Virulence Factors and Antibiogram of Bacteria Isolated from Fresh Aquatic Produce Sold at Open Air Market Centre in Okepedi, Itu, Akwa Ibom State

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ABSTRACT

The study on virulence factors and antibiogram of bacteria from fresh aquatic produce sold in the open market centre of Okepedi fishing settlement, Itu, Akwa Ibom State was investigated using standard microbiological techniques and analytical procedures. The skin, gills and intestine of *Tilapia guinensis* and *Marcusenius senegalensis* as well as that of viscera of shrimps (*Macrobranchium rosenbergii*, *Crangon vulgaris* and *Paneaus monodon*) were all investigated. The study revealed that all the fishes and shrimps obtained from the Okepedi open market centre were contaminated with microorganisms. The heterotrophic bacterial density accumulated by the fishes and crustaceans exceeded 1.2×10^5 cfu/g recommended in fresh fish and shrimps. The high faecal coliform load of the fishes and shrimps has shown that the river surrounding the market is highly contaminated with faecal matter. Bacterial contaminant isolated from fishes and shrimps used in this study included *Micrococcus* sp, *Streptococcus* sp, *Staphylococcus aureus*, *Klebsiella pneumonia*, *Enterobacter aerogene*, *Salmonella* sp, *Vibrio cholera*, *Bacilus subtilis*, *Escherichia coli*, *Enterococus* sp, *Pseudomonas aeruginosa*, *Proteus vulgaris* and *Serratia* sp. Among these

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isolates, multiple antibiotics resistant bacteria were identified. *Bacilus subtilis* was resistant to all the tested antibiotics (100% MAR index) while *Micrococcus* sp had the least index. Plasmid profile study of *Enterobacter aerogene*, *E. coli Bacilus subtilis* and *Staphylococcus aureus* revealed that isolates were plasmid-coded in plasmid of about 300 bp. The presence of this plasmid suggests that the resistant genes could be harboured in the plasmids. The occurrence of plasmid mediated multidrug resistant in bacteria in these aquatic produce lightens the public health concern and therefore, efforts should be make to maintain and enforce adequate sanitation practices in the habitats of these produce. The present study however challenge scientists on the need for development of new antibiotics to combat the infections caused by these resistant strains.

1. INTRODUCTION

The world's aquaculture industry has in recent times grown rapidly to satisfy the demand for seafood, which cannot be met by wild fisheries harvesting as this is currently in a state of decline because of over-fishing, pollution and marine habitat destruction. Aquaculture production is increasing at about 9.25% per year [1] and the FAO had previously estimated that half of the world's seafood demand will be met by aquaculture in 2020 [2]. Aquaculture is an emerging industrial sector which requires continued research with scientific, technical developments, and innovation. The world aquaculture production in 2001 was approximately of 37.9 million tons, which represents about 41% of that obtained from extensive captures for human consumption [1]. Aquaculture has the potential to make a significant contribution to the increasing demand for aquatic food in most world regions. However in order to achieve this goal, the sector will have to face significant challenges, including the production intensification, the disease control and the prevention of the environmental deterioration [3]. Aquaculture is increasing fast in several places of the world. Because the products of aquaculture are important sources of food, it is economically important [4]. In rural Brazil, aquaculture is widely developed in environments in which domestic animals (pigs, ducks, poultry or cattle) are common. In this context, if husbandry is conducted incorrectly, animal feces can pollute the water and jeopardize human and animal health by the presence of undesirable pathogens.

Aquatic produce are water based and can be found in freshwater, brackish or marine (mariculture) environments. Aquatic produce has to do with the process of farming aquatic organisms like fish, crab, shrimp, frog, turtle, insect, reptiles etc which encompasses a wide range of aquatic farming processes differing by species, environment and systems used. Fish and shrimps are among the major aquatic produce encountered in the Niger Delta of Nigeria. Fish are generally defined as aquatic vertebrates that use gills to obtain oxygen from water and have fins with variable number of skeletal elements called fin rays [5]. Fish is known for its high nutritional quality, relatively low fat content, saturated fat, cholesterol and high polyunsaturated fatty acids, protein and minerals such as calcium, phosphorus, sodium, potassium and magnesium [6]. On the other hand, shrimps are marine and freshwater crustaceans that are found on the bottom of the water in nearly every environment around the world. Shrimps are generally tiny in size, with some species of shrimp being so small. There are more than 2,000 different species of shrimp worldwide, all of which are invertebrates. This implies that shrimp do not have a backbone, but a hard exoskeleton (the shell of the shrimp) which is often transparent and colourless making shrimp difficult to see in the water. Shrimp lives on the river beds and ocean floors around the world, filtering sand and particles in the water.

Fish is one of the sources of proteins, vitamins and minerals, and it has essential nutrients required for supplementing both infants and adults diet [7]. In Nigeria, fish is eaten fresh and smoked and form a much cherished delicacy that cut across socio-economic, age, religions and educational barriers [8]. As an important source of protein to the large teaming population of Nigeria, fish provides 40% of the dietary intake of animal protein to the average Nigerian. According to Adekoya and Miller [9], fish and fish products constitute more than 60% of the total protein intake in adults especially in rural areas. According to FAO [1], to maintain the present per capital fish consumption level of 13 kg per year, 2.0 million metric tons of fish food would be required. It has been noted by some researchers

that the only means of meeting up with this annual fish demand for the country would be through a pragmatic option of intensive fish farming [10].

Advantages of fish as a food are its easy digestibility and high nutritional value [11]. These important attributes makes the commodity readily susceptible to microbial attack. The appearance and development of fish disease is the result of the interaction among pathogen, host and environment. Therefore, only multidisciplinary studies involving the characteristics of potential pathogenic microorganisms for fish, aspects of the biology of the fish hosts as well as a better understanding of the environmental factors affecting such cultures, will allow the application of adequate measures to prevent and control the main diseases limiting the production of fishes. Fishes are known to have many non-specific and specific, humoral and cellular mechanisms to resist bacterial diseases. Non-specific humoral factors include growth inhibiting substances, e.g. transferrin and antiproteases; lysins, e.g. lysozyme, C-reactive protein (CRP), bactericidal peptides and most importantly, complement which has lytic, pro-inflammatory, chemotactic and opsonic activities thus making a link with non-specific phagocyte responses. However, the penetration and colonization of bacteria in different fish tissues and organs, such as the gastrointestinal tract, gills, muscle, kidney and bladder, have been reported in polluted aquatic environments. Although *E. coli* is not an indigenous inhabitant of the gut microbiota of fish, this bacterium has been often isolated from the stomach and gut of fish [12].

