ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 14, Issue 3, pp: (82-94), Month: July - September 2026, Available at: www.researchpublish.com

ANTIBIOGRAM OF ESBL-PRODUCING ENTEROBACTERIACEAE ISOLATED FROM FISH COLLECTED FROM INTEGRATED POULTRY-FISH FARMS AT ILA ORANGUN, OSUN STATE, NIGERIA

[*1]Adeluwoye-Ajayi, Olayemi Amos., [2]Akinnibosun, Faith Iguodala., [3]Akinnibosun, Henry Adewale., [4]Iyekowa, Osaro., [5]Akinnibosun, Adeyemi Osarodion

[1]Department of Science Laboratory Technology (Microbiology Option), Rufus Giwa Polytechnic, Owo, P.M.B 1019, Ondo State Nigeria.

[2] Department of Microbiology, Faculty of Life Sciences, University of Benin, P. M. B. 1154, Benin City, Nigeria.

[3] Department of Plant Biotechnology, Faculty of Life Sciences, University of Benin, P. M. B. 1154, Benin City, Nigeria.

[4] Department of Chemistry, Faculty of Physical Sciences, University of Benin, P. M. B. 1154, Benin City, Nigeria.

[5]School of Medicine, College of Medical Sciences, University of Benin, P. M. B. 1154, Benin City, Nigeria.

DOI: https://doi.org/10.5281/zenodo.21847996

Published Date: 08-August-2026

Abstract: **Antimicrobial resistance (AMR) is widely recognized as a silent pandemic, threatening decades of medical progress and complicating the treatment of infectious diseases worldwide. Among the myriad resistance determinants, extended-spectrum β-lactamases (ESBLs) have emerged as a major driver of therapeutic failure in clinical medicine. This study is aimed at determining the pattern of resistance of ESBL-producing enterobacteriaceae isolated from fish collected from integrated poultry-fish farms at Ila Orangun, Osun State, Nigeria. From November 2024 to April 2025, a total of 18 catfish were aseptically and randomly collected from six poultry-fish farms, processed and bacteriologically analysed using standard microbiological techniques. Isolated isolates were conventionally characterized, ESBL-production in them were confirmed via double-disc synergy test, and antibiogram of the confirmed ESBL+ isolates were carried out via Kirby-Bauer disc diffusion method. Out of 47 isolates recovered from the catfish, 2 (4.26%) were ESBL+. The ESBL+ isolates were totally resistance (100%) to the beta-lactam combination, the cephems and the monobactam respectively tested in this study but totally susceptible (100%) to the carbapenem. They were totally (100%) multidrug-resistance (MDR) with 50% of them resisting 5 and 4 classes of antibiotics respectively, and showing 0.6 and 0.4 multiple antibiotic resistance index (MARI) respectively. The results confirm integrated agro-ecosystems as important reservoirs of MDR, ESBL-producing Enterobacterales. Urgent interventions in the farming practices, surveillance, and public education are needed to mitigate MDR, ESBL-producing enterobacteriaceae spread from farms produce to human through the food chain.**

Keywords: **Enterobacterales, Antimicrobial resistance, Extended-spectrum β-lactamases, Pandemic, Catfish.**

1. INTRODUCTION

Fish is low in cholesterol and contains all essential amino acids and is estimated to provide roughly 60% of the world's protein requirements, with 60% of the developing world obtaining more than 30% of their animal protein from fish (Kuton *et al*., 2021). In addition to being a good source of protein, fish is high in vitamins, unsaturated fatty acids, and essential minerals (Rajeshkumar & Li, 2018). The world's focus has shifted towards fish to address the deficiency of animal protein,

particularly in light of the increasing global population and depletion of natural resources (Du Thanh *et al*., 2017). The high consumption of fish also stems from the fact that it is cheaper and generally accepted without any religious bias giving it an added benefit over other sources of proteins (Aliyu *et al*., 2018). The Food and Agriculture Organization (FAO) estimates an increase in fish production in Nigeria by 2030 to about 18.2%, while export will increase by about 6.6% (FAO, 2018).

Cases of fish-borne disease caused by pathogenic microorganisms reported in several parts of the world heightened consumers fear regarding fish meat and fish products (Li *et al*., 2019; Yanestria *et al*., 2019). The use of organic manure also leads to the release of high concentration of opportunistic and pathogenic microorganisms into the ponds which pose a threat not only to fish health but the environment (Abdel-Wahed *et al*., 2018). Also, these microorganisms in fish and fish ponds portend grave consequences for public health (Li *et al*., 2019; Yanestria *et al*., 2019).

Antimicrobial resistance (AMR) is widely recognized as a silent pandemic, threatening decades of medical progress and complicating the treatment of infectious diseases worldwide (Murray *et al*., 2022). Both pathogenic and non-pathogenic bacteria can exhibit AMR. Multiple antibiotic resistance (MAR) is a worldwide problem caused by the abuse of antibiotics (Sharma *et al*., 2015). Multidrug resistance (MDR) has increased all over the world and is considered a public health threat (Catalano *et al*., 2022). The presence of MDR bacteria in fish can endanger public health because fish are thought to be potential carriers of foodborne bacterial diseases (Hassen *et al*. 2020). From investigations reported, the emergence of multidrug-resistant bacterial pathogens from different origins, increases the necessity of the proper use of antibiotics, routine applications of the antimicrobial susceptibility testing to detect the antibiotic of choice, and screening of emerging MDR strains (Abolghait *et al*., 2020; Algammal *et al*., 2020; Algammal *et al*., 2022).

Among the myriad resistance determinants, extended-spectrum β-lactamases (ESBLs) have emerged as a major driver of therapeutic failure in clinical medicine as they hydrolyze a broad spectrum of β-lactams, including third-generation cephalosporins such as cefotaxime, ceftriaxone, and ceftazidime, leaving limited treatment options (Bush & Bradford, 2020), thus ESBL-producing bacteria can exhibit co-resistance to multiple classes of antibiotics (Tacão *et al*., 2014). Infarct, the emergence of ESBL-producing *Enterobacteriaceae* became one of the crucial public health concerns, and subsequently fewer treatment options (Castanheira *et al*., 2021).

Historically, ESBL-producing Enterobacterales were considered a nosocomial concern; however, in the past two decades, their epidemiology has shifted, with increasing prevalence in community, animal, and environmental settings (Bevan *et al*., 2021). This reflects the interconnected nature of AMR as described in the One Health framework, which emphasizes the interdependence of human, animal, and environmental health (Robinson *et al*., 2016). Food production systems, particularly integrated farms where poultry and fish intersect, are emerging as high-risk hotspots for resistance dissemination (Van Boeckel *et al*., 2019). High organic loads and possible antibiotic residues from veterinary use in this system create selective pressures that favor resistant population (Gothwal & Shashidhar, 2020).

