Magna Scientia Advanced Biology and Pharmacy

eISSN: 2582-8363

Cross Ref DOI: 10.30574/msabp

Journal homepage: https://magnascientiapub.com/journals/msabp/

(RESEARCH ARTICLE)

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Fish sellers' knowledge and practice of hygiene practices, antibiogram and detection of extended spectrum β-lactamase positive *Escherichia coli* in commercially available frozen fish in Awka metropolis

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Magna Scientia Advanced Biology and Pharmacy, 2025, 14(02), 059-069

Publication history: Received on 08 January 2025; revised on 11 March 2025 accepted on 13 March 2025

Article DOI: https://doi.org/10.30574/msabp.2025.14.2.0023

Abstract

The detection of extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* in frozen fish is an indication of a major public health challenge. The current study aims to identify ESBL-producing *E. coli* from frozen fishes. A total of 310 frozen fish samples purchased from the selected markets were screened for *E. coli* contamination using MacConkey agar. Confirmed isolates were screened phenotypically and molecularly for ESBL coding genes using standard protocols. A total of 100 (32.25%) were confirmed to be contaminated with *E. coli*. Varying resistance patterns were recorded: cefixime (100%), ceftazidime (100%), cefuroxime (99%), amoxicillin-clavulanate (100%), ofloxacin (96.0 %), ciprofloxacin (97.0 %) and gentamicin (99%). Moreover, Nitrofurantoin was the most active antibiotic. Also, 21 isolates expressed ESBL resistance. Of the 21 ESBL-positive isolates, 11 (52.4%) and 7 (33.3%) isolates had the TEM and SHV genes respectively. Many of the fish sellers (participants) claimed to have a good knowledge of some hygiene practices adopted in food handling such as washing hands (67.2%), use of gloves (56.7%), aprons (53.7%) and hair covers (73.1%). However, 38.8% of the participants do not know that fish stored at room temperature can cause disease when consumed. This study revealed that although the fish sellers had the basic education and knowledge of good hygiene, they seldom practiced it and thus are implicated as the primary source of contamination of the fish. Thus, strict compliance to appropriate policies should be routinely monitored to ensure proper implementation by the sellers.

Keywords: Frozen; Fish; Extended Spectrum Beta Lactamase; Hygiene; *Escherichia coli*

1. Introduction

The increased development and transmission of multidrug-resistant (MDR) organisms constitute a great health challenge, especially in emergent nations like Nigeria. This has affected the World Health Organization's (WHO) 'One Health' policy resulting from an increased rate of transfer of these resistance genes to humans and animals [1]. Ampicillin Class C beta-lactamases (AmpC beta-lactamases) can induce resistance among cephalosporins, including beta-lactamase inhibitors [2]. The presence of these enzymes (ESBL and AmpC) in pathogenic bacteria reduces the antibiotic activity in therapy [3]. These pathogens can contaminate food and food-producing animals and are a potential source for a wide spread of antibiotic resistance [2]. The increase in demand for convenient foods has given rise to a high demand for animal protein consumption [4, 5]. This has made the fish business very common and lucrative.

In Nigeria, fish is a major component of many Nigerian dishes and so, it's in high demand. In the process of preserving fishes, they are exposed to various handling techniques which may introduce contaminants particularly microbial

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contaminants to the fish which may lead to infection upon consumption of such contaminated fish. Antibiotics and vaccines that are used in aquaculture to treat common bacterial diseases partly contribute to the evolution of drug-resistant bacteria observed at the community level and may also be involved in the selection and transmission of AmpC-producing *Enterobacteriaceae* in animals [6]. Determining the prevalence rate of AmpC-producing *Enterobacteriaceae* using appropriate susceptibility tests is important in reducing the possible risks of infections caused by this class/group of bacteria.

