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# Multi-Drug 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 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 faecal samples. A total of twenty-eight composite (n = 5) faecal samples were collected from farms in four different Local Governments 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, Antibiotics Sensitivity Test (AST) 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%), Amoxicillin-Clavulanate (100%). Of the 22 positive *E. coli* isolates, 8 (36.4%) were ESBL-producing and 17 (60.7%) were multi-drug resistant. The ESBL enzymes share the ability to hydrolyze third-generation Cephalosporin and this makes ESBL-producing *E. coli* exhibit resistance to antibiotics (especially the Cephalosporins). The result shows the possibility of AMR becoming a looming pandemic, globally. The presence of multi-drug resistant ESBL *E. coli* in cattle in Ibadan was established. The resistance to third-generation Cephalosporin antibiotics is of public health significance. Ensuring Antimicrobial stewardship and prescription-based medication, alternatives therapies to antibiotics and adoption of a collaborative approach are measures to preventing AMR pandemic.

**Keywords:** *E. coli*; ESBL; cattle feacal 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 (Christie et al., 2018, Ranyal et al., 2023) This global expansion has indirectly led to an increase in the usage of antimicrobials by farmers as growth boosters and treatment of animal illnesses (Thomas et al., 2015, Hosain et al., 2021). Antibiotic resistance has occurred from increased use and inadequate antimicrobial stewardship, which has resulted in inefficient treatment of certain bacterium illnesses (CDC, 2022, Banan, et al., 2023).

Misuse and overuse of antimicrobials are the main drivers in the development of drug-resistant pathogen. Antimicrobial resistance (AMR), is a growing concern for public health and global economy. Resistant infections are predicted to become the leading cause of death which will reach 10 million per year by 2050 (WHO 2021). This contributes to the emergence of antimicrobial resistance (AMR), which WHO has declared as one of the top 10 global public health threats facing humanity (WHO 2021).

The antimicrobial resistance of bacterial species originating from food-producing animals also influences human health through the transfer of resistant organisms or genes via food chain and this is a grown public health challenge (Hosain et al., 2021). This study therefore determined the prevalence of commensal and ESBL producing *E. coli* and also assessed their antibiogram.

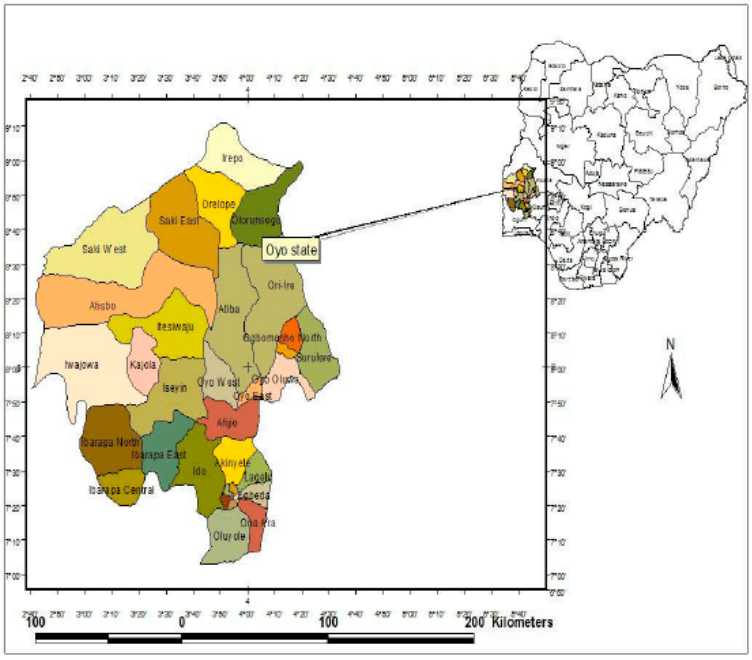
*Escherichia coli* is one of the deadliest bacteria organisms. A major mechanism through which *E. coli* builds resistance to antibiotics is by genetic mutation which has led to the production of Extended spectrum beta lactamase enzymes.

Extended-spectrum beta-lactamases (ESBL) 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 (Ben-Ami et al., 2009). Because 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) (Braford et al., 2001).

## 2. Materials and Methods

### *2.1. Sample Collection*

Using a cross-sectional study design, feacal 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) faecal 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).




A map of Oyo state indicating the geographical location of the studied Local Government Areas.

We collected the samples within the first two weeks and laboratory analysis was done during the period of January to April 2022. The total duration of the study lasted from November 2021 to June 2022. The study population consists 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 d = level of precision (5%)

*Therefore, for Escherichia coli it has a prevalence rate of 89.5% (Eyitayo et al., 2015)*

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

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

n = 0.361 0.0025 n = 144.4 approx. 144

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

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*2.3. Microbiological Analysis*

Fresh feacal samples were collected aseptically in composite 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 was the inoculated unto 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 for 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 ECC. Isolates that were catalase positive, indole positive and oxidase negative were confirmed to be *E. coli*.