Although integrated poultry-fish farming systems are economically efficient but may inadvertently recycle resistant bacteria and ARGs across compartments, creating opportunities for horizontal gene transfer (HGT) and amplification of resistance determinants. The generation of extended-spectrum β-lactamase (ESBL) by Enterobacteriaceae can intensify this challenge (Iredell *et al*., 2016).

There is insufficient data on the presence and spread of drug resistant ESBL-producing enterobacteriaceaea in integrated poultry-fish farms in Nigeria hence this study aimed at determining the pattern of resistance of ESBL-producing enterobacteriaceae isolated from fish collected from integrated poultry-fish farms at Ila Orangun, Osun State, Nigeria so as to enhance antibiotic resistance control efforts**.**

2. MATERIALS AND METHODS

Description of Study Area

The study was conducted in commercialised integrated poultry-fish farms system located at Ila Orangun, Osun State, Nigeria. Ila Orangun, the headquarters of Ila Local Government Area in Osun State Nigeria, has an area of approximately 303km^2 and a population of 62,049 (2006 census). However, according to the information obtained from GeoNames geographical database, the population of Ila Orangun is 179, 192. The latitude and longitude coordinate of this town are 8.019116 and 4.901962 respectively. A common traditional profession of the indigenes of the town is palm-wine tapping. The people of Ila speak the distinctive dialect of the Yoruba language called Igbomina (or Igbonna).

Consideration of Ethical Issues

The study's purpose was explained to the integrated poultry-fish farmers, who consented to sampling on the condition that their identities not be published, hence the sampling farms are anonymous in this report. This study was performed in accordance with guidelines of the University of Benin research committee and the guidelines for the care and use of Agricultural Animals in research and teaching (Grandin *et al.*, 2020).

Samples Collection

Between November 2024 and November 2025, with the help of farms' manager, eighteen of five to six months old (adult) live apparently healthy African catfish (*Clarias gariepinus*) with approximately similar wet weight (1.5kg to 2.5kg) and length (29cm to 35cm) were randomly collected with the aid of cast net into bowls containing water from the fishponds of origin. Samples were collected once in two months (to give room for samples processing, bacteria isolation and identification) in triplicates using triangular method of sampling from the same integrated system on each of the six farms early in the morning (06:00am to 09:00am) for the period of sampling. All samples collected from each of the three integrated system per farm were well labelled and transported to the laboratory for immediate processing and bacteriological analysis.

Sample Preparation for Microbiological Study

The sample was prepared by making dilutions in distilled water.

Bacteriological Analysis

Bacterial isolation was done according to the method described by Cheesbrough (2016). The media used were prepared according to manufacturer's instruction. The glass wares such as Petri dishes, conical flasks, test tubes, beakers and bijou bottles were thoroughly washed and sterilized in a hot air oven at 160 °C for an hour. The inoculating loop was sterilized by flaming in the Bunsen burner until it turns red hot. Similarly, microbial load on the working surfaces were reduced by the application of disinfectant solution (70% ethanol).

Maintenance of Bacteria Stock Culture

Each of the isolates were maintained as stock cultures for further studies on nutrient agar (Oxoid, UK) slants in Bijou bottles. This was done by streaking the isolates on the agar slants, and incubating at 37 ^{o}C for 24 hrs. After incubation, the inoculated slants were stored in the refrigerator at ambient temperature and these stock cultures served as source of each isolate for further bacteriological studies.

Identification of Bacterial Isolates

Morphological Characterization of Isolates

A 24-hour old pure culture of the isolates was characterized and the different morphologies were recorded.

Gram-Staining and Microscopic Examination

This was carried out according to the method described by (Becerra *et al*, 2016)

Biochemical Characterization of the Isolates

The biochemical tests such as motility test, catalase test, citrate utilization test, indole test, methyl red test, voges-proskauer test, and sugar fermentation (glucose, lactose and maltose) test were carried out according to Cheesbrough (2016).

Screening for Potential Extended Spectrum Beta-Lactamase Producing Enterobactericeae

All the isolates were assessed for the production of extended spectrum beta-lactamase. Mueller-Hinton agar plates were inoculated with homogeneous inoculum of the test isolates and separate antibiotic discs that contain ceftazidime (30 µg) and cefotaxime (30 µg) respectively were positioned aseptically on the agar surface at a distance of 30 mm apart. The plates were placed on workbench for about half-hour to allow the antibiotics to diffuse into the agar, and then they were incubated at 37 ^{o}C for 24 hrs. Thereafter, meter rule was used to measure the zones of inhibition. A reduced susceptibility/resistance to either of the two antibiotics used for the screening was taken as suspected case of extended spectrum beta-lactamase production (CLSI, 2024).

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 14, Issue 3, pp: (82-94), Month: July - September 2026, Available at: www.researchpublish.com

Recognition of Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae

An 18 hrs old culture of isolates that shows resistance to one or more of the third generation cephalosporins were inoculated into test tubes which contain sterile normal saline, then the turbidity of the content was adjusted to 0.5 MacFarland standards. The bacterial suspension was inoculated on prepared Mueller Hinton agar plates by uniformly swabbing the entire surface of the agar plates. The double disc synergy test was done using discs of augmentin alongside the discs of ceftazidime (30 µg) and cefotaxime (30 µg); which were placed at 20 mm equidistance from augmentin disc. Then, the plates were incubated for 24 hrs at 37 ºC, the plates were observed and the organisms was considered to be producing extended spectrum beta-lactamase when the zone of inhibition is ≤5 mm in diameter of either cefotaxime or ceftazidime in combination with augmentin against its zone when tested alone (CLSI, 2024)

Antibiogram of the ESBL-producing Bacteria

This was done using the standard Kirby-Bauer disc diffusion (Jayabarath, 2015). The ESBL-producing bacteria inoculum was prepared by separately suspending the freshly grown bacteria in 5 ml sterile nutrient broth and its turbidity adjusted to 0.5 McFarland standards. The antimicrobial susceptibility testing was performed separately on Mueller-Hinton agar using the following antibiotics: beta-lactam combination agent (augmentin 20/10µg), cephem (cefotaxime 30µg, ceftazidime 30µg, cefixime 5µg), carbapenem (imipenem 10µg), aminoglycosides (gentamicin 10µg), fluoroquinolone (ciprofloxacin 5µg, ofloxacin, 5µg), monobactam (aztreonam 30µg) and nitrofurans (nitrofurantoin 300µg). The plates were incubated aerobically at 37 ºC for 24 h. The zones of inhibition were measured with a metre rule and the results were recorded and interpreted according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (2024). Isolates showing resistance to at least three different classes of antibiotics were considered as multidrug resistant strains (Magiorakos *et al*., 2012). *E. coli* ATCC 25922 was used as a quality control organism

