Original Research Article

Detection of Resistance Genes in *Escherichia coli* Isolated from Fishes and Shellfishes in Creek Road/Bonny Estuary, Port Harcourt, Nigeria

ABSTRACT

Resistance bacteria may be transferred to humans through the consumption of fishes and shellfishes. Hence, this study characterised *E. coli* isolates from fishes and shellfishes in Creek Road/Bonny Estuary, with the use of molecular techniques. Seventy two (72) fishes and shellfishes were examined for the presence of *E. coli*. Most probable number (MPN) technique, Eijkman test and molecular techniques were used for the isolation and identification of *E. coli*. The analysis was performed seasonally, which was during the end of rainy season (November), start of rainy season (April) and middle of rainy season (July). Eleven (11) isolates of *E. coli* (randomly selected) recovered from the fishes and shellfishes were screened for resistance genes in the pathgotypes of *E. coli* (EHEC, EPEC, EAEC, ETEC and EIEC, including SHV, CTX-M, TEM and MCR genes. The results showed that both coliforms and *E. coli* were recovered from the fishes and shellfishes. The distribution of the resistance genes in the *E. coli* isolates from fishes and shellfishes is: fishes – SHV (9.5%); CTX-M (11.1%); TEM (7.9%); MCR (0%); str1 and str2 (6.3%); esV and bfA (3.2%); aaiC (7.9%); elt (6.3%); invE (4.7%) and shellfishes - SHV (5.6%); CTX-M (5.6%); TEM (7.4%); MCR (0%); str1 and str2 (3.7%); esV and bfA (0%); aaiC (0%); elt (0%); invE (3.7%). In fishes and shellfishes, the most detected of all the genes are CTX-M and TEM, respectively. The data from this study suggest that fishes and shellfishes can be reservoirs of antibiotic resistance genes. These genes can easily be transmitted to humans through the consumption of raw or improperly cooked fishes and shellfishes, creating public health problem.

Keywords: *Escherichia coli*; Pollution; Genes; Resistance; Fishes; Shellfishes; Water bodies

INTRODUCTION

"In Nigeria, the pollution of natural water bodies with waste effluents and heavy metals from different industries, and oil through spills have been recorded. That being so, there is the likelihood that toxicants from these sources may affect plants, enter the food chain and affect a larger human population, as well as cause mutations in microorganisms found in water bodies" [1, 2, 3, 4].

"It has been noted that the presence of *Escherichia coli* (*E. coli*) in water environment could be as a result of human activities in and around the water body. Also, it has been revealed that citing of waste dumps, discharge of sewage, building of toilets on water bodies and the use of human and animal excreta as manure, could contribute to the presence of *E. coli* in water bodies" [3, 4, 5]. "The bacterium, *E. coli* is commonly found in the gut of endotherms and can be easily spread in different ecosystems via the food chain and water. *Escherichia coli* exchanges genetic material with other species of bacteria and it is possible that this bacterium may transfer antibiotic resistance genes to transient pathogenic bacteria that cause disease in humans" [6, 7].

[8] stated that "class 1, 2 and 3 integrons have been involved in the dissemination of antimicrobial resistance genes. These integrons are important contributors in the dissemination of antimicrobial resistance in Gram-negative bacteria. They are known as genetic structures able to capture, excise and express genes, frequently included into mobile elements as plasmids that allow their spread among bacteria" [8]. According to [9], the existence and spread of antimicrobial resistance (AMR) in bacteria in natural environments and wildlife are linked to agricultural and livestock activities.

"It was reported in the news that there is rapidly rising antimicrobial resistance rates in *E. coli*. The news was reported in BBC News London, by James Gallagher (21 December, 2015). He stated that another strain of *E. coli* that is resistant to the "last-resort" antibiotic called colistin (polymyxins), has been discovered and the gene identified to be responsible is the Mobilized Colistin Resistance gene (MCR gene). The most recently discovered strain has been isolated from humans and food animals/poultry" [10, 11].

