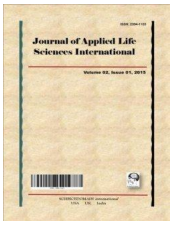

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Virulence Factors of Bacteria Isolated from Fish Sold at Open Air Market Centre in Okepedi, Itu, Akwa Ibom State, Nigeria

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

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ABSTRACT

The study on the virulence factors of bacteria isolated from fish 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 *Clarias gariepinus*, *Oreochromis niloticus* and *Tilapia guinensis* were all investigated. The results revealed that all the fishes obtained from the Okepedi open market centre were contaminated with microorganisms. The densities of heterotrophic bacteria accumulated by the fishes exceeded 1.2 x 10^5 cfu/g recommended fresh fish samples and the loads of fecal coliform (*Escherichia coli*) in the fishes. Analysis of variance revealed a significant difference in the mean load of bacteria in the fishes at significance level of $p \leq 0.05$. The high faecal coliform load of the fishes has shown that the rivers surrounding the market are highly contaminated with faecal matter. Contaminant bacteria isolated from the fishes 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. The analysis revealed that the isolates exhibited varying degree of virulence. *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.

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Salmonella sp, *E. coli*, *Proteus vulgaris*, *Klebsiella pneumonia, Streptococcus* sp, *Enterococus* sp, *Pseudomonas aeruginosa*, *Bacilus subtilis*, *Staphylococcus aureus* and *Vibrio* sp were 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 occurrences of pathogenic bacteria in these fishes lighten the public health concern and therefore, efforts should make maintaining and enforce adequate sanitation practices in the habitats of these produce.

Keywords: Virulence; Bacteria; Clarias gariepinus; Oreochromis niloticus and Tilapia guinensis.

1. INTRODUCTION

Fish is one of the sources of proteins, vitamins and minerals, and it has essential nutrients required for supplementing both infants and adults diet [1]. In Nigeria, fish is eaten fresh and smoked and form a much cherished delicacy that cut across socio-economic, age, religions and educational barriers [2]. 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 [3], fish and fish products constitute more than 60% of the total protein intake in adults especially in rural areas. According to FAO [4], 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 [5].

There are various reasons for the merits of eating fish. One such reason is that fish is less tough and more digestible when compared with beef, mutton, chicken and bush meat. This is possible because of the greater ratio of muscles protein to connective tissues in fish in relation to other animals thus making fish acceptable by infant and adults. Because of its greater digestibility, fish is usually recommended to patients with digestive disorders such as ulcers [6]. Fish product has a nutrient profile superior to all terrestrial meats of beef, pork and chicken etc., being an excellent source of high quality animal protein and highly digestible energy. It is a good source of sulphur and essential amino acids such as lysine, leucine, valine and arginine. It is therefore suitable for supplementary diets of high carbohydrate contents [7]. Attention has been focused recently on the relationship between fish consumption and reduced incidence of cardiovascular disease. The benefit has been attributed to the nature of the fats in fish. Unlike other fats in other food, it is the only type of fat that supplies omega-3 poly unsaturated fatty acids (PUFA). PUFAs are essential in lowering blood cholesterol level and high blood pressure. It is able to migrate to alleviate platelet of (cholesterol) aggregation and various arteriosclerosis conditions in adult population. [8]. It reduces the risk of sudden death from heart attack and reduced rheumatoid arthritis. Omega-3 fatty acid also lower the risk age related muscular degeneration and vision impairment, decrease the risk of bowel cancer, and reduce insulin resistance in skeletal muscle. Fish is abundant to some extent and occur free in nature. This may account for its relatively low cost compare with other meats. Fish is available in most market as fresh, smoked, dried, canned, chilled or frozen and as such the problem of scarcity is removed. The place of aquatic product in the food basket of the nation cannot be over-emphasized.

Advantages of fish as a food are its easy digestibility and high nutritional value [9]. 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 [10].

