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Proceeding Paper

Multidrug Resistance in Extended-Spectrum Beta Lactamase (Esbl)-Producing *Escherichia coli* Isolates from Selected Cattle Farms in Ibadan, Oyo State †

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Abstract: Antimicrobial resistance (AMR) has been recorded as a fast-growing One Health challenge globally. The major driver of AMR is the inappropriate use of antibiotics in humans and animals. A substantial volume of antimicrobials is consumed by the animal industry for the treatment of *Escherichia coli* (*E. coli*) infections, which is a challenge; therefore, the understanding of AMR in animals is critical in solving this rising One Health problem. This study assessed the resistance level of some critically important antibiotics to *E. coli* bacteria isolates from cattle fecal samples. A total of twenty-eight composite (n = 5) fecal samples were collected from farms in four different Local Government Areas (LGAs) within Ibadan: Akinyele LGA:7, Ibadan north LGA:12, Ido LGA:4, and Lagelu LGA:5. Standard microbiological methods were used for isolation, antibiotic sensitivity tests (ASTs), and ESBL production. A total of 22 (78.6%) *E. coli* isolates were recovered, and the results showed resistance to critically important antibiotics in ascending order; streptomycin (0.00%), meropenem (0.00%), gentamicin (4.55%), ceftazidime (18.8%), sulphamethazole (22.73%), cefotaxime (54.55%), ampicillin (63.64%), pefloxacin (81.82%), and amoxicillin–clavulanate (100%). Of the 22 positive *E. coli* isolates, 8 (36.4%) were ESBL-producing and 17 (60.7%) were multidrug-resistant. ESBL enzymes share the ability to hydrolyze third-generation Cephalosporin, and this makes ESBL-producing *E. coli* exhibit resistance to antibiotics (especially Cephalosporins). The results show the possibility of AMR becoming a looming pandemic globally. The presence of multidrug-resistant ESBL-producing *E. coli* in cattle in Ibadan was established. Resistance to third-generation Cephalosporin antibiotics is of public health significance. Ensuring antimicrobial stewardship and prescription-based medication, alternative therapies to antibiotics, and the adoption of a collaborative approach are measures to preventing an AMR pandemic.

Keywords: *E. coli*; ESBL; cattle fecal sample; antimicrobial resistance

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1. Introduction

The worldwide need for animal protein is growing, which has resulted in the global spread of intensive farming [1]. This global expansion has indirectly led to an increase in the usage of antimicrobials by farmers as growth boosters and treatments of animal illnesses [2,3]. Antibiotic resistance has resulted from increased use and inadequate antimicrobial stewardship, which has resulted in the inefficient treatment of certain bacterial illnesses [4,5].

The misuse and overuse of antimicrobials are the main drivers in the development of drug-resistant pathogens. Antimicrobial resistance (AMR) is a growing concern for public health and the global economy. Resistant infections are predicted to become the leading

cause of death, which will reach 10 million per year by 2050 [6]. This contributes to the emergence of antimicrobial resistance (AMR), which the WHO has declared as one of the top 10 global public health threats facing humanity [7]

The antimicrobial resistance of bacterial species originating from food-producing animals also influences human health through the transfer of resistant organisms or genes via the food chain, and this is a growing public health challenge [3]. This study, therefore, determined the prevalence of commensal and ESBL-producing *E. coli* and assessed their antibiogram.

Escherichia coli is one of the deadliest bacteria organisms. A major mechanism through which *E. coli* builds resistance to antibiotics is by genetic mutation, which has led to the production of extended-spectrum beta lactamase enzymes.

