Antimicrobial susceptibility patterns and plasmid profile of *vibrio* species from processed and unprocesssed food grade crab

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Abstract

Antimicrobial susceptibility patterns and plasmid profiles of *Vibrio* species isolated from edible crabs (*Callinectes sapidus*) were evaluated. A total of 40 crabs and the obtained water samples collected from four different markets in Lagos State were examined. Morphological and biochemical techniques were used to identify the *Vibrio* spp present in the samples. Susceptibility of bacteria isolates to 8 antibiotics was studied using a disc diffusion method and plasmid profile was determined by agarose gel electrophoresis. The total viable *Vibrio* count in unprocessed samples ranged from 3.30 to 5.80 Log_{10}cfu/g while the processed ones was 1.80 to 2.5 Log_{10}cfu/g. The identified *Vibrio* spp included*, V. cholerae, V. harveyi. V. mimicus, V. parahaemolyticus, V. furnissii* and *V. fluvalis*. Results revealed that *Vibrio* isolates from the samples expressed total (100%) antibiotic resistance to amoxcycilin and augumentin, 83.3% to cotrimoxazole, 72.2% to tetracycline, 66.6% to nitrofurantoin, 61.6% to gentamicin, 33.3% to nalidilixic acid and no resistance to ofloxacin. The isolates had a multiple antibiotic resistance (MAR) index ranging from 0.25–0.88. Plasmid profile of 10 isolates which showed multiple resistance to antibiotics revealed that 7 harboured plasmid with bands ranging from 23130 bp – 20240 bp as molecular weight. Isolation of *Vibrio* spp from seafood of this nature is considered as a potential danger for consumers.

Keywords: Crab, *Vibrio* spp, plasmid, Antibiotic resistance

1. Introduction

Crustaceans are important sources of animal protein for humans worldwide. In 2006, an estimated 144 million tons of fish and crustaceans were obtained from fisheries and aquaculture operations, and the majority of this production (110 million tons) was destined for human consumption (Traore *et al.*, 2012). These animals readily harbor pathogenic microorganisms because of their filter feeding nature and also their microbe laden habitat they inhabit. Shellfish pose a serious threat to public health when contaminated with pathogenic bacteria.

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A part of the natural biota of fish and shellfish is formed by some *Vibrio* species (Raissy *et al.*, 2012). Vibrios are natural inhabitants of both marine and estuarine environments and the consumption of raw or insufficiently cooked seafood may lead to their transmission from environment to humans. *Vibiro* species have been recognized as human enteropathogens and are the causative agents of shellfish-vectored illnesses ranging from gastroenteritis to septicemia and wound infection (Sudha *et al.*, 2012). Transmission of the bacteria may occur through exposure of wounds to warm seawater but primarily involves direct entry into the digestive tract through the consumption of seafood, mainly raw or undercooked crustaceans.

According to Gaber and Samy (2014), three *Vibrio* species: *V. cholerae*, *V. parahaemolyticus* and *V. vulnificus*, are well-documented human pathogens primarily associated with foodborne gastrointestinal illness. *V. mimicus* is a recognized pathogen with similar characteristics to *V. cholerae*, except an ability to ferment sucrose. Other species within the genus, such as *V. alginolyticus*, *V. fluvialis*, *V. furnissii*, *V. metschnikovii* and *V. hollisae* are occasional human pathogens. Antimicrobial resistance is one of the most important public health problems that directly relates to disease management and control (Raissy *et al.*, 2012). Antibiotic resistance in enteric pathogens is becoming a critical area of concern in developing countries threatening the public health. This situation actually brings human to new medical dilemma and the consequences can be severe in a thickly populated country where there is wide availability of antibiotics, without controls on over-the-counter use. Resistant bacteria can make their way directly to humans through consumption of contaminated food or indirectly through the transfer of their resistance genes into fish pathogenic bacteria or human pathogens (Sudha *et al.,* 2012). The presence of such non-clinical resistant bacteria poses a risk to humans and the environment as they may act as resistance reservoirs, contributing to the maintenance and spread of antibiotic resistance genes (Chikwendu *et al.,* 2011).

Plasmids are double stranded extra-chromosomal genetic element which have been identified in many bacteria but are sometimes found in eukaryotic organisms. These mobile genetic elements can replicate autonomously and are stably inherited. The genes encoding microbial resistance are often carried on plasmids that have the ability to replicate and perhaps the potential for self transmission. In fact plasmids carry genes that may be useful periodically to enable the cell to exploit particular environmental stress situations. Thus they harbor genes that confer antibiotic resistance, resistance to a number of toxic heavy metals as well as virulence determinants and genes for nutritional expansion and stability (Gu and Zang, 2005 ; Bennett, 2008).

Most plasmids range in size from those with 2 or 3 genes (2–3 kb) to elements that accommodate 400 or more genes. The largest plasmid carries 1674 genes (Bennett, 2008). Plasmids allow the movement of genetic material, including antimicrobial resistance genes between bacterial species and genera (Akinjogunla and Enabulele, 2010). Plasmids are widely distributed throughout the prokaryotes. The plasmid content of a cell normally comprises less than 5% of the total DNA (Olushola and Benjamin 2009; Bennett, 2008).

