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Antibiogram Profile of ESBL-Producing Enterobacteriaceae Isolated from Poultry Droppings Collected from Integrated Poultry-Fish Farms at Ila Orangun, Osun State, Nigeria

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Abstract
Antibiotic resistance (AR) is globally an urgent veterinary and public health threats. Extended-spectrum β-lactamase (ESBL) production is a major mechanism enhancing bacteria resistance to multiple antibiotics thus limiting treatment options of such bacteria diseases and resulting in high mortality hence, the focus of this present study on evaluating antibiogram profile of ESBL-producing enterobacteriaceae (ESBL+EN) harboured in poultry droppings collected from integrated poultry-fish farms at Ila Orangun, Osun State, Nigeria. From November 2024 to April 2025, a total of 162 poultry droppings 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 161 isolates recovered from the poultry droppings, 60 (37.27%) were probable ESBL+ while 29 (18.01%) were confirmed ESBL+. Overall, the ESBL+EN exhibited highest resistance (93.1%) to aztreonam and lowest resistance (0%) to imipenem. Individual members of the enterobacteriaceae showed varying degree of sensitivity to the tested antibiotics. Among all the ESBL+ isolates, 22 (75.86%) with MARI greater than 0.2 were multidrug-resistant (MDR) indicating these isolates originated from potentially dangerous sources where antibiotics are regularly used and depicting how difficult it will be to treat infections resulting from consuming chicken meat from the studied farms. This study alarms chickens as a potential reservoir of MDR, ESBL+EN, which might shed and contaminate the fishponds water, the fish and the environment through faecal matter. Prudent use of antibiotics should be implemented to manage antibiotic resistance in integrated poultry-fish farms.

Keywords: *Enterobacteriaceae, Antibiotic resistance, Extended-spectrum β-lactamases, antibiogram, Multiple Antibiotic Resistance Index, Antibiogram*

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I. INTRODUCTION

Over time, antibiotics have become widely used in various fields such as medicine, agriculture and industries, as they play important roles in the survival of humans against various infectious diseases. (McEwen and Collignon, 2017; WHO, 2018). The excessive use of antibiotics has led to the rapid emergence of bacteria resistant otherwise called "superbugs" which latter becomes a major threat to humanity as they make the treatment of bacterial diseases very difficult (Smith *et al*., 2021). In 2019, about 4.95 million death was recorded as a result Antibiotic Resistance (ABR) across the globe (Fernandez, 2022). If measures are not taken to fight against antibiotic resistance by 2050, more mortality rate rising to 10 million people annually could be recorded, which resultantly can affect gross domestic product (GDP) declining by 2% to 4% (O'neill, 2014).

Extended-Spectrum β-Lactamases (ESBLs) have been identified as one of the major causes of therapeutic failure in clinical medicine among other resistance determinants, as they are capable of hydrolizing a broad spectrum of β-lactams, including third-generation cephalosporins such as ceftriaxone, cefotaxime and ceftazidime, leaving limited treatment options (Buss and Bradford, 2020), thus ESBL-producing bacteria can exhibit co-resistance to multiple classes of antibiotics (Tacão *et al*., 2014). The emergence of ESBL-producing *Enterobacteriaceae has* become one of the major public health concerns, with limited treatment options (Castanheira *et al*., 2021). In several studies, many clinical samples have displayed Extended-Spectrum β-lactamase (ESBL)-producing Enterobacteriaceae , but there is insulficient information on their prevalence in nonclinical samples (Laudy *et al*., 2017; Dehbashi *et al*., 2020)

ESBL-producing Enterobacterales were sometimes ago regarded a nosocomial concern, however, in the past two decades, their epidemiology has shifted, which is more common in community, animal, and environmental settings (Bevan *et al*., 2021). This substantiate the interconnection with AMR as described in the One Health framework, emphasizing the relationship between human, animal, and environmental health (Robinson *et al*., 2016).

Food production systems, particularly poultry and fishery, are one of the sectors with high-risk of antibacterial resistance transmission (Van Boeckel *et al*., 2019). The level of organic matter and possible antibiotic residues in this sector could create selective pressures that favour resistant population (Gothwal and Shashidhar, 2020). Although this farming system (integrated poultry-fish) are beneficial economically, but may facilitate the movement of resistant bacteria and antimicrobial resistance genes (ARGs) unintentionally between different sections which may expand the possibility of horizontal gene transfer (HGT) and the spread of resistance determinants. The production of extended-spectrum β-lactamases (ESBLs) by Enterobacteriaceae may further worsen this problem (Iredell et al., 2016).

Conducting a continuous surveillance becomes very important for addressing the growing threat of antibiotic resistance effectively. However, antibiotic resistance surveillance studies in sub-Saharan Africa have largely concentrated on human populations, with limited attention given to animals and the environment (Adzitey, 2020; Donkor et al., 2019; Appiah et al., 2020; Dayie et al., 2021; Kotey et al., 2022; Baah et al., 2022; Addae-Nuku et al., 2022; Donkor et al., 2023). To help tackle the antibiotic resistance menace efficiently, it is important to continuously conduct surveillance. However, most antibiotic resistance surveillance studies, particularly, those conducted in sub-Saharan Africa, have mainly focused on humans and rarely on animals and the environment (Adzitey, 2020).

There is limited information on the occurrence and spreading of drug-resistant ESBL-producing Enterobacteriaceae in integrated poultry and fishery in Nigeria. To address this gap, this study focuses on determining the antibiotic resistance patterns of ESBL-producing Enterobacteriaceae isolated from poultry droppings collected from integrated poultry-fish farms in Ila Orangun, Osun State, Nigeria, with the aim of contributing to efforts to control antibiotic resistance.

II. 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 be concealed, therefore, the farms where the samples were taken are not disclosed in this study. 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,, 2026).

Samples Collection and Transportation

A longitudinal sampling protocol spanning from November 2024 to April 2025 (six months) yielded 6 composite discrete poultry droppings. At each integrated farm, nine freshly passed poultry droppings of approximately equal mass (10g) were aseptically and randomly retrieved from three well-spaced spots (the middle and both ends of the cage) per integrated system and three integrated system in each farm. Clean and Sterile spatula was used in the samples collection which later was placed into well labelled sterile universal

sampling bottles containing 90 ml buffer peptone water (BPW) (Himedia, India M614). The samples were mixed to form a composite (pooled) sample and were immediately placed in a cooler (icebox) containing ice packs to maintain a temperature of 4°C. The samples were collected very early in the morning (06:00am to 09:00am), once in a month (to give room for samples processing, bacteria isolation and identification). They were taken to the laboratory within 24 hours for immediate processing and bacteriological analysis.

Sample Processing and Bacteria Isolation

One (10) grams of each composite sample was separately weighed and introduced into separate 90ml of sterile distilled water contained in cotton-plugged sterile conical flask. Each mixtures was homogenized by shaking. Six fold serial dilutions were made by withdrawing 1ml of the mixtures into test tubes containing 9ml of distilled water. Second, 4^{th} and 6^{th} dilution factors were respectively used to inoculate different sterile growth media (Nutrient Agar, Eosin Methylene Blue, Salmonella/Shigella and MacConkey agars) using the pour plate method of inoculation. Inoculated plates were incubated at 37ºC for 24-48 h in an incubator. Distinct colonies were sub-cultured into fresh sterile medium using the streak plate method and further incubated for 24 h to obtain pure cultures.