Multiple Antibiotic Resistance Index of Isolates

As described by Ekwealor *et al*. (2016), the calculation of the Multiple Antibiotic Resistance Index (MARI) involved employing the provided equation below. This index was derived by assessing whether a zone of inhibition was present or absent. The MARI value was determined by dividing the total count of antibiotics to which an isolate demonstrated resistance by the total count of antibiotics administered to each strain.

$$MARI = \frac{\text{Number of antibiotics strain resistant}}{Number\ of\ antibiotics\ strain\ subjected}$$

3. RESULTS

Morphological, Macroscopic, Cultural and Staining Characteristics of the Bacteria Isolated from the Catfish Collected from the Integrated Poultry-Fish Farms

Table 1 below showed the morphological, macroscopic, cultural and staining characteristics of the bacteria isolated from catfish collected from the six integrated poultry-fish farms at Ila Orangun, Osun State, Nigeria. Inferences drawn from the bacteria characteristics are as depicted in the table.

Biochemical Characteristics of the Bacteria Isolated from the Samples Collected from the Integrated Poultry-Fish Farms

Table 2 below showed the biochemical characteristics of the bacteria isolated from catfish collected from the six integrated poultry-fish farms at Ila Orangun, Osun State, Nigeria. Inferences drawn from the biochemical characteristics were used in getting the identity of the isolates as depicted in the table.

Preliminary Screening for ESBL Producing Isolates from Catfish Samples from the Integrated Poultry-fish Farms

The preliminary screening for ESBL production by *E. coli, K. pneumonia, K. aerogenes, Salmonella sp., Enterobacter sp. and Proteus sp.* isolated from catfish is shown in Table 3 where it was revealed that 7 (41.18%), 1 (8.33%), 1 (33.33%), 1 (25.0%), 1 (20.0%) and 1 (16.67%) of the respective isolates were suspected to be ESBL producers since they showed resistance to cefotaxime.

Confirmatory Screening for Suspected ESBL-producing Isolates from Catfish Collected from Poultry-fish Pond Integrated Farms

The confirmatory screening for ESBL production by isolates from catfish indicated that 2 (16.67) out of the 12 suspected ESBL positive isolates from catfish were confirmed to be ESBL positive. Furthermore, out of all the bacteria isolated only *E. coli* produced ESBL which was just 2 (28.57) of the 7 suspected E. coli (Table 4).

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 14, Issue 3, pp: (82-94), Month: July - September 2026, Available at: www.researchpublish.com

Prevalence of ESBL-Producing Enterobacterales in the Catfish from the Integrated Poultry-Fish Farms

Table 5 below showed that out of the 47 enterobacterales recovered from the fish sampled, only *E. coli* produced ESBL and its prevalence in relation to the total number of isolates isolated was just 4.26%

Antibiogram of the ESBL-Producing *E. coli* (n = 2) Isolated from Catfish Collected from Integrated Poultry-Fish Farms

Table 6 showed the antibiogram of the only ESBL-producing enterobactericeae (i.e. *E. coli)* isolated from catfish collected from the farms to the tested antibiotics. The ESBL-producing *E. coli* isolates from the sample were totally resistance (100%) to the beta-lactam combination (augmentin), the cephems (cefotaxime, ceftazidime and cefixime) and the monobactam (aztreonam) respectively tested in this study but totally susceptible (100%) to the carbapenem (imipenem).

Antibiotype of ESBL Producing *E. coli* (n = 2) Isolated from Catfish Collected from

Integrated Poultry-Fish Farms

The antibiotypes of the ESBL producing *E. coli* showed that 1 (50%) of the two isolates that produced ESBL resisted five classes of antibiotics (CIP-CAZ-NIT-GEN-OFL-AUG) and four classes of antibiotics (CIP-CAZ-GEN-AUG) respectively, and showing 0.6 and 0.4 multiple antibiotic resistance index (MARI) respectively. The ESBL-producers were totally (100%) multidrug-resistant (Table 7).

Table 1: Morphological, Macroscopic, cultural and Staining Characteristics of the Bacteria Isolates from the Samples Collected from the Integrated Poultry-fish farms

Colony characteristics on MacConkey Agar	Morphology (Staining characters)	Probable organism
Smooth, circular, flat and non-mucoid, colonies that are pink to reddish-pink due to lactose fermentation	Gram-negative (pink/red colour), small (1.0–2.0 µm long and 0.5 µm wide), rod-shaped, arranged in single or paired short, motile, Non-sporing, some are capsulated	*E. coli*
Smooth, large (often 4-6 mm), round, convex (dome-shaped), and shiny colonies that are pink to dark pink/red due to lactose fermentation, appearing mucoid slimy, glistening, and sticky because of their prominent polysaccharide capsule.	Gram-negative (pink/red colour), small rod-shaped, arranged in single or paired short , Non-motile, Non-spore-forming, capsulated	*Klebsiella* sp.
small to medium (2-3 mm), convex (dome-shaped), smooth, and can have slightly serrated or entire margins**,** colorless or pale, translucent colonies because they are non-lactose fermenters	Gram-negative (pink/red colour), small (2-5 µm long, 0.8-1.5 µm wide) rod-shaped, arranged in single or paired short ,motile, Non-spore-forming, capsulated	*Salmonella* sp.
Pink to red/rose-red colonies due to lactose fermentation (Lac+) and produce a capsule, making the colonies appear moist, mucoid (slimy/sticky and sticky, often surrounded by a hazy zone**.**	Gram-negative (pink/red colour), motile, small **(**1–5 µm long) straight rod-shaped bacteria **(**bacilli**)** appearing as singly, dispersed or scattered**,** facultative anaerobic, and non-spore forming, capsulated bacteria.	*Enterobacter* sp.
Translucent, colorless, non-lactose fermenting colonies that often exhibit a characteristic swarming (forming a thin, filmy layer) growth pattern, spreading across the plate in concentric rings with a fishy or ammonia-like smell, though they may appear as smaller, smooth, pale or grayish-white colonies if motility is inhibited.	Gram-negative (pink/red colour)**,** motile, non-spore-forming, non-encapsulated, rod-shaped (bacilli) bacteria that are sometimes pleomorphic	*Proteus* sp.