There is insufficient data on the prevalence rate of AmpC-producing (*Enterobacteriaceae) Escherichia coli* isolated from frozen fishes in southeastern Nigeria. Thus, this study sought to determine the prevalence rate of ESBL and AmpC resistance by *E. coli* isolated from frozen fishes sold at some/selected open markets in the Awka metropolis. The study was also intended to ascertain to what extent to which the fish mongers maintain and observe hygiene in their daily activities.

2. Materials and Methods

2.1. Study Area

The study was conducted in Awka, the capital city of Anambra State, where samples were obtained from the biggest market 'Eke-Awka market' in the city. The market serves over 90% of the city's populace. Within the market, different species of ice-frozen fish including tilapia which was the focus of the study. A total of seventy-six retailers were selected for this study.

2.2. Data Collection

Well-standardized questionnaires were employed as instruments for data collection. This was developed to assess the knowledge of hygiene and safety hygiene practices adopted by fish sellers in within the study site in Awka metropolis. Data were collected from 67 frozen fish sellers who voluntarily participated in the study. The questionnaires contained three sections that assessed the participants' demographic characteristics, knowledge of hygiene and safety, and practice of hygiene.

2.3. Ethical Approval and Sampling Method

Approval for the study was obtained from the State Ministry of Agriculture following the screening and approval of the study design and objectives. The study made use of a random sampling technique for the purchase of 310 frozen fish. Study samples were obtained from retailers placed in sterile inverted plastic bags and immediately transported in ice boxes to the research laboratory for analysis. The willingness of each retailer to participate in the research was obtained.

2.4. Questionnaire Survey and Observation

A questionnaire survey that accompanied direct observation was carried out to ascertain the cleanliness (personal hygiene) and the safety of public health through the application of appropriate food storage practices aimed at discouraging any form of cross-contamination of uncooked fish among retailers at the Eke-Awka market. The questionnaires were categorized into questions that evaluated educational status, the knowledge and practice of personal hygiene, fish handling practices, the food safety/storage knowledge.

Each questionnaire was completed by a direct interaction with each respondent that was identified as a fish retailer. The standard guidelines as presented in the Codex Alimentarius Commission of Food and Agriculture organization were adopted in the management of the questionnaires and checklists.

2.5. Isolation and Identification of *Escherichia coli* contaminants

The identification of the fish samples was according to the protocols presented in Bacteriological Analytical Manual methods and other published protocols with slight modifications. Briefly, the surface of each fish sample was aseptically swabbed with a sterile swab stick inoculated into a freshly prepared sterile nutrient broth (Hi-Media, UK), and incubated (Genlab, UK) for 18-24 hrs at 35°C. Isolation by streak plate method was carried out on Mac-Conkey agar (Hi-Media, UK) and incubated for 18-24 hours at 35°C.

After incubation on Mac-Conkey agar, colonies suspected to be *Escherichia coli* were confirmed by combining colony morphology, gram stain, and response to selected biochemical tests which included indole test, citrate test, and catalase test [7, 8].

2.6. Antimicrobial Susceptibility Testing

The susceptibility to selected antibiotics from different classes was determined using the modified Kirby Bauer disc diffusion technique on Mueller- Hinton agar (Hi-Media, UK) plates as recommended by the National Committee for Clinical Laboratory Standards [9]. The bacterial strains were tested against 8 antibiotics (Oxoid, UK) which includes the following discs; ceftazidime (30 ug), Amoxicliin-clavulanic acid (30 ug), cefuroxime (30 ug), ciprofloxacin (5 ug), gentamicin (10 ug), cefixime (5 ug), ofloxacin (5 ug), and nitrofurantion (300 ug).

2.7. ESBL Detection

Following the assessment of Multiple Antibiotic Resistance Indices (MARI) which is an epidemiological tool to monitor drug resistance in the isolated *E. coli* strains. All the isolates observed to be Multi-Antibiotic Resistant using the calculation were further screened for ESBL production. Phenotypic ESBL expression was detected in the resistant isolates as observed by the demonstration of synergistic action between the Ceftazidime (30 µg) and Cefotaxime (30 µg)

antibiotics and Augmentin (Amoxicillin 20 µg and Clavulanic acid 10 µg), placed 15 mm apart on a pre-inoculated lawn on of Muller Hinton Agar [10, 7].

