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## FOOD SCIENCE & TECHNOLOGY | RESEARCH ARTICLE

# Prevalence and antimicrobial susceptibility of *Vibrio parahaemolyticus* isolated from seafoods in Lagos Lagoon Nigeria

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**Abstract:** In this study, a total of 90 seafood samples; croaker fish (*Pseudotolithus senegalensis*), shrimps (*Penaeus notialis*) and blue crab (*Callinectes sapidus*) collected from landing sites along the Lagos Lagoon in Nigeria were examined for the prevalence of *Vibrio parahaemolyticus* using both biochemical and molecular methods. Biochemical identification of the isolates was confirmed by Polymerase Chain Reaction (PCR). The presence of the virulence-associated *tdh* (thermostable direct haemolysin), *trh*<sub>1</sub> (thermostable-related haemolysin) and *trh*<sub>2</sub> genes in the *V. parahaemolyticus* isolates was also detected by the PCR method. PCR products from the V.16S primers were sequenced. Antibiotics susceptibility of the isolates was also determined. About, eight isolates were presumptively identified as *V. parahaemolyticus*, PCR identified five and none of the isolates were positive for the genes *tdh* or *trh*. The five isolates sequenced were identified as different strains of *V. parahaemolyticus*. *V. parahaemolyticus*\_RIMD\_2210633 = 2MKSHA remained resistant to all antimicrobials tested. However, only *V. parahaemolyticus*\_MP-2\_AY911391 = TBSHy showed strong sensitivity to all the antimicrobials with ampicillin (minimum inhibitory concentration-4 µg/ml). In addition, the other three isolates showed sensitivity for Tetracycline, Ciprofloxacin, Gentamicin and Ceftazidime. Ampicillin resistance in most of the isolates suggests low efficiency of ampicillin in management of *V. parahaemolyticus* infection.

**Subjects:** Agriculture and Food; Environment & Agriculture; Fisheries Science; Food Science & Technology; Microbiology

**Keywords:** seafoods; *V. parahaemolyticus*; antibiotic susceptibility; virulence-associated gene; primers; minimum inhibitory concentration (MIC)

### ABOUT THE AUTHORS

The principal investigator C. Oramadike designed and performed experiments, analysed data and wrote the paper while S.T. Ogunbanwo supervised the project. The research findings included in this publication may be of public and health sector interest.

### PUBLIC INTEREST STATEMENT

The safety of seafood is important to the farmers, marketers and consumers of seafoods to avoid adverse human health implication. Thus, prevalence and antibiotic sensitivity of *V. parahaemolyticus* in seafood samples from Lagos Lagoon Nigeria will be very informative. This article, described the methods of isolation of *Vibrio* species from seafoods, different methods of characterization and effects of antibiotics on *V. parahaemolyticus*. This research work will help in isolation, characterization and in determination of the most effective antibiotics for treatment in case of occurrences.

## 1. Introduction

The latest consciousness on health benefit in seafood consumption has made seafood an important component in the human diet worldwide (Gillet, 2008). The seafoods especially shellfish is a substrate for some zoonotic *Vibrios* of which these micro-organisms also cause food poisoning and diarrhoea in human. Seafoods are prone to bacterial contamination and could cause health risk to human consumers (Lutz, Erken, Noorian, Sun, & Mcdougald, 2013).

*Vibrio* species are natural inhabitants of estuarine, coastal waters and marine sediments throughout the world (Farmer, Janda, & Birkhead, 2003; Heidelberg, Heidelberg, & Colwell, 2002; Miyoshi, 2013). Several cultivation-dependent and independent studies have showed that *Vibrio* species appear at particularly high densities in and/or on marine organisms, e.g. corals (Rosenberg & Ben-Haim, 2002), fish (Huys et al., 2001), molluscs, shrimps and zooplankton (Sawabe et al., 2003; Suantika et al., 2001; Vandenberghe, Thompson, Gomez-Gil, & Swings, 2003). *Vibrio* account for a significant number of foodborne infections from the consumption of foods and water contaminated with human faeces or sewage, raw fish or improperly cooked contaminated fish and shellfish (Oyster, Clams, Mussels, Crabs, Shrimps, periwinkles and prawns) (Eja et al., 2008; Farmer et al., 2003; Lutz et al., 2013).

