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Carbapenem-Resistant and ESBL-Producing Salmonella in Poultry: A One Health Threat Driven by CTX-M–Mediated Multidrug Resistance in Owerri, Imo – State, Nigeria

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Abstract **Original Research Article**

The emergence of multidrug-resistant (MDR) and extended-spectrum β-lactamase (ESBL)–producing *Salmonella* in poultry represents a critical public health threat due to potential food-chain transmission. This study assessed antimicrobial resistance (AMR) patterns, ESBL production, and associated resistance genes in *Salmonella* isolates recovered from chicken cloacal swabs (n = 50) and fecal samples (n = 50). The isolates exhibited alarmingly high resistance to β-lactam antibiotics, including ampicillin, cefotaxime, and ceftazidime, as well as carbapenems. Cloacal swab isolates showed resistance rates of 98% to ampicillin, 94% to cefotaxime, 72% to ceftazidime, and 76–80% to carbapenems, while fecal isolates demonstrated complete resistance to ampicillin (100%) and markedly higher resistance to imipenem (86%), meropenem (86%), and ertapenem (96%). Fluoroquinolone resistance was substantial, with ciprofloxacin resistance ranging from 68% to 74% and levofloxacin resistance from 36% to 78%. Aminoglycoside resistance was also notable, particularly to gentamicin (54–70%). ESBL production was confirmed in 31 isolates, predominantly from fecal samples 18(29%) compared to cloacal swabs 13(21%). Molecular characterization of representative isolates identified CTX-M as the most prevalent ESBL gene (80%), followed by TEM (60%) and SHV (20%). The high prevalence of MDR and ESBL-producing *Salmonella* in poultry underscores a significant AMR burden and highlights the urgent need for enhanced surveillance, antimicrobial stewardship, and biosecurity interventions to mitigate zoonotic transmission.

Keywords: *Salmonella*, Antimicrobial resistance, ESBL, CTX-M, TEM, Poultry, Livestock.

Introduction

Antimicrobial resistance (AMR) is a rapidly escalating global health crisis that threatens the sustainability of modern medicine, food security, and economic development. The World Health Organization has identified AMR as a top global health priority, with disproportionate impacts in low- and middle-income countries where

regulatory oversight of antimicrobial use is often weak. The convergence of human, animal, and environmental reservoirs of resistant bacteria underscores the urgency of adopting a One Health framework to address AMR comprehensively.

Salmonella spp. remain a leading cause of foodborne infections worldwide, particularly non-typhoidal *Salmonella* (NTS), which disproportionately affects children, the elderly, and immunocompromised individuals. In sub-Saharan Africa, salmonellosis is a major contributor to morbidity and mortality, with poultry and poultry products serving as primary sources of zoonotic transmission (Okoro *et al*., 2012; Kuang *et al*., 2018). In Nigeria and other West African countries, the rapid expansion of intensive poultry production, often accompanied by unregulated antibiotic use, has amplified the risk of disseminating multidrug-resistant (MDR) *Salmonella* through the food chain and the environment (Ejikeukwu *et al.*, 2017; Amaechi & Nwankwo, 2015).

Of growing concern is the emergence of *Salmonella* strains producing extended-spectrum β-lactamases (ESBLs), enzymes that confer resistance to penicillins and third-generation cephalosporins, which are classified as critically important antimicrobials for human medicine. ESBL-producing *Salmonella* frequently exhibit co-resistance to fluoroquinolones, aminoglycosides, and carbapenems, thereby severely limiting therapeutic options. Among ESBL families, CTX-M-type enzymes have become globally dominant, replacing earlier TEM and SHV variants and driving widespread resistance in both community and hospital settings (Cantón *et al*., 2012). Recent studies from Nigeria and neighboring West African countries have increasingly reported CTX-M-producing Enterobacteriaceae in poultry, food products, and clinical samples, highlighting the regional expansion of plasmid-mediated resistance (Akinyemi *et al*., 2015; Saeed *et al.*, 2020).

Poultry cloacal swabs and feces constitute critical environmental reservoirs for resistant *Salmonella*, facilitating the spread of resistance genes through soil, water systems, crops, and human contact. This environmental dimension of AMR is particularly relevant in Nigeria, where poultry waste is commonly reused as manure or disposed of without adequate treatment, increasing opportunities for cross-sector transmission. Despite this risk, systematic surveillance of ESBL-producing *Salmonella* at the animal–environment interface remains limited, constraining effective policy formulation and enforcement of antimicrobial stewardship (Nnabugwu *et al.*, 2024).

