

Prevalence of bacterial pathogens in bivalve shellfish harvested from the brackish waters of Niger Delta, Nigeria

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ABSTRACT: The presence of bacterial pathogens in bivalve shellfish harvested from the marine ecosystem globally has continued to raise serious food safety concerns, thereby undermining the nutritional and health benefits derived from their consumption. Four species of bivalve shellfish: Bloody cockle (*Anadara senilis*), Donax clam (*Donax rugosus*), Knife clam (*Tagelus adansonai*) and Mangrove oyster (*Crassostrea gasar*) harvested from the brackish waters of the Niger Delta were assessed for their bacterial pathogens. Standard methods of analysis were employed to assess microbiological hazards accumulated by shellfish species. The results of the microbiological analysis revealed that the total viable count (TVC) was lower than the recommended limit (5.7 log cfu/g) for fresh bivalve shellfish while *Vibrio cholerae*, *Vibrio parahaemolyticus*, *Salmonella* spp, *Shigella* spp, *Listeria monocytogenes* and *E. coli* were far above the stipulated FDA standard for molluscan shellfish. The loads of microbiological hazards in bivalve species indicated that in all the locations, Bloody cockle and Donax clam accumulated more bacterial pathogens while the samples harvested from Ibeno and Iko Town brackish waters were lower in pathogenic loads when compared to shellfish harvested from Andoni and Bonny location. The result obtained in this study is of public health importance since, it will serve as a guide towards improved processing techniques and stimulate actions towards the development of novel surveillance as well as, prevention and control strategies, that will help to reduce foodborne disease outbreaks associated with shellfish.

Keywords: Bacterial pathogen, bivalve shellfish, brackish water, food safety, Nigeria.

INTRODUCTION

The presence of pathogenic bacteria in the marine ecosystem has continued to raise serious concerns among researchers and consumers with regard to food safety due to their potential to cause foodborne disease outbreaks depending on the environmental conditions (Letchumanan *et al.*, 2014). Microbial hazards are important with respect to bivalve shellfish quality and safety because they are prone to contamination by a variety of microorganisms from the environment. The number and species of pathogenic bacteria in estuarine and brackish waters depend on factors such as climatic conditions, seasonal changes and anthropogenic activities (Vernocchi *et al.*, 2007). According to Lee and Rangdale (2008), bacterial

groups found in bivalves can be classified into three groups; Those that are indigenous to marine or salty waters, the non-indigenous/enteric bacteria that are present as a consequence of faecal contamination and those from cross-contamination during food preparation and processing. The retention of bacteria by bivalve depends on several factors, such as its form and dimension that influence bacteria adsorption and capture (Ukwo *et al.*, 2020). The feeding physiology of bivalves also determines the accumulation of pathogenic bacteria filtered from the overlying water (Burkhardt and Calci, 2000), which may partly be responsible for geographical and seasonal differences that are noted in bacterial

content in bivalve species. Environmental parameters, such as temperature, salinity, dissolved oxygen and turbidity can limit the filtration activity, also conditioning the retention of bacteria by bivalve shellfish. Therefore, bacteria may accumulate in bivalve species at higher concentrations than those in the environment they inhabit. The accumulation factor is a measure usually used to assess sanitary risk associated with bivalve consumption and corresponds to the ratio between the geometric mean indicator concentration of bacteria in bivalves and the geometric mean concentration of bacteria in the water body (Derolez *et al.*, 2013). According to the data provided by the FAO (2018), bivalve shellfish are consumed by inhabitants of all five continents. However, in spite of this reason, reports of bivalve-associated disease outbreaks from Africa are lacking. It is not true that outbreaks have not occurred in the warm climatic conditions prevailing in Africa. It is therefore believed that the research represents under reporting instead of the absence of bivalve associated infections (Potasman *et al.*, 2002). Bivalve shellfish are well-established food commodity in the global market. Demand for the products has continued to increase annually with supply unable to march the growing interest. Consequently, bivalve products have evolved from being a relatively low-priced product to rather high-priced product with a world export value of US 29.2 billion in 2016, from US 1.41 billion in 2002 representing an increase of over 95.1% during the period (FAO, 2018). The world production of bivalve molluscs: oysters, clams (including cockle and ark shells), scallops and mussels have been on a steady increase from 9013.5 tonnes in 1995 to reaching a new recent record of 17.1 million tonnes in 2016 (FAO, 2018).