Edible crab (*Cancer pagurus*) is one of the most important crustaceans consumed, either as boiled or steamed cooked product. The stomach-filling protein in crab satiates the appetite and is used to build and repair body tissue. Crab is a great source of beneficial long-chain omega-3 fatty acids which have anti-inflammatory properties. Research suggests they help lower blood pressure, protect against heart disease, improve cognitive function, and reduce conditions such as psoriasis and ulcerative colitis. Minerals in crab, such as copper, zinc and selenium, support the immune system. Steamed, baked or boiled, crab is an excellent source of A, C and B vitamins, as well as minerals like copper and zinc. Unlike other seafood, crab contains the mineral chromium that enhances the action of insulin, a hormone that helps move energy from food out of the bloodstream and into our cells (Maulvault *et al.,* 2012).

Up to now, the majority of studies assessing health benefits and risks associated with seafood consumption have been carried out on raw products, despite being generally cooked before consumption (Marhual *et al.,* 2012). Since the habitats of crustaceans include brackish lagoons, shallow coastal and inland waters, considerable risk exists for their contamination with environmental pollutants and infectious agents such as *Salmonella*, *Clostridium botulinum*, *Clostridium perfringens*, and bacteria naturally occurring in aquatic environments, such as members of the genus *Vibrio*, some of which can be pathogenic for humans. Vibrios can survive and multiply in aquatic animals (Traore *et al.,* 2012).

Bacterial resistance to antibiotics constitutes an emerging clinical problem owing to the wide availability of antibiotics and often, their misuse (Okoh, 2012). The emergence of antibiotic resistant pathogenic bacteria in clinical environments has become a serious problem worldwide. However, drug resistant bacteria have also been detected from natural environments, where no direct exposure to antibiotics is known. The presence of such non-clinical resistant bacteria poses risk to humans and the environment as they may act as resistance reservoirs, contributing to the maintenance and spread of antibiotic resistance genes (Chikwendu *et al.,* 2011). While it is clear that antibiotics are pivotal in the selection of bacterial resistance, the spread of resistance genes and of resistant bacteria also contributes to the problem. Resistance to antibiotics is encoded in DNA, the genetic blue print for life. Bacteria

are able to exchange DNA, especially in the form of plasmids and pass resistance very rapidly (Eleonor and Leobert, 2001).

According to the Centre for Disease Control (CDC), approximately 70% of infections that people acquire while hospitalized are now resistant to at least one antibiotic (Stuart, 2002). Resistance to antibiotics is rapidly outpacing the ability to synthesize new drugs. In order to curb the resistance problem, we must encourage the return of the susceptible commensal flora. They are our best allies in combating antibiotic resistance (Stuart, 2002; Wang *et al*., 2006). This research was conducted to ascertain the antimicrobial susceptibility patterns and plasmid profile of *Vibrio* species associated with processed and unprocessed food-grade crabs (*Callinectes sapidus*) commonly consumed in Nigeria.

2. Materials and Methods

2.1 Crab sample collection

A total of 40 crabs (20 live crabs and 20 cooked crabs) were obtained from four different markets in Lagos state, Nigeria. The crabs were obtained from: Ikorodu, Bariga, Makoko – Makoko and Morocco markets. The water samples from these locations were also collected for examination. The five live crabs obtained from Ikorodu were labeled IK1, IK2, IK3, IK4, IK5 and IKW for the water sample. The five live crabs obtained from Bariga were labeled B6, B7, B8, B9, B10 and BW for the water sample. The five live crabs obtained from Makoko were labeled MK11, MK12, MK13, MK14, MK15 and MKW for the water sample. The five live crabs obtained from Morocco Market were labeled MC16, MC17, MC18, MC19, MC20 and MCW for the water sample. Cooked samples from these areas where also collected, labeled accordingly and subjected to analysis. The water and crab samples were transported in sterile containers in ice-box to the microbiology laboratory in the Nigerian Institute of Medical Research (NIMR), Yaba, Lagos for examination.

2.2 Bacterial enrichment and isolation

One gram (1g) each of the different parts of the crabs including the gills, gut, mouth parts and flesh were inoculated into 9ml of Alkaline Peptone Water according to the labeled bottles in duplicate. The set-up was incubated at 37°C. After 5 h colonies were sub-cultured onto freshly prepared Thiosulphate citrate bile salt sucrose (TCBS) agar and allowed to grow for 18–24 h at 37°C. The plates with yellow growth indicated presence of sucrose fermenting *Vibrio* species and green growth the presence of non-sucrose fermenting *Vibrio* species. Single growth colonies of the sucrose fermenters and non-sucrose fermenters were picked separately using a sterile wire loop and sub-cultured onto fresh TCBS plates and allowed to grow for 18–24 h at 37°C for proper distinctive growth. Single colonies were sub-cultured onto Tryptose soy

agar (which contains no inhibitory factor) for the purpose of biochemical tests and allowed to grow for 18–24 h at 37°C.

