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Assessment of Antibiotic-Resistant *E. coli* in Cowshed Environments in Owo, Nigeria: Implications for Public Health

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Abstract

This study assessed the microbial load, and antibiotic resistance profiles of *Escherichia coli* (*E. coli*) isolated from cowshed soil and wastewater in Owo metropolis, Nigeria. The total microbial count ranged from 2.73×10^4 to 1.66×10^8 cfu/mL, with an average of 2.41×10^7 cfu/mL, indicating significant bacterial contamination. All isolates were Gram-negative, oxidase-negative, and predominantly indole- and citrate-positive. PCR amplification targeting the *uidA* gene confirmed the identity of E. coli in 16 of 25 phenotypically suspected isolates, reinforcing the reliability of molecular diagnostics. Antibiotic susceptibility testing revealed that all isolates exhibited 100% resistance to penicillin, doripenem, and amikacin. High resistance was also recorded for ceftazidime (89%) and cefepime (78%), while norfloxacin showed the highest susceptibility (89%). Wastewater isolates demonstrated slightly higher resistance levels, with 100% resistance to ceftazidime and MARI values ranging from 0.5 to 0.875, compared to soil isolates with MARI values between 0.375 and 0.75. These findings suggest that cowshed environments serve as reservoirs for multidrug-resistant *E. coli*, likely due to the indiscriminate use of antibiotics in livestock and inadequate waste disposal practices. The presence of resistant *E. coli* in environmental matrices poses a serious public health threat, particularly in communities using nearby water sources for domestic purposes and underscores the urgent need for stricter regulation of antimicrobial use in animal farming, and continuous environmental surveillance to curb the spread of antibiotic resistance and protect both human and environmental health.

Keywords: Antibiotic-Resistant; Cowshed; *E. coli;* Environments; Public Health.

1.0 Introduction

Antibiotic resistance is one of the major public health concerns globally. This is commonly demonstrated in bacteria such as *E. coli*, found on the WHO global priority list of antibiotic-resistant bacteria to guide research, discovery, and development of new antibiotics (WHO, 2018; Ramadan *et al.*, (2019)). Although the discovery

of antimicrobials leads to various expectations, it has been influenced by the emergence of resistant bacterial strains against antibiotics or therapeutics. Twenty to fifty percent (20-50%) of humans are using antibiotics unnecessarily and 40-80% agricultural usages of antibiotics are highly suspicious (Wise *et al.,* 2015; Dankar *et al.,* 2023). On account of irrational use of antimicrobials in the last few decades, present clinically important bacteria have been converted from susceptible to resistance to single and multiple antibiotics which has become a threat in public health sector in Nigeria (Adegoke *et al.,* 2020) as well as whole world in general (Santos-Lopez *et al.,* 2019).

Cowshed wastewater can be a potential risk factor for public health and ecological balance, since it contains various hazardous components including pathogenic microorganisms (Sharpe, 2017). Moreover, owing to heavy antibiotic use in animal husbandry cowshed waste waters contain high numbers of antibiotic-resistant microorganisms than domestic wastewaters (Morinigo *et al.,* 2015). Cowshed wastewater carries pathogenic multidrug resistant microorganisms and are responsible for the spread of these organisms to the environment. Sometimes, a treated cowshed waste water can also spread multidrug resistant microorganisms (Rahman *et al.,* 2016). Cowshed waste effluents contaminate aquatic environments leading to human, fish and other animals that depend on the water are dangerously affected. Some researchers have reported that almost 80 percent of reared animals contained multidrug-resistant bacteria and these were identical with the specimens collected from hospital patients (Overdevest *et al.,* 2014; Bendary *et al.,* 2022). Moreover, it has been reported that, the irrigation water system also has been contaminated by multidrug resistant bacteria which have a chance of entering into the food chain directly.

However, the data on impact of environmental contamination with antimicrobial resistant *E. coli* for human health are increasing. In Nigeria, especially in Owo, Ondo State, there is limited data on the multidrug resistance profile of *E. coli* from cowshed wastewater and soil. Therefore, this study is aimed to assess the prevalence and antibiotics susceptibility of *E. coli* isolated from cowshed waste water in Owo metropolis.

2.0 Materials and Methods

2.1 Sample Collection

Soil and wastewater samples were collected from three different cowshed locations within Owo metropolis for microbial analysis. Surface soil samples were obtained at three different points during each sampling using a sterilized shovel and placed in Ziploc bags. Similarly, three untreated wastewater samples were collected from within the cowsheds using 500 mL pre-sterilized plastic bottles during each sampling session. Sampling was conducted over a period of three months, and all samples were transported to the laboratory in ice-cooled containers.

