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Prevalence of Potentially Pathogenic *Vibrio* species in Sea-foods obtained from Markets in Lagos

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Abstract

Sea-foods are regarded as reservoirs for potentially pathogenic *Vibrio* species. This study investigated the prevalence of medically important *Vibrio* species from sea-foods obtained from markets in Lagos. A total of 174 samples made up of 58 crabs, 44 shrimps and 35 fishes were collected from major markets in Lagos metropolis. Isolation was done on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar. Suspected colonies from the TCBS agar were subjected to Gram's reaction, oxidase test, motility test, triple sugar iron, lactose fermentation and salt tolerance tests. Toxigenic capabilities of isolated species were determined using the rabbit ileal loop assay. Antibiotics susceptibility pattern of all the isolates was investigated. Out of the 174 samples, 137 (78.74%) were positive for *Vibrio species*, all the crabs were contaminated with *Vibrios*, while 44 (58%) of the shrimps and 35 (87.5%) of the fishes were reservoirs of *Vibrio spp*. *Vibrio parahaemolyticus* was the most predominant specie with 45 (32.85%) of the total isolated species, this was followed by *Vibrio alginolyticus* 28 (20.44%). The result of the rabbit ileal loop assay of the isolated strains showed that all the *V.mimicus* strains possessed toxigenic ability, while 39.29% of *V.alginolyticus* and 31.58% of *Vibrio cholerae* exhibited fluid inducible capabilities. Antibiotics sensitivity pattern revealed total susceptibility of strains to ofloxacin, pefloxacin and ciprofloxacin, while strains showed varying susceptibilities to amoxicillin, augmentine, cotrimoxazole and tetracycline. Proper cooking of sea-foods before consumption could reduce the risk factor of exposure to *Vibrio* species infections.

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Introduction

Vibrio species are a group of gram negative, usually curved rods, motile bacteria in the family *Vibrionaceae*. The commonest of the *Vibrio spp* is the *Vibrio cholerae*, which has been implicated as the causative agent of cholera (Prescott *et al*., 2005). Cholera is primarily a water borne infection (Idika *et al*., 2000) and its rich history spanning over decades from the earliest recorded pandemic to the emergence of serogroup 0139 has been well documented (Dhamodaran *et al*., 1995; Faruque *et al*., 1998). In recent times the incidence of non-01 *Vibrio* infections is gradually receiving attention (Agarwal *et al*., 1994).

These non-cholera *Vibrio* infections have been associated with the consumption of sea-foods and shellfishes (Rippey, 1994). Aside *Vibrio cholerae* 01 and non - 01, these medically significant *Vibrio* species are *Vibrio parahaemolyticus* (Makino *et al*., 2003), *Vibrio vulnificus (Miceli et al*., 1993), *V. alginolyticus*, *V.fluvalis*, *V.furnisii, V. hollisae*, *V.damsela*, *V.carchariae, V.metschnikovii* (Hansen *et al*., 1993) and *V.mimicus* (Ramamurthy *et al*., 1994). *Vibrio parahaemolyticus*, *V.alginolyticus* and *V.vulnificus* are known to cause sea-foods borne infection such as septicemia and wound infections (Elliott *et al*., 1998).

Ingestion of raw or improperly cooked sea-foods has been fingered as the pre-disposing factor of infections. Sea-foods like crabs, lobsters, shrimps and fishes (Vieiria and Iaria, 1993; Elhadi *et al*., 2004) have been implicated as sources of non-cholera *Vibrio* infections. Sea-foods associated food poisoning outbreaks have been reported over the years (Klontz *et al*., 1993; Eberhert-Phillips *et al*., 1996) which affirms the public health relevance of this group of bacteria. No major study has been undertaken to evaluate the prevalence of potentially pathogenic *Vibrio* species in sea-foods from Lagos markets. Therefore the aim of this study is to determine the prevalence of potentially pathogenic *Vibrio* species in sea-foods obtained from major markets in Lagos, using cultural, physiological and toxigenicity methodologies to characterize isolated strains.