2.2.1 Enumeration of *Vibrio* species

Vibrio were isolated and enumerated following the method employed by Gaber and Samy (2014) but with some modifications. A weighed 12.5 g of the sample was homogenised with 112.5 ml Alkaline Peptone Water (APW) with 3% NaCl in a sterile polythene stomacher bag (Masticator, IUL instruments, Spain) for 1 min and enriched by incubation at 37°C for 18–24 h. Following incubation aliquots (0.1ml) of diluted enrichment broth was pour plated using Thiosulphate citrate bile salt sucrose (TCBS) agar. The plates were incubated at 37°C for 18–24 h. The total viabl cell count was the expressed as Log10cfu/g for crab samples. For water, 1 ml was added to 9 ml of APW incubated and pour plated as for crab samples on TCBS. However the baacteria count was expressed as Log10cfu/ml. Cheesbrough, 2000; Collins and Lyne 2004).

2.2.2 Percentage occurrence

This was expressed as the ratio of number of times a bacteria species was isolated to total number of samples examined multiplied by 100.

$$n \div N \times 100$$

Where n is number of occurrence, N is total no of sample examined

2.3 Identification of *Vibrio* species

A battery of biochemical tests and API20E system (BioMerieux, France) were used to identify *Vibrio* organisms to species level.

2.3.1 Oxidase test

For the oxidase test bacterial colonies were transferred with a sterile glass rod to filter paper moistened with oxidase reagent. Rapid appearance of a dark purple color within few seconds was considered a positive reaction (Cheesbrough, 2000).

2.3.2 Sodium ion requirement test: 0% and 6% NaCl test

Cells grown in the presence of 0 and 6% (Wt/ Vol) NaCl in 1% tryptone broth were used to determine the requirement for Na^{+}. The medium was inoculated and incubated at 37°C for 18–24 h in a shaking water bath (SBS40/2, Stuart Equipment, UK) at 30 rpm. Positive results were determined by examining the turbidity (Cheesbrough, 2000).

2.3.3 Voges-Proskauer test

Voges-Proskauer (VP) test assay was perfomed by using a culture grown in MR – VP medium and incubated at 37°C for 48 h. A yellow brown colour was indicative of a negative result while a cherry red colour showed a positive result (Cheesbrough, 2000).

2.3.4 Lysine decarboxylase test

The method used for Lysine decarboxylase (LDC) test was based on Bergey's manual. A 24 h - old culture was inoculated into LDC broth and incubated at 37°C for 24–48 h. Positive reactions were indicated by a dark purple color throughout the medium, whereas negative reactions indicated by a yellow color throughout the medium (Choopun *et al*., 2002).

2.3.5 Test for ONPG (O-nitrophenyl-beta-d-galactosidase)

For the ONPG test, a 24 h – old culture was inoculated into tubes containing physiological saline solution. The ONPG disks were added and the tubes were incubated at 37°C for 24 h. Positive test was indicated by a development of yellow colour (Cheesbrough, 2000).

2.3.6 String test

A sterile wire loop was used to place a drop of freshly prepared 0.5% Sodium deoxycholate solution on a sterile glass slide. An overnight bacterial growth was placed on the slide by the use of a sterile wire loop and mixed with the solution. After mixing, a positive reaction was indicated by the appearance of a viscous mass that could be drawn in a string from the slide with a bacteriological loop. A negative result was recorded when cells remained evenly dispersed in the deoxycholate solution and no string was formed. String test is a confirmatory test of *Vibrio* spp because the reagent used has the ability to lyse the cell wall of *Vibrio* thereby releasing the cell constituents into the solution and causing it to stretch (Keast and Riley, 1997).

2.3.7 Serogroup typing

This was done using *Vibrio cholerae* antisera Polyvalent 01 (Inaba, Ogawa). A drop of the reagent was placed on a sterile slide, growth colony was picked from the plate and emulsified with the drop of the reagent. When a smooth emulsification was obtained, the emulsification was allowed to stand and observed for any form of agglutination. The presence of agglutination was a confirmation that the organism is 01 positive and negative in the absence of agglutination (Saswat *et al*., 2007).

2.3.8 Serotyping

Polyvalent antiserum reagents of both Ogawa and Inaba were dropped on separate sterile glass slides and emulsified with growth that was positive for serogroup typing. In this test, 0.85% saline solution was used as control. The reagents was mixed by tilting the slide back and forth for 60 sec and viewed under indirect light against a dark background. The occurrence of agglutination was regarded as positive. The test without clumping was regarded as negative. Specimens that showed agglutination with Inaba antiserum were

reported as *Vibrio cholerae* O1 serovar Inaba. The same went for the Ogawa strain (Saswat *et al*., 2007; Shimada, 1993).

2.4 Antibiotic susceptibility test

Antibiotic susceptibility of the *Vibrio* isolates was studied using a disc diffusion method on Mueller-Hinton agar (Oxoid) according to the instruction of Clinical Laboratory Standards Institute (CLSI, 2007). Discs (Oxoid) contained the following antibiotics: AMX – amoxcycilin (25 mg), COT - cotrimoxazole (20 mg), NIT – nitrofurantoin (30 mg), GEN – getamicin (10 mg), NAL – nalidixic acid (30 mg), OFL – ofloxacin (30 mg), AUG – augumentin (30 mg), TET - tetracycline (30 mg). The results were recorded as resistant or susceptible by measurement of the inhibition zone diameter according to the standard of CLSI (2007).