2.2 Enumeration and isolation of *E. coli* from Soil Samples Collected from Cowshed

Soil samples (1g) were enriched in peptone water (pH 8.6) and incubated at 37ºC for 24 h after which was serially diluted using 7-fold dilution. After dilution, 0.1 mL each of the suitable dilution were placed in the centre of well labelled dried plates of Eosin Methylene Blue Agar (EMB) plates. Immediately a glass spreader was used to spread the sample evenly all over the surface of the plate. The plates were then incubated in an inverted position at 35 ^{O}C for 24 h. Green metallic sheen colonies on each plate were selected and representative distinct colonies were re-streaked on non-selective media and stored on nutrient agar slants for further analysis.

Water samples (1L) were enriched in 9ml peptone water (pH 8.6) and incubated at 37ºC for 24 h after which was serially diluted using 7-fold dilution. After dilution, 0.1 mL each of the suitable dilution were placed in the centre of well labelled dried plates of Eosin Methylene Blue Agar (EMB) plates. Immediately a glass spreader was used to spread the sample evenly all over the surface of the plate. The plates were then incubated in an inverted position at 35^{0}C for 24hr. Green metallic sheen colonies on each plate were selected and representative distinct colonies were re-streaked on non-selective media and stored on nutrient agar slants for further analysis

2.3 Identification of presumptive *E. coli*

After the 24h of incubation, the target bacteria colonies on the agar plate were identified based on the manufacturer's instructions. Presumptive *E. coli* colonies appeared as dark centered and flat, a metallic sheen. The presumptive colonies of *E. coli* were confirmed by the standard biochemical tests: Indole, Citrate, methyl red, Voges-Proskauer and Gram staining.

2.5 DNA template Preparation for PCR Amplification

Genomic DNA of *E. coli* isolates were extracted by suspending multiple colonies from overnight cultures grown on Nutrient Agar plates into 100 µL of 1X Tris-EDTA buffer. The suspension was vortexed and subsequently boiled at 100 °C for 10 minutes, following the method described by Adesiyan *et al.* (2019). The resulting lysate (boilate) was immediately transferred to a freezer at -20 °C for 10 minutes, then allowed to return to room temperature. After vortexing, the mixture was centrifuged at 10,000 rpm for 10 minutes. The supernatant containing the extracted DNA was carefully collected, stored at 4 °C, and used as the DNA template for PCR analyses.

2.6 Molecular Identification of *E. coli*. through PCR amplification of 16S rRNA gene

All organisms suspected to be *E. coli* by their phenotypic characteristics were confirmed as *E. coli* by amplifying their *16S rRNA* gene (as described by Hassan *et al*. 2014 (Table 1). *E. coli* strain ATCC 25922 was used as the positive control while sterile distilled water was used as the negative control. A 12.5 µl reaction mixture contained 6.25 uL of One *Taq* Quick-Load 2XMaster mix with Standard Buffer (Bio Labs, New England), 0.25

pmol each of the forward and reverse primers (Inqaba, Biotec, South Africa), 2 µl of the DNA template and made up with 3.75ul nuclease free water (BioConcept, Switzerland). Amplification conditions were as follows: Initial denaturation at 95°C for 5 min; 35 cycles of denaturation at 94°C for 45 s, annealing at 45°C for 45s, and extension at 72°C for 1 min; followed by a final extension at 72°C for 5 min. Each amplicon (10µL) was electrophoresed on a 1.5% agarose gel (Cleaver Scientific, United Kingdom) pre stained with 0.5µg/mL Ethidium bromide in 1X Tris-Acetate-EDTA(TAE) buffer and viewed with a UVitec transilluminator (Avebury, Cambridge UK).

Table 1: DNA Sequence of *E. coli* Primer

Type	Primer Designation	Primers (5 to 3)	Target gene	Amplicon size (bp)
ECO	ECO-1	GACCTCGGTTTAGTTCACAGA	*16SrRNA*	585
	ECO-2	CACACGCTGACGCTGACCA		

2.7 Antibiotic Susceptibility Test

The antibiotic susceptibility of *E. coli* isolates was determined using the disc diffusion method. Fresh cultures (18–22 hours old) of the isolates were transferred into test tubes containing 5 mL of 0.85% sterile physiological saline. The turbidity of the bacterial suspension was adjusted to match the 0.5 McFarland standard (equivalent to 1.5×10^8 CFU/mL). Sterile swabs were dipped into the suspension and evenly spread across the surface of Mueller-Hinton agar plates. Antibiotic discs were then placed on the agar surface, and the plates were incubated at 35 ± 2 °C for 18 to 24 hours. After incubation, zones of inhibition were measured and recorded. Results were classified as susceptible, intermediate, or resistant, based on the Clinical and Laboratory Standards Institute (CLSI) guidelines (2018). A total of eight antibiotics were tested against the confirmed *E. coli* isolates: Penicillin (10 µg), Doripenem (10 µg), Ceftazidime (30 µg), Cefepime (30 µg), Kanamycin (30 µg), Streptomycin (300 µg), Norfloxacin (30 µg), and Amikacin (30 µg).