Materials and methods

Sample collection

A total of 174 sea-food samples were obtained from five major markets in Lagos metropolis. The samples were collected from February 2014 to May 2014. The collected samples were 58 crabs, 76 shrimps and 40 fishes. Each was collected into a sterile container and immediately transported to the Nigerian Institute of Medical Research (NIMR), Yaba Lagos for analysis.

Isolation and identification

Each sea-food was dissected and was placed in a blender for grinding to achieve homogenization. One gram of each sample was placed in 9 ml distilled water and serial dilution was carried out. Plating was done on Thiosulphate Citrate Bile Salt Sucrose (TCBS) agar (Oxoid, England), was incubated at 37°C for 24 hours. Characteristic yellow and green colonies were considered for further identifications. Suspected colonies were subjected to Gram's reactions, oxidase test, triple sugar iron agar, motility test and lactose fermentation. (Adebayo-Tayo *et al*., 2011).

Salt tolerance test

Isolated colonies were incubated into nutrient broth (Oxoid, England) supplemented with varying percentages of sodium chloride (0% to 10%) at 37°C for 24 hours. After the 24 hours incubation, re-plating was done on nutrient agar (Oxoid, England) to confirm the presence of colonies. (CDC, 2013).

Serology assay for Vibrio cholerae 01

Diagnostic commercially available cholera antisera 01 and serotypes Inaba and Ogawa (CDC, USA) were used to assay for *Vibrio cholerae* through slide agglutination method involving ratio 1:3 of the antisera with normal saline. The titre value of the agglutination reaction as read after 60 seconds. Each test isolate was directly compared with normal saline without antisera as control. (Shimada *et al*., 1994).

Antibiotics susceptibility test

Antimicrobial susceptibility of the isolates was tested using multi-discs diffusion method. The following antibiotics were tested for its efficacy against the isolates: Augumentin (25 µg), Amoxicillin (25 µg), Tetracycline (50 µg), Ofloxacin (5 µg), Cotrimozazole (25 µg), Ciprofloxacin (10µg) and Pefloxacin (5 µg). (Enabulele *et al*., 2006).

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Toxigenicity bioassay

Isolates were cultured overnight on tryptic soy broth, this was followed by centrifugation at 3000 rpm for 30 minutes. The supernatant obtained was used in toxigenicity test using the rabbit ileal loop model. Adult rabbits were starved for 48 hours after which were knocked unconscious with formaldehyde, the abdominal region was surgically opened, and the prepared supernatant from each test isolate was injected into the 2-3 cm tied knots in the ileal region of the small intestine. Each test loop was replicated with normal saline adjacent loop as a control. The abdominal region of the test animal was sealed and the rabbit was allowed to live. After 6-8 hours the test animal was sacrificed and the ileal loop examined for any abnormally swollen loop comparatively with the adjacent control loop. (Al-Hissnawy *et al.*, 2012)

Statistical evaluation

All statistical calculations were done using the Microsoft Excel version 2007. Analysis of variance (ANOVA) and Least Significance Difference (LSD) were employed for comparing mean values of results.

Table 1: Prevalence of some Vibrio spp contamination of sea-foods from markets in Lagos, Nigeria

Source	Number positive for *Vibrio* spp	Total number of samples
Crabs	58 (100)	58
Shrimps	44 (58.0)	76
Fishes	35 (87.5)	40
Total	137 (78.74)	174

*Figures in bracket represent percentages

Results

The prevalence of the *Vibrio* spp in sea-foods obtained from major markets in Lagos South-west Nigeria is presented in Table 1. Out of the 174 sea-food samples collected 137 (78.74%) were contaminated with potentially pathogenic *Vibrio* species. All the 58 crabs sampled were positive for *Vibrio* contamination while 44 (58%) of the 76 shrimps contain *Vibrio* spp and 35 (87.5%) of the 40 fishes showed presence of *Vibrios*. The total plate count in cfu/ml of *Vibrio* species was $8.02 \pm 0.20 \times 10^4$ for crabs, shrimps was $2.49 \pm 0.13 \times 10^4$ and fishes were $4.28 \pm 0.07 \times 10^4$.