In fish farming, the widespread use of antibiotics as prophylactic and therapeutic agents to control bacterial diseases has been associated with the emergence of antibiotic resistance in bacterial pathogens and with the alteration of the microbiota of the aquaculture environment [13]. For example shrimps can be a unique source of the antioxidant and anti-inflammatory carotenoid nutrient astaxanthin. This resulted in the ban of antibiotic usage as animal growth promoters in Europe and stringent worldwide regulations on therapeutical antibiotic applications. This scenario has led to an ever-growing interest in the search and development of alternative strategies for disease control, within the frame of good husbandry practices, including adequate hygiene conditions, vaccination programmes and the use of probiotics and immunostimulants [14]. Recently, novel strategies to control bacterial

infections in aquaculture have emerged, such as specific killing of pathogenic bacteria by bacteriophages, growth inhibition of pathogen by short-chain fatty acids and polyhydroxyal-kanoates, and interference with the regulation of virulence genes (quorum sensing disruption). Probiotics are live microbial adjuncts which have a beneficial effect on the host by:

(i) modifying the host-associated or ambient microbial community;
(ii) improving feed use or enhancing its nutritional value;
(iii) enhancing the host response towards disease; and/or
(iv) improving its environment.

The microorganisms intended for use as probiotics in aquaculture should exert antimicrobial activity and be regarded as safe not only for the aquatic hosts but also for their surrounding environments and humans.

The water bodies where fishes are harvested play an important role in the post-harvest contamination of fresh produce [15]. Contaminated water bodies have been implicated in several reported outbreaks linked to consumption of contaminated fishes. Surface water can be fecally contaminated by agricultural runoff, livestock and wildlife fecal material, wastewater discharge and septic leakage. Furthermore microorganisms found in fishing waters can be passed on to soil and fresh fishes in the marketing environment.

The consequences of fish spoilage are far reaching, and more than just the loss of protein. Studies have shown different modes of transmission of pathogenic microorganisms to fresh produce including poor handling and contamination during exposure for sales at market centers. In all the modes the fishes are rendered unsafe for consumption. There have been great economic losses reported due to foodborne illness as the result of consuming contaminated fish. The microbial association with fish compromises safety and the quality for human consumption; particularly when the microorganisms are opportunistic and/or pathogenic in nature [16]. Considering the problems relating to poor handling and insufficient and improper storage facilities on the streets, the risks of contracting food-borne diseases by consumers may be high.

Aquatic produce are perishable food that needs proper handling and preservation if it is to have a long shelf life and retain a desirable quality and nutritional value. Fish and shrimps are the main source of protein in wetland or riverside areas [17]. The central concern of fish processing is to prevent fish from deteriorating. The most obvious method for preserving the quality of fish is to keep them alive until they are ready for cooking and eating. There are few or no reports on the microbial quality of fishes marketed in the Nigerian Fishing Settlements. This study can fill in for lack of information for factors affecting fresh fish produce contamination at the post-harvest level. It is expected that the results obtained from this research will be used to create awareness and educate the public on the bacteriological quality of aquatic produce in open market centres and the risks associated with consumption of contaminated and improperly cooked fresh aquatic produce. This study is designed to investigate the "Virulence factors and antibiogram of bacteria isolates from some aquatic produce sold in open market Centre at Okepedi Fishing Settlement, Itu, Akwa Ibom State.

2. MATERIALS AND METHODS

2.1 Source and Collection of Samples

The fresh aquatic produce investigated in this study include fresh samples of *Tilapia guinensis* (Plate 1) *Marcusenius senegalensis* (Plate 2) and shrimps, mainly *Macrobranchium rosenbergii* (Plate 3) *Crangon vulgaris* (Plate 4) and *Paneaus monodon* (Plate 5) were obtained on display from fish vendors at the Okopedi Open Market Centre. The market centre is situated within Okopedi Fishing Settlement, located in Itu Local Government Area of Akwa Ibom State, Nigeria (Fig. 1). The fishing settlement lies within latitude 4°30' to 4°45'N and longitude 7°30' to 8°E. and within the Cross River Basin in the Niger Delta Region of Nigeria.

Apparently four (4) fresh samples each of fishes and shrimps were obtained directly from the fish sellers. The samples were collected into sterile polythene bags, preserved in ice-packed coolers and immediately transported to the Postgraduate laboratory of the Department of Microbiology, University of Uyo for analysis.

Plate 1. *Tilapia guinensis*

Plate 2. *Marcusenius senegalensis*

Plate 3. *Macrobranchium rosenbergii*

Plate 4. *Crangon vulgaris*
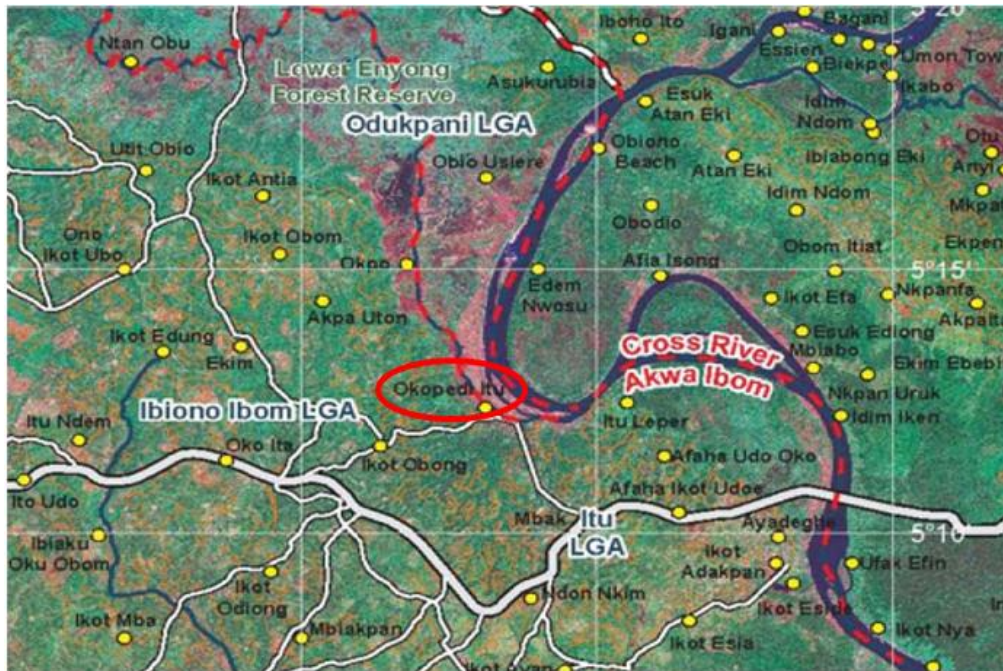
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Plate 5. *Paneaus monodon*

Fig. 1. Map Okopedi fishing settlement

2.2 Preparation of Fish and Shrimp for Analysis

For the analysis of internal organs of the fish and shrimps, the fish was placed on a dissection pan. With the aid of sterile scalpel, a vertical incision along the centre of the fish was made, to divide it into equal halves. Two horizontal incisions were also made across the fish. This incision was somewhat deeper, going through the skin and the muscle below it. Using forceps and scalpel, the skin were removed and pinned to the dissection tray. The muscle flaps were then pulled apart using forceps, in order to expose the internal organs. The fish internal anatomy was examined and the organ of interest (intestine, gills and skin) were derived for analysis. Similarly the shrimp was placed on dissection pan and dissected using sterile scalpel. Then the visceral of the shrimps was removed and crushed for bacteriological analysis.

