Research Article

Antibiotic Sensitivity Pattern of *Escherichia coli* Isolates from Poultry Litter and Soil

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ABSTRACT

Antibiotic usage in poultry farms is causing a rise in concerns since it acts as a breeding ground for antimicrobial resistance in infectious bacteria. The antibiotic resistance profiles of *Escherichia coli* (*E. coli*) in poultry litter and the soil around the farm that generates this litter were examined in this study. *E. coli* was isolated and identified using a biochemical assay on Eosin Methylene blue agar. The disc diffusion method was used to assess antibiotic susceptibility against 12 antibiotics, and the multiple antibiotic resistance indexes were established. Resistance to cefotaxime, imipenem, gentamycin, and levofloxacin was found in 50% of the 36 *E. coli* isolates obtained from all samples. Of the 76% that demonstrated resistance to at least three medications, 100% were multidrug resistant (MDR). Cefotaxime (100%), imipenem, augmentin, and cefuroxime (94% each) had the highest proportion of resistance. The lowest resistance rates were 39%, 17%, 14%, and 8% for nalidixic acid, ceftriaxone, nitrofurantoin, and ofloxacin, respectively. The majority of the 100% (36/36) MDR (72%) were isolated from chicken litter, while the least (28%) were isolated from soil samples. The poultry litter had a higher mean MAR index than the soil. Although there was no statistically significant difference, comparison of resistance pattern in *E. coli* isolates from surrounding soil of the poultry farm to profiles of *E. coli* produced from poultry litter on the farm showed that they were substantially comparable. This indicates that poultry farms could serve as a source of antimicrobial-resistant *E. coli* strains.

 Keywords: Poultry, antibiotics, *E. coli,* multidrug resistance, soil, chicken litter

Introduction

Antibiotic resistance is a developing issue that concerns contemporary healthcare across the world. Resistance has historically been seen as a clinical issue, but non-clinical contexts have lately been identified as a major component in the spread of antibiotic resistance genes (ARGs) (Berglund 2015). *Escherichia coli (E. coli),* a prevalent microbiological contamination of retailed chicken products and a prominent flora of the digestive tract of poultry (Agyare *et al*., 2019). *Escherichia coli* is a Gram-negative organism that has long been recognized to readily and often share genetic material with other bacterial species through horizontal gene transfer.

The use of antibiotics in poultry and livestock production benefits farmers and the economy because it improves poultry output efficiently and commercially. However, the likely propagation of antibiotic resistant strains of infectious and not transmissible, organisms into the environment and their subsequent transfer to humans via the food chain could

have grave implications for public health (Apata, 2009). Furthermore, this might lead to a selection advantage and an increase in the quantity of resistant bacteria in animals, their waste, and the environment around them (Heuer and Smalla, 2007). In chicken waste (Adelowo *et al*., 2009; Furtula *et al*., 2010), poultry grown in commercial settings (Nilsson *et al*., 2009), and poultry products (Fortini *et al*., 2011), antibiotic resistant bacteria have been found. The majority of food animal producing farms in Nigeria do not have a waste treatment facility. Waste from these farms is either deposited in piles on farmlands or in secluded locales, typically inside or near bodies of water. Waste is sometimes utilized as organic fertilizer or feed additions in fish ponds. This raises the possibility that people living near the farm and/or animal waste disposal will be exposed to antibiotic-resistant bacteria in the waste. Furthermore, a strong relationship between animals and the human environment is widespread in Nigeria, with livestock housed near or inside human houses.

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This practice has been linked to high rates of zoonotic *E. coli* transmission between cattle and people in rural Uganda (Rwego *et al*., 2008).

Antimicrobials utilized for human and animal therapeutics enter the natural world via manure, wastewater, agriculture, and other means. To estimate the occurrence and range of ARBs and ARGs in environmental strains, numerous studies have been carried out. The findings show unequivocally that the natural environment is the oldest and most extensive storage for prospective antibiotic resistance genes and that soil bacteria serve as a reservoir of resistance-determining factors that can be mobilized and transferred to pathogenic bacteria (Allen *et al*., 2010; D'Costa *et al*., 2011).

Despite the fact that soil is well known in nature as a reservoir of a variety of microorganisms, one of the methods by which soils get infested with infectious and resistant strains of certain microbes is through the use of animal manure as fertilizer in agricultural operations (Fatoba *et al*., 2022). The purpose of this research is to determine the variety of antibiotic resistance in soil and chicken litter, with the goals of identifying resistance patterns, the MAR index, and MDR in chicken litter and soil from the farm's surrounding region.