The continuing challenges of *Vibrio* infections in the early twenty-first century are integrally linked to the nature of the global changes around us and antibiotic resistance. Global changes are invariably intertwined and cannot be separated from epidemiology of diseases and emerging diseases associated with many specific factors such as ecological modification, microbial adaptation, human demographic changes and behaviour (Lee, 2001; Sharma, Sachdeva, & Viridi, 2003). This results in adverse environmental effects, emergence of antibiotic resistance and persistence of chemical residues in fish tissues (Karunasagar et al., 2005).

Pathogenic non-cholera *Vibrio* species, especially *Vibrio parahaemolyticus*, represent an increasing medical threat in Europe and sub-Saharan Africa (Baker-Austin, Stockley, Rangdale, & Martinez-Urtaza, 2010). They are also defined as emerging cause of different diseases, either by exposure of open wound, aquatic environment or consumption of contaminated seafoods. It can cause mild to moderate gastrointestinal infections, which are usually self-limiting and critical. The pathogenicity factors of *V. parahaemolyticus* are known to be caused by the presence of thermostable direct haemolysin (*tdh*) and thermostable direct haemolysin-related haemolysin (*trh*) genes (Raghunath, Acharya, & Bhanumathi, 2008).

Many outbreaks of foodborne infection, especially in Asia countries, have been frequently reported to be due to the presence of these bacteria. However, an incidence of *V. parahaemolyticus* infection was reported by Utsalo, Mbotto, Gemade, and Nwangwa (1988) in Calabar Nigeria, however, is not as frequent as in Asia, several outbreaks have also been reported in the United States and Europe (McLaughlin et al., 2005; Ottaviani, Santarelli, & Bacchiocchi, 2005).

The present study was conducted to investigate the prevalence and antimicrobial susceptibility of *V. parahaemolyticus* in seafoods from Lagos Lagoon Nigeria, due to limited information available on prevalence of *V. parahaemolyticus* associated with seafoods from Lagos Lagoon Nigeria.

## 2. Materials and methods

### 2.1. Description of the study area

Lagos Lagoon was the study area for this project. The Nigerian coastline is between longitude 02° 53'E to 08°14'E and latitude 06°21'N 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 and 27,780 km with a total area of 41,000 km<sup>2</sup>. 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<sup>2</sup> (Ajao, 1990). These lagoons over the years have gradually more

exposed to land-based anthropogenic activities leading to their use as sinks and their resulting deterioration are of the view that pollution of these waters has continued unabated through unregulated discharges of wastes (Chukwu, 2002; Nwankwo, 2004; Onyema, Otudeko, & Nwankwo, 2003).

## 2.2. Sample collection

A total of 90 seafood samples were investigated for the presence of *V. parahaemolyticus*. These samples (included croaker fish (*Pseudotolithus senegalensis*), shrimps (*Penaeus notialis*) and blue crab (*Callinectes sapidus*) were obtained from landing sites in three villages (Liverpool, Makoko and Takwa Bay) along the Lagos Lagoon in Nigeria. Samples were collected monthly for two years (February, 2011–February, 2013). Samples 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 analysed at most 2 h after collection.