Extended-spectrum beta-lactamases (ESBLs) are enzymes that impart resistance to beta lactam antibiotics such as penicillin, cephalosporins, and aztreonam, a monobactam. Infections used by ESBL-producing organisms have been linked to poor clinical outcomes. ESBL-producing Enterobacteriaceae from the community and hospitals are common globally [8]. Because the reliable identification of ESBL-producing organisms in clinical laboratories can be difficult, their prevalence is most likely understated. Beta-lactamases are enzymes that break apart the beta lactam ring, rendering the antibiotic inactive. In the 1960s, the first plasmid-mediated beta-lactamase in Gram-negative bacteria was identified in Greece. It was termed TEM after the patient who isolated it (Temoniera) [9].

2. Materials and Methods

2.1. Sample Collection

Using a cross-sectional study design (Figure 1), fecal samples were collected from selected commercial cattle farms in four Local Government Areas (Lagelu, Ona Ara, Ibadan North, and Akinyele). A total of twenty-eight composite (n = 5) fecal samples were collected from Akinyele Local Government Area (LGA) (n = 7), Ibadan north LGA (n = 12), Ido LGA (n = 4), and Lagelu LGA (n = 5).

We collected the samples within the first two weeks, and laboratory analysis was performed during the period of January to April 2022. The total duration of the study lasted from November 2021 to June 2022. The study population consisted of fresh fecal samples of cattle from the farms.

2.2. Sample Size Estimation

The minimum sample size was calculated using this formula by Thrusfield (2005): Sample size, n = z2p(1 − p)/d2, where n = minimum sample size calculated, z = score for a given confidence interval at 95% is 1, p = known or estimated of prevalence rate, and d = level of precision (5%).

Therefore, for Escherichia coli, it has a prevalence rate of 89.5% [10]

$$n = \frac{1.962 \times 0.085(1 - 0.0895)}{0.052}$$

$$n = \frac{3.8416 \times 0.0895 \times 0.105}{0.0025}$$

$$n = 0.361 \times 0.0025$$

n = 144.4 approx. 144

The sample size is 144 fecal materials. 28 composite fecal samples were collected, with each composite sample comprising 5 fecal materials (n = 28 × 5). This gives a total of n = 140 fecal samples.

2.3. Microbiological Analysis

Fresh fecal samples were collected aseptically in composites of 5, and a total of 28 composite samples were collected (n = 140). Samples were incubated overnight in 1% buffered peptone water. Aliquots of overnight culture were inoculated onto MacConkey agar without cefaxamine and MacConkey agar with cefaxamine to identify commensal

E. coli and presumptive ESBL-producing *E. coli*. Two isolates from each plate were selected for biochemical confirmation of *E. coli*. The selected isolates were purified to obtain discrete colonies. Pure isolates were inoculated on Eosin Methylene Blue (EMB) and Chromogenic (ECC) agars. *E. coli* gives a green metallic sheen color on EMB agar plates and blue colonies on ECC. Isolates that were catalase-positive, indole-positive, and oxidase-negative were confirmed to be *E. coli*.