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 14, Issue 3, pp: (82-94), Month: July - September 2026, Available at: www.researchpublish.com

Table 2: Biochemical Characteristics of the Bacteria Isolated from the Samples Collected from the Integrated Poultry-fish Farms

MOT	CAT	CIT	IND	MR	VP	GLU	LAC	MAL	ISOLATES IDENTITY
+	+	-	+	+	-	+	+	+	*Escherichia coli*
-	+	+	-	-	+	+	+	+	*Klebsiella pneumoniae*
+	+	+	-	-	+	+	+	+	*Klebsiella aerogenes*
+	+	+	-	+	-	+	-	+	*Salmonella* sp.
+	+	+	-	-	+	+	+	+	*Enterobacter* sp.
+	+	+	-	+	-	-	-	+	*Proteus* sp.

KEY: MOT = Motility, CAT = Catalase, CIT = Citrate, IND = Indole, MR = Methyl Red, VP = Voges-Proskauer, GLU = Glucose, LAC = Lactose, MAL = Maltose, - = negative, + = positive, +/- = variable

Table 3: Preliminary Screening for ESBL-producing Isolates from Catfish Samples of Integrated Poultry-fish Farms

Name of isolate	No. of isolate	Antibiotics used	No. (%) resistant	No. (%) sensitive
E. coli	17 (36.2)	Cefotaxime	7 (41.18)	10 (58.82)
		Ceftazidime	6 (35.29)	11 (64.71)
K. pneumoniae	12 (25.5)	Cefotaxime	1 (8.33)	11 (91.67)
		Ceftazidime	0 (0)	12 (100.0)
K. aerogenes	3 (6.4)	Cefotaxime	1 (33.33)	2 (66.67)
		Ceftazidime	0 (0)	3 (100.0)
Salmonella sp.	4 (8.51)	Cefotaxime	1 (25.0)	3 (75.0)
		Ceftazidime	0 (0)	4 (100.0)
Enterobacter sp.	5 (10.6)	Cefotaxime	1 (20.0)	4 (80.0)
		Ceftazidime	0 (0)	5(100)
Proteus sp.	6 (12.8)	Cefotaxime	1 (16.67)	5 (83.33)
		Ceftazidime	0 (0)	6 (100.0)
TOTAL	47			

KEY: No. = Number, % = Percentage

Table 4: Confirmatory Screening for Suspected ESBL-producing Isolates from Catfish Samples collected from Poultry-fish Pond Integrated Farms

Name of Isolate Screened	No. of Isolates Screened	No. of ESBL + (%) Isolates	No. of ESBL - (%) Isolates
E. coli	7	2 (28.57)	5 (71.43)
K. pneumoniae	1	0 (0)	1 (100.0)
K. aerogenes	1	0 (0)	1 (100.0)
Salmonella sp.	1	0 (0)	1 (100.0)
Enterobacter sp.	1	0 (0)	1 (100.0)
Proteus sp.	1	0 (0)	1 (100.0)
TOTAL	**12**	**2 (16.67)**	**10 (83.33)**

KEY: No. = Number, % = Percentage

ISSN 2348-313X (Print)
International Journal of Life Sciences Research ISSN 2348-3148 (online)
Vol. 14, Issue 3, pp: (82-94), Month: July - September 2026, Available at: www.researchpublish.com

Table 5: Occurrence of ESBL Producers among the Bacteria Isolated from the Fish Samples Collected from Poultry-fish Pond Integrated Farms

Isolate	Isolate No	No. (%) of Probable ESBL Producers	No. (%) of Confirmed ESBL Producers	No. (%) of Non ESBL Producers
E. coli	17	7 (41.18)	2 (11.76)	15 (88.24)
K. pneumoniae	12	1 (8.33)	0 (0)	12 (100.0)
K. aerogenes	3	1 (33.33)	0 (0)	3 (100.0)
Salmonella sp.	4	1 (25)	0 (0)	4 (100.0)
Enterobacter sp	5	1 (20)	0 (0)	5 (100.0)
Proteus sp.	6	1 (16.67)	0 (0)	6 (100.0)
Total	**47**	**12 (25.53)**	**2 (4.26)**	**45 (95.74)**

KEY: No. =Number; % = percentage

Table 6: Antibiogram of ESBL-producing *E. coli* (n = 2) Isolated from Catfish Collected from Integrated Poultry-fish Farms

Family of Antibiotics Tested	Name of Antibiotics Tested	Antibiotics Disc Code	Antibiotics Disc Concentrations (µg)	Interpretive Categories and Zone Diameter Breakpoint, Nearest Whole Number (mm)		Antibiogram	
				S	R	S No. (%)	R No. (%)
Beta-lactam combination	Augmentin	AUG	30	≥ 18	≤ 13	0 (0)	2 (100)
Cephems	Cefotaxime	CTX	30	≥ 26	≤ 22	0 (0)	2 (100)
	Ceftazidime	CAZ	30	≥ 18	≤ 14	0 (0)	2 (100)
	Cefixime	CXM	5	≥ 16	≤ 20	0 (0)	2 (100)
Carbapenem	Imipenem	IMP	10	≥ 19	≤ 15	2 (100)	0 (0)
Aminoglycosides	Gentamicin	GEN	10	≥ 15	≤ 12	1 (50)	1 (50)
Fluoroquinolones	Ciprofloxacin	CIP	5	≥ 21	≤ 15	1 (50)	1 (50)
	Ofloxacin	OFL	5	≥ 16	≤ 12	1 (50)	1 (50)
Monobactam	Aztreonam	AZT	30	a≥ 22	≤ 15	0 (0)	2 (100)
Nitrofuran	Nitrofurantoin	NIT	300	≥ 17	≤ 14	1 (50)	1 (50)

KEY: n/No. = Number, % = percentage, S = Susceptible, R = Resistant

Table 7: Antibiotype of ESBL-producing *E. coli* (n = 2) Isolated from Catfish Collected from Integrated Poultry-fish Farms

Pattern No.	Antibiotypes	No. of Antibiotics (Classes)	No. of Antibiotics tested	No. (%) ESBL+ *E. coli*	MAR Index
1	NIT-IMP-CIP-CAZ-GEN-AUG-AZT	7 (7)	10	0 (0)	0
2	CIP-IMP-AZT-GEN-CRX-AUG	6 (6)	10	0 (0)	0
3	CIP-CAZ-NIT-GEN-OFL-AUG	6 (5)	10	1 (50)	0.6
4	NIT-CIP-CXM-GEN-AUG	5 (5)	10	0 (0)	0
5	CIP-CRX-GEN-OFL-AUG	5 (4)	10	0 (0)	0
6	IMP-CXM-GEN-OFL	4 (4)	10	0 (0)	0
7	NIT-CXM-IMP-AUG	4 (4)	10	0 (0)	0