2.4.1 Multiple antibiotic resistance (MAR) determination

The multiple antibiotic resistance index of *Vibrio* isolates was calculated as the ratio between number of antibiotics for which an isolate is resistant and the total number of antibiotics to which the isolate was exposed (Akinjogunla and Enabulele, 2010).

2.5 Plasmid isolation profiling and electrophoresis

Small-scale plasmid preparations were obtained by a slight modification of the method described previously by Trevors (1984). Pure isolates were inoculated on Mueller Hinton broth and incubated overnight at 37°C. The grown cells were harvested and suspended in 200 µl of solution A (100 mM glucose-50 mM Tris hydrochloride (pH 8)-10 mM EDTA). 400 µl of freshly prepared 1% sodium dodecyl sulfate in 0.2 N NaOH was added and the samples were mixed by inverting tubes. 300 µl of a 30% potassium acetate solution (pH 4.8) was added and the samples were mixed by vortex for 30 sec. After incubating on ice for 5 min, the debris was removed by a 5-min centrifugation in a centrifuge (Model 5415R; Eppendorf) at 1000rpm. The supernatant was removed and extracted once with a phenol-chloroform mixture (1:1) and precipitated with an equal volume of isopropanol. The plasmid DNA was then dissolved in 50 µl of Tris EDTA (TE) buffer.

Electrophoresis of the DNA was carried out on a 0.8% agarose gel in a 1X concentration of Tris-Borate-EDTA (TBE) buffer. The agarose gel was prepared by boiling 0.8 g of agarose powder in 100 mls of 1X TBE buffer. After boiling, the solution was allowed to cool and 10µl of ethidium bromide was added to the cooled agarose solution. This was poured into a casting tray with a comb placed across its rim to form wells. The gel was allowed to set for 30 min and the comb was removed. 20 µl of the plasmid DNA samples were then loaded into the wells after mixing with 2 µl of bromophenol blue. A DNA molecular weight marker was also loaded into one of the wells. The gel was then electrophoresed in a horizontal tank at a constant voltage of 60°C for 90 min. After electrophoresis, plasmid DNA bands were viewed by

fluorescence under a short wave ultraviolet light transilluminator and the photograph were taken using a photo documentation system. The molecular weights of the plasmid were extrapolated and calculated using the standard molecular weight marker preloaded in the wells.

3. Results and Discussion

In Table 1 is shown the phenotypic characteristics of *Vibrio* isolates recovered from the crab and water samples. A total of 24 *Vibrio* isolates which underwent morphological and biochemical tests where characterized as 7 *Vibrio* species which included *V. cholerae, V. harveyi, V. mimicus, V. parahaemolyticus, V. furnissii, V. fluvialis* and *V. vulnificus.* The occurrence of *Vibrio* species is shown in Table 2. While all unprocessed crab samples showed the presence of foodborne pathogens only 4 processed samples contained *Vibrio* organisms. All water samples exhibited the presence of *Vibrio* species (Table 3). The total viable *Vibrio* count in the unprocessed samples ranged from 3.30 to 5.80 Log_{10}cfu/g while that of the processed was 1.80 to 2.5 Log_{10}cfu/g (Table 2). For water samples, while only sample IKW had *Vibrio cholerae* plus other *Vibrio* species isolated in this study, the total viable count ranged from 1.64 to 1.88 Log_{10}cfu/ml with the highest being water from sample IKW and lowest water from MCW**.** The percentage occurrence of the different *Vibrio* species is depicted in Table 4. Of the seven species, *V. harveyi* and *V. fluvialis* where most prevalent with a percentage occurrence of 13.63%. This was closely followed by *V. mimicus* (9.09%). *Vibrio parahaemolyticus* showed modest isolation of 6.82%. The least encountered organism was *V. cholerae* which was isolated from water at a rate of 2.27%.

