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Prevalence and antibiogram of bacteria isolated from *Tilapia zilli* sold in markets in port-harcourt metropolis

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Abstract
Fish is a good source of food to man and its flesh is rich in proteins, minerals and fat. It is however, the fishes are susceptible to a wide variety of bacterial pathogens which are capable of causing diseases. The study was carried out to determine prevalence and antibiogram of the bacterial flora of *Tilapia zilli* sold in three markets in Port Harcourt. The flesh, gills and intestine were aseptically dissected using and subjected to standard microbiological techniques. A total of seven (7) bacterial species was isolated and identified as *Listeria spp Salmonella spp*, *Bacillus spp*, *Shigella spp*, *Klebsiella spp*, *Vibrio spp* and *Escherichia coli*. The results indicated that Borokiri had the highest total heterotrophic bacterial (THB) count (6.60±0.14 cfu/g) from the flesh, (5.50±0.14 cfu/g) from the gills and (3.15±0.07 cfu/g) from the intestine while the least THB count was found in Iwofe with (1.08±0.7 cfu/g) from the fish flesh (1.77±0.02 cfu/g) gills and (1.90±0.14 cfu/g) intestine. It was also recorded that Iwofe had the no *Pseudomonas* (TPC) count in the different parts of the fish. Borokiri had the highest prevalence of the bacterial isolate (25%) followed by Eagle Island (15%) and Iwofe (10) bacterial isolates. The susceptibility pattern indicated that *Listeria spp*, *Shigella spp*, *Salmonella spp* were resistant to all antibiotics used (amoxicillin, erythromycin, nitrofurantoin, augmentin, chloramphenicol, gentamicin, tetracycline and ciprofloxacin (100%). *Klebsiella species* was the only organism that was 100% susceptible to erythromycin.

Keywords: Antibiogram, prevalence, antibiogram, *Tilapia zilli*

Introduction
Fish is an important component of diets around the world. About 1 billion people rely on fish as their main source of animal protein [1]. Fish provides the much needed protein to people living in developing countries at affordable prices. It feeds millions of people daily and sustains many through employment in services related to fish and fish products. The nutritional attributes of fish are highly praised as it is rich in the essential amino acids, has high quality vitamins and its fatty acids fraction have well established health benefits. Therefore, its availability in many developing countries should enable fish to contribute significantly to a healthy and balanced diet in these countries. The risks of food borne disease associated with fishes are related to inland or coastal ecosystems, where the potential of environmental contamination is greater when compared with capture fisheries. Most of the food safety hazards associated with products from aquaculture can be controlled by good fish farm management practices and appropriate consumer education regarding such risks as eating raw or partially cooked products that may contain pathogenic bacteria [2].

Fish is an essential source of protein providing 16% of the animal protein consumed by different people in the world. It is estimated that close to one billion people world- wide rely on fish as their primary source of animal protein (FAO, 2000). Some of the bacterial species found in fish poses serious diseases when eaten by humans, the colonization of bacteria can be seen on fish skin and gills due to constant exposure to contaminated water, while the digestive tract may be affected due to contaminated feed or water. When these fishes which are contaminated are being consumed by humans, it causes serious diseases such as, Cholera which is a highly contagious disease caused by infection of the small intestines with *Vibrio cholera*. It is characterized by massive acute diarrhoea, vomiting and dehydration and *Vibrio cholerae* is often transmitted by water or by eating improperly cooked fish or fish products that have been in contact with contaminated water, *Listeria monocytogenes* which is the causative agent of listeriosis, a foodborne infection in humans poses serious health implications. Contamination of seafood may often occur from contaminated coastal areas

and from contaminated surroundings. *Salmonella species* can also contaminate water sources due to poor sanitation and incorrect disposal of human and animal waste. The risks of food borne disease associated with fishes are related to inland or coastal ecosystems, where the potential of environmental contamination is greater. These diseases associated with fish and water are usually caused by anthropogenic activities, therefore, it is important to properly cook fish before consumption. Food products that show evidence of faecal contamination are generally regarded as a greater risk to human health, as they are likely to contain human-specific enteric pathogens. The rivers that harbour the fish may be the source of contaminants due to indiscriminate deposition of human, animal excreta and other environmental wastes into natural water, land and during the rainy season especially, as the faecal matter from various sources are washed from contaminated land into different water bodies. Free roaming animals and pets especially dogs also contribute to faecal contamination of surface water. Run-off from roads, parking lots and yards can carry animal wastes into natural water course and ponds. Birds can also be a significant source of bacteria. Swans, Geese and other water fowl can all elevate bacteria counts in water bodies and ponds [3]. Due to the contamination of these organisms in the fish and water bodies, it is important to check the prevalence and antibiogram of *Tilapia zilla* to minimize the level contamination as well as reduce health problems to also to ensure a better understanding of ecology and distribution of pathogens in the food chain.