Materials and Methods

A total of 100 samples were collected, 50 of which were poultry litter samples and 50 of which were surrounding soil samples around the poultry farm.

Bacteria culture and identification

Each poultry litter and soil sample were inoculated onto separate sterile EMB agar plates after basic sample preparation techniques. These plates were incubated at 37°C aerobically and tested for bacterial growth for 24 hours. The organisms were identified using normal laboratory procedures (Cheesbrough, 2006). All suspected *E. coli* isolates were subjected to conventional bacteriological identification procedures and validated using biochemical testing. Positive *E. coli* isolates had a green metallic sheen on EMB agar (Cheesbrough, 2006).

Antibiotic susceptibility test

The disc diffusion method was used to test antibiotic susceptibility with 12 antibiotic agents (ceftriaxone, cefotaxime, amoxicillin/clavulanic acid, cefuroxime, cefixime, gentamicin, nalidixic acid, ofloxacin,

nitrofurantoin, ampiclox, levofloxacin, and imipenem (Oxoid, Cambridge, UK). For each isolated *E. coli* strain, 12 antibiotic discs were put on each plate and incubated at 37 °C for 24 hours. The inhibitory zone diameter throughout the disc was measured with a caliper and interpreted according to Clinical and Laboratory Standard Institute (CLSI) criteria (CLSI, 2020). Susceptible (S), intermediate (I), or resistant (R) values were obtained. MDR isolates were identified as those with resistance to more than three types of antimicrobials examined (Magiorakos *et al*., 2012).

Multiple antibiotic resistance index (MAR)

The multiple antibiotic resistance (MAR) index was calculated for each isolate using the formula MAR = a/b, where a represents the number of antibiotics to which the test isolate was resistant and b represents the total number of antibiotics to which the test isolate was tested for susceptibility (Cookey and Otokunefor, 2016).

Statistical analysis

The data on *E. coli* counts were log-transformed and analyzed using Microsoft Excel 2016.

Results

Bacterial Identification

A total of 36 *E. coli* isolates were isolated from diverse samples of chicken litter and soil, with the bulk (26%) coming from fowl, followed by soil (10%), and other non-*E. coli* bacteria (64%).

Antibiotic susceptibility profile

Antibiotic susceptibility profiles of *E. coli* bacteria from soil and poultry litter were classified as "Resistant," "Intermediate," and "Susceptible" (Figures 1 and 2). The test of all strains against 12 different antibiotics using Kirby-Bauer disc diffusion was standardized and evaluated using National Committee for Clinical Laboratory Standards procedures. The percentile distribution was obtained for the prevalence of resistance of isolates to different classes of antibiotics, including penicillin (amoxicillin clavulanate and ampicillin), aminoglycosides (gentamycin), fluoroquinolones (ofloxacin, levofloxacin, and nalidixic acid), nitrofuran (nitrofurantoin), extended spectrum cephalosporins (cefuroxime, cefotaxime, cefixime, and ceftriaxone),

and carbapenem (imipenem) (Table 1). The study compared *E. coli* resistance patterns in soil and poultry litter (Table 1). The MAR indices of *E. coli* isolates are shown in Table 2. A comparison of *E. coli* MAR indices from both sources. All of the isolates tested positive for MDR for at least three of the antibiotic classes utilized (Figure 3). Susceptibility analysis of both groups of isolates indicated a total of 24 distinct resistance profiles (Table 3). Of these 24 antibiograms,