Despite the numerous advantages of seafood-based diets, adverse health effects can also exist and seafood harvested from polluted aquatic environments can contain biological and chemical contaminants (FAO/WHO, 2011). Bivalve molluscs are sedimentary filter feeders, they feed by opening their shells for absorption of food particles. Due to their feeding pattern, they filter tiny particles of aquatic plants, animals and inorganic matter. It also accumulates the diversities of other contaminants from the surrounding seawater (Lee and Rangdale 2008; Ukwu *et al.*, 2020). The feeding pattern of bivalve shellfish also determines the accumulation of pathogenic bacteria filtered from the overlying water (Burkhardt and Calci, 2000). According to Lees *et al.* (2010), hazards posed by the routes of transmission from the environment to humans include the consumption of raw, uncooked or lightly cooked bivalve shellfish, representing a significant health risk to the consumers. Also, the bioaccumulation of harmful microorganisms in shellfish is compounded by the traditional consumption of certain bivalve shellfish in raw or only mildly cooked dishes. The most notable examples include *Vibrio* species, which account for 20% of all outbreaks of disease (Potasman *et al.*, 2002). According

to FAO/WHO (2011), it is estimated that over one billion people around the globe rely on seafood products as their main source of animal protein. Fishing is also a major occupation of the people of the riverine communities of Nigeria, and various fisheries resources are also important delicacies including bivalve molluscs which are common among small-scale fisheries. Contamination of the aquatic ecosystem in the Niger Delta region cannot be overemphasized as most of the contaminants can bioaccumulate and become significant along the food chain giving concern about seafood safety to consumers (Davidson *et al.*, 2006). The objective of the study was to detect and characterize microorganisms from bivalve shellfish harvested from the brackish waters of the Niger Delta in order to assess the prevalence and diversity of selected pathogenic bacteria (*Salmonella*, *Shigella*, *E. coli*, *Listeria*, and *Vibrios*).

MATERIALS AND METHODS

Study location

The study location lies along the Atlantic coastline in the Niger Delta region of Nigeria. Four locations were chosen for this study (Andoni, Bonny, Ibeno and Iko Town). The locations were chosen because of their popularity in artisanal fishing activities particularly on bivalve shellfish which also served as an important delicacy and food for the locals. Shellfish also served as a major source of income and employment for the people in these communities. The locations are essentially estuarine in nature with brackish water characterized by fine sandy beaches surrounded by mangrove swamps and intertidal mudflats in which *Nypa* vegetation dominates. The area is also naturally endowed with abundance of rivers, creeks and streams which receive water and waste from the hinterland into the Atlantic Ocean. Also, this coastal environment has continued to suffer from environmental degradation occasioned by the exploration and production of petroleum, liquefied natural gas production and spillage of petroleum products.

Sample preparation and treatments

Samples of bivalve shellfish mostly consumed in these localities were harvested manually by fishermen during low tide from intertidal estuarine or brackish waters of the different study locations. The bivalve specimens collected were: Bloody cockle (*Anadara senilis*), Donax clam (*Donax rugosus*), Knife or Razor clam (*Tagelus adansonai*) and Mangrove oyster (*Crassostrea gasar*). They were identified at the Department of Fisheries and Aquatic Environmental Management, University of Uyo. At each sampling site, twenty (20) samples of each bivalve specimen were collected and transferred to the laboratory within 24 h of collection in plastic containers washed with

5% nitric acid and rinsed with distilled water before use. At the laboratory, the bivalves were promptly cleaned of incrustations and washed in distilled water to remove all dirt. Samples were shucked with a sterile scalpel to extract the flesh and intravalvular fluid into a sterile container. The extracted tissues were homogenized for 60 seconds in a stomacher (Seward Laboratory Stomacher 400, England) and taken for microbiological analysis

Microbiological analysis

Microbiological analysis includes enumeration and isolation of pathogenic microorganisms in fresh bivalve tissues. For the purpose of this study, microorganisms indigenous to marine or estuarine environments and those from faecal contamination or enteric origin were assessed.

Determination of total viable count (TVC)

The total viable count or aerobic plate counts were carried out using the Pour plate Method as outlined by Hatha *et al.* (2005). The homogenate was serially diluted up to 10^{-5} using 9 mL sterile dilution blanks and 1 mL of the 10^{-3} and 10^{-4} dilutions were transferred to sterile Petri dishes, plated in triplicate in standard plate count agar (PCA, OXOID). The plates were incubated at 37°C for 48 hours. After incubation, the plates with 30-300 colonies were chosen for counting and total plate count bacteria were expressed in log colony-forming units per gram of bivalve ($\log \text{CFUg}^{-1}$).

Enumeration of *Escherichia coli*

The isolation and enumeration of *E. coli* was carried out according to the standard method of the Food and Drug Administration (FDA) as outlined by Feng *et al.* (2002). A 25 g homogenated sample was incubated in a pre-enrichment medium Brain Heart infusion (BHI) broth at 35°C for 3 hours to facilitate the resuscitation of sublethally injured cells. The pre-enrichment was then transferred into 225 mL of tryptone phosphate broth and incubated at 44°C for 20 hours. A loopful of the isolate from the enrichment broth was then plated on Eosine Methylene Blue (EMB) agar and MacConkey agar. These plates were incubated for 18-24 hours at 37°C. The plates were examined for green metallic sheen colonies on the EMB agar plate and brick red colour on the MacConkey agar plates. Colonies on the EMB plates were plated on plate count agar (PCA) slants incubated for 18-24 hours at 35°C and used for Gram staining and IMViC reactions.