The distribution of the potentially pathogenic *Vibrio* species associated with sea-foods obtained from markets in Lagos is shown in Table 2. *Vibrio parahaemolyticus* was most occurring with 45 (32.85%) of the 137 isolated species. This was followed by *V.alginolyticus* with 28 (20.44%) of the total strains. In the salt tolerance tests only *Vibrio alginolyticus* is able to withstand 10 % saline stress, *V.parahaemolyticus* withstood up to 8 % sodium chloride enriched medium, *V.cholerae* saline stress limit could not exceed 3 %. All the 19 *Vibrio cholerae* strains isolated in the study recorded negative to the slide agglutination test with the commercial antisera for serogroup 01.

The result of the enterotoxigenic capability of the strains through rabbit ileal loop assay is shown in Table 3. Except for *Vibrio mimicus* which showed 100% fluid inducible capability, all other species tend to have less strains with fluid producing abilities in the adult rabbit ileum.

Table 2: Distribution of some potentially pathogenic *Vibrio spp* in sea-foods from various markets in Lagos

Source	VP	VV	VM	VC	VA	VMT	Total
Crabs	18	8	2	8	13	9	58^b
Shrimps	14	7	0	8	9	6	44^a
Fishes	13	6	1	3	6	6	35^a
Total	45^b	21^a	3^b	19^a	28^b	21^a	137

*Figures in bracket represent percentages

VP = *Vibrio parahaemolyticus*, VV= *Vibrio vulnificus*, VM = *Vibrio mimicus*, VC = *Vibrio cholera*e, VA = *Vibrio alginolyticus*, VMT = *Vibrio metschnikovii*

(a) shows no 95% significant difference in the means using least significance difference, while, (b) shows there was significant difference when comparing means.

Table 3: The summary of the toxigenicity bioassay of some *Vibrio* species using the rabbit ileal loop model

Vibrio species	Number of strains with fluid inducible capabilities	Number of strains without fluid inducible capabilities
Vibrio parahaemolyticus	15 (33.33)	30 (66.67)
Vibrio vulnificus	7 (25)	14 (75)
Vibrio mimicus	3 (100)	0 (0)
Vibrio cholerae	6 (31.58)	13 (68.42)
Vibrio alginolyticus	11 (39.29)	17 (60.71)
Vibrio metschnikovii	10 (47.62)	11 (52.38)

*Figures in bracket represent percentages

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Table 4: Antibiotics susceptibility patterns of some *Vibrio species* associated with sea-foods

	Number of resistant strains						Number of sensitive strains					
	VP	**VV**	**VM**	**VC**	**VA**	**VMT**	**VP**	**VV**	**VM**	**VC**	**VA**	**VMT**
Amx	35 (77.78)	16 (76.19)	2 (66.7)	13 (68.42)	21 (75)	15 (71.43)	10 (22.22)	5 (23.81)	1 (33.33)	6 (31.58)	7 (25)	6 (28.57)
Aug	30 (66.67)	13 (61.9)	2 (66.7)	11 (57.89)	19 (67.86)	11 (52.38)	15 (33.33)	8 (38.1)	1 (33.33)	8 (42.11)	9 (32.14)	10 (47.62)
Cot	15 (33.33)	12 (38.1)	2 (66.7)	11 (57.89)	11 (39.29)	6 (28.57)	30 (66.67)	13 (61.9)	1 (33.33)	8 (42.11)	17 (60.71)	15 (71.43)
Tet	5 (11.11)	2 (9.52)	0 (0)	3 (15.79)	2 (7.14)	2 (9.52)	40 (88.89)	19 (90.48)	3 (100)	16 (84.21)	26 (92.86)	19 (90.48)
Pef	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	45 (100)	21 (100)	3 (100)	19 (100)	28 (100)	21 (100)
Ofl	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	45 (100)	21 (100)	3 (100)	19 (100)	28 (100)	21 (100)
Cip	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	45 (100)	21 (100)	3 (100)	19 (100)	28 (100)	21 (100)

*Figures in bracket represent percentages

Amx = Amoxicillin, Aug= Augmentin, Cot = Cotrimoxazole, Tet = Tetracycline, Pef = Pefloxacin, Ofl = Ofloxacin, Cip = Ciprofloxacin. VP = *Vibrio parahaemolyticus*, VV= *Vibrio vulnificus*, VM = *Vibrio mimicus*, VC = *Vibrio cholera*e, VA = *Vibrio alginolyticus*, VMT = *Vibrio metschnikovii*

Table 4 shows the antibiotics sensitivity pattern of the species. All the isolated strains were sensitive to ofloxacin, pefloxacin and ciprofloxacin. While, varying degree of susceptibility was observed in amoxicillin, augmentin (amoxicillin-clavulanate), cotrimoxazole and tetracycline.