8	CIP-CAZ-GEN-AUG	4 (4)	10	1 (50)	0.4
9	CIP-CXM-OFL-AUG	4 (4)	10	0 (0)	0
10	CIP-CAZ-GEN-AUG	4 (4)	10	0 (0)	0
11	CAZ-OFL-AUG	3 (3)	10	0 (0)	0
12	CXM-GEN-AUG	3 (3)	10	0 (0)	0
13	IMP-CAZ-CTX	3 (2)	10	0 (0)	0
Total MDR of ESBL+ Isolates No. (%)				2 (100)	

KEY: n/No. = Number, % = percentage

4. DISCUSSION

Antibiotic resistance has been causing serious problems to human medicine and also to animal, livestock, and veterinarians (Lawson, 2008). The heavy and off-label use of antibiotics has been reported to be a risk factor for the development and spread of beta-lactamase producing organisms (Chishimba *et al*., 2016; Economou and Gousia, 2015). Therefore, the present study focus on investigating the pattern of resistance of ESBL-producing enterobacteriaceae (ESBL-PEN) isolated from fish collected from integrated poultry-fish farms at Ila Orangun, Osun State, Nigeria.

The morphological, macroscopic, cultural and staining characteristics of the bacteria isolated from the fishponds water in this present study were in agreement with previous researchers report (Akinnibosun *et* al., 2024; Osuntokun *et al*., 2020; Okpalaji *et al*., 2025; Nwabueze & Nwabueze, 2011; Ali, 2025; Bello *et al*., 2019; Agwaranze *et al*., 2025; Kanu *et al*., 2024; Ajayi, 2012; Fakorede *et al*., 2020).

Also, the biochemical characteristics of the members of enterobacteriaceae isolated from the fishponds water in this present study were in agreement with previous researchers report (Akinnibosun *et* al., 2024, Osuntokun *et al*., 2020; Okpalaji *et al*., 2025; Nwabueze & Nwabueze, 2011; Ali, 2025; Bello *et al*., 2019; Agwaranze *et al*., 2025; Kanu *et al*., 2024; Ajayi, 2012; Fakorede *et al*., 2020).

The results of this study indicating the presence of ESBL positive (11.76%) *E. coli* is in agreement with the earlier reports of Elhadi (2016) in Saudi Arabia, Hinthong *et al*. (2024) in Thailand and Dayie *et al*. (2024) in Ghana each of whom respectively reported the presence of ESBL-producing *E. coli* (27.2 %, 5.7 %, and 7.4 % respectively) in fish samples. However, the 11.76% ESBL producing *E. coli* (ESBL-PEC) observed in this study is lower than 21.2% but higher than 5.7% and 7.4% respectively reported by same authors. The reason for this disparity could be due the number of isolates studied, the species of fish studied and the level of animal exposure to antibiotics.

The overall ESBL-producers (4.26%) in this present study is in agreement with the 1.3% reported by Dayie *et al*. (2024) in Ghana. However, the 4.26% ESBL producers observed in this study is a little bit higher than the 1.3% reported by same author. The reason for this disparity could be due to differences in the method of screening for ESBL producers, due the number of isolates studied, the species of fish studied and the level of animal exposure to antibiotics. In addition, none of the other isolates in this present study produced ESBL, a report which negate the report of Singh *et al*. (2017) and Dayie *et al*. (2024) both of whom reported the production of ESBL by the other bacteria species. The reason for this disparity could be due the number of isolates studied, the species of fish studied and the level of animal exposure to antibiotics.

The presence of ESBL-PEC in catfish from the farms is of public health importance as it point toward a great health risk for the consumers in the area of study. The contamination of fish meant for public consumption raises a potential risk of infection among the consumers as well as the handlers of these fish with ESBL producing *E. coli* particularly when these fish are eaten without adequate cooking. It is important to note that catfish are used in making pepper soups in the studied area, a recipe which undergo minimal heat processing which are incapable of neutralizing the microbial contaminants on the catfish. On the other hand, the type of resistance caused by ESBLs usually enables the organism to be resistant to other forms of antibiotics, and this in effect makes antibiotic therapy ineffective (Reist *et al*., 2013). Deaths due to ESBL-producing *E. coli* infections are reported to be three-times greater than those caused by *E. coli* that are incapable of producing ESBLs (Tekiner & Özpınar, 2016; Melzer & Petersen, 2007).

Antibiotic resistance presents a significant public health concern, as antibiotic-resistant bacteria can cause severe illness or even death, and can be challenging to treat (Blaak *et al*., 2015; García-Vello *et al*., 2020). This present study revealed

different degree of resistance by the only isolated ESBL-producers (i.e. *E. coli*) among the isolates to the tested commercially available antibiotics. Out of all the tested antibiotics, the isolated ESBL-PEC showed total resistance (100%) to the beta-lactam combination (augmentin), the cephems (cefotaxime, ceftazidime and cefixime) and the monobactam (aztreonam) respectively tested in this study but totally susceptible (100%) to the carbapenem (imipenem).

The total resistance (100%) to cefotaxime and ceftazidime respectively observed in this present study support the report of Singh *et al*. (2017) where 97.63% and 91.12% of the ESBL-producing isolates were respectively resistance to these antibiotics. The total resistance (100%) of the ESBL-PEC to ceftazidime observed in this present study is parallel to the same value of resistance to same antibiotic earlier reported by Chancelle *et al*. (2023). Also, the total resistance (100%) of the ESBL-PEC to cefotaxime observed in this present study is parallel to the same value of resistance to same antibiotic earlier reported by Chancelle *et al*. (2023). However, the total resistance (100%) of the ESBL-PEC to cefotaxime is far higher than the values earlier reported in previous research studies (Dayie *et* al., 2024; Adinortey *et al*., 2020; Chibuike *et al.*, 2021; Agbeko *et al*., 2022).

The total resistance (100%) shown by the ESBL-PEC in this present study to aztreonam and augmentin respectively is far higher than the 65.08% and 38.46% resistance respectively shown by isolated bacteria to these antibiotics as reported by Singh *et al*. (2017). The total resistance (100%) shown by the ESBL-PEC in this present study to aztreonam support the report of Chibuike *et al.* (2021) and Chancelle *et al*. (2023) who reported 100% resistance and 89.5% resistance respectively to same antibiotic. Also, the total resistance (100%) shown by the ESBL-PEC in this present study to augmentin is in conformity with the same value of resistance to same antibiotic reported by Chibuike *et al.* (2021).