Enumeration of *Salmonella* and *Shigella* species

The detection of *Salmonella* spp. and *Shigella* spp. were

performed according to the ISO 6579 (ISO, 2002), with some modifications. A 225 mL of buffered peptone water was added to 25 g of homogenized sample and incubated at 37°C for 18–24 hours. Afterwards, isolation and enumeration of presumptive colonies were carried out using a selective and differential medium, *Salmonella Shigella* agar. *Salmonella* do not ferment lactose but produce hydrogen sulphide resulting in colonies appearing colourless but black in centres while *Shigella* spp. does not ferment lactose or produce hydrogen sulphide hence the colour appears colourless on the media. An appropriate serial dilution was carried out using 1 g of the homogenated bivalve samples. These were plated on the media and the plate incubated aerobically at 35-37°C for 24 hours. Based on the colour and morphology, a loopful was further plated on PCA and incubated for 24 hours at 35-37°C. Cellular morphology and biochemical characteristics of the microbial isolates were used for further identification of the isolates according to Garrity and Holt (2001).

Enumeration and identification of *Vibrio* species

Analysis for *Vibrio parahaemolyticus* and *Vibrio cholerae* were performed according to the ISO/TS 21872-1 method (ISO, 2001). The most common selective media is thiosulphate citrate bile salts sucrose (TCBS), a highly selective differential medium that is widely used not only for *Vibrio cholerae* but all other pathogenic *Vibrios*. TCBS is a selective system consisting of ox bile (0.8%), NaCl (1%) and alkaline (pH 8.6) which suppresses the growth of other interfering gram-positive organisms. A 25 g of the homogenized bivalve sample was added to 22 mL of alkaline peptone water (APN Oxoid Ltd, Basingstoke; Hampshire, UK) and incubated at 41.5°C for 18 hours. The APN serves as a suitable enrichment to resuscitate injured cells. Appropriate serial dilutions of samples in APN were incubated on thiosulphate citrate bile salt sucrose agar (TCBS; Oxoid Ltd. Basingstoke, Hampshire, UK) at 37°C for 24 hours. The green characteristic colonies were presumptively selected as *Vibrio parahaemolyticus* while yellow characteristic colonies were selected as *V. cholerae*. The isolates were ascertained for their purity through morphological characteristics such as colony shape, elevation, edge, colour and consistency. Biochemical analyses such as Gram staining, catalase, motility, oxidase, indole, methyl red, sucrose and Voges Proskauer tests were also conducted to ascertain the organism.

Detection and enumeration of *Listeria monocytogenes*

This was done according to the method of ISO11290-1 (ISO, 2004). A 25 g of homogenated sample was added to

225 mL sterile Frasier broth (Oxoid Ltd Basingstoke, Hampshire, UK) and incubated at 30°C for 24 hours. The broth serves as an enrichment medium for the resuscitation of injured cells. Appropriate decimal dilutions were prepared for enumeration of *L. monocytogenes*. The spread plate method was used in which 0.1 ml of 10⁻⁴, 10⁻³ were spread plated in triplicate on PALCAM agar (Oxoid Ltd. Basingstoke, Hampshire UK) and incubated at 30°C for 24-72 hours for observation of growth. The isolates were ascertained for their purity through morphological characteristics such as colony shape, elevation, edge, colour and consistency. Biochemical analyses such as Gram staining, catalase, motility, oxidase, indole, methyl red, sucrose and Voges Proskauer tests were also conducted to identify the organism.

Experimental design and data analysis

A two factor (4 x 4) factorial experiment with location and species of bivalve samples being factors A and B respectively was used to study the prevalence of bacterial pathogens in four species of bivalve shellfish harvested from four locations in the Niger Delta. Data obtained from analyses were subjected to a two-way analysis of Variance (ANOVA) to evaluate the effect of location and species on bivalve molluscs. The level of significance was set at $p < 0.05$. Means with significant differences were separated using the Duncan-multiple Range Test. All experiments were conducted in triplicate and data were analysed using XLSTAT – Pro software program, Addinsoft, Boston (USA) Version 2018.7.