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 ºC 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 test such as glucose, lactose and maltose were carried determined following the method described by Cheesbrough, 2016. The validation was carried out by incorporating established reference strains typically *Escherichia coli* ATCC 25922, *Klebsiella pneumoniae* ATCC 13883, *Enterobacter aerogenes* ATCC 13049, *Salmonella typhimurium* ATCC 14028 and *Proteus mirabilis* ATCC 43071.

Screening of Potential Extended Spectrum Beta-Lactamase Producing Enterobactericeae

All the isolates were screened for the production of extended-spectrum β-lactamases (ESBLs). Mueller-Hinton agar plates were inoculated with a homogeneous suspension of each test isolate. Separate antibiotic discs containing ceftazidime (30 µg) and cefotaxime (30 µg), were aseptically placed on the surface of the inoculated agar, with a distance of 30 mm between the discs. The plates were allowed to stand on the workbench for approximately 30 minutes to facilitate antibiotic diffusion into the agar and were subsequently incubated at 37 °C for 24 hours. After incubation, the zones of inhibition were measured using a meter rule. Reduced susceptibility or resistance to either of the antibiotics used for screening was considered indicative of suspected ESBL production (CLSI, 2024).

Recognition of Extended Spectrum Beta-Lactamase Producing Enterobacteriaceae

An 18-hour-old culture of isolates showing resistance to one or more third-generation cephalosporins was transferred into test tubes containing sterile normal saline. The turbidity of each suspension was adjusted to the 0.5 McFarland standard. The prepared bacterial suspension was then inoculated onto Mueller-Hinton agar plates by uniformly swabbing the entire agar surface. The double-disc synergy test was performed using an Augmentin disc together with ceftazidime (30 µg) and cefotaxime (30 µg) discs. The ceftazidime and cefotaxime discs were positioned at an equidistance of 20 mm from the Augmentin disc. The plates were subsequently incubated at 37 °C for 24 hours. Following incubation, the plates were examined, and an isolate was considered to be an ESBL producer when the zone of inhibition showed a difference of ≤5 mm between cefotaxime or ceftazidime tested in combination with Augmentin and the corresponding antibiotic tested alone (CLSI, 2024).

Antibiotic Susceptibility Test of the ESBL-Producing Bacteria

The test for Antibiotic Susceptibility of the ESBL-Producing Bacteria was done using the standard Kirby-Bauer disk 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] shown in Table 1. However, those isolates that show intermediate resistance to the antibiotics were designated resistant [Magiorakos *et al*., 2012]. *Escherichia coli* ATCC 25922, was used as a quality control organism.

Table 1: Concentrations and Interpretation Breakpoints of the Various Antibiotics Used in this Study [CLSI, 2024]

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)	
				S	R
Beta-lactam combination	Augmentin	AUG	30	≥ 18	≤ 13
Cephems	Cefotaxime	CTX	30	≥ 26	≤ 22
	Ceftazidime	CAZ	30	≥ 18	≤ 14
	Cefixime	CXM	5	≥ 16	≤ 20
Carbapenem	Imipenem	IMP	10	≥ 19	≤ 15
Aminoglycosides	Gentamicin	GEN	10	≥ 15	≤ 12
Fluoroquinolones	Ciprofloxacin	CIP	5	≥ 21	≤ 15
	Ofloxacin	OFL	5	≥ 16	≤ 12
Monobactam	Aztreonam	AZT	30	≥ 22	≤ 15
Nitrofuran	Nitrofurantoin	NIT	300	≥ 17	≤ 14

Rates of Antimicrobial Resistance (AMR)

The following calculation was used to determine the percentage of resistant isolates for each antibiotic.

$$\% \text{ rate} = \frac{\text{Number of resistant isolates } \times 100}{Number\ of\ tested\ isolates}$$

According to Papadopoulos *et al*. [2021] and Adebowale *et al*. [2022], rates (%) of resistance were classified into % rate >70%, >50 to 70%, >20 to 50%, >10 to 20%, >1 to 10%, 0.1 to 1% and <0.1% as extremely high, very high, high, moderate, low, very low and rare, respectively.

Multidrug Resistance (MDR) Patterns

Briefly according to Sweeney *et al*. [2018], isolates that were non-susceptible to at least three antibiotics classes were grouped as MDR.

The Multiple Antibiotic Resistance Index (MARI)

The formula: a/b, was used to calculate and interpret the multiple antibiotic resistance index (MARI), where "a" represents how many antibiotics an isolate proved resistance to, and "b" represents how many antibiotics were tested in total (Adegoke *et al*., 2020; Adzitey *et al*., 2012; Titilawo *et al*., 2015). MARI values were compared to the threshold value of 0.2. A MARI value >0.2 implies that the original sources of isolates likely involved heavy antibiotic use or misuse of antibiotics (Elshebrawy *et al*., 2022), whereas <0.2 denotes samples from sources with less antibiotic usage or low risk (Afunwa *et al*., 2020).

III. RESULTS

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

Table 2 below showed the morphological, macroscopic, cultural and staining characteristics of the bacteria isolated from poultry droppings 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 Poultry Droppings Collected from the Integrated Poultry-Fish Farms

Table 3 below showed the biochemical characteristics of the bacteria isolated from poultry droppings 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 Poultry Droppings Collected from the Integrated Poultry-Fish Farms

The preliminary screening for ESBL production by *E. coli, K. pneumonia, K. aerogenes, Shigella sp., Salmonella sp., Enterobacter sp., Serratia sp. and Proteus sp.* isolated from the poultry dropping samples are shown in Table 4 where 24 (68.57%), 10 (33.33%), 4 (33.33%), 2 (16.67%), 9 (30%), 3 (27.27%), 3 (60%) and 5 (19.23%) of the respective isolates were suspected to be ESBL producers since they showed resistance to cefotaxime (Table 4).

Confirmatory Screening for Suspected ESBL-producing Isolates from Poultry Droppings Collected from the Integrated Poultry-Fish Farms

The confirmatory screening for ESBL production by isolates from the poultry dropping samples indicated that 29 (48.33%) out of the 60 suspected ESBL positive isolates from the poultry dropping samples were confirmed to be ESBL positive. Of all the bacteria isolated *Shigella sp., Enterobacter sp.* and *Serratia sp* were ESBL negative, others were positive with E. coli showing the highest occurrence (79.17%) of the ESBL positive isolates in the poultry dropping samples (Table 5).

