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Full Length Research Paper

Vibrio species are gram negative, faculative anaerobic motile asporogenous rod shaped bacteria of singlepolar flagellum,nigeria

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Most *Vibrio* infections are associated with the consumption of raw or undercooked sea foods or exposure of wounds to warm seawater. In this study, 63 samples of a variety of sea foods viz: shrimps, periwinkle, prawn, crayfish collected from two major household markets in Port-Harcourt were cultivated using standard bacteriological method on Thiosulphate Citrate Bile Sucrose (TCBS) agar. A total of 63 *Vibrios* belonging to seven different species were isolated with *Vibrio fluvialis* recorded as the highest percentage frequency occurrence and the most predominant species, 30 (47.6%), followed by *Vibrio paraheamolyticus* with 19 (30.2%), *Vibrio vulnificus* with 4 (6.34%), *Vibrio metschnikovi* with 4 (6.34%), *Photobacterium* spp. with 3 (4.76%), *Vibrio cholerae* with 2 (3.17%) and the least was *Vibrio mimicus* with 1 (1.60%). Statistically, there was a significant different (P< 0.05) in prawn sold in Mile 1 market as compared to prawn sold in Mile 3 markets. These isolates were subjected to their susceptibility patterns using agar diffusion method, which recorded susceptible to most antibiotics used. The presence of these pathogenic strains of *Vibrios* in commonly consumed sea foods is of public concern.

Key word: Vibrios, sea foods, Nigeria.

INTRODUCTION

Vibrio species are Gram negative, facultative anaerobic motile asporogenous rod or curved rod -shaped bacteria with a single polar flagellum. The genus contains at least twelve species pathogenic to human, eight of which can cause or are associated with food-borne illness (Dickinson et al., 2013). The majority of the food-borne illness is caused by *Vibrio paraheamolyticus, Vibrio vulnificus, Vibrio fluvialis and Vibrio cholerae. V. vulnificus* is responsible for 95% of sea food related deaths while immune suppressed individuals are most susceptible to other *Vibrio* infection **(**Miyoshi , 2013). Marine *Vibrios* are ubiquitous in the marine environment; therefore, it is not surprising that many are pathogenic for various seafood hosts which are harvested for human consumption (Kaysner and DePaola, 2004).

Most countries have guidelines for detecting *V. parahaemolyticus, V. cholera* 0_1 and 0_{139} in seafood, whereas few have guidelines for *V. vulnificus.* Thus, there are routine microbiological analysis of seafood includes testing for *V. paraheamolyticus,* V. *cholera* 0_1 and 0_{139},

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Table 1. Mean *Vibrio* species from sea foods bought in Mile 1 and Mile 3 markets in Port Harcourt represented as log_{10} cfu/ml.

Sample	Mile 1	Mile 3
Periwinkle	67.0±44.2	20.4±14.5
Shrimp	52.0±21.6	21.0±11.1
Prawn	24.4±13.9	49.4±41.6
Crayfish	15.0±9.8	17.7±11.5

but seldom for *V. vulnificus* (Park et al., 2013).

Some species are primarily associated with gastrointestinal illness (*V. cholerae* and *V. parahaemolyticus*) while others can cause non-intestinal illness, such as septicemia (*V. vulnificus*). In tropical and temperate regions, disease-causing species of *Vibrio* occur naturally in marine, coastal and estuarine environments and are most abundant in estuarine. Pathogenic *Vibrios,* in particular *V. cholerae,* can also be recovered from freshwater reaches of estuaries (Lutz et al., 2013). The occurrence of these bacteria does not generally correlate with numbers of fecal coli forms and depurations of shellfish may not reduce their numbers. However, a positive correlation between fecal contamination and levels of *V. cholerae* may be found in areas experiencing cholera outbreaks (Amita et al., 2003).

Cholera epidemics outbreaks have killed millions of people and continue to be the major public health concern worldwide (Islam et al., 2013). Fluid and electrolytes replacements are the major treatment of cholera patients, however, patients with severe disease require antibiotic treatment to reduce the duration of illness and reduce replacement fluid intake. Like most bacteria of clinical and public health significance, *V. cholerae* is continuously becoming resistance to a variety of antimicrobial agents, necessitating use of newer drugs which are more expensive and have more adverse effect. Spread of cholera epidemics worldwide has been associated with the emergence of multiple drug resistance among a large number of *V. cholera* strains (Dunstan et al., 2013).