In alignment with national and global AMR action plans, evidence-based data on resistance prevalence, resistance mechanisms, and transmission pathways are urgently needed to inform policy and intervention strategies. Therefore, the objectives of this study are to (i) determine the antimicrobial resistance profiles of *Salmonella* isolates recovered from chicken cloacal swabs and fecal samples; (ii) assess the phenotypic production of ESBLs among the isolates; and (iii) characterize the distribution of key ESBL genes (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}) using molecular methods.

Materials and Methods

2.1 Sample Collection: *Salmonella* isolates were obtained from poultry farm, 100 samples comprising chicken cloacal swabs (n=50) and chicken feces (n=50) were collected. Chicken cloacal swabs and fecal samples were collected from the Imo State University Poultry Farm using sterile containers, following the method of Ejikeukwu *et al* .(2017). Chicken cloacal swabs and feces were collected using sterile spatulas and placed in 20 mL sterile plastic bottles as described by Amaechi & Nwankwo (2015).

Ten antibiotics representing six classes—aminoglycosides, carbapenems, penicillins, fluoroquinolones, cephalosporins, and monobactams—were tested: Gentamicin (10 µg), Imipenem (10 µg), Ampicillin (10 µg), Levofloxacin (5 µg), Ceftazidime (30 µg), Aztreonam (30 µg), Cefotaxime (30 µg), Ciprofloxacin (5 µg), Meropenem (10 µg), and Ertapenem (10 µg). Resistance breakpoints (µg/mL) were defined according to CLSI (2019).

2.2. Isolation and Characterization of *Salmonella*

Samples were enriched in brain heart infusion broth for 24 h at 30 °C. Enriched cultures were subcultured by streaking a loopful onto freshly prepared *Salmonella Shigella* agar plates and 2–3 morphologically distinct colonies were purified on nutrient agar. Isolates were confirmed using Gram staining and IMViC tests as described by Cheesbrough (2003).

2.3 Antimicrobial Susceptibility Testing

The Kirby–Bauer disk diffusion method (Cheesbrough, 2003) was used. Isolates were standardized to 0.5 McFarland, spread on Mueller–Hinton agar, and antibiotic discs applied using sterile forceps. Plates were incubated at 35 °C for 24 h, and zones of inhibition were measured to classify isolates as resistant or susceptible based on CLSI (2019) breakpoints.

2.4. Determination of ESBL-Producing Isolates

2.4.1 Phenotypic Screening

Isolates resistant to cefotaxime (30 µg) or ceftazidime (30 µg) were screened for ESBL production using the Combination Disk Test (CLSI, 2009). Resistant strains were further assessed via the modified Double Disk Synergy Test (DDST) (Mchanty *et al.,* 2010). Lawn cultures were prepared on Mueller–Hinton agar, with an amoxicillin–clavulanate disk (20/10 mg) at the center and cephalosporin disks 15–20 mm apart (Paterson, 2006). Enhancement or distortion of inhibition zones toward the clavulanate disk indicated ESBL production.

2.4.2 Molecular Detection of ESBL Genes

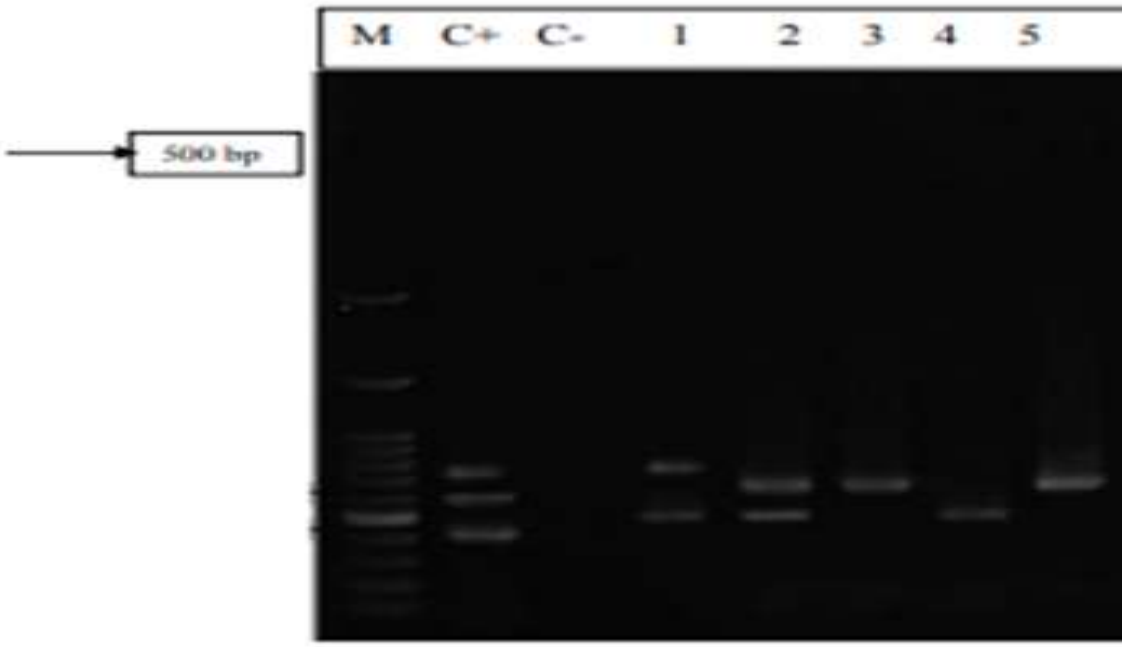
Genomic DNA was extracted by boiling (Akinyemi *et al*., 2015). Overnight cultures were centrifuged at 10,000 rpm for 5 min, the pellet resuspended in 200 µL TE buffer (10 mM Tris-HCl, 1 mM EDTA, pH 8.0), heated at 100 °C for 10 min, centrifuged, and 100 µL supernatant stored at −20 °C. DNA concentration and purity were measured using a BIO-RAD SmartSpec™ 3000 spectrophotometer.