Discussion

The prevalence study of potentially pathogenic *Vibrio* spp. obtained from major markets in Lagos showed that crabs have the highest risk factor of infection because all the examined crabs were contaminated with *Vibrios*. This finding is in agreement with Vieiria *et al.* (2004). The distribution pattern of *Vibrio* species in different sea-foods shown in Table 2 suggested that *V.parahaemolyticus* is the most prevalent in all the sea-foods with 45 (32.85%) of the total isolated species. Statistically, the values obtained for *V. parahaemolyticus* exhibited significance difference at 95% in means values when directly compared to others. This invariably means *V.parahaemolyticus* is one of the predominant species among the *Vibrio* species associated with sea-foods. This finding agreed with Srinivasan & Ramasamy (2009). Another medically significant *Vibrio* specie is *V.vulnificus*, a lactose fermenting *Vibrio* showed 21 (15.33%) of the total isolates. The presence of *V.vulnificus* further confirms the emerging threats this bacterium poses to the public as it has been implicated as another major cause of sea-food borne outbreaks (Hlady *et al.*, 1993; Hau and Ho, 2011). All the *V. cholerae* strains isolated in this study were non-01 serogroup, this result confirms the fact that the non-01 *V.cholerae* strains are more predominant in the environment and our observation is in agreement with past report (Shimada *et al.*, 1994).

The toxigenic capacity of the strains isolated in this study demonstrated that some of the strains isolated from sea-foods from major markets in Lagos are capable of causing symptom of clinical relevance. Although, the basis of the toxigenicity in other Vibrio species is still not fully understood, extensive study on the molecular basis of the pathogenesis of V.cholerae revealed that some of its strains are not toxigenic and that toxigenicity features is acquired through horizontal gene transfer most especially in use of a vector, a bacteriophage specific for bacterium (Waldor and Mekalanos, 1996; Miller, 2003).

Epidemiological surveillance of antimicrobial resistance is pertinent for treatment of infections and in preventing the spread of antimicrobial resistant microorganisms. This study revealed varying degree of antibiotic resistance of *Vibrio* isolates from sea-foods to Amoxicillin, Augmentin, cotrimoxazole and tetracycline. Studies carried out in the Horns of Africa had established the presence of multiple drug resistant *Vibrio* spp to some traditional antibiotics (Coppo *et al.*, 1995), this assertion of increased multiple drug resistance was confirmed in our study.

Elsewhere in Italy, Ottaviani *et al.* (2001) studied the susceptibility patterns of pathogenic halophylic *Vibrio* species isolated from sea foods and observed that while some were sensitive to Trimethoprim, Sulphamethozazole, Cefotamime, Ciprofloxacin, others were resistant to Lincomycin and other antibiotics screened. The high resistance observed in amoxicillin and augmentin in this study is in agreement with observation of Adeleye *et al.* (2008), in which they opined that these traditional antibiotics might have been over exposed to the organisms and its effectiveness over the years has diminished. While Amita *et al.* (2003) demonstrated the horizontal transfer of tetracycline resistant genes among *Vibrio* species which was proffered as the molecular basis of *Vibrio* drugs resistance. On the other hand the high susceptibility of strains to pefloxacin, ofloxacin and ciprofloxacin demonstrated the fact that these are newer generation drugs and the level of adaptation of the strains to these drugs is relatively lower when compared to the traditional antibiotics in which the strains have developed adaptive mechanism of resistance.

Conclusion

This study has demonstrated that potentially pathogenic *Vibrio* species are present in the various sea-foods obtained from markets in Lagos. In addition, these organisms could possess toxin producing ability which could lead to clinical symptoms of the infection. It is however recommended that proper cooking of sea-foods should be encouraged before consumption.

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