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Incidence of *Vibrio* species in seafood samples collected from Lagos Lagoon, Nigeria

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Abstract
Vibrio species are natural inhabitant of the marine and estuarine environment which often causes food-borne gastroenteritis. A total of 90 seafood samples, croaker fish (*Pseudotolithus senegalensis*), shrimps (*Penaeus notialis*), and blue crab (*Callinectes sapidus*), were collected from landing sites along the Lagos Lagoon, Lagos State Nigeria. Physicochemical parameters of water samples were also monitored using standard methods. Samples were collected for 2 years between 2011- 2013. They were examined for incidence of *Vibrio* species using both biochemical and molecular methods. Biochemical identification of the isolates was confirmed with Polymerase Chain Reaction (PCR) and nucleotide sequencing using V. 16S-700F and V. 16S- 325R primers. 27 isolates were obtained from the samples examined. Dissolved oxygen and conductivity showed a significant difference ($P < 0.05$) in the Lagos lagoon. 16 isolates were presumptively identified as *Vibrio* species. PCR identified 7 isolates as *Vibrio* species; DNA sequencing identified the 7 isolates as *V. parahaemolyticus* (5), *V. fluvialis* (1) and *Shewanella algae* (1). The results reveals incidence of *Vibrio* species in seafood samples from Lagos lagoon.

1. Introduction

Vibrio species are Gram-negative, curved rod shape, oxidase positive and non spore forming bacteria (Austin, 2010). They are ubiquitous that are naturally present in the marine environment, and are particularly resistant to high salt concentrations. A number of species within the genus *Vibrio* are associated with food borne infections and food spoilage. However, only a few of the species are more specifically pathogenic to humans, such *as V. cholera, V. mimicus, V. metschnikovii, V. cincinnatiensis, V. hollisae, V. damsel, V. fluvialis, V. furnissii, V. alginolyticus, V. parahaemolyticus, V. vulnificus, V. carchariae* (Igbinosa, 2010; Adagbada *et al.*, 2012; Farmer *et al*., 2003). Some species such as *V. Parahaemolyticus*, *V. cholera* and *V. vulnificus* are more specifically pathogenic to humans causing severe intestinal diseases (Farmer *et al*., 2003).

Vibrio species are known to cause food-borne illnesses especially in Asia and Africa regions (McLaughlin *et al*., 2005). Utsalo *et al*. (1992) also reported *Vibrio* infection in Calabar Nigeria. However, there is a significant number of reports on food borne infections from the consumption of raw fish or improperly cooked contaminated fish and shellfish (Oyster, Clams, Mussels, Crabs, Shrimps, periwinkles and prawns) (Farmer *et al*., 2003; Eja *et al*., 2008; Lutz *et al*., 2013). Seafood constitute an important food

component for a large section of world population (Lutz *et al*., 2013).The rise in seafood consumption and the global warming, resulting in increased ocean surface temperatures, may cause higher prevalence of *Vibrio* species, and increase the risk of *Vibrio* food borne infections (Hassan *et al*., 2012). The isolation of *Vibrio* species with Thiosulphate citrate bile sucrose agar (TCBS) has been extensively used and to date is still the most commonly used selective medium for the isolation of *Vibrio* species from natural environments and clinical sources (Martinez-Urtaza *et al*., 2008). Nevertheless, several limitations associated with the use of the biochemical microbiological techniques for the detection of *Vibrio* species are however, slow, laborious and often require several days to be performed. Moreover, this phenotypic identification method, characterized by a low sensitivity, may fail to detect strains of bacteria present in the samples or give an incorrect identification (Martinez-Urtaza *et al*., 2008; Thompson *et al*., 2004).

Molecular methods for the identification of *Vibrio* species have increased lately, especially the use of Polymerase Chain Reaction (PCR)-based techniques to amplify specific DNA sequences, as well as digestion of these fragments with restriction enzymes. The most frequently used molecular methods to identify *Vibrio* species are the Amplified Fragment Length Polymorphism (AFLP), Fluorescence *In Situ* Hybridization, Microarrays, Multilocus Enzyme Electrophoresis (MLEE), Multilocus Sequence Typing (MLST), Real-Time PCR, Restriction Fragment Length Polymorphism (RFLP) and Ribotyping (Rademaker, *et al*., 2000; Gurtler and Mayall, 2001; Thompson and Swings, 2006; Thompson, *et al* ., 2009; Chan, *et al*., 2012).