"The presence of multidrug-resistant (MDR) *E. coli* of faecal origin isolated from seafood is a serious concern. Seafood containing MDR *E. coli* can serve as a medium for the transfer of resistant bacteria to consumers" [12]. For this reason, this study carried out the detection of

resistance genes in *Escherichia coli* isolated from fishes and shellfishes in Creek Road/Bonny Estuary, Port Harcourt, Nigeria.

MATERIALS AND METHODS

Description of Study Area

Rivers State is where oil and gas industries are located and at present, the only oil and gas-producing region in Nigeria. There is the Oil and Gas Free Zone, Fertilizer Company, Petroleum Refining Companies, Petrochemical Company, Harbours and as a result, it is exposed to activities of Oil Multinational Companies, which have been linked to the degradation of the natural environment, pollution and low agricultural productivity. Finally, by reason of the numerous industrial activities and increase in population in the State, there is an increase in generation of both domestic and industrial wastes that are having their tow on the Water bodies. Ultimately, the unplanned development of the area has led to destruction of forests and biodiversity with the State being exposed to effects of radiation [13, 14].

Study Design

This study was carried out for the period of 1 year. The choice of the water body was based on human activities in and around the water body and their close proximity to the markets. The water body is exposed to sewage, direct faecal materials (arising from building toilets on the water body), crude oil (from spills and bunkering activities), agricultural products and other products discharged into the water body from domestic activities.

Data from HPC, TCC, and TEC were used to count the number of bacteria. The TCC and TEC were performed using the most probable number (MPN) technique, which was obtained statistically using the MacCrady table. **Sample Collection**

Altogether, 40 fishes and 32 shellfishes were obtained from the water body. Seven different types of fishes were used, while six different types of shellfishes were used. In total, the number of fishes and shellfishes was 72.

The fishes used for this study were: Mackerel (*Rastrelliger brachysoma*), Catfish (*Clarias gariepinus*), Mullet (*Mugil cephalus*), Sardine (*Sardina pilchardus*), Tilapia (*Oreochromis niloticus*), Mudskipper (*Periophthalmus gracilis*) and Codfish (*Gadus morhua*). Besides, the following shellfishes were used: Periwinkle (*Melahaphe neritoides*), Oyster (*Crassosterea rhizophorae*), Crab (*Liocarcinus vernalis*), Prawn (*Penaeus monodon*), Periwinkle Snail (*Littorina littorea*) and Clams (*Spisula solidissima*).

Fishes and shellfishes were immediately placed in a clean cooler containing ice packs, and transported to the laboratory. Testing was started within 3 to 6 hours after sampling.

Analysis of Fishes and Shellfishes for Bacterial Detection

The fishes and shellfishes were chosen for this study because they are the commonest and most consumed in the State. The fish and shellfish were properly cleaned with sterile water before being dissected with a sterile dissecting equipment. Fish gills and intestines were chosen because they come into direct touch with reservoir water. Gills and gut samples were aseptically taken for the detection of bacteria. Sterile swab sticks were used to collect samples from the gills, while for the gut samples, the fish abdomen was opened and the content emptied into 4 oz. (118.3 ml) sterile specimen containers. After that, sterile normal saline was poured into the containers before the analysis was carried out [15].

Some of the shellfishes were opened up using sterile knives, while the ones that had softer skin were opened up with the hand, with the use of hand gloves. The samples of the shellfishes were put into sterile containers and sterile normal saline added before the

samples were analysed. Heterotrophic Plate Count (HPC), Total Coliform Count (TCC) and Total *E. coli* Count (TEC) were carried out on both the samples of fishes and shellfishes.

Cultivation of Microorganisms

Media such as nutrient agar, MacConkey agar, MacConkey broth, brilliant green lactose (bile) broth and CHROMagar for *Escherichia coli* and other coliform (CHROMagar ECC) were prepared and used following the manufacturers' instructions and directions. After the preparation of the media, the media plates were labelled clearly and stored appropriately in the refrigerator at 4-6 ^{o}C.