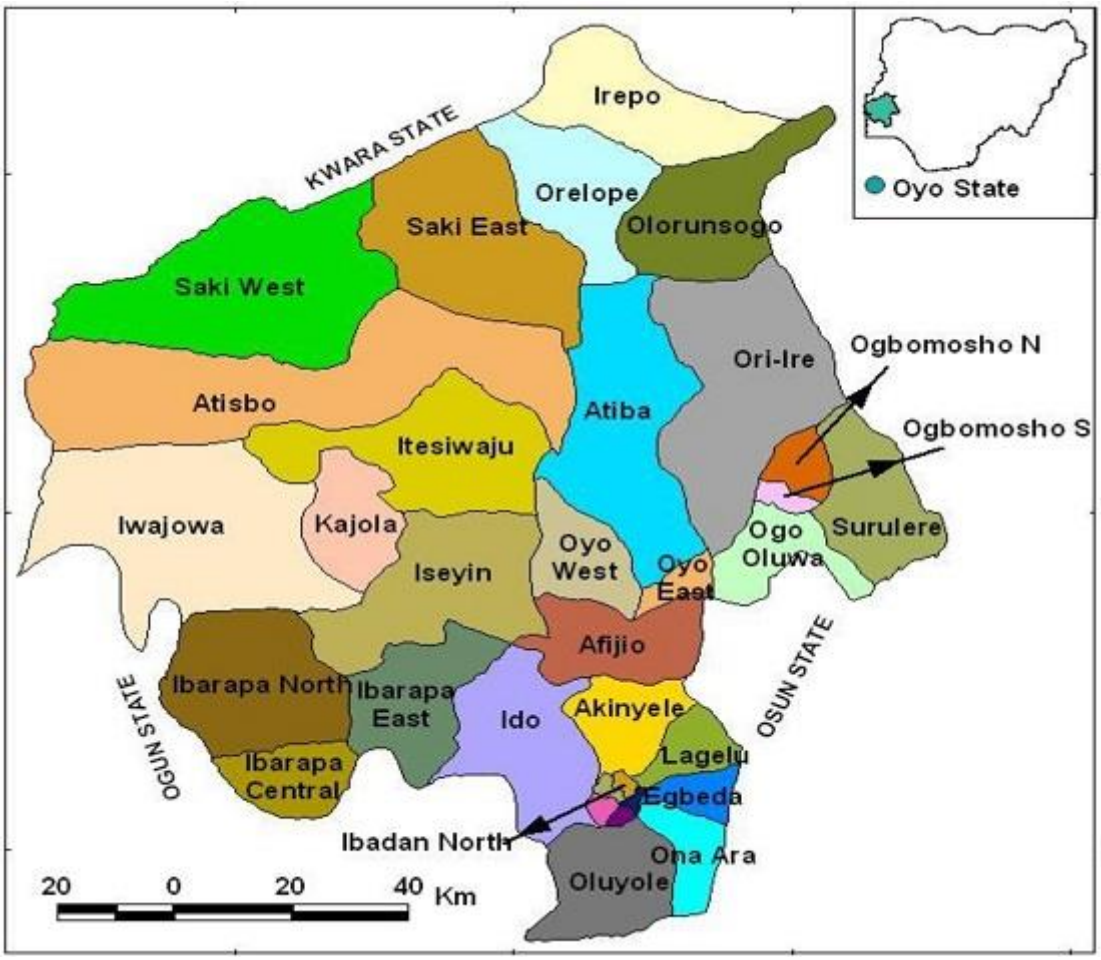


Figure 1. A map of Oyo State indicating the geographical location of the studied Local Government Areas.

2.4. Antibiotic Susceptibility Tests (ASTs)

Antibiotic susceptibility tests were carried out using the disc diffusion method described by Kirby Bauer et al. (1996). The antibiotic discs used included the following: chloramphenicol (C30), pefloxacin (PEF5), gentamicin (CN10), sulphamethazole (SXT25), tetracycline (TE30), ampicillin (AMP10), cefotaxime (CTX30), ceftazidime (CAZ30), streptomycin (S300), amoxicillin + clavulanic acid (AMC30), and meropenem (MEM10). The zones of inhibition were measured and interpreted based on Clinical and Laboratory Standards Institute [11].

2.5. The Detection of Esbl-Producing E. coli Using the Double-Disk Synergy Test

All the cefotaxime- and ceftazidime-resistant *E. coli* isolates were selected as presumptive ESBL-producing *E. coli* isolates for confirmatory ESBL detection using the double-disk synergy test previously described by Lu et al. (2010). The test was carried out using an ESBL kit and a combination of cefotaxime (CTX 30) and clavulanic acid (CTL) placed at a distance of 15–20mm from each other (center to center). The plates were observed after incubating at 37 °C for 18–24 h.

ESBL-producing *E. coli* isolates were confirmed by subtracting the CTX diameter from the CTL diameter (CTX − CTL). A zone of diameter that is >15 mm confirms the presence of ESBL-producing *E. coli* isolates.