Occurrence of ESBL Producers in the Poultry Droppings Collected from the Integrated Poultry-Fish Farms

From table six, 60 (37.27%) of the 161 bacteria isolated from the poultry dropping samples were suspected to be ESBL producers, 29 (18.01%) were confirmed to be ESBL producers while 31 (19.26%) were non-ESBL producers (Table 6)

Prevalence of Enterobacterales and the ESBL-producing Enterobacterales in the Poultry Droppings from the Integrated Poultry-Fish Farms

Table 7 below showed that out of 161 Enterobacteriaceae isolates identified, the most predominant isolate was *E. coli* 35 (21.74), followed by *K. pneumoniae* 30 (18.63), *Salmonella sp.* 30 (18.63), *Proteus sp.* 26 (16.15), *K. aerogenes* 12 (7.45), *Shigella sp.* 12 (7.45), *Enterobacter sp.* 11 (6.83) and *Serratia sp.* 5 (3.11).

Also, the table showed that out of the 161 enterobacterales recovered from the poultry droppings in the present study, the ESBL-positive was 29 (18.01%). Of these, the prevalence of *E. coli, K. pneumonia, K. aerogenes, Salmonella sp.,* and *Proteus sp.* were 19 (54.29), 5 (16.67), 1 (8.33), 3 (10), and 1 (3.85), respectively.

Antibiotic Resistance Profile of ESBL-producing Enterobacteriaceae Isolated from Poultry Droppings Collected from Integrated Poultry-Fish Farms

Table 8 showed the antibiotic resistance profile of the ESBL-producing enterobactericeae isolated from poultry droppings collected from the farms to the tested antibiotics. The ESBL-producing Enterobacterioceae showed highest (93.1%) resistance to aztreonam and lowest resistance (0%) to imipenem with 89.5% of *E. coli*, 100% of *K.* pneumonia, 100% of *K. aerogenes*, 100% of *Salmonella* sp. and 100% of *Proteus* sp., respectively resisting aztreonam and none resist imipenem

Multidrug Resistance Profiles of ESBL producing Enterobacteriaceae (N = 29) Isolated from Poultry Dro ppings Collected from Integrated Poultry-Fish Farms

Table 9 showed a total of 7 antibiotics from 7 classes (beta-lactam combination agent, cephem, carbapenem, aminoglycosides, fluoroquinolone, monobactam and nitrofurans) were used to assess the MDR patterns of the ESBL positive Enterobacteriaceae. The overall MDR prevalence in this study was 22/29 (75.86%). Regarding the MDR rate of individual bacterial isolates *K. aerogenes*, *Salmonella* sp. and *Proteus* sp. were totally (100%) MDR followed by *E. coli* (73.68%) and K. pneumonia (60%). Overall, 1/29 (3.45%) of the enterobacteriacea resisted highest number of antibiotic classes which was 6 (CIP-CAZ-NIT-GEN-OFL-AUG) while 5/29 (17.24%) resisted the lowest which was 3 (CAZ-OFL-AUG and CXM-AZT-AUG, respectively). As regards the individual member of the enterobacteriaceae, 1/19 (5.26%) of *E. coli* resisted highest number of antibiotic classes which was 6 (CIP-CAZ-NIT-GEN-OFL-AUG) while 4/19 (21.05%) resisted the lowest which was 3 (CXM-AZT-AUG); 1/5 (20%) of *K. pneumonia* resisted highest number of antibiotic classes which was 4 (CIP-CRX-GEN-OFL-AUG and NIT-CAZ-AZT-AUG, respectively) while same value resisted the lowest which was 3 (CAZ-OFL-AUG); 1/3 (0.33%) of *Salmonella sp.* resisted highest number of antibiotic classes which was 4 (CIP-CRX-GEN-OFL-AUG) while same value resisted the lowest which was 3 (CAZ-OFL-AUG); 1/1 (100%) of *K. aerogenes* and *Proteus* sp. resisted 4 (CIP-CRX-GEN-OFL-AUG) and 3 (CXM-AZT-AUG) classes of antibiotics, respectively

Multiple Antibiotic Resistance (MAR) Index of ESBL producing Enterobacteriaceae (N = 29) Isolated from Poultry Droppings Collected from Integrated Poultry Fish Farms

Table 10 showed the Multiple antibiotic resistance (MAR), defined here as resistance to at least two (2) antibiotics. MAR was observed in 22 (75.86%) of the 29 ESBL+EN isolates. Ten (45.46%), 6 (27.27%), 5 (22.73%), 5 (22.73%) and 1 (4.55%) of the enterobacteriaceae showed 0.3, 0.4, 0.5 and 0.6 MAR index, respectively. Regarding the MAR index rate of individual bacterial isolate: Four (28.57%), 2 (14.29%) and 1 (7.14%) of *E. coli* showed 0.4, 0.5 and 0.6 MAR index respectively; 1 (33.33), 1 (33.33) and 1 (33.33) of *K pneumoniaea* and the *Salmonella* sp. showed 0.3, 0.4 and 0.5 MAR index, respectively; All the *K. aerogenes* and the *Proteus* sp. showed 0.5 and 0.3 MAR index respectively.

Table 2: Morphological, Macroscopic, Cultural and Staining Characteristics of the Bacteria Isolates from Poultry Droppings 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.
Small (1-2 mm), smooth (entire), circular, smooth, low convex colonies that are colorless or whitish or clear (non-lactose fermenters), often translucent, and may have slightly rough edges	Gram-negative (pink/red colour), small (1 to 3 µm long and 0.4 to 0.6 µm wide), non-motile, non-spore-forming, non-encapsulated, rod-shaped bacteria (bacilli).	*Shigella* 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,	Gram-negative (pink/red colour)**,** motile, non-spore-forming, non-encapsulated, rod-shaped (bacilli) bacteria that are sometimes pleomorphic	*Proteus* sp.

smooth, pale or grayish-white colonies if motility is inhibited.		
Smooth, circular, raised, and can become mucoid, Initially the colonies appear colorless or pale at 24 hours but later turns pink/red at 48 hour due to late/slow lactose fermentation and production of prodigiosin	Gram-negative (pink/red colour), motile, non-spore-forming, encapsulated, rod-shaped bacteria appearing as single cells or arranged in short chains typically straight or slightly curved, measuring approximately 0.5 to 0.8 µm in diameter and 1 to 2 µm in length.	*Serratia* sp.

Table 3: Biochemical Characteristics of the Bacteria Isolated from Poultry Droppings 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.
-	+	-	+/-	+	-	+	-	+/-	*Shigella* sp.
+	+	+	-	-	+	+	+	+	*Enterobacter* sp.
+	+	+	-	+	-	-	-	+	*Proteus* sp.
+	+	+	-	-	+	+	+	+	*Serratia* 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 4: Preliminary Screening for ESBL-producing Isolates from Poultry Droppings Collected from the Integrated Poultry-Fish Farms