Heterotrophic Plate Count (HPC)

The HPC, also known as the standard plate count, is a procedure for estimating the number of live heterotrophic bacteria in water. This test provides useful information about water quality and supporting data on the significance of coliform test results. The standard plate count employs serial dilution technique.

A row of tubes was arranged in a test tube rack and the tubes held 9 ml of diluent (sterile normal saline) each. Using a sterile pipette, 1 ml of the original water sample was transferred to tube 1 (9 ml of diluent and 1 ml of sample). Then, using another sterile pipette, 1 ml of the diluted solution in tube 1 was transferred to tube 2 and so on, until 1ml of the content of tube 3 was transferred to tube 4 (10^4). Further, 0.1 ml was transferred from diluted solution in the tubes to the appropriate media plate (nutrient agar) and spread (spread plate technique) using a sterile glass spreader. Finally, the plates were incubated at 37 ^{o}C for 18 to 24 hours in incubator and afterwards, the visible colonies on the culture plates were counted and reported [16].

The Most Probable Number (MPN) Technique

In carrying out the MPN technique, a series of tubes containing a MacConkey broth culture medium were inoculated with test portions of the samples (3 tube method). After a specified incubation time at a given temperature, each tube showing gas formation and yellow colour was regarded as "presumptive positive" since the gas indicates the possible presence of coliforms. Although, gas may also be produced by other microorganisms, and so a subsequent confirmatory test was imperative; the two tests are known as the *presumptive test* and the *confirmatory test*, respectively. For the confirmatory test, a more selective culture medium, brilliant green lactose (bile) broth was inoculated with materials taken from the positive tubes. Following an appropriate incubation time, the tubes were examined for gas formation as before.

Afterwards, "the MPN of bacteria present was estimated from the number of tubes inoculated and the number of positive tubes obtained in the test, using specially devised statistical table (MacCrady tables)" [17, 18, 19]. Moreover, for confirmation of thermotolerant coliform bacteria (*E. coli)*, positive tubes obtained in the confirmatory test were further incubated in water bath at 44.5 ^{o}C for 24 hours (the Eijkman test) [19]. In addition, in order to further confirm, CHROMagar ECC (CHROMagar for *E. coli* and other coliforms) was inoculated with materials taken from the positive tubes (positive tubes incubated at 44.5 ^{o}C). Following an appropriate incubation time, the culture plates were examined for the presence of *E. coli* (showed a blue colour) [20] and the Gram-staining technique was performed to confirm further. Finally, the pure cultures of *E. coli* were inoculated in nutrient agar slants. Then, implicated genes in the *E. coli* isolates were characterized using molecular analysis.

Molecular Analysis

A total of 11 (7 from fishes and 4 from shellfishes) isolates of *E. coli* were subjected to molecular typing after antibiotic susceptibility testing. The steps taken during the molecular

analysis were as follows: DNA extraction, DNA quantification, 16S rRNA amplification, amplification of SHV genes, amplification of TEM genes, amplification of CTX-M genes, amplification of MCR genes and pathotyping of *E. coli* by multiplex PCR and sequencing.

DNA Extraction

An overnight Luria Bertani (LB) broth culture of each of the *E. coli* isolates (5 ml) was centrifuged at 14000 rpms for 3 minutes and the supernatant was discarded into a disinfectant jar. To the cells in each tube, 500 µl of normal saline was used to resuspend them (cell pellet) and they were heated at 95 ^{o}C for 20 minutes. Then, the heated bacterial suspension was cooled on ice and spun for 3 minutes at 14000 rpms. The supernatant containing the DNA was transferred into a clean 1.5 ml microcentrifuge tube and stored at -20 ^{o}C for other downstream reactions till required for polymerase chain reaction (PCR).