2.6. Data Analysis

Data were analyzed by using descriptive statistics, proportions, and percentages and presented in the form of tables and figures. The level of significance was set at $p \leq 0.05$. ANOVA (Analysis of Variance) values were used to ascertain significance in the antibiotic sensitivity testing.

2.7. Ethical Considerations

The consent of the owners of these farms was sought before taking samples from their cattle. All contaminated materials and media were decontaminated by autoclaving and or incineration.

3. Results

3.1. The Occurrence of Escherichia coli

The occurrence of *E. coli* was 22/28 (78.6%). All isolates showed multidrug resistance, and seven (25.01%) were confirmed to be positive ESBL-producing isolates.

3.2. The Antibiotic Susceptibility of E. coli in Cattle

A zone of clearance, which signifies bacterial susceptibility, and lack of clearance, which signifies bacterial resistance, were observed (Table 1).

Table 1. Antibiogram profile of *Escherichia coli* isolates (%) from cattle within Ibadan, Nigeria.

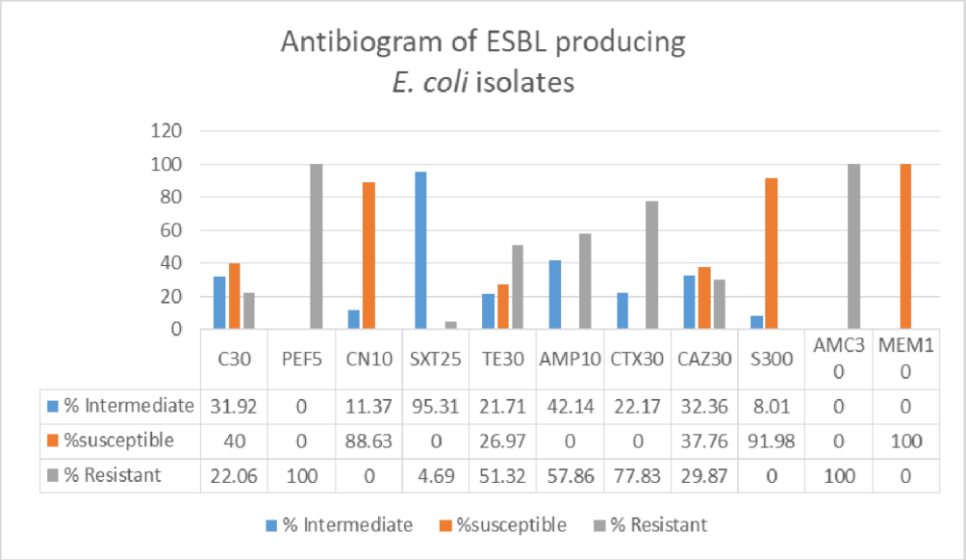
Subclass	Antibiotics	Percentage Age-Susceptible (n = 22)	Percentage Intermediate (n = 22)	Percentage Resistant (n = 22)
Aminoglycosides	Gentamicin CN10	81.82	13.64	4.55
	Streptomycin S300	95.45	4.55	0.00
Cephalosporin III	Cefotaxime CTX30	13.64	31.82	54.55
	Ceftazidime CAZ30	54.55	27.27	18.18
Quinolones	Pefloxacin PEF5	18.18	0.00	81.82
Aminopenicillin	Ampicillin AMP10	18.18	18.18	63.64
Folate Pathway Inhibitor	Sulphamethazole/ trimethoprim SXT25	22.73	54.55	22.73
Phenicols	Chloramphenicol C30	68.18	18.18	13.64
Tetracyclines	Tetracycline TE30	27.27	18.18	54.55
Carbapenems	Meropenem MEM10	90.91	9.09	0.00
B-lactam lactamase +B-	Amoxicillin + clavulanic acid	0.00	0.00	100.00

3.3. The Prevalence of Esbl-Producing E. coli Isolates

Of the 12 (54.5%) presumptive ESBL-producing *E. coli* isolates, 7 (25.01%) were recorded as confirmed ESBL-producing isolates using the double-disk synergy test.