Materials and Methods

Description of Study Area

The stud area was three markets in Port Harcourt (Eagle Island, Iwofe and Borokiri). Eagle island is located on the Southern west of Port Harcourt between longitude 040 46743'N and 0070 00557; latitude 040 48217'N and 0060 48989'E. Iwofe is situated in Obio Akpor LGA with geographical coordinates 404846.551'N and 605612.0906'E. Borokiri with coordinates 404451.0475'N and 70242.2074'E. These sample locations were selected because they serve as the major markets were fish can be easily bought and they are bounded by rivers from which the fishes are caught. Fig 2.1 shows a map of Rivers State highlighting the three (3) sample locations.

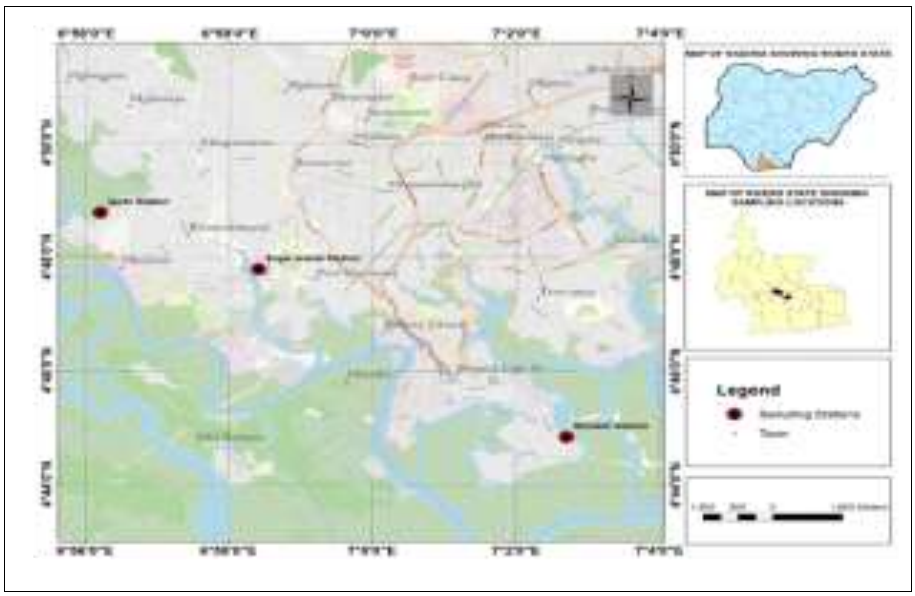

Fig 1: Map showing sampling locations

Sample Collection

The fish (*Tilapia zilli*) samples were bought from the three (3) markets and stored in sterile polythene bags. The samples were put in ice packed and were conveyed to the microbiology laboratory in Rivers State University within 4 hours of sample collection. Once in the laboratory, the fish was aseptically dissected to get the gills, intestine and flesh for microbiological analyses.

Microbiological Analyses

Bacterial Enumeration

The gills, flesh and intestines of the fish (*Tilapia zilli*) were used in the stock preparation. The parts were dissected and weighed, five grams each of the parts were separately added to 45ml of 0.1% of peptone water to give $10^{-1.}$

After thorough shaking, further 10 fold (v/v) serial dilutions were made by transferring 1ml of the original solution to fresh peptone water to give a range of 10^{-2} dilutions. This was repeated until a dilution of 10^{-6} was reached. Aliquot (0.1 ml) of the appropriate dilutions were inoculated unto fresh agar plates in duplicate and spread using a glass spreader. Inoculated plates were incubated at 37 °C for 24-48 hours.

Bacterial Isolation/Identification and Preservation

Pure cultures of bacteria were obtained by aseptically streaking representative colonies of different morphological types on freshly prepared nutrient agar plates. Discreet colonies were transferred into 10% sterile glycerol solution and preserved for further analysis, this served as pure culture for further biochemical test such as catalase, oxidase, citrate utilization, indole production, methyl red test, sugar fermentations and starch hydrolysis [4, 5]. The identification of bacterial isolates was confirmed by comparing them with Bergey's Manual of Determinative Bacteriology.

Antibiotic Susceptibility Test

The standard agar-disc-diffusion method (Kirby Bauer technique) was used to examine the antibiotic susceptibility pattern of the bacteria using Mueller-Hinton agar (Difco, Detroit, MI). Disc impregnated with Ampicillin, Erythromycin, Tetracycline, Gentamicin, Ciprofloxacin, Amoxicillin were used. After inoculation of the standardized bacteria the disc are placed on the plate allowing it to make contact with the agar. The plates were then inverted and incubated at 37 °C for 24 h. After incubation, the plates were examined, and the zone of inhibition was measured in mm [6].