DNA Quantification

Nanodrop 1000 spectrophotometer was used to quantify the extracted genomic DNA. The software of the equipment was lunched by clicking twice on the nanodrop icon. Then, the equipment was initialized with 2 µl of sterile distilled water; it was blanked using the normal saline. Two microlitres of the extracted DNA was loaded onto the lower pedestal and the upper pedestal was brought down to contact the extracted DNA on the lower pedestal. Finally, the DNA concentration was measured by clicking on the "measure" button.

16S rRNA Amplification

The 16S rRNA region of the rRNA genes of the *E. coli* isolates were amplified using the 27F: 5'-AGAGTTTGATCMTGGCTCAG-3' and 1492R: 5'-CGGTTACCTTGTTACGACTT-3' primers on an ABI 9700 Applied Biosystems thermal

cycler at a final volume of 40 microlitres for 35 cycles [21]. The PCR mix were: the X2 Dream Taq Master Mix supplied by Inqaba, South Africa (Taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and the extracted DNA as template. The following were the PCR conditions: Initial denaturation, 95 ºC for 5 minutes; denaturation, 95 ºC for 30 seconds; annealing, 52 ºC for 30 seconds; extension, 72 ºC for 30 seconds for 35 cycles and final extension, 72 ºC for 5 minutes. The product was resolved on a 1 % agarose gel at 130 V for 25 minutes and visualized on a blue light transilluminator.

Amplification of Sulfhydryl Variable (SHV) Genes

Sulfhydryl Variable (SHV) genes from the isolates of *E. coli* were amplified using the SHV F: 5' CGCCTGTGTATTATCTCCCT-3' and SHV R: 5'-CGAGTAGTCCACCAGATCCT-3' primers [22] on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 30 µl for 35 cycles. The PCR mix were: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and 50 ng of the extracted DNA as template. The following were the PCR conditions: Initial denaturation, 95 ºC for 5 minutes; denaturation, 95 ºC for 30 seconds; annealing, 56 ºC for 40 seconds; extension, 72 ºC for 50 seconds for 35 cycles and final extension, 72 ºC for 5 minutes. The product was resolved on a 1 % agarose gel at 120 V for 25 minutes and visualized on a UV transilluminator for a 200 bp product size [22].

Amplification of CTX-M Genes

Primers, such as CTX-MF: 5'-CGCTTTGCGATGTGCAG-3' and CTX-MR: 5'-ACCGCGATATCGTTGGT-3' were used to amplify CTX-M genes from the isolates [23] on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation,

95 ºC for 5 minutes; denaturation, 95 ºC for 30 seconds; annealing, 52 ºC for 30 seconds; extension, 72 ºC for 30 seconds for 35 cycles and final extention, 72 ºC for 5 minutes. The product was resolved on a 1 % agarose gel at 120 V for 25 minutes and visualized on a UV transilluminator for a 550 bp product size.

Amplification of MCR Genes

All isolates were screened for the presence of the MCR using the MCR gene. MCR-F 5'-CGGTCAGTCCGTTTGTTC-3' and MCR-R 5'-CTTGGTCGGTCTGTAGGG-3'. A 25 µl PCR reaction was carried out with the following amplification conditions 94 ºC 60 sec. with a final extension of 72 ºC for 10 min. PCR generated products were run on a 1.5 % agarose gel at 120 V for 25 min. and visualised on a blue light transilluminator.

Amplification of Temoniera (TEM) Genes

Temoniera (TEM) genes from the isolates were amplified using the TEMF: 5'-ATGAGTATTCAACATTTCCGTG-3' and TEMR: 5'-TACCAATGCTTAATCAGTGAG-3' primers [22] on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 40 microlitres for 35 cycles. The PCR mix included: the X2 Dream taq Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4 M and 50 ng of the extracted DNA as template. The PCR conditions were as follows: Initial denaturation, 95 ºC for 5 minutes; denaturation, 95ºC for 30 seconds; annealing, 58 ºC for 30 seconds; extension, 72 ºC for 30 seconds for 35 cycles and final extention, 72 ºC for 5 minutes. The product was resolved on a 1 % agarose gel at 120 V for 25 minutes and visualized on a UV transilluminator for a 980 bp product size.