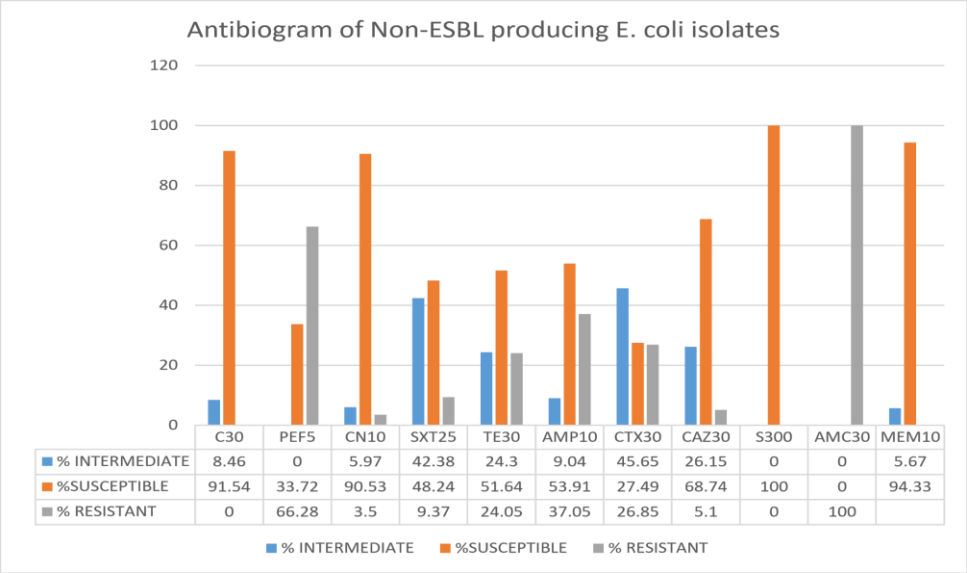
3.4. The Antibiogram of Esbl- and Non-Esbl-Producing E. coli

Figures 2 and 3 show graphical charts of the antibiotic susceptibility pattern of the confirmed ESBL-producing *E. coli* isolates and the non-ESBL-producing isolates.



	C30	PEF5	CN10	SXT25	TE30	AMP10	CTX30	CAZ30	S300	AMC30	MEM10
% Intermediate	31.92	0	11.37	95.31	21.71	42.14	22.17	32.36	8.01	0	0
%susceptible	40	0	88.63	0	26.97	0	0	37.76	91.98	0	100
% Resistant	22.06	100	0	4.69	51.32	57.86	77.83	29.87	0	100	0

Figure 2. A chart showing antibiogram of ESBL-producing *E. coli* isolates.



	C30	PEF5	CN10	SXT25	TE30	AMP10	CTX30	CAZ30	S300	AMC30	MEM10
% INTERMEDIATE	8.46	0	5.97	42.38	24.3	9.04	45.65	26.15	0	0	5.67
%SUSCEPTIBLE	91.54	33.72	90.53	48.24	51.64	53.91	27.49	68.74	100	0	94.33
% RESISTANT	0	66.28	3.5	9.37	24.05	37.05	26.85	5.1	0	100	

Figure 3. A chart showing the antibiogram of non-ESBL-producing *E. coli* isolates.

From Figure 1 below, the ESBL-producing isolates show higher antibiotic resistance compared to the non-ESBL-producing isolates in Figure 3. This is an expected outcome as the ESBL-producing isolates are the resistant genes that render antibiotics ineffective against the bacteria.

4. Discussion

4.1. ESBL-Producing E. coli

In this study, of the 12 (54.5%) presumptive ESBL-producing *E. coli* isolates, 7 (25.01%) were recorded as confirmed ESBL-producing isolates. The *E. coli* isolates showed multidrug resistance to the antibiotics used at various degrees, with the ESBL-producing isolates having resistance to more antibiotics than the non-ESBL-producing isolates. This result agrees with the findings of other researchers who reported multidrug resistance among *E. coli* isolates [12,13].