Detection of bla_TEM, bla_SHV**,** and bla_CTX-M genes was performed via multiplex PCR (M-PCR) using primers from Promega Corp. (Germany) (Asgin *et al.,* 2019; Al-Hashimy *et al.,* 2020). PCR reactions (20 µL) contained 10 µL 1× PCR buffer, 1.5 mM $MgCl_2$, 200 nM dNTPs, 0.5 µL of 40 pmol forward and reverse primers, 1 µL genomic DNA (~100 ng), 1.25 U Taq polymerase, and nuclease-free water. Cycling conditions were: 94 °C for 3 min, 25 cycles of 94 °C for 30 s, 54 °C for 30 s, 72 °C for 1 min, and final extension at 72 °C for 7 min. PCR products (5 µL) were resolved on 2% agarose gels, stained with ethidium bromide (0.5 µg/mL), and visualized under UV light. A 100-bp DNA ladder (Fermentas, Canada) was used for sizing.

Table 1. Primers used for PCR amplification of ESBL genes

Target	Primer	Sequence (5'–3')	Product (bp)	Reference
bla_TEM	TEM-F	TCCGCTCATGAGACAATAACC	931	Asgin *et al.,* 2019
	TEM-R	TTGGTCTGACAGTTACCAATGC		
bla_SHV	SHV-F	TGGTTATGCGTTATATTCGCC	868	Asgin *et al*., 2019
	SHV-R	GGTTAGCGTTGCCAGTGCT		
bla_CTX-M	CTX-F	TCTTCCAGAATAAGGAATCCC	909	Al-Hashimy *et al*., 2020

Target	Primer Sequence (5'–3')	Product (bp)	Reference
	CTX-R CCGTTTCCGCTATTACAAAC		



The results of M-PCR from left to right: M; bp 100 ladder, postive control (*Salmonella tphi*), negative control (*Esccherichia coli 25923 ATCCC*), bla SHV (bp 747), bla TEM (bp 455) and bla CTX-M (bp 593), 11-1; clinical strains and veterinary strains of *Salmonella*

RESULT

3. Antimicrobial Resistance Profile of Salmonella Isolates

The antimicrobial resistance patterns of *Salmonella* isolates from chicken cloacal swabs (n = 50) and chicken feces (n = 50) are presented in Tables 2 and 3. High levels of resistance were observed for β-lactam antibiotics, particularly ampicillin, cefotaxime, and ceftazidime and carbapenems. In cloacal swab isolates, resistance was highest against ampicillin (98%), cefotaxime (94%), and ceftazidime (72%), Aztreonam (80%), Imipenem (76),Meropenem (80%), Gentamycin (70%) while fecal isolates exhibited complete resistance to ampicillin (100%) and high resistance to cefotaxime (96%) and ceftazidime (94%) ciprofloxacin (74%),imipenem(86%),meropenem (86%),ertapenem (96%), lavoflaxacin (78%).Resistance to carbapenems varied between sample types. Cloacal swab isolates showed 76% resistance to imipenem, 80% to meropenem, and 52% to ertapenem, whereas fecal isolates demonstrated higher resistance to imipenem (86%), meropenem (86%), and ertapenem (96%). Fluoroquinolone resistance was moderate to high, with ciprofloxacin resistance ranging from 68% in cloacal swabs to 74% in fecal isolates, and levofloxacin resistance

from 36% to 78%, respectively. Aminoglycoside resistance was also significant, with gentamicin resistance observed in 70% of cloacal swab isolates and 54% of fecal isolates. Aztreonam resistance differed markedly between cloacal swab (80%) and fecal isolates (52%).