Data Analyses

The data obtained was analysed using analysis of variance (ANOVA) to test for significance and where differences occur, Duncan multiple range test was used to separate the means using the Statistical Package for Social Science (SPSS) version 25 [7].

Results and Discussion

Mean Microbial Count of *Tilapia zilli* in Iwofe

Results of the mean value of the microbial counts isolated from the different parts of the fish (gill, flesh and intestine) from Iwofe is presented in table 1. Total heterotrophic bacterial (THB) count was highest in the fish intestines ($1.90x10^6$ cfu/g) and the least THB count was in the fish flesh $1.08 x10^6$ cfu/g. In Salmonella-shigella agar (SSA), the fish intestine had the highest count ($2.07x10^5$cfu/g) while flesh had no growth. The Total coliform count (TCC) was highest in the intestine ($1.90x10^5$cfu/g) and the least count was found in the flesh ($1.48 x10^5$cfu/g). For total *Vibrio* counts, the intestines had the highest count of $4.50x10^3$ Cfu/g while the flesh had the least count of $2.85x10^3$cfu/g. The intestines had the highest *Staphylococcus* count (TSC) ($1.80x10^3$cfu/g) and the least count was found in the flesh ($1.14x10^3$ Cfu/g).

Table l: Mean value of Bacterial counts (CFU/g) of different parts of the fish collected from Iwofe

SAMPLES	THB×10^6	SSA×10^5	TCC×10^5	TCBS ×10^3	TPC ×10^3	TSC ×10^3
Flesh	1.08±0.7	0.00±0.00^a	1.48±0.07^a	2.85±0.07^a	0.00±0.00^a	1.14±0.01^a
Gills	1.77±0.02	1.36±0.14^b	1.55±0.02^a	3.70±0.14^a	0.00±0.00^a	1.23±0.16^a
Intestines	1.90±0.14	2.07±0.04^b	1.90±0.07^b	4.50±0.71^b	0.00±0.00^a	1.80±0.14^b

Means with same alphabet across the column shows no significant difference ($p \geq 0.05$)
Key: THB = Total Heterotrophic Bacteria, SSA = *Salmonella- Shigella Agar*, TCC = Total Coliform Count, TCBS = Thio-sulphate Citrate Bile Salt Sucrose Agar, TPC = Total *Pseudomonas* Count, TSC = Total *Staphylococcus* Count.

Mean Bacterial Count of *Tilapia zilli in E*agle Island

Results of the mean bacterial counts of the different parts of the fish (gill, flesh and intestine) bought in Eagle Island is presented in Table 2. THB counts was highest in the fish intestines ($2.24x10^6$cfu/g) and the lowest in the flesh ($1.38x10^6$cfu/g). The intestine had the highest *Salmonella-Shigella* count (SSA) ($8.85x10^5$cfu/g) while the least count was recorded in the flesh ($4.85x10^5$cfu/g). The TCC was highest in the flesh ($8.75x10^5$cfu/g) while the least count was recorded in the intestine ($1.09 x 10^5$cfu/g). The *Vibrio* counts showed that the fish intestines had the highest ($3.85x10^3$cfu/g) counts while the least *Vibrio* count was recorded in the flesh ($1.15x10^3$cfu/g). The intestines had the highest TPC ($1.10x10^3$cfu/g) while the least TPC count was recorded in the fish flesh. The TSC was highest in the intestine ($3.25x10^3$cfu/g) while the least TSC was recorded in the flesh ($1.15x10^3$cfu/g).

Table 2: Mean value of Bacterial counts (CFU/g) of different parts of the fish collected from Eagle Island

SAMPLES	THB×10^6	SSA×10^5	TCC×10^5	TCBS×10^3	TPC×10^3	TSC×10^3
Flesh	1.38±0.01^a	4.85±0.02^a	8.75±0.21^a	1.15±0.07^a	0.00±0.00^a	1.15±0.21^a
Gills	1.87±0.01^b	6.35±0.07^b	1.85±0.07^b	2.20±0.14^b	1.05±0.07^a	2.05±0.07^b
Intestines	2.24±0.014^c	8.85±0.07^c	1.09±0.04^c	3.85±0.07^c	1.10±0.14^b	3.25±0.21^c

Means with same alphabet across the column shows no significant difference ($p \geq 0.05$)
Key: THB = Total Heterotrophic Bacteria, SSA = *Salmonella- Shigella Agar*, TCC = Total Coliform Count, TCBS = Thio-sulphate Citrate Bile Salt Sucrose Agar, TPC = Total *Pseudomonas* Count, TSC = Total *Staphylococcus* Count