## 2.3. Detection and identification of *V. parahaemolyticus*

The procedure of Elliot, Kaysner, Jackson, and Tamplin (1998) and Adeleye, Eyinnia, Nwanze, Smith, and Omonigbehin (2008) was adopted. Alkaline peptone water (APW) at pH 8.4 and Alkaline Peptone Salt Broth (APSB) i.e. (APW supplemented with 3% NaCl) at pH 8.4 were used for the enrichment of the samples. Twenty-five grams of the samples (crab, croakers fish and shrimps) each were scaled and blended separately with sterile blender, 25 g of sediment was weighed into 225 ml of sterile APW and APSB each (i.e. 1:10 dilution). The APW homogenate was incubated at 37°C for 6 h. And after incubation, 1 ml of the broth culture showing positive growth (turbidity) was transferred to a sterile petri dish, then to a sterile cooled TCBS agar (pour plate method) to obtain discrete colonies for each of the different samples. The petri dishes were incubated overnight for 18 h at 37°C after which only the green colonies were subcultured to obtain pure isolates on dried Tryptone soy agar (TSA) supplemented with 3% NaCl and incubated overnight for further identification of the isolates. Typical colonies of *V. parahaemolyticus* are green, 2–3 mm on TCBS. Typical colonies of presumptive *V. parahaemolyticus* were subcultured onto dried TSA supplemented with 3% NaCl for presumptive identification (oxidase test, Gram staining, morphology and motility). The colonies being oxidase positive, Gram-negative, rod curve shaped and motile were then identified further for the species level using Polymerase Chain Reaction (PCR) and nucleotide sequencing. The colonies suspected as *V. parahaemolyticus* were confirmed using nucleotide sequence.

## 2.4. Molecular identification of isolates (DNA extraction)

The presumptively identified *V. parahaemolyticus* were grown overnight in Tryptone soy broth supplemented with 3% NaCl, the cultured broth was centrifuged at 1,000 rpm for 60 s and the supernatant was 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 (Trois, Coulon, de Combret, Martins, & Oxarango, 2010).

## 2.5. Oligonucleotide primers

Oligonucleotide primers used to amplify all *Vibrio* species, specific genes and pathogenicity genes are all listed in Tables 1 and 2. All primers were synthesized by Biomers Germany.

## 2.6. PCR conditions

The PCR was run in a 20 µl volume of the reaction mixture consisting of 4 µl Master Mix (PCR buffer, deoxynucleoside Triphosphate (dNTP), MgCl<sub>2</sub> Taq DNA polymerase), an additional 0.1 µl of AmpiTaq Gold (Applied Biosystems, Foster City, CA), primer concentration of 0.05 µM for each of the primers listed in Table 1, 5 µl of the DNA template and nuclease-free water making up the total volume. The thermal cycling profile was 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.

For the detection of *V. parahaemolyticus* virulence genes, *tdh*, *trh*<sub>1</sub> and *trh*<sub>2</sub> primers are listed in Table 2. The reactions were carried out each in a total volume of 20 µl, consisting of 4 µl Master Mix (PCR buffer, deoxynucleoside Triphosphate (dNTP), MgCl<sub>2</sub> Taq DNA polymerase), primer

**Table 1. List of primers used in PCR identification of *V. parahaemolyticus***

Target species	Primer	Sequence (5–3)	Concentration (µM)	Amplicon size (bp)
All <i>Vibrio</i> species	V.16S-700F	CGG TGA AAT GCG TAG AGA T	0.05	663
	V.16S-325R	TTA CTA GCG ATT CCG AGT TC		
<i>V. parahaemolyticus</i>	VpFlaE-79F	GCA GCT GAT CAA AAC GTT GAG T	1	897
	VpFlaE-34R	ATT ATC GAT CGT GCC ACT CAC		

Source: Amin and Salem (2012).

**Table 2. List of primers used for virulence genes determination**

Target species	Primer	Sequence
<i>V. parahaemolyticus</i>	V p <i>tdh</i> _fw	CCA TCT GTC CCT TTT TCC TG
	V p <i>tdh</i> _re	TAC GGT TTG TCC AAA AGT CAG A
	V p <i>trh</i> <sub>1</sub> _fw	AAA AGC GTT CAC GGT CAA TC
	V p <i>trh</i> <sub>1</sub> _re	CCA GAA AGA GCA GCC ATT GT
	V p <i>trh</i> <sub>2</sub> _fw	CCC CAG TTA AGG CAA TTG TG
	V p <i>trh</i> <sub>2</sub> _re	AGG CGC TTA ACC ACT TTG AA

Source: Modified annealing temperature (Messelhauser et al., 2010).

concentration of 0.05 µM for each of the primers, 5 µl of the DNA template and nuclease-free water making up the total volume as described with annealing temperature modification by Messelhäuser et al. (2010). The thermal cycling profile was as follows: a 15-min soak at 93°C followed by 35 cycles of 92°C for 40 s, 52°C for 1 min and 72°C for 1.5 min and a final soak at 72°C for 7 min.