The observed 50% resistance to gentamicin in this study is far higher than the values earlier reported in previous studies (Hinthong *et al*., 2024; Dayie *et al*. 2024; Singh *et al*., 2017; Agbeko *et al*., 2022; Adinortey *et al*., 2020; Chancelle *et al*., 2023). The 50% resistance shown by the isolate to ofloxacin in this present study is similar but lower than the 57% and the 94.7% resistance shown by same organism to same antibiotic reported by Adinortey *et al*. (2020) and Chancelle *et al*. (2023) respectively. Also, the 50% resistance shown by the isolate in the present study to ciprofloxacin is similar but a little bit lower than the 63.2% resistance shown by same organism to same antibiotic reported in earlier study by Chancelle *et al*. (2023). However, the observed 50% resistance to ciprofloxacin in this present study by the isolate negate the 0% resistance shown by same organism to same antibiotic reported by Mwanza *et al*. (2021).

The none resistance (0%) shown by the ESBL-PEC in this present study to the carbapenem (imipenem) is parallel to the value of resistance to same antibiotics earlier reported in previous research (Dayie *et al*., 2024; Chibuike *et al.*, 2021). However, the zero resistance shown by the ESBL-PEC to the tested carbapenem (imipenem) in this present study is lower than the 10.65% and 18.2% resistance respectively reported by Singh *et al*. (2017) and Chancelle *et al*. (2023). The reason for the disparity in the level of antibiotics activity observed in the present study in relation to previous studies could be due the number of isolates studied and the level of the fish exposure to antibiotics. The reasonably high level of activity of the imipenem against the ESBL-PEC isolates in this study support the view of carbapenems being the standard drug for the management of infections occasioned by some Gram negative bacteria including those provoked by extended-spectrum-β-lactamases (Dahiya *et al*., 2015).

All the ESBL-PEC in this study were multidrug-resistant with 50% of them resisting five (CIP-CAZ-NIT-GEN-OFL-AUG) and four (CIP-CAZ-GEN-AUG) classes of antibiotics respectively supporting the report of Kimera *et al*. (2021) whose studied showed eight isolates resisting eight classes of antimicrobials; and the report of Homeier-Bachmann *et al*. (2021) where all the 25 ESBL-producing Enterobactericeae were reported as multidrug-resistant.

The ESBL-PEC in this study showed 0.6 and 0.4 Multiple Antibiotic Resistance Index (MARI) respectively. According to Thenmozhi *et al*. (2014), Multiple Antibiotic Resistance Index (MARI) determines the level of an isolate's resistance to the antibiotics tested, and a value of ≥ 0.2 indicates a high risk of contamination from sources with high antibiotic use hence it could be stated that catfish from the source studied is of high risk of antibiotics contamination and is not safe for human consumption.

5. CONCLUSION

This study showed the presence of multidrug resistant (MDR), ESBL-PEC in the catfish gotten from the farms. Highest resistance (100%) shown by this isolate was to augmentin, cefotaxime, ceftazidime, cefixime and aztreonam respectively while the least resistance (0%) was to imipenem.

6. RECOMMENDATION

Urgent interventions in the farming practices, surveillance, and public education are needed to mitigate MDR, ESBL-producing enterobacteriaceae spread from catfish to human through the food chain.

REFERENCES

[1] Abdel-Wahed, R. K., Shaker, I. M., Elnady, M. A. and Soliman, M. A. M. (2018). Impact of Fish farming Management on Water Quality, Plankton Abundance and Growth Performance of Fish in Earthen Ponds. *Egyptian Journal of Aquatic Biology and Fisheries 22*: 49–63

[2] Abolghait, S. K., Fathi, A. G., Youssef, F. M. and Algammal, A. M. (2020). Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from chicken meat and giblets often produces staphylococcal enterotoxin B (SEB) in non-refrigerated raw chicken livers. *International journal of food microbiology 328*: 108669

[3] Adinortey, C. A., Aheto, D. W., Boateng, A. A. and Agbeko, R. (2020). Multiple antibiotic resistance-coliform bacteria in some selected fish farms of the central region of Ghana. *Scientifica 2020*(1): 6641461

[4] Agbeko, R., Aheto, D. W., Asante, D. K., Asare, N. K., Boateng, A. A. and Adinortey, C. A. (2022). Identification of molecular determinants of antibiotic resistance in some fish farms of Ghana. *Heliyon 8*(9)

[5] Agwaranze, D. I., Gabriel, U. P. and Ukeachu, N. F. (2025). Microbiological analysis of some fish pond water in Wukari metropolis. *Journal of Research in Agriculture and Food Sciences 1*(3): 115-115

[6] Ajayi, A. O. (2012). Bacteriological study of catfish, *Claria gariepinus*, from fish pond sources in Akungba-Akoko community, Nigeria.

[7] Akinnibosun, F. I., Akinnibosun, H. A., Iyekowa, O. and Adeluwoye-Ajayi, O. A. (2024). Determination of Extended Spectrum Beta-lactamase-producing Enterobactericeae in Integrated Poultry-fish Farming System in Ila Orangun, Osun State, Nigeria. *Nigerian Journal of Life Science* 14 (2): 6-10

[8] Algammal, A. M., Mabrok, M., Ezzat, M., Alfifi, K. J., Esawy, A. M., Elmasry, N. and El-Tarabili, R. M. (2022). Prevalence, antimicrobial resistance (AMR) pattern, virulence determinant and AMR genes of emerging multi-drug resistant *Edwardsiella tarda* in Nile tilapia and African catfish. *Aquaculture 548*: 737643

[9] Algammal, A. M., Mabrok, M., Sivaramasamy, E., Youssef, F. M., Atwa, M. H., El-Kholy, A. W..... and Hozzein, W. N. (2020). Emerging MDR-*Pseudomonas aeruginosa* in fish commonly harbor opr L and tox A virulence genes and bla TEM, bla CTX-M, and tet A antibiotic-resistance genes. *Scientific reports 10*(1): 15961

[10] Ali, J. F. (2025). Isolation, Identification and Antibiogram of Bacteria from Imported Frozen Fish at Public Markets in Mosul City, Iraq. *Egyptian Journal of Veterinary Sciences 56*(1): 91-100.