RESULTS AND DISCUSSION

The microbiological compositions of bivalve tissue harvested from the brackish waters of the Niger Delta are shown in Table 1 and Figure 1. The Total Viable Count (TVC) results indicated significant differences ($p < 0.05$) among the bivalve species, and the species from different locations. Bloody cockle from the Bonny location had the highest TVC (3.95 log cfu/g) while knife clam from Iko Town had the lowest TVC (3.25 log cfu/g). Values for *Vibrio cholerae* were highest in bloody cockle harvested from Andoni (3.30 log cfu/g), Bonny (3.37 log cfu/g) and Iko (3.46 log cfu/g) locations but *Vibrio cholerae* was not detected in knife clam and bloody cockle samples harvested from Andoni and Ibeno location. *Vibrio parahaemolyticus* was not observed in mangrove oysters obtained from Ibeno, Iko and knife clam species obtained from Andoni, Ibeno and Iko locations. Also, the highest value of 3.36 log cfu/g was recorded in cockle from Ibeno. *Salmonella* spp. was detected in almost all species except in knife clam and mangrove oysters harvested from the Iko location. *Shigella* was also detected in most bivalve

samples analysed, with the highest *Shigella* count of 3.26 log cfu/g recorded in bloody cockle from Ibeno coastal water. Results from the analysis for *Listeria monocytogenes* indicated that the bacteria was not detected in bloody cockle and Donax clam harvested in Andoni and Bonny as well as knife clam and mangrove oysters obtained from Ibeno and Iko town locations. *Escherichia coli* was detected in all bivalve samples harvested from all the study locations. There were no significant differences ($p > 0.05$) in *E. coli* count in all samples analysed. The highest count of *E-coli* 3.30 logcfu/g was observed in Donax clam from Ibeno and the lowest count (2.19 log CFU/g) in mangrove oysters from Andoni.

The loads of microbiological hazards in bivalve samples as shown in Figure 1 indicated that bloody cockle and Donax clam accumulated more bacteria and samples harvested from Ibeno and Iko Town coastal locations were lower in microbial loads when compared to bivalve harvested from Andoni and Bonny locations. Bioaccumulation of harmful micro-organisms in bivalve molluscs has been attributed to various outbreaks of bivalve-associated infections globally. The threats posed by microbial hazards are compounded by the traditional consumption of certain bivalve species in raw or mildly cooked dishes. The total viable count (TVC) provides a quantitative estimate of both viable and non-viable cells in bivalve tissues. As regards current legislation, the TVC for bivalve shellfish harvested from the Niger Delta coastal waters were lower than the recommended limit (5.7 log CFU/g) for good quality fresh bivalve molluscs as outlined by the Centre For Food Safety and Applied Nutrition (CFSAN, 2003) of the US Food and Drug Administration. The TVC recorded in this present study was similar to values obtained by Anaclato *et al.* (2013) in Portugal but lower when compared to values reported by Hatha *et al.* (2005) and Adjei-Boateng *et al.* (2009) in Suva, Fiji Island and Ghana respectively.

Vibrios are primarily associated with estuarine and coastal marine environments (Hervio-Heath *et al.*, 2002). *Vibrio cholerae* and *Vibrio parahaemolyticus* have been implicated in food poisoning associated with seafood consumption, especially in countries with high ambient temperatures and where shellfish are consumed raw (Lee and Rangdale, 2008). According to Hatha *et al.*, (2005), *Vibrios* are likely to be concentrated in the bodies of bivalve shellfish during filter feeding. The result obtained in this study indicated that 88% and 63% of the bivalve samples analysed were positive for *V. cholerae* and *V. parahaemolyticus* respectively. The *Vibrios* are involved in the transmission and epidemiology of disease leading to outbreaks at endemic, epidemic and pandemic levels (Goel *et al.*, 2010). For *Vibrios*, an input of nutrients to brackish waters by runoff and other input sources could be in favour of higher growth of these bacteria and with regards to the marine bacteria, the incidence of total *Vibrio*

Table 1. Effect of location and species on microbiological composition (log CFU/g) of bivalve tissue.