Statistical analysis revealed significant variation in resistance patterns across antibiotics for cloacal swab isolates ($X^2 = 20.77$; $p = 0.014$; SD = 9.15), while differences for fecal isolates were not statistically significant ($X^2 = 16.26$; $p = 0.062$; SD = 8.59). ANOVA LSD values were 5.234 and 4.91 for cloacal swab and fecal isolates, respectively. Resistance was uniformly high across β-lactams, cephalosporins, carbapenems, monobactam, amynoglycosides and fluoroquinolones, indicating extensive multidrug resistance (MDR). Differences between antibiotics were not statistically significant ($p > 0.05$), reflecting widespread resistance in all tested drugs.These findings indicate widespread multidrug resistance (MDR) among *Salmonella* isolates from poultry, consistent with previous reports highlighting the extensive use of antibiotics in animal husbandry as a key driver of resistance (Ejikeukwu et al., 2017; Cheesbrough, 2003). The high resistance to β-lactams and carbapenems is particularly concerning, as these are critical drugs for both human and veterinary medicine.

A total of 50 Salmonella isolates from chicken cloacal swabs exhibited diverse multidrug-resistant patterns, with 37 distinct resistance combinations identified CTX+CAZ+ETP+IPM+MEM+CIP+AMP+ ATM. (Table 6). The most frequent patterns involved simultaneous resistance to β-lactams (ampicillin, cephalosporins), carbapenems, fluoroquinolones, aminoglycosides, and monobactams. Majority of isolates (≥7) showed resistance to all β-lactams,carbapenems, fluoroquinolones, aminoglycosides, and monobactams. The extensive multidrug resistance indicates selective pressure from widespread antibiotic use in poultry production. Among 50 isolates from chicken waste, 22 distinct resistance patterns were observed, with the most common pattern being CAZ CTX ETP LEV ATM IPM CN CIP MEM (Table 6). This indicates co-resistance across β-lactams, carbapenems, fluoroquinolones, aminoglycosides, and monobactams. The predominance of the pattern CAZ CTX ETP LEV ATM IPM CN CIP MEM (5 isolates) indicates high-level multidrug resistance in environmental samples. The co-resistance to β-lactams, carbapenems, fluoroquinolones, and aminoglycosides reflects potential risks for human transmission through contact with poultry feces.

Extended-spectrum β-lactamase (ESBL) production was assessed in *Salmonella* isolates from chicken cloacal swabs and fecal samples (Table 4). Out of 100 isolates tested (50 cloacal swabs and 50 feces), 31 isolates (31%) were confirmed as ESBL producers. Specifically, 13/50 isolates (26%) from cloacal swabs and 18/50 isolates (36%) from chicken feces were positive.

The higher prevalence of ESBL producers in fecal samples compared to cloacal swabs suggests that the gastrointestinal tract of chickens may harbor a more diverse and abundant population of ESBL-producing *Salmonella*. This may reflect prolonged exposure to antibiotics through feed, water, or prophylactic treatments in poultry farms. The overall detection rate of 50% (combined cloacal and fecal samples) is indicative of widespread dissemination of β-lactamase-producing *Salmonella* within poultry populations.

This finding aligns with previous studies demonstrating that poultry can serve as a significant reservoir for ESBL-producing Enterobacteriaceae, highlighting the risk of transmission to humans through direct contact, handling, or consumption of contaminated products. The detection of ESBL producers in both cloacal swabs and feces emphasizes the potential for environmental contamination and spread of resistance genes in farm settings.

A subset of five ESBL-producing *Salmonella* isolates was analyzed for the presence of major ESBL genes: bla_TEM, bla_SHV, and bla_CTX-M (Table 5). The results indicated that:bla_CTX-M was the most prevalent gene, detected in 2 isolates (40%).bla_TEM alone was

detected in 1 isolate (20%).Co-occurrence of bla_TEM and bla_CTX-M was observed in 1 isolate (20%).One isolate harbored bla_TEM, bla_SHV, and bla_CTX-M simultaneously (20%).The predominance of bla_CTX-M is consistent with global reports, where CTX-M-type enzymes have increasingly supplanted TEM and SHV variants as the dominant ESBLs in Enterobacteriaceae, including *Salmonella*. The presence of multiple ESBL genes within single isolates indicates the potential for horizontal gene transfer, contributing to the rapid dissemination of multidrug resistance within bacterial populations.

The chi-square analysis ($\chi^2 = 0.6$; $p = 0.896$) suggests no statistically significant difference in the distribution of these genes among the small number of isolates tested. Nevertheless, the observed gene combinations reflect the genetic diversity of ESBL mechanisms in poultry-associated *Salmonella*, which is concerning from both veterinary and public health perspectives.