7 were unique to soil isolates and 16 to poultry litter isolates, whereas there was one antibiogram shared by both groups of isolates. No isolate was totally susceptible to any of the 12 medications, and none was completely resistant to any of them. The antibiogram data revealed that both groups of isolates had a comparable amount of variety, with poultry litter isolates having a greater level of diversity.
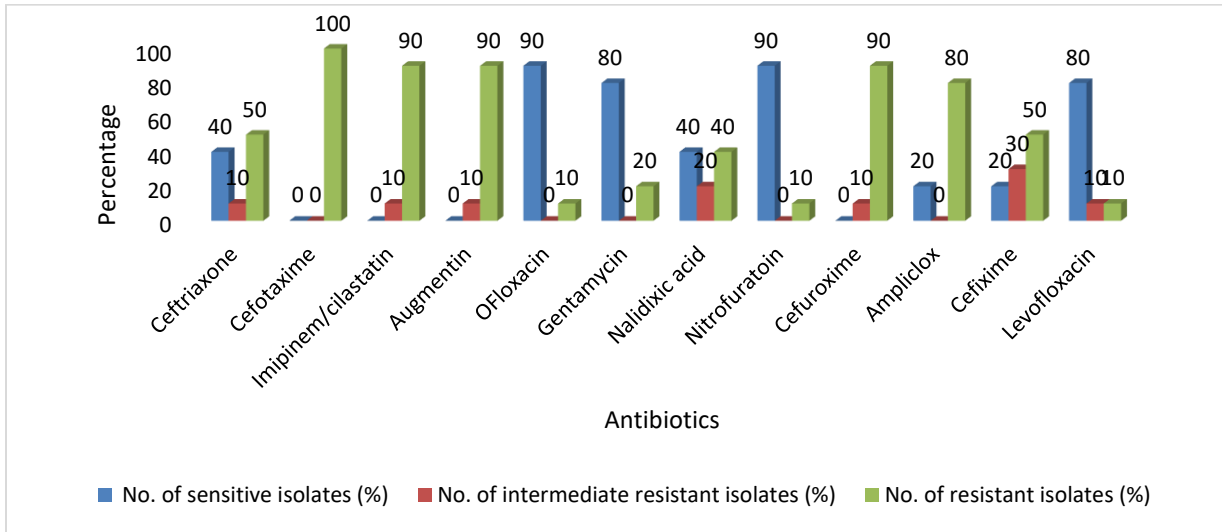

Fig. 1: Sensitivity pattern to different antibiotics of *E. coli* isolated from poultry farm surrounding soil
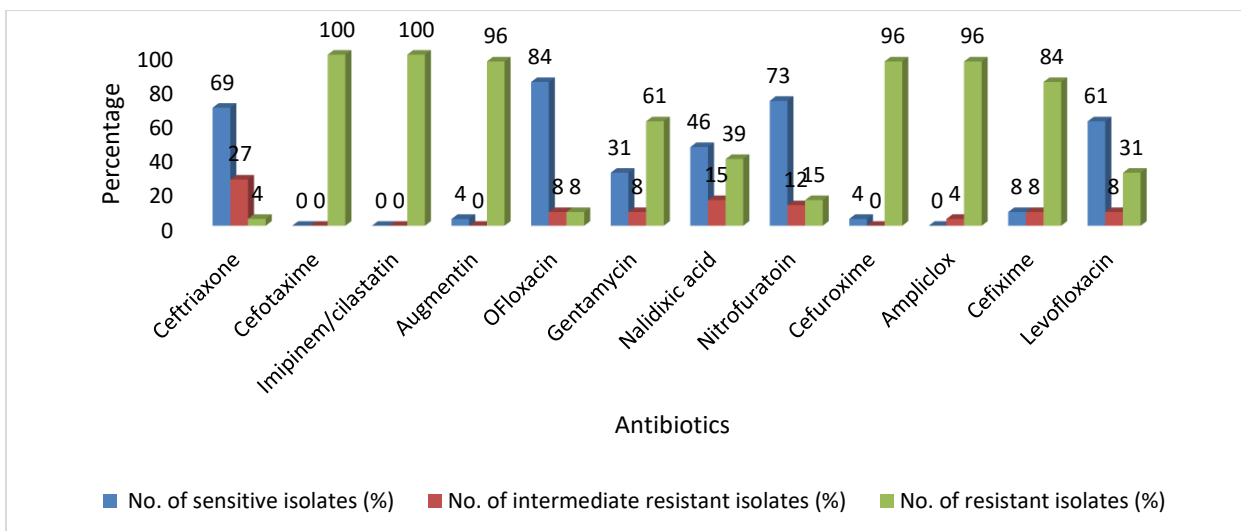

Fig. 2: Sensitivity pattern to different antibiotics of *E. coli* isolated from poultry litter