Location	Species	Total viable count	<i>Vibrio cholerae</i>	<i>Vibrio parahaemolyticus</i>	<i>Salmonella</i>	<i>Shigella</i>	<i>Listeria monocytogenes</i>	<i>E. coli</i>
Andoni	Cockle	3.85±0.02 ^c	3.30±0.04 ^a	3.08±0.07 ^{ab}	2.10±1.83 ^a	3.11±0.07 ^a	ND	3.13±0.16 ^{ab}
	Donax clam	3.80±0.04 ^e	3.10±0.17 ^{ab}	3.10±0.35 ^{ab}	3.01±0.02 ^a	3.03±0.05 ^a	ND	3.04±0.13 ^{ab}
	Knife clam	3.66±0.07 ^h	ND	ND	3.23±0.13 ^a	2.83±0.16 ^a	3.02±0.12 ^a	2.97±0.06 ^{ab}
	Oyster	3.84±0.04 ^d	2.89±0.11 ^{ab}	2.03±1.76 ^{bc}	3.30±0.09 ^a	3.04±0.23 ^a	2.86±0.24 ^a	2.19±1.89 ^b
Bonny	Cockle	3.95±0.01 ^a	3.37±0.06 ^a	3.29±0.03 ^a	3.26±0.07 ^a	3.13±0.16 ^a	ND	3.16±0.02 ^a
	Donax clam	3.83±0.02 ^d	3.29±0.11 ^a	3.30±0.00 ^a	2.16±1.87 ^a	ND	ND	3.17±0.20 ^a
	Knife clam	3.90±0.01 ^{ab}	3.16±0.15 ^{ab}	3.30±0.00 ^a	3.10±0.17 ^a	2.10±1.83 ^a	3.03±0.05 ^a	3.10±0.26 ^{ab}
	Oyster	3.77±0.02 ^f	2.20±1.91 ^b	2.87±0.51 ^{ab}	3.10±0.17 ^a	ND	3.03±0.26 ^a	3.00±0.00 ^{ab}
Ibeno	Cockle	3.89±0.01 ^{ab}	ND	3.36±0.10 ^a	3.30±0.00 ^a	3.26±0.07 ^a	ND	3.25±0.05 ^a
	Donax clam	3.86±0.04 ^{ac}	2.97±0.58 ^{ab}	1.67±1.53 ^c	2.87±0.75 ^a	ND	2.98±0.03 ^a	3.30±0.00 ^a
	Knife clam	3.71±0.03 ^g	3.19±0.16 ^{ab}	ND	2.10±1.83 ^a	2.87±0.51 ^a	ND	3.07±0.40 ^{ab}
	Oyster	3.55±0.05 ⁱ	3.10±0.17 ^{ab}	ND	2.93±0.13 ^a	ND	ND	3.13±0.16 ^{ab}
Iko Town	Cockle	3.91±0.01 ^{ab}	3.46±0.15 ^a	ND	3.26±0.24 ^a	2.10±1.83 ^a	2.26±0.24 ^b	3.21±0.12 ^a
	Donax clam	3.82±0.03 ^d	3.26±0.24 ^a	2.00±0.00 ^{bc}	2.63±0.59 ^a	2.83±0.46 ^a	2.26±0.24 ^b	3.29±0.13 ^a
	Knife clam	3.49±0.04 ^j	2.95±0.05 ^{ab}	ND	ND	2.84±0.06 ^a	ND	3.18±0.03 ^a
	Oyster	3.25±0.04 ^k	2.56±0.07 ^{ab}	ND	ND	2.68±0.17 ^a	ND	2.97±0.03 ^{ab}

Means with different superscripts along the same column are significantly different (Duncan's test) $p < 0.05$; Values are means \pm standard deviation of triplicate samples; ND= Not detected.

parahaemolyticus was similar in shellfish and seawater samples and equally distributed between the three sites as reported by Rincé *et al.* (2018). Microbiological standards for shellfish as outlined by FDA (2009), indicated that *Vibrio cholerae* should be absent in seafood for human consumption while the count of *Vibrio parahaemolyticus* should not exceed (3 log cfu/g).

In this study, the prevalence of *Salmonella* spp. was similar to those observed in market oysters in the United States (DePaola *et al.*, 2010) but lower than those from other studies of molluscs in Northern Ireland, of oysters from 36 United States

bays and of mussels in Morocco (Rincé *et al.*, 2018). *Salmonella* and *Shigella* are non-indigenous but enteric bacteria that occur due to faecal contamination of the estuarine waters. These enteric pathogens from the polluted environment can enter the bivalve during feeding when seawater is being filtered into their gut. *Salmonella* has been described as a highly persistent micro-organism in the environment near farms and human waste and has the ability to produce biofilms on surfaces and to persist and grow in the external environment and on animal feed (Hayward *et al.*, 2016). Consumption of

bivalves with these bacteria usually results in infectious diseases such as typhoid fever and other serious salmonellosis in the case of *Salmonella* spp and bacillary dysentery or shigellosis in the case of *Shigella* spp (Potasman *et al.*, 2002). The result of this study indicated that 86% and 75% of bivalve samples were positive for *Salmonella* and *Shigella* species respectively. Similar findings were observed in bivalves (clams) by Anacleto *et al.* (2013) and in mangrove oysters by de Farias *et al.* (2010). From the public health point of view, the current legislation (FDA, 2009) requires the total absence of *Salmonella* and *Shigella* spp. in 25 g of

