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Bacteriological load and antibiotic susceptibility patterns of bacterial isolates from aquatic resources harvested from Okwan Obolo estuary, Akwa Ibom State

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Abstract

This study was conducted to determine bacteriological load and antibiotic susceptibility profile of isolates from aquatic resources harvested from Okwan Obolo estuary, Akwa Ibom state, using standard microbiological techniques. The aquatic resources used were the fresh land crab *(Gecarinus quadratus)*, Pallid Ghost crab *(Ocypode pallidulla)* and fishes *(Sadinella maderensis)* samples**.** The bacterial load of each sample was determined using serial dilution and pour plating techniques. Antibiotic sensitivity test was conducted using Kirby-Bauer Agar-disc diffusion method. The results indicated the highest mean of total heterotrophic bacterial counts(THBC), total *Vibrio* count (TVC), total coliform count (TCC) and total fecal coliform count(TFCC) ranges of 1.15 x10^5 CFU/g, 4.9x10^5 CFU/g 6.9 x10^5 CFU/g, 5.25 x10^5 CFU/g for *Gecarinus quadratus 1,* 1.16 x10^5 CFU/g, 4.4 x10^5 CFU/g, 6.5 x10^5 CFU/g, 5.75 x10^5 CFU/g for *Ocypode pallidulla 1.* Mean bacterial load from different anatomical sites of *Sadinella maderensis* ranged from 4.1 x 10^5 CFU/g, 1.6 x 10^5 CFU/g, 3.3 x 10^5 CFU/g and 2.6x 10^5 CFU/g for *Sadinella maderensis*1, 4.4 x 10^5 CFU/g, 2.6x 10^5 CFU/g, 2.8x 10^5 CFU/g, 2.0 x 10^5 CFU/g for *Sadinella maderensis*2 respectively. The isolates recovered were *Vibrio parahaemolyticus, Vibrio vulnificus, Escherichia coli, Enterococcus spp, Staphylococcus aureus, Salmonella spp, Micrococcus spp, Coagulase- negative Staphylococcus (CON Staphylococcus), Bacillus* spp, *Enterobacter spp, Pseudomonas spp and Proteus species***.** However, guts samples had the highest number of bacterial loads and isolates. *Staphylococcus aureus had the highest percentage of occurrence (*14.1%), closely followed by *Escherichia coli and Micrococcus spp (12.5%) respectively*. All the isolates recorded 100% sensitivity to amoxicillin-clavulanic antibiotics. CON *Staphylococcus and Proteus spp* recorded 100% sensitivity to all drugs tested while *Escherichia coli* recorded 100% *to* imipenem, gentamicin, ceftriazole and amoxicillin-clauvalanic antibiotics respectively. Proper cooking of aquatic resources is encouraged, and good water quality should be maintained in the estuary.

Keywords: Bacterial load, crabs, fishes, estuary, pathogens, Estuary, Akwa Ibom State