The results highlight high prevalence of ESBL producers in poultry, both cloacal swabs and fecal samples showed substantial ESBL production, indicating that poultry farms may act as significant reservoirs of resistant *Salmonella*. The dominance of bla_CTX-M emphasizes the global trend of CTX-M as the principal ESBL type and suggests selective pressure from β-lactam antibiotic use in poultry production. The Co-existence of multiple ESBL genes detection of isolates carrying two or three ESBL genes underscores the potential for multidrug resistance and the risk of gene transfer to other pathogenic bacteria.The presence of ESBL-producing *Salmonella* in poultry implies potential transmission through the food chain, highlighting the need for antibiotic stewardship, biosecurity, and regular surveillance in farm environments. These findings provide critical evidence of the prevalence and genetic diversity of ESBL-producing *Salmonella* in poultry, reinforcing the urgent need for integrated One Health approaches to monitor and control antimicrobial resistance.

Table 1. Antimicrobial Resistant Profile of *Salmonella* Isolated from Chicken Cloacal Swab Samples

ANTICIOTICS	**No(%) of Resistant Isolates** ***Salmonella*** ***(n=50)***
Ampicillin	49(98)
Cefotaxime	47(94)
Ceftazidime	36(72)
Ciprofloxacin	34(68)
Imipenem	38(76)
Meropenem	40(80)
Aztreonam	40(80)
Gentamycin	35(70)
Ertapenem	26(52)

Lavofloxacin	18(36)
SD	9.15
X^2	20.77; p = 0.014
ANOVA LSD	5.234

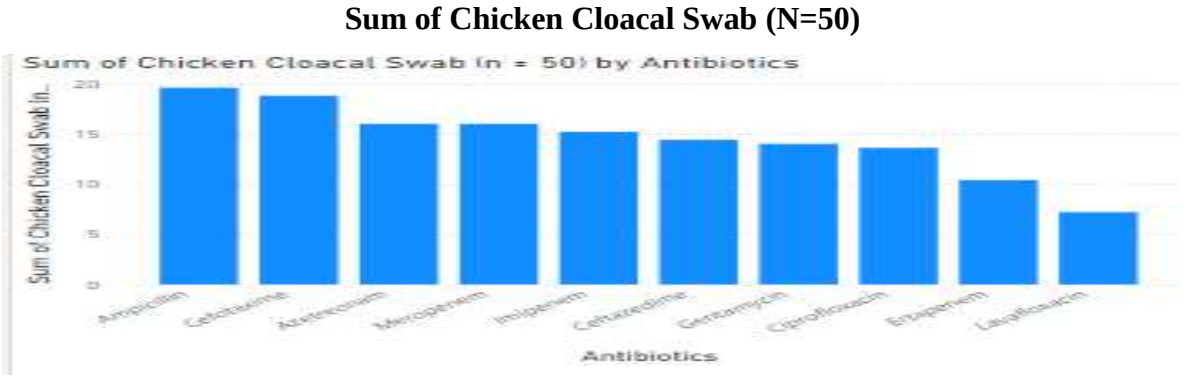


FIG 1**.** The bar chart shows the sum of Chicken Cloacal Swab (n = 50) by different antibiotics. The Y-axis represents the sum of the chicken cloacal swab in a numeric scale, and the X-axis lists various antibiotics.

Table 2. Antimicrobial Resistant Profile of *Salmonella* Isolated from Chicken Feces Samples.

ANTICIOTICS	**No(%) of Resistant Isolates *Salmonella* *(n=50)***
Ampicillin	50(100)
Cefotaxime	48(96)
Ceftazidime	47(94)
Ciprofloxacin	37(74)
Imipenem	43(86)
Meropenem	43(86)
Aztreonam	26(52)
Gentamycin	27(54)
Ertapenem	48(96)
Lavofloxacin	39(78)