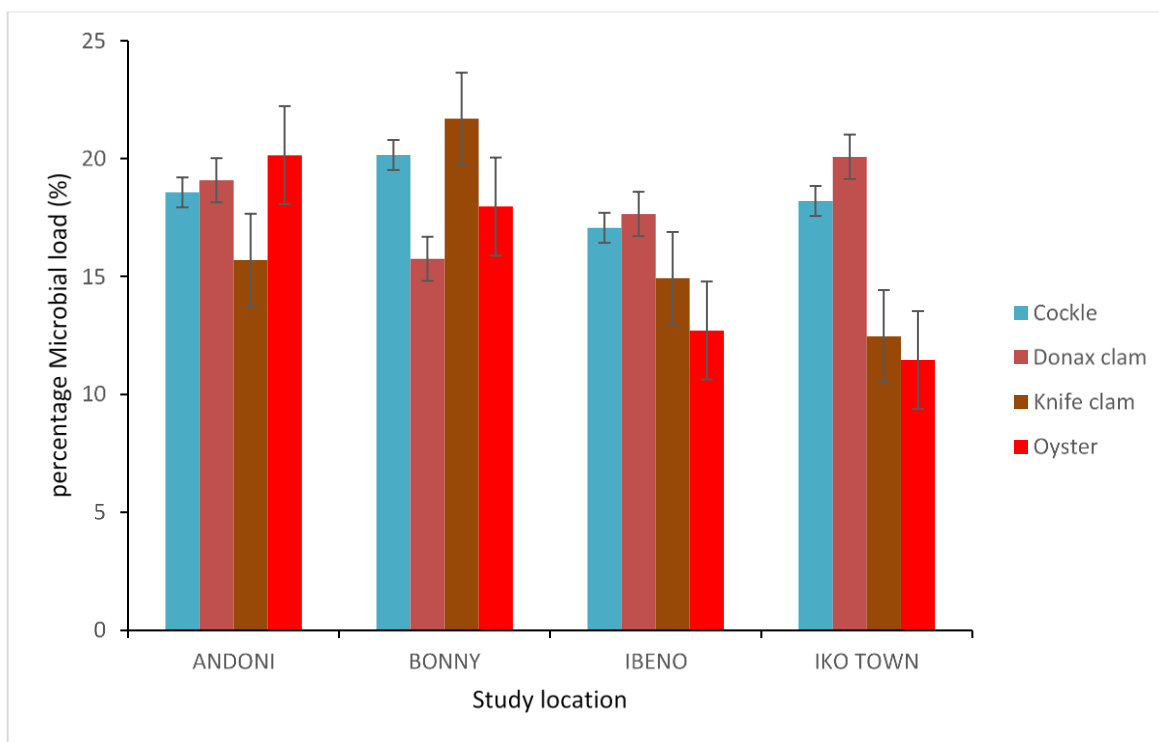


Figure 1. Loads of Microbial hazards in bivalve species from the study locations.

bivalves so as to ensure safe products.

Listeria monocytogenes is indigenous to marine environments and can be present in bivalves eaten raw or fermented. *L. monocytogenes* have been implicated as a causative agent in invasive listeriosis, meningitis and febrile gastroenteritis (Ezeama and Efiuvwevwere, 2007; Lee and Rangdale, 2008). *L. monocytogenes* usually occur widely in the estuarine environment but the level of risk associated with it in seafood consumption is relatively low. *E. coli* is an enteric bacteria which is usually used as an indicator of poor food safety and sanitary conditions. The presence of this bacterium in food therefore serves as an indicator of the presence of pathogenic microorganisms. *E. coli* was detected in all bivalve samples analysed indicating that contamination is associated with human activities as the major source of pollutants in this area. The result from this study was similar to the findings observed by Udoh *et al.* (2017) on freshwater clam from Cross River, in Nigeria. *E. coli* has been implicated in acute diarrhea and gastroenteritis particularly serotypes such as 0148, 0157 and 0124. According to FDA (2009), *E. coli* in bivalves should equal or less than 230MPN/100g and it was observed that the result from this study exceeded the standard. The coastal environment is surrounded by residential houses and shanties for fishermen who do not have toilet facilities and according to Efiuvwevwere and Ezeama (2004), the microbiological safety of seafood is

closely related to the feeding pattern and sanitary condition, of the water as well as untreated wastes from the surrounding. Therefore, the high microbial loads in bloody cockle and knife clams in some locations may be connected to their benthic nature (Souza *et al.*, 2012). According to Ezeama and Efiuvwevwere (2007), the differences in the microbiological composition are directly related to human activities that are prevailing in that environment and hence the high bacteria counts in samples from Bonny can be attributed to the dense population and other human related activities of that location. From the results of this study, bivalve molluscs harvested from the coastal waters of the Niger Delta are not considered safe for human consumption without any treatment or proper processing and cooking time. This is due to the high accumulation of pathogenic bacteria which posed serious health risks to the consumers.