Mean Bacterial Count of *Tilapia zilli in* Borokiri

Results of the mean bacterial count of the different parts of the fish (gill, flesh and intestine) bought in Eagle Island is presented in table 3. THB count was highest in the flesh ($6.60x10^6$cfu/g) while the least THB count was recorded in the intestines ($3.15x10^6$cfu/g). The fish intestine had the highest SSA count ($6.50x10^5$cfu/g) while the least count was recorded in the flesh ($1.85x10^5$cfu/g). The TCC was highest in the gills ($4.85x10^5$cfu/g) while the least count was recorded in the flesh ($3.30x10^5$cfu/g). The intestines had the highest *Vibrio* count ($3.00x10^3$cfu/g) while the least *Vibrio* count was recorded in the flesh ($1.80x10^3$cfu/g). The TPC count was highest in the intestines ($1.60x10^3$cfu/g) while the least count was recorded in the flesh. The TSC count was highest in the flesh ($5.50x10^3$cfu/g) while the least count was recorded in the intestines ($1.70x10^3$cfu/g)

Table 3: Mean Bacterial Counts (CFU/g) of different Parts of the Fish Collected from Borokiri

SAMPLES	THB×10^6	SSA×10^5	TCC×10^5	TCBS×10^3	TPC×10^3	TSC×10^3
Flesh	6.60±0.14^C	1.85±0.07^a	3.30±0.14^a	1.80±0.00^b	0.00±0.00^a	5.50±0.71^c
Gills	5.50±0.14^b	2.30±0.14^a	4.85±0.07^c	2.20±0.14^c	0.00±0.00^a	3.80±0.14^b
Intestines	3.15±0.07^a	6.50±0.71^b	3.50±0.71^b	3.00±0.00^a	1.65±0.07^b	1.70±0.14^a

Means with same alphabet across the column shows no significant difference ($p \geq 0.05$)
Key: THB = Total Heterotrophic Bacteria, SSA = *Salmonella- Shigella* Agar, TCC = Total Coliform Count, TCBS = Thio-sulphate Citrate Bile Salt Sucrose Agar, TPC = Total *Pseudomonas* Count, TSC = Total *Staphylococcus* Count

Prevalence of Bacteria in *Tilapia zilli*

Results of the prevalence of bacteria isolated from Borokiri, Eagle Island and Iwofe are presented in (Fig. 2-4) *Escherichia coli Bacillus* had the highest prevalence of (27.3%;33.3% and 42.9%) in the fish intestine and the least occurrence is the *Listeria spp*, *Shigella* spp, *Salmonella spp* and *Shigella* having no occurrence. In the fish gills, *Salmonella species Vibrio*, *Bacillus* and *Listeria species* had the highest occurrence of (25%; 33.3% and 60%) and the least occurring bacteria are *Vibrio spp*, *Escherichia. Coli*, *Listeria spp*, *Salmonella spp*, *E. coli*, *Shigella spp* and *Klebsiella* spp with no occurrence. In the fish flesh, *Bacillus* spp, *Listeria* spp and *Vibrio spp* had the highest occurrence (40%; 100% and 66.7%) and the least occurrence is the *Salmonella spp*, *Vibrio spp*, *Escherichia. coli Salmonella spp*, *Vibrio spp*, *Shigella spp*, *Klebsiella spp* and *Bacillus spp*.