Table 1: Frequency of *E. coli* soil and poultry resistant to the antibiotics used

| Class of antibiotics	R (%) in soil	R (%) in poultry	R (%) in general
Cephalosporins			
Ceftriaxone	5(50%)	1(4%)	6(17%)
Cefotaxime	10(100%)	26(100%)	36(100)
Cefuroxime	9(90%)	25(96%)	34(94%)
Cefixime	5(50%)	22(84%)	27(75%)
Penicillin			
Ampiclox	8(80%)	25(96%)	33(92%)
Augmentin	9(90%)	25(96%)	34(94%)
Carbapenem			
Imipenem/cilastatin	9(90%)	26(100%)	35(97%)
Aminoglycoside			
Gentamycin	2(20%)	16(61%)	18(50%)
Fluoroquinolones			
Levofloxacin	10(10%)	8(31%)	18(50%)
Nalidixic acid	4(40%)	10(39%)	14(39%)
Ofloxacin	1(10%)	2(8%)	3(8%)
Nitrofuran			
Nitrofurantoin	1(10%)	4(15%)	5(14%)

Key: R- resistance
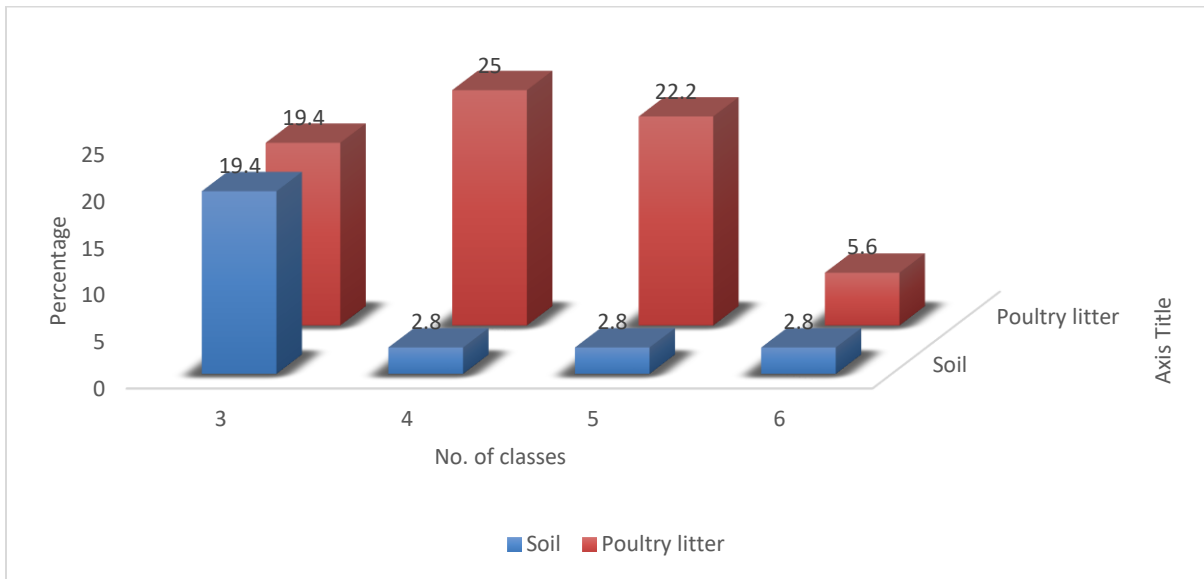
Fig. 3: Distribution of multidrug resistance classes across the two samples

Key:3(Cephalosporins-Penicillin-Carbapenem); **4** (Cephalosporins-Penicillin-Carbapenem Aminoglycoside/Fluoroquinolone/Nitrofurantoin); **5** (Cephalosporins-Penicillin-Carbapenem-Fluoroquinolones-Aminoglycoside); **6** (Cephalosporins-Penicillin-Carbapenem- Fluoroquinolones-Aminoglycoside- Nitrofurantoin)

Table 2: MAR index of *E. coli* from Soil and Poultry Litter in general

MAR index	Soil (%)	Poultry litter (%)	Prevalence (%)
0.2	10	0	2.8
0.3	10	3.8	2.8
0.4	20	7.7	11.1
0.5	30	19.2	22.2
0.6	20	23.1	22.2
0.7	70	27	19.4
0.8	20	15.4	16.7
0.9	0	3.8	0
1	0	0	2.8

Table 3: Antibiogram of antibiotic resistance in soil and poultry (n= 37)