2.8 Antibiotics Resistance indexing

According to the result of the antibiotics susceptibility testing, the frequencies, percentages and pattern of antibiotics resistance were obtained and also the multiple antibiotic-resistant phenotypes (MARPs) for isolates that showed resistance to more than two antibiotics at each sampling location were calculated. The MAR index is key indicator to identify the risk source of contamination potentially hazardous to human (Titilawo *et al.*, 2015) and it is expressed mathematically as

$$MAR\ index = {}^{a}/_{b} \quad (2)$$

Where a, is the number of antibiotics to which the isolate was resistant and b, the total number of antibiotics against which an individual isolate was tested.

3.0 Results

3.1 Colony Count

Sample 1- The colony count on the EMB Agar as shown in Table 2 depicts a total of one hundred and fifty-three (153) colonies were recorded on from the soil samples collected from the cowshed. Highest growth was recorded on SCE 2 with 25 colonies while the lowest colony count was recorded on cowshed soil sample 4 and sample 9 (SCE 4, SCE 9). No growth was observed on SCE 5 and SCE 13 respectively. SCE 10 and SCE 11 soil samples were found to be contaminated

Sample 2 - A total of four untreated cowshed waste water samples collected from different locations were cultured for total microbial count. It was found that 87.5% of the samples had bacteria growth while 12.5% samples had no growth and these were omitted in the subsequent data analysis. The results showed that the total microbial count ranged from 2.73×10^4 to 1.66×10^8cfu/ml. Mean TMC was $(2.41 \pm 4.1) \times 10^7$cfu/ml.

Table 2: Presumptive *E. coli* Count on EMB Agar

Sample Code	Total Colony Count on Eosin Methylene Blue Agar (EMB)
SCE 1	13
SCE 2	25
SCE 3	16
SCE 4	10
SCE 5	No Growth
SCE 6	11
SCE 7	12
SCE 8	14
SCE 9	10
SCE 10	Contaminated
SCE 11	Contaminated
SCE 12	12
SCE 13	No Growth
SCE 14	16
SCE 15	14

KEY: SCE (Soil from cowshed *E. coli*)

3.2 Biochemical Characteristics of the Isolates

Sample 1- Fifteen distinct (15) colonies were subjected to biochemical tests. The Gram stain reaction test revealed that all *E. coli* isolate from soil samples were Gram negative, oxidative negative and indole positive. Further revealed in Table 3, eleven (11) out the 15 (fifteen) isolates were citrate positive while 3 (three) were indole negative.

Sample 2- Pure colonies were identified according to their Gram staining and other microscopically characteristic. The Bacterial isolated were Gram negative rod in shape, non-spore forming, with peritrichous flagella. Bacterial isolated suspected to be E. coli according to microscopical characteristics were subjected to the related biochemical test. Results illustrated in Table 4 below showed that all the ten isolates were positive for catalase, indole and metyl red, but negative test for oxidase, citrate and vogas-proskauer. Seven isolates were subjected to PCR

Table 3: Sample 1-Biochemical Tests Results of *E. coli* spp in Soil Samples

Samples code	**Gram's Reaction**	**Oxidase test**	**Citrate test**	**Indole test**
SCE 1	-	-	+	+
SCE 2	-	-	+	+
SCE 3	-	-	+	+
SCE 4	-	-	-	+
SCE 5	-	-	+	+
SCE 6	-	-	+	+
SCE 7	-	-	-	+
SCE 8	-	-	-	+
SCE 9	-	-	+	+
SCE 10	-	-	+	+
SCE 11	-	-	+	+
SCE 12	-	-	+	+
SCE 13	-	-	-	+
SCE 14	-	-	+	+
SCE 15	-	-	+	+

Key: Positive (+), Negative (-) **SCE** (Soil from cowshed *E. coli*)

Table 4: Sample 2- Biochemical Characterization of *E. coli* isolates from Cowshed waste water

Biochemicaltests	**Isolates**									
	SW 001	**SW 002**	**SW 003**	**SWa a1**	**SW aa2**	**SW aa3**	**SW bb1**	**SW bb2**	**SW cc1**	**SW cc2**
Gram Reaction	-	-	-	-	-	-	-	-	-	-
Catalase test	+	+	+	+	+	+	+	+	+	+
Oxidase test	-	-	-	-	-	-	-	-	-	-
Indole test	+	+	+	+	+	+	+	+	+	+

Citrate utilization test	-	-	-	-	-	-	-	-	-	-
Methyl-red test	+	+	+	+	+	+	+	+	+	+
Voges-Proskauer	-	-	-	-	-	-	-	-	-	-