2.3 Bacteriological Analysis of the Fish and Shrimp Samples

Bacteriological analyses of the fish and shrimps organs were conducted based on standard microbiological methods.

2.3.1 Culture media preparation and sterilization

The media used for the study were: Nutrient Agar (NA), MacConkey Agar (MCA), Eosine

Methylene Blue Agar (EMBA), Thiosulphate – Citrate – Bile salts – Sucrose agar (TCBS) and Salmonella–Shigella agar (SSA) for the enumeration and isolation of heterotrophic bacteria, total coliform, feacal coliform (*Escherichia coli*), vibro and Salmonella and Shigella species respectively. They were aseptically prepared according to the manufacturer's instructions, sterilized by autoclaving at 121°C for 15 minutes.

2.3.2 Enumeration of bacteria loads

Crushed and homogenized organ of the fish samples each from skin, intestine and gills and visceral of the shrimp were used to carry out microbiological analysis by homogenizing 1.0 g of the blended organ in 9ml of sterile water. A ten-fold serial dilution using physiological saline (Oxoid) was prepared (each from skin, intestine and gills of the fish as well as the viscera of the shrimp) and one ml of the desired dilution levels were plated in triplicates on the appropriate media using the pour plate method.

The density of heterotrophic and potential pathogens was determined using standard analytical procedures. *Vibro, Escherichia coli* (fecal coliform), coliform and Salmonella and Shigella loads on the samples was determined using the pour plate technique. All inoculated plates were incubated at 37°C for 24 hours.

After 24 hours, discrete colonies that appeared on the culture plates were enumerated with the aid of a Quebec colony counter and recorded as Colony Forming Units (CFU) per gram of fish sample.

2.3.3 Characterization and identification of the bacterial isolates

The pure bacterial isolates were grouped into recognizable taxonomic units and characterized to their generic level using standard procedures. The pure isolates were examined for colonial morphology, cultural and biochemical characteristics according to the methods of Cowan and Steel [18] and Chessbrough [19].

2.4 Antimicrobial Testing

2.4.1 Preparation of inocula

Preparation of microbial inoculums for antimicrobial sensitivity screening was carried out according to the method described by Adeshina et al. [20]. In this method, colonies of pure bacteria isolates from their stock cultures was transferred into prepared nutrient broth (NB) using sterile inoculating wire-loop and incubated at 37°C for 24 h.

2.4.2 Standardization of inocula

The 24 h broth cultures of the bacterial isolates were appropriately diluted by carefully and asceptically adding freshly prepared nutrient broth. The density (turbidity) of the diluted cultures was standardized by comparing with 0.5% Barium chloride solution (Mcfarland standard) as described by Cheesbrough, [19] to obtain an inoculum size of approximately 1.0×10^6 cfu/ml [20].

2.4.3 Antimicrobial susceptibility testing

The Kirby-Bauer method was used to screen for the antimicrobial susceptibility pattern. The isolates were inoculated on to Muller Hinton Agar plates by streaking. The multiple antibiotic discs were applied to each plate with sterile forceps with lowest concentration towards the center of the agar plate. The plates were incubated at 37°C for 24 hours. The zones of inhibition of the growth were measured by the use of scale ruler in centimeter, which then was transferred to millimetre ruler for the value. Clear zone of inhibition indicated susceptibility of the organisms while absence of such zone was also reported.

2.5 Determination of Multiple Antibiotics Resistance (MAR) Index

The Multiple Antibiotic Resistance (MAR) index was determined for each of the selected bacterial isolate by dividing the number of antibiotics to which the isolate was resistant by the total number of antibiotics tested [21]. Multi-resistant Antibiotic bacteria were sent to the Molecular Biology Laboratory of the National Veterinary Research Institute (NVRI), Jos for plasmid profiling.

2.5.1 Plasmid profiling of the multi-resistant antibiotic strains

Plasmid analysis was carried out on representative isolates selected on the basis of their antibiotic resistance phenotypes. The modified alkaline lysis method for plasmid extraction described by [22] was used for extraction of plasmid.

2.5.2 Extraction of plasmid

Detection and extraction of plasmid was carried out using the alkaline lysis method described by Odeyemi et al. [22]. Organisms were grown in 2.5 ml of nutrient broth and incubated at 35℃ for 18h. After incubation, 0.5 ml of each culture was transferred into 1.5 ml Eppendorf tubes for plasmid extraction and glycerol was added to the remaining culture and stored at 4℃. The Eppendorf tubes were centrifuged at 6,000 rpm for 15 seconds after which the supernatant was carefully removed with the use of fine-tip automatic micropipette and the cell pellet was thoroughly suspended in the 100 µl of lysozyme solution. The pellet-lysozyme mixture was incubated at 0℃ for 30 mins after which 200 ml of the alkaline sodium doedecyl sulphate (SDS) solution was added and gently vortexed. The tubes were maintained for 5 mins at 0℃ and then 150 µl of sodium acetate solution was added. The content of each tube was gently mixed for about six to seven seconds during which clots of DNA were observed in each tube. The tubes were maintained at 0℃ for 60 mins to allow most of the protein, high molecular weight RNA and chromosomal DNA to precipitate. The tubes were centrifuged for 5mins at 15,000 rev/minute to yield a clear supernatant. About 0.4 ml of the supernatant was removed from each tube and transferred into smaller centrifuge tubes. One millilitre of cold ethanol was added and held at -20℃ for 30O mins. The precipitate was then collected by centrifugation at 6000 rev/min for 2 mins and the supernatant was removed by aspiration. The pellet was dissolved in 100 µl of 0.1 M sodium acetate/0.05 Tris HCl (pH 8) and re-precipitated in 2vols of cold ethanol. After 10 min, at -20℃, the precipitate was again collected by centrifugation as described earlier. The pellet was dissolved in 40 µl of water and then 10 µl of sample buffer was added. Between 10-20 µl of plasmid DNA in solution was applied to an agarose gel for electrophoresis.

2.5.3 Agarose gel electrophoresis

One percent agarose was prepared and loaded into electrophoresis chamber containing between 12-18 wells. The electrophoresis buffer that was used contained 40 mM Tris, 20 mM sodium acetate, 2 mM EDTA, adjusted to pH 7.8 with acetic acid. The sample buffer contained 25% sucrose, 5 mM sodium acetate, 0.05% bromophenol blue and 0.1% SDS. Electrophoresis was allowed to proceed at room temperature until bands become visible at the positive end of the chamber. After electrophoresis, gels were stained with ethidium bromide (1 µl/ml) and viewed under UV trans illumination. The molecular marker that was used was the bacteriophage *Hind III* digest.

2.6 Evaluation of Virulence Factors Producing Potentials of the Isolates

2.6.1 Determination of hemolytic activity

Hemolytic activity of the strains was determined on blood agar with sheep blood as described by Citak et al. [23] and Jurkovic et al. [24] to detect α, β, as well as γ haemolysis.