	Antibiotic resistance pattern	Soil	Poultry	Total (%)
1	CTX-ACX-IMP	1	0	1(2.7%)
2	CTX-CXM-ACX-IMP	0	1	1(2.7%)
3	CTX-CXM-AUG-ACX-IMP	2	1	3 (8.3%)
4	CTX-CXM-ZEM-AUG-IMP	0	1	1(2.7%)
5	CTX-CXM-ZEM-AUG-ACX-IMP	0	4	4 (11.1%)
6	CRO-CTX-CXM-AUG-ACX-IMP	1	0	1(2.7%)
7	CRO-CTX-CXM-ZEM-AUG-IMP	2	0	2 (5.6%)
8	CRO-CTX-CXM-ZEM-AUG-ACX-IMP	1	0	1(2.7%)
9	CTX-ZEM-AUG-ACX-IMP-GN	0	1	1(2.7%)
10	CTX-CXM-ZEM-AUG-ACX-IMP-GN	0	4	4 (11.1%)
11	CRO-CTX-CXM-ZEM-AUG-ACX-IMP-GN	0	1	1(2.7%)
12	CTX-CXM-AUG-ACX-IMP-NA-LBC	1	0	1(2.7%)
13	CTX-CXM-ZEM-AUG-ACX-IMP-NA-LBC	0	2	2 (5.6%)
14	CTX-CXM-ZEM-AUG-ACX-IMP-NF	0	1	1(2.7%)
15	CTX-CXM-AUG-ACX-IMP-LBC-GN	0	1	1(2.7%)
16	CTX-CXM-AUG-ACX-IMP-NA-LBC-GN	0	1	1(2.7%)
17	CTX-CXM-ZEM-AUG-ACX-IMP-NA-GN	0	2	2 (5.6%)
18	CTX-CXM-ZEM-AUG-ACX-IMP-OFX-NA-GN	0	1	1(2.7%)
19	CTX-CXM-ZEM-AUG-ACX-IMP-NA-LBC-GN	0	2	2 (5.6%)
20	CRO-CTX-CXM-ZEM-AUG-ACX-IMP-NA-GN	1	0	1(2.7%)
21	CTX-CXM-ZEM-AUG-ACX-IMP-GN-NF	0	1	1(2.7%)
22	CTX-CXM-ZEM-AUG-ACX-OFX-NA-GN-NF	1	0	1(2.7%)
23	CTX-CXM-ZEM-AUG-ACX-IMP-NA-LBC-GN-NF	0	1	1(2.7%)
24	CTX-CXM-ZEM-AUG-ACX-IMP-OFX-NA-LBC-GN-NF	0	1	1(2.7%)

CRO-Ceftriaxone sulbactam; **CTX-**Cefotaxime; **IMP-**Imipenem/cilastatin; **AUG-**Augmentin; **OFX-O**floxacin; **GN-**Gentamycin; **NA-**Nalidixic acid; **NF-**Nitrofurantoin; **CXM-**Cefuroxime; **ACX-**Ampiclox; **ZEM-**Cefixime; **LBC-**Levofloxacin.

Discussion

As the epidemic of antimicrobial drug resistance spreads over the world, creating an ever-increasing global health hazard, increasing amounts of research are being directed towards minimizing the growth of drug resistant microorganisms and reversing this disastrous trend. Among the techniques used to identify potential sources of antibacterial antibiotic resistance and examine their potential consequences for humans. The chicken environment is one such setting that has the potential to operate as a repository of antimicrobial drug resistance due to the use of significant doses of antibiotics as growth boosters, prophylactics, and treatments. With the poultry environment producing up to 6.69 kg of chicken litter a day (Ogundiran *et al.*, 2015), *Escherichia coli* is the leading indicator bacterium for fecal contamination, and its presence in poultry is concerning. It does, however, indicate a serious concern regarding utilizing poultry litter as natural fertilizer and alludes to one of the likely pathways of antibiotic-resistant bacterium transfer to people via contaminants in plants. Vegetables and fruits have been linked to the majority of interstate outbreaks of foodborne illnesses in the United States (Crowe *et al.*, 2015), and organic compost has been identified as a likely source of contamination for these commodities (Atidegla *et al.*, 2016).