2.8. Molecular detection of β-lactamase Genes

Selected β-lactamase genes TEM, SHV, and CTX-M were amplified in single PCRs using the primers for their identification. Using the Solis Biodyne 5X FIREPol Blend Master mix, ESBL genes were identified in the DNA extracted from the *E. coli* cultures via boiling method [11].

2.9. Data Analysis

The data generated from the questionnaires and laboratory results were prepared using Microsoft Excel and analysed. The data obtained was summarized using descriptive statistics such as frequencies and percentages. The knowledge of respondents was evaluated using questions that covered contamination, handling and storage of fish and the respondents were scored based on their response.

3. Results and Discussion

3.1. Occurrence of *Escherichia coli* contaminants

Of 310 frozen fish samples analyzed, 100 (32.25%) fish samples were contaminated with *E. coli.*

3.2. Demographic Details of the Participants

Data collected from the respondents interviewed in the study area are presented in (Table 1; Figure 1).

More female participants/sellers (74.2%) than males (26.9%) participated in the survey in the selected study area. Most were below the age of (52.2%) while (48.48%) were 30 years and above. Also, (58.2%) of participants were married while (41.8%) were not. The majority had basic education Senior School Certificate Examination (SSCE) as the highest level of education (55.2%). This was followed by those that had tertiary education (37.3%) whereas, (7.5%) had FSLC First School Leaving Certificate (FSLC). Furthermore, (64.2%) were business owners while (35.8%) were employees. Also, (55.2%) have less than 5 years of business experience, (32.8%) have between 6-10 years of experience while (11.9%) have more than 10 years' experience as shown in Table 1. The majority of the participants were located in Amenyi (29.85%), followed by Eke Awka (28.36%). Furthermore, we recorded a varied number of respondents for other study areas: in Kwata (17.91%), (10.45%) in Awka, (8.96%) in the Ifite market, (2.99%) in Agulu, and (1.49%) in Amaihe location Figure 1.

Table 1 Demographic Characteristics of the Participants (n = 67)

Demographics		**No (%)**
Gender	Male	18 (26.9 %)
	Female	49 (74.2 %)
Age	<30	35 (52.2 %)
	≥30	32 (48.48 %)
Marital status	Married	39 (58.2 %)
	Single	28 (41.8 %)
Education level	FSLC	5 (7.5 %)
	SSCE	37 (55.2 %)
	Tertiary	25 (37.3 %)
Ownership	Owners	43 (64.2 %)
	Employees	24 (35.8 %)
Years of Business Experience	< 5 years	37 (55.2 %)
	6 -10 years	22 (32.8 %)
	10 years	8 (11.9 %)

FSLC (First School Leaving Certificate), SSCE (Senior School Certificate Examination)