SD	8.59
X^2	16.26;p = 0.062
ANOVA LSD	4.91

Sum of Chicken Feces (n=50) by Antibiotics

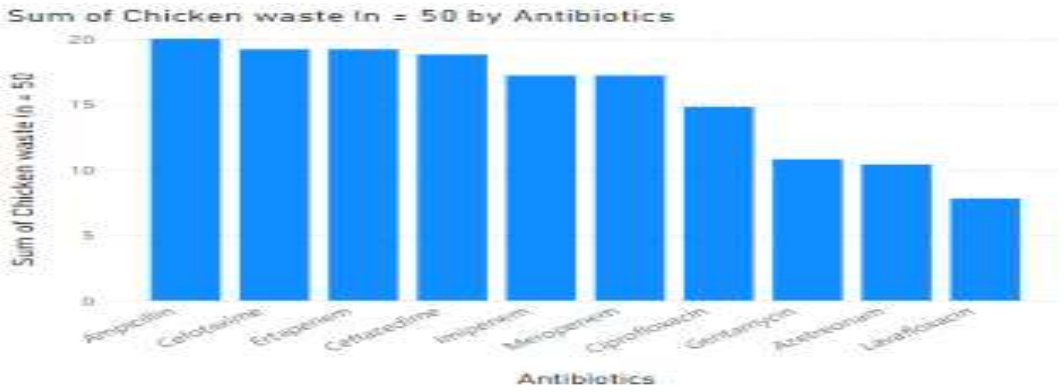


Table 3. Antimicrobial resistant patterns of *Salmonella* from chicken cloacal swab and chicken feces

Organism	Sources	No of pattern expressed	No of organisms expressing patterns	Predominant patterns
Salmonella	Chicken cloacal swab	37	8	CTX+CAZ+ETP+IPM+MEM+CIP+AMP+ ATM.
	Chicken feces	22	9	CAZ CTX ETP LEV ATM IPM CN CIP MEM

Table 4: Frequency (%) of ESBL Producers from the Different Sample Types

Sampling Sources	Total
Chicken cloacal Swab(n=50)	13(21)
Chicken feces (n=50)	18 (29)

TOTAL (100)	**31(50.0)**

Table 5. Distribution of TEM, SHV, and CTX-M genes in *Salmonella* isolates (n = 5).

ESBL genotype	Number of isolates (%) Salmonella
TEM	1(20)
CTX-M	2 (40)
TEM and CTX-M	1 (20)
TEM, SHV, and CTX-M	1 (20)
Total	5 (100)
SD	0.5
X^2	0.6;p = 0.896
ANOVA LSD	0.333

KEY WORDS –

TEM= Temoneira

CTX-M: Cefotaximase- Munich

SHV= SulfydrylVariable enzyme

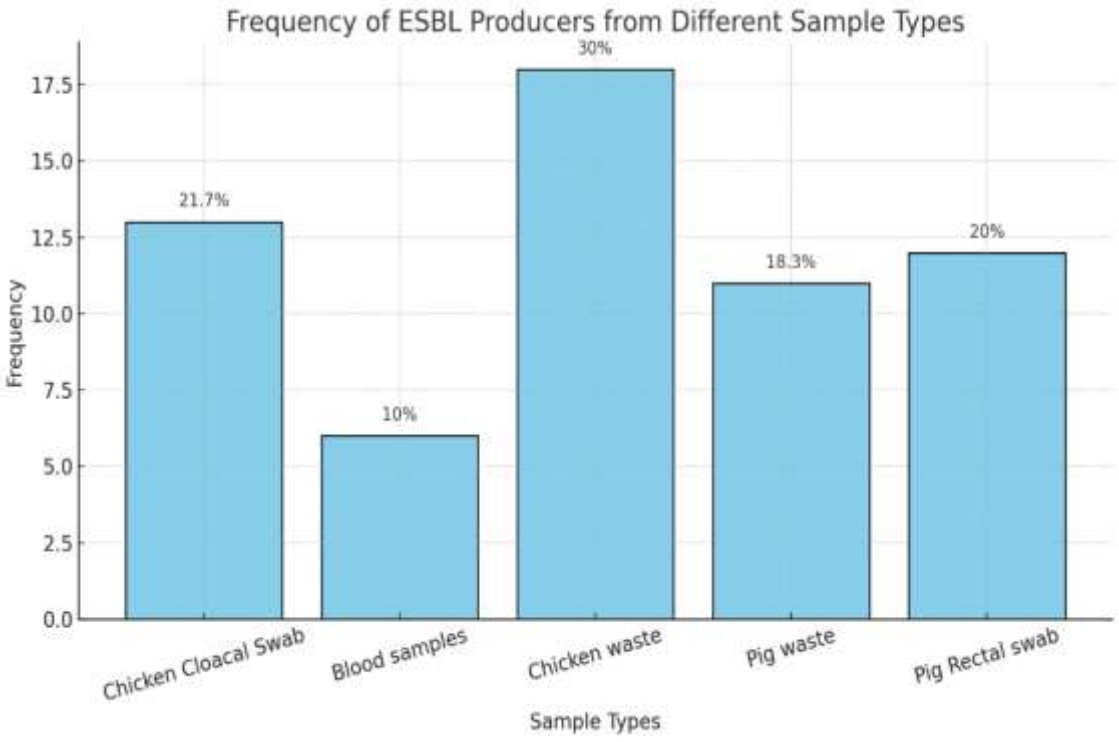


FIG 2.The Bar chart of ESBL Producers from the Different Samples

4. Discussion

Antimicrobial resistance is one of the most important public health concerns worldwide and contributes to the economic burden of both developed and developing countries through increasing the costs associated with treatment, hospitalization and infection control procedures (Antonelli *et al*., 2019).