### 2.7. Visualization of PCR products in agarose gels and fragment analysis

The DNA amplicon was observed by running the PCR reactions on 1.5% (w/v) agarose gel (Amersham Pharmacia Biotech) in 1 × 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 was 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 and 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 (Espiñeira, Atanassova, Vieites, & Santaclara, 2010).

### 2.8. 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, earlier described by Trois 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 analysed using Chromas Version 1.43 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/>).

### 2.9. Determination of antibiotic susceptibility

Antibiotic susceptibility of the isolates to Tetracycline (TC), Ciprofloxacin (CL), Gentamicin (GM), Ceftazidime (TZ) and ampicillin (AM) was determined using *E*-test strips (AB bio merieux, solna, Sweden) according to the manufacturer's instructions and these antimicrobials were chosen in accordance with the CLSI M45-A guidelines and they represent antimicrobials of clinical importance

to non-*Vibrio cholera*. Breakpoints for non-*cholera Vibrio* species were used in accordance with the Clinical and Laboratory Standards Institute (CLSI) document M45-A guidelines (CLSI, 2010). The minimum inhibitory concentration (MIC) MIC<sub>50</sub> was calculated as described by Hamilton-Miller (1991).

### 3. Results and discussion

Ninety seafood samples were investigated for the presence of *V. parahaemolyticus*. Table 3 summarizes the results of the prevalence study. *V. parahaemolyticus* was detected in eight seafood samples using cultural method, while five samples mainly shrimps were confirmed having *V. parahaemolyticus* using PCR and nucleotide sequencing methods. The prevalence of *Vibrio* species has been studied by various research groups. However, in some instances, phenotypic identification was not able to differentiate *V. parahaemolyticus* from *V. vulnificus* due to the sharing of serological markers and phenotypic characters of these *Vibrio* species (Sathiyamurthy, Baskaran, & Kumar, 2013). Therefore, PCR-based detection which targets the specific chromosomal loci of different *Vibrio* species is necessary for identification of these *Vibrio* species (Blackstone, Nordstrom, & Bowen, 2007; Hassan, Kamruzzaman, Mekalanos, & Faruque, 2010). This result is not in agreement with Melo et al. (2011) that reported detection of 10 isolates of *V. parahaemolyticus* phenotypically and also confirmed the 10 isolates genotypically without difference in the two methods of identification.

Thirty samples each of the seafoods (shrimps, crabs and fish) revealed 4(50%), 2(25%) and 2(25%) of *V. parahaemolyticus*, respectively, presumptively identified using cultural method, while PCR method showed 4(80%), 1(20%) and 0(0%), respectively. Furthermore, of all the five isolates of *V. parahaemolyticus* were obtained, none was positive for *tdh* or *trh*. This result is in agreement with Hassan, Zwartkruis-Nahuis, and de Boer (2012). The predominant of *V. parahaemolyticus* in shrimps may be attributed to water bodies' contamination and their mode of feeding (filter feeders).

Gene Bank published sequences of *Vibrio* species as shown in Table 4 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; 2LvSHc was identical to *V. parahaemolyticus* ECGS020801\_AY456924 at 100% similarity. Isolate Mc b/b was also identical to *V. parahaemolyticus* O3\_K6\_BA00 and TBSHy sequence was identical to *V. parahaemolyticus* MP-2\_AY911391. It contained five *Vibrionaceae* related isolates; (five strains of *V. parahaemolyticus*). The sequences of known species, mainly type strains, were obtained from Gen Bank (<http://www.ncbi.nlm.nih.gov/BLAST/>). This study has undertaken to accurately identify and differentiate the isolated seafood *Vibrio* species as previously reported by Oramadike and Ogunbanwo (2014).