Name of isolate	No. (%) of isolate	Antibiotics used	No. (%) resistant	No. (%) sensitive
E. coli	35 (21.74)	Cefotaxime	24 (68.57)	11 (31.43)
		Ceftazidime	22 (62.86)	13 (37.14)
K. pneumoniae	30 (18.63)	Cefotaxime	10 (33.33)	20 (66.67)
		Ceftazidime	8 (26.67)	22 (73.33)
K. aerogenes	12 (7.45)	Cefotaxime	4 (33.33)	8 (66.67)
		Ceftazidime	2 (16.67)	10 (83.33)
Shigella sp.	12 (7.45)	Cefotaxime	2 (16.67)	10 (83.33)
		Ceftazidime	1 (8.33)	11 (91.67)
Salmonella sp.	30 (18.63)	Cefotaxime	9 (30)	21 (70.0)
		Ceftazidime	7 (23.33)	23 (76.67)
Enterobacter sp.	11 (6.83)	Cefotaxime	3 (27.27)	8 (72.73)
		Ceftazidime	2 (18.18)	9 (81.82)
Serratia sp.	5 (3.11)	Cefotaxime	3 (60)	2 (40.0)
		Ceftazidime	2 (40.0)	3 (60.0)
Proteus sp.	26 (16.15)	Cefotaxime	5 (19.23)	21 (80.77)
		Ceftazidime	4 (15.39)	22 (84.61)
TOTAL	**161**	**Cefotaxime**	**60 (37.27)**	**101 (62.73)**
		Ceftazidime	**101 (62.73)**	**60 (37.27)**

KEY: No. = Number, % = Percentage

Table 5: Confirmatory Screening for Suspected ESBL-producing Isolates from Poultry Droppings Collected from the Integrated Poultry-Fish Farms

Name of isolate screened	No. of isolates screened	No. of ESBL + (%) isolates	No. of ESBL - (%) isolates
E. coli	24	19 (79.17)	5 (20.83)
K. pneumoniae	10	5 (50.0)	5 (60.0)

K. aerogenes	4	1 (25.0)	3 (75.0)
Shigella sp.	2	0 (0)	2 (100.0)
Salmonella sp.	9	3 (33.33)	6 (66.67)
Enterobacter sp.	3	0 (0)	3 (100.0)
Serratia sp	3	0 (0)	3 (100.0)
Proteus sp.	5	1 (20.0)	4 (80.0)
TOTAL	**60**	**29 (48.33)**	**31 (51.67)**

KEY: No. = Number, % = Percentage

Table 6: Occurrence of ESBL-producers in the Poultry Dropping 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	35 (21.74)	24 (68.57)	19 (54.29)	16 (45.71)
K. pneumoniae	30 (18.63)	10 (33.33)	5 (16.67)	25 (83.33)
K. aerogenes	12 (7.45)	4 (33.33)	1 (8.33)	11 (91.67)
Shigella sp.	12 (7.45)	2 (16.67)	0 (0)	12 (100.0)
Salmonella sp.	30 (18.63)	9 (30.0)	3 (10.0)	27 (90.0)
Enterobacter sp	11 (6.83)	3 (27.27)	0 (0)	11 (100.0)
Serratia sp.	5 (3.11)	3 (60.0)	0 (0)	5 (100.0)
Proteus sp.	26 (16.15)	5 (19.23)	1 (3.85)	25 (96.15)
Total	**161**	**60 (37.27)**	**29 (18.01)**	**31 (19.26)**

KEY: No. =Number; % = percentage

Table 7: Prevalence of Enterobacterales and the ESBL-producing Enterobacterales in Poultry Droppings Collected from Poultry-Fish Pond Integrated Farms

Isolate	Isolate No. (%)	No. (%) of Probable ESBL Producers	No. (%) of Confirmed ESBL Producers
E. coli	35 (21.74)	24 (68.57)	19 (54.29)
K. pneumoniae	30 (18.63)	10 (33.33)	5 (16.67)
K. aerogenes	12 (7.45)	4 (33.33)	1 (8.33)
Shigella sp.	12 (7.45)	2 (16.67)	0 (0)
Salmonella sp.	30 (18.63)	9 (30)	3 (10)
Enterobacter sp.	11 (6.83)	3 (27.27)	0 (0)
Serratia sp.	5 (3.11)	3 (60)	0 (0)
Proteus sp.	26 (16.15)	5 (19.23)	1 (3.85)
Total	**161 (100)**	**60 (37.27)**	**29 (18.01)**

KEY: No. =Number; % = percentage

Table 8: Antibiotic Resistance Profile of ESBL-producing Enterobacteriaceae Isolated from Poultry Droppings Collected from Integrated Poultry-Fish Farms

Antibiotics Disc Code	ESBL-producing Enterobacteriaceae (n = 29)											
	E. coli (n = 19)		*K. pneumonia* (n = 5)		*K. aerogenes* (n = 1)		*Salmonella* sp. (n = 3)		*Proteus* sp. (n = 1)		Total (n = 29)	
	S No. (%)	R No. (%)	S No. (%)	R No. (%)	S No. (%)	R No. (%)	S No. (%)	R No. (%)	S No. (%)	R No. (%)	S No. (%)	R No. (%)
AUG	3 (15.7)	16 (84.2)	1 (20)	4 (80)	0 (0)	1 (100)	1 (33.3)	2 (66.7)	0 (0)	1 (100)	5 (17.2)	24 (82.8)
CTX	7 (36.8)	12 (63.2)	2 (40)	3 (60)	1 (100)	0 (0)	2 (66.7)	1 (33.3)	0 (0)	1 (100)	12 (41.4)	17 (58.6)
CAZ	6 (31.6)	13 (68.4)	2 (40)	3 (60)	1 (100)	0 (0)	2 (66.7)	1 (33.3)	0 (0)	1 (100)	11 (37.9)	18 (62.1)
CXM	5 (26.3)	14 (73.7)	1 (20)	4 (80)	1 (100)	0 (0)	2 (66.7)	1 (33.3)	0 (0)	1 (100)	9 (31)	20 (69)

IMP	19 (100)	0 (0)	5 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	1 (100)	0 (0)	29 (100)	0 (0)
GEN	12 (63.2)	7 (36.8)	3 (60)	2 (40)	1 (100)	0 (0)	2 (66.7)	1 (33.3)	1 (100)	0 (0)	19 (65.5)	10 (34.5)
CIP	9 (47.4)	10 (53.6)	2 (40)	3 (60)	1 (100)	0 (0)	2 (66.7)	1 (33.3)	1 (100)	0 (0)	15 (51.7)	14 (48.3)
OFL	8 (42.1)	11 (57.9)	2 (40)	3 (60)	1 (100)	0 (0)	2 (66.7)	1 (33.3)	1 (100)	0 (0)	14 (48.3)	15 (51.7)
AZT	2 (10.5)	17 (89.5)	0 (0)	5 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	1 (100)	2 (6.9)	27 (93.1)
NIT	18 (94.7)	1 (5.3)	5 (100)	0 (0)	1 (100)	0 (0)	3 (100)	0 (0)	1 (100)	0 (0)	28 (96.6)	1 (3.4)

KEY: ESBL = Extended-spectrum beta-lactamase, No. / n = number, % = percentage, S = susceptible, R = resistance, ANT = antibiotics

Table 9: Multidrug Resistance Profiles of ESBL producing Enterobacteriaceae Isolated from Poultry Dro ppings Collected from Integrated Poultry-fish Farms

