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Antibiotic susceptibility profile of Escherichia coli isolated from poultry settings

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Abstract

Poultry production contributes significantly to environmental antibiotic resistance, particularly through manure-amended soils. This study evaluated the antibiotic susceptibility of Escherichia coli isolated from soils amended with conventional poultry manure (Site A), local poultry droppings (Site B), and unamended control soils (Site C). Isolates were confirmed via Gram staining and biochemical tests, and then subjected to disc diffusion testing using ten antibiotics, as per the CLSI 2024 guidelines. E. coli from Site A exhibited significantly reduced susceptibility to trimethoprim-sulfamethoxazole (11.5 ± 1.50 mm), streptomycin (14.1 ± 3.08 mm), and sparfloxacin (16.1 ± 2.85 mm) compared to Site C ($p < 0.05$). Site C consistently showed higher inhibition zones across most antibiotics, indicating lower resistance to these antibiotics. While Site B isolates showed intermediate resistance, differences from Site C were not statistically significant. Two-way ANOVA confirmed significant effects of both antibiotic type ($p = 0.0003$) and sample site ($p < 0.0001$) on resistance patterns. These findings underscore the role of poultry farming practices, particularly conventional systems, in promoting soil-based antimicrobial resistance, warranting stricter regulation and improved manure management.

Keywords: Antibiotic Resistance, Escherichia coli, Organic Poultry, Structure-Activity Relationship.

INTRODUCTION

The global expansion of poultry production to meet rising demands for meat and eggs has intensified the use of antibiotics in animal agriculture, particularly within conventional systems where drugs are routinely administered for disease prevention and growth promotion (FAO, 2017; USDA, 2020). These antibiotics are often only partially metabolized and are excreted in droppings, which are frequently used as organic fertilizers (Meng *et al*., 2022). This practice contributes to the introduction of antibiotic residues, antibiotic-resistant bacteria (ARB), and antibiotic resistance genes (ARGs) into soil ecosystems, thereby altering native microbial communities and promoting horizontal gene transfer (HGT) via mobile genetic elements such as plasmids and integrons (Xu *et al*., 2021).

The resulting spread of multidrug-resistant organisms in the environment has been identified as a critical threat to global public health, with projections estimating that up to 10 million AMR-related deaths could occur annually by 2050 if unchecked (Zhang *et al*., 2024). Soils enriched with poultry manure have been shown to harbour resistant pathogens, including *Escherichia coli*, *Salmonella*, and *Campylobacter*, with high levels of resistance to beta-lactams, sulfonamides, and fluoroquinolones (Furlan & Stehling, 2021; Fatoba *et al*., 2022; Muhtar *et al*., 2022). These findings suggest that poultry manure serves as a vector for disseminating clinically significant resistance determinants into the broader environment.

Less attention, however, has been paid to locally bred or "organic" poultry systems, especially in low- and middle-income countries like Nigeria (Maikasuwa *et al*., 2011). These systems generally avoid routine antibiotic use, yet birds are often reared in close proximity to households and fed on kitchen scraps or wastewater, which are sources that may contain non-antibiotic chemical contaminants such as disinfectants, detergents, and personal care products (Muhammad *et al*., 2024). Compounds like triclosan and quaternary ammonium compounds, though not classified as antibiotics, can exert selective pressure through oxidative stress and structural mimicry of antimicrobial agents, thereby inducing cross-resistance via structure-activity relationships (Scaccia *et al.*, 2021; Caioni *et al.*, 2023).

E. coli, a common inhabitant of poultry guts and an established faecal indicator, is particularly suited for tracking environmental AMR due to its ability to rapidly acquire and disseminate ARGs (Abubakar *et al.*, 2023; Beshiru *et al.*, 2024). Studies have reported its prevalence in both clinical and agricultural settings in Nigeria, where multidrug-resistant strains, including ESBL-producing and AmpC β-lactamase-harboring types, are increasingly isolated from soils and water (Igbinosa *et al.*, 2023; Fashola *et al.*, 2024).