Foodborne illness elicited by non-typhoidal *Salmonella* (NTS) remains a great threat to the health of children, immunocompromised and the elderly mainly in resource-limited countries in sub-Saharan Africa (Kuang *et al*, 2018., Okoro , *et al;* 2012). NTS infection is predominantly zoonotic since outbreaks have been linked to consumption of contaminated foods majorly from animal origin (Fernandez, *et al;*2018).

Owing to the importance of *Salmonella* serotypes in human public health and necessity of information about epidemiology of *Salmonella* antibiotic resistance patterns to launch infection control programs, different isolated bacteria from various veterinary and clinical samples were selected, and the multidrug-resistant ones were confirmed before bacterial plasmid extraction, analysis, and curing using SDS, phenotypic and genotypic analysis of ESBL and succesptibility of plant extracts to isolated *Salmonellla*

The high prevalence of multidrug-resistant *Salmonella* observed in both cloacal swabs and feces is consistent with reports from poultry-producing regions, highlighting the potential risk of transmission through the food chain (Ejikeukwu et al., 2017; Amaechi & Nwankwo, 2015). Resistance to β-lactams, particularly ampicillin and third-generation cephalosporins (cefotaxime and ceftazidime), carbapenem is alarming, as these antibiotics are commonly used for treatment of salmonellosis in humans and animals. The complete resistance to ampicillin in fecal isolates underscores the selective pressure imposed by indiscriminate antibiotic use in poultry production.

The observed carbapenem resistance, especially ertapenem (up to 96% in fecal isolates), is concerning, as carbapenems are considered last-

resort antibiotics for treating multidrug-resistant Gram-negative infections. This may indicate the presence of plasmid-mediated carbapenemase genes or co-resistance mechanisms, as previously reported in poultry-associated *Salmonella* (Paterson, 2006).

Fluoroquinolone resistance, notably ciprofloxacin and levofloxacin, was substantial, suggesting the potential risk of therapeutic failure in human infections. Aminoglycoside resistance (gentamicin) was also moderately high, indicating that multiple resistance mechanisms may coexist within these isolates, possibly mediated by plasmids, integrons, or transposons.

Overall, the data demonstrate a multidrug-resistant profile among *Salmonella* isolates from chicken sources, with resistance patterns generally higher in fecal isolates compared to cloacal swabs. This may reflect differences in bacterial load, environmental exposure, or antibiotic usage patterns. Widespread use of antibiotics in livestock and poultry production for different purposes such as treatment, growth promotion and prophylaxis has led to the emergence of antibiotic-resistant serotypes, causing treatment of *Salmonella* infections more difficult with each passing year (Rahmani *et al*., 2013). The findings highlight the urgent need for prudent antibiotic stewardship in poultry production and continuous surveillance to mitigate the spread of resistant. These resistance rates are in agreement with the results reported from United States, reporting 63% and 26% resistance to tetracycline and streptomycin, respectively (Velasquez *et al.*, 2018).

Given that Ampicillin and third generation cephalosporins are classified by the World Health Organization (WHO) as “critically important” antibiotics—used in treating serious human infections and diseases caused by organisms transmitted from non-human sources or capable of acquiring resistance genes—the resistance levels noted in this study, particularly to Ampicillin,cefotaxime and Ceftazedime raise concerns (WHO,2019).

More than 60.0% of isolates were resistant to third-generation cephalosporins. In line with the current results, Saeed *et al*., 2020 from Pakistan, reported resistance rates of 96.3%, and more than 50.0% against carbapenems, trimethoprim-sulfamethoxazole, and third-generation cephalosporins, respectively. El-Tayeb *et al*.,2017 from Saudi Arabia, revealed a high resistance rate of *S. Typhi* isolates against nitrofurantoin. As a result of nitrofurans being used as a feed supplement or treatment by veterinary professionals, particularly those working in the poultry industry, *Salmonella* has exhibited high resistance to this antibiotic. Indiscriminate application of antibiotics in human and animal health, and food production and subsequently leaching of the antibiotics into the environment are contributing to the increased AMR bacteria.

Among the five ESBL-producing *Salmonella* isolates analyzed, blaCTX-M was the most prevalent gene (40%), followed by blaTEM alone (20%), co-occurrence of blaTEM and blaCTX-M (20%), and the combined presence of blaTEM, blaSHV, and blaCTX-M (20%). Notably, blaSHV was not detected as a sole ESBL determinant in any isolate. Although the chi-square analysis ($\chi^2 = 0.6$; $p = 0.896$) indicated no statistically significant difference in gene distribution—likely due to the small sample size—the findings clearly demonstrate substantial genetic diversity of ESBL determinants among the isolates.