MDR Pattern	No. of Antibiotics (Classes)	No. of Antibiotics tested	ESBL-producing Enterobacteriaceae					
			E. coli (n = 19)	K. pneumoniae (n = 5)	K. aerogenes (n = 1)	*Salmonella sp.* (n = 3)	*Proteus sp.* (n = 1)	Total No. (%) (n = 29)
			No. (%)	No. (%)	No. (%)	No. (%)	No. (%)	
NIT-IMP-CIP-CAZ-GEN-AUG-AZT	7 (7)	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CIP-IMP-AZT-GEN-CRX-AUG	6 (6)	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
CIP-CAZ-NIT-GEN-OFL-AUG	6 (6)	10	1 (5.26)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.45)
CIP-CAZ-NIT-GEN-AZT-AUG	5 (5)	10	1 (5.26)	0 (0)	0 (0)	0 (0)	0 (0)	1 (3.45)
CIP-CRX-GEN-OFL-AUG	5 (4)	10	1 (5.26)	1 (20)	1 (100)	1 (0.33)	0 (0)	4 (13.79)
IMP-CXM-GEN-OFL	4 (4)	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NIT-CXM-IMP-AUG	4 (4)	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
NIT-CAZ-AZT-AUG	4 (4)	10	1 (5.26)	1 (20)	0 (0)	0 (0)	0 (0)	2 (6.90)
CIP-CXM-OFL-AUG	4 (4)	10	1 (5.26)	0 (0)	0 (0)	1 (0.33)	0 (0)	2 (6.90)
CIP-CAZ-GEN-AUG	4 (4)	10	2 (10.53)	0 (0)	0 (0)	0 (0)	0 (0)	2 (6.90)
CAZ-OFL-AUG	3 (3)	10	3 (15.79)	1 (20)	0 (0)	1 (0.33)	0 (0)	5 (17.24)
CXM-AZT-AUG	3 (3)	10	4 (21.05)	0 (0)	0 (0)	0 (0)	1 (100)	5 (17.24)
IMP-CAZ-CTX	3 (2)	10	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Total Non-MDR of ESBL+ Isolates No. (%)			**5 (26.32)**	**2 (40)**	**0 (0)**	**0 (0)**	**0 (0)**	**7 (24.14)**
Total MDR of ESBL+ Isolates No. (%)			**14 (73.68)**	**3 (60)**	**1 (100)**	**3 (100)**	**1 (100)**	**22 (75.86)**

KEY: ESBL = Extended-spectrum beta-lactamase, No. /n = Number, % = percentage, MDR = Multidrug-resistant,

Table 10. Multiple Antibiotic Resistance (MAR) index of ESBL producing Enterobacteriaceae Isolated fr om Poultry Droppings Collected from Integrated Poultry-fish Farms Nigeria

No. of Antibiotics [a]	No. of Antibiotics tested [b]	MAR Index (a/b)	ESBL-producing Enterobacteriaceae					
			E. coli (n = 14)	K. pneumoniae (n = 3)	K. aerogenes (n = 1)	*Salmonella sp.* (n = 3)	*Proteus sp.* (n = 1)	Total No. (%) MAR (n=22)
			No. (%) MAR	No. (%) MAR	No. (%) MAR	No. (%) MAR	No. (%) MAR	
10	10	1	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
9	10	0.9	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
8	10	0.8	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
7	10	0.7	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
6	10	0.6	1 (7.14)	0 (0)	0 (0)	0 (0)	0 (0)	1 (4.55)
5	10	0.5	2 (14.29)	1 (33.33)	1 (100)	1 (33.33)	0 (0)	5 (22.73)
4	10	0.4	4 (28.57)	1 (33.33)	0 (0)	1 (33.33)	0 (0)	6 (27.27)
3	10	0.3	7 (50)	1 (33.33)	0 (0)	1 (33.33)	1 (100)	10 (45.46)
2	10	0.2	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)

KEY: ESBL = Extended-spectrum beta-lactamase, No. /n = Number, % = percentage, MAR = Multiple Antibiotic Resistance,

IV. DISCUSSION

Antibiotic-resistant bacteria emerges as a major threat to public health as they the capable of spreading among humans animals and the environment alongside their mobile genetic elements (Robinson *et al*., 2016). Misuse of antibiotics has been published as a major risk factors for the development and spreading of beta-lactamase producing organisms (Chishimba *et al*., 2016; Economou and Gousia, 2015). Therefore, the present study focus on investigating the resistance patterns of ESBL-producing enterobacteriaceae (ESBL-PEN) isolated from poultry droppings collected from integrated poultry-fish farms at Ila Orangun, Osun State, Nigeria.

A total of 161 bacterial isolates were isolated from the collected poultry droppings (n=162) in this present study. The morphological, macroscopic, cultural and staining characteristics of the bacteria isolates were in agreement with previous researchers report (Adeluwoye-Ajayi *et al*., 2026; 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 were in agreement with previous researchers report (Adeluwoye-Ajayi *et al*., 2026; Agwaranze *et al*., 2025).

In the current study, 161 Enterobacteriaceae isolates were identified, and the most predominant isolate was *E. coli* 35 (21.74), followed by *K. pneumoniae* 30 (18.63), *Salmonella sp.* 30 (18.63), *Proteus sp.* 26 (16.15), *K. aerogenes* 12 (7.45), *Shigella sp.* 12 (7.45), *Enterobacter sp.* 11 (6.83) and *Serratia sp.* 5 (3.11). Similarly, it was equally reported from Northwest Ethiopia, Gordar City (Tigabie *et al*., 2023), Souk at Ahras region, Algeria (Kamel *et al*., 2024), Ila Orangun at Osun State, Nigeria (Akinnibosun *et al*., 2024) and Southwest, Ethiopia (Bushen *et al*., 2021). The predominance of *E. coli* in this and many other studies may be because *E. coli* is a widely distributed commensal bacterium that is majorly found as normal floral in the gastrointestinal tracts of animals and humans (Torres, 2010).

Generally, Gram-negative bacteria (GNB) produce β-lactamases enzymes that render antibiotics useless. Beta-lactamases confer resistance to β-lactam antibiotics by splitting the four-membered ring of such antibiotics with the release of an inactive product (Toth *et al*., 2016). The results of this study indicating the presence of ESBL positive (18.01%) enterobacteriaceae in poultry droppings conforms with 25% reported by Nossair *et al*., 2022 from Egypt, 29% by Falgenhauer *et al*., 2019 from Ghana, and 18.7% by Sardari *et al*., 2024 from Iran. However, other studies from different countries of the world have reported far higher prevalence of ESBL+EN in poultry farms, such as Germany (100%) (Geser *et al*., 2012), Denmark (93%) [Agersø *et al*., 2014] and Saudi Arabia (97.5%) (Alpakistany *et al*., 2024) while Tigabie et al. (2023) reported a lower prevalence of ESBL+EN (8.4%) from Gondar City, Northwest Ethiopia.