Antibiotic resistance is the obtained ability of an organism to bear the effect of antibiotics to which it is normally susceptible. Antibiotic-producing bacteria are able to transmit naturally occurring resistance genes to other bacteria through genetic exchange, enabling them to destroy the antibiotics with which they are challenged (Chythanya, Nayak, & Venugopal, 1999). The sudden rise in the surfacing of multi-antibiotics-resistant bacteria in recent years is worrisome (Adeleye et al., 2008; Jun et al., 1999). The

**Table 3. Prevalence of *V. parahaemolyticus* isolated from seafood samples from Lagos Lagoon landing sites by cultural method and PCR method**

Locations	Total number (%)	Number of samples positive using cultural method (%)			Total number (%)	Number of samples positive using PCR method (%)		
		Shrimps	Fish	Crab		Shrimps	Fish	Crab
Liverpool	3(37.5)	3(75.0)	0(0.0)	0(0.0)	1(20.0)	1(25.0)	0(0.0)	0(0.0)
Makoko	5(62.5)	1(25.0)	2(100.0)	2(100.0)	3(60.0)	2(50.0)	0(0.0)	1(100.0)
Takwa Bay	0(0.0)	0(0.0)	0(0.0)	0(0.0)	1(20.0)	1(25.0)	0(0.0)	0(0.0)
Total n = 90	8(100.0)	4(50.0)	2(25.0)	2(25.0)	5(100)	4(80.0)	0(0.0)	1(20.0)

Notes: Results expressed as the number of positive sample; the numbers in bracket indicate the percentage.

**Table 4. Identification of *V. parahaemolyticus* after analysing the nucleotide sequences of the V16S rRNA**

<i>Vibrio</i> isolate	Identification	Gene bank accession number
2MKSHa	<i>V. parahaemolyticus</i>	RIMD_2210633
3mkSHg	<i>V. parahaemolyticus</i>	SW-1_AY911394
2LvSHc	<i>V. parahaemolyticus</i>	ECGS020801_AY456924
Mc b/b	<i>V. parahaemolyticus</i>	03_K6_BA00
TBSHy	<i>V. parahaemolyticus</i>	MP-2_AY911391

susceptibility patterns of different strains of *V. parahaemolyticus* tried against 12 different antibiotics were summarized in Table 5. Only one of the isolates *V. parahaemolyticus*\_MP-2\_AY911391 = TBSHy was strongly susceptible to all the antibiotics tested, ampicillin, Tetracycline, Ciprofloxacin, Gentamicin and Ceftazidime and revealed the following sensitive breakpoints MIC-4 µg/ml, MIC-0.023 µg/ml, MIC-0.125 µg/ml, MIC-0.25 µg/ml and MIC-6 µg/ml, respectively. *V. parahaemolyticus*\_03\_K6\_BA00 = MCB/b revealed moderate sensitivity breakpoint to ampicillin (MIC-16 µg/ml) and strong sensitivity to Tetracycline, Ciprofloxacin, Ceftazidime and Gentamicin with MIC-0.125 µg/ml, MIC-0.125 µg/ml, MIC-0.50 µg/ml and MIC-6 µg/ml, respectively. However, *V. parahaemolyticus*\_RIMD\_2210633 = 2MKSHa strongly resisted the entire five antimicrobials, ampicillin, Tetracycline, Ciprofloxacin, Gentamicin and Ceftazidime and revealed the following breakpoints MIC-64 µg/ml, MIC-256 µg/ml, MIC-16 µg/ml, MIC-32 µg/ml and MIC-48 µg/ml, respectively. Furthermore, *V. parahaemolyticus*\_SW 1\_AY911394 = 3MKSHg and *V. parahaemolyticus*\_E\_GS020801\_AY456924 = 2LVSHc revealed strong sensitivity to Tetracycline, Ciprofloxacin, Ceftazidime and Gentamicin, respectively, showing (MIC-0.75 µg/ml and MIC-3 µg/ml), (MIC-0.38 µg/ml and MIC-0.50 µg/ml), (MIC-0.75 µg/ml and MIC-2 µg/ml) and (MIC-6 µg/ml and MIC-8 µg/ml) with strong resistance breakpoints to ampicillin (MIC-32 µg/ml and MIC-48 µg/ml).