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INTRODUCTION

Aquatic ecosystems are being threatened by various forms of pollutants which may be microbiological, biological and chemical. These pollutants may find themselves into waters through water runoff, industrial wastes, agricultural wastes, wildlife and birds droppings, domestics waste, discharging of sanitary wastewater from boats, and human activities including untreated or partially treated human sewage (Ibe and Okplenge,2005). These pollutants carry enormous effects on the aquatic environment which ultimately affect the sustainability of aquatic lives resulting in negative impacts such as economic losses and disease. Thus, water pollution and other form of contamination is a serious issue for almost all types of ambient water bodies (U.S.EPA,2012). Some of these microorganisms can possess virulence factors which are molecules or properties, some are found in the gene products produced by microorganisms and these properties enable them to establish themselves on or within a host of a particular species and enhance their potential to cause disease (Webb and Kahler, 2008). They can cause diseases in humans, animals or plants which may range from diarrhea, gastroenteritis, salmonellosis, shigellosis and pneumonia just to mention but a few. People may become infected through direct contact with or ingestion of contaminated water or by eating partially cooked shellfishes harvested from contaminated waters. The risks may be further complicated, since many of these bacteria remain viable in chilled products, most especially seafood. The habit of consuming partially cooked aquatic resources increases bacterial-associated risks (World Health Organisation, 2015; Karunasagar, 2014; Khora, 2014). Consumption of raw or undercooked aquatic resources such as crab and oyster has been linked to waterborne diseases including the outbreak of cholera, gastro-enteritis accompanied by diarrhea, and other related extra-intestinal diseases such as cholecystitis (inflammation of the gall bladder) and peritonitis (inflammation of the peritoneum) caused by pathogenic microorganisms (Faruque and Mekalanos, 2003: Sack *et al.,* 2004).Currently, the emergence of waterborne diseases and antibiotic resistance, particularly the multidrug resistance strains possess a public health concern because certain bacteria are not disrupted by conventional treatment and this leads to prolonged illness and a greater risk of death (Petrosinos *et al*.,2013). Of late, some bacteria isolated from food prepared from aquatic source such as fish, crab, and other shellfishes have been reported to be resistant to certain antibiotic (Akinjogunla *et al*., 2011; Magiorakos *et al*., 2012). Therefore, this work aimed at investigating bacteriological loads and antibiotic susceptibility patterns of bacterial isolates from aquatic resources harvested from Okwan Obolo estuary, Akwa Ibom State.

MATERIALS AND METHODS

Study location

The study area was Okwan Obolo estuary in Eastern Obolo Local Government Area of Akwa Ibom State. The area is mostly riverine and the inhabitantsare involved in fishingand peasant farming**.**

Collection of Samples

The two species of crabs *(Gecarinus quadratus and Ocypode pallidulla**)*** and two sardine fishes (*Sadinella maderensis*) used in this research work were harvested from Okwan Obolo estuary and were collected from landing site of fishers. The samples were collected using sterile containers and stored in an ice-packed cooler and transported to Department of Fisheries and Aquaculture, Faculty of Agriculture, University of Uyo, Uyo, Akwa Ibom State for identification. Later, the samples were taken to Microbiology laboratory, Department of Microbiology, University of Uyo, Uyo for further analysis.

Processing of Samples

The crab parts namely the crab gut and meat were carefully cut using a sterile scalpel and each sample was homogenized separately using a sterile mortar and pestle. Fish parts namely, the gill, flesh and intestine were likewise aseptically cut and homogenized.

Bacteriological analysis

Serial Dilution

Serial dilution method employed in this work was as described by Fawole and Oso, (2001). A ten-fold serial dilution was carried out using standard tube method. Each test tube was well labeled. To each sample, 1g was measured using a weighing

balance and macerated in 9ml of distilled water in a test tube to form aliquot. Serial dilution was then carried out using 1ml which was diluted serially in ten-fold on the original sample of the homogenate from the initial tube 10^{-1} to 10^{-10}, each tube contained 9 ml of distilled water. The test tubes were agitated vigorously to ensure equal distribution of microbial cells from the homogenate for determination of microbial load.

Determination of bacterial loads

The pour plate technique was employed by aseptically pipetting and transferring 1ml of each sample 10^{-5} dilution into sterile petri dishes. Thereafter, Nutrient Agar (Oxoid, USA), Eosin Methylene Blue Agar (Oxoid, USA). Thiosulphate citrate bile-salt Agar (Oxoid, USA) and MacConkey Agar Oxoid, USA) already prepared were poured into each petri dish and swirled so as to mix properly with the sample, and was allowed to solidify. The method of Clarence *et al.,* (2009) was used. All plates wereincubatedat37^0Cfor24 hours. After which the bacterial loads of each plated sample was estimated by counting all the colonies on the plates using colony counter. The number of viable colonies for each plate was multiplied by the reciprocal of the dilution factor. The mean for each sample analyzed was calculated.