Fish is a perishable protein food. Freezing does not prevent spoilage of fish because of autolytic activities and chemical changes occurring in fish after harvest [11]. The degradation of fish is accelerated by microorganisms associated with aquatic environments as well as contaminants during post-harvest handling. When fish dies, microorganisms on the surface as well as gut and gills begin to utilize the fish protein and food nutrients resulting in loss of nutritional value. Microbial activities create undesirable changes like off-flavors, texture and appearance [12]. Rate of bacterial spoilage is dependent on the initial microbial load, ambient temperature and improper handling. Therefore, proper storage is critical in maintaining a high standard of safety when processing fish [13]. When a fish dies under water with high ambient temperatures (water temperature 18-21°C), the spoilage begins right under water. In addition to these problems faced by small scale fishermen in developing countries, there is lack of investments in landing sites processing and selling sites, resulting in poor sanitation and hygiene [14]. These problems lead to cross contamination and multiplication of microorganisms and hence poor quality of fish are presented to the consumers.

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 [16]. 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 [17]. 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.

Previous studies of fresh produce contamination in Asia have shown parasitic and microbial contamination of produce at the market or postharvest level [18]. Fishery products have been recognized as a major carrier of food-borne pathogens [19] and human infections caused by organisms transmitted from fish are common depending on the season, the patients' contact with fish, dietary habits, and the immune status of the exposed individual. Foodborne pathogens take a serious toll on public health, while many agricultural products including fish are cooked prior to eating, many people, especially the Southeast Asian culture consume uncooked or partially cooked produce either directly or as fresh condiments to dishes. Consumption of contaminated fresh produce including fresh fishes poses a serious threat to health, especially to children, elderly, pregnant women and immunocompromised people. These circumstances and the growing demands for fish prompted this research to look into the safety and quality of fish sold at open market centers in Nigerian coastal fishing settlement. There are few or no reports on the microbial quality of fishes marketed in the Nigerian Fishing Settlements thus, the results that will be obtained from this research will be used to create awareness and educate the public on the bacteriological quality of fish sold in open air market centre in Okepedi, Itu, Akwa Ibom State, Nigeria and the risks associated with consumption of contaminated and improperly cooked fresh fish.

2. MATERIALS AND METHODS

2.1 Source and Collection of Samples

The fishes investigated in this study include fresh samples of *Clarias gariepinus* (Plate 1), *Oreochromis niloticus* (Plate 2), *Tilapia guinensis* (Plate 3) 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. 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. The Okopedi River where the fishes were harvested is a typically freshwater in which the social, economic and cultural lives of the people living the catchment are significantly linked to.

Apparently twelve samples of three different fishes were obtained directly from the fish sellers. The samples were collected according to the method described by Mhango et al. [20] in which the fish were collected into sterile polythene bags, preserved in ice-packed coolers and immediately transported to laboratory of the for analysis.

2.2 Preparation of Fish for Analysis

For the analysis of internal organs, fish was placed on 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.

2.3 Bacteriological Analysis of the Fish

Bacteriological analyses of the fish organs were conducted based on standard microbiological

Plate 1.*Clarias gariepinus*

Plate 2. *Oreochromis niloticus*

Plate 3. *Tilapia guinensis*

methods. The analysis was conducted in line with the submissions and approved quality assurance and quality control plan for microbial studies. The quality control and quality assurance policy adopted covers all aspects of the activities from sample collection, to accurate preservation techniques through laboratory analysis to data validation. Every sample was aseptically collected and preserved appropriately, and analyzed using scientifically accepted techniques and high quality standard non-expired reagents and culture media.

2.4 Enumeration of Bacteria Loads

Crushed and homogenized organ of the fish samples each from skin, intestine and gills 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) and one ml of the desired dilution levels were plated in triplicates on the appropriate media using the pour plate method.

2.4.1 Total heterotrophic bacterial count

The total heterotrophic bacteria counts (THBC) of the samples was determined by plating 1.0 ml of 10^{-4} dilution of the samples on nutrient agar (NA) incubated at 37°C for 24 h using pour plate technique, a method recommended by Harrigan and McCance [21].