[11] Aliyu, A., Ibrahim, Y. K. and Oyi, R. A. (2018). Bacteriological and Elemental Quality of *Clarias gariepinus* (cat fish) Samples from River Lavun, Bida Niger state, Nigeria. *Niger Journal of Pharmaceutical Research 12*: 139–147

[12] Becerra, S. C., Roy, D. C., Sanchez, C. J., Christy, R. J. and Burmeister, D. M. (2016). An optimized staining technique for the detection of Gram positive and Gram negative bacteria within tissue. *BMC research notes 9*(1): 216

[13] Bello, O. O., Oni, M. O., Bello, J. O. and Bello, T. K. (2019). The incidence of extended-spectrum beta-lactamase (ESBL)-producing bacteria in salad vegetables in Ondo City, Nigeria

[14] Bevan, E. R., Jones, A. M. and Hawkey, P. M. (2021). Global epidemiology of CTX-M β-lactamases: Temporal and geographical shifts in genotype. *Journal of Antimicrobial Chemotherapy 76*(7): 1596–1604

[15] Blaak, H., van Hoek, A. H., Hamidjaja, R. A., van der Plaats, R. Q., Kerkhof-de Heer, L., de Roda Husman, A. M. and Schets, F. M. (2015). Distribution, numbers, and diversity of ESBL-producing *E. coli* in the poultry farm environment. *Public Library of Science One 10*(8): e0135402.

[16] Bush, K. and Bradford, P. A. (2020). Epidemiology of β-lactamase-producing pathogens. *Clinical Microbiology Revieew 33*(2): e00047-19

[17] Castanheira, M., Simner, P. J. and Bradford, P. A. (2021). Extended-spectrum β-lactamases: an update on their characteristics, epidemiology and detection. *JAC-antimicrobial resistance 3*(3): dlab092

[18] Chancelle, D., Akami, M., Xavier, E. F. and Francioli, K. K. (2023). Antimicrobial resistance pattern of *Escherichia coli* strains isolated from meat and fish products collected in retail market in Douala, Cameroon. *American Journal of Microbiological Research 11*(4): 97-105

[19] Cheesbrough (2016). District Laboratory Practice in Tropical Countries Part 2 Second Edition. Cambridge university press

[20] Chibuike, K. U., Iroha, I. R., Moses, I. B., Chukwunwejim, C. R., Peter, I. U., Edemekong, C. I.... and Okonkwo-Uzor, N. J. (2021). Phenotypic screening of multidrug-resistant *E. coli* from water and fish collected from different fish farms within Abakaliki metropolis, Nigeria. *Scientific Research and Essays 16*(2): 15-19.

[21] Chishimba, K., Hang'Ombe, B. M., Muzandu, K., Mshana, S. E., Matee, M. I., Nakajima, C. and Suzuki, Y. (2016). Detection of extended-spectrum beta-lactamase-producing *Escherichia coli* in market-ready chickens in Zambia. *International journal of microbiology 2016*(1): 5275724.

[22] Clinical and Laboratory Standard Institute (CLSI) (2024). Performance Standards for Antimicrobial Susceptibility Testing. Twenty-eighth Information Supplement CLSI document M100-S26. Wayne, P.A. 38 (3): 1-296

[23] Dahiya, S., Singla, P., Chaudhary, U. and Singh, B. (2015). Carbapenemases: a review. *International Journal of Advanced Health Sciences 2*(4): 11-17

[24] Dayie, N. T., Nathan-Mensah, F. N., Kotey, F. C., Tabi, B. K. A., Kabotso, D. E., Odoom, A….. and Donkor, E. S. (2024). Multidrug-resistant bacteria in aquaculture systems in Accra, Ghana. *Environmental Health Insights 18*: 11786302241299369

[25] Du Thanh, H., Phan Vu, H., Vu Van, H., Le Duc, N., Le Minh, T. and Savage, G. (2017). Oxalate content of taro leaves grown in Central Vietnam. *Foods 6*(1): 2

[26] Economou, V. and Gousia, P. (2015). Agriculture and food animals as a source of antimicrobial-resistant bacteria. *Infection and drug resistance* 49-61

[27] Ekwealor, P. A., Ugwu, M. C., Ezeobi, I., Amalukwe, G., Ugwu, B. C., Okezie, U., Stanley, C. and Esimone, C. (2016). Antimicrobial evaluation of bacterial isolates from urine specimen of patients with complaints of urinary tract infections in Awka, Nigeria. *International journal of Microbiology* 1-6

[28] Elhadi, N. (2016). Prevalence of extended-spectrum-β-lactamase-producing *Escherichia coli* in imported frozen freshwater fish in Eastern Province of Saudi Arabia. *Saudi Journal of Medicine* and *Medical Sciences 4*(1): 19-25

[29] Fakorede, C. N., Fatokun, E. N., Philip-Kantiok, B., Iwu, C. J. and Jaja, I. F. (2020). Bacteriological quality and antibiotics' susceptibility profile of small-medium scale commercial fish farms in Nigeria. *The Open Agriculture Journal 14*(1)

[30] Food and Agriculture Organization (FAO). (2018). *The state of world fisheries and aquaculture-meeting the sustainable development goals*; Food and Agriculture Organization of the United Nations: Rome

[31] García-Vello, P., González-Zorn, B. and Saba, C. K. S. (2020). Antibiotic resistance patterns in human, animal, food and environmental isolates in Ghana: a review. *Pan African Medical Journal* 35: 37

[32] Gothwal, R. and Shashidhar, T. (2020). Antibiotic pollution in the environment: A review. *Clean Technologies and Environmental Policy 22*: 1175–1191

[33] Grandin, T. (Ed.). (2020). *Improving animal welfare: A practical approach*. Cabi.

[34] Hassen, B., Jouini, A., Elbour, M., Hamrouni, S. and Maaroufi, A. (2020). Detection of Extended-Spectrum β-Lactamases (ESBL) Producing Enterobacteriaceae from Fish Trapped in the Lagoon Area of Bizerte, Tunisia. *Biomedical Sciences and Medicine Research International 2020*(1): 7132812

[35] Hinthong, W., Thaotumpitak, V., Sripradite, J., Indrawattana, N., Srisook, T., Kongngoen, T.... and Jeamsripong, S. (2024). Antimicrobial resistance, virulence profile, and genetic analysis of ESBL-producing *Escherichia coli* isolated from Nile tilapia in fresh markets and supermarkets in Thailand. *Public Library of Science One 19*(1): e0296857.