The results of this study show that *E. coli* bacteria had significant resistance rates to routinely used antibiotics such as cefotaxime (100%), imipenem, augmentin, and cefuroxime (94% each). Resistance to ampiclox (92%) and cefixime (75%), respectively, was likewise strong. as well as moderate resistance to gentamycin (50%) and levofloxacin (50%), with relatively lower resistance rates to nalidixic acid (39%), ceftriaxone (17%), nitrofurantoin (14%), and ofloxacin (8%); these findings are consistent with those from neighboring countries, such as Kenya (Stanley *et al.*, 2018). It is possible that the high resistance to imipenem, a carbapenem that is frequently considered a "last-resort" treatment, is generated by the activity of the AmpC enzyme, which is also responsible for resistance to cephalosporins and penicillin in this research.

Cefotaxime resistance was highest in *E. coli,* followed by imipenem, augmentin, cefuroxime, and ampiclox resistance, all of which ranged from 80–100% in soil samples, and very moderate resistance to ceftriaxone and cefixime, all of which were 50%. Isolates from chicken litter also had the greatest resistance to cefotaxime (100%), imipenem (100%), augmentin, cefuroxime, and ampiclox (96%), and cefixime (84%). This shows that the resistance mechanisms in chicken litter isolates and soil are comparable and that there is a possibility of mobile genetic transmission of these genes responsible for antibiotic resistance from the poultry farm to the surrounding environment. This process can be initiated by waste water runoff, manure, or by farmers and locals that visit the farm. A considerable resistance occurred with the cephalosporins of this study, similar to that in *E. coli* from poultry litter in the study by Cookey and Otokunefor (2016), who reported resistance to ampiclox (95%), penicillin (100%). Adelowo *et al.* (2014) discovered ampiclox resistance (36% in poultry and 22% in soil).

However, soil isolates were susceptible to nitrofurantoin (90%), ofloxacin (90%), and levofloxacin (80%); isolates from poultry litter were susceptible to ofloxacin (84%), nitrofurantoin (84%), ceftriaxone (69%), and levofloxacin (61%). Because fluoroquinolones and nitrofuran are rarely used in chicken production, these isolates are unlikely to have been exposed to them. Fatoba *et al.* (2021) showed comparable susceptibility patterns to fluoroquinolones and nitrofuran.

This study also discovered that *E. coli* had the highest multidrug resistance, including resistance to all six antibiotic classes, at a rate of 2.8% (1) in soil and 5.6% (2) in poultry litter. All of the isolates shared resistance to three antibiotic classes: cephalosporin, penicillin, and carbapenem. The MAR index is an accurate, trustworthy, and economically viable tool for identifying the origins of microbes that are resistant to antibiotics (Sandhu *et al.*, 2016). The isolates from poultry litter had the highest MAR index, and 1 isolate (2.8%) was resistant to 11 antibiotics. The MAR index of 0.8 was found in soil isolates, which varies from another study that found an index of 0.6 (Fatoba *et al.*, 2021).

With MAR indices larger than 0.2 in both sources of the isolates, there is a substantial potential for infiltration from poultry into the soil due to the widespread implementation of antibiotics in chicken as boosters of growth or for medicinal uses (Davis and Brown, 2016; Oliveira, 2012). The average MAR index

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of 0.9 found in the chicken litter isolates in this investigation attests to the poultry farm's heavy use of antibiotics. Furthermore, the relatively large percentage (90%) of isolates with a MAR index >0.2 in soil indicates cross-contamination with *E. coli* via sources that utilize a lot of antibiotics. Antibiotic resistance in soil can be caused by the discharge of animal and human microorganisms from anthropogenic point sources such as sewage and animal husbandry, along with selection pressure in medicine and agriculturally developed antibiotic substances (Lebreton *et al*., 2017). The similarity in resistance patterns in both sources supports this assertion in our investigation. The consistently higher levels of resistance found in isolates from poultry litter as opposed to soil isolates may suggest that poultry serves not only as a reservoir for bacteria that are resistant to antibiotics but also as the ideal setting for the emergence of such resistance.

In conclusion, this investigation has highlighted the relationship between antibiotic resistance and *E. coli* isolated from poultry litter and soil at a farm in Choba, Port Harcourt, Rivers State. Given the ability of mobile genetic components to transmit resistance genes from one creature to another, these results, notably in cephalosporins, carbapenems, and penicillin, might have serious public health ramifications. An examination of the relatedness of these isolates would be great to identify if these resistance characteristics are spreading continuously. In this example, antibiogram typing has been found to be ineffective, most likely due to the link between drug resistance patterns in both sources.

Acknowledgement

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