*Vibrio* species is widely distributed in the coastal waters of many regions of the world (Eyisi, Nwodo, & Iroegbu, 2013). Some of these species are pathogenic to human and represent a possible health threat as a result of raw or undercooked seafood. Since 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 with knowledge of its drug sensitivity (Frans et al., 2011; Tantillo, Fontanarosa, di Pinto, & Musti, 2004). Thus, the coastal waters of Lagos State Nigeria are not expected to be an exception.

*V. parahaemolyticus* can be isolated from seafoods in Lagos Lagoon, Lagos State Nigeria (Adeleye, Daniel, & Enyinnia, 2010; Eyisi et al., 2013). In this investigation, all short curved rod, Gram-negative, green, 2–3 mm on TCBS, motile and oxidase-positive bacteria were presumptively identified as *V. parahaemolyticus*. Generally, the cultural and biochemical properties of the isolates agreed with the Bergey’s Manual of Systematic Bacteriology (Holt, Krieg, Sneath, Staley, & Williams, 1994).

**Table 5. Antibiotic susceptibility patterns of *V. parahaemolyticus***

Test groups	Antimicrobial agent	Antimicrobial agent concentration range (µg/ml)	MIC breakpoint (µg/ml)			Number of sensitive strains	Number of intermediate strains	Number of resistant strains	MIC <sub>50</sub> mean values	Range of MIC values (µg/ml)
			S	I	R					
Penicillin	Ampicillin	0.016–256	≤8	16	≥32	1	1	3	32	4–64
Tetracyclines	Tetracycline	0.016–256	≤4	8	≥16	4	0	1	0.75	0.023–256
Quinolones	Ciprofloxacin	0.016–32	≤1	2	≥4	4	0	1	0.38	0.125–16
Aminoglycosides	Gentamicin	0.016–256	≤4	8	≥16	4	0	1	0.75	0.25–32
Cephems	Ceftazidime	0.016–256	≤8	16	≥32	4	0	1	6	6–48

Notes: Breakpoints as recommended by the CLSI M45-A (2010). S, I and R stand for susceptible, intermediate and resistant, respectively. MIC<sub>50</sub> value means where growth of 50% of the isolates is inhibited (Hamilton-Miller, 1991).

The prevalence of *V. parahaemolyticus* in seafoods studied had the highest prevalence rate in the shrimp (50%). Gopal, Otta, and Kumar (2005) reported dominance of *V. alginolyticus* followed by *V. parahaemolyticus* from east and west coast of India multiple shrimp farms environment screened for abundance of *Vibrio* species. Hassan et al. (2012) reported 38 positive samples for *V. parahaemolyticus* out of 200 in the Netherlands, India (55%) (Chakraborty, Surendran, & Joseph, 2008) and Italy (24.3%) (Ottaviani et al., 2005). Nwachukwu (2006) also studied pathogenic characteristics of *Vibrio* species isolated from seafoods in Nigeria. Adebayo-Tayo et al. (2011) and Nsofor, Kemajou, and Nsofor (2014) reported prevalence rate of (30.2%) for *V. parahaemolyticus* from seafoods sold in Portharcourt Nigeria. Adeleye et al. (2010) also reported prevalence of *V. parahaemolyticus* in Lagos State Nigeria. The percentage of *V. parahaemolyticus* in this study was higher in most of these studies cited in this work. The differences in the percentages of *V. parahaemolyticus* could be due to seasonal effects, difference in seafoods examined, different analytical methods used and possibly the different hygienic practices applied during the handling of seafood products.