In this study, the prevalence of ESBL-producing *E. coli of 54.29%* is in line with earlier reports from North-western Romanania (55%) (Rus *et al*., 2025), Keffi, Nigeria (46.9%) (Tama *et al*., 2019), Dar es Salaam, Tanzania (65.3%) (Kimera *et al*., 2021), Northern Tailand (54.5%) (Tanzawai *et al*., 2026) and Lebanon (60%) (Mikhayal *et al*., 2021). However, other studies from different countries of the world have reported higher prevalence of ESBL-producing *E*. coli in poultry farms, such as Malaysia (84.5%) (Lemlem *et al*., 2024) and Nasarawa, Nigeria (83.3%) (Tama *et al*., 2021) while lower prevalence of ESBL-producing *E. coli* in poultry farms of 32%, 3%, 12.6% and 26.32% were reported from Maiduguri, Nigeria (Kwoji *et al*., 2019), Malaysia (Chai *et al*., 2022), Gordar City, North-western, Ethiopia (Tigabie *et al*., 2023) and Yaounde capital city of Cameroon (Djuikoue *et al*., 2022) respectively.

In this study, the result of 16.67% prevalence of ESBL-producing *K. pneumoniae* is comparatively similar but higher than the earlier reports from Malaysia (14%) (Veloo *et al*., 2022), Ethiopia (9.1%) (Tigabie *et al*., 2023), Saudi Arabia (5%) (Alpakistany *et al*., 2024), Algeria (6.4%) (Chenouf *et al*., 2021). The variation in prevalence rates of ESBL-producing enterobacteriaceae and the members of the family when compare with other studies could be due to difference in ESBL screening methods used, poor animal management practices, hygienic conditions and number of isolates studied.

The results of this present study and other previous studies have demonstrated the widespread distribution of ESBL in Nigeria and other parts of the world. This is worrisome because these poultry birds can serve as a source of meat for humans, and ESBL bacterial pathogens are known to be multidrug-resistant and are responsible for a number of nosocomial and community infections. Summarily, the presence of ESBL+EN in the poultry droppings of the study area could be due to selective antibiotic pressure caused by the extensive use of third-generation cephalosporins by the integrated poultry-fish farmers [Cantón *et al*., 2008]. Consequently, to prevent the development and spreading of ESBL+EN, adopting a One Health approach through antibiotic resistance surveillance programs are crucial needs in poultry farms.

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). Some reports have indicated high incidence of antibiotic resistant bacteria in integrated fish farming; a practice that combines aquaculture with other livestock farming operations and use of their manure for fish feed (Shah *et al*., 2012; Neela *et al*., 2015; Watts *et al*., 2017). Resistant bacteria are capable of transferring and conferring resistance to commensal bacteria through horizontal gene transfer using mobile genetic elements (MGE) such as conjugative plasmids, transposons, and phages (Al-Tawfiq *et al*., 2017).

Individual members of the family of enterobacteriacea producing ESBL in this present study showed varying levels of resistance to the tested commercially available antibiotics. The resistance rate of *E. coli* to cefotaxime (63.2%) and ceftazidime (68.4%) observed in this study is lower than the values reported by previous researchers (Djuikoue *et al*., 2022; Kwoji *et al*., 2019; Mezalira *et al*., 2019; Mikhayel *et al*., 2021; Nossaie *et al*., 2022; Le *et al*., 2015; Tsitsos *et al*., 2025). However, the observed rate of resistance by this bacterium to these drugs were higher than the reports from study conducted by earlier researchers (Tigebie *et al*., 2023; Tama *et al*., 2021; Lemlem *et al*., 2023; Tama *et al*., 2019; Kamel *et al*., 2024).

Also, the resistance rate of *E. coli* to gentamicin (36.8%) and ciprofloxacin (53.6%) observed in this study is lower than the values reported by previous researchers (Djuikoue *et al*., 2022; Le *et al*., 2015). However, the observed rate of resistance by this bacterium to these antibiotics were higher than the reports from study conducted by earlier researchers (Tigebie *et al*., 2023; Kimera *et al*., 2024; Tama *et al*., 2019; Tansawai *et al*., 2026). Furthermore, the resistance rate of *E. coli* to augmentin (84.2%) and aztreonam (89.5%) observed in this study is higher than the values reported by previous researchers (Djuikoue *et al*., 2022; Lemlem *et al*., 2023; Tansawai *et al*., 2026). However, the observed rate of resistance by this bacterium to the augmentin is lower than the 88.8% reported by Mikhayel *et al*. (2021). Likewise, the observed rate of resistance by this bacterium to the aztreonam is lower than the 98.5% reported by Kwoji *et al*. (2019).

The observed rate of resistance (57.9%) by the isolated *E. coli* to ofloxacin is lower than the 90.9% and 59.8% reported by Djuikoue *et al*. (2022) and Lemlem *et al*. (2023) respectively. The observed no resistance (0%) shown by this bacterium to imipenem is consistent with the same value reported by earlier researchers (Rus *et al*., 2025; Djuikoue *et al*., 2022; Rizal *et al*., 2024; Mezalira *et al*., 2019; Mikhayel *et al*., 2021; Tansawai *et al*., 2026; Hiroi *et al*., 2012; Tsitsos *et al*., 2025). This observed resistance exhibited by this bacterium against imipenem is similar but lower than the 22.2%, 17.5%, 12% and 12.5% reported by Tama *et al*. (2021), Lemlem *et al*. (2023), Tama *et al*. (2019) and Nossair *et al*. (2022) respectively.

Regarding the resistance rate exhibited by the isolated *K. pneumonia* to the tested antibiotics, this isolate showed no resistance (0%) to cefotaxime and ceftazidime, respectively. This finding is in agreement with the report of Kamel *et al*. (2024) who reported same value of resistance to these antibiotics. However, this result negate the 36.4% to 100% resistance rate exhibited by this organism against these antibiotics reported by earlier researchers (Tigebie *et al*., 2023; Nossair *et al*., 2022; Tsitsos *et al*., 2025; Mikhayel *et al*., 2021). The 0% resistance rate showed by this isolate to imipenem corroborate the same value of resistance exhibited by this organism to same antibiotic reported by earlier researchers (Nossair *et al*., 2022; Tsitsos *et al*., 2025; Mikhayel *et al*., 2021). This study revealed that this isolate showed no resistance to gentamicin and ciprofloxacin. This was far lower than the values reported by Tigebie *et al*. (2023) and Tsitsos *et al*. (2025). Also, the 100% resistance shown by this bacterium to augmentin is in agreement with previous researchers report (Tsitsos *et al*., 2025; Mikhayel *et al*., 2021]) However, this observed value of resistance exhibited by this bacterium to augmentin is higher than the 28.57% and the 33.33% reported by Nossair *et al*., (2022) and Kamel *et al*. (2024), respectively. Furthermore, the 100% resistance rate exhibited by this bacterium to aztreonam is higher than the 79.3% reported by Mikhayel *et al*. (2021).

Regarding the resistance rate exhibited by the isolated *Salmonella* sp. to the tested antibiotics, the observed 33.3% resistance rate to cefotaxime, ceftazidime and gentamicin was higher than the 0% resistance rate reported by Andre *et al*. (2020). This value of resistance exhibited by this bacterium against cefotaxime was higher than the 0%, 6.3%, and 21.7% resistance rate reported by Kamel *et al*. (2024), Karim *et al*. (2023) and Tigabie *et al*. (2023), respectively but lower than the 38.1% resistance rate reported by Garaibeh *et al*. [2024]. Also, this value of resistance exhibited by this bacterium against ceftazidime was higher than the 14.29%, 21.7% and 7.9% resistance rate reported by Kamel *et al*. (2024), Tigabie *et al*. (2023) and Garaibeh *et al*. (2024), respectively but lower than the 36.4% resistance rate reported by Bushen *et al*. (2021). Furthermore, the observed 33.3% resistance rate exhibited by this bacterium against cefuroxime is lower than the 50% resistance rate reported by Andre *et al*. (2020).