Pathotyping of *E. coli* by Multiplex PCR

Multiplex PCRs were performed on an ABI 9700 Applied Biosystems thermal cycler at a

final volume of 40 microlitres for 35 cycles. The PCR mix included: the X5 Multiplex Master mix supplied by Inqaba, South Africa (taq polymerase, DNTPs, MgCl), the primers at a concentration of 0.4M and 50ng of theextracted DNA as template. The following cycling conditions: 95 ºC /5 min, 15 cycles of 95°C/1 min, 62 ºC /2 min, and 72 ºC /2 min, 20 cycles of 95 ºC /1 min, 58 ºC /2.5 min, and 72 ºC /1 min and final extension at 72 ºC /7 min. PCR products were separated on 2.5 % agarose gel at 130V for 25min..

RESULTS

Table 1. Seasonal Comparison of Heterotrophic Plate Count (HPC) for Fishes

Table 1 shows the results of the HPC of fishes from all the Seasons at Creek Road/Bonny Estuary. The variance in the mean was compared statistically using ANOVA and according to the results, there was a significant difference in the values from Catfish ($p=0.005$; $t=14.094$), Tilapia ($p=0.017$; $t=8.666$) and Mudskipper ($p=0.040$; $t=5.779$). To see which group is the variation significant, Tukey Multiple Comparison Test was performed and the results showed that there was a significant difference between group A and B for Catfish, Tilapia and Mudskipper; A and C, Catfish and Tilapia; B and C, none.

Table 2. Heterotrophic Plate Count (HPC) for Shellfishes

As shown in Table 2, the HPC of Seafood was carried out in November, April and July at Creek Road/Bonny Estuary. The variance in the mean was compared statistically using ANOVA. According to the results, there was a significant ($p<0.05$) difference in the values from Periwinkle, Crab, Prawn and Clams. In order to see which group is the variation significant, Tukey Multiple Comparison Test was performed and the result shows that there was a significant difference between group A and B for Periwinkle; A and C, all the seafood; B and C, only Prawn.

Table 3. Total Coliform Count (TCC) and Total *E. coli* Count (TEC) Present in Fishes

Total coliform count and total *E. coli* present in fishes at Creek Road/Bonny Estuary in the three Seasons are presented in Table 3. The TCC was highest in July (233 MPN/100 ml) and lowest in November (100 MPN/100 ml). In November, the percentage of the TEC ranged from 19.0 % to 57.1 %; in April, the percentage of the TEC ranged from 7.7 % to 36.4 %; in July, the percentage of the TEC ranged from 5.3 % to 27.3 %.

Table 4. Total Coliform Count (TCC) and Total *E. coli* Count (TEC) Present in Shellfishes

Table 4 shows that the TCC was highest in July (92 MPN/100 ml) and lowest in November (61 MPN/100 ml). In November, the percentage of the TEC was 0 % to 42.8 %; in April, the percentage of the TEC ranged from 0 % to 27.3 %; in July, the percentage of the TEC ranged from 0 % to 35.5 %.

Table 5 and 6. Molecular Analysis of the Isolates of *E. coli*

Table 5 and 6 show the distribution of the resistance genes detected in *E. coli* isolates. The gene detected most in the *E. coli* isolated from fishes and shellfishes was CTX-M (7 %), followed by SHV (9.5 %). Mobilised Colistin Resistance (MCR) gene was not detected in any of the *E. coli* isolates. Amongst the Fishes, Mudskippers had the highest number of resistance genes (50.6 %), while amongst the Shellfishes, Crabs had the highest number of genes (20.5 %).