[36] Homeier-Bachmann, T., Heiden, S. E., Lübcke, P. K., Bachmann, L., Bohnert, J. A., Zimmermann, D. and Schaufler, K. (2021). Antibiotic-resistant Enterobacteriaceae in wastewater of abattoirs. *Antibiotics* 10(5): 568.

[37] Iredell, J., Brown, J. and Tagg, K. (2016). Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications. *British Medical Journal 352*

[38] Jayabarath, J. (2015). Isolation and Characterization of Antibiotic Resistant Bacteria from Lutjanus campechanus. *World Journal of Pharmaceutical Research* 5: 4-8.

[39] Kanu, N. V., Orji, S. L., Okafor, H. A., Adama, R. I., Mustapha, F. A., Oluwafemi, M. P.... and Ekwe, E. O. (2024). Molecular Detection of Beta Lactamase Genes from *Salmonella Typhimurium* Isolated from Poultry Droppings in Nyanya, Abuja. *International Journal of Research and Innovation in Applied Science 9*(8): 211-222

[40] Kimera, Z. I., Mgaya, F. X., Misinzo, G., Mshana, S. E., Moremi, N. and Matee, M. I. (2021). Multidrug-resistant, including extended-spectrum beta lactamase-producing and quinolone-resistant, *Escherichia coli* isolated from poultry and domestic pigs in dar es salaam, Tanzania. *Antibiotics* 10(4): 406

[41] Kuton, M. P., Ayanda, I. O., Uzoalu, I. A., Akhiromen, D. I., George, A. and Akinsanya, B. (2021). Studies on heavy metals and fish health indicators in *Malapterurus electricus* from Lekki Lagoon, Lagos, Nigeria. *Veterinary and Animal Science 12*: 100169.

[42] Lawson, M. A. (2008). The antibiotic resistance problem revisited. *The American Biology Teacher 70*(7): 405-410

[43] Li, Y., Pei, X., Yan, J., Liu, D., Zhang, H., Yu, B., Li, N. and Yang, D. (2019). Prevalence of foodborne pathogens isolated from retail freshwater fish and shellfish in China. *Food Control 99*: 131–136

[44] Magiorakos, A. P., Srinivasan, A., Carey, R. B., Carmeli, Y., Falagas, M. E., Giske, C. G., ... & Monnet, D. L. (2012). Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clinical microbiology and infection*, *18*(3), 268-281.

[45] Mathers, A. J., Peirano, G. and Pitout, J. D. D. (2015). The role of epidemic resistance plasmids and international high-risk clones in the spread of multidrug-resistant Enterobacteriaceae. *Clinical Microbiology Reviews 28*(3): 565–591

[46] Melzer, M. and Petersen, I. (2007). Mortality following bacteraemic infection caused by extended spectrum beta-lactamase (ESBL) producing *E. coli* compared to non-ESBL producing *E. coli*. *Journal of Infection 55*(3): 254-259

[47] Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A.… and Naghavi, M. (2022). Global burden of bacterial antimicrobial resistance in 2019: A systematic analysis. *The Lancet 399*(10325): 629–655

[48] Mwanza, F., Komba, E. V. G. and Kambarage, D. M. (2021). Occurrence and determination of antimicrobial resistant *Escherichia coli* isolates in fish and vegetables as indicator organism of faecal contamination in Dar es Salaam, Tanzania. *International Journal of Microbiology 2021*(1): 6633488.

[49] Nwabueze, A. A. and Nwabueze, E. O. (2011). Microbial flora of fish feeds sold in Asaba, Southern Nigeria. *American Journal of Experimental Agriculture 1*(2): 27

[50] Okpalaji, N. C., Okonkwo, A. P., Okonkwo, J. C., Ezenyilimba, B. N., Ejivade, O. M., Okafor, E. C. ... and Nwankwo, C. A. (2025). *Pathogen profile of poultry industries in Oyi local government area of Anambra State, Nigeria. Agrobiological Records 19: 24-34*

[51] Osuntokun, O. T., Muniru, A. A. and Morenike, K. T. (2020). Bacteriological assessment of African catfish (*Clarias gariepinus*) isolated from earthen and concrete fish pond. *Asian Journal of Biochemistry, Genetics and Molecular Biology 6*: 17-34

[52] Rajeshkumar, S. and Li, X. (2018). Bioaccumulation of heavy metals in fish species from the Meiliang Bay, Taihu Lake, China. *Toxicology reports 5*: 288-295

[53] Reist, M., Geser, N., Hächler, H., Schärrer, S. and Stephan, R. (2013). ESBL-producing Enterobacteriaceae: occurrence, risk factors for fecal carriage and strain traits in the Swiss slaughter cattle population younger than 2 years sampled at abattoir level. *Public Library of Science One 8*(8): e71725.

[54] Robinson, T. P., Bu, D. P., Carrique-Mas, J., Fèvre, E. M., Gilbert, M., Grace, D.… and Woolhouse, M. E. (2016). Antibiotic resistance is the quintessential One Health issue. *Transactions of the Royal Society of Tropical Medicine and Hygiene 110*(7): 377–380

[55] Sharma, I., Rabha, D., Das, S. and Ningombam, D. (2015). Hemolytic activity and antibiotic resistance of *Aeromonas* sp. isolated from marketed fish. *European Journal of Pharmaceutical Medical Research 2*(4): 304-312

[56] Singh, A. S., Lekshmi, M., Prakasan, S., Nayak, B. B. and Kumar, S. (2017). Multiple antibiotic-resistant, extended spectrum-βlactamase (ESBL)-producing Enterobacteria in fresh seafood. Microorganisms 5. *Published online*.

[57] Tacão, M., Moura, A., Correia, A. and Henriques, I. (2014). Co-resistance to different classes of antibiotics among ESBL-producers from aquatic systems. *Water research 48*: 100-107.

[58] Tekiner, İ. H. and Özpınar, H. (2016). Occurrence and characteristics of extended spectrum beta-lactamases-producing Enterobacteriaceae from foods of animal origin. B*razilian journal of microbiology 47*(2): 444-451.

[59] Van Boeckel, T. P., Pires, J., Silvester, R., Zhao, C., Song, J., Criscuolo, N. G. … and Laxminarayan, R. (2019). Global trends in antimicrobial resistance in animals in low- and middle-income countries. *Science 365*(6459): eaaw1944.

[60] Yanestria, S. M., Puspita Rahmaniar, R., Wibisono, F. J. and Effendi, M. H. (2019)**.** Detection of invA gene of *Salmonella* from milkfish (*Chanos chanos*) at Sidoarjo wet fish market, Indonesia, using polymerase chain reaction technique. *Veterinary World 12*: 170–175