



Safety of Retailed Poultry: Analysis of Antibiotic Resistance in *Escherichia coli* From Raw Chicken and Poultry Fecal Matter From Selected Farms and Retail Outlets in Accra, Ghana

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ABSTRACT

PURPOSE: To assess the safety of retailed poultry using the prevalence of antibiotic resistance in *Escherichia coli* (*E. coli*), a dominant intestinal microflora.

METHODS: Two medium-scale farms and 8 well-known retail outlets within the La-Nkwantanang Madina municipality in Accra were purposefully selected for sampling from January to March 2020. We randomly sampled raw chicken (n = 25) and poultry fecal matter (n = 50). All samples were immediately transported on ice to the laboratory for analysis within 12 hours after collection. Conventional culture techniques, biochemical tests, and matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF-MS) were used for isolation and identification. The antimicrobial susceptibility of isolated *E. coli* strains (n = 36) was tested using the Kirby Bauer disk diffusion method.

RESULTS: Antimicrobial resistance in *E. coli* ranged from 10.7 % (cefotaxime) to 82.1% (tetracycline) in fecal matter and 0% (gentamicin & cefotaxime) to 62.5% (tetracycline) in chicken. The prevalence of antimicrobial resistant *E. coli* in fecal samples was higher than in chicken for almost all antibiotics tested, except for cefoxitin, cefuroxime, and ceftazidime. Multidrug resistance was 57.1% in *E. coli* from fecal samples compared to 62.5% in chicken.

CONCLUSION: The high level of resistance to *E. coli* in fecal matter is of public health concern because cross-contamination often occurs during slaughter and processing. This calls for close surveillance and strict adherence to Hazard Analysis and Critical Control Point (HACCP) principles in the chicken production chain to prevent the transmission of antimicrobial-resistant *E. coli* strains through the food chain.

KEYWORDS: retailed poultry, food safety, one health, cross contamination, antibiotic use, antimicrobial susceptibility tests

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Introduction

Escherichia coli (*E. coli*), a dominant flora of the intestinal tract of poultry and a common microbial contaminant of retailed poultry products^{1–4} has been implicated in many foodborne infections.^{4–6} Chicken is the most consumed poultry in Ghana with annual national demand of 400 000 metric tonnes (mt) far outstripping production in the country (58 000 mt). The shortfall is complemented by imports worth more than US\$ 300 million (about 180 000 mt).⁷

Antibiotics are widely used in poultry farming for prophylaxis, to treat infection, and enhance growth.^{1,2,8} This exploitation of antibiotics in food animal farming has implications for

public health due to the potential increase in the occurrence of resistant bacterial strains in the food chain over the years. The use of antibiotics in the poultry sector in Ghana to meet the high demand for fresh chicken, is of great concern, as it is often unregulated and without recourse to veterinary services.³ This contributes to the global crisis of antibiotic resistance, which affects not only the health of poultry but also humans and the environment. Antibiotic-resistant bacteria, which can survive and even proliferate in the presence of antibiotics, are one of the biggest threats to global health.^{1,2,8–12} This has been enhanced by the widespread plasmid-mediated resistant gene among bacteria species in the gastrointestinal tract.^{1,4,13} This



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often occurs through the transfer of resistant plasmids between unrelated bacteria, conferring resistance to more antibiotics.^{3,14-17} Drug-resistant *E. coli* found in the gut have become a reservoir of resistant genes in humans and animals. Antibiotic resistant intestinal *E. coli* have become an important etiology for extraintestinal infections that are usually difficult to treat.^{1,2}

Antibiotic resistant bacteria have been detected in poultry waste, poultry products, and poultry environment.^{4,13,18,19} Resistant strains from the intestinal tract of poultry readily contaminate poultry carcasses or eggs and, when consumed, may alter the normal flora of the human intestinal tract. This resistant *E. coli* may be acquired by humans through the consumption of contaminated poultry products.^{4,13,20} When this happens, *E. coli* related infections that are usually easily treatable by antibiotics become difficult to treat. This takes an economic toll on patients.¹³ These patients may become carriers of these resistant bacteria and may contaminate the environment through poor hygiene practice.^{1,18,19} Continuous surveillance in the poultry industry using a one-health approach that determines resistance of bacterial isolates from animals, the environment, and humans to commonly used antibiotics is required to inform both human medical and veterinary practice.