Table 1 Phenotypic characteristics of isolates

Isolate code	Growth on TCBS AGAR	Oxidase test	String test	Growth in 0% NaCl	Growth in 6% NaCl	LDC	D-Cellobiose	VP test	ONPG	Serogroup polyvalent	Serotype Ogawa	Probable organism
IK1	NSF	+	+	-	+	+	-	-	-	-	-	*Vibrio parahaemolyticus*
IK2	NSF	+	+	-	+	+	-	-	-	-	-	*Vibrio parahaemolyticus*
IK3	SF	+	+	-	+	+	-	-	-	-	-	*Vibrio harveyi*
IK4	NSF	+	-	+	-	+	-	-	+	-	-	*V. mimicus*
IK5	NSF	+	-	-	+	+	-	-	-	-	-	*V. parahaemolyticus*
IKW	SF	+	+	-	-	+	-	-	-	+	+	*V. cholerae*
B6	SF	+	+	-	+	-	+	-	+	-	-	*V. fluvialis*
B7	SF	+	+	-	+	-	+	-	+	-	-	*V. fluvialis*
B8	SF	+	+	-	+	-	+	-	+	-	-	*V. fluvialis*
B9	SF	+	+	-	+	+	-	-	-	-	-	*V. harveyi*
B10	SF	+	+	-	+	-	-	-	+	-	-	*V. furnissii*
BW	SF	+	+	+	-	+	-	-	+	-	-	*V. mimicus*
MK11	SF	+	+	-	+	+	-	-	-	-	-	*V. harveyi*
MK12	SF	+	+	-	+	+	-	-	-	-	-	*V. harveyi*
MK13	SF	+	-	-	+	-	-	-	-	-	-	*V. fluvialis*
MK14	SF	+	+	-	+	-	-	-	-	-	-	*V. fluvialis*
MK15	SF	+	+	-	+	+	-	-		-	-	*V. harveyi*
MKW	NSF	+	+	+	-	+	-	-	+	-	-	*V. mimicus*
MCW	SF	+	+	-	+	+	-	-	-	-	-	*V. harveyi*
MC16	NSF	+	+	-	+	+	-	-	-	-	-	*V. vulnificus*
MC17	NSF	+	+	-	+	+	-	-	-	-	-	*V . vulnificus*
MC18	SF	+	+	-	+	-	-	-	+	-	-	*V. furnissii*
MC19	SF	+	+	+	+	-	+	-	+	-	-	*V. fluvialis*
MC20	N SF	+	+	+	-	+	-	-	+	-	-	*V. mimicus*

Note: IK1–IK5 – crabs obtained from Ikorodu, B6-B10 – crabs obtained from Bariga market, MK11-MK15 – crabs obtained from Makoko market, MC16-MC20 – crabs from Morocco Market, SF – Sucrose fermenters, NSF – Non-sucrose fermenters, + = Positive, - = Negative. IKW = water from Ikorodu, BW = water from Bariga, MKW= water from Makoko, MCW = water from morocco.

Table 2 Occurrence of *Vibrio* species in crab samples

Sample code	Unprocessed crab	Bacterial count (log_{10}cfu/g)	Processed crab	Bacterial count (log_{10}cfu/g)
IK1	+	5.65	+	2.50
IK2	+	5.13	-	0.00
IK3	+	4.71	-	0.00
IK4	+	4.32	-	0.00
IK5	+	4.95	-	0.00
B6	+	3.41	-	0.00
B7	+	3.70	-	0.00
B8	+	4.60	-	0.00
B9	+	5.80	+	2.00
B10	+	5.20	-	0.00
MK11	+	4.60	-	0.00
MK12	+	3.80	-	0.00
MK13	+	4.10	-	0.00
MK14	+	5.00	-	0.00
MK15	+	3.90	-	0.00
MC16	+	5.58	+	1.85
MC17	+	5.53	+	1.80
MC18	+	4.80	-	0.00
MC19	+	4.20	-	0.00
MC20	+	3.30	-	0.00

KEY: IK1-IK5 – crabs obtained from Ikorodu, B6-B10 – crabs obtained from Bariga market, MK11-MK15 – crabs obtained from Makoko market, MC16-MC20 – crabs from Morocco Market, + = present, - = not detected

Table 3 Occurrence of Vibrio species in crab water samples

Water sample	Occurrence	Bacterial count (Log_{10}cfu/ml
IKW	++	1.88
BW	+	1.72
MKW	+	1.83
MCW	+	1.64

Note: ++ =*Vibrio cholerae* plus other vibrio spp, + = other *Vibrio* species

IKW = Water from Ikorodu, BW = Water from Bariga

MKW= Water from Makoko, MCW = Water from Morocco

Table 4 Percentage occurrence of *Vibrio* species in crab and water samples

Microbial isolates	Occurrence	Percentage (%) occurrence
Vibrio parahaemolyticus	3	6.82
Vibrio harveyi	6	13.6
Vibrio mimicus	4	9.09
Vibrio cholerae	1	2.27
Vibrio fluvialis	6	13.63
Vibrio furnissii	2	4.55
Vibrio vulnificus	2	4.55

Note: N = 44

Various factors pose a condition of risk to seafood safety today. This range from the environment where it is caught up to contamination by the processor, the retailers and then the consumer before eating (Vieira, 1989). Research has indicated that crab may contain many bacteria of public health significance such as *Vibrio* spp.

The presence of such pathogens in crab beyond certain limits renders it unfit and dangerous for human consumption. Butt *et al*. (2004) noted that consumption of raw or undercooked seafood is the factor most commonly associated with infection. The number and types of *Vibrio* organisms isolated from crab in this study calls for public health concern. Seven different species of *Vibrio* isolated were : *V. cholerae, V. harveyi, V. mimicus, V. parahaemolyticus, V. furnissii, V. fluvialis* and *V. vulnificus.* Raw or unprocessed crab was found to contain *Vibrio* in the range of 3.30 to 5.80 Logcfu/g. Sudha *et al.* (2012) reported that *Vibrio* species from harvested shrimps ranged between 1.30×10^{3} cfu/g and 1.44×10^{5} cfu/g which is in concord to the finding of the present study. While the raw samples had high levels of *Vibrio*, the cooked or processed samples had significantly low levels or none detected in

some cases. This low levels or none detection of *Vibrio* species in the processed samples can be attributed to the effect of heat which must have killed the organisms. The major sources of these *Vibrio* species in the sample may be sediment, water and unhygienic conditions of different stages. In fact indiscriminate disposal of organic matter such as sewage, houshold wastes, faeces and animal remains can engender pollution of water bodies. Surface run-offs, flood, storm and rain water percolation may have contributed immensely to the contamination of the water sources of the crab in this study.