Note: (+) Positive results (-) negative results

3.3 Molecular Identification of *E. coli* through PCR Amplification of 16S rRNA Gene

All organisms suspected to be *E. coli* by their phenotypic characteristics were confirmed as *E. coli*. Lane 1-7,9-11 confirmed *E. coli* as Plate 1. The presumptive *E. coli* isolates were confirmed via PCR as *E. coli* as they were positive for the *uid*gene. Figure 1 below shows a gel picture of the amplicons of the expected size of 585 bp for some positive isolates. As it is observable, the Molecular weight marker (100bp); lane 2: positive control; lane 8: negative control; lane 1-7,9-11 confirmed *E. coli*. Seven of the isolates were confirmed positive for *E – coli*

Table 5: Presumptive *E. coli* Count on EMB Agar

Sample Code	**Total Colony Count on Eosin Methylene Blue Agar (EMB)**
SCE 1	13
SCE 2	25
SCE 3	16
SCE 4	10
SCE 5	No Growth
SCE 6	11
SCE 7	12
SCE 8	14
SCE 9	10
SCE 10	Contaminated
SCE 11	Contaminated
SCE 12	12
SCE 13	No Growth
SCE 14	16
SCE 15	14

KEY: SCE (Soil from cowshed *E. coli*)

Table 6: Presumptive *E. coli* Count on EMB Agar

Sample Code	**Total Colony Count on Eosin Methylene Blue Agar (EMB)**
SCE 1	65
SCE 2	80
SCE 3	71
SCE 4	88
SCE 5	No Growth
SCE 6	85
SCE 7	48
SCE 8	62
SCE 9	74
SCE 10	Contaminated
SCE 11	Contaminated
SCE 12	54
SCE 13	No Growth
SCE 14	47
SCE 15	68

KEY: SCE (Soil from cowshed *E. coli*)

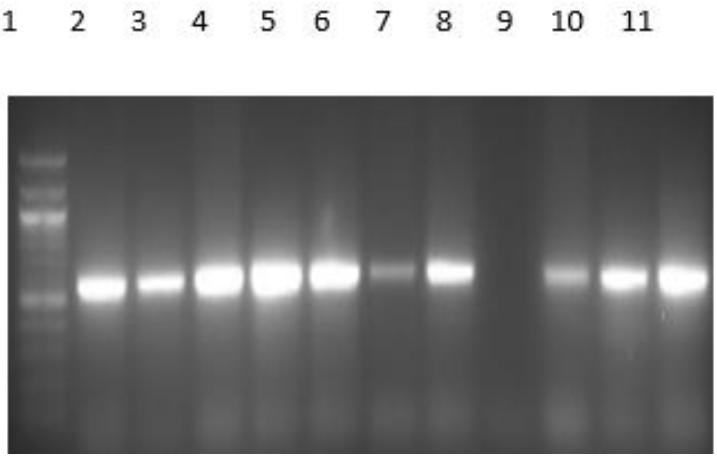

Plate 1: Amplification of 16S rRNA of *Escherichia coli*. L: 100bp ladder; Lane 2: positive control, Lane 8 negative control, Lane 1-7,9-11 confirmed *E.coli*

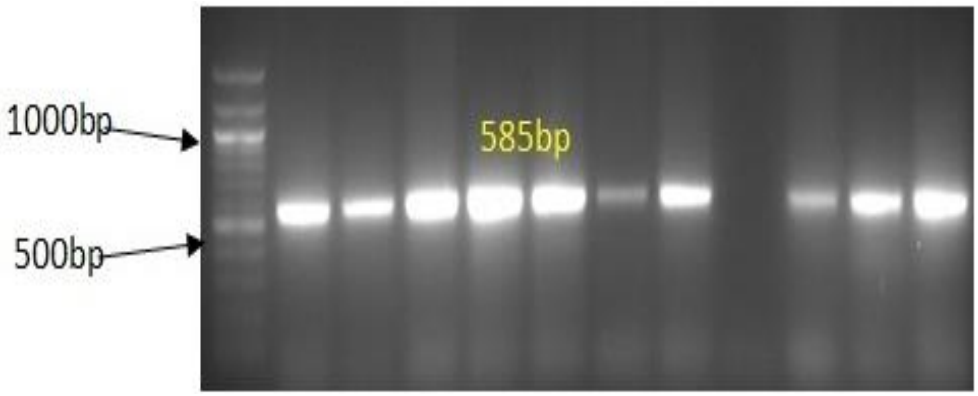

Plate 2: Amplification of 16S rRNA of *Escherichia coli*. L: 100bp ladder; Lane 2: positive control, Lane 8 negative control, Lane 1-7,9-11 confirmed *E.coli*

3.4 Antibiotics Resistance Pattern of *E. coli* species from Soil of Cowshed

The antibiotic resistance pattern of the *E. coli* isolate revealed that all the isolates were resistant against penicillin and doripenem Majority of the *E. coli* isolates were susceptible to norfloxacin except SCE 2 samples which was resistant to norfloxacin. Also, almost all the *E. coli* isolateswere resistant against ceftazidime except for sample SCE 10. Five (5) out of the 9 (nine) *E. coli* isolateswere susceptible to kanamycin, SCE7. SCE 9 and SCE 15 were resistant to kanamycin while only SCE 14 show no resistant against kanamycin. SCE 2, SCE 3, SCE 15 were all resistant against streptomycin, SCE 6, SCE 7, SCE 9 and SCE 14 *E. coli* were not resistant against streptomycin while SCE 10 and SCE 12 were susceptible to streptomycin. Furthermore, *E. coli isolates*; SCE 3, SCE 6, SCE 7, SCE 9, SCE 10, SCE 11 and SCE 15 were resistant against cefepime while *E. coli* isolates SCE 2 and SCE 14 were not resistant against cefepime as shown in Table 7. The antibiotic distribution pattern of the *E. coli* from the cowshed soil samples showed that all isolates were resistant against penicillin, doripenem and amikacin 9(100%) respectively. 1(11%) of the *E. coli was* resistant to norfloxacin, majority 8(89%) were susceptible to ceftazidime, as shown in Figure 1. Relatively, majority of the *E. coli* 8(89%) were highly resistant to ceftazidime while 1(11%) were susceptible to ceftazidime. The *E. coli* isolatewere also observed to show low resistance 3(33%) against kanamycin and high susceptibility 5(56%) against kananmycin. Almost all the *E. coli* isolates 7(78%) were highly resistant against cefepime and low resistant against streptomycin 3(33%) was observed as shown in Table 8.

Table 7: Antibiotics Resistance Pattern of *E. coli* species from soil of cowshed

Sample Code	**Antimicrobial Agents**								**Antibiotic Resistance**
	P	**DOR**	**NOR**	**CAZ**	**K**	**S**	**FEP**	**AK**	
SCE 2	R	R	R	R	S	R	I	S	5/8
SCE 3	R	R	S	R	S	R	R	S	5/8
SCE 6	R	R	S	R	S	I	R	S	4/8
SCE 7	R	R	S	R	R	I	R	S	5/8

SCE 9	R	R	S	R	R	I	R	S	5/8
SCE 10	R	R	S	S	S	S	R	S	3/8
SCE 12	R	R	S	R	S	S	R	S	4/8
SCE 14	R	R	S	R	I	I	I	S	3/8
SCE 15	R	R	S	R	R	R	R	S	6/8

Key: R- Resistance, I- Intermediate, S – Susceptible SCE- Soil Cowshed *E. coli*

Table 8: Percentage Antibiotics Susceptibility Profile of *E. coli* species from cowshed soil samples

SAMPLE CODE	No. of Isolates	R (%)	I (%)	S (%)
P	9	100(9)	0(0)	0(0)
DOR	9	100(9)	0(0)	0(0)
NOR	9	11(1)	0(0)	89(8)
CAZ	9	89(8)	0(0)	11(1)
K	9	3(33)	1(11)	5(56)
S	9	3(33)	4(45)	2(22)
FEP	9	7(78)	2(22)	0(0)
AK	9	100(9)	0(0)	0(0)

KEY

P – Penicillin, DOR – Doripenem, NOR – Norfloxacin, CAZ – Ceftazidime, K- Kanamycin, S-Streptomycin, FEP – Cefepime, AK - Amikacin

Table 9: Antibiotic resistance pattern assessment of isolated *E. coli* wastewater

Antibiotic	No. of Isolates	Resistant (%)	Intermediate (%)	Susceptible (%)
Penicilin	9	7(100%)	0 (0%)	0 (0%)
Amikacin	7	7(100%)	0 (0%)	0 (0%)
Norfloxacin	7	1(14.3%)	2 (28.6%)	4 (57.1%)
Kanamycin	7	0 (0%)	2(28.6%)	7 (71.4%)
Doripenem	7	7(100%)	0 (0%)	0 (0%)
Cefepime	7	1(14.3%)	2 (28.6%)	4 (57.1%)
Streptomycin	7	3(42.9%)	0 (0%)	4 (57.1%)
Ceftazidime	7	7(100%)	0 (0%)	0 (0%)