The observed 33.3% resistance rate to gentamicin and ciprofloxacin was higher than the values reported by earlier researchers (Karim *et al*., 2023; Tigabie *et al*., 2023). This value of resistance exhibited by this bacterium against gentamicin was higher than the 32.6% resistance rate reported by Garaibeh *et al*. [2024] but lower than the 36.4% resistance rate reported by Bushen *et al*. (2021). Also, this value of resistance exhibited by this bacterium against ciprofloxacin was higher than the 27.3% resistance rate reported by Bushen

et al. (2021) but lower than the 100% and 91% resistance rate reported by Kamel *et al*. (2024) and Garaibeh *et al*. (2024), respectively.

The observed 66.7% resistance rate to augmentin was higher than the 0% and 54.5% resistance rate reported by Andre *et al*. (2020) and Bushen *et al*. (2021), respectively but lower than the 71.43% resistance rate reported by Kamel *et al*. (2024).

Regarding the resistance rate exhibited by the isolated *Proteus* sp. to the tested antibiotics, the observed 100% resistance rate to ceftazidime and augmentin was higher than the resistance rates reported by earlier researchers (Kamel *et al*., 2024; Mezalira *et al*., 2019; Bushen *et al*., 2021; Mikhayel *et al*., 2021). This value of resistance exhibited by this bacterium against ceftazidime was higher than the 77.8% resistance rate reported by Tigabie *et al*. (2023). Also, the 100% resistance rate exhibited by this bacterium against cefotaxime was consistent with the same value reported by Mikhayel *et al*. (2021) but higher than the 0% and 87.5% resistance rate reported by Kamel *et al*. [2024] and Mezalira *et al*., (2019), respectively. Furthermore, the 100% resistance rate exhibited by this bacterium against cefuroxime was higher than the 77.8% resistance rate reported by Tigabie *et al*. (2023).

The observed 0% resistance rate to gentamicin and ciprofloxacin was extremely lower than the values reported by earlier researchers (Tigabie *et al*., 2023; Bushen *et al*., 2021). This value of resistance exhibited by this bacterium against gentamicin was in agreement with same value reported by Mezalira *et al*. (2019) but extremely lower than the 100% resistance rate reported by Mikhayel *et al*. (2021). Also, this value of resistance exhibited by this bacterium against ciprofloxacin was extremely lower than the 85.7% and 75% resistance rate reported by Kamel *et al*. (2024) and Mezalira *et al*. (2019), respectively.

The observed 0% resistance rate to imipenem was in agreement with same value reported by previous researchers (Mezalira *et al*., 2019; Mikhayel *et al*., 2021). The 100% resistance rate exhibited by the bacterium to azttreonam is higher than the 60% resistance rate reported by Mikhayel *et al*. (2021).

The observed high resistance rates [augmentin (82.8%), cefotaxime (58.6%) and ceftazidime (62.1%)] exhibited by the isolated ESBL+EN are in accordance with the 90%, 70% and 87.5% resistance rate to augmentin, cefotaxime and ceftazidime, respectively reported by Alpakistany *et al*. (2024). This value of resistance exhibited by this bacteria family against augmentin was higher than the 61.3% resistance rate reported by Bushen *et al*. (2021). Also, this value of resistance exhibited by group of bacteria against cefotaxime was higher than the 21.7% resistance rate reported by Tigabie *et al*. (2023). Furthermore, this value of resistance exhibited by this bacteria family against ceftazidime was higher than the 33.9% and 21.7% resistance rate reported by Bushen *et al*. (2021) and Tigabie *et al*. (2023), respectively. The 69% resistance rate showed by the ESBL+EN against cefuroxime was higher than the 55% resistance rate reported by Alpakistany *et al*. (2024).

The observed resistance rates [gentamicin (34.5%) and ciprofloxacin (48.3%)] exhibited by the isolated ESBL+EN were higher than the values reported by previous researchers (Bushen *et al*., 2021; Tigabie *et al*., 2023; Alpakistany *et al*., 2024). This study revealed 93.1% and 3.4% resistance rate to aztreonam and nitrofurantoin respectively. These values were higher than the 52.5% and 0% resistance rate to these respective antibiotics reported by Alpakistany *et al*. (2024). Also, observation from this present study revealed 0% resistance rate to imipenem. This result is similar to but lower than the 9.1% resistance rate against meropenem, a carbapenem belonging to same class as imipenem reported by Tigabie *et al*. (2023). However, this 0% resistance rate showed by the bacteria group against imipenem was far lower than the 47.5% resistance rate reported by Alpakistany *et al*. (2024).

This none resistivity to imipenem observed across all the members of the enterobacteriaceae producing ESBL justifies the use of this drug as the last β-lactam antibiotic of choice for treatment of infections caused by Gram-negative Enterobacteriaceae including those provoked by extended-spectrum-β-lactamases (Dahiya *et al*., 2015). The low resistivity of isolates in this present study to some of the antibiotics tested may be due to the fact that the drugs are very expensive, and are likely not abused. The high resistivity of isolates in this present study to some of the antibiotics tested may be due to misuse, abuse or use of substandard antibiotics in the study area. Also, the relatively high level of resistance of bacteria to some of the antibiotics observed in this study connotes the existence of some antibiotic residues in the poultry droppings. Antibiotics may have been directly or indirectly introduced through feeds used for poultry birds. The disparity observed in the level of antibiotics activity in the present study in relation to previous studies could be due the number of isolates studied and the level of antibiotics usage in the environment.

In addition to Antimicrobial Resistance (AMR) being a global public health problem, Nigeria also faces a precarious situation of poor antibiotic prescription monitoring and prescription-only medicines (POM), including antimicrobials that are routinely sold Over-The-Counter (OTC) in pharmacies and by patent proprietary medicines vendors (PPMVs). Easy access to critical antibiotics of last resort by human being increases its imprudent use, further worsening the problem of AMR. Currently, there are no available studies outlining the full burden of AMR and its health and economic impact on Nigerians (Use, 2017). Hence, the

implication of the finding of this study could be far-reaching and could be used as a baseline study on AMR in the environment.