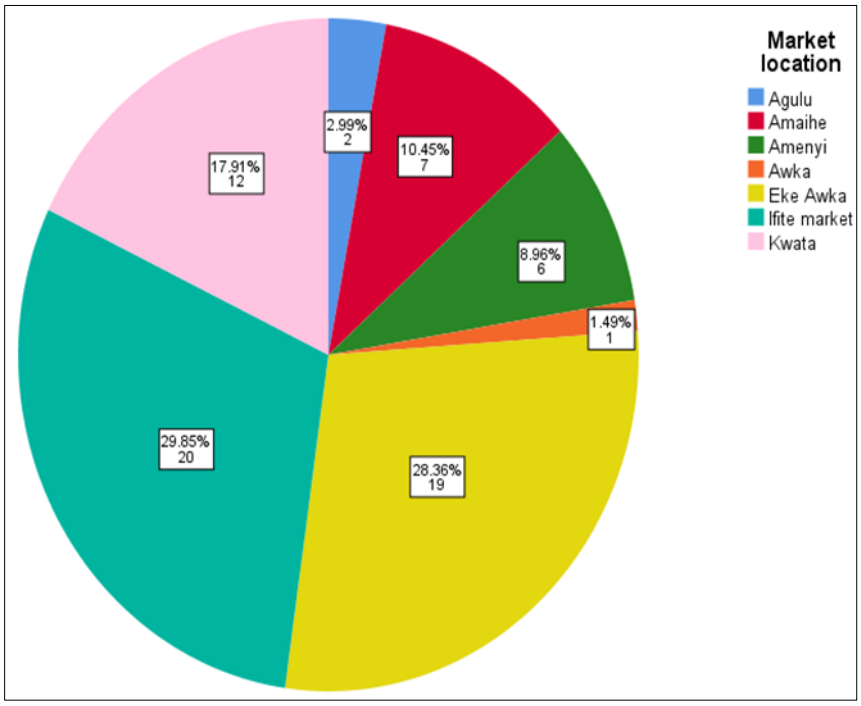

Figure 1 Percentage distribution of study areas

3.3. Knowledge of Hygiene and Safety

The majority of the participants (67.2%) had good knowledge of washing hands before work and were aware that it reduces the risk of fish contamination. However, (13.4%) did not know about hand hygiene. Few of the interviewed respondents expressed good knowledge of how to use and properly discard hand gloves during work to reduce contamination (56.7%), while 19 (28.4%) and 9 (13.4%) disagreed and were indifferent about the use of hand gloves. More than half of the respondents (53.7%) acknowledged that constant use of aprons can reduce the risk of food

contamination while (34.3%) had a negative view and (11.9%) were indifferent. Our results also showed that more than 50 % of them indicated that hair covering during work reduces food contamination risk, whereas (17.9%) disagreed and (9.0%) indicated uncertainty. An almost equal distribution among the respondent's views concerning infected handlers was observed, where (41.8%) affirmed this, (34.3%) and (23.9%) disagreed and indicated uncertainty respectively. The majority of the fish handlers expressed a positive food safety attitude. A good percentage (80.6 %) has adequate knowledge and attitudes about proper storage using a refrigerator. However, only (11.9%) and (7.5%) indicated disagreement and uncertainty respectively. Furthermore, (61.2%) of the participants indicated that storage of fish at room temperature results in contamination, whereas (26.9%) and (11.9%) of the respondents expressed disagreement and uncertainty Table 2.

Table 2 Knowledge of Hygiene practices among the Participants and the Safety (n = 67)

Variables	True no (%)	False no (%)	Not sure no (%)
1. Properly washed hands before handling the fishes eliminates the risk of contamination	45 (67.2)	9 (13.4)	9 (13.4)
2. Constant use of gloves during work eliminates the risk of fish contamination	38 (56.7)	19 (28.4)	9 (13.4)
3. Using apron during work eliminates the risk of food contamination	36 (53.7)	23 (34.3)	8 (11.9)
4. Hair covering during work eliminates the risk of food contamination	49 (73.1)	12 (17.9)	6 (9.0)
5. If you have diarrhea, it is necessary to stay away from handling fish for consumption/ business	28 (41.8)	23.34.3)	16 (23.9)
6. If you have skin wound on hands, it is necessary to cover it when handling fish	51 (76.1)	14 (20.9)	2 (3.0)
7. The refrigerator is the safest fish storage facility	54 (80.6)	8 (11.9)	5 (7.5)
8. Using caps eliminates risk of food contamination	33 (49.3)	19 (28.4)	15 (22.4)
8. Fish kept at room temperature can be contaminated	41 (61.2)	18 (26.9)	8 (11.9)
9. Fish stored at room temperature when consumed can cause disease	28 (41.8)	26 (38.8)	11 (16.4)

3.4. Hygienic practices of Frozen Fish Sellers

Most of the fish sellers practiced food safety and hygienic measures. The survey showed that less than 50 % of the respondents always use gloves during work, the majority of them (50.7%) always wash their hands before using the gloves, 50.7% wear aprons, 52.2% wear face masks, 43.3% always cover their hair, more than 50% of them always clean the contact surface before and after business, and the majority of them indicated that they sometimes wash their hands before and after handling fish Table 3.