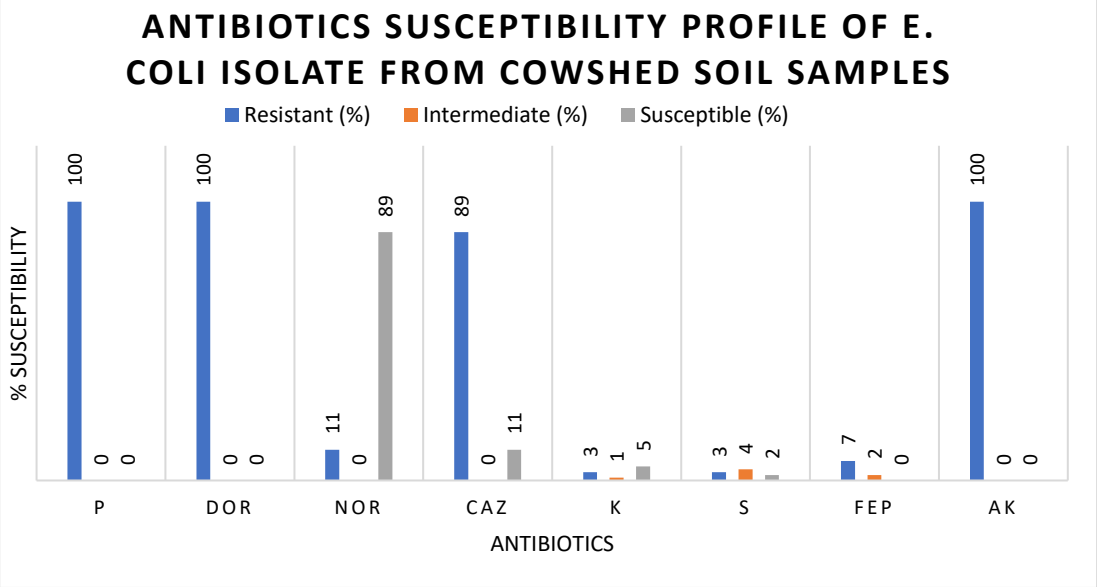

Figure 1: Antibiotic Susceptibility Profile of *E. coli* isolates from Cowshed soil

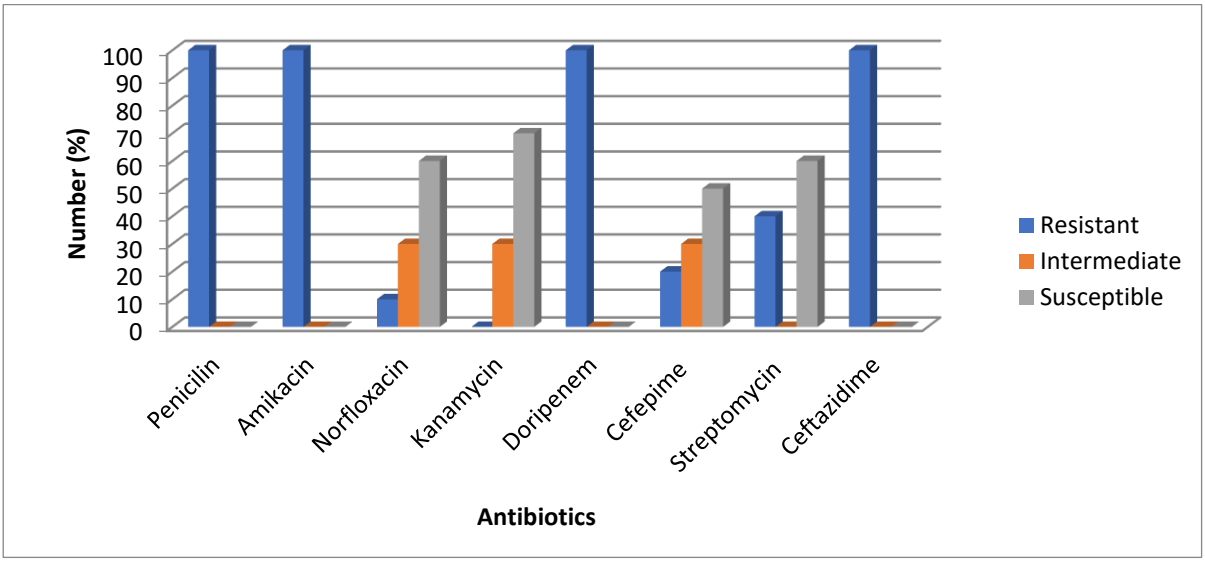

Figure 2: Antibiotic resistance pattern of *E. coli* isolates from wastewater

Table 10: Multiple Antibiotics Resistance Pattern and Index Profile of *E. coli* isolates from cowshed soil samples

Isolates	**Antibiotics Resistance Pattern**	**Multiple Antibiotics Resistance Index (MARI)**
SCE 2	P-DOR-NOR-CAZ-S	0.625
SCE 3	P-DOR-CAZ-S-FEP	0.625
SCE 6	P-DOR-CAZ-FEP	0.500
SCE 7	P-DOR - CAZ-K-FEP	0.625
SCE 9	P-DOR-CAZ-K-FEP	0.625
SCE 10	P-DOR-FEP	0.375
SCE 12	P-DOR-CAZ-FEP-	0.500
SCE 14	P-DOR-CAZ	0.375
SCE 15	P-DOR-CAZ-K-S-FEP	0.750