Due to increase in seafood consumption and the global warning, which may cause a higher prevalence of *Vibrio* species and increase in the risk of *Vibrio* borne infections, this present study was conducted to monitor the incidence of *Vibrio* species in sea foods from Lagos Lagoon Nigeria.

2. Materials and Method

2.1. Description of the Study Area

Lagos Lagoon was the study area for this project. The Nigerian coastline is between longitude 02° 53′ to 08° 14′ E and latitude 06° 21′ to 03° 55′N, covering a distance of 85 km and lies in between the Gulf of Guinea. Lagos coast is a narrow coastal shelf and lies between 14, 816 km and 27,780 km with a total area of 41,000 km2. The Lagoon extends from the coast to about 37 km north and about 48 km east where it narrows and continues as the Lekki Lagoon. The estimated area of the main body of the Lagoon is 150.56 km^2 (Ajao, 1990).

2.2. Collection of Seafoods and Water Analysis

Seafoods samples, (blue crab *Callinectes sapidus*; croaker fish *Pseudotolithus senegalensis*; shrimps *Penaeus notialis*) were collected from landing sites along three villages (Takwa bay, Liverpool and Makoko) in Lagos Lagoon. The seafoods were collected from local fishermen in sterile stainless plates and transported in cooler boxes to Nigerian Institute for Oceanography and Marine Research Victoria Island Lagos. Samples were analyzed at most 2h after collection. Samples were collected from each site monthly for a period of two years (24 Months), February, 2011- February, 2013 covering the rainy and dry seasons. A total of 90 samples (30 samples each from the seafoods) were collected during the period.

2.3. Physico- Chemical Parameter Determination

Salinity, pH, turbidity, dissolved oxygen and conductivity were measured *in situ* with a Horiba U-10 multi-parameter Water Quality Checker (Nkwoji *et al*., 2010). Water temperature was measured in situ with a mercury thermometer (Nkwoji *et al*., 2010).

2.4. Isolation of *Vibrio* Species from Seafood Samples

The procedure of Elliot *et al.* (1998) and Adeleye *et al*. (2008) were adopted. Using Alkaline peptone water (APW), pH 8.4, and Alkaline Peptone Salt Broth (APSB) i.e. (APW supplemented with 3% NaCl) at pH 8.4 for enrichment of the samples. 25 grams of the samples (crab, croakers fish and shrimps) each scaled and blended separately with sterile blender, 25 grams of sediment were weighed into 225ml of sterile APW and APSB each (i.e. 1:10 dilution). The APW homogenate was incubated at 37^{o}C for 6 hours. And after incubation, 1 ml of the broth culture showing positive growth (turbidity) was transferred to a sterile petri dish, on a sterile TCBS agar to obtain discrete colonies for each of the different samples. The petri dishes were incubated overnight for 18 hours at 37^{o}C after which only the yellow colonies were sub cultured to obtain pure isolates on dried Tryptone soy agar (TSA) supplemented with 3% NaCl and incubated overnight for further identification of the isolates (6 to 8 hours of incubation is specific to check for the presence of *Vibrio cholera*).

For the APSB homogenate, a 3-tube most probable number (MPN) method was also adopted (Elliot *et al*., 1998). After incubation of the inoculated tubes with the samples, 1 ml of the broth culture showing positive growth (turbidity) was transferred to a sterile petri dish, on a sterile TCBS agar to obtain discrete colonies for each of the different samples. The petri dishes were incubated overnight at 37^{o}C, after which round, opaque, greenish and yellowish colonies was sub cultured on dried TSA supplemented with 3% NaCl plates and incubated at 37^{o}C overnight (Elliot *et al*., 1998). The isolates were then sub cultured into 1% Tryptone broth supplemented with 3% sodium chloride and incubated at 37^{o}C for 24 hours.

2.5. Characterization of Isolates Suspected to be *Vibrio* Species

The randomly-selected colonies were first Gram stained to determine the curved rod morphology of *Vibrio* species before purification by three successive streaking and re-isolations on TSA-3% NaCl. The purified isolates were stored on Tryptone soy broth (TSB)-3% NaCl. They were incubated at 37^{o}C for 24 h and stored at room temperature until further use. From the stock culture, each isolate was assayed for oxidase, urease and indole production, motility and salt tolerance tests using the method described by of Elliot *et al*. (1998). All isolates preliminarily identified to be *Vibrio species* from the above assay results were further confirmed using PCR and nucleotide sequencing.