Table 1: Seasonal Comparison of Heterotrophic Plate Count (HPC) for Fishes at Creek Road/Bonny Estuary (CFU/ml)

	Mackerel (10^3)	**Catfish (10^4)**	**Mullet (10^3)**	**Sardine (10^3)**	**Tilapia (10^4)**	**Mudskipper (10^4)**	**Codfish (10^3)**
November (A)	5.87 ± 0.76	5.00 ± 0.26	6.27 ± 0.38	3.47 ± 0.21	4.47 ± 0.72	4.97 ± 0.55	3.27 ± 0.38
April (B)	5.07 ± 0.15	6.67 ± 0.64	6.17 ± 0.99	3.17 ± 0.21	6.40 ± 0.70	4.07 ± 0.35	3.93 ± 0.45
July (C)	5.47 ± 0.57	6.70 ± 0.36	7.10 ± 0.95	3.53 ± 0.45	6.17 ± 0.38	5.00 ± 0.10	3.90 ± 0.26
p-value	0.287	0.005	0.373	0.369	0.017	0.040	0.122
F-value	1.548	14.094	1.166	1.184	8.666	5.779	3.048
Remarks	NS	S	NS	NS	S	S	NS
Post hoc (Tukey) A and B		0.009 (S)			0.021 (S)	0.062 (NS)	
A and C		0.008 (S)			0.035 (S)	0.994 (NS)	
B and C		0.995 (NS)			0.892 (NS)	0.055 (NS)	

Table 2: Seasonal Comparison of Heterotrophic Plate Count (HPC) for Seafood in Creek Road/Bonny Estuary (CFU/ml)

	Periwinkle (10^3)	**Oyster (10^3)**	**Crab (10^5)**	**Prawn (10^5)**	**Periwinkle Snail (10^3)**	**Clams (10^3)**
November (A)	3.47 ± 0.57	7.67 ± 0.84	3.13 ± 0.15	7.13 ± 0.38	6.70 ± 0.10	3.77 ± 0.35
April (B)	5.13 ± 0.32	8.57 ± 0.78	4.50 ± 0.40	7.43 ± 0.60	7.03 ± 0.35	4.63 ± 0.49
July (C)	5.77± 0.31	8.63 ± 1.12	5.27 ± 0.87	8.70 ± 0.26	7.60 ± 0.60	5.20 ± 0.26
p-value	0.001	0.415	0.010	0.010	0.087	0.010
F-value	24.429	1.021	11.102	10.792	3.777	10.740
Remarks	S	NS	S	S	NS	S
Post hoc (Tukey) A and B	0.006 (S)		0.056 (NS)	0.695 (NS)		0.071 (NS)
A and C	0.001 (S)		0.008 (S)	0.011 (S)		0.009 (S)
B and C	0.229 (NS)		0.290 (NS)	0.028 (S)		0.242 (NS)

Table 3: Total Coliform Count (TCC) and Total *E. coli* Present in Fishes in Creek Road/Bonny Estuary

S/N	Types of Fishes	November		April		July	
		TCC MPN/100 ml	TEC MPN/100 ml (%)	TCC MPN/100 ml	TEC MPN/100 ml (%)	TCC MPN/100 ml	TEC MPN/100 ml (%)
1	Mackerel (*Rastrelliger brachysoma*)	7	3 (42.9)	11	4 (36.4)	11	3 (27.3)
2	Catfish (*Clarias gariepinus*)	15	3 (20.0)	21	7 (33.3)	39	3 (7.7)
3	Mullet (*Mugil cephalus*)	7	4 (57.1)	14	3 (21.4)	28	3 (10.0)
4	Sardine (*Sardina pilchardus*)	7	4 (57.1)	11	4 (36.4)	11	3 (27.3)
5	Tilapia (*Oreochromis niloticus*)	28	7 (25 .0)	39	3 (7.7)	48	7 (14.6)
6	Mudskipper (*Periophthalmus gracilis*)	21	4 (19.0)	39	3 (7.7)	75	4 (5.3)
7	Codfish (*Gadus morhua*)	15	3 (20.0)	11	3 (27.3)	21	4 (19.0)
	Total	100		107		233	