In this present study, from PCR-based analysis, the conserved housekeeping genes (16S-rRNA) used as a source of specific marker for *V. parahaemolyticus* used on the eight isolates revealed only five isolates as *V. parahaemolyticus*. None of them were found *tdh* and *trh* positive. DePaola, Nordstrom, Bowers, Wells, and Cook (2003) and Deepanjali, Kumar, and Karunasagar (2005) reported a frequency range of 0–12% of *tdh*-positive *V. parahaemolyticus* in both the environmental and seafood samples. In India, Gopal et al. (2005) reported 2 of 47 isolates that were *tdh* positive and 1 of 47 isolates *trh* positive. In a study reported from China, 2 of 28 *V. parahaemolyticus* isolated from fresh shrimp were *tdh* positive, and none was *trh* positive (Yang et al., 2008). Raghunath et al. (2008) also reported that 25% of *V. parahaemolyticus* isolated from shrimp (*Penaeus monodon*) were *trh* positive, whereas no *tdh*-positive sample was found. In Italy, Di Pinto, Ciccacese, and De Corato (2008) also reported that 33.3% of *V. parahaemolyticus* isolated from seafood were *tdh* positive, and no *trh*-positive sample was reported. The differences in the prevalence of total and virulent *V. parahaemolyticus* isolates from the different samples reported in different studies may be a result of different sampling techniques, seasonal effects and molecular identifications especially in the primers used (Di Pinto et al., 2008; Hayat Mahmud, Kassu, & Mohammad, 2006; Ottaviani et al., 2005).

This study was undertaken to accurately identify and differentiate the isolated seafood *V. parahaemolyticus* 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 from a highly conserved region of the 16S-rRNA gene and this primer was adopted and modified from Amin and Salem (2012). 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.

The growing problems with antimicrobial drug resistance are beginning to wear down our antibiotic abilities to fight antibiotic resistance, and thus limiting therapeutic options to present-day clinicians (Igbinsosa, 2010). The epidemiological observation of antimicrobial resistance is crucial for treatment of infections and in preventing the spread of antimicrobial-resistant micro-organisms. This study revealed susceptibility of *V. parahaemolyticus* to the majority of antimicrobials tested and high ampicillin resistance. Interestingly, ampicillin resistance in *V. parahaemolyticus* is not a pristine occurrence. A study that was carried out in the Jakarta, Indonesia, reported over 90% of 160 *V. parahaemolyticus* are resistant to ampicillin and exhibited Lactamase activity (Joseph, DeBell, & Brown, 1978). Zanetti et al. (2001) reported 80% of ampicillin resistance in 8 *V. parahaemolyticus*. Maluping et al. (2005) also reported 12 out of 14 *V. parahaemolyticus* are resistant to ampicillin in the Philippines and Thailand. Vaseeharan, Ramasamy, Murugan, and Chen (2005) also reported 100% ampicillin resistance from *V. parahaemolyticus* isolated from *P. monodon* hatcheries and ponds. Akinbowale, Peng, and Barton (2006) also reported ampicillin resistance from Austria. This finding was also in agreement with a number of literatures from all around the world Malaysia and Nigeria (Adeleye et al., 2008; Marlina, Cheah, Suhaimi, & Zunita, 2007; Orlando, 2003).

#### 4. Conclusion

In conclusion, the presence of *V. parahaemolyticus* in shrimps and crab determined in this study suggests that shrimps and crab may be potential sources of *V. parahaemolyticus* in the Lagos State, Nigeria. The ampicillin-resistant patterns in this study suggest that treatment of *V. parahaemolyticus* infection with ampicillin needs to be reconsidered. Hence, the data obtained in this study are expected to give valuable information on the microbiological safety of seafoods from Lagos Lagoon, Lagos State Nigeria.

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#### Competing interests

The authors declare no competing interest.

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