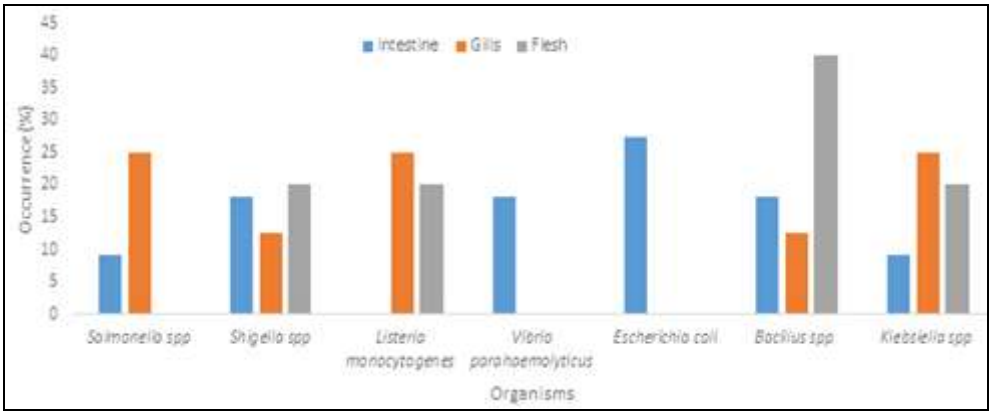

Fig 2: Prevalence of Bacteria from Borokiri

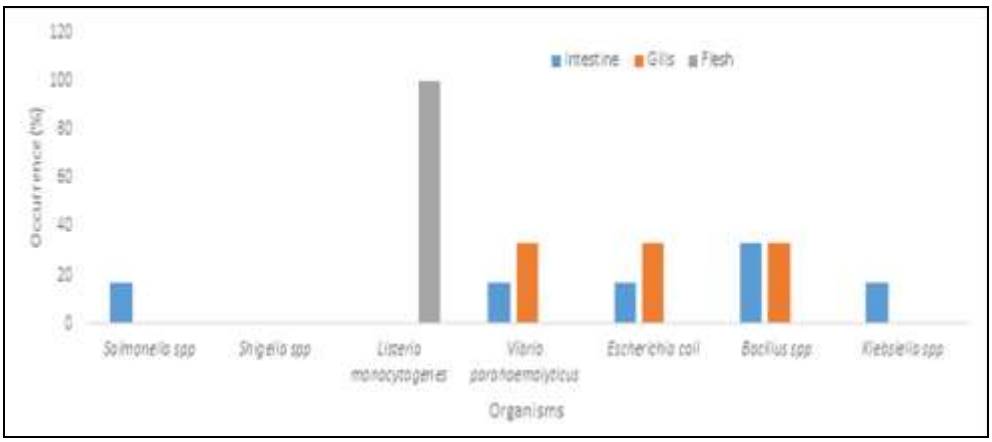

Fig 3: Prevalence of Bacteria from Eagle Island

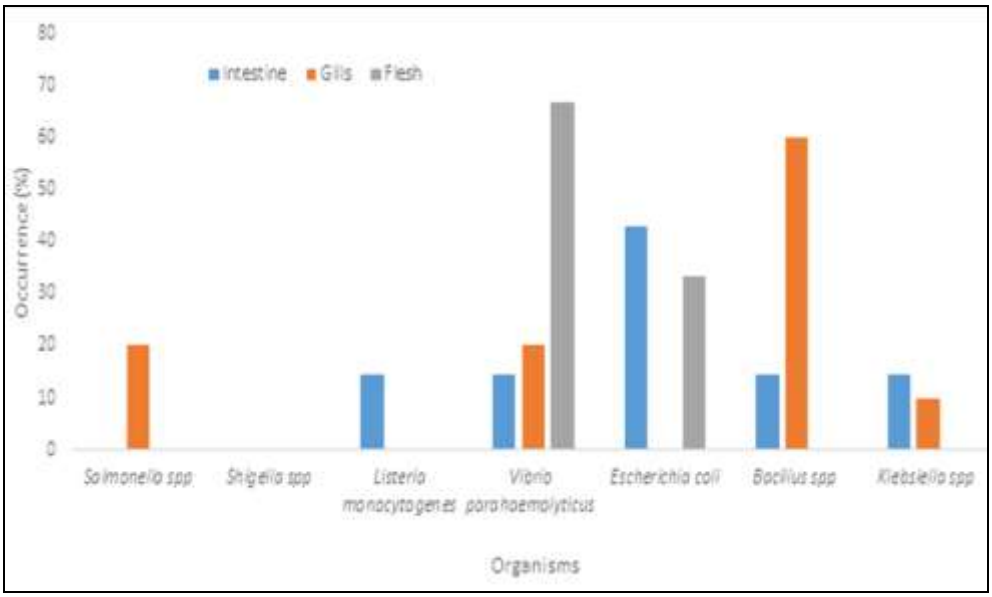

Fig 4: Prevalence of Bacteria from Iwofe

3.5 Susceptibility Pattern of *Listeria* and *Shigella* isolated from *Tilapia zilli*

Results of the susceptibility pattern of *Listeria species* and *Shigella species* are presented in table 4. *Listeria species* was highly resistant to amoxicillin, erythromycin, nitrofurantoin, augmentin, chloramphenicol, gentamicin, tetracycline and ciprofloxacin (100%). Also *Shigella species* was highly resistant to amoxicillin, erythromycin, nitrofurantoin, augmentin, chloramphenicol, gentamicin, tetracycline (100%). Results of susceptibility pattern of *E. coli* and *Klebsiella species* are presented in table 5. *Escherichia coli* was highly resistant to amoxicillin, erythromycin, nitrofurantoin, augmentin, chloramphenicol, gentamicin, and ciprofloxacin (100%). *Klebsiella species* was highly resistant to amoxicillin, nitrofurantoin, augmentin, chloramphenicol, gentamicin and ciprofloxacin (100%) but was highly susceptible to erythromycin (100%). Results of susceptibility Pattern of *Vibrio species* and *Bacillus species* isolated from *Tilapia zilli* are presented in table 6. *Vibrio species* was highly resistant to amoxicillin, nitrofurantoin, gentamicin, tetracycline (100%) and 75% resistant to erythromycin, augmentin and chloramphenicol and susceptible to ciprofloxacin. *Bacillus spp* was highly resistant to amoxicillin, nitrofurantoin, gentamicin, tetracycline (100%) and was (66.7%) resistant to both erythromycin and ciprofloxacin, 3.10. Susceptibility Pattern of *Salmonella* isolated from *Tilapia zilli.* The susceptibility of *Salmonella spp* to antibiotics is presented in table 7, indicating that they were highly resistant to amoxicillin, erythromycin, nitrofurantoin, augmentin, chloramphenicol, gentamicin, tetracycline and ciprofloxacin (100%).