According to Magiorakos *et al.* (2012), multidrug resistance is defined as showing resistance to at least one agent in three or more antimicrobial categories. The observed prevalence of MDR ESBL+ *E. coli* 14 (73.68%) in this present study was similar but a little bit lower than the reports from Gorda City, Northwest Ethiopia (83.9%) (Tigabie *et al*., 2023), Tanzania (86.8%) (Kiiti *et al*., 2021), Zambia (85.7%) (Chishimba *et al*., 2016), and Cameroon (83.1%) (Moffo *et al*., 2021) and far lower than the study in Daula City, Cameroon (94.9%) (Chancelle *et al*., 2023), in Malaysia (93.8%) (Lemlem *et al*., 2023), in Albania (95%) (Alcaine *et al*., 2016), and in Malaysia (100%) (Ibrahim *et al*., 2021). However, it was far higher than a study conducted in Northern Thailand (57.1%) (Tansawai *et al*., 2026), in Bahir City, North-western Ethiopia (62.2%) (Geta & Kibret, 2022), in Dar es Salaam, Tanzania (48%) (Mwanza *et al*., 2021), in Malaysia (3.3%) (Chai *et al*., 2022), in Jimma, Ethiopia (54.2%) (Bushen *et al*., 2021), Tanzania (69.3%) (Mgaya *et al*., 2021), and Egypt (57.8%) (AbdelRahman *et al*., 2020). The disagreement of results might be due to the use of different types and concentrations of antibiotics in the poultry industry in the different countries.

The multidrug resistance rate of ESBL+ *Salmonella* spp. was 3 (100%). This was far higher than a study conducted in Gorda City, Northwest Ethiopia (73.9%) (Tigabie *et al*., 2023), in Debre Zeit, Ethiopia (86.0%) (Asfaw *et al*., 2020), Albania (82%) (Alcaine *et al*., 2016), and Malaysia (82%) (Ibrahim *et al*., 2021), in Jimma, Ethiopia (44.4%) (Bushen *et al*., 2021). This discrepancy could be due to the inappropriate use of antibiotics on the farms represents a selective pressure for resistant bacteria which can develop cross-resistance between several classes of antibiotics (Chantziaras, 2017).

The prevalence of MDR ESBL+ *K. pneumonia* and *Proteus* sp. in this study was 60% and 100%, respectively. This is in line with the study conducted in Bangladesh *P. mirabilis* 83% (Nahar *et al*., 2014). These findings were higher than a study conducted in Jimma, Ethiopia, where 57.1% of *K. pneumoniae* and 50.0% of *P. mirabilis* were reported as MDR (Bushen *et al*., 2021); a study conducted in Gorda City, Northwest Ethiopia where 81.8% of *K. pneumoniae* and 77.8% of *P. mirabilis* were reported as MDR (Tigabie *et al*., 2023); 53.57% of *K. pneumoniae* and 83% of *P. mirabilis* were also reported as MDR by a study from Indonesia (Permatasari *et al*., 2020) and Bangladesh (Nahar *et al*., 2014), respectively. These bacteria may develop ABR via acquired mechanisms. The acquired resistance occurs through horizontal gene transfer such as conjugation, transduction, and transformation from other resistant bacteria. Additionally, mutations in the gene could also cause this MDR when the bacteria are constantly under pressure after being exposed to antibiotics (Tenover, 2006).

The general prevalence of MDR ESBL+ Enterobacteriaceae was 22 (75.86). This was higher than the obtained values from Sohuthwest Ethiopia (52.5%) (Bushen *et al*, 2021), Makkah Province, Saudi Arabia (22.5%) (Alpakistany *et al*., 2024), Bahir City, North-western Ethiopia (52.6%) (Geta & Kibret, 2022) and Bangladesh (39%) (Nahar *et al*., 2014) but lower than the report from Gorda City, Northwest Ethiopia (81.1%) (Tigabie *et al*., 2023), Souk Ahras redion, Algeria (100%) (Kamel *et al*., 2024) and China (88.2%) (Yassin *et al*., 2017). This difference may be due to the types of commercial chicken, and the number of farms included in the study (Daehre *et al*., 2018).

Infections caused by multidrug resistant organisms may be difficult to manage or can lead to high mortality since these organisms have multiple resistance mechanisms which enables them to inactivate antibiotics (Magiorakos *et al*., 2012). Hence, the observed trend of multidrug resistant strains poses a major public health concern globally and there is need to come with effective policies and implementation plans to address these concerns.

Multiple Antibiotic Resistance Index (MARI) is recognized as an efficient and cost effective method for bacteria source tracking (Osundiya *et al*., 2013).

A significant observation from this study is that MAR index of 22/29 (75.86%) of the ESBL+EN ranged from 0.3-0.6, which was above 0.2 implying the origination of the bacteria from an environment where misuse and abuse of antibiotics is common. This present results support the findings of previous researchers (Fakorede *et al*., 2020; Adinortey *et al*., 2020; Chibuike *et al*., 2021) where values of MARI reported for their isolates were above 0.2.

Observation from this present study regarding MARI exhibited by the isolates indicated these bacteria originated from potentially dangerous sources where antibiotics are regularly used and were possibly introduced through faecal contamination of human or animal origin supporting the view of Kathleen *et al*., (2016)

The handling and subsequent consumption of the chickens carrying ESBL producing multidrug-resistant bacteria just as revealed in this present study represent a public health threat as these bacteria may cause various human diseases that might be difficult to treat. Another probable likelihood is that antibiotic-

resistant genes harboured by the resistant bacteria could be passed on to other bacteria in the gastrointestinal tract of consumers and this will result in possible life threatening infectious diseases.

Data generated from this study will help/assist in monitoring chickens of integrated poultry-fish farms and it will serve as a guide for application of antimicrobial stewardship program in all sectors to increase the level of knowledge of physician and farmers with regards to judicious use of antibiotics.

V. CONCLUSION

This study revealed that the poultry droppings were contaminated with potentially pathogenic bacteria that could affect chicken health and chicken meat consumers sourced from the study area. These organisms could lower chicken yield, cause diseases and economic loss, and equally endanger public health, particularly if the chicken meat gotten from the study area are not properly cooked before consumption. Discharging of contaminated poultry droppings into ponds water and from the ponds water to the environment could further enhance the transfer of resistant bacteria to the environment. Findings from this study showed 75.86% of the ESBL+EN gotten from the poultry droppings of the randomly selected farms were MDR. Overall highest resistance (93.1%) by the enterobacteriaceae was to AZT and lowest resistance (0%) was to IMP

VI. RECOMMENDATION

1. It is important to implement prudent and responsible use of antibiotics in the poultry section of the integrated poultry-fish farms practices, in line with regulatory guidelines and best management practices, to minimise the selection and spread of antibiotic resistance.
2. There is need to increase awareness about risks associated with the misuse and overuse of antibiotics by humans and the potential risk of spread of multi-drug resistant-bacteria in the environment.
3. Extensive research that will cover other regions of the country is required to obtain a clear understanding of ways to effectively and sustainably tackled antibiotic resistance in the poultry section of the integrated poultry-fish farms.
4. More elaborate studies especially multicentre studies should be carried out to determine the impact of integrated fish farming on antimicrobial resistant bacteria in ponds on a national level in order to enable formulation of guidelines for monitoring and establishing preventive programmes.
5. Regular monitoring of poultry droppings for microbial contamination is necessary to maintain chicken and public health
6. Implementation of policies to control or reduce the abuse of these antibiotics will go a long way in checking abuse and misuse
7. Regular and proper surveillance is needed
8. There is a need for serious campaigns and awareness programs as well as sanctions against abuse of antibiotics in the poultry industry.

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