Characterization and identification of bacterial isolates

The bacterial isolates were sub-cultured, purified, characterized and identified as described by Holt *et al.,* (1994). The characterization and identification were based on features which include colonial morphology and pigmentation on plates as determined by visual observation while cell shapes were examined microscopically. The following biochemical tests were used to characterize the organisms; Gram stain reaction, catalase test, coagulase test**,** oxidase test, urease test, motility test, methyl-red, Voges-Proskauer and sugar fermentation tests.

Antibiotic susceptibility test

The Kirby-Bauer method was used to screen for the antibiotic susceptible pattern. The method is as described by Cheesbrough, (2000) and CLSI (2006). Single disc antibiotics were bought commercially from the manufacture. The antibiotics used were imipemem (10µg), amoxicillin (30µg), gentamicin (30µg), ceftriaxole (10µg) and amoxicillin-clavulanic acid(10µg). The isolates were inoculated onto Mueller Hinton agar plate. The antibiotic disc was picked with a sterile forceps, placed onto the agar and was pressed to have a direct contact with the agar. The plates were incubated at 37^0C for 24 hours, after which the zones of inhibitions were measured from the plates using a metre ruler.

RESULTS

Table 1 shows the bacterial loads of the fresh land crab *(Gecarinus quadratus)* analyzed. The r results indicated the mean total heterotrophic bacteria count (THBC), total *Vibrio* count (T(TVC), total coliform count (TCC) and total faecal coliform count (TFCC) as $1.15x10^5$ CFU/g, $4.9x10^5$CFU/g $6.9x10^5$ CFU/g,$5.25x10^5$ CFU/g for *Gecarinus quadratus* 1 and$1.04x10^5$ CFU/g, $3.05x10^5$ CFU/g, 5.95 $x10^5$CFU/g, $4.1x10^5$ CFU/g recorded for *Gecarinus quadratus* 2 respectively.

Bacterial loads of the Pallid Ghost crab *(Ocypode pallidulla)* studied is presented in Table 2. The results show mean THBC, TVC, TCC and TFCC recorded for *Ocypode pallidulla*1as $1.16x10^5$ CFU/g, $4.4x10^5$ CFU/g, 6.5 $x10^5$ CFU/g, $5.75x10^5$ CFU/g while $1.08x10^5$ CFU/g, $5.2x10^5$ CFU/g, $6.35x10^5$ CFU/g and $4.8x10^5$ CFU/g recorded for *Ocypode pallidulla 2.* Bacterial load in different anatomical sites of Fish *(Sadinella maderensis)* samples studied is shown on Table 3. The results indicated the mean THBC, TVC, TCC, and TFCC ranged as follows: 4.1 x 10^5 CFU/g, 1.6 x 10^5 CFU/g, 3.3x 10^5 CFU/g and 2.6 x 10^5 CFU/g for *Sadinella maderensis* 1, 4.4x 10^5 CFU/g, 2.6x 10^5 CFU/g, 2.8x 10^5 CFU/g, 2.0 x 10^5 CFU/g for *Sadinella maderensis* 2 respectively.

Table 4 presents the occurrences of bacterial isolates from different anatomical parts of *Gecarinus quadratus* and *Ocypode pallidulla* analysed. A total of 29 isolates comprising *Vibrio parahaemolyticus, Vibrio vulnificus, Escherichia coli, Enterococcus spp, Staphylococcus spp, Salmonella spp, Micrococcus spp, CON Staphylococcus, Bacillus* spp, and *Enterobacter spp were obtained from the crab samples studied.* Higher number of 10 different isolates were gotten from the gut of *Ocypode pallidulla* while the least number of 4 isolates was recorded from *Gecarcinus quadratus* meat.