2.6.2 Production of hydrolytic enzymes

Bacterial isolates were screened for production of hydrolytic enzymes such as lipase, urease, and gelatin using agar diffusion method with specific substrates. The basal mineral agar medium (pH 7.0) contained (%): KH2PO4 0.1, (NH4)2SO4 0.5, MgSO4 .7H2O 0.01, NaCl 0.01, and agar 2.0. Inoculated plates were incubated for 3 - 5 days at room temperature. The growth of cultures, zones of clearing around the colonies or color of diffusion zones on respective specific media were used as an indication of the presence of the relevant enzyme activity [24].

2.7 Data Analysis

Data gathered from the microbiological assessment of fresh produce were subjected to single factor analysis of variance (ANOVA) using SPSS package version 20.0 with Duncan Multiple Range Test (DMRT) for post-hoc determinations of significant differences ($\alpha = 0.05$).

3. RESULTS AND DISCUSSION

3.1 Results

3.1.1 Bacterial loads of fresh aquatic resources sold in Okopedi open market centre

The mean bacterial load of the skin, gills and intestine of *Tilapia guinensis* and *Marcusenius senegalensis* as well as that of the viscera of shrimps (*Macrobranchium rosenbergii*, *Crangon vulgaris* and *Paneaus monodon*) are presented in Tables 1- 3 respectively. The results revealed

varying levels of microbial contamination of the fresh produce.

The bacterial properties of *Tilapia guinensis* presented in Table 1 showed that the loads of hetrerotrophic bacteria was high (7.03 x 10^5 ± 0.06) cfu/g in intestine of the fish while coliform count was high in the gills (4.80 x 10^4 ± 0.10) cfu/g. In this fish, faecal coliform was not found in the gills of the fish and no *salmonella/shigella* was found in the gills and intestine of the fish. Intestine had the highest Vibrio count (3.13 x 10^3 ± 0.06) cfu/g although it was not detected on the skin of the fish. The organs (Table 2) of *Marcusenius senegalensis* haboured relatively high bacterial loads. Heterotrophic bacteria counts were high in all the three organs but slightly higher (6.27 x 10^5 ± 0.06) cfu/g in the intestine. Contamination with coliform and faecal coliform were also high with the highest density obtained from intestine (5.07 x 10^4 ± 0.06)cfu/g while the highest faecal coliform count was found in gills (3.30 x 10^3 ± 0.10)cfu/g. No *salmonella/shigella* was found in the skin. Vibrio

count was also remarkable with highest of 3.03 x 10^3 ± 0.06) cfu/g in gills which did not differ greatly from others.

Among the shrimps sample (Table 3) *Crangon vulgaris* had the highest heterotrophic bacteria count (7.13 x10^5 ± 0.06) cfu/g followed by *Macrobranchium rosenbergii* (6.50 x10^5 ± 0.10) cfu/g. The entire microbial groups in this study were found to be present in the entire shrimp sample under study. *Paneaus monodon* commonly called giant tiger prawn or Asian tiger shrimp widely reared for food had the highest coliform and faecal coliform count of 2.87 x10^4 ± 0.06) cfu/g and (5.10 x 10^3 ± 0.10) cfu/g respectively. The vibrio counts of the shrimp were high in all the three shrimp samples with *Macrobranchium rosenbergii* having the highest (3.13 x 10^3 ± 0.06)cfu/g count. *Paneaus monodon* had the highest count (2.27x10^2 ± 0.06) cfu/g of *Salmonellae/Shigellae* although the values (*Salmonella/Shigella* counts) recorded were comparatively lower than those recorded for the fishes.

Table 1. Microbial loads (cfu/g) of *Tilapia guinensis* Sold in Okopedi Open Market Centre

Fish type	THBC(×10^5)	TCC(×10^4)	FCC(×10^3)	SSC(×10^3)	VC(×10^3)
Skin	5.27±0.06^a	4.17±0.12^a	3.87±0.06^c	4.10±0.01^b	0.00±0.00^a
Gill	5.30±0.01^a	4.80±0.10^b	0.00±0.00^a	0.00±0.00^a	1.77±0.06^b
Intestine	7.03±0.06^b	4.30±0.10^a	2.70±0.01^b	0.00±0.00^a	3.13±0.06^c

Value reported in the form means ±SD, similar letter mean not significantly different (p>0.05). Different letters mean significantly different (p<0.05).
Key: THB = Total heteotrophic bacteria count, TCC = Total coliform count, FCC = Faecal coliform count, SSC = Salmonella shigella count, VC = Vibrio count.

Table 2. Microbial loads (cfu/g) of *Marcusenius senegalensis* sold in Okopedi Open Market Centre

Fish type	THBC(×10^5)	TCC(×10^4)	FCC(×10^3)	SSC(×10^3)	VC(×10^3)
Skill	4.17±0.06^a	3.87±0.06^a	2.20±0.10^a	0.00±0.00^a	2.20±0.10^a
Gill	4.70±0.10^b	4.30±0.10^b	3.30±0.10^b	4.70±0.10^b	3.03.±0.06^b
Intestine	6.27±0.06^c	5.07±0.06^c	2.87±0.06^c	3.80±0.10^c	2.13±0.06^a

Value reported in the form means ±SD, similar letter mean not significantly different (p>0.05). Different letters mean significantly different (p<0.05).
Key:THB = Total heteotrophic bacteria count, TCC = Total coliform count, FCC = Faecal coliform count, SSC = Salmonella shigella count, VC = Vibrio count.

Table 3. Microbial loads (cfu/g) of viscera of shrimps sold in Okopedi Open Market Centre

Shrimp type	THBC(×10^5)	TCC(×10^4)	FCC(×10^3)	SSC(×10^3)	VC(×10^3)
Macrobranchium rosenbergii	6.50±0.10^a	2.63±0.06^a	3.53±0.06^a	3.00±0.01^a	3.13±0.06^a
Crangon vulgaris	7.13±0.06^b	2.70±0.10^a	4.00±0.10^b	3.77±0.06^b	2.83±0.06^b
Paneaus monodon	5.80.±0.10^c	2.87±0.06^b	5.10±0.10^b	4.17±0.06^c	2.27±0.06^c

Value reported in the form means ±SD, similar letter mean not significantly different (p>0.05). Different letters mean significantly different (p<0.05).
Key: THB = Total heteotrophic bacteria count, TCC = Total coliform count, FCC = Faecal coliform count, SSC = Salmonella shigella count, VC = Vibrio count

3.1.2 Bacterial species isolated from fresh aquatic resources sold in Okopedi open market centre

The morphological and biochemical characteristics of the bacterial isolates from skin, gills and intestine of *Tilapia guinensis* and *Marcusenius senegalensis* as well as that of the viscera of shrimps (*Macrobranchium rosenbergii*, *Crangon vulgaris* and *Paneaus monodon*) revealed the following bacterial species; *Serratia* sp, *Enterobacter aerogene*, *Shigella* sp, *E. coli*, *Klebsiella pneumonia*, *Proteus vulgaris*, *Streptococcus* sp, *Micrococcus* sp, *Salmonella* sp, *Pseudomonas aeruginosa*, *Vibrio sp*, *Staphylococcus aureus, Bacilus subtilis and Enterococus* sp.