2.6. Molecular Identification of Isolates

DNA extraction: The presumptively identified *V.* species were grown overnight in Tryptone soy broth (TSB) supplemented with 3% NaCl, the cultured broth were centrifuged at 1000 rpm for 60 seconds and the supernatant discarded the pellets were then suspended in 500 µℓ of sterile distilled water and the DNA was extracted with Zymo bacteria DNA extraction kit according to the manufacturer's instructions (Troisa *et al*., 2010).

Table 1. *Primers used in PCR identification of Vibrio species*

Target species	Primer	sequence (5to 3)	Concn. (µM)	Amplicom Size (bp)
Vibrio species	V.16S-700F	CGG TGA AAT GCG TAG AGA T	0.05	663
	V.16S-325R	TTA CTA GCG ATT CCG AGT TC		

Source: (Amin and Salem, 2012).

Oligonucleotide Primers: Oligonucleotide Primers used to amplify *Vibrio* species are all listed in Table 1. All primers were synthesized by Biomers Germany.

PCR conditions: The PCR cycle targeting the different Amplicon sizes with the primers as stated in Table 1 as described by Amin and Salem, (2012). The reactions were carried out each in a total volume of 20 µℓ, the reaction contained an additional 0.1µℓ of AmpiTaq Gold (Applied Biosystems, Foster City, CA), Master Mix consists of (PCR buffer, deoxynucleoside Triphosphate (dNTP), $MgCl_2$ Taq DNA polymerase) 4 µℓ, primer concentration of 0.05 for each of the primers (Table 1) 5 µℓ of template DNA and nuclease free water to make up the volume. The thermal cycling profile were as follows: a 15-min soak at 93°C followed by 35 cycles of 92°C for 40 s, 57°C for 1 min and 72°C for 1.5 min and a final soak at 72°C for 7 min.

Visualization of PCR Products in Agarose Gels and Fragment Analysis: The DNA Amplicon were observed by running the PCR reactions on 1.5% (w/v) Agarose gel (Amersham Pharmacia Biotech) in1×TE (Tris phosphate EDTA) buffer at 80 V, 200 mA and 100 W for 1 h. The molecular maker 100 bp DNA ladder (Biomer, Germany) was loaded as negative control. In the case of Agarose gel analysis, 5 µl of PCR product were loaded by lane. The result for each isolate was determined by comparison of the Amplicon size for all *Vibrio* species with reference to standards as described by Amin & Salem, (2012). Bands were observed using an Upland CA 9178 USA transilluminator Model M5. Gel photos were taken using the Vilber Lourmat camera with Vida max screen **(**Espeñeira *et al*., 2010).

Sequencing of PCR products: Sequencing of PCR products to confirm the identification of isolated strains was carried out by extraction of DNA, amplified with V. 16S-700F and V. 16S- 325R primers, as described earlier (Troisa *et al*., 2010). PCR products were purified with a Qiaquick PCR purification kit (Qiagen Iberia S.L., Madrid, Spain) according to the manufacturer's instructions, and these products were used for nucleotide sequencing. Sequencing of DNA was determined according to the procedure of GATC Biotech Germany. Sequences were analyzed using Chromas Version1.43 software program (Technelysium, Tewantin Qld, Australia). The homology of the amplified sequences with the correspondent *Vibrio* species was determined by a BLAST alignment for the isolates. Sequences were compared with all *Vibrio* 16S rRNA sequences available in GenBank using BLAST software (http://www.cnbi.nlm.nih.gov/BLAST/).

2.7. Statistical Analyses

Data were analysed using descriptive statistics (Mean and $\pm$ SD) with PAST (Paleontological Statistics Software Package for Education and Data analysis, Version 1.49) statistical software. T- test were performed on the data, the least significant difference were used to compare differences among individual means, differences were considered as significant at 95% confidence level, $P< 0.05$ (Hammer *et al*, 2001).

3. Results

The surface water temperature at the different localities of Lagos Lagoon (Makoko, Liverpool and Takwa bay) study sites during the dry season was observed in 2011 to range from 27.7 ± 1.5 to 28.7 ± 0.6 and 25.2 ± 0.8 to 26.0 ± 1.0 ^{0}C in rainy season while it ranged from 28.2 ± 1.9 to 29.3 ± 0.8^{0}C in dry season and 26.0 ± 2.0 to 29.7 ± 1.5 ^{0}C in rainy season from February, 2012 to February, 2013. This result revealed that there was no significant difference ($P > 0.05$) in temperature monitoring between the periods considered (Table 2). Similarly, the same trend was observed in the pH monitoring in the different localities within the periods (Table 2).