Conclusion

This study has demonstrated a high prevalence of potential pathogenic bacteria in bivalve shellfish harvested from the brackish water of the Niger Delta. Microbiological analysis indicated that the bivalves were loaded with both indigenous marine bacteria vibrios (*Vibrio cholera* and *Vibrio parahaemolyticus*), *Listeria monocytogenes* and

enteric bacteria *Salmonella spp.*, *Shigella spp.* and *E. coli*. The bacteria count of each bivalve sample was higher than the limit stipulated by FDA for shellfish quality. Therefore, the bivalve samples harvested from the locations are not considered safe for human consumption without any further treatment or proper processing. This will help reduce the levels of pathogenic bacteria which may pose health risks to the consumers. The result obtained in this study is of public health importance since it will serve as a guide and stimulate actions toward the development of novel surveillance as well as, prevention and control strategies, which will help to reduce foodborne disease outbreaks associated with shellfish consumption

CONFLICTS OF INTEREST

The authors declare that they have no conflicts of interest.

REFERENCES

- Adjei-Boateng, D., Amisah, S., & Quagraine, K. K. (2009). Bacteriological contamination of the freshwater clam (*Galatea paradoxa*) from the Volta estuary, Ghana. *African Journal of Microbiological Research*, 3(7), 396-399.
- Anacleto, P., Pedro, S., Nunes, M. L., Rosa, R., & Marques, A. (2013). Microbiological composition of native and exotic clams from Tagus estuary: Effect of season and environmental parameters. *Marine Pollution Bulletin*, 74(1), 116-124.
- Burkhardt, W. I., & Calci, K. R. (2000). Selective accumulation may account for shellfish associated viral illness. *Applied and Environmental Microbiology*, 66(4), 1375-1378.
- Centre for Food Safety and Applied Nutrition (CFSAN) (2003). National Shellfish Sanitation Program. Guide for the Control of Molluscan Shellfish. Pp. 357-359.
- Davidson, V. J., Ryks, J., & Fazil, A. (2006). Fuzzy risk assessment tool for microbial hazards in food systems. *Fuzzy Sets and Systems* 157(9), 1201-1210.
- DePaola, A., Jones, J. L., Woods, J., Burkhardt III, W., Calci, K. R., Krantz, J. A., ... & Nabe, K. (2010). Bacterial and viral pathogens in live oysters: 2007 United States market survey. *Applied and Environmental Microbiology*, 76(9), 2754-2768.
- Derolez, V., Soudant, D., Fiandrino, A., Cesmat, L., & Serais, O. (2013). Impact of weather conditions on *Escherichia coli* accumulation in oysters of the Thau Lagoon (the Mediterranean, France). *Journal of Applied Microbiology*, 114(2), 516-525.
- Efiuwewere, B. J. O., & Ezeama, C. F. (2004). The bacteriological profiles of freshwater snail (*Pila ovata*) subjected to microcosms simulating local storage conditions. *World Journal of Microbiology and Biotechnology*, 20(4), 359-363.
- Ezeama, C. F., & Efiuwewere, B. (2007). Microbiological assessment of fresh water snail (*Pila ovata*) and their different habitat locations and the physico-chemical qualities of the habitats. *Asian Journal of Microbiology, Biotechnology and Environmental Sciences*, 9(3), 469-475.
- FAO (2018). Global Aquaculture Updates. In: FAO Aquaculture Newsletter No. 58. Fisheries and Aquaculture Department. Rome, Italy.
- FAO/WHO (2011). Food and Agriculture Organisation of the United Nations. Report of the Joint FAO/WHO Expert Consultation on the Risk and Benefit of fish consumption. FAO Fisheries and Aquaculture Report No. 978 Rome. 1-63.
- de Farias, M. F., Rocha-Barreira, C. D. A., de Carvalho, F. C. T., Silva, C. M., dos Reis, E. M. F., Costa, R. A., & Vieira, R. D. F. (2010). Microbiological conditions of *Tagelus plebeius* (Lightfoot, 1786) (Mollusca: Bivalvia: Solecurtidae) and the water in the Ceará River estuary in Fortaleza-CE. *Boletim do Instituto de Pesca*, 36(2), 135-142.
- Food and Drug Administration (FDA) (2009). Seafood nutrition facts. Food and Drug Administration. Retrieved 10th August 2018 from <https://www.fda.gov/downloads/Food/GuidanceRegulation/ucm063478.pdf>.
- Feng, P., Weagant, S. D., & Grant, M. A. (2002). Enumeration of *Escherichia coli* and the coliform bacteria. In: *Bacteriological Analytical Manual*. US Food and Drug Administration (FDA). Retrieved 08 May 2018 from <http://www.fda.gov/Food/FoodScienceResearch/LaboratoryMethods/ucm064948>.
- Garrity, G. M., & Holt, J. G. (2001). *Begey's Manual of Systematic Bacteriology*. 2nd Edition, Springer. New York, USA.
- Goel, A. K., Jain, M., Kumar, P., & Jiang, S. C. (2010). Molecular characterization of *Vibrio cholerae* outbreak strains with altered El Tor biotype from southern India. *World Journal of Microbiology and Biotechnology*, 26(2), 281-287.
- Hatha, A. A. M., Christi, K. C., Singh, R., & Kumar, S. (2005). Bacteriology of the fresh water bivalve clam *Batissa violacea* (Kai) sold in the Suva market. *The South Pacific Journal of Natural Science*, 23, 48-50.
- Hayward, M. R., Petrovska, L., Jansen, V. A., & Woodward, M. J. (2016). Population structure and associated phenotypes of *Salmonella enterica* serovars Derby and Mbandaka overlap with host range. *BMC Microbiology*, 16, Article number 15
- Hervio-Heath, D., Colwell, R. R., Derrien, A., Robert-Pillot, A., Fournier, J. M., & Pompepuy, M. (2002). Occurrence of pathogenic vibrios in coastal areas of France. *Journal of Applied Microbiology*, 92(6), 1123-1135.
- ISO (2001). Microbiology of food and animal feeding stuffs – horizontal method of the enumeration of potentially enteropathogenic Vibrios Part 1. Detection of *Vibrio parahaemolyticus* and *Vibrio cholerae*. International Organization for Standardization, Geneva, Switzerland. p. 26.
- ISO (2002). *Microbiology of food and animal feeding stuffs – horizontal method for detection of Salmonella spp.* International Organization for Standardization, Geneva, Switzerland, 8, 34.
- ISO (2004). *Microbiology of food and animal feeding stuffs. Horizontal method for the detection and enumeration of L. monocytogenes. Part 1: Detection method.* International Organization for Standardization, Geneva, Switzerland.
- Lee, R. J., & Rangdale, R. E. (2008) Bacterial pathogens in seafood. In: Borresen, T. (ed.). *Improving seafood products for the consumer*. Woodhead publishing series in Food Science, Technology and Nutrition. No. 158.
- Lees, D., Younger, A., & Dore, B. (2010). Depuration and relaying. In: Rees, G., Pond, K., Kay, D., Bartram, J., & Santo Domingo, J. S. (eds.). *Safe management of shellfish and harvest waters*. World Health Organization (WHO), IWA Publishing, London, UK. Pp. 145-181.