The various strains of *Vibrio* isolated in this study pose public health threat. Of the seven species isolated, three: *V. cholerae, V. parahaemolyticus,* and *V. vulnificus.* are known to cause human disease. The clinical signs may range from gastroenteritis to wound infection, otitis media and septicemia depending on the species (Ulusarac and Carter, 2004). *V. vulnificus* can cause a spectrum of illness which vary from gastroenteritis to primary sepsis and necrotizing fascitis. The infection largely result from handling or consumption of contaminated seafood or exposure of open wounds or broken skin to contaminated salt or brackish water (Jones and Oliver, 2009). Previous report indicates the isolation of *V. parahaemolyticus* from seafood. This organism when ingested with raw or undercooked seafood causes watery diarrhoea often with abdominal cramps, nausea, vomitting, fever and chills within 24h. *Vibrio cholerae* causes cholera disease characterized by painless diarrhoea and vomitting. Dobson and Carper (1993) reported that fewer than 10% of cases develop the severe form of the disease, which if not treated can be rapidly life threatening due to dehydration and shock. In severe cases the diarrhoea can be very watery and profuse leading to loss of fluid.

Table 5 shows the antibiotic susceptibility pattern of eighteen (18) isolates screened for susceptibility to eight (8) antibiotics. The results showed that 18 (100%) were resistant to amoxcycilin, 15 (83.3%) were resistant to co-trimoxazole, 12 (66.6%) were resistant to nitrofurantoin, 11 (61.1%) were resistant to gentamicin, 6 (33.3%) were resistant to nalidilixic acid, none (0%) was resistant to ofloxacin, 18 (100%) was resistant to augumentin and13 (72.2%) were resistant to tetracycline. Table 6 shows the multiple antibiotic resistance (MAR) index of the isolates which ranged from 0.25–0.88 with the highest being *V. parahaemolyticus* P1 and the lowest *V. furnissii* S1. Seven (7) of these isolates harbored plasmids with molecular weight ranging from 20240 bp–23130 bp.

Figure 1 shows the photograph of the plasmid DNA of multidrug resistant *Vibrio* species with their corresponding molecular weight sizes viewed by fluorescence under a short wave ultraviolet light transilluminator. Figure 2 shows the percentage resistance of the isolates to the antibiotics used for the susceptibility test.

Table 5 Sensitivity pattern of *Vibrio* species to antibiotics

Microbial isolate	AMX	COT	NIT	GEN	NAL	OFL	AUG	TET
Vibrio fluvialis F1	R	R	R	R	S	S	R	R
Vibrio furnissii S1	R	S	S	S	S	S	R	S
Vibrio harveyi H1	R	R	R	S	R	S	R	R
Vibrio harveyi H2	R	R	R	R	S	S	R	R
Vibrio fluvialis F2	R	R	R	R	S	S	R	R
Vibrio harveyi H3	R	R	R	R	S	S	R	R
Vibrio harveyi H4	R	R	S	S	S	S	R	R
Vibrio mimicus M1	R	R	R	R	S	S	R	S
Vibrio vulnificus	R	R	S	R	S	S	R	R
Vibrio furnissii S2	R	S	S	S	S	S	R	R
Vibrio harveyi H5	R	R	R	S	S	S	R	S
Vibrio fluvialis F3	R	R	R	R	R	S	R	R
Vibrio fluvialis F4	R	S	R	R	S	S	R	R
Vibrio fluvialis F5	R	R	R	R	R	S	R	R
Vibrio harveyi H6	R	R	S	S	S	S	R	R
V. parahaemolyticus P1	R	R	R	R	R	S	R	R
V. parahaemolyticus P2	R	R	S	S	R	S	R	S
Vibrio cholerae	R	R	R	R	R	S	R	R

Note: 13 mm and above are regarded as sensitive (S), 12 mm and below are regarded as resistant, (R), , R – Resistant, S – Sensitive, AMX – Amoxcycilin, COT - Cotrimoxazole, NIT – Nitrofurantoin, GEN – Getamicin, NAL – Nalidixic acid, OFL – Ofloxacin, AUG – Augumentin, TET -tetracycline.