Table 2. *Seasonal mean values of physicochemical parameters from the Lagos Lagoon from February, 2011- February, 2013*

Sampling station	Seasons	Surface H_2O temperature (0^C)	pH	salinity(‰)	Dissolved Oxygen(mg/L)	Conductivity (µs cm-1)
Makoko	Dry	28.3 ± 2.1	7.9 ± 0.1	13.4 ± 8.1	4.9 ± 1.0	13.9 ± 10.0
2011-2012	Rainy	26.3 ± 1.5	8.4 ± 0.1	22.9 ± 8.3	3.5 ± 1.1	22.7 ± 18.7
2012-2013	Dry	29.3 ± 0.8	8.2 ± 0.1	27.0 ± 2.7	5.3 ± 0.6	43.5 ± 3.7
	Rainy	29.7 ± 1.5	8.3 ± 0.2	22.2 ± 11.2	7.3 ± 5.0	51.6 ± 1.1
Liverpool	Dry	27.7 ± 1.5	8.0 ± 0.1	20.2 ± 4.5	5.3 ± 0.6	17.0 ± 15.6
2011-2012	Rainy	26.0 ± 1.0	8.3 ± 0.2	19.3 ± 4.7	3.7 ± 2.2	7.3 ± 5.5
2012-2013	Dry	28.3± 1.6	8.3 ± 0.1	19.4 ± 6.3	5.7 ± 1.2	30.9 ± 9.4
	Rainy	26.0 ± 2.0	8.3 ± 0.2	22.2 ± 11.2	7.9 ± 1.9	26.5 ± 8.2
Takwabay	Dry	28.7 ± 0.6	8.0 ± 0.1	20.2 ± 4.5	5.2 ± 1.1	9.2 ± 2.2
2011-2012	Rainy	25.2 ± 0.8	8.3 ± 0.1	23.0 ± 8.3	3.7 ± 2.2	18.6 ± 15.8
2012-2013	Dry	28.2 ± 1.9	8.3 ± 0.1	30.1 ± 0.3	6.9 ± 1.6	47.2 ± 1.7
	Rainy	26.0 ± 2.0	8.4 ± 0.1	14.1 ± 7.4	7.8 ± 1.9	51.5 ± 0.9

Data represent Mean ± SD of three different sampling periods.

The mean salinity value was observed in 2011 to range from 13.4 ± 8.1 to 20.2 ± 4.5‰ in dry season and 19.3 ± 4.7 to 23.0 ± 8.3‰ in rainy season while in the following year, the mean salinity value ranged between 19.4 ± 6.3 to 30.1 ± 0.3 ‰ in dry season and 14.1 ± 7.4 to 22.2 ± 11.2‰ in rainy season.

The results of this study also show that, of all the parameters, only dissolved oxygen and conductivity were statistically significant ($P< 0.05$) with the dissolved oxygen ranging from 4.9 ± 1.0 to 5.3 ± 0.6 mg/L in dry season and 3.5 ± 1.1 to 3.7 ± 2.2 mg/L in rainy season in 2011 while in the following year, the dissolved oxygen ranged from 5.3 ± 0.6 to 6.9 ± 1.6 mg/L in dry season and rainy season ranged from 7.3 ± 5.0 to 7.9 ± 1.9 mg/L. However, conductivity ranged from 9.2 ± 2.2 to 17.0 ± 15.6µS cm^{-1} in dry season and 7.3 ± 5. 5 to 22.7 ± 18.7µS cm^{-1} in rainy season while between February, 2012 to February 2013 the conductivity recorded 30.9 ± 9.4 to 47.2 ± 1.7µS cm^{-1} in dry season and 26.5 ± 8.2 to 51.6 ± 1.1 µS cm^{-1} in rainy season (Table 2).

Vibrio colonies on TCBS agar appeared either yellow (showing that they are sucrose fermenters) or green or bluish-green (meaning non-sucrose fermenters). A total of 27 bacteria were isolated from the seafood samples. Out of the 27 isolates, only 16 were colony morphology type showed that they were predominantly curved -shaped Gram-negative rods, thus presumptively identified as *Vibrio* species. The *Vibrio* counts were obtained from shrimp, crab, and fish collected from the three landing sites (Liverpool, Makoko and Takwa bay) along the Lagos lagoon from February, 2011- February, 2013. Liverpool showed 5 (5.6%) with 10%, 6.7% and 0 of shrimps, fish and crab respectively, 9 (10.0%) from Makoko with 10% of shrimps, fish and crab respectively and 2 (2.2%) in Takwabay with 3.3%, 3.3% and 0 of shrimps, fish and crab respectively (Table 3).