- Letchumanan, V., Chan, K. G., & Lee, L. H. (2014). *Vibrio parahaemolyticus*: a review on the pathogenesis, prevalence, and advance molecular identification techniques. *Frontiers in microbiology*, 5, Article number 705.
- Potasman, I., Paz, A., & Odeh, M. (2002). Infectious outbreaks associated with bivalve shellfish consumption: a worldwide perspective. *Clinical Infectious Diseases*, 35(8), 921-928.
- Rincé, A., Balière, C., Hervio-Heath, D., Cozien, J., Lozach, S., Parnaudeau, S., Le Guyader, F. S., LemHello, S., Giard, J-C., Sauvageot, N., Benachour, A., Strubbia, S., & Gourmelon, M. (2018). Occurrence of bacterial pathogens and human noroviruses in shellfish-harvesting areas and their catchments in France. *Frontiers in Microbiology*, 9, Article number 2443.
- Souza, D. S., Ramos, A. P., Nunes, F. F., Moresco, V., Taniguchi, S., Leal, D. A., Sasaki, S. T., Bicego, M. C., Montone, R. C., Durigan, M., Teixeira, A. L., Pilotto M. R., Delfino N., Franco R. M., Melo C. M., Bainy A. C., & Barardi C. R., (2012). Evaluation of tropical water sources and molluscs in southern Brazil using microbiological, biochemical, and chemical parameters. *Ecotoxicology and Environmental Safety*, 76, 153-161.
- Udoh, D. I., Udo, I. U., & Udoh, E. I. (2017). Microbiological analysis of the freshwater clam (*Galatea paradoxa*, BORN 1778) caught from Cross River, Nigeria. *Nigerian Journal of Agriculture, Food and Environment*, 13(3), 59-64.
- Ukwo, S. P., Ezeama, C. F., & Obot, O. I. (2019). Microbiological safety and toxic element contaminants in bivalve shellfish from intertidal mudflats of IKO estuary, Niger Delta, Nigeria. *South Asian Journal of Food Technology and Environment*, 5(2), 846-854.
- Vernocchi, P., Maffei, M., Lanciotti, R., Suzzi, G., & Gardini, F. (2007). Characterization of Mediterranean mussels (*Mytilus galloprovincialis*) harvested in Adriatic Sea (Italy). *Food Control* 18(12), 1575-1583.