Sea foods especially shellfish is a substrate for some zoonotic *Vibrios* of which these microorganisms, cause food poisoning and diarrhea in human. Sea foods are prone to bacterial contamination and could cause health risk to human consumers (Lutz et al., 2013). *Vibrio*s are associated with live sea foods as they form part of the indigenous micro flora of the marine environment. *V. parahaemolyticus* is the top causative agent among all the reported seafood poisoning outbreaks in Nigeria (Eyisi et al., 2013). There is large number of public frozen seafood processing services distributed in the country, where a considerable number of people buy their fresh sea food products daily. Serious consequences relating to national productivity and development can arise from lack of hygiene (Eyisi et al., 2013). In this study, we evaluated the incidence and antibiotic susceptibility pattern of *Vibrio* spp. isolated from commonly consumed sea foods in Port-Harcourt, Nigeria.

MATERIALS AND METHODS

Sample collection, cultivation and identification of *Vibrio* spp.

Fresh samples of variety of sea foods including prawn, periwinkles, crayfish and shrimp were collected randomly from different sellers at the two major household markets (Mile 1 and Mile 3) in Port-Harcourt city, Rivers State, Nigeria. The samples were transported to the laboratory immediately in a screw cap bottles containing enrichment medium (alkaline peptone water) with alkalinity ranging from 8.6- 9.0 and incubated at 37°C for 8-10 h. The samples was divided into 2 sets, one set of the samples was directly cultured onto the Thiosulphate Citrate Bile Salt Agar and the other set was carried out with a pour plate technique using a 10 fold serial dilution method with thiosulphate citrate bile salt agar. The inoculated plates were incubated overnight at 37°C, no antibiotics were added to the TCBS plates. *Vibrio* spp. were identified using conventional microbiological tests: Oxidase positive, rapid and darting motility, sugar fermentation (which differentiates the various *Vibrio* spp.) and salt tolerance (Cheesbrough, 2000).

Antibiotics susceptibility testing

The antibiotics susceptibility pattern of the isolates was determined using the disk diffusion method (Cheesbrough, 2000), on Mueller-Hinton agar (Oxoid, England). Inhibition zone diameter values were interpreted using standard recommendations of the Clinical Laboratory Standard Institute (CLSI, 2006). Susceptibility was tested against Ofloxacin (10 µg), Gentamycin (10 µg), Ciprofloxacin (10 µg), Ampicillin (30 µg), Streptomycin (30 µg), Pefloxacin (10 µg), Augmentin (30 µg), Septrin (30 µg), Ceporex (10 µg) (Oxoid, England). *Escherichia coli* ATCC 25922 was included as a reference strain.

Statistical analysis

Analysis of variance (ANOVA) and students T-test were used to compare means, and values were considered significant at $p< 0.05$. Post hoe multiple comparisons for the ANOVA were done using least significant difference (LSD).

RESULTS AND DISCUSSION

In this study, 63 samples of sea foods: 15 crayfishes, 18 prawns, 15 shrimps and 15 periwinkles were analyzed following standard microbiological method on TCBS Agar (Oxoid, England). The mean bacterial counts of the sea foods obtained from the two markets are shown in Table 1. Table 2 shows that there was no significant difference ($P> 0.05$) in the frequency of occurrence of *Vibrio* species in the sea food collected from Mile 1 and Mile 3 markets. Table 3 shows the antibiotic susceptibility pattern of the *Vibrio* isolates from sea foods bought in Mile 1 and Mile 3 market were significantly ($P< 0.05$) more susceptible to Ciprofloxacin, Ofloxacin, Pefloxacin, Gentamycin and

Table 2. Frequency of occurrence of *Vibrio* species isolated from prawns, periwinkle, crayfish and shrimp purchased in Mile 1 and Mile 3 markets.

Isolates	Mile 1					Mile 3			
	TNI	PR	PE	CR	SH	PR	PE	CR	SH
V. cholerae	2	0(0.00)	0(0.00)	0(0.00)	1(50)	1(50)	0(0.00)	0(0.00)	0(0.00)
V. fluvialis	30	5(16.7)	4(13.3)	3(10)	2(6.67)	4(13.3)	5(16.7)	4(13.3)	3(10)
V. paraheamolyticus	19	3(15.9)	1(5.3)	3(10)	2(10.5)	3(15.9)	3(15.9)	1(5.3)	3(15.9)
V. vulnificus	4	1(25)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(50)	1(50)	0(0.00)
V. mimicus	1	0(0.00)	1(50)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
V. metschnikovi	4	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)
Photobacterium spp.	3	1(33.3)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	0(0.00)	1(33.3)	0(0.00)

TNI = Total number of isolates; PR = Prawn; PE = periwinkle; CR = crayfish; SH = shrimp.

Table 3. The antibiotic susceptibility pattern of *Vibrio* species isolated from sea foods bought from Mile 1 and Mile 3 markets.