Table 3. *Distribution and frequency of occurrence of Vibrio species from Lagos lagoon landing sites*

Locality (Lagos lagoon)	No. Tested (%)	Liverpool	Makoko	Takwa bay
Samples		No. positive for *Vibrio* spp. (%)	No. positive for *Vibrio* spp. (%)	No. positive for *Vibrio* spp. (%)
Shrimps	30(33.3)	3(10.0)	3(10.0)	1(3.3)
Fish	30(33.3)	2(6.7)	3(10.0)	1(3.3)
Crab	30(33.3)	0(0.0)	3(10.0)	0(0.0)
Total	90(100.0)	5(5.6)	9(10.0)	2(2.2)

Out of 16 isolates identified phenotypically as *Vibrio* species, only 7 isolates were confirmed by PCR at 663 Amplicon size (bp) chromosomal loci specific for *Vibrio* species.

Table 4. *Identification of Vibrio species after analyzing the sequences of the V16S r RNA*

Isolate Code	Identification
2MKSHa	*V. parahaemolyticus*_ RIMD_ 2210633
3MKSHg	*V. parahaemolyticus* _SW 1_ AY911394
MCb/b	*V. parahaemolyticus*_03_K6_BA00
TBSHy	*V. parahaemolyticus*_MP-2_AY911391
3MKCR3	*V. fluvialis*_CCMM_8664_FR695475
2LVSH_	*V. parahaemolyticus* _E_GS020801_AY456924
2LVSH_	*Shewanella algae*_BRY_X81621

Gene Bank published sequences of *Vibrio* species revealed an alignment of 98.9% sequence similarities of isolate code 2mkSHa to V.16SrRNA sequence to *V. parahaemolyticus* RIMD_2210633. However, 3mkSHg showed 98.9% similarity to *V. parahaemolyticus* SW-1_AY911394; $3mkCR_3$ revealed 99.3% similarity to *V. fluvialis* CCMM_8664_FR695475, 2LvSHc was an identical to *V parahaemolyticus* ECGS020801_AY456924 at 100% similarity. Isolate Mc b/b was also an identical to *V parahaemolyticus* 03_K6_BA00 and TBSHy sequence was identical to *V parahaemolyticus* MP-2_AY911391. Then isolate code 2LvSH sequence revealed a 99.5% similarity to *Shewanella algae* BRY_X81621 which also belongs to the same phylum "Proteobacteria" and class Gammaproteobacteria with the family *Vibrionaceae*. A Dendogram was constructed to group the sequenced isolates.

It contained 6 *Vibrionaceae* related isolates; (5 strains of *V parahaemolyticus* and 1 *V. fluvialis*) and a *Shewanella algae* as shown in Fig.1. The sequences of known species, mainly type strains, were obtained from Gen Bank (http://www.cnbi.nlm.nih.gov/BLAST/).

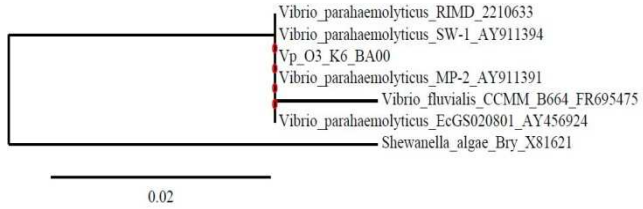

Fig 1. *The Dendogram showing the grouping of the Vibrio species from the seafoods*

*V .parahaemolyticus*_ RIMD_ 2210633 = 2MKSHa, *V .parahaemolyticus* _SW 1_ AY911394 = 3MKSHg, *V .parahaemolyticus*_03_K6_BA00 = MCb/b, *V .parahaemolyticus*_MP-2_AY911391 = TBSHy, *V .fluvialis*_CCMM_8664_FR695475 = 3MKCR3, *V .parahaemolyticus* _E_CGS020801_AY456924 = 2LVSH_C, Shewanella algae_BRY_X81621 = 2LVSH

4. Discussion

Vibrio species can be pathogenic to human and represent a possible health threat as a result of eating raw or undercooked seafood (Eyisi *et al*., 2013). Seeing that pathogenic *Vibrio* species has become a major hurdle in the public health and safety of the human food supply, a rapid and effective detection method would be needed in order to monitor its presence in seafoods (Tantillo *et al* ., 2004; Frans *et al*., 2011). Thus, the coastal waters of Lagos State Nigeria are not expected to be an exception.