2.4.2 Coliform count

The total coliform counts of the samples was determined by plating 1.0 ml of 10^{-4} dilution of the samples on MacConkey agar incubated at 37°C for 24 h using pour plate technique [22].

2.4.3 *Escherichia coli* (faecal coliform count)

The faecal coliform counts of the samples was also determined by plating 1.0 ml of 10^{-4} dilution of the samples on Eosine Methylene Blue (EMB) agar incubated at 37°C for 24 h using pour plate technique [22].

2.4.4 *Salmonellae and Shigellae* count

The *salmonella*e and *shigellae* counts of the samples was determined by plating 1.0 ml of 10^{-4} dilution of the samples on *salmonella shigella* agar (SSA) incubated at 37°C for 24 h using pour plate technique [22]. After incubation, the plates were counted and recorded in cfu/g.

2.4.5 *Vibrio* count

The *Vibrio* count of the samples was determined by plating 1.0 ml of 10^{-4} dilution of the samples on Thiosulphate – Citrate – Bile salts – Sucrose agar (TCBS), incubated at 37°C for 24 h using pour plate technique (Cheesbrough, 2000). After incubation, the plates were counted and recorded in cfu/g.

2.5 Isolation and Maintenance of Stock Cultures of Pure Bacterial Isolates

Discrete colonies of bacterial isolates were respectively and repeatedly sub-cultured onto Petri dishes containing freshly prepared nutrient agar to obtain pure (cultures) isolates. Thereafter, the pure microbial isolates were stocked in McCartney bottles containing 10% of sterilized Glycerol solution (autoclaved at the temperature of 121°C for 15 minutes) and kept refrigerated at 4°C for subsequent characterization and further use.

2.6 Characterization of 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 [23]. The biochemical tests used for characterization of the isolates include Citrate, Oxidase, indole, Urease, Coagulase, Catalase, Methyl red and VogesProskauer, Motility, Starch hydrolysis, Carbohydrate fermentation tests (Glucose, Sucrose, Lactose, Maltose, Fructose, Galactose and Dextrose).

2.7 Evaluation of Virulence Factors Producing Potentials of the Isolates

2.7.1 Determination of hemolytic activity

Hemolytic activity of the strains was determined on blood agar with sheep blood as described by Citak et al. [24] and Jurkovic et al. [25] to detect α, β, as well as γ haemolysis.

2.7.2 Production of hydrolytic enzymes

Bacterial isolates were screened for production of hydrolytic enzymes such as lipase, urease, and gelatinase 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 [25].

2.8 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 (α = 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 *Clarias gariepinus*, *Oreochromis niloticus* and *Tilapia guinensis* are presented in Tables 1- 3 respectively while Fig. 1. shows the mean counts of the various bacterial group encountered in the fish. The results revealed varying levels of microbial contamination of the fish samples. The skin of *Clarias gariepinus* had the highest bacterial load ($8.10 \times 10^5 \pm 0.06$) cfu/g. followed by the intestine ($6.12 \times 10^5 \pm 0.03$)cfu/g. while the intestine haboured the highest densities of coliform ($3.27 \times 10^4 \pm 0.06$)cfu/g and faecal coliform ($3.87 \times 10^3 \pm 0.06$) cfu/g. No faecal coliform was found in the gills of *Clarias gariepinus.* The skin and intestine of the fish did not habour any viable cell of *Vibrio* while remarkable loads of *salmonellae/shigellae* were encountered. Similar observations were made for *Oreochromis niloticus* (Table 2) where high numbers of heterotrophic bacteria were found on the skin ($6.30 \times 10^5 \pm 0.10$) cfu/g and intestine ($3.33 \times 10^5 \pm 0.06$)cfu/g of the fish. Total coliform count was also high ($3.87 \times 10^4 \pm 0.06$) cfu/g in the fish intestine. No faecal coliform was found in the gill and intestine of the fish however *Salmonella* and *Shigella* count ($5.03 \times 10^3 \pm 0.06$) cfu/g was remarkable in gill but absent in the intestine of the fish.

The bacterial properties of *Tilapia guinensis* presented in Table 3 showed that the loads of hetrerotrophic bacteria was high ($7.03 \times 10^5 \pm 0.06$) cfu/g in intestine of the fish while coliform count was high in the gills ($4.80 \times 10^4 \pm 0.10$) 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 ($3.13 \times 10^3 \pm 0.06$) cfu/g although it was not detected on the skin of the fish.

3.1.2 Bacterial species isolated from fish sold in okopedi open market centre

The morphological and biochemical characteristics of the bacterial isolates from skin, gills and intestine of *Clarias gariepinus*, *Oreochromis niloticus* and *Tilapia guinensis* are presented in Table 4. A total of 13 bacterial isolates were characterized. Their incidence and distribution varied with the fish as presented in Fig. 2.

For *Clarias gariepinus* (Table 5), 9 bacterial species were isolated from the fish skin, 6 from gills and 9 from intestine. *Salmonella* sp (100%) was found to be the most abundant bacterial species in *Clarias gariepinus. Oreochromis niloticus* (Table 6) on the other hand recorded 5 bacterial species from skin, 4 from gills and 6 from intestine with *Staphylococcus aureus* (100%) being the most prevalent bacterial species. Also in *Tilapia guinensis* (Table 7), 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.

Table 1. Microbial loads (cfu/g) of *Clarias gariepinus* sold in Okopedi open market centre

Fish organs	THBC($\times 10^5$)	TCC($\times 10^4$)	FCC($\times 10^3$)	SSC($\times 10^3$)	VC($\times 10^3$)
Skiln	8.10 ± 0.10^a	3.13 ± 0.06^a	3.00 ± 0.10^a	2.20 ± 0.04^a	0.00 ± 0.00^a
Gill	2.70 ± 0.01^b	1.77 ± 0.06^b	0.00 ± 0.00^b	2.03 ± 0.06^b	1.13 ± 0.06^b
Intestine	6.12 ± 0.03^c	3.27 ± 0.06^a	3.87 ± 0.06^c	2.37 ± 0.06^a	0.00 ± 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 2. Microbial loads (cfu/g) of *Oreochromis niloticus* sold in okopedi open market centre

Fish type	THBC(×10^5)	TCC(×10^4)	FCC(×10^3)	SSC(×10^3)	VC(×10^3)
Skin	6.30±0.10^b	3.73±0.06^b	2.80±0.06^b	4.70±0.08^b	2.20±0.10^b
Gill	3.13±0.06^a	2.27±0.06^a	0.00±0.009^b	5.03±0.06^b	1.33±0.06^a
Intestine	3.33±0.06^a	3.87±0.06^b	0.00±0.00^a	0.00±0.00^a	3.10±0.10^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 3. 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

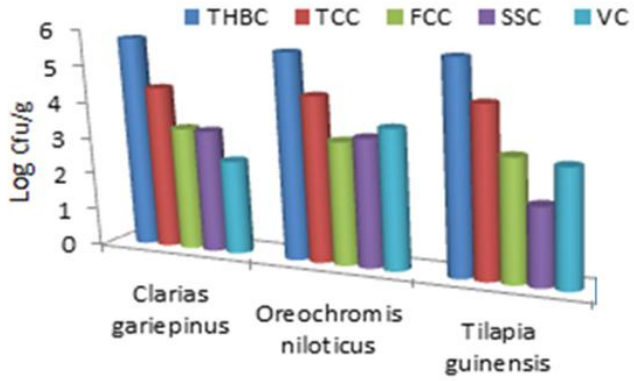

Fig. 1. Summary of the mean count of the various bacterial groups in the different fish

Key: THB = Total heteotrophic bacteria count, TCC = Total coliform count, FCC = Faecal coliform count, SSC = Salmonella shigella count, VC = Vibrio count.

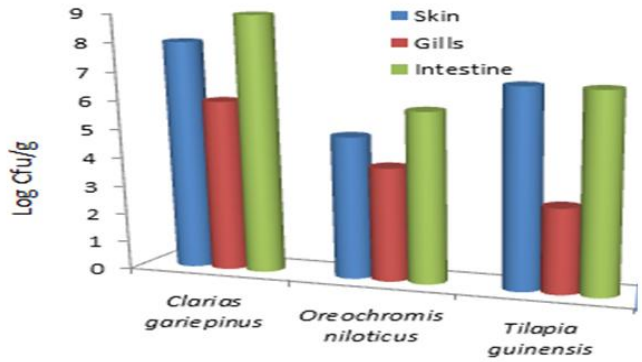

Fig. 2. Summary of occurrence of microbial species in fishes from okopedi open market in itu local government area of akwa ibom state

Table 4. Biochemical characterization of the bacterial isolates

Isolate code	Gram's stain	Shape	Catalase	Coagulase	Citrate	Motility	Indole	MR	VP	Oxidase	Sugar fermentation						Probable organism
											Glucose	Lactose	Sucrose	Manitol	Galactose	Maltose	
MM_2I	-	Rod	+	+	+	-	+	-	+	-	A	AG	AG	A	AG	AG	*Salmonella* sp
EP_3I	-	Rod	+	-	+	+	-	+	+	-	A	A	A	A	A	A	*Serratia* sp
NM_IG	-	Rod	+	-	+	-	-	-	+	-	AG	AG	-	A	-	-	*Enterobacter aerogene*
SP_2I	-	Rod	+	-	-	-	-	-	-	-	-	-	-	AG	-	AG	*Shigella* sp
EM_3I	-	Rod	+	-	-	+	+	+	-	-	AG	AG	AG	AG	AG	AG	*E. coli*
EP_2I	-	Rod	+	-	+	-	-	-	+	-	AG	A	A	A	A	A	*Klebsiella pneumonia*
EM_2G	-	Rod	+	-	+	+	-	+	-	-	AG	-	AG	-	A	-	*Proteus vulgaris*
EP_1S	+	Cocci	-	-	-	+	+	-	+	-	AG	AG	AG	AG	AG	AG	*Streptococcus* sp
MM_2G	+	Cocci	+	-	+	-	+	-	-	+	AG	-	AG	AG	-	AG	*Micrococcus* sp
NP_2S	+	Cocci	-	-	+	+	-	+	-	-	AG	AG	AG	AG	-	A	*Enterococus* sp
NM_1I	+	Rod	+	-	+	+	-	-	+	-	A	-	A	A	A	A	*Bacilus subtilis*
NM_2S	+	Cocci	+	+	-	-	-	+	-	-	AG	AG	AG	AG	AG	AG	*Staphylococcus aureus*
NM_2G	-	Comma	+	-	-	+	-	-	-	-	AG	AG	AG	-	AG	AG	*Vibrio sp*
NM_23	-	Rod	+	+	-	-	-	-	+	+	AG	-	A	AG	A	-	*Pseudomonas aeruginosa*

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

Organisms	Skin	Gills	Intestine	Occurrence rate (%)
Micrococcus sp	+	-	+	66.7
Streptococcus sp	-	+	-	33.3
Staphylococcus aureus	+	+	-	66.7
Klebsiella pneumonia	+	-	+	66.7
Enterobacter aerogene	+	-	+	66.7
Salmonella sp	+	+	+	100
Shigella sp	+	-	-	33.3
Vibrio sp	-	+	-	33.3
Bacilus subtilis	-	-	+	33.3
Escherichia coli	+	-	+	66.7
Enterococus sp	-	+	+	66.7
Pseudomonas aeruginosa	+	-	-	33.3
Proteus vulgaris	+	-	+	66.7
Serratia sp	-	+	+	66.7
Species richness	9	6	9	

Table 6. Occurrence of the diverse bacterial species in *Oreochromis niloticus* sold in Okopedi open market centre

Organisms	Skin	Gills	Intestine	Occurrence rate (%)
Micrococcus sp	-	-	+	33.3
Streptococcus sp	-	+	-	33.3
Staphylococcus aureus	+	+	+	100
Enterobacter aerogene	+	-	+	66.7
Salmonella sp	+	-	-	33.3
Shigella sp	-	-	+	33.3
Bacilus subtilis	+	-	-	33.7
Escherichia coli	+	-	-	33.3
Enterococus sp	-	+	+	66.7
Proteus vulgaris	-	-	+	33.3
Serratia sp	-	+	+	66.7
Species richness	5	4	7	

Table 7. 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 8. Virulence factors producing potential of the diverse bacterial isolates

Organism	Haemolysis	Lipase	Gelatin	Urease
Salmonella sp	A	+	-	-
Serratia sp	A	-	-	-
Shigella sp	A	-	-	-
Enterobacter aerogene	A	-	-	-
E. coli	A	+	+	-
Klebsiella pneumoniae	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	+	-	-

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 8) 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.

3.2 Discussion

The health conscious consumers made fresh aquatic produce a growing industry. However, the industry is facing new challenges, including the protection of consumers against microbiological hazards [26]. The present study has shown that fresh fishes 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. However their densities varied with the type of fresh produce and between the various organs analyzed. The highest heterotrophic bacterial load was obtained from *Tilapia guinensis* with mean count of 5.86 x 10^5±0.83 cfu/g while *Oreochromis niloticus* had the least count of 2.21 x 10^3±1.46 cfu/g. However the values recorded for the exposed fishes skin and intestine were remarkably higher than 1.2 x10^2 cfu/g reported by Pastuszka et al. [27] for fresh produce. Coliform count was as high as 3.87 x 10^4 ± 0.06 cfu/g in the fish intestines. 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. There was a significant difference in the mean load of microorganisms isolated from the fishes. This result agrees with the work of Donderski et al. [28] where there was variation in bacterial loads in different fishes.

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.

This study has revealed the prevalence of *E. coli* in many of the samples analyzed. It implies that fish from open markets in Okopedi Fishing settlement are heavily contaminated with enteric bacteria. More than 60% of the samples were contaminated with *E. coli.* 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.

For fresh produce, *E. coli* is currently the best available indicator of fecal contamination. *E. coli* was isolated from many fresh produce and all the items have similar prevalence of *E. coli* contamination. Other authorities [29] have reported positive rates of *E. coli* in fresh produce. There are various ranges of bacterial counts from previous studies from other countries [29]. *Salmonella* has also been isolated from fresh, frozen, canned and sun dried marine fish products [30]. 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 occurrence in fresh seafood has previously been reported [31]. Their occurrence varied with the type of produce and organ analyzed. *Clarias gariepinus* haboured more species of bacterial contaminants and more bacterial species were encountered in the fish intestine. *Staphylocccus aureus*, *Micrococcus* sp and *Vibrio* sp were the most occurring bacterial species (100%) in the fresh aquatic produce. Ebinyo et al. [32] assessed the microbial quality of *Trachurus trachurus* sold in some markets of three South-south States of Nigeria. The results obtained showed diverse microbial contaminants including *Staphylococcus aureus*, *Escherichia coli, Bacillus, Salmonella, Shigella, Pseudomonas, Micrococcus, Proteus* and *Streptococcus* sp as well as fungal contaminants.

This study provides the very first data about pathogenic potential contaminants in raw fishes sold in Okepedi open air market centre, Itu, Akwa Ibom State. The ability of pathogenic bacteria to cause disease in a susceptible host is influenced by their ability to produce virulence factors. 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. Pathogenicity is the ability to produce disease in a host organism. Microbes express their pathogenicity by means of their virulence, a term which refers to the degree of pathogenicity of the microbe. Hence, the determinants of virulence of a pathogen are any of its genetic or biochemical or structural features that enable it to produce disease in a host. *Salmonella* sp, *Serratia* sp, *Enterobacter aerogene*, *E. coli, Proteus vulgaris, Micrococcus* sp, *Bacilus subtilis*, *Staphylococcus aureus* and *Vibrio* sp showed α haemolytic activity while *Klebsiella pneumonia*, *Streptococcus* sp, *Enterococus* sp and *Pseudomonas aeruginosa* showed β haemolytic activity in this research. *Salmonella* sp, *E. coli, Proteus vulgaris, Klebsiella pneumonia, Streptococcus* sp, *Enterococus* sp, *Pseudomonas aeruginosa*, *Bacilus subtilis*, *Staphylococcus aureus* and *Vibrio* sp were 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* sp showed urease producing activity. *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. [33] 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 [34]. Species of *Enterococcus,* e.g. *E. faecalis* can cause endocarditis as well as bladder, prostate and epidermal infections [34, 35]. Although commonly found in the lower intestine of warm-blooded organisms, most strains of *Escherichia coli* are harmless but some serotypes can cause serious food poisoning in human and are occasionally responsible for product recalls due to food contamination [36]. *E. coli* is a Gram-negative, rod-shaped bacterium commonly found in the intestinal tract of humans. It is also the most implicated pathogen on diarrheal cases worldwide [37]. To date there are five pathogenic classes of *E. coli* recognized: these are the Enterotoxigenic *E. coli* (ETEC), Enteroinvasive *E. coli* (EIEC), Enteropathogenic *E. coli* (EPEC), Enteroaggregative *E. coli* (EAEC) and Enterohemorrhagic *E. coli* (EHEC). ETEC produces enterotoxins and can mostly attack infants and travelers. EIEC has the ability to attack epithelial cells and can cause dysentery-like diarrhea with fever. EPEC can cause lesions to the intestinal mucosa that could lead to watery, and sometimes bloody diarrhea. EAEC is a cause of persistent diarrhea and malnutrition in children, with the ability to attach to tissues in an aggregative manner. EHEC can produce verotoxin or shiga-like toxins (*stx*) and is the primary cause of bloody diarrhea and hemorrhagic colitis [38]. The presence of *E. coli* indicates fecal contamination in the environment. However, they are released into the atmosphere when the fecal matter is disturbed [28]. Thermotolerant or fecal coliform *E. coli* are able to grow at higher temperatures typically 44.5 - 45.5°C for 24 - 48 hours. This potential poses a serious risk if aquatic produce are not properly cooked before consumption.

Staphylococcus aureus is associated with a wide among 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 [34]. According to ICMSF [39], *Salmonella* and *Vibrio cholerae* should not be found in sea food products. However, in the present study, high numbers of *Salmonella* was also 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 [40]. *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 [41] and in 41% of cases during 1992 -2000 in the UK. 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.

4. CONCLUSION

Fishes are among the major aquatic produce encountered in the Niger Delta of Nigeria and are the main source of protein in wetland or riverside areas. This study is among the few reported cases of bacterial contamination of aquatic produce in the Niger Delta region of Nigeria. The study has revealed the presence of indicator microorganisms of fecal pollution, opportunistic and pathogenic bacteria to humans in the fish and shrimp samples. The study has specifically revealed that the fish and shrimps were contaminated with faecal matter as depicted by the high densities of *E. coli* as well as pathogenic microorganisms such as *Staphylococcus aureus* and *Streptococcus* sp in the aquatic produce and there is potential risk of eating these fishes and shrimps if not properly cooked. The ability of bacteria to cause disease depends to a large extent on the expression of virulence factors, which help them to invade the host, produce pathological effects and evade host defenses. The study has clearly shown:

(i) The unsanitary conditions in which aquatic produce are handled and displayed for sales in our fishing settlements

(ii) That fresh aquatic produce are heavily contaminated with bacteria including fecal coliform and potential pathogens

(iii) That a good number of the bacterial contaminants associated with the fresh fish and shrimps can elaborate virulent factors and may cause infections in compromised host immune systems.

(iv) The research findings suggest that there is a potential food safety risk from fresh produce on sale at open markets in Okopedi Fishing Settlement.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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