Table 4: Susceptibility Pattern of *Listeria* and *Shigella* isolated from *Tilapia zilli*

Antibiotics	Conc. (µg)	*Listeria*			*Shigella*		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
A	30 µg	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
E	10 µg	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
NI	30 µg	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
AU	10 µg	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
CH	30 µg	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
G	20 µg	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
T	20 µg	2 (100)	0 (0.0)	0 (0.0)	2 (100)	0 (0.0)	0 (0.0)
C	20 µg	2 (100)	0 (0.0)	0 (0.0)	1 (50)	1 (50)	0 (0.0)

Keys: A = Amoxicillin, E = Erythromycin, NI = Nitrofurantoin, AU = Augmentin, CH = Chloramphenicol, G = Gentamicin, T = Tetracycline, C = Ciprofloxacin

Table 5: Susceptibility Pattern of *E. coli* and *Klebsiella* isolated from *Tilapia zilli*

Antibiotics	Conc. (µg)	*E. coli*			*Klebsiella*		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
A	30 µg	2(100)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)
E	10 µg	2(100)	0(0.0)	0(0.0)	0(0.0)	0(0.0)	2(100)
NI	30 µg	2(100)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)
AU	10 µg	2(100)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)
CH	30 µg	2(100)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)
G	20 µg	2(100)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)
T	20 µg	1(50)	1(50)	0(0.0)	1(50)	1(50)	0(0.0)
C	20 µg	2(100)	0(0.0)	0(0.0)	2(100)	0(0.0)	0(0.0)

Keys: A = Amoxicillin, E = Erythromycin, NI = Nitrofurantoin, AU = Augmentin, CH = Chloramphenicol, G = Gentamicin, T = Tetracycline, C = Ciprofloxacin

Table 6: Susceptibility Pattern of *Vibrio* and *Bacillus isolated* from *Tilapia zilli*

Antibiotics	Conc. (µg)	*Vibrio*			*Bacillus*		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)	Resistant n (%)	Intermediate n (%)	Susceptible n (%)
A	30 µg	4 (100)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
E	10 µg	3 (75)	0 (0.0)	1 (25)	3 (66.7)	0 (0.0)	1 (33.3)
NI	30 µg	4 (100)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
AU	10 µg	3 (75)	1 (25)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
CH	30 µg	3 (75)	0 (0.0)	1 (25)	3 (100)	0 (0.0)	0 (0.0)
G	20 µg	(100)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
T	20 µg	4 (100)	0 (0.0)	0 (0.0)	3 (100)	0 (0.0)	0 (0.0)
C	20 µg	2 (50)	1 (25)	1 (25)	2 (66.7)	0 (0.0)	1 (33.3)

Keys: A = Amoxicillin, E = Erythromycin, NI = Nitrofurantoin, AU = Augmentin, CH = Chloramphenicol, G = Gentamicin, T = Tetracycline, C = Ciprofloxacin.

Table 7: Susceptibility Pattern of *Salmonella* isolated from *Tilapia zilli*

Antibiotics	Conc. (µg)	*Salmonella*		
		Resistant n (%)	Intermediate n (%)	Susceptible n (%)
A	30 µg	2(100)	0(0.0)	0(0.0)
E	10 µg	2(100)	0(0.0)	0(0.0)
NI	30 µg	2(100)	0(0.0)	0(0.0)
AU	10 µg	2(100)	0(0.0)	0(0.0)
CH	30 µg	2(100)	0(0.0)	0(0.0)
G	20 µg	2(100)	0(0.0)	0(0.0)
T	20 µg	2(100)	0(0.0)	0(0.0)
C	20 µg	2(100)	0(0.0)	0(0.0)

Keys: A = Amoxicillin, E = Erythromycin, NI = Nitrofurantoin, AU = Augmentin, CH = Chloramphenicol, G = Gentamicin, T = Tetracycline, C = Ciprofloxacin

Discussion

Fish are susceptible to a wide variety of bacterial pathogens. Many of these bacteria capable of causing disease are considered by some to be saprophytic in nature. These bacteria only become pathogen when fishes are physiologically unbalanced, nutritionally deficient, or there is other stressor, i.e., poor water quality, overstocking, which allows opportunistic bacterial infection to proceed [8].