The predominance of blaCTX-M observed in this study is consistent with global and regional reports indicating that CTX-M enzymes have largely supplanted TEM and SHV as the dominant ESBLs in *Enterobacteriaceae* (Cantón *et al*., 2012; Asgin *et al*., 2019; Al-Hashimy *et al*., 2020). CTX-M-type ESBLs have been widely reported in both hospital-acquired and community-associated infections and are now considered the most rapidly disseminating ESBL family worldwide. The detection of blaTEM as the second most frequent ESBL gene aligns with previous studies, although a substantial proportion of TEM-positive isolates also harbored blaCTX-M, highlighting the increasing trend of multi-gene carriage.

The absence of blaSHV alone and its detection only in combination with other ESBL genes

mirrors findings from several regional studies, suggesting a declining independent role of SHV-type enzymes in ESBL epidemiology. However, variations in ESBL gene distribution have been widely reported. While some studies have documented a predominance of TEM with lower frequencies of CTX-M and SHV (Caubey & Suchitra, 2018), others similar to the present findings have reported CTX-M as the dominant genotype (Bajpai *et al*., 2017; Mahmoudi *et al.*, 2019).

A particularly important observation in this study is the co-existence of multiple ESBL genes within single isolates, including TEM + CTX-M and TEM + SHV + CTX-M combinations. The detection of all three bla genes in 20% of the isolates suggests the presence of multiple resistance plasmids and underscores the role of horizontal gene transfer in the dissemination of antimicrobial resistance. Similar co-existence patterns have been documented in *Salmonella* isolates from poultry and human sources, indicating that these strains may serve as efficient reservoirs for resistance determinants (Mahmoudi *et al*., 2019).

Phenotypically, ESBL-producing *Salmonella* isolates exhibited high levels of resistance to ampicillin, β-lactam/β-lactamase inhibitor combinations, third-generation cephalosporins (cefotaxime and ceftazidime), carbapenems (imipenem and meropenem), and the monobactam aztreonam. This resistance profile is alarming and consistent with previous reports attributing such patterns to the extensive and often indiscriminate use of these antibiotics in both human medicine and animal husbandry (Bindayna *et al*., 2010; Hassan & Abdalhamid, 2014).

The coexistence of ESBL production with resistance to non-β-lactam antibiotics, including quinolones and aminoglycosides, further compounds the therapeutic challenge. ESBL genes are frequently located on plasmids that also harbor resistance determinants for multiple antibiotic classes, leading to multidrug-resistant (MDR) phenotypes (Jacoby & Sutton, 1991; Paterson *et al*., 2000). In the present study, ESBL-producing isolates demonstrated resistance to up to ten different antibiotics, significantly limiting treatment options and increasing the risk of therapeutic failure.

The high prevalence of MDR and ESBL-producing *Salmonella* in poultry-associated samples, including cloacal swabs and chicken waste, represents a serious public health concern. Chicken waste, in particular, constitutes a critical environmental reservoir that facilitates the dissemination of resistant strains through soil, water, and the food chain, thereby increasing the risk of zoonotic transmission. If such strains infect humans, treatment options for invasive salmonellosis may be severely restricted.

Overall, these findings emphasize the urgent need for robust antibiotic stewardship programs, enhanced biosecurity measures, and continuous surveillance of antimicrobial resistance in poultry production systems. Accurate and rapid detection of ESBL-producing *Salmonella* from food animals, animal products, and environmental sources is essential to prevent further spread, reduce treatment costs, and minimize morbidity and mortality in both veterinary and human settings. Integrating these efforts within a One Health framework is critical for mitigating the growing threat of antimicrobial resistance at national and regional levels.

Conclusion

Poultry in Imo State is a significant reservoir of multidrug-resistant and ESBL-producing *Salmonella*. The high prevalence of bla_CTX-M and co-occurrence of multiple ESBL genes indicate potential for rapid dissemination of resistance. These findings highlight the urgent need for surveillance, prudent antibiotic use, and environmental control measures to mitigate public health risks.

Recommendations

1. Implement strict antibiotic stewardship in poultry production.
2. Conduct routine surveillance of antimicrobial resistance and ESBL genes in poultry farms.

3. Improve farm biosecurity, hygiene, and waste management to prevent environmental contamination.
4. Educate poultry farmers on zoonotic risks and judicious antibiotic use.
5. Integrate One Health strategies linking human, animal, and environmental health for AMR mitigation.

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