Materials and Methods

Study location

This cross-sectional study was conducted from January 2020 to March 2020. The study was carried out on 2 medium scale farms and 8 popular retail stores in the La-Nkwantanang Madina municipality in the Greater Accra region of Ghana. The municipality covers a total area of 70.887 square kilometers with a population of 137 975.²¹ As an emerging municipality, it hosts several poultry farms close to human settlements making the threat of zoonotic transmission of antibiotic-resistant enterobacteria a huge concern. The farms purposively selected, were the 2 biggest and most patronized in the municipality.

Sample collection

In all 75 samples, including 50 fecal and 25 chicken samples were collected. For the fecal sample collection from the farms, a plastic scoop was used to pick fresh fecal samples into 50 ml sterile Falcon tubes and then covered. For chicken samples, retail stores in the study area were randomly selected and chicken samples were purchased from each. All samples were immediately transported on ice to the Bacteriology Laboratory of the Noguchi Memorial Institute for Medical Research for analysis within 12 hours after collection.

Bacteria culture and identification

For each fecal sample, buffered peptone water (BPW) was added up to the 45 ml mark of the 50 ml Falcon tube and

vortexed. One (1 ml) of the mixture was transferred to 9 ml of freshly prepared tetrathionate broth and incubated at 37°C for 24 hours. After 24 hours, a 10 ul loopful of incubated tetrathionate broth was streaked on MacConkey agar (Oxoid).

For each chicken sample, 25 g was weighed and placed in a sterile empty wide mouth container with screw cap followed by the addition of 9 parts (225 ml) of buffered peptone water (BPW) and incubated at 35°C for 24 hours. A loopful of BPW and sample mixture were streaked on MacConkey agar and incubated at 37°C for 24 hours.

Well isolated pinkish colonies on MacConkey agar, typical of *E. coli* were sub cultured on fresh MacConkey agar plates and identified using the Analytical Profile Index (API 20E, BioMérieux, France) isolate identification according to established protocol. All isolates were further confirmed using Matrix-Assisted Laser Desorption-Ionization Time of Flight Mass Spectrometry (MALDITOF-MS).

Antimicrobial susceptibility test

Antibiotic susceptibility tests were performed on all isolates using the Kirby Bauer disc diffusion method according to Clinical Laboratory Standards Institute (CLSI) guidelines.²² *Escherichia coli* ATCC 25922 was used as a control. Antibiotics tested included Ampicillin (AM, 10 µg), Cefotaxime (CTX, 30 µg), Cefoxitin (FOX, 10 µg), Ceftazidime (CAZ, 30 µg), Cefuroxime (CXM, 30 µg), Chloramphenicol (C, 30 µg), Ciprofloxacin (CIP, 5 µg), Gentamicin (CN, 30 µg), Nalidixic Acid (NA, 30 µg), Co-trimoxazole (SXT, 25 µg), Tetracycline (TET, 30 µg) (Oxoid, UK). The isolates were classified as susceptible, intermediate, and resistant according to the recommendations of the zone diameter interpretative standards of the CLSI guidelines. Intermediate resistant isolates were deemed resistant. Multidrug resistance (MDR) was defined as any isolate showing resistance to at least 2 classes of antibiotics²³

Data management and analysis

All data were entered and cleaned with Microsoft Office excel 2020 and transported to GraphPad Prism V 9.1.2 for analysis. Data were presented as frequencies and percentages and compared using the chi-square test for categorical values. Mann-Whitney U test was used to determine significant differences in the degrees of resistance of *E. coli* isolates between chicken and fecal samples. A *P*-value of less than .05 at a 95% confidence level was considered significant.

Ethical considerations

Ethical clearance was received from the Institutional Review Board of the Noguchi Memorial Institute for Medical Research (NMIMR), University of Ghana (NMIMR-IRB CPN 103/17-18).

Table 1. Resistance patterns of recovered *Escherichia coli* isolates.

ANTIBIOTICS	RESISTANCE	
	FECAL SAMPLES	CHICKEN SAMPLES
	N (%)	N (%)
Gentamicin	11 (39.3)	0 (0.0)
Cefoxitin	10 (35.7)	4 (50.0)
Co-trimoxazole	19 (67.9)	3 (37.5)
Chloramphenicol	13 (46.4)	3 (37.5)
Cefotaxime	3 (10.7)	0 (0.0)
Ciprofloxacin	15 (53.6)	1 (12.5)
Tetