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Occurrence of potentially pathogenic *Vibrio species* in some edible molluscs from Kalarugbani creek in Okirika LGA, River State, Nigeria

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ABSTRACTS

The objective of this study was to determine the potentially pathogenic Vibrio species associated with Tympanotonus fuscatus (Periwinkle) and Crassostrea sp (oyster) and its habitants. The Vibrio counts ranges from 6.1x10^3 - 2.1X 10^4 for oyster, 5.7 x10^3 – 1.2x10^4 for periwinkle, 1.4 x10^3 -6.7x10^3 for sediments and 3.1 x10^3 - 1.2x10^5 for the overlying water. Statistical analysis for the Vibrio counts of REP, RSO, OLW and MFS showed a great significant difference at P<0.05 between the various samples and also at P< 0.05 there was a significant difference in the counts obtained for various months sampled. One-way ANOVA was used in comparing between RSO, REP and OLW, there was a slight significant difference at P<0.05, between RSO, REP, and MFS, there was also a great significant difference at P<0.05. The study confirms the risk associated with the consumption of these shellfish and the need for proper storage and preparation condition prior to the consumption. In addition, in order to prevent the health risks associated with these shellfish heat treatment has to be applied prior to consumption.

Keywords: *Crassostrea sp* (oyster), edible molluscs, shellfish, *Tympanotonus fuscatus* (Periwinkle), *Vibrio species*, Vibrio counts.

Abbreviations: *RSO: Raw shucked oyster, REP: Raw extracted periwinkle, MFS: Mudflat sample, OLW: Overlying water.*

INTRODUCTION

Members of the genus Vibrio are defined as gram negative asporogenous rods that are straight or have a single rigid curve and are motile with a single polar flagellum when grown in liquid medium [1]. The importance of *Vibrio spp* as a contaminant of raw or under cooked seafood has been well established [2]. *Vibrio species* are known to occur naturally in marine and fresh water environments and thus are commonly associated with seafood and or food of fresh water origin [3-4]. Many species can cause gastrointestinal diseases. *Vibrio paraheamolyticus* has been frequently involved in out break of food borne disease worldwide [5-6]. *Vibrio cholerae* also constitutes a very important risk. The serogroups can cause less severe diarrhea [7-8]. *Vibrio*

vulnifus is another organism of great concern in seafood safety due to the severity of the disease and the high mortality rate it can cause [9-10]. Other species that have been increasingly recoginized as food pathogens in recent years are *V. mimicus* and *V. alginolyticus. V. mimicus* has genetic and many biochemical similarities to *V. cholerae* and its pathogencity involves several toxins including that of *V. choreae*. Many foods borne outbreak cases involving *V.mimicus* have been reported [11-12]. *V. alginolyticus* is one of the most common *Vibrio species* occurring in the marine environments and seafood [2, 4, 13]. This is an opportunistic pathogen [14] and its pathogencity is thought to be similar to that *V. paraheamolyticus*.

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 [15-17]. However most survey are quantitative which causes difficulties in evaluating the risk relating to *Vibrio spp* in raw seafood can also affect survival of the organisms through processing. For processed and ready to eat seafood (including ready to eat product) that are intended for raw consumption such as raw oyster [18-19], the presence and level of *Vibrio spp* has a direct impact on food safety.

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 out break worldwide [20-21]. In the Asian region, *Vibrio spp* have been recognized as the leading cause of food borne outbreak in many countries Japan [22-24], India [2, 25], China [24,26], Taiwan[23], Korea [27], Thailand [28], and Iran [29]. Investigation shows that many out break were cause by consumption of contaminated seafood [30]. Cases of food borne out break resulting from consumption of ready to eat seafood dishes especially those supplied by food catering food service establishments continually occur [31-32].

Vibrio species are distributed world wide in sea water and is associated with the resident aquatic organisms. The aim of this study was to investigate the counts of these pathogenic *Vibrio spp* of public health concern and the seasonal variation of the occurrence of these *Vibrio spp* from these shellfish, River water and sediment from Kalarugbani creek in Okirika LGA, River State, Nigeria.

MATERIALS AND METHODS

A total of 150 samples of oyster and perwinkle were obtained over a period of seven months (July, 2010- January, 2011) from Kalarugbani creek in River state along with the water sample and sediments. During the collection all the samples were placed in sterile labelled sealed plastic except the water sample that was obtained with a sterile 1 litre gallon prior to transportation to the laboratory. Statistical analysis of the Vibrio counts of RSO, REP, OLW and MFS was done using one way ANOVA to determine if there is any significant difference between these samples.