The occurrence of each bacterial isolates from different anatomical parts of *Sadinella maderens is analysed* is shown on Table 5. A total of 35 isolates were recovered from the fish samples. The isolates comprising *Staphylococcus aureus, spp Micrococcus spp, Enterococcus spp, Pseudomonas spp, Vibrio parahaemolyticus, Vibrio vulnificus, Escherichia coli, Proteus spp,* and *Enterobacter spp.* Highest number of 8 different isolates were isolated from the gills of the two fish samples studied while the least number of

3 isolates was recorded respectively for flesh part of the fish samples studied.

Percentage of occurrences of bacterial isolates from anatomical parts of all samples analyzed is presented on Table 6. Results obtained showed *Staphylococcus aureus* with the highest percentage of occurrence of 14.1%, this was closely followed by *Escherichia coli* and *Micrococcus spp* with12.5% respectively.

Table 1: Bacteriological load of fresh land Crab (*Gecarinus quadratus)*

Sample code	THBC ($\times10^5$CFU/g)	TVC ($\times10^5$CFU/g)	TCC ($\times10^5$CFU/g)	TFCC ($\times10^5$CFU/g)
GQ1	1.16	5.6	7.0	5.7
GQ1b	1.14	4.2	6.8	4.8
MCGQ1	1.15	4.9	6.9	5.25
GQ2	1.08	4.1	6.1	4.7
GQ2b	1.00	2.0	5.8	3.5
MCGQ2	1.04	3.05	5.95	4.1

Key:GQ1**:** *Gecarcinus quadratus* 1 gut, GQ1b: *Gecarcinus quadratus 1 meat***,** GQ2: *Gecarcinus quadrates 2 gut* GQ2b: *Gecarcinus quadrates* 2 meat, MCGQ1= Mean bacterial count for *Gecarcinusquadratus* 1**,** MCGQ2 =Mean bacterial count for*Gecarcinus quadratus* 2**,** THBC =Total Heterotrophic Bacteria Count,TVC: Total Vibrio count, TCC = Total Coliform Count, TFCC = Total Faecal Coliform Count

Table 2: Bacteriological load of fresh Pallid Ghost Crab (*Ocypode pallidulla)* studied

Sample code	THBC ($\times10^5$CFU/g)	TVC ($\times10^5$CFU/g)	TCC ($\times10^5$CFU/g)	TFCC ($\times10^5$CFU/g)
OP1	1.21	3.6	7.4	5.9
OP1b	1.11	5.2	5.6	5.6
MCOP1	1.16	4.4	6.5	5.75
OP2	1.16	6.0	7.2	5.3
OP2b	1.00	4.4	5.5	4.3
MCOP2	1.08	5.2	6.35	4.8

Key: OP1: *Ocypode pallidulla* 1 gut, OP1b::*Ocypode pallidulla* 1 meat**,** OP2::*Ocypode pallidulla 2 gut***,**OP2b::*Ocypode* pallidulla *2* meat**,**MCOP1= Mean bacterial count for *Ocypode* pallidulla 1, MCOP2= Mean bacterial count for *Ocypode pallidulla* 2, THBC =Total Heterotrophic Bacteria Count, TVC: Total *Vibrio* count, TCC = Total Coliform Count, TFCC =Total Faecal Coliform Count

Table 3: Bacteriological load of different anatomical parts of fish *(Sadinella maderensis)* analyzed

Anatomical site /code	THBC ($\times10^5$CFU/g)	TVC ($\times10^5$CFU/g)	TCC ($\times10^5$CFU/g)	TFCC ($\times10^5$CFU/g)
FF1	5.0	2.3	3.8	2.7
FG1	3.2	1.0	2.5	2.1
FI1	4.1	1.5	3.6.	3.0
MCF1	4.1	1.6	3.3	2.6
FF2	3.8	2.1	3.0	2.6
FG2	4.7	3.6	2.8	1.6
FI2	4.6	2.0	2.6	1.8
MCF2	4.4	2.6	2.8	2.0