The fish flesh in Borikiri had the highest bacterial count which is in agreement with the study of Adedeji *et al.* [9], were eleven different organisms were isolated, with nine different types from skin samples, and five from stomach. The high bacterial counts from flesh in Borikiri could be as a result of the high intake of pollutants and contaminants from various sources such as fecal substances, oil spills that are being discharge into the bodies of water. The high counts from the different samples could be as a result of mass pollution of the environments, hence higher bacterial load observe [10]. The presence of bacteria in the aquatic environment can be used to evaluated its sanitary and bacteriological state as well as the state of fish in it the survival of this bacteria is dependent on the prevailing condition in the aquatic environment and fish are often the hosts [11]. Eagle Island had the highest total coliform count on the flesh which indicates that there was a high level of faecal contamination in the water bodies than the other areas. This is in line with (Omonona *et al.* [12], where the high microbial counts in the surface water may be attributed to different anthropogenic activities around the coastal water such as direct defecation into the open rivers. Results of the analysis showed that there was no significant difference between the bacterial counts at $p \geq 0.05$.

Iwofe had the least bacterial count and there was no significant difference between the microbial counts at $p \geq 0.05$. The low bacterial count is an indication that there was little contamination in the water bodies and the handing activities compared to the other locations.

Eagle Island also had the highest *Salmonella- Shigella* count in the three fish parts i.e. gills, flesh and intestine which indicates the presence of faecal contamination in the water from where the fishes were harvested and sold in the markets and also it indicates that there exists an inherent risk of contamination by *Shigella species* present in the environment. The microorganisms isolated in this study may have entered into the water bodies where these fishes live as a result of direct discharge of domestic wastes into the open water body as well as anthropogenic activities around the rivers. Coliforms were one of the most common organisms found in the aquatic environments where these fishes live. The dominant bacteria belonging to the genera *Listeria species*, *Bacillus species*, *Salmonella species*, *Klebsiella species*, *Vibrio species*, *Shigella species*, and *E. coli* was isolated from the different bodies of water. This affirms the study of Ogbonna *et al.* [13] where *Bacillus*, *Vibrio*, *E. coli*, *Klebsiella* were also isolated from there research (Antibiogram of Bacteria Flora of *Tilapia zilli* from creeks Around Port Harcourt). Apart from the fact that microorganisms are described to be ubiquitous, Ogbonna *et al.* [14] also affirmed that their presence especially in an aquatic environment depends upon the nature of materials being added during natural storm water run offs as well as soil erosion and discharge from sewage effluents. In addition to this observation, the study areas were described as very busy with activities of human settlement resulting in pollution from domestic and industrial sources which brought about increase in the amount of organic matter as well as nutrients for the microorganisms [15, 16].

Borokiri had the highest number of isolates. *E.coli* had the highest percentage occurrence of 27.3% in the fish intestine, this is due to the fact that it is part of the normal bacterial flora of the intestine and is an indicator organism of fecal contamination while *Listeria species* had the least percentage occurrence (0) in the intestine because the conditions in the intestine was not favourable for its growth from the gills, *Salmonella species* and *Listeria species* had the highest percentage occurrence (25%) and the least is *Vibrio* species and *E. coli.* Fishes usually serve as a host to variety of organisms including *Salmonella species. Salmonella species* which is not a fish pathogen, due to the presence of *Salmonella species* in the river as the fishes drink the water, contaminated with *Salmonella species*, these organism remains in their gills as the fishes are filter feeders. The least is *Vibrio species* and *E.coli* with no (0) percentage occurrence due to the fact that the environmental conditions was not favourable for their growth.

From the fish flesh, *Bacillus species* had the highest percentage occurrence of (40%) and the least was *Salmonella species*, *Vibrio species*, *E. coli* with no (0) percentage occurrence. The presence of *Bacillus species* is as a result of soil particles, dust particles on the body of the fish. *Bacillus species* are always abundant in dust and soil particles. Iwofe had a total of 10 isolated *E.coli* having the highest percentage occurrence (42.9%) in the fish intestine. This is due to the fact that *E.coli* is part of the normal bacterial flora of the intestine and serves as indicator organism for fecal contamination whereas the *Salmonella species* and *Listeria species* had the least percentage occurrence (0) as the conditions in the fish intestine was not favourable to their growth. *Bacillus species* had the highest percentage occurrence of 60% because as *Bacillus species*