Table 3 The Practice and Perceptions of Hygiene by the Fish Vendors (n = 67)

VARIABLES	Always no (%)	Sometimes no (%)	Never no (%)
Do you wear gloves during work?	27 (40.3)	31 (46.3)	7 (10.4)
Do you wash your hands before wearing gloves?	34 (50.7)	13 (19.4)	19 (28.4)
Do you wear clean apron during work?	34 (50.7)	30 (44.8)	2 (3.0)
Do you put on a face mask during work?	24 (35.8)	35 (52.2)	6 (9.0)
Do you cover hair during work?	29 (43.3)	32 (47.8)	5 (7.5)
Do you clean contact surfaces before you start business?	42 (62.7)	24 (35.8)	1 (1.5)
Do you clean contact surfaces after the end of the days/business?	42 (62.7)	24 (35.8)	1 (1.5)

Do you wash your hands before handling the raw fish?	25 (37.3)	39 (58)	2 (3.0)
Do you wash your hands after handling raw fish?	38 (56.7)	39 (58.2)	2 (3.0)
Do you wash your hands after you finish work at the end of the day?	49 (73.1)	17 (25.4)	1 (1.5)
Do you eat or drink while at your business place?	32 (47.8)	30 (44.8)	3 (4.5)
How often do you consume fish from your business?	30 (44.8)	34 (50.7)	2 (3.0)
Do you store left over fish at room temperature for sale the next day?	26 (38.8)	26 (38.8)	14 (20.9)
Do you further process left over fish for sale the next day?	33 (49.3)	29 (43.3)	2 (3.0)

3.5. The Antibiotic Susceptibility Profile of Isolates

All the isolated *E. coli* strains in this study showed complete resistance to the cephalosporins tested: cefixime (100%), ceftazidine (100%) and cefuroxime (99%). Also, the sensitivity of the isolates revealed a 100% resistance to Amoxicillin clavulanate. A high percentage antibiotic resistance rate was also recorded for the fluoroquinolone: ofloxacin (96.0 %), ciprofloxacin (97.0%), and the macrolides like Gentamicin (99%). However, the least resistance was recorded for Nitrofurantoin having a susceptibility of 61% Table 4. Out of the 100 strains of *E. coli* isolated in this study, more than 90 % displayed resistance to beta-lactam antibiotics recorded in the preliminary antibiotic susceptibility screening test and thus are potential ESBL producers

Table 4 Antibiotics Susceptibility Profile

S/N	Antibiotics	Resistant n, (%)	Intermediate N, (%)	Susceptibility N, (%)
1	Cefixime (CXM)	100 (100.0)	0 (0.0)	0 (0.0)
2	Ofloxacin (OFX)	96 (96.0)	2 (2.0)	2 (2.0)
3	Amoxicillin Clavulanate (AMC)	100 (100.0)	0 (0.0)	0 (0.0)
4	Nitrofurantoin (NIT)	16 (16.0)	22 (22.0)	61 (61.0)
5	Ciprofloxacin (CPR)	97 (97.0)	2 (2.0)	1 (1.0)
6	Ceftazidine (CAZ)	100 (100.0)	0 (0.0)	0 (0.0)
7	Gentamicin (GEN)	99 (99.0)	1 (1.0)	0 (0.0)
8	Cefuroxime (CRX)	99 (99.0)	0 (0.0)	1 (1.0)

Table 5 Multiple Antibiotics Resistance Index (MARI) of the Isolates

MARI	Number of Isolates	Percentage (%)
0.12	0	0.00
0.25	0	0.00
0.38	0	0.00
0.50	0	0.00
0.63	8	8.00
0.75	14	14.00
0.88	44	44.00
1.00	34	34.00

3.6. Screening of Isolates for ESBL Production

Out of 100 isolated *E. coli* strains, 21% of the isolates phenotypically expressed ESBL-mediated resistance. This was determined by the Double Disk Synergy Test (DDST) (Table 6, Plate I).