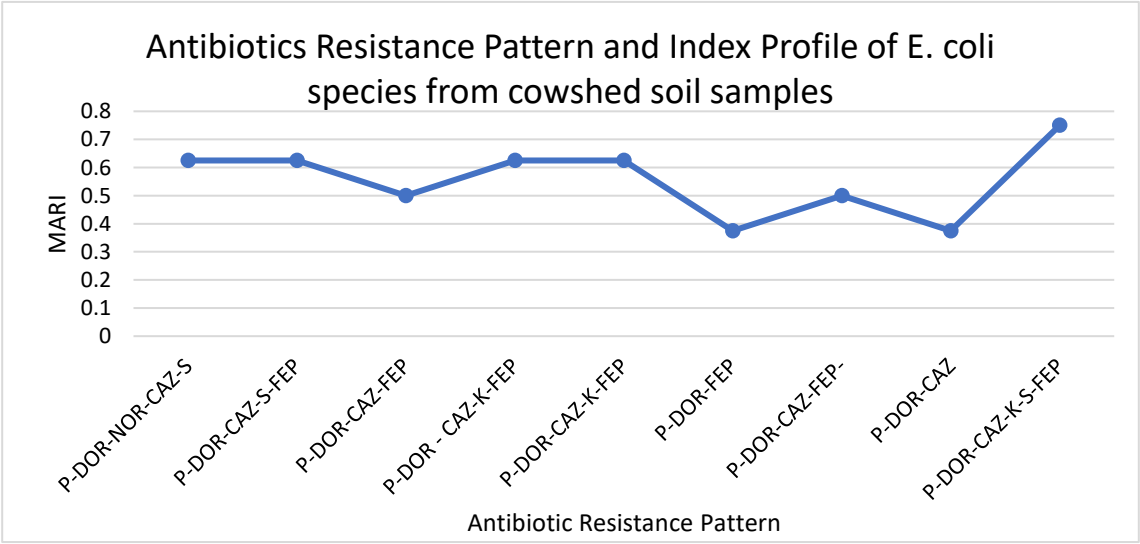

Figure 3: Multiple Antibiotic Resistance Index of *E. coli* Species from Cowshed

3.5 Multiple Antibiotics Resistance Pattern and Index Profile of *E. coli* Isolated from Cowshed waste water, Owo.

A MAR index higher than 0.2 indicated high-risk exposure to antibiotics. In this study, the MAR index ranged between 0.5 and 0.875. All the isolates showed MAR index of higher than 0.20 threshold (Isolate with the highest MARI are CSWOO1, CSWOO3, CSWaa1, CSWbb2, CSWcc1 while those with the lowest MARI are CSWOO2, CSWaa2, CSWaa3, CSWbb2, CSWcc2). Table 11 below show the MAR index of *E. coli*isolated from Cowshed waste water.

Table 11: Multiple Antibiotics Resistance Pattern and Index Profile of *E. coli* Cowshed waste water

Isolates Codes	**Antibiotics Resistance Pattern**	**Multiple Antibiotics Resistance Index (MARI)**
SW 001	P-AMK- DOR-S-CEZ	0.625
SW 002	P-AMK- DOR-CEZ	0.500
SW 003	P-AMK- NOR -DOR-CEF-S-CEZ	0.875
SW aa1	P-AMK -DOR-CEF-S-CEZ	0.750
SW aa2	P-AMK -DOR-CEZ	0.500
SW aa3	P-AMK -DOR-CEZ	0.500
SW bb1	P-AMK -DOR-CEZ	0.500
SW bb2	P-AMK -DOR-S-CEZ	0.625
SW cc1	P-AMK -DOR-S-CEZ	0.625
SW cc2	P-AMK -DOR-CEZ	0.500

*****P-Penicilin, AMK-Amikacin, NOR- Norfloxacin, DOR-Doripenem, CEF-Cefepime,S-Streptomycin, CEZ-Ceftazidime,

4.0 Discussion

The prevalence of *Escherichia coli* (*E. coli*) isolates from cowshed soil and wastewater samples observed in this study aligns with the findings of Mohamed and Habib, 2023 and Mohammed et al. (2016) who both reported that *E. coli*, although primarily an enteric bacterium in animals, can survive in the environment, including dairy products and fecal-contaminated materials. *E. coli* is one of the most significant foodborne pathogens, commonly found in dairy cattle and capable of causing serious infections in both animals and humans (Halimi *et al.,* 2025).

Our findings revealed a high concentration of *E. coli* in both cowshed wastewater and soil samples collected from Owo metropolis. Given the elevated counts observed, the release of such wastewater into the environment could pose serious public health risks. Diseases resulting from contact with contaminated water include gastrointestinal illnesses, as well as skin, ear, respiratory, eye, neurologic, and wound infections (CDC, 2015). Common symptoms reported include stomach cramps, diarrhea, nausea, vomiting, and low-grade fever.