Key: FS1 = Fish Flesh 1, FG1 = Fish Gills 1, FI1 = Fish Intestine 1, FF2 = Fish Flesh2, FG2 = Fish Gills 2, FI2= Fish Intestine 2, MCF1 =Mean bacterial count for fish 1, MCF2= Mean bacterial count for fish

Table 4: Occurrence of bacterial isolates from different anatomical parts of *Gecarcinus quadratus* and *Ocypode pallidulla* analyzed

Isolates	GQQ	GQM	OPQ	OPM
Vibrio parahaemolyticus	+	-	+	-
Vibrio vulnificus	+	-	+	+
Esherichia coli	+	+	+	+
Enterococcus spp	+	+	+	+
Staphylococcus aureus	+	+	+	+
Salmonella spp	-	-	+	-
Micrococcus spp	-	-	+	+
CON *Staphylococcus*	+	+	+	+
Bacillus spp	+	-	+	+
Enterobacter spp	+	-	+	-
Total no. of isolates	8	4	10	7

Keys: GQQ**:** *Gecarcinus quadratus* guts, GQM: *Gecarcinus quadratus meat***, OPQ:** *Ocypode pallidulla* guts, OPM: *Ocypode pallidulla* meat**.**

Table 5: Occurrence of bacterial isolates from different anatomical parts of *Sadinella maderensis analyzed*

Isolates	FF1	FG1	FI1	FF2	FG2	F12
Staphylococcus aureus	+	+	+	-	+	+
Micrococcus spp.	+	+	+	+	+	+
Pseudomonas spp	+	+	-	+	+	-
Vibrio parahaemolyticus	-	+	+	+	+	+
Vibrio vulnificus	-	+	+	-	+	+
Escherichia coli	-	+	+	-	+	+
Proteus spp	-	+	+	-	+	-
Enterobacter spp	-	+	+	-	+	+
Total no. of isolates	3	8	7	3	8	6

Key: FF1 =Fish flesh 1, FG1=Fish gills 1, FII = Fish intestine1, FF2=Fish Flesh2, Fish gills 2, FI2= Fish intestine 2.

Table 7 shows antimicrobial susceptibility of the isolates obtained in the study. Greater numbers of isolates obtained in the study were highly sensitive to routine antibiotics used in the study. Nearly all the isolates recorded 100% sensitive to one or more antibiotics tested. All the isolates recorded 100% sensitivity to Amoxicillin-Clavulanic antibiotics. CON *Staphylococcus and Proteus spp* recorded 100% sensitivity to all drugs tested while *Escherichia coli* recorded the same 100% to Imipenem, Gentamicin, Ceftriazole and Amoxicillin- Clavulanic antibiotics respectively.

DISCUSSION

The results of this work have revealed the bacterial contamination status of aquatic resources harvested from Okwan Obolo estuary in Eastern Obolo, Akwa Ibom State. The mean THBC, TVC, TCC and TFCC values show the land crab *(Gecarinus quadratus) and* Pallid Ghost crab *(Ocypode pallidulla)* analyzed had no statistical difference in terms of bacterial load carriage ($p< 0.05$) but there was statistical difference in the mean bacterial load carriage by Fish *(Sadinella maderensis)* samples studied. Additionally, crab samples were observed with highest bacterial loads as compared to that of fish. However, the bacteriological loads recorded is not as high as one recorded from crabs from mangrove in which the total bacterial load ranged between $0.7 \pm 0.49 \times 10^6$ and $8.9 \pm 0.13 \times 10^6$ CFU/g of crabs' gut sample and with maximum load of $8.9 \pm 0.13 \times 10^6$ CFU/g in the gut (Sivasubramanian *et al*., 2017).