Table 6 Confirmation of ESBL Producing Isolates (n = 100)

Samples screened	Confirmed *E. coli* strains	ESBL Producers
310	100	21 (21.0%)

Figure 2 Plate 1 Petri dish showing positive ESBL isolates S2 (a) and S63 (b)

Table 7 Molecular detection of the co-expression of ESBL-genes (CTX, TEM, and SHV) in *E. coli* isolated from fresh fish in selected retail markets in Awka metropolitan.

Co-expressed ESBL Genes	Number of isolates (%)
CTX, TEM, and SHV	0

Frozen foods have been identified as potential sources of food borne pathogens like *Escherichia coli* [12]. The production of Extended Spectrum Beta-lactamases (ESBL) is observed mostly in Gram-negative bacteria especially in the *Enterobacteriaceae* family, for example, *E. coli* among others have an important mechanism of resistance that enables them to withstand the antimicrobial onslaught of some potent antibiotics. ESBLs are important enzymes responsible for conferring resistance by organisms that have them to beta-lactam antibiotics. The degree of dissemination of the genes CTX, TEM, SHV, etc

Responsible for conferring resistance is very worrisome. Hence this study investigated the presence of ESBL-producing *E. coli* strains that contaminated frozen fish samples in Awka, Anambra State, South-eastern Nigeria, and assessed the fish sellers' knowledge and practice of common hygiene practices.

Bacteriological investigation revealed that 100 (32.25%) *E. coli* organisms were obtained from the 310 frozen fish samples. This result conforms with previous observations by Taiwo *et al., [13]* who reported *E. coli* as one of the predominant bacterial contaminants in fish samples. Also, in this study, the observed level of contamination by *E. coli* is similar to previously established *E. coli* contamination of fish in different study areas [14]. A previous study by Ekici and Dumen, [15], showed food poisoning caused by the spread of small numbers of *E. coli* with a high probability to cause death. It is one of the most implicated bacterial etiological agents associated with childhood diarrhea [8]. The prevalence of *E. coli* reported in this study is in line with the findings by Yohans *et al.,*[16] who reported a prevalence rate of 36 (20%). However, this study identified a higher prevalence rate than that recorded by Awot *et al.,*[17]who recovered 9