Gram staining of the isolates confirmed that all *E. coli* strains were Gram-negative, oxidase-negative, and indole-positive, which is consistent with the results of Mohammed *et al.* (2016). Presumptive *E. coli* isolates identified through biochemical tests were further subjected to molecular confirmation using PCR targeting the *uidA* gene,

which encodes β-glucuronidase, a marker specific to *E. coli*. Of the isolates tested, 70% were positive for *uidA*, affirming their identity. This supports the assertion that PCR offers higher specificity than culture-based methods, a finding similarly reported by Moghaddam *et al.* (2023). The molecular confirmation underscores the potential danger posed by stagnant wastewater, as it may serve as a medium for the transmission of resistant pathogens to humans and surface water bodies.

Antibiotic susceptibility testing revealed that all *E. coli* isolates were resistant to penicillin and doripenem (100%), while most showed susceptibility to norfloxacin. A high level of resistance (89%) was also observed against ceftazidime. This is not unexpected, as these antibiotics are commonly used in animal treatment and may be transmitted to humans or the environment through manure application on agricultural fields (Herrera *et al.,* 2021; Wichmann *et al.,* 2023). The emergence of antimicrobial resistance (AMR) in food-producing animals is a growing concern due to the zoonotic nature of such bacteria, which can enter the human population through the food chain (Anes *et al.,* 2020).

The indiscriminate use of antibiotics in recent years has significantly contributed to the rise in antibiotic-resistant bacteria, with developing countries like Nigeria being disproportionately affected (Neupane *et al.,* 2016). In this study, *E. coli* isolates exhibited diverse resistance patterns, suggesting the potential for horizontal gene transfer to other pathogenic organisms within the stagnant water. The increasing resistance trend presents a looming public health threat, especially for residents in Owo.

Further antibiotic resistance profiling of *E. coli* from cowshed soil samples showed 100% resistance to penicillin, doripenem, and amikacin. This could be attributed to the specific antimicrobials tested, many of which are primarily used for human infections, unlike other studies that focused on veterinary antibiotics. The variety of resistance patterns observed, and the number of antimicrobials to which isolates were resistant, raise concerns about possible treatment failures even with multidrug therapy (Tanwar et al., 2020).

Consistent with Moghaddam *et al.* (2015) study of multidrug resistant *E. coli* isolates from north of Iran, this study found that *E. coli* isolates displayed variable antibiotic resistance patterns. Notably, 100% resistance was recorded against penicillin, amikacin, doripenem, and ceftazidime; 42.9% against streptomycin; and 14.3% against cefepime. The total resistance to penicillin aligns with Breijyeh *et al.* (2020), who noted that Gram-negative bacteria, including *E. coli*, are more resistant to penicillin than Gram-positive organisms. In contrast, a study by Zumaya-Estrada *et al.* (2017) in Mexico found that 73% of *E. coli* isolates from diarrheal patients were resistant to ampicillin. Ampicillin, ceftazidime, cefotaxime, tetracycline, chloramphenicol, gentamycin, ciprofloxacin, and azithromycin have been widely documented for use in hospital treatments. It is believed that isolates from cowshed wastewater and soil may have higher resistance potential compared to isolates from other sources.

Supporting our findings, Sabir *et al.* (2014) reported high levels of resistance in *E. coli* isolates in Pakistan—cefotaxime (89.7%), ceftazidime (73.8%), gentamicin (59.8%), ciprofloxacin (54.2%), penicillin (97.3%), streptomycin (30%), and kanamycin (98%). Similarly, Mandal *et al.,* (2022) found that *E. coli* from hospital wastewater was resistant to more than three antibiotic classes, a trend also evident in our study.

All tested isolates in this study exhibited multiple antibiotic resistance (MAR), showing resistance to two or more antibiotics. This observation aligns with reports by Hameli *et al.* (2017) and Sayah *et al.* (2017). The rise in multidrug resistance poses a serious challenge to the treatment of infectious diseases as it limits therapeutic options and prolongs infectious periods, thereby increasing the potential for resistant bacteria to spread within communities.

5.0 Conclusion

The findings of this study highlight the significant public health risks posed by the presence of antimicrobial-resistant *E. coli* in cowshed soil and wastewater within the Owo metropolis. As cattle are a major source of meat and surrounding water bodies are widely used for domestic purposes, the contamination of these environmental matrices raises serious concerns. The resistance of *E. coli* isolates to clinically important, first-line antibiotics underscores the potential for treatment failure and the spread of resistant pathogens in the community. These results emphasize the need for stricter regulation of antimicrobial use in food-producing animals, alongside the

implementation of effective waste and wastewater treatment systems to limit the release of antibiotic residues and resistant bacteria into the environment. Continuous environmental surveillance and targeted risk assessment strategies are essential to protect public health and preserve environmental integrity.

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