificus* is indigenous to estuarine waters and shellfish worldwide [51]. Despite the frequently lethal consequences of *V. vulnificus* infections, the growth rates of the various biotypes and their response to environmental changes are not well characterized [52].

Consumption of seafood (primarily raw oysters) containing *V. vulnificus* can result in a severe, fulminant systemic infection [53]. Characteristics of this disease include fever, chills, nausea, hypotensive septic shock and the formation of secondary lesions on the extremities of patients [53]. However, many literatures reported other pathogenic *Vibrio* in different seafood. Hidalgo *et al.* [39] found *V. fluvialis* in mollcan shellfish in Spain. Regarding the public health hazard, vibrios have been implicated in food poisoning and gastroenteritis; *V. parahaemolyticus* [54], *V. fluvialis* [55] and *V. vulnificus* [50].

The study has shown that samples of fresh fish and shellfishes examined were grossly contaminated by microorganisms and thus, constitute potential health hazard to the public. The presence of these organisms in the fresh sea foods reflects the unhygienic nature of fish handling that predisposes fish to contamination by bacteria. Owing to the potential hazard of some pathogenic *Vibrio*, it is clearly necessary to put more emphasis on food hygiene. Therefore surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health.

**REFERENCES**

5. Adeleye, I.A., F.V. Daniels and V.A. Enyinnia, 2010. Characterization And Pathogenicity Of *Vibrio Spp*. *Vibrio vulnificus* is indigenous to estuarine waters and shellfish worldwide [51]. Despite the frequently lethal consequences of *V. vulnificus* infections, the growth rates of the various biotypes and their response to environmental changes are not well characterized [52].

Consumption of seafood (primarily raw oysters) containing *V. vulnificus* can result in a severe, fulminant systemic infection [53]. Characteristics of this disease include fever, chills, nausea, hypotensive septic shock and the formation of secondary lesions on the extremities of patients [53]. However, many literatures reported other pathogenic *Vibrio* in different seafood. Hidalgo *et al.* [39] found *V. fluvialis* in mollcan shellfish in Spain. Regarding the public health hazard, vibrios have been implicated in food poisoning and gastroenteritis; *V. parahaemolyticus* [54], *V. fluvialis* [55] and *V. vulnificus* [50].

The study has shown that samples of fresh fish and shellfishes examined were grossly contaminated by microorganisms and thus, constitute potential health hazard to the public. The presence of these organisms in the fresh sea foods reflects the unhygienic nature of fish handling that predisposes fish to contamination by bacteria. Owing to the potential hazard of some pathogenic *Vibrio*, it is clearly necessary to put more emphasis on food hygiene. Therefore surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health.