*2.4. Antibiotics Susceptibility Tests (AST)*

Antibiotics susceptibility tests were carried out using the disc diffusion method described by Kirby Bauer et al (1996). The antibiotics discs used include: Chloramphenicol (C30), Pefloxacin (PEF5), Gentamicin (CN10), Sulphamethazole (SXT25), Tetracycline (TE30), Ampicillin (AMP10), Cefotaxime (CTX30), Ceftazidime (CAZ30), Streptomycin (S300), Amoxicillin + Clauvinic acid (AMC30), Meropenem (MEM10). The zone of inhibition were measured and interpreted based on Clinical and Laboratory Standards Institute (CLSI) (2021).

*2.5. Detection of Esbl Producing E. coli Using Double Disk Synergy Test*

All the Cefotaxime and Ceftazidime resistant *E. coli* isolates were selected as presumptive ESBL *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 ESBL-kit, 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). The zone of diameter that is >15 mm confirms ESBL producing *E. coli* isolates.

### *2.6. Data Analysis*

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

### *2.7. Ethical Considerations*

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

## 3. Results

### *3.1. Occurrence of Escherichia coli*

Occurrence of *E. coli* was 22/28 (78.6%). All isolates showed multi-drug resistance and 7 (25.01%) were confirmed to be positive ESBL producing isolates.

### *3.2. Antibiotic Susceptibility of E. coli in Cattle*

A zone of clearance which signifies bacteria susceptibility and lack of clearance which signifies bacteria resistant were observed.

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

| Subclass | Antibiotics | Percentage 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 Path way Inhibitor | Sulphamethazole/Tri methopr im 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 +B-lactamase | Amoxicillin + Clavanalic Acid | 0.00 | 0.00 | 100.00 |

**Table 2.** Antimicrobial-resistant pattern of *Escherichia coli* isolates (%) from cattle in different location in Ibadan, Nigeria.

| Subclass | Antibiotics | Akinyele (n = 19) | Ibadan North (n = 44) | Ido (n = 4) | Lagel u (n = 2) |
|---|---|---|---|---|---|
| Aminoglycosides | Gentamicin CN10 | 0.0 | 72.7 | 0.0 | 0.0 |
| | Streptomycin S300 | 0.00 | 0.00 | 0.00 | 0.00 |
| Cephalosporin III | Cefotaxime CTX30 | 80.0 | 36.4 | 75.0 | 50.0 |
| | Ceftazidime CAZ30 | 0.0 | 18.2 | 25.0 | 50.0 |
| Quinolones | Pefloxacin PEF5 | 80.0 | 72.7 | 100.0 | 100.0 |
| Penicillin | Ampicilin AMP10 | 60.0 | 36.4 | 75.0 | 100.0 |
| Folate Pathway Inhibitor | Sulphamethazole/Trimetho prim SXT25 | 20.0 | 9.1 | 25.0 | 50.0 |
| Amphenicols | Chloramphenicol C30 | 0.0 | 9.1 | 50.0 | 0.0 |
| Tetracyclines | Tetracycline TE30 | 80.0 | 18.2 | 75.0 | 50.0 |
| Carbapenems | Meropenem MEM10 | 0.0 | 0.0 | 0.0 | 0.0 |

### *3.3. Prevalence of Esbl Producing E. coli Isolates*

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

### *3.4. Antibiogram of Esbl and Non-Esbl Producing E. coli*

The Figures 1 and 2 gives a graphical chart on the antibiotics susceptibility pattern of the Confirmed ESBL *E. coli* isolates and the Non-ESBL producing isolates.

From the Figure 1 below, the ESBL producing isolates shows higher antibiotic resistance compared to the non-ESBL isolates in Figure 2. This is an expected outcome, as the ESBL isolates are the resistant genes that renders antibiotics ineffective against the bacteria.