For *Tilapia guinensis* (Table 4), 7 bacterial species were isolated from skin, 3 from gills and 7 from intestine and *Micrococcus* sp (100%) was the most occurring bacterial species in the fish. The result presented in Table 5 revealed that 5 bacterial species were isolated from skin of *Marcusenius senegalensis*, 5 from gills and 7 from intestine. *Vibrio* sp (100%) was the most occurring bacterial species in *Marcusenius senegalensis.* Among the studied shrimps, 5 bacterial species were isolated from *Macrobranchium rosenbergii*, 3 from *Crangon vulgaris* and 4 from *Paneaus monodon Micrococcus* sp (100%) was found to be the most abundant bacterial species in the shrimps.

3.1.3 Virulence factors producing potential of the bacterial isolates

The results of the test on the ability of the bacterial isolates to cause infection (Table 7) have shown that *Salmonella* sp, *Serratia* sp, *Enterobacter aerogene*, *E. coli, Proteus vulgaris,* *Micrococcus* sp, *Bacilus subtilis*, *Staphylococcus aureus* and *Vibrio cholerae* demonstrated α haemolytic activity while *Klebsiella pneumonia*, *Streptococcus sp*, *Enterococus* sp and *Pseudomonas aeruginosa* showed β haemolytic activity. *Salmonella* sp, *E.coli, Proteus vulgaris, Klebsiella pneumonia, Streptococcus* sp, *Enterococus* sp, *Pseudomonas aeruginosa*, *Bacilus subtilis, Staphylococcus aureus* and *Vibrio* sp capable of producing lipase while *E.coli, Proteus vulgaris, Klebsiella pneumonia*, *Micrococcus* sp, *Streptococcus* sp and *Staphylococcus aureus* exhibited gelatinase activity. *Proteus vulgaris*, *Pseudomonas aeruginosa*, *Enterococus* sp, and *Vibrio cholera* showed urease producing activity. The *Shigella* sp strains obtained failed to elaborate virulence factors.

3.1.4 Antibiogram, multiple antibiotics resistance (MAR) index of bacteria and isolates from fresh aquatic resources sold in Okopedi open market centre

In this study, the antibiotic resistance/susceptibility was tested for gram negative bacteria (Table 8) and gram positive (Table 9). *Enterobacter aerogene* was resistant to the entire gram negative antibiotic except Gentamycin while *E. coli* was susceptible to only Septrin. *Salmonella* sp and *Proteus vulgaris* were resistant to Streptomycin, Ceponex and Nalidixic while *Klebsiella pneumonia* was susceptible to all the tested antibiotics on the gram negative disc. *Serratia* sp was susceptible to all the antibiotics. On the other hand, on the gram positive disc (Table 9), *Bacilus subtilis* and *Staphylococcus aureus* showed resistance against all the tested antibiotics, although *Staphylococcus aureus* was less resistant to Ampiclox and Erythromycin. *Streptococcus* sp was susceptible to all the

Table 4. Occurrence of the diverse bacterial species in *Tilapia guinensis* Sold in Okopedi open market centre

Organisms	Skin	Gills	Intestine	Occurrence rate (%)
Micrococcus sp	+	+	+	100
Streptococcus sp	-	-	+	33.3
Staphylococcus aureus	+	-	-	33.3
Klebsiella pneumonia	+	-	+	66.7
Salmonella sp	+	-	-	33.3
Shigella sp	-	-	+	33.3
Vibrio sp	-	+	+	66.7
Escherichia coli	+	-	+	66.7
Enterococus sp	+	+	-	66.7
Proteus vulgaris	-	-	+	33.3
Serratia sp	+	-	+	66.7
Species Richness	7	3	8	

Table 5. Occurrence of the diverse bacterial species in *Marcusenius senegalensis* sold in Okopedi open market centre

Organisms	Skin	Gills	Intestine	Occurrence rate (%)
Micrococcus sp	-	+	+	66.7
Staphylococcus aureus	+	-	+	66.7
Klebsiella pneumonia	+	-	+	66.7
Enterobacter aerogene	+	-	-	33.3
Salmonella sp	-	+	+	66.7
Vibrio sp	+	+	+	100
Escherichia coli	+	-	+	66.7
Enterococus sp	-	+	-	33.3
Proteus vulgaris	-	+	+	66.7
Species Richness	5	5	7	

Table 6. Occurrence of the Diverse Bacterial Species in Shrimps Sold in Okopedi Open Market Centre

Organisms	*Macrobranchim rosenbergii*	*Crangon vulgaris*	*Paneaus monodon*	Occurrence rate (%)
Micrococcus sp	+	+	+	100
Salmonella sp	+	+	-	66.7
Vibrio sp	+	-	+	66.7
Escherichia coli	-	+	+	66.7
Proteus sp	+	-	-	33.3
Serratia sp	+	-	+	66.7
Species Richness	5	3	4	

Table 7. Virulence factors producing potential of the diverse bacterial isolates

Organism	Haemolytic activity	Lipase	Gelatin	Urease
Salmonella sp	**A**	+	-	-
Serratia sp	A	-	-	-
Shigella sp	A	-	-	-
Enterobacter aerogene	A	-	-	-
E.coli	A	+	+	-
Klebsiella pneumonia	B	+	+	+
Proteus vulgaris	A	+	+	+
Streptococcus sp	B	+	+	-
Micrococcus sp	A	-	+	-
Enterococus sp	B	+	-	+
Bacilus subtilis	A	+	+	-
Staphylococcus aureus	A	+	+	-
Vibrio sp	A	+	-	+
Pseudomonas aeruginosa	B	+	-	-

Key: A = α haemolytic activity, B = β haemolytic activity, + = positive, - = negative

antibiotics on the gram positive disc. *Micrococcus* sp showed resistance to only ampiclox while *Enterococus faecalis* showed resistance to rifampicin and erythromycin.

Multiple Antibiotics Resistance (MAR) indices of 0.9, 0.9, 1.0 and 0.7 were recorded for *Enterobacter aerogenes*, *E. coli*, *Bacilus subtilis* and *Staphylococcus aureus* respectively. *Bacilus subtilis* had the highest index and was 100% resistant to all the tested antibiotics while *Micrococcus* sp has least index of 0.1 (Table 10).

3.1.5 Plasmid profile of multi-antibiotic resistant (MAR) bacteria isolated from the aquatic Produce

Fig. 2 shows the plasmid profile patterns of four multi-antibiotic resistant bacteria. The isolates profiled were *Enterobacter aerogene* (S1), *E. coli*

(S2), *Bacillus subtilis* (S3) and *Staphylococcus aureus* (S4). The result revealed the resistance of all the MAR bacteria but *Staphylococcus aureus* were coded in plasmids of about 300 bp.

It implies that their resistance was plasmid mediated as the bacteria lost their antibiotic resistance when they lost 300 pb plasmid (Fig. 3).