Table 6 Multiple antibiotic resistance (mar) and plasmid profile

Microbial isolate	MAR	Number of plasmids	Plasmid size (bp)
Vibrio fluvialis F1	0.75	1	20240
Vibrio fumissi S1	0.25	0	-
Vibrio harveyi H1	0.75	0	-
Vibrio harveyi H2	0.75	1	23130
Vibrio fluvialis F2	0.75	0	-
Vibrio harveyi H3	0.75	1	23130
Vibrio harveyi H4	0.50	1	20240
Vibrio mimicus M1	0.63	1	20240
Vibrio vulnificus	0.63	0	-
Vibrio fumissi S2	0.34	0	-
Vibrio harveyi H5	0.50	0	-
Vibrio fluvialis F3	0.88	1	20240
Vibrio fluvialis F4	0.75	0	-
Vibrio fluvialis F5	0.55	1	20240
Vibrio harveyi H6	0.50	0	-
V. parahaemolyticus P1	0.88	0	-
V. parahaemolyticus P2	0.50	0	-
Vibrio cholerae	0.88	0	-

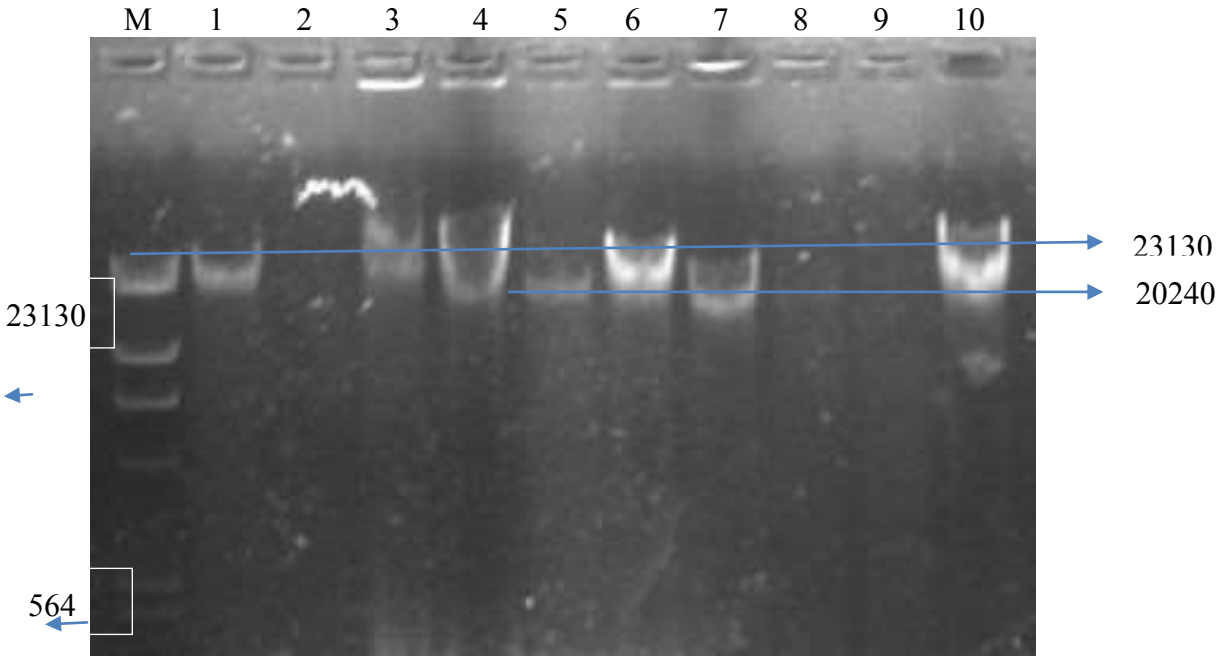

Figure 1 Plasmid profiles of multidrug resistant *Vibrio* species with their corresponding molecular weight sizes. Lane 1- *Vibrio harveyi* H2, lane 2- *Vibrio furnissii* S1, lane 3- *Vibrio harveyi* H3, lane 4- *Vibrio harveyi* H4, lane 5- *Vibrio parahaemolyticus* P1, lane 6- *Vibrio fluvialis* F1 , Lane 7- *Vibrio fluvialis* F5, lane 8- *Vibrio vulniificus*, lane 9- *Vibrio cholerae*, lane 10- *Vibrio mimicus* M1, lane M- Hind III DNA molecular weight marker.