(9.4%) *Escherichia coli* strains from 96 fish samples sold at retail shops in Mekelle City, Ethiopia. The isolation rate of *E. coli* obtained from the study area in this study is lower than that reported by Tilahun and Engdawork, [18] who reported an 80 (23.3%) prevalence rate of *Escherichia coli* that contaminated 343 fish samples sourced from Lake Hawassa, Southern Ethiopia. The presence of *Escherichia coli* in the sample studied in our study might be due to poor water treatment used in the aquaculture leading to contamination. The high prevalence rate may be linked to inadequate hygiene and poor handling of the fish resulting in contamination and eventually affecting the health of the consumers upon consumption. This is corroborated by the study carried out by Ohalete *et al*., [19] who reported an *E. coli* contamination rate of 58.3% of fried fish sold in Owerri, Nigeria. Furthermore, this may lead to a higher infection rate with *E. coli*. Poor hygienic practices by food handlers may be the reason why they should be regarded as potential sources of food contamination. Also, in this study, the findings presented show that poor personal hygiene during packaging and as well as during transportation may also be a potential route for contamination with *E. coli* strains. Thus the minimum basic requirement of all food handlers should be to adhere to strict compliance of personal cleanliness. The antibiotic sensitivity profiling showed that all the isolates had complete resistance to cephalosporins like cefixime (100%), ceftazidine (100%), and cefuroxime (99%). Also, amoxicillin clavulanate recorded complete resistance (100%). This resistance rate was followed by fluoroquinolone: ofloxacin (96.0%), ciprofloxacin (97.0%), and the macrolides like Gentamicin (99%). The observed antibiotic resistance is similar to the observations of Elhadi, [20] who reported high rates of resistance to cefotaxime, chloramphenicol, ceftriaxone, and ciprofloxacin antibiotics. Also, our findings corroborated with the observation by Tilahun and Engdawork, [18] who reported resistance of *E. coli* isolated from frozen fish to cefoxitin. However, in a study carried out by Ali *et al.,* [21], they observed 33.3% of the *E. coli* isolates from fish samples displayed resistance to gentamicin. In addition to the varying pattern of resistance by the *E. coli* isolates recorded in this study, all the isolates also expressed multidrug resistance. A study carried out in the eastern province of Saudi Arabia by Elhadi, [20] demonstrated high phenotypic expression of multi-resistant patterns to 21 antibiotics expressed by *E. coli* isolates isolated from three different fish samples which included tilapia, mrigal, and catfish, imported from Thailand and milkfish imported from Vietnam. However, Nitrofurantoin recorded the least resistance case as its susceptibility level is greater than half (61%). According to Heller & Spence, [22], resistance determinants have been confirmed in *E. coli* and certain *Enterobacteriaceaes.* These resistant determinants confer resistance to antibiotics of the class aminoglycosides, fluoroquinolones, and sulfonamides, and are transmissible both intra- and inter-strains. The isolates involved in this study were highly resistant to the cephalosporins and Aminoglycosides (Table 4). Although there is not much available surveillance data on food-borne disease in frozen fish in Nigeria, this study confirmed the presence of multidrug-resistant *E. coli* in frozen fish. This study also supports the work by Ali *et al.,* [21] carried out in Maiduguri Nigeria, who reported that 75.7% of the *E. coli* isolates resisted four or more different antibiotic agents.

These results indicated that the amplified prevalence of multidrug ESBL-producing *E. coli* in fish samples may be linked to the high use of antibiotics in the fishery or may be due to exposure to a contaminated environment due to poor power supply to preserve the fish. These antibiotic-resistant bacteria in aquaculture products like frozen fish are a potential health threat since plasmids can transfer these resistant genes to other bacteria.

The presence of Extended Spectrum Beta-lactamase (ESBL) was phenotypically confirmed in only 21 (21%) of the *E. coli* strains. The result showed a moderately high prevalence of ESBL-producing *E. coli* in frozen fish within the study areas. However, this is not in agreement with the findings by Chibuike *et al.,[23]*who observed that all the *E. coli* isolates from water and fish samples lacked the potential for ESBL production. Of the 21 phenotypically confirmed ESBL-positive isolates, PCR analysis of β-lactamase genes revealed 11 (52.45) ESBL-producing *E. coli* isolates harboring TEM genes. This, however, is in line with the work of Tanko *et al.,[24]* whose predominant genes were TEM genes. However, Elhadi, [20] identified *bla*CTX-M as the most prevalent ESBL gene in imported freshwater fish (52.2%, *n* = 117). Also, 7(33.3%, n = 21) of the isolates had SHV genes. This is quite low compared to the expressed *bla*CTX-M genes. Similarly, the low prevalence rate of SHV (2.2%, *n* = 5) was reported by Elhadi, [20]. The results of this study further validate existing reports on antibiotic resistance arising from frozen fish and animals in communities. This reveals that antibiotics are unduly used in the communities. Moreover, none of the isolates in this study co-expressed the ESBL genes. Similarly, Elhadi, [20] identified ESBL gene combinations *bla*CTX-M/*bla*SHV present in only two strains isolated from catfish imported from Thailand. Of the isolates, 79% did not express any of the β-lactamase genes. Similarly, Elhadi, [20] observed that strains of E. coli isolated from rohu and mrigal fishes imported from Thailand lacked the expression of β-lactamase genes. The presence of ESBLpositive isolates in frozen fish indicates serious public health implications since these isolates could serve as a medium through which resistance traits could spread undetected.