Table 8. Antibiogram of gram negative bacterial isolates (mm) from fresh aquatic resources sold in Okopedi Open Market Centre

Isolate	OFX	PEF	CPX	AU	CN	S	CEP	NA	SXT	PN
Salmonella sp	20	16	24	20	18	6	8	10	14	22
Serratia sp	22	28	24	10	12	22	24	14	26	10
Enterobacter aerogene	8	8	6	10	12	8	8	8	8	8
E.coli	8	10	10	8	8	8	6	6	14	8
Klebsiella pneumonia	22	22	18	20	22	18	24	22	24	20
Proteus vulgaris	14	20	18	18	16	12	12	10	10	14

Key:OFX = Tarivid (10 µg), PEF = Refacine (10 µg), CPX = Ciproflox (10 µg), AU = Augmentin (30 µg), CN = Gentamycin (10 µg), S = Streptomycin (30 µg), CEP = Ceponex (10 µg), NA = Nalidixic (30 µg), SXT = Septrin (30 µg), PN = Ampicilin (30 µg)
Standard resistant range: 1-12
Standard susceptible range:13-30

Table 9. Antibiogram of gram positive bacterial isolates (mm) from fresh aquatic resources sold in Okopedi Open Market Centre

Isolate	CPX	NB	CN	AMX	S	RD	E	CH	APX	LEV
Streptococcus sp	20	18	14	20	20	14	20	16	16	18
Micrococcus sp	24	16	18	20	18	20	14	12	10	22
Enterococus faecalis	24	24	20	22	20	8	8	20	12	18
Bacilus subtilis	6	6	6	6	6	6	6	6	6	6
Staphylococcus aureus	12	6	8	6	6	8	12	6	12	10

Key:CPX = Ciproflox (10 µg), NB = Norfloxacin (10 µg), CN = Gentamycin (10 µg), AML = Amoxil (20µg), S = Streptomycin (30 µg), RD = Rifampicin (20 µg), E = Erythromycin (30 µg), CH = Chloramphenicol (30 µg), APX = Ampiclox (20 µg), LEV = Levoflox (20 µg).
Standard resistant range: 1-12
Standard susceptible range:13-30

Table 10. Multiple antibiotics resistance (MAR) index of bacteria isolates from fresh aquatic resources sold in Okopedi Open Market Centre

S/N	Isolates	Total number of antibiotics tested	Number of resistance	MAR Index
	Gram negative			
1	*Salmonella* sp	10	3	0.30
2	*Serratia* sp	10	2	0.20
3	*Enterobacter aerogene*	10	9	0.90
4	*E.coli*	10	9	0.90
5	*Klebsiella pneumonia*	10	0	0.00
6	*Proteus vulgaris*	10	2	0.20
	Gram positive			
7	*Streptococcus* sp	10	0	0.00
8	*Micrococcus* sp	10	1	0.10
9	*Enterococus faecalis*	10	2	0.20
10	*Bacilus subtilis*	10	10	1.00
11	*Staphylococcus aureus*	10	7	0.70

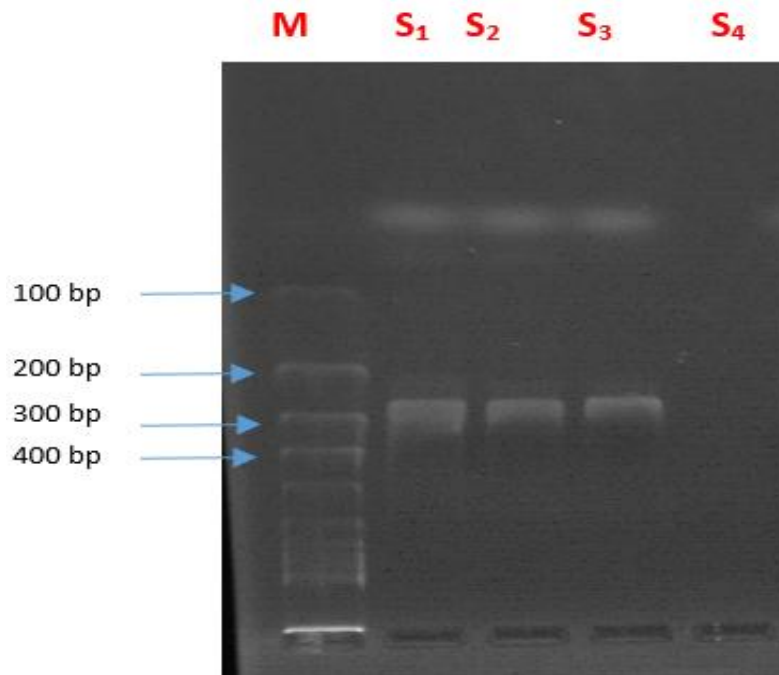Key: MAR- Multiple Antibiotics Resistance

Fig. 2. Uncured plasmid profile of MAR bacterial isolatesKey: M – Control Marker is Bacteriophage Hind III digest Plasmid DNA of *Enterobacter aerogene*; S2 – *Escherichia coli,* S3- *Bacillus subtilis* and S4 = *Staphylococcus aureus*
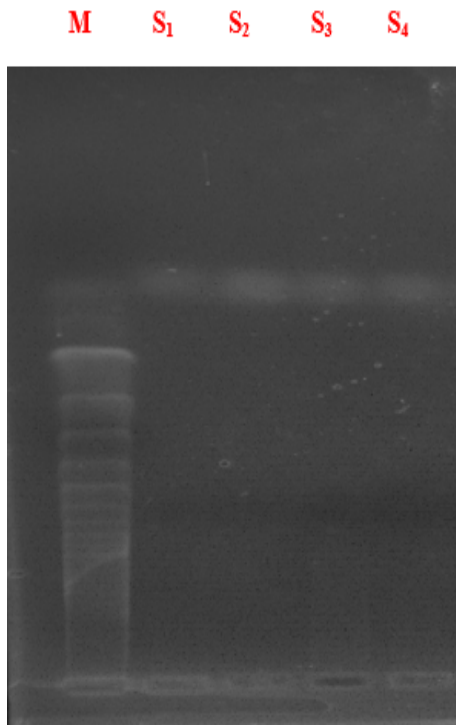

Fig. 3. Electrophoretic gel of cured plasmids bands of MAR bacterial isolates

3.2 Discussion

Beuchat [25] reported new challenges faced by the aquaculture industry including the protection of consumers against microbiological hazards. The present study has shown that fresh fishes and shrimps sold in Okopedi Open Air Markets are heavily contaminated with microorganisms including enteric bacteria and potential pathogens. Analysis has shown that viable cells of heterotrophic bacteria, coliform and faecal coliform as well as *Salmonella, Shigella* and *Vibrio* sp were encountered on the displayed fishes and shrimps. No fecal coliform was found in the gill and intestine of the fishes although *Salmonella* and *Shigella* were isolated from the gills of some fishes but absent in the intestine. Fecal coliform was not detected in the gill samples from *Tilapia guinensis* while *Salmonella* and *Shigella* were absent in both the gills and intestine of the fish.

Among shrimp samples analyzed, the viscera from *Crangon vulgaris* had the highest heterotrophic bacteria count of $(7.13 \times 10^5 \pm 0.06)$ cfu/g followed by *Macrobranchium rosenbergii* which had $(6.50 \times 10^5 \pm 0.10)$ cfu/g of hetrotrophic bacteria. *Paneaus monodon* (commonly called

giant tiger prawn or Asian tiger shrimp) which is widely reared for food had the highest coliform and faecal coliform count of $(2.87 \times 10^4 \pm 0.06)$ cfu/g and $5.10 \times 10^3 \pm 0.10$ cfu/g respectively. The densities of *Vibrio* sp in the shrimps were generally high with count of $(3.13 \times 10^3 \pm 0.06)$ cfu/g recorded for *Macrobranchium rosenbergii* while *Paneaus monodon* haboured the highest number $(4.17 \times 10^2 \pm 0.06$ cfu/g) of *Salmonella* and *Shigella.*

The high contamination level observed in this study indicates gross contamination during the period of exposure by the fish sellers in the market. Higher coliform load was also found in all the fishes and the source of this coliform may be the water in which the fishes were harvested. It implies that contamination of waters for aquaculture, compounded by poor handling during distribution, can have negative impact on public health. During processing in the food supply chain, many opportunities exist for food to be contaminated. Fecal pollution is the main contaminant, and impacted water supplies can also serve as a vehicle to transmit pathogens to foodstuff. Several factors could have contributed to the contamination of the fresh produce. Among the sources of pollution discernable were unsanitary human activities and indiscriminate disposal of organic wastes including faecal matter. Humans themselves have been reported to act as carriers of saprophytic and pathogenic micro-flora causing illness in individual [26].

This study has revealed the prevalence of *E. coli* in many of the samples analyzed. It implies that fresh produce from open markets in Okopedi Fishing settlement are heavily contaminated with enteric bacteria. This high level of contamination indicates potential breakdown of hygiene at various stages of the produce handling and distribution chain. These results suggest potential of foodborne disease caused by consumption of inadequately processed produce.

E. coli is currently the best available indicator of fecal contamination for fresh produce. *E. coli* was isolated from many fresh produce and all the items have similar prevalence of *E. coli* contamination. Other authorities [27] have reported positive rates of *E. coli* in fresh produce. There are various ranges of bacterial counts from previous studies from other countries [27]. *Salmonella* has also been isolated from fresh, frozen, canned and sun dried marine fish products [28]. *Salmonella* is apparently carried by fresh shrimp and other seafood products

especially if they are cultured or washed in sewage polluted water or by contamination during processing. The large variation in counts between studies could be due to differences in microbiological methodologies, the types of produce tested, the sources of the samples and geographical locations. Other bacterial isolates encountered, include *Serratia* sp, *Enterobacter aerogene*, *E. coli, Proteus vulgaris, Micrococcus* sp, *Bacilus subtilis*, *Staphylococcus aureus* and *Vibrio* sp and *Shigella* sp *Klebsiella pneumoniae, Streptococcus* sp and *Pseudomonas aeruginosa.* Their occurrences in fresh seafood have previously been reported (29. Less number of isolates was found in the gills of the fishes as well as the viscera of the shrimps. *Staphyloccus aureus*, *Micrococcus* sp and *Vibrio* sp were the most occurring bacterial species (100%) in the fresh aquatic produce.

Virulence factors acting individually or together may induce infection depending on the host resistance. These factors compromised the host's defense mechanisms resulting in successful colonization and establishment of infection. All but one of the bacteria isolated from the fresh produce exhibited variable forms of virulence and may capable of inducing foodborne infections. *Staphylococcus aureus* which haemolysis red blood cells in this study (α haemolytic activity) is arguably the most prevalent pathogen of humans, may cause up to one third of all bacterial diseases ranging from boils and pimples to food poisoning, to septicemia and toxic shock.

Among the microorganism isolated, *Staphylococcus aureus, Salmonella* sp*, Streptococcus* sp*, Bacillus* sp and some strains of *Esherichia coli* are known to be pathogenic. In a related study, Ibrahim et al. [30] evaluated the occurrence and antimicrobial susceptibility profiles of *Salmonella* serovars from fish in Maiduguri, sub-Saharah, Nigeria. A total of 23 isolates were positive for *Salmonella* out of the 200 samples (11.5%) analyzed. The study revealed that *Salmonella* serovars are the pathogens associated with fish contamination in the region and constitute serious health risks for the human population and need to be controlled by targeted interventions. Some species of *Streptococcus* have also been reported to be associated with several infections. For instance, *Streptococcus pyogenes* is associated with sore throat. *Streptococcus pneumoniae* is associated with pneumococcal pneumonia, pink eye, meningitis, endocarditis and other respiratory

tracts diseases [31]. Species of *Enterococcus,* e.g. *E. faecalis* can cause endocarditis as well as bladder, prostate and epidermal infections [31].

Staphylococcus aureus can cause a wide range of illness ranging from minor skin infections such as gastroenteritis, pimples, impetigo, boils (furuncles), cellutitis, folliculitis, carbuncles, scaled skin syndrome and abscesses to life threatening diseases such as pneumonia, meningitis, oseteomyelitis, endocarditic, toxic shock syndrome (TSS), chest pain and sepsis [31]. According to ICMSF [32], *Salmonella* and *Vibrio cholerae* should not be found in sea food products. However, in the present study, high numbers of *Salmonella* were isolated from the fresh shrimps. This level of contamination is unacceptable for human consumption although, most shrimp are cooked prior to consumption and therefore, cause negligible health risks to the consumers except for cross contamination in the kitchens [33]. *Salmonella* is a Gram negative, rod shaped bacterium. *Salmonella* sp are the most commonly identified etiological agent associated with fresh produce -related infection, isolated in 45 cases between 1973 and 1997 in USA [34] and in 41% of cases during 1992 -2000 in the UK [35]. In this study, about 33 aquatic produce samples were positive for *Salmonella* sp. The high detection rate of *Salmonella* sp can be alarming as many foodborne outbreaks have been associated with these microorganisms.

Water and food-borne infections in the State and the Niger Delta region has continued largely due to the low literacy level, poor hygienic conditions, historical neglect, apathy, pollution, socio-ecological green washing of the exploration and allied companies operating in the region and the abysmal poverty level of the inhabitants who cannot afford clean, safe drinking water and medicare [36]. The gastrointestinal illnesses prevalent in the area ranged from mild to severe stomach distress with symptoms including abdominal cramps, diarrhea, occasional vomiting and dehydration [37]. These illnesses although preventable, are often undiagnosed, life threatening, sometimes fatal and unreported. Food borne infection and intoxication in the study area as a result of consumption of sea foods is particularly worrisome because of the unhygienic environment, handling of the harvested aquatic biota and the mild heat applied during the smoking process which could be stimulatory to the microorganisms. Exposure to food borne infection and intoxication is usually more pronounced in avid consumers who are in the habit of eating uncooked or improperly cooked aquatic produce such as fishes and shrimps harvested from the estuarine ecosystem [36].