In the present study, the physico – chemical parameters such as salinity, pH, surface water temperature, dissolved oxygen and conductivity were monitored for the two seasons in 24 months at the study site. A significant difference ($P< 0.05$) was observed in the dissolved oxygen and conductivity in all the different Lagoon study site while the salinity , pH and water surface temperatures showed no significant difference ($P> 0.05$). The fluctuations in the water conductivity and the dissolved oxygen in the Lagos lagoon might be as a result of water influx from the rain runoffs and the anthropogenic activities in the lagoon. Similar observations were made by Ajao, (1990), Nkwoji *et al*. (2010) in studies on Lagos lagoon, Adejuwon and Adelakun, (2013) in Ogun state and Ideriah *et al*. (2010) portharcourt Nigeria.

Members of the genus *Vibrio* can be isolated from seafoods even in Lagos State Nigeria (Adeleye *et al*., 2010). In this investigation, all short curved rod, gram negative and oxidase positive bacteria were presumptively identified as *Vibrio* species. Generally, the cultural and biochemical properties of the isolates agreed with the Bergey's Manual of Systematic Bacteriology (Holt and Krieg, 1994).

In this study, the samples of seafood phenotypically analysed showed varying levels of *Vibrio* contamination. The total frequency of occurrence of *Vibrio* species was 17.8% in all the samples, in shrimps, crab and fish showed 23.3, 20.0 and 10.0 % respectively. The study also showed that shrimps and crab may be potential sources of *Vibrio* species in the Lagos lagoon. This study is in agreement with Gopal *et al.* (2005) that reported varying rates of *Vibrio* species from multiple shrimp farm environments from the east and west coast of India. While, in Iran, only 2.1% of studied shrimp samples harboured *Vibrio* species as reported by (Hosseini *et al*., 2004). The predominant of *Vibrio* species especially *V. parahaemolyticus* in shrimps may be attributed to water bodies contamination, their mode of feeding, (filter feeders), and high salt tolerance ability of the organism. Nwachukwu (2006) also studied pathogenic characteristics of *Vibrio* species isolated from seafoods in Nigeria. Adebayo-Tayo *et al*. (2011) and Nsofor *et al*. (2014), reported dominance of *V. fluvialis* (47.6%) followed by *V. parahaemolyticus* (30.2%) from seafoods sold in Portharcourt Nigeria.

The incidence of *Vibrio* species in seafood samples has been studied by various research groups. However, in some cases phenotypic identification /biochemical tests were not able to differentiate *V. cholera* from *V. mimicus* and *V. parahaemolyticus* from *V. vulnificus* due to the sharing of serological markers and phenotypic characters of these *Vibrio* species (Sathiyamurthy *et al*., 2013). Therefore PCR- based detection which targets the specific chromosomal loci of different *Vibrio* species is necessary for identification of these *Vibrio* species (Hasan *et al*., 2010; Balckston *et al*., 2007). In this present study, from PCR-based analysis, the conserved housekeeping genes (16S-rRNA) used as a source of specific marker for *Vibrio* species used on the 16 isolates, revealed only 7 isolates as *Vibrio* species. The genomic sequencing revealed 6 isolates as *Vibrio* species. The differences in the molecular identifications could be as a result of the primers used (Ottaviani *et al*., 2005; Mahmud *et al.*, 2006; Di Pinto *et al*., 2008).

This study has undertaken to accurately identify and differentiate the isolated seafood *Vibrio* species and the 16S – rRNA gene loci have drawn a considerable attention as one such means to accomplish this particular goal. The V.16S rRNA gene primer was generated form a highly conserved region of the 16S- rRNA gene and this primer was adopted from (Amin and Salem, 2012).

5. Conclusion

The presence of *Vibrio* species in seafood may cause gastroenteritis and this can lead to a great human health implication. The results show that 16S rRNA sequencing analysis approach becomes even more powerful in the identification of *Vibrio* species and consequently, proves important for differentiation of species within a very complex *Vibrio* genus and for characterization of outbreak strains and isolates found in suspected food samples.

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