The average multiple antibiotic resistance index of the isolates recorded was 0.83. Bacteria having a MAR index > 0:2 are considered to come from a high-risk source of contamination where several antibiotics or growth enhancers are used while values < 0:2 indicate bacteria from the source with minimal antibiotic use [25]. The study reported completely resistant isolates (MARI of 1.0) in thirty-four isolates. All the *E. coli* isolates expressed a MAR index that is

greater than 0.25 indicating heightened resistance to the antibiotics used. This is in line with the reports by Datok *et al.,*[26] who recorded *E. coli* isolates with a MAR Index of = 0.5 with the most frequent MAR Index being 0.8 with 37.5% occurrence. This suggests a high incorporation of antibiotics in animal production, and this requires the supervision and regulation of all relevant stakeholders in Nigeria. Also, *E. coli* isolates from frozen fish with a MAR index of 0.4 and above may be attributed to human contamination, [25]. The increased prevalence of ESBL-producing *E. coli* isolated from the fish samples from the study area is an indication of the high usage of antibiotics in fish farms in these areas. This may be attributed to the absence of regulations and policies for fish farming or aquaculture which poses a potential health threat related to antibiotic resistance currently being faced in the world.

The farming processes, fish farmers as well as sellers are all implicated in the contamination of the fish. Although the level of contamination is quite high, this may be due to the level of basic education of the sellers having only the basic (SSCE) level of education as the only education attained. In addition, the study outcome showed that the knowledge of the common hygiene practices employed in the fish market by the majority of the fish sellers in Awka metropolis was below average. This is evident by the percentage of sellers who are aware that the use of an apron minimizes contamination and that inadequate storage will become contaminated and may eventually cause diseases when consumed. Our study also showed that the practice of hygiene and safety of fish sellers was below average. Furthermore, there was no strict adherence to some of the

hygienic practice measures as some of the sellers seldom engage in them. For example; the percentages of the fish sellers that observed covering of hair (43.3%), using gloves (40.3%) and face masks (35.8%) during work and washing of hands before touching raw fish (37.3%) were not up to half of the population. Some of these inefficiencies suggest the high occurrence of E. coli contaminants and the presence of ESBL-producing *E. coli*

4. Conclusion

The rate of prevalence of *E. coli* and ESBL-producing *E. coli* isolates from frozen fish samples collected from the study area validates *E. coli* as an important contaminant. This is a serious threat facing public health practice, as the isolates can develop resistance to other antibiotics and also transfer the antibiotic resistance determinants to other previously susceptible bacteria that have been identified to be clinically significant to humans. Therefore, a mechanism that ensures routine surveillance and monitoring of *E. coli* contaminants as well as ESBL producers should be activated and strictly enforced. Fish sellers should be educated on the need for personal hygiene and hygienic handling of fish. Clean aprons, table knives, gloves, and bags. In this study, most of the antibiotics used such as ciprofloxacin, gentamicin, and ofloxacin were not active against the *E. coli* isolates. Finally, the findings from this study indicate unsatisfactory microbiological quality of fish within the study areas. This calls for further investigation of the possible presence of uncharacterized strains and beta-lactam resistance.

Compliance with ethical standards

Acknowledgments

Authors are grateful to Mr. Ukwuoma for funding this research and to Pharmaceutical Microbiology and Biotechnology Laboratory, Faculty of Pharmaceutical Sciences, Nnamdi Azikiwe University Awka, Anambra State, Nigeria, for the support provided.

Disclosure of conflict of interest

The authors declare no conflict of interest

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