Table 6: Percentage of occurrence of bacterial isolates from anatomical parts of all samples analyzed

Isolates	GQ (n=4)	OP (n=4)	FI (n=3)	F2 (n=3)	Total No .(n=10)	%
Vibrio parahaemolyticus	1	1	2	3	7	10.9
Vibrio vulnificus	1	2	2	2	7	10.9
Escherichia coli	2	2	2	2	8	12.5
Enterococcus spp	2	2	-	-	4	6.25
Staphylococcus aureus	2	2	3	2	9	14.1
Salmonella spp	-	1	-	-	1	1.5
Micrococcus spp	-	2	3	3	8	12.5
CON *Staphylococcus*	2	2	-	-	4	6.25
Proteus spp	-	-	2	1	3	4.7
Pseudomonas spp	-	-	2	2	4	6.25
Enterobacter spp	1	1	2	2	6	9.4
Bacillus spp	1	2	-	-	3	4.7
Total no of isolates	12	17	18	17	64	100
Mean	3	4.25	6	5.7	6.4	

Key: GQ= Crabs *(Gecarcinus quadratus)* OP= Crabs *(Ocypode pallidulla*), F1= Fish 1 (*Sadinella maderensis)*, F2=Fish 2(*Sadinella maderensis)*

Table 7: Antimicrobial susceptibility of the isolates obtained in the study

Isolates	No of isolates tested	Antibiotics used / No. sensitive (%) IMI	AMX	GEN	CEFT	AMX-CLAV
Vibrio parahaemolyticus	7	6(85.7)	3(42.9)	4(57.1)	7(100)	7(100)
Vibrio vulnificus	7	7(100)	2(28.6)	7(100)	7(100)	7(100)
Escherichia coli	8	4(50)	2(25)	6(75)	5(62.5)	7(87.5)
Enterococcus spp	4	4(100)	4(100)	3(75)	4(100)	4(100)
Staphylococcus aureus	9	7(77.8)	3(33.3)	5(55.6)	7(77.8)	9(100)
Salmonella spp	1	1(100)	0(0.00)	1(100)	1(100)	1(100)
Micrococcus spp	8	8(100)	6(75)	7(87.5)	8(100)	8(100)
CON *Staphylococcus*	4	4(100)	4(100)	4(100)	4(100)	4(100)
Proteus spp	3	3(100)	3(100)	3(100)	3(100)	3(100)
Pseudomonas spp	4	3(75)	3(75)	2(50)	3(75)	4(100)
Enterobacter spp	6	4(66.7)	3(50)	4(66.7)	6(100)	6(100)
Bacillus spp	3	3(100)	1(33.3)	1(33.3)	2(66.7)	2(66.7)

Key: IMI= Imipenem, AMX= Amoxicillin,GEN= Gentamicin, CEFT= Ceftriazole, AMX-CLAV= Amoxicillin- Clavulanic,14 ≥ High sensitivity, 11 – 13 = Intermediate, 0 ≤ 10 = Resistant

In a study carried out in Aby Lagoon, Kambire *et al.* (2016) observed that crabs had a higher bacteriological load compared to fish samples studied. The researcher noted that the difference in terms of bacterial carriage by the aquatic resources could be related to the level of water column frequented by each species in the lagoon. Moreover, the researchers also stressed the fact that the water column contributes greatly to the microbial carriage. Another study revealed that due to the enrichment of nutrition in the gut of mangrove crabs, this condition creates opportunity for other microorganisms to accumulate in the guts, and therefore, the highest total diversity and abundance of the

intestinal bacteria in the crabs were also linked likely to the effect of their feeding methods (Li *et al*., 2007). Fish swim at the interface water-sediment and crabs are in permanent contact with the sediments. Indeed, in the aquatic environment, crabs swim deeper and are in permanent contact with the sediments. Therefore, the high bacterial loads of the crabs and fish samples may be attributed to these activities. High bacterial loads in crab can also be attributed activities of the inhabitants of the area such as indiscriminate dumping of domestic waste and sewage into the water body. This waste carries enormous loads of microbes to the estuarine environment and as well as negative impact on the aquatic resource.