Processing of samples

Samples were categorized according to the method of processing and collection namely; Raw shucked oyster (RSO), Raw extracted periwinkle (REP), Mudflat sample (MFS), Overlying water (OLW). Raw oyster was shucked aseptically with a sterile stainless steel instrument (Knife) which was inserted between the shell about 2cm from the hinge area the knife will be pushed into the shellfish the fluid and meat removed from the shell and placed in a sterile container as described by APHA [33]. The raw periwinkle meat in their shell was cracked using a small sterile hammer on the improvised sterile anvil then the meat was extracted individually

from the broken shell using a sterile forceps and transferred into a sterile container as described by APHA [33] as modified by Odu et al. [34].

Physiochemical parameter of the overlying water

The physiochemical parameter of the overlying water, salinity of the overlying water was determine using a refract meter (Antergo 28). A drop of the test water was placed on the lens of the instrument while the meter was held horizontally. The test water was allowed to remain for about five minutes and the salinity was then read off from the eyepiece and recorded in parts per thousand. Water temperature was measured in situ using mercury –in glass thermometer and the sampling site. The thermometer was immersed in water to about 6cm below the water surface and left to stabilize for about five minutes and the average values recorded in degree centigrade. Hydrogen- ion pH was taken immediately at the sampling site. A multiple meter, model U-10 micro from Horiba Limited Japan was used to determine the pH. The electrode was immersed into the beaker of water sample and the values recorded after 5 minutes to stabilize.

Bacteriological analysis

Analysis of *Vibrio spp* in these seafoods were carried out in duplicates on 25g of oyster and perwinkle meat. For each seafood sample, 25g were homogenised in 225ml sterile 0.1% peptone water in a Stomacher 400 Circulator Homogeniser at 120rev/min for 2 minutes. A 10 fold serial dilution in sterile 0.1% peptone water was prepared using standard bacteriological analytical methods. Spread plate method was carried out using 10^{-2} and 10^{-3} dilutions on Thiosulphate Citrate Bile Salt Sucrose agar (TCBS). Analysis of *Vibrio spp* in the overlying water sample was carried out in duplicates, 1ml of the overlying water was dispensed into 9 ml of 0.1% peptone water. A ten fold serial dilution of the water sample was done, 10^{-2} and 10^{-3} was spread plated on TCBS agar. Analysis of *Vibrio spp* in the mud flat sample was carried in duplicates on 25g of mud flat was placed in 225ml of 0.1% peptone water shaked imitates. A ten fold serial dilution of the mud flat sample was done, 10^{-2} and 10^{-3} was spread plated on TCBS agar. The TCBS plates were incubated at 37^0C for18-24 hours and counts were made for each colony type. For *Vibrio spp* identification for each sample, 10-20 representative colonies of each of Green and yellow colony type were selected from TCBS plates containing 20-200 colonies. A total of 210 isolates was subjected to biochemical tests and sodium chloride tolerance leading to the species characteristics of human pathogenic Vibrionaceae commonly encountered in seafood listed in BAM [35].

RESULTS AND DISSCUSSION

A total of 210 isolates were obtained in this study and identified as species characteristics of human pathogenic Vibrionaceae commonly encountered in seafood listed in BAM [35]. These include; *Vibrio paraheamolyticus, Vibrio chloreae, Vibrio vulinifus, Vibrio mimicus,* and *Vibrio alginolyticus.* Table 1 shows a detailed result of the *Vibrio spp* counts of RSO, REP, OLW and MFS. Total vibrio counts in the samples $6.1x10^3$-$2.2x10^4$, $5.7x10^3$-$1.2x10^4$, $3.1x10^3$-$1.2x10^4$, $2.3x10^3$-$6.7x10^3$ respectively. The RSO had the highest counts while MFS had the least counts. Oysters are filter feeder and are also able to accumulate bacteria in their tissues to level of 4-7 times higher than that of the surrounding [36]. The higher Vibrio count may also be due to the ability of oyster to concentrate *Vibrio spp,* 100 fold compared with the amount found in the surrounding water through filteration [37]. As reported by Chen *et al*. [26], the food hygiene regulation of Japan requires *V. paraheamolyticus* level to be below $<10^2$ MPN/g in seafood for raw consumption. The level of concern established by Food and Drug Administration (FDA) for *V. paraheamolyticus* in molluscan shellfish is 10^4MPN/g. Also, for ICMSF the level of concern is 10^3 MPN/g. Seasonal variation of the Vibrio counts in the various samples was observed from

Table 1. It shows high level of Vibrio count during the dry season between November 2010 to January 2011 and decrease in the Vibrio count during the rainy season. This corresponds to the studies of Neumann*et al*. [38] and Deepanjali *et al.* [39].

Table 1: **Vibrio counts in the various samples**

Sample	Vibrio count (CFU/g)						
	July, 2010	**August, 2010**	**Sept. 2010**	**Oct. 2010**	**Nov. 2010**	**Dec. 2010**	**Jan. 2011**
RSO	6.9×10^3	9.3×10^3	8.9×10^3	6.1×10^3	9.1×10^3	1.9×10^4	2.2×10^4
REP	6.1×10^3	7.5×10^3	5.7×10^3	6.1×10^3	8.4×10^3	9.8×10^4	1.2×10^4
OLW	3.9×10^3	4.7×10^3	3.1×10^3	4.9×10^3	6.6×10^3	7.4×10^3	1.2×10^4
MFS	3.8×10^3	4.9×10^3	2.3×10^3	1.4×10^3	4.4×10^3	6.1×10^4	6.7×10^4

Fig 1 shows the pH values ranges from 6.5-6.8 in the wet season and 6.8-7.0 in the dry season. Seasonal variation of pH of the overlying water was observed in this study is in agreement with the results of previous studies in Bonny River where the highest pH values were recorded in the dry season and lower value of pH in the wet season and other studies conducted. This may be due to the influx and decay debris in the area as well as imbalance level of hydrogen ions inputs from surface runoff during the rainy season.

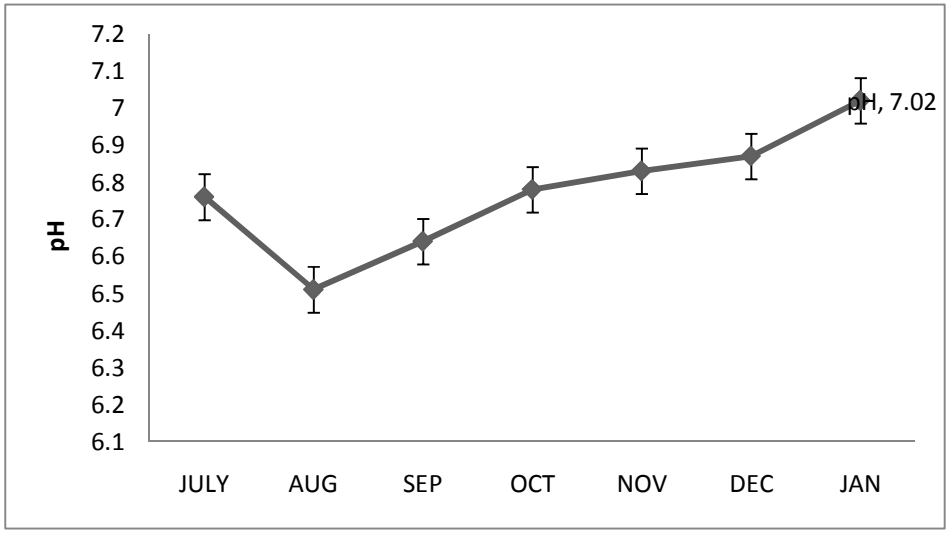

Figure 1: A graph showing the monthly variation in the pH of the overlying water.
Values represent the mean of the determination two determinations. Bar indicates standard errors.

Figure 2 shows the temperature values ranges from 24.8- 26.1ºC for the wet season and 26.9-27.6ºC in the dry season. Seasonal variation in the ambient temperature was observed in Kalarugbani creek. Dry season was slightly higher than that of the wet season. Higher temperature values recorded in the dry season are expected since heat from the sunlight increases temperature of surface water, similarly the drop in the water temperature in the rainy season is attributed to heavy rainfall experienced during the period. This is similar to the observation of previous workers.

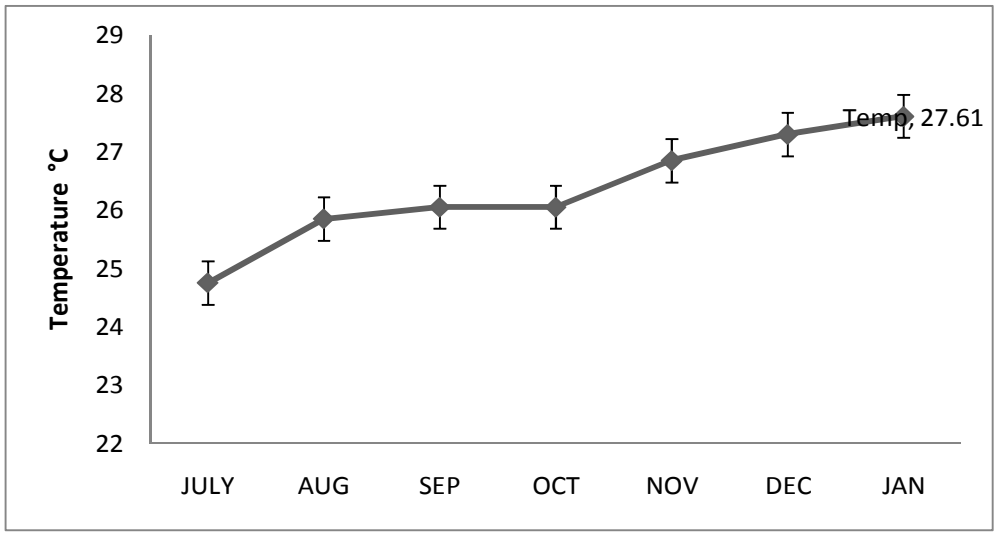

Figure 2: A graph showing the monthly variation in the Temperature of the overlying water.
Values represent the mean of the determination two determinations. Bar indicates standard error.

Fig 3 shows the salinity values ranges from 1.1- 2.7% in the wet season and 2.9-3.8% in the dry season. Salinity has been viewed as one of the most important variables influencing the utilization of the organisms in estuaries. Seasonal variation was observed, high salinity values was recorded during the dry season than the wet season. This is because during the wet season high volumes of fresh water are discharged into coastal or estuarine water that lowers or dilute the water. Similarly, some studies have reported that rain fall could cause dilution of estuaries and hence cause reduction in salinity, while heat generated by sun light in dry season months would cause evaporation of the surface water making it saltier and hence more saline.

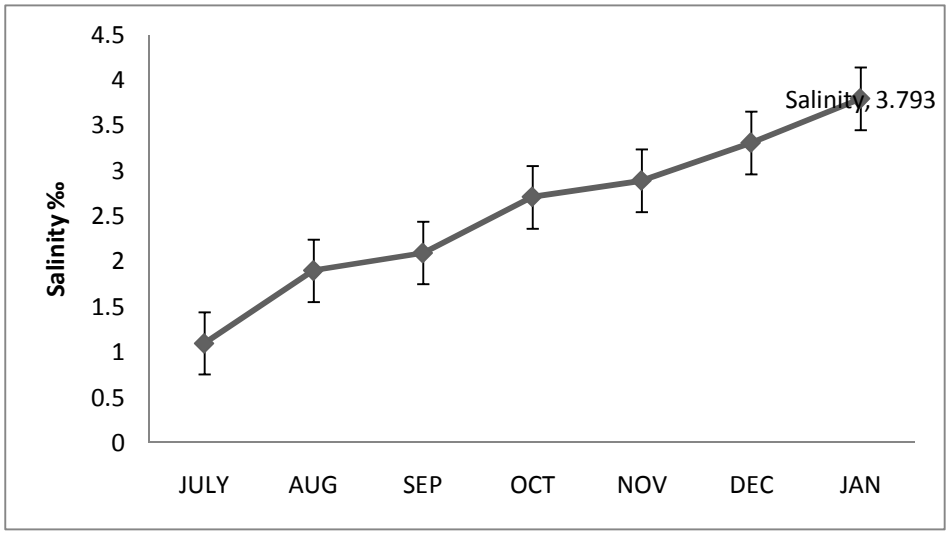

Figure 3: A graph showing the monthly variation in the Salinity of the overlying water.
Values represent the mean of the determination two determinations. Bar indicates standard errors.

Fig 4 shows the percentage frequency of occurrence of the *Vibrio spp* isolated from the various samples. These *Vibrio spp* occurred at varying percentage in RSO, REP, MFS and OLW. *Vibrio paraheamolyticus* occurred in all samples at varying percentage and the least of occurrence in the various samples is *Vibrio vulnifus*. *Vibrio paraheamolyticus, Vibrio chloreae, Vibrio mimicus, Vibrio vulnifus,* and *Vibrio alginolyticus* occurred in the oyster and perwinkle. This is in

agreement with the studies of Gopal *et al*. [2] and Colakogu *et al.* [13]. Vibrio *paraheamolyticus*, *Vibrio chloreae, Vibrio alginolyticus*, *Vibrio mimicus* was present in the overlying water and mudflat sample. This corresponds to the studies of Amirmozafari et al. [40] and Ouseph et al. [41].

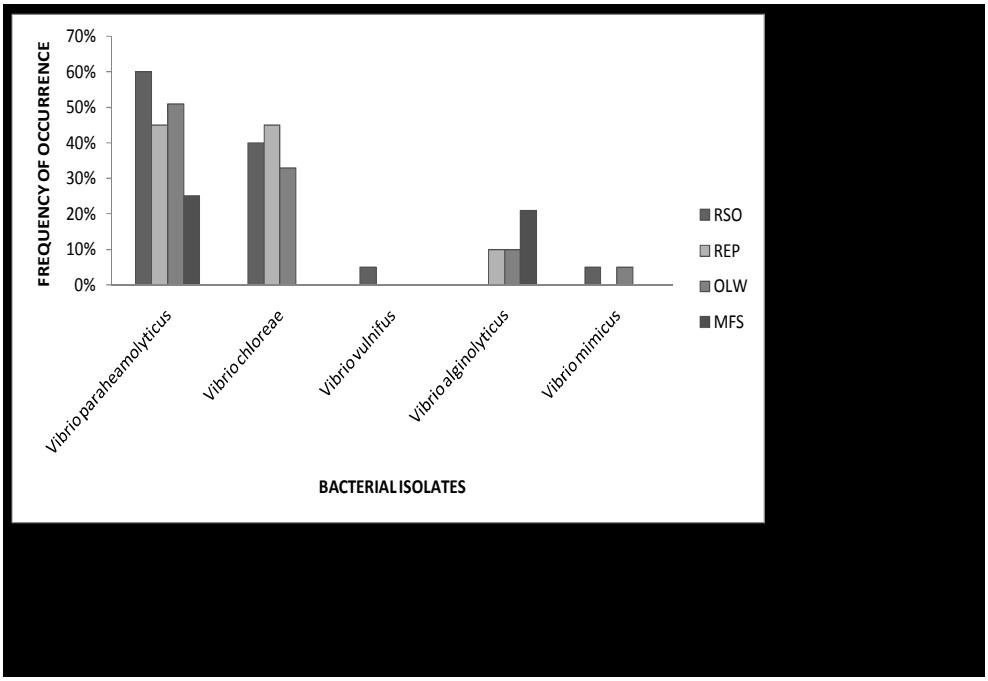

Figure 4: Frequency of occurrence of *Vibrio spp* in the various samples

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 in this study suggests that food borne illness could arise, if these seafoods are 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 world wide. It has been found that fresh water as well as brackish water and marine environments may support the growth of these organisms which are also pathogenic to humans [42].

V. paraheamolyticus was first recognised as the cause of food borne illness Osaka Japan in 1951 and was identified as a common cause of food borne illness due to consumption of seafood in many Asian countries [20]. The finding of this study with regards to the high contamination of *V. paraheamolyticus* in these seafoods and in the overlying water and mud flats is in concurrence with studies of Ismail and Bilal [43]; Deepanjali *et al.* [39]; Thararat *et al*. [28]; and Ali [29], since *Vibrio spp* are widely distributed in marine environment, and has been studied extensively by various researcher [44].

It is well recognised that *V. cholerae* is part of the natural bacteria flora of the aquatic environments. *Vibrio paraheamolyticus* disease are usually associated with the ingestion of raw or insufficiently cooked seafood, improper post harvest storage conditions or poor handling of seafood during preparation [45]. From the findings of this study, it is imperative that monitoring and routine screening of seafood sample for the presence of *Vibrio spp* infection, since *Vibrio spp* occur naturally in aquatic environment. In addition to prevent possible adverse effects of

microrganism living in polluted water, necessary hygienic measures are to be taken and additional heat treatment during cooking process should be efficiently done in order to minimize food borne diseases.

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