This study has shown that aquatic foods may be a major source of foodborne infections. Infectious diseases are the leading cause of death worldwide. Not only are new infectious diseases emerging, but the re-emergence of deadly infectious diseases, and the increasing prevalence of antimicrobial resistant strains, presents a formidable threat to public health and welfare [31]. The antibiotics susceptibility assay of the bacterial contaminants showed high degree of resistance. This is in agreement with the report of Idika [38] who studied *Vibrio cholerae* during an outbreak of cholera in Lagos in 1997 and reported the isolates were resistant to Tetracycline and Gentamicin. This study has shown that the bacterial contaminants exhibited less resistance to Streptomycin and Ampiclox but highly resistant to Ciproflox, Septrin and Refacine. These resistance rates are quite high compared to those previously described among organisms isolated from river water where resistance to Amikacin was less than 10% and resistance to Gentamicin was less than 25%. In a recent study in Poland, high resistance to erythromycin was observed among the gram positive organisms isolated from surface water and fishes from the same water, reaching resistance level above 60% [39]. Increase in antibiotics resistance has been observed among organisms isolated from shrimps in Brazil where high indices of resistance to Ampicillin and Tetracycline were observed [40]. Ash et al. [41] also reported high level of resistance in Gram-negative bacteria isolates from rivers in the United States. The same authors reported that the isolates exhibited multiple resistant to a number of commonly used antibiotics such as Ampicillin, Tetracycline and Penicillin.

Majority of isolated bacteria from the fresh aquatic produce demonstrated high level of resistance to several antibiotics. *Bacilus subtilis* and *E. coli* showed high rate of resistant to all the antibiotics tested with MAR index of 1.0 and 0.9 respectively. The antibiotic resistance pattern encountered in this study is in agreement with the findings of Odeyemi et al. [42] on the least resistance of the test isolates to Ciprofloxacin. Since most of these pathogens are of human origin, human activities in the water body (Okopedi River) where these aquatic produce are gotten may have contributed to their resistance to antibiotics. As earlier reported by Ajayi and

Akonai [43], the discharge of wastewater through erosion and human activities in the water body could enhance the ingestion of resistance strain of bacteria by these fishes and shrimps. The uncontrolled use of antibiotics for empirical treatment of infectious diseases has been implicated as a cause of high prevalence of these antibiotics resistance [44].

Plasmid profiling of the multidrug resistant bacteria (*Enterobacter aerogene*; *E. coli*; *Bacilus subtilis* and *Staphylococcus aureus*) isolates revealed that three (*Enterobacter aerogene*; *E. coli*; *Bacilus subtilis*) out of the four isolates had plasmid of about 300 bp. This then prompted curing the bacteria of its plasmids to determine if the MDR was plasmid or genetically mediated. The result of the analysis revealed that the organisms lost its resistant once it lost its plasmid thus, ascertaining that the MDR potentials of the *Enterobacter aerogene*; *E. coli* and *Bacilus subtilis* was plasmid mediated and encoded on the 300 bp plasmid. On the other hand, *Staphylococcus aureus* had no plasmid and it is possible that its potential to resist multiple antibiotics is genetically encoded. The MAR trait in the bacterial isolates was plasmid mediated and is in agreement with earlier report by Akinyemi et al. [45]. It implies that the resistance trait can be easily spread through trait transfer from one organism to another. This is a serious risk knowing that some of the isolates in this study are carry plasmid(s). Plasmids are infectious. They can be transferred between bacteria of the same or different genera. Plasmids are major mechanism for the spread of antibiotic resistant genes in bacterial populations. *Enterobacter aerogene*, *E.coli*, *Bacilus subtilis* and *Staphylococcus aureus* were found to contain R plasmid. Multidrug resistance and plasmid were observed in the bacterial isolates.

Aja et al. [46] in their study of *Vibrio* strains isolated from cultured shrimps reported that some strains were resistant to four antibiotics, others were resistant to two antibiotics and all contained one plasmid of 21.2 kb. They suggested that resistance to antibiotics could be encoded in some strains in plasmids and in others in the chromosomes. Mirza et al. [47] reported that antimicrobial resistance was transferable from *Salmonella* sp to *Escherichia coli* as well as between other members of the intestinal normal flora. Plasmids are a major mechanism for the spread of antibiotic resistant genes in bacterial populations [48]. Conjugation occurs by F-plasmids that can transfer genes encoded for multiple resistance and mobilize other non-conjugative plasmids to host cells [49]. Multiple resistance genes are harboured on R-plasmids some of which are conjugative [50]. *Escherichia coli* have been reported to transfer the antibiotic resistant genes to enteric pathogens such as *Salmonella* sp and *Proteus* sp and normal flora bacteria [51]. Usually all functions required for plasmid transfer, including synthesis of pili, are encoded by genes on the plasmid. Thus, after transfer to a second host, these genes may enable a newly formed trans-conjugant to become a donor in another round of conjugation [31]. This process may be repeated several times.

4. CONCLUSION

Virulence factor and antibiogram of bacteria associated with fresh aquatic produce sold in the open market centre of Okepedi fishing settlement, Itu, Akwa Ibom State was investigated. Several pathogenic bacteria such as *Staphylococcus aureus, Streptococcus* sp, *Bacillus* sp, *Escherichia coli* as well as *Enterococci* have been found to be associated with fishes and shrimps displayed for sale in open market. The contaminants demonstrated strong potential to elaborate virulence factors and therefore serious health hazards could result from consumption of these fishes and shrimps if not properly cooked. These bacteria were found to be resistant to most of the existing antibiotics and pose a serious health threat. It is of significant public health concern that most of the isolates had multidrug-resistant strains and may constitute a potential reservoir of resistance plasmids that could be transferred to hitherto non-pathogenic bacteria. The occurrence of plasmid mediated multidrug resistant in bacteria in this aquatic produce heightens the public health concern. The research findings support studies that suggest the existence of a reservoir of antibiotic resistance genes. Infections with multidrug-resistant pathogens limit the options available to treat infectious disease of animals and humans. The high prevalence of multidrug-resistant bacteria observed in this study suggests the need for improved education and communication on the issue of antibiotic used. The results established in this research may be of use to farmers, retailers, food safety educators, and policy makers in improving the microbiological quality and safety of fresh produce in our markets and in preventing the occurrence of diseases associated with it. To achieve the above, it is recommended that:

(i) Wastes including human fecal matter should be properly disposed or managed to avoid contamination of the market environ as well as water bodies where the produce are harvested.

(ii) The fishes and shrimps should be properly cooked before consumption to get rid of potential disease causing agent before they are consumed.

(iii) Steps must be taken to control the overuse of antibiotics in the region and Nigeria in general, as well as in other developing countries.

(iv) The present study however challenge scientists on the need for development of new antibiotics to combat the infections caused by these resistant strains.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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