A study carried out on dams and calves in Germany revealed that the mean farm ESBL/AmpC-*E. coli* prevalence in calves was almost 3.5 times higher (mean = 63.5%) than

that of the dams (mean = 18.0%). Nearly 14% of the calf–cow pairs were positive for ESBL/AmpC-E. coli (Weber et al., 2021). The high prevalence in the calves could be due to their low immunity development, which could predispose them to infections.

4.2. Antibiogram of ESBL- and Non-ESBL-Producing E. coli Isolates

The comparison of the antibiograms of ESBL- and non-ESBL-producing *E. coli* isolates shows a significant resistance pattern. From Figure 1, the ESBL-producing isolates show low susceptibility and high resistance to the antibiotics, while the reverse was the case with the non-ESBL-producing isolates (Figure 2). The high resistance displayed by the ESBL-producing isolates was expected as ESBL genes are produced by *E. coli* bacteria to protect them from destruction by antibiotics.

The indiscriminate use of antibiotics is the main cause of the emergence, selection, and discrimination of drug-resistant genes in bacteria important in both veterinary and human medicine [14]. In Nigeria, veterinary drugs are sold and used without much control. This indiscriminate usage and non-adherence to withdrawal periods are responsible for the spread of resistant genes like ESBLs within the bacterial population in food animals and humans by extension. These resistant bacteria are rendering second- and third-line antibiotics ineffective, and this may return us to the pre-antibiotic era [15].

4.3. Conclusions

In Nigeria, large number of antimicrobials go into use without veterinarians' prescriptions, particularly as antibiotics are available over the counter (OTC) [16]. Farmers or cattle handlers resort to self-medication due to the lack of adequate—and the high cost of—veterinary services, increasing the chances of antimicrobial resistance as a result.

5. Recommendations

To reduce and/or eliminate pathogens in cattle, the following are therefore recommended:

1. Highly multidrug-resistant isolates are of public health importance. The need for a One Health approach involving collaborations with microbiologists, veterinarians, cattle breeders, public health practitioners, and other One Health-related professionals in Nigeria for the containment of AMR is highly urgent.
2. Alternative medication can be used in place of antibiotics, such as probiotics, bacteriophages, and Therapy.
3. Biosecurity measures should be strictly adhered to, and the management of farm animals and their environment should be of the utmost priority.
4. Proper antimicrobial stewardship by veterinary professionals should be ensured before any drug is recommended and administered.
5. Farmers should employ good hygiene in the management of their cattle farms.
6. There should be increased awareness among farmers towards the likelihood of cattle as a potential source of foodborne diseases and how to preven.t them. Government and extension agents could work to achieve this.
7. The withdrawal period after drug administration is important to prevent antibiotic resistance in humans, which can be fatal upon occurrence. Farmers should ensure that they do not sell cattle that has just been administered antimicrobial drugs until the withdrawal period elapses.
8. Carriers or clinically sick animals should be isolated and treated separately by veterinary professionals.
9. A ban on the usage of antimicrobial drugs which are no longer effective in the treatment of these pathogens should be implemented. This will help prevent bacterial organisms from building more resistant genes.
10. Further research work involving the use of molecular characterization to determine resistant genes, as well as whole-genome sequencing, should be conducted. This will ensure that researchers are at the cutting edge and are constantly ready to curb any form of disease outbreak.

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Institutional Review Board Statement: Ethical review and approval were waived for this study due to the fact that the samples used in this study were not obtained directly from the bodies of the animals. The fecal samples collected were fresh feaces which had just been dropped on the floor and environment within the cattle farm.

Informed Consent Statement: Not applicable.

Data Availability Statement: The data presented in this study are available on request from the corresponding author, (E.E.A.).

Conflicts of Interest: The authors declare no conflict of interest.

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