Isolates	TNI	OFX	PEF	CN	AU	CPX	SXT	S	PN	CEP
V. cholerae	2	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	2(100)	1(50)
V. fluvialis	30	27(91.3)	28(93.3)	27(90)		29(96.4)	30(100)	23(76.7)	30(100)	26(86.7)	26(86.7)
parahemolyticus	19	19(100)	19(100)	17(89.5)	16(84.4)	19(100)	15(78.9)	19(100)	12(63.2)	17(89.5)
V. vulnificus	4	4(100)	4(100)	4(100)	2(50)	4(100)	4(100)	3(75)	2(50)	4(100)
V. mimicus	1	1(100)	1(100)	1(100)	1(100)	1(100)	0(0.00)	1(100)	1(100)	0(0.00)
V. metschnikovi	4	2(50)	2(50)	4(100)	2(50)	4(100)	0(0.00)	4(100)	2(50)	2(50)

TNI: Total number of isolates; OFX: ofloxacin (10 µg); CN: Gentamycin (10 µg); CPX: Ciprofloxacin (10 µg); PN: Ampicillin (30 µg) S: Streptomycin (30 µg); PEF: Pefloxacin (10 µg); AU: Augmentin (30 µg); SXT: Septrin (30 µg); CEP: Ceporex (10 µg).

Streptomycin as compared to Augmentin, Septrin, Ampicillin and Ceporex.

This study indicates high occurrence of *V. fluvialis* and *V. paraheamolyticus* in the sea foods studied. Similar observations were made by Nwachukwu (2006) in studies on the pathogenic characteristics of non 0_1 Vibrio cholera isolated from sea foods in Nigeria and in Malaysia by Elhadi et al. (2004). High occurrence of V. cholerae has also been reported in Bangladesh by Faraque et al. (2003). This high prevalence may be attributed to their high salt tolerance ability. In contrast, Caldini et al. (1997) reported that 150 *Vibrio* species isolated from the Aron River basin in Italy, recorded *V. cholerae* non 0_1 as the most prevalent specie with 82% occurrence. This was attributed to the fact that River Basin is fresh water.

In Tanzania, data collected during cholera epidemics in 1990 and 1991 showed that all *V. cholerae* 0_1 strains were sensitive to Streptomycin, Gentamycin, Ciprofloxacin (Webber et al., 1998). Rajkarnitar (2000) reported that 25 strains of *V. fluvialis* were susceptible to Tetracycline, Streptomycin while 84% were resistance to Ampicillin, the results are in line with this research.

However, it is important to note that *V. fluvialis*, *V. paraheamolyticus, V. vulnificus, V. metschnikovi, V. cholerae, V. mimicus* and *Photobacterium* spp. in periwinkles, prawns, crayfish and shrimp and other sea foods that are rampant in Port-Harcourt might be significantly responsible of gastroenteritis and severe diarrhea, wound infection cases among the population of the city considering the sources and high occurrence among the sea foods that were studied in this research.

Based on this research, the *Vibrio* species isolated showed different susceptibility to the antibiotics used. It was recorded that the *Vibrio* species isolated from the sea food sample showed 100% susceptibility to Streptomycin and Ciprofloxacin, followed by Ofloxacin, Pefloxacin, Gentamycin, Ceporex, Septrin and Augmentin and the least susceptible was Ampicillin. The antimicrobial susceptibility pattern of *Vibrio* species can be predicted easily, due to control in prescription of antimicrobial agents. Therefore it is clearly necessary to put more emphasis on food hygiene; thereby surveillance of potential contaminant bacteria in harvested seafood is crucial for sustenance of public health.

To prevent *Vibrio* infections, proper hygiene cooking, treatment of water supply and avoidance of eating raw food should be encouraged seriously; there should be establishment of a well separated sewage treatment infrastructure. Warning on possible cholera or other *Vibrio* spp. contamination should be reported around contaminated water sources with direction on how to decontaminate the water and sea foods for possible use. Effective sanitations practices should be practiced, if instituted and adhered to in time they are usually sufficient to stop an epidemic. Patients who present

diarrhea symptoms should be referred to the health centre or hospital for immediate diagnosis and treatment.

In conclusion, the *Vibrio* species isolated from sea foods (periwinkles, prawns, crayfish and shrimp) showed high incidence in *V. fluvialis*, followed by *V. paraheamolyticus* while the least incidence was observed in *V. mimicus* among sea foods sold in some market in Port-Harcourt, Nigeria. It is important to note that this research is laying serious emphasis on the need for high level of hygiene and proper cooking of sea foods before eating. It is also important to note the need to establish regular nationwide antibiotic susceptibility surveillance of *Vibrio* species in different parts of the country so as to provide guidance on the best option in different situation.

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