The present work revealed that greater numbers of isolates were obtained from the guts of the two crabs. Sivasubramanian, *et al.,* (2017) likewise reported high bacterial loads obtained from crab guts studied. Several species of mangrove crabs are recognized and known to be as herbivorous Ravichandran *et al*., (2007a), thus, crab receives bacteria in the gut from their aquatic environment through water and food that are populated with bacteria. Being rich in nutrient, the environment of the crab gut confers favourable conditions for the microorganisms. They engage themselves in consuming a good quantity of leaf litter falling from different mangrove species. Thus these crabs perform important role in the process of leaf degradation and making mangrove leaves more rapidly available to other tiny form of life (Ravichandran *et al*., 2007b).

In this study, *Vibrio parahaemolyticus, Vibrio vulnificus, Escherichia coli, Enterococcus spp, Staphylococcus spp, Salmonella spp, Micrococcus spp, CON Staphylococcus, Bacillus* spp, *Enterobacter spp* were bacteria associated with *Gecarinus quadratus* and *Ocypode pallidulla* analysed. Other researchers likewise isolated various bacterial species from crabs studied. For instance, Liu *et al.,* (2003) likewise isolated several bacterial genera such as *Bacteroides*, *Acinetobacter*, *Flavobacterium*, *Chryseobacterium* and *Porphyrobacter* from in crab guts, and reported that some species belonging to these genera can cause disease especially some Bacteroides species are opportunistic pathogens that normally associate with a variety of soft tissue and other infections. Therefore, there are some variations in microbial diversity from aquatic resources based on the level of pollution of the water in which the aquatic lives are found.

The isolation of some human pathogens such as *Enterococcus spp, Staphylococcus spp, Micrococcus spp, Pseudomonas spp, Vibrio parahaemolyticus, Vibrio vulnificus, Escherichia coli, Proteus spp, and Enterobacter spp,* from fish samples studied is not surprising but of public health concern. All crabs and fish samples analyzed were contaminated by Staphylococcus species, and coliforms. This also agrees with the work by Kambire *et al., (*2016). Who further explained that the contamination by bacterial could be derived from the direct action of the population through defecation or relate to contamination during post-catch handling. The same observation is noticed in this present study. Eze *et al*., (2011) reported that *Escherichia coli,* is an indicator of faecal contamination since this organism is a normal flora of the intestinal tract of all warm-blooded animals and it is particularly useful as an indicator of faecal contamination when recovered in smaller numbers or as an indicator of mishandling when appeared in large numbers. Oliveira *et al*., (2011) likewise isolated pathogenic bacteria such as *Salmonella spp., Vibrio cholerae, Shigella spp., V. vulnificus, Vibro parahaemolyticus, Escherichia coli, Micrococcus spp., Staphylococcus aureus, Klebsiella spp, Streptococcus spp*, from freshly caught fishes and crustaceans, which were most likely contaminated by unhygienic harvest waters and also contamination partly as a result of their feeding pattern. According to China *et al,* (2003), the main microbiological indicators in the polluted water consist of bacteria of the genera *Escherichia*, *Salmonella* and *Clostridium*, main pathogens such *Vibrio* and *Clostridium,* and secondary pathogens such as *Campylobacter*, *Staphylococcus* and *Aeromonas.* Olsen *et al.* (2000) affirmed that 25% of food borne diseases in the United States, were associated with fish consumption.

Therefore, the isolation of *S. aureus*, *Escherichia coli* and *Enterobacter* species in this work is of concern. This is because enterotoxin producing strain of *S. aureus* is a leading cause of food intoxication (Williams, 2002; Nema *et al. (*2007). *Escherichia coli* and *Enterobacter* species isolated in the study are enteric organisms. They are normal flora of the intestine in humans and animals and are widely distributed in the environment contaminating food and water. Their presence is generally traceable to fecal