S/N	Types of Seafood	November	April	July

***This was carried out in triplicate**

Table 4: Total Coliform Count (TCC) and Total *E. coli* Present in Seafood in Creek Road/Bonny Estuary

		TCC MPN/100 ml	TEC MPN/100 ml (%)	TCC MPN/100 ml	TEC MPN/100 ml (%)	TCC MPN/100 ml	TEC MPN/100 ml (%)
1	Periwinkle (*Melahaphe neritoides*)	3	0 (0)	3	0 (0)	3	0 (0)
2	Oyster (*Crassosterea rhizophorae*)	14	3 (21.4)	14	3 (21.4)	21	3 (14.3)
3	Crab (*Liocarcinus vernalis*)	15	3 (20.0)	20	4 (20.0)	20	7 (35.0)
4	Prawn (*Penaeus monodon*)	15	3 (20.0)	14	3 (21.4)	23	4 (17.4)
5	Periwinkle Snail (*Littorina littorea*)	7	0 (0)	11	3 (27.3)	14	3 (21.4)
6	Clams (*Spisula solidissima*)	7	3 (42.9)	11	3 (27.3)	11	3 (27.3)
	Total	61		73		92	

***0 Denotes No TEC was Detected**

Table 5: Molecular Analysis of the Isolates of *E. coli*

E. coli Isolates	SHV	CTX-M	TEM	MCR (Colistin)	EHEC	EPEC	EAEC	ETEC	EIEC
Isolates from fishes									
Mudskipper-1	+	+	+		+			+	
Mudskipper-2	+	+			+		+	+	+
Mudskipper-3	+	+	+			+	+		
Mudskipper-4	+	+	+		+	+	+		
Mudskipper-5	+	+			+		+	+	+
Mudskipper-6	+	+	+		+			+	
Catfish-1		+	+				+		+

Isolates from shellfishes	SHV	CTX-M	TEM	MCR (Colistin)	EHEC	EPEC	EAEC	ETEC	EIEC
Crab-1	+	+	+		+				
Crab-2		+	+						+
Crab-3	+	+	+		+				
Prawn-1	+		+						+

	No. (%) of Resistance Genes								
	SHV	**CTX-M**	**TEM**	**MCR (Colistin)**	**EHEC**	**EPEC**	**EAEC**	**ETEC**	**EIEC**
F**ishes** (63 Isolates)									
Mudskipper	6(9.5)	6(9.5)	4(6.3)	0	4(6.3)	2(3.2)	4(6.3)	4(6.3)	2(3.2)
Catfish	0	1(0.9)	1(0.9)	0	0	0	1(0.9)	0	1(0.9)
Total Genes	6(9.5)	7(11.1)	5(7.9)	0	4(6.3)	2(3.2)	5(7.9)	4(6.3)	3(4.7)
	SHV	**CTX-M**	**TEM**	**MCR (Colistin)**	**EHEC**	**EPEC**	**EAEC**	**ETEC**	**EIEC**
S**hellfishes** (54 Isolates)									
Crab	2(3.7)	3(5.6)	3(5.6)	0	2(3.7)	0	0	0	1(1.9)
Prawn	1(1.9)	0	1(1.9)	0	0	0	0	0	1(1.9)
Total Genes	3(5.6)	3(5.6)	4(7.4)	0	2(3.7)	0	0	0	2(3.7)

Table 6: Distribution of Resistance Genes in the Isolates of *E. coli*

DISCUSSION

Generally, this study revealed that *Escherichia coli* (*E. coli*) could be isolated from fishes and shellfishes. Aside from *E. coli*, other coliforms could also be found from fishes and shellfishes. The presence of the bacteria in both fishes and shellfishes suggests that the water body could be polluted with faecal materials. This could be possible, as during sampling, it was observed that toilets were built on the water body. Also, the bacteria may have been introduced into the water body by birds and other animals, as they were found in and around the water body. Waste dumps and runoffs are other means through which the bacteria may

have found their ways into the water body. This is because waste dumps were spotted around the river; some of the wastes looked as if they were directly disposed into the water body.