are always found in air, dust particles and soil particles, when these fishes breath through their gills, these spore forming *Bacillus species* enters and remains in the gills. The *Listeria species, E.coli, Shigella species* and *Klebsiella species* had the least percentage occurrence (0%) because the environmental condition was not favourable to their growth. *Vibrio species* had the highest percentage occurrence of 66.7% in the fish flesh as a result of pollution of the water where these fishes live and through run off into the water bodies while *Salmonella species, Shigella species, Klebsiella species, Bacillus species, Listeria species* has no (0) percentage occurrence. Eagle Island had a total of 15 isolates with *Bacillus species* having the highest percentage occurrence of 33.3% in the fish intestine and *Listeria species* and *Shigella species* had no (0) percentage occurrence. In the fish gills, *Vibiro species, Bacillus species* and *E.coli* had the highest percentage occurrence of (33.3%) while the least occurrence were *Listeria species Salmonella species, Shigella species* and *Klebsiella species* with no (0) percentage occurrence. From the fish flesh, *Listeria species* had the highest percentage occurrence (100%) as a result of food processing, skinning and slicing of the fish as it is mostly found in the environment. *Salmonella species, Vibrio species, Shigella species, Klebsiella species, Bacillus species* and *E-coli* had no (0) percentage occurrence as conditions in the flesh was not favourable for their growth. This indicates that most of the major contributors to the pollution of the rivers are the households that throw their wastes materials into the river and also whose wastewater eventually end up in the rivers, also contamination as a result of illegal refineries.

In this study, antibiotic susceptibility pattern of the identified bacteria, were performed by disc diffusion method against eight (8) different antibiotics, they include: Amoxicillin (30 µg), Nitrofurantoin (30 µg), Chloramphenicol (30 µg), Ciprofloxacin (20 µg), Augmentin (10 µg), Tetracycline (2 µg), Gentamicin (20 µg), and Erythromycin (10 µg). The resistance pattern of the isolated from the bodies of water are presented in table for 4.6 to 4.10 in this study Amoxicillin, Erytromicin, Nitrofurantin, Gentamicin were the antibiotics to which most of the organisms tested, irrespective of the source were resistant. The Resistance by these organisms to this antimicrobial agent may be because the drug is not used as frequent as other chemotherapy agent on account of its new photo toxic side-effects [17]. The level of resistance among *Escherichia coli* and other isolate to antibiotics tested in this present study is similar to that observed by other workers Al-Jebouri [18] and Antai and Anozie [19] have variously reported a high level of resistance among *E. coli* strains from raw sewage and teaching hospitals and pediatric clinics in Port Harcourt respectively. Some factors may also account for the level of assistance particularly as many of their solid tested we are multiple resistance it is known that he coli strains are rarely found in soil, vegetation or water in the absence of excremental contamination [20]. It could be said therefore the *E. coli* strain tested in the present study was of fecal origin with the uncontrolled use of antibiotics and common practice of self-medication typical of the Nigerian settings [19]. Antibiotic prescription in hospitals is given without clear evidence of infection or adequate medical indication. Broad spectrum drugs are sometimes given in place of narrow spectrum drugs as a substitute for culture and sensitivity testing with consequential risk being super infections and the selection of drug resistant mutants. The consumption of antibiotics is enormous, and it has been estimated that the antibiotic market consumption worldwide lies between 100,000-200,000 tons [21]. Different level of resistance of the bacterial isolated to individual antibiotics and various degree of sensitivity were obtained among isolates. This could be so because strains of the same microorganisms isolated from the different sources may present diverse level of resistance as a means of surviving [22]. Multiple drug resistance is an extremely serious public health concern and has been found to be associated with the outbreak of major epidemics throughout the world [23]. Resistance to some β- lactam drugs such as Amoxicillin by Enterobacteria specially *Shigella species, E.coli, Klebsiella species, Salmonella species* has been reported as a result of frequent use of these antibiotics [24]. Erythromycin was the only antibiotic to which *Klebsiella species* was sensitive to (100%). The high level of resistance to antimicrobial agents may be as a result of indiscriminate, length and widespread use of the antibiotics in the treatment of the fishes during rearing [25]. Thus the multiple drug resistance shown by these bacteria is worrisome because of the public health implications.

Conclusion and Recommendation

Fish is mostly contaminated with foodborne pathogens such as *Salmonella species, Listeria monocytogenes, Vibrio species,* which is being reflected on the microflora of the surrounding water body, contamination of the natural habitat of fish may affect not only the health of the fish alone, but will also bring about public health concerns as fish and fish products can be a main source of human pathogenic bacteria. Various factors such as anthropogenic activities, contaminated water sources, and poor hygiene during capture, handling, and transportation of fish could affect the prevalence of bacteria in fish and surrounding water body. The hazard of these microorganisms is being increased with the specific abilities of these bacteria to survive in the environment. The emergence of bacteria also due to the fact that fish often miss the heat treatment procedure before consumption, which has dramatic effects on human health. Pathogens via contaminated fish and fish products may enter the food chain, and processing of fish may lead to cross-contamination of equipment, and end-product, facilitating the distribution of pathogenic bacteria. Most times, humans develop resistance to antimicrobial agents after eating the fishes which may be due to indiscriminate, widespread and lengthy use of antimicrobial drugs in the treatment of the fishes during rearing. Therefore, good hygienic practice is a measure to avoid contamination and to provide the safety of fish.

Conflict of Interest

There was no conflict of interest in the research

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