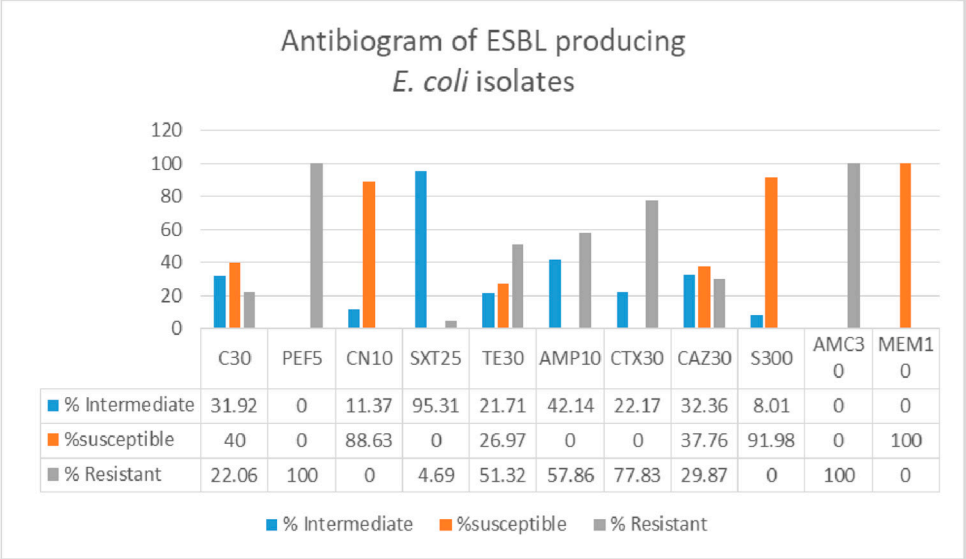


**Figure 1.** A chart showing the Antibiogram of ESBL producing *E. coli* isolates.

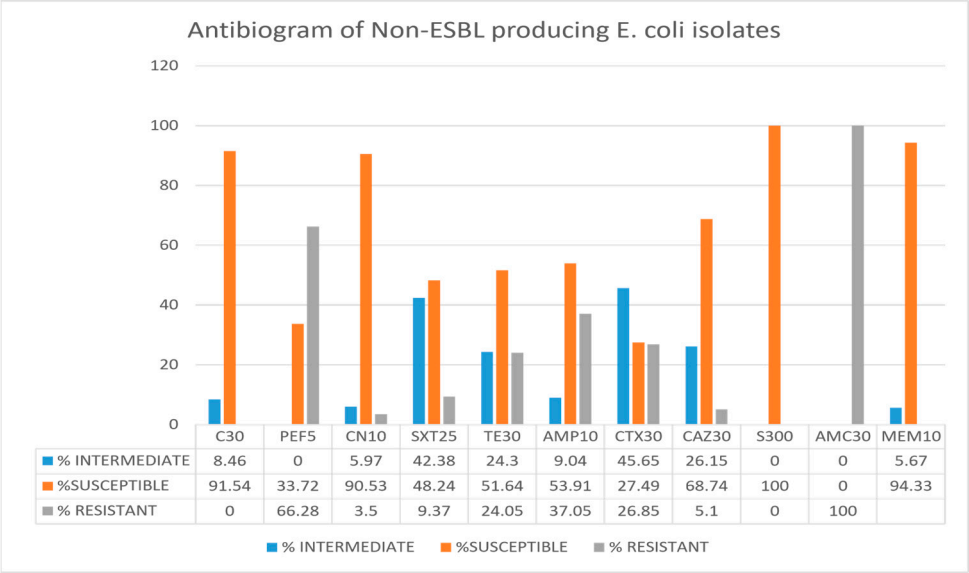


**Figure 2.** A chart showing the Antibiogram of Non-ESBL producing *E. coli* isolates.

## 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 confirmatory as ESBL producing isolates. *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 (Schroeder et al., 2002 and Ojo et al., 2009).

A study carried out on the dam 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 E. coli Isolates*

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

The indiscriminate use of antibiotics is the main cause of emergence, selection and discrimination of drug-resistant genes in bacteria important in both veterinary and human medicine (Smith et al., 2005). In Nigeria, veterinary drugs are sold and used without much control. This indiscriminate usage and non-adherence to withdrawal periods is responsible for the spread of resistant genes like the ESBLs within the bacteria population in food animals and humans by extension. These resistant bacteria are rendering the second- and third-line antibiotics ineffective and this may return us to the pre-antibiotic era (WHO, 2000).

*4.3. Conclusions*

In Nigeria, large number of antimicrobials goes into use without Veterinarians' prescriptions particularly as antibiotics are available over-the-counter (OTC) (Ojo et al., 2014). Farmers or cattle handlers result to self-medication due to lack of adequate and high cost of veterinary services as a result increasing the chances of antimicrobial resistance.

**5. Recommendation**

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

1. The high 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 containment of AMR is highly recommended.
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 management of farm animals and their environment should be of 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 n management of their Cattle farms.
6. There should be increased awareness among farmers on the likelihood of Cattle as a potential source of foodborne diseases and how to prevent it. Government and extension agents could work to achieve this.
7. Withdrawal period after drug administration is important to prevent antibiotics resistance in humans, which can be fatal on occurrence. The farmers should ensure not to 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 the veterinary professionals.
9. A ban in the usage of antimicrobial drugs which are no longer effective in treatment of these pathogens. This will help prevent the bacteria organisms from building more resistant genes.
10. Further research work to involve the use of molecular characterization to determine resistant genes, and whole-genome sequencing. This will ensure the researchers are at the cutting edge and are constantly ready to curb any form of diseases outbreak.

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