A study has also reported the presence of *E. coli* in the same water body where the fishes and shellfishes were harvested for this present study [5, 24, 25, 26, 27] have as well, reported the presence of *E. coli* in other water bodies. "*Escherichia coli* is one of the indicator bacteria that if detected, may suggest the presence of faecal material in food, soil, as well as water environment" [28].

Furthermore, resistance genes were detected in the *E. coli* isolated from fishes and shellfishes. This result is in accordance with the reports by other researchers [29, 12]. Although, the resistance genes associated with *E. coli* in this study are not the same as the ones reported by the other researchers. Nonetheless, [5] reported the presence of SHV, CTX-M, TEM, stx1 and stx2, esV and bfA, aaiC, elt and invE in their study. According to the researchers, the most commonly detected gene was CTX-M, which is in conformity with the result from this present study. As found in this study, the gene detected most in the *E. coli* isolated from the fishes and shellfishes was CTX-M (7 %), followed by SHV (9.5 %).

Studies carried out by [24, 25] also revealed the presence of resistance genes in *E. coli* isolated from water bodies. In this study, the resistance genes detected were: fishes – SHV (9.5%); CTX-M (11.1%); TEM (7.9%); MCR (0%); str1 and str2 (6.3%); esV and bfA (3.2%); aaiC (7.9%); elt (6.3%); invE (4.7%) and shellfishes - SHV (5.6%); CTX-M (5.6%); TEM (7.4%); MCR (0%); str1 and str2 (3.7%); esV and bfA (0%); aaiC (0%); elt (0%); invE (3.7%). Mobilised Colistin Resistance (MCR) gene was not detected in any of the *E. coli* isolates. Amongst the fishes, mudskippers had the highest number of resistance

genes (50.6 %), while amongst the shellfishes, crabs had the highest number of genes (20.5 %).

A study in 2012 revealed that *E. coli* isolated from fishes and seafood (including shellfishes) had no resistance to amikacin, amoxicillin/clavulanic acid and cefoxitin. There are similarities between this study and the present study, as it also reported resistance genes in *E. coli* isolates. The genes reported were tetracycline resistance genes (tetB and tetD), beta-lactam resistance gene bla(TEM) and aminoglycoside resistance gene (aadA). It was stated that the genes were carried in isolates of *E. coli* that were resistant to one or more antibiotics [29].

CONCLUSION

This research shows that fishes and shellfishes may act as a reservoir for multidrug-resistant bacteria, including *Escherichia coli* and make easier the dissemination of the resistance genes. In other words, resistance or multidrug-resistant bacteria may be transferred to humans via the consumption of fishes and shellfishes. This could be possible when toilets are built on water bodies. Sad to say, this is a common practice in some rural areas in Rivers State, Nigeria. Sewage and agricultural runoff could also be sources of *Escherichia coli* (*E. coli*) in water bodies. The latter is possible when human and animal excreta are used as manure.

RECOMMENDATIONS

It is therefore recommended that the building of toilets on water bodies and the dumping of wastes in and around water bodies be discouraged. Also, it is important that fishes and shellfishes be cooked properly before consumption.

DISCLAIMER

Authors have declared that no competing interests exist. The products used for this research are commonly and predominantly use products in our area of research and country. There is absolutely no conflict of interest between the authors and producers of the products because we do not intend to use these products as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the producing company rather it was funded by personal efforts of the authors.

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