contamination either direct or indirect means. It can produce extremely potent gastrointestinal toxin. *Escherichia coli* and *Enterobacter* species have been implicated in the ability to initiate the pathogenic cascade of sepsis leading to septic shock (Prescott *et al.,* 2002). Notably is the fact that *Enterobacter* species are bacteria commonly known to further cause gastroenteritis, meningitis, and infection in the bladder (Nester *et al.,* 1995). More so, an enterotoxigenic strain of *E. coli* is the most common cause of traveler's diarrhea and some strains of this pathogen can cause a wide variety of infections such as other forms of diarrhea and other associated gastrointestinal problems especially in a community setting (Donnenberg *et al.,* 2005). Thus, aquatic resources that contain *E. coli* and other enteric pathogens if not properly cooked could serve as a vehicle for transmission of infections and of course, in its infective dose can be a continuous source of infections leading to complications and death especially among children and immunosuppressed individuals (Ternhag *et al.,* 2008). The isolation of *Vibrio* species namely *V. vulnificus, Vibro parahaemolyticus* from the aquatic resources is not surprising. These organisms are normally found in marine and estuarine environments throughout the world (McLaughln, 1995; Udoh and Itah, 2012). However, this organism can be agent of diarrhoael diseases in which the major mode of transmission is through contaminated water and food, or person-to-person spread in the overcrowded and unhygienic environment. According to Baffone*et al*., (2000) and Traoré *et al*., (2011), several reports have implicated *Vibrio* species seafood infections in Côte d'Ivoire, and bacteriological studies identified fish, crabs, and shrimp harvested in the Ebrié Lagoon revealed as reservoirs of *Vibrionaceae*. However, the non-isolation of *Vibrio cholerae* which is the major pathogen implicated in diarrhoeal illness in this study shows the good quality of water which should be encouraged by maintaining proper hygiene and sanitation by the inhabitants of the estuary.

The result of the antimicrobial assays revealed that bacteria from the aquatic resources were highly sensitive to the routine antibiotics. It was quite encouraging to record 100% sensitivity of *Proteus* species (4) to all antibiotics used in the study, *Vibrio parahaemolyticus* (7) to Ceftriazole and Amoxicillin- Clavulanic antibiotics. All test isolates recorded 100% sensitivity to Amoxicillin-Clavulanic antibiotics. Pathogenic organisms such as *Enterococcus spp* recorded the 100% sensitivity to multiple antibiotics used. This is a rare record in this era of multiple drugs resistance. This is in contrast to many popular reports of multidrug resistant organisms from many researchers ((Akinjokunla *et al.,* 2011; Udoh *et al.,* 2017). The economic loss and health problems caused by this shellfish contamination are further complicated by development of antibiotic resistant among some of the isolates. Therefore, this antibiotics susceptibility result provides evidence that fish and crabs in the study location do not harbor antibiotic resistance isolates.

CONCLUSION

Enteric bacteria and other human pathogens were found to have associated with the crabs and fish samples studied in Okwan Obolo estuary. Although, *Vibrio cholera*e was not isolated in the estuary, but the presence of *E. coli* and other intestinal pathogens strongly suggest feacal contamination of the water. Therefore, public awareness programs should be carried out in the communities to educate residents and fishers on proper processing and cooking methods of fish and crabs from aquatic resources, hygiene, effect of open defecation into water bodies, and other preventive measures to avoid disease outbreak in the study area. Additionally, continuous screening and surveillance are encouraged so as to prevent the emergence of multiple drugs resistant isolates in the study area. The present study wishes to suggest for further studies, the use of serotyping kits for the identification of all isolates to species as well as the application of metagenomics to identify non- culturable microorganisms from fish and crabs in Okwan Obolo estuary.

Conflict of interest

There is no conflict of interest in this work.

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Author Contribution

UDI designed the study, supervised the laboratory work and wrote the protocol. UIU engaged in field work, did literature searches and wrote part of the first draft of the manuscript. USJ participated in sample collection, laboratory work and data management. UDI critically revised the manuscript for intellectual content. All authors read and approved the final manuscript.

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