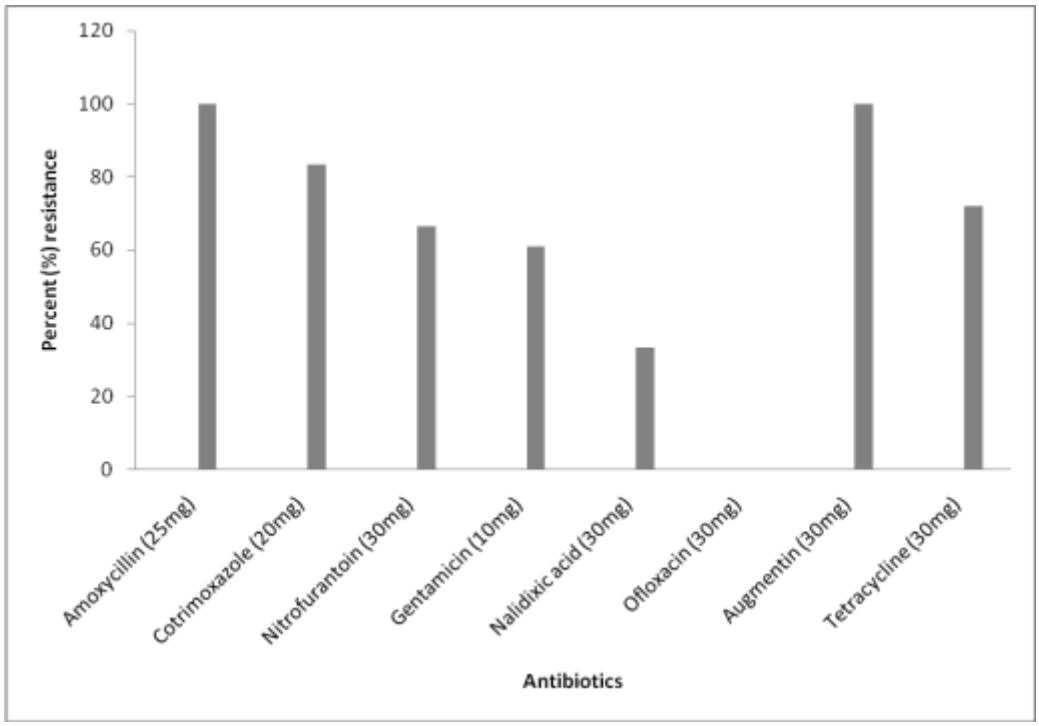

Figure 2 Percent distribution of antibiotic resistance of *Vibrio* species from crab

The results of antibiotic resistance in this study indicated that majority of the *Vibrio* spp showed antibiotic resistance to one or more antibiotics. Similar results were reported from studies on *Vibrio* spp from clinical samples, shrimp and ponds (Eleonor and Leobert, 2001). Highest incidence of antibiotic resistance in this report was evident against amoxycillin, augumentin and cotrimoxazole. These antibiotics are commonly used to prevent diseases in human beings. Therefore, terrestrial bacteria entering into seawater with antibiotic resistant plasmids might have contributed to the prevalence of the resistance in genes in the marine environment, which is concordant with earlier reports (Chikwendu *et al*., 2011).

In this current study, we observed that *Vibrio* isolates exhibited expanding resistance to nitrofurantoin and cotrimoxazole as also shown by Okoh (2012). Thungapathra *et al*. (2002) indicated that in a total number of 94 isolates of *V. cholera,* 43 strains contained R-plasmids and exhibited resistances to ampicillin, neomycin, tetracycline, gentamicin, streptomycin, sulfonamide, furazolidone and chloramphenicol.

In spite of the fact that in some previous studies streptomycin and tetracycline were considered to be effective against *Vibrio* species (Li *et al*., 1999), it was found that there was resistance to tetracycline antibiotic in the examined *Vibrio* isolates in this study. Another study indicated that 43.0% of *Vibrio* isolates from shrimp are resistant to this antibiotic (Roque *et al*., 2001).

Antibiotic resistance plasmids can harbour genes that confer resistance to most if not all clinically significant antibiotic classes such as macrolides, tetracyclines, cephalosporins, fluoroquinolines, aminoglycosides and β-lactamss. The accumulation of different antibiotic resistance genes on plasmids may be enhanced in the environmental microbes (Bennett, 2008). All the isolates that had multiple resistance to the antibiotics were subjected to plasmid isolation and profiling and the results obtained showed that most of the strains had plasmids which coded for the antibiotic resistance genes and molecular weight that ranged between 23130bp to 20240bp. The profile identifications are useful to determine the serotype-specific reference patterns that are responsible for detecting certain strain with possible variation in plasmid content. Studies of Ji and Ruifu, (2005) showed high frequency of plasmid in *Vibrio* spp of both polluted and pristine environment. High frequency of plasmid in *Vibrio* may be ecologically important for the survival of these bacteria in the environment. It is also reported that there is a correlation between possessions of the plasmid with antibiotic resistance and in some cases; their involvement in resistance to many antibiotics has been proven (Marhual *et al.,* 2012). The results revealed that multi-drug resistant *Vibrio* spp present in seafood, obtain antibiotic resistance via plasmids and they can transfer the resistance via transformation, conjugation and other mobile elements such as integrons. The presence of both resistance of

antibiotics and the large plasmid in *Vibrio* isolates may have significant ecological and public health implications (Gu and Zhang, 2005). Bacterial resistance is usually associated with the presence of plasmids and the ability of plasmids for transconjugation. In general, plasmids which could be transconjugated usually possess a high molecular weight (Li *et al.,* 1999). Extensive use of antibiotics has resulted in antibiotics resistance in bacteria (Zulkifli *et al.,* 2009).

Moreover, *Vibrio* species are capable of transferring the plasmid-encoded resistance into other bacterial genera, which can be transferred to human either directly or indirectly (Raissy *et al.,* 2012). The exceedingly increase and emergence of multidrug resistance pathogens in the developing countries can be attributed to indiscriminate use of antibiotics, complex socio-economic, behavioral antecedents and dissemination of drug-resistant pathogens in human medicine (Okeke *et al*., 1999).

4. Conclusion

Isolation of *Vibrio* spp from crab raises public health concern, therefore, further studies on establishing the role of antibiotics and distribution of antibiotic resistance in seafoods are needed.

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