In this study, we investigated the antibiotic resistance profiles of *E. coli* isolated from soils amended with droppings from conventionally raised poultry, organically reared local poultry, and unamended control soils. Our objective was to compare the extent to which each manure source contributes to the dissemination of resistance and to explore the potential co-selective role of household-derived non-antibiotic chemicals in shaping resistance outcomes. This work aims to inform environmental AMR surveillance strategies and support sustainable waste management practices in poultry farming systems.

MATERIALS AND METHODS

Soil samples were collected from three distinct settings: poultry farms in the Dutse metropolis, households rearing local (organic homebred) chickens, and control sites at the Federal University Dutse that are free from poultry droppings. In total, 12 samples were obtained, four from each setting. All samples were clearly labeled with their collection site and date, following standard protocols (Cheesbrough, 2006). Group A (A1-A8) represents soils impacted by conventional poultry droppings, Group B (B1-B8) comprises soils from local poultry droppings, and Group C (C1-C8) includes control sites with no poultry droppings. Soil suspensions were prepared by adding 1 g of soil to 9 mL of normal saline and performing serial dilutions up to 10^{-6} as described by Cheesbrough (2006).

Isolation and characterization of *Escherichia coli*

For the isolation of *E. coli*, 0.1 mL aliquots from the 10^{-5} and 10^{-6} dilutions were spread onto MacConkey Agar plates using aseptic techniques. The plates were incubated at 37°C for 24h (Khamari *et al.*, 2021). Presumptive *E. coli* colonies were subcultured on Nutrient Agar to obtain pure cultures and then identified by Gram staining and a series of biochemical tests—including Indole Test, Methyl Red Test, Voges-Proskauer Test, Citrate Utilization Test and Oxidase Test. (Begum *et al.*, 2020; Prayekti *et al.*, 2021).

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing was performed according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2024) using the disc diffusion method on Mueller-Hinton Agar. Pure cultures of *E. coli* were adjusted to the 0.5 McFarland standard in Nutrient Broth. A sterile swab was used to evenly spread the standardized bacterial suspension onto Mueller-Hinton agar plates, which were then allowed to dry for 5 minutes. Antibiotic discs were then placed on the surface using sterile forceps. The antibiotic susceptibility testing was performed using commercially prepared Gram-negative antibiotic discs (Abtek Biologicals Ltd) containing Ofloxacin (OFX), Pefloxacin (PEF), Gentamicin (CN), Amoxicillin-clavulanic acid (AU), Ciprofloxacin (CPX), Trimethoprim-sulfamethoxazole (SXT), Streptomycin (STR), Chloramphenicol (CH), Sparfloxacin (SPX) and Ampicillin (AM). The plates were then inverted and incubated at 37 °C ± 2 °C for 24 hours. Following incubation, the zones of inhibition were measured in millimeters and compared to CLSI 2024 interpretive charts to determine bacterial susceptibility (Igbinosa *et al.*, 2023).

RESULTS

Presumptive *Escherichia coli* colonies isolated from soil samples on MacConkey agar appeared round, pink, flat, and dry—non-mucoid and typical of lactose fermenters. Gram staining confirmed them as Gram-negative rods, occurring singly or in pairs. Biochemical tests (Indole, Methyl Red, Voges-Proskauer, Citrate, and Oxidase) were used for confirmation, and only isolates matching the standard biochemical profile of *E. coli* were retained for antibiotic susceptibility testing. The antibiotic susceptibility testing using the disc diffusion method revealed variations in inhibition zones across the three sites (Table 1).

Isolates from Site A (conventional poultry manure) showed the lowest susceptibility. For instance, the mean inhibition zone for trimethoprim-sulfamethoxazole was 11.5 ± 1.50

mm, indicating a high level of resistance. Sparfloxacin (15.5 ± 2.73 mm) and streptomycin (16.75 ± 2.22 mm) also showed low efficacy. In contrast, ciprofloxacin (23.8 ± 2.48 mm) and ampicillin (22.13 ± 2.42 mm) demonstrated moderate activity.

Site B (local poultry droppings) exhibited intermediate susceptibility patterns. The highest mean inhibition zone was observed for ciprofloxacin (24.25 ± 1.71 mm), followed by ofloxacin (22.25 ± 2.22 mm) and amoxicillin-clavulanic acid (21.38 ± 3.32 mm). Lower susceptibility values were recorded for sparfloxacin (17.29 ± 3.53 mm) and pefloxacin (18.5 ± 2.28 mm), though still higher than those in Site A.

Site C (control) showed the highest overall susceptibility. Amoxicillin-clavulanic acid had the largest mean inhibition zone (23.57 ± 3.33 mm), followed by ciprofloxacin (23.38 ± 2.94 mm) and streptomycin (22.63 ± 3.00 mm). Ofloxacin recorded the lowest mean inhibition zone at this site (19.88 ± 5.13 mm), but still within the susceptible range. Pefloxacin remained consistently ineffective across all sites, with inhibition zones below the CLSI 2024 susceptibility threshold.

Based on CLSI 2024 interpretive standards, isolates from Site A had a higher frequency of intermediate and resistant profiles, particularly against trimethoprim-sulfamethoxazole, sparfloxacin, and streptomycin. Site B displayed mixed profiles, while Site C showed predominantly susceptible isolates, indicating that less selective pressure was present in the absence of poultry manure.

A two-way ANOVA revealed that both antibiotic type and isolate site significantly influenced inhibition zones. The interaction between antibiotics and site was not statistically significant (F(18, 60) = 1.62, p = 0.0828), but accounted for 19.08% of the total variance. The type of antibiotic had a highly significant effect (p = 0.0003), contributing 25.07% of the variance, while the isolate site was also significant (p < 0.0001), explaining 16.67% of the total variance. Post hoc comparisons showed statistically significant differences between Site A and Site C, particularly for sparfloxacin, trimethoprim-sulfamethoxazole, and streptomycin. In each case, inhibition zones were significantly lower at Site A, suggesting a possible influence of conventional poultry

Table 1: Antibiotic Susceptibility Profiles of *E. coli* Isolates

	Mean Zone of Inhibition ±SD									
Site	*OFX*	*SPX*	*PEF*	*CN*	*AUG*	*CPX*	*SXT*	*STR*	*AMX*	*CH*
A	20±2.68	14.25±2.86$_a$	15.67±0.94	16±0	22±1	23.8±2.48	11.5±1.5$_b$	13.71±2.76$_c$	22±2.12	20.29±3.53
B	23±2.53	17.29±3.53	21.25±3.83	17.8±5.19	18±3.56	24.25±1.71	17.8±3.49	19.6±3.61	20±3.82	21.88±2.52
C	22.62±2.87	23.5±1.5$_a$	19.88±5.13	21±3.70	23.57±3.33	22.88±3.41	21.29±3.81$_b$	21.14±3.52$_c$	21.63±4.03	23±3.46

KEY:SD= Standard deviation, $_a$=Indicates statistically significant differences in sparfloxacin susceptibility between Sites A and C, $_b$=Indicates statistically significant differences in trimethoprim-sulfamethoxazole (trimethoprim-sulfamethoxazole) susceptibility between Sites A and C, $_c$=Indicates statistically significant differences in streptomycin susceptibility between Sites A and C.

manure on the development of antibiotic resistance in soil *E. coli* populations.

DISCUSSION

This study demonstrates that manure from conventional poultry farming (Site A), which is likely to contain antibiotic residues, exerts a strong selective pressure on soil *E. coli* populations. This was reflected in consistently lower inhibition zones for several antibiotics, especially streptomycin, trimethoprim-sulfamethoxazole, and sparfloxacin. These findings align with studies by Mecik *et al.* (2023) and Kaviani *et al.* (2023), who reported that antibiotic-enriched manure promotes the dissemination of resistance genes via mobile genetic elements and nutrient-fueled horizontal gene transfer (Manaia *et al.*, 2024). By contrast, the control site (Site C) exhibited broader inhibition zones, suggesting preserved baseline susceptibility in unamended soils.

Ciprofloxacin showed moderate efficacy across all sites, with Site B (local poultry) performing slightly better. The literature on ciprofloxacin resistance remains mixed (Fatoba et al., 2022; Wang et al., 2023; Ainyakou-Sanga et al., 2025), suggesting that environmental and usage-specific factors modulate its resistance profile. Ofloxacin, on the other hand, was consistently effective, especially in Site B. This may possibly reflect the reduced selective pressure or different co-selective stressors, as opined in similar research by Tian et al. (2021) and Merchant et al. (2012).Trimethoprim-sulfamethoxazole showed markedly reduced efficacy at Site A, consistent with reports that sulfonamides persist in soils fertilized with antibiotic-contaminated manure (Zhang *et al.*, 2022). Chloramphenicol remained effective across all sites, particularly in the control, suggesting it is less influenced by manure-driven selection.

Pefloxacin exhibited poor performance across all sites, consistent with widespread resistance reported in manure-amended environments (Ainyakou-Sanga *et al.*, 2025; Emmanuel-Akerele *et al.*, 2021). Gentamicin showed superior activity at the control site, consistent with the findings of Fatoba et al. (2022), which suggest that litter-free soils harbor more susceptible *E. coli* populations.Amoxicillin-clavulanic acid maintained good activity at all sites, especially the control, though this contrasts with previous reports of high resistance (Fatoba *et al.*, 2022). This discrepancy may reflect regional variations in antibiotic usage or resistance mechanisms.Sparfloxacin was notably less effective at Site A, supporting the notion that intensive antibiotic use promotes resistance (Tian *et al.*, 2021). Ampicillin's unexpectedly strong performance at all sites, despite reports of high resistance elsewhere (Ngene *et al.*, 2021; Wang *et al.*, 2023), may reflect sampling limitations or localized patterns of antibiotic use.

Two-way ANOVA revealed significant effects of both site and antibiotic on inhibition zones. Although the site-antibiotic interaction was not statistically significant ($p = 0.0828$), post hoc tests confirmed differences between Site A and Site C for sparfloxacin, streptomycin, and trimethoprim-sulfamethoxazole. These results reinforce that conventional poultry manure significantly contributes to AMR in soils. Interestingly, *E. coli* isolates from Site B also exhibited elevated resistance compared to the control. Although not statistically distinct from Sites A or C, these findings suggest that local poultry droppings, even in the absence of direct antibiotic supplementation, may still promote resistance. This may stem from exposure to non-antibiotic household contaminants (e.g., disinfectants, detergents) that promote cross-resistance through structure-activity relationships (Caioni *et al.*, 2023). Thus, the perceived safety of organic poultry waste warrants reevaluation.

CONCLUSION

In conclusion, this study underscores the significant impact of poultry manure on antibiotic resistance in soil *E. coli* populations. The conventional poultry site, characterized by antibiotic-supplemented feed, exhibited reduced susceptibility to several key antibiotics, highlighting the strong selective pressure exerted by such practices. While the control site maintained higher susceptibility, the concerning observation of elevated resistance in the local poultry environment, even in the absence of direct antibiotic supplementation, suggests that alternative drivers of resistance, such as co-selection, may be at play. These findings underscore the need for a critical reevaluation of poultry litter management in agriculture and call for further research into the specific mechanisms driving resistance in various poultry farming systems to safeguard public health and ensure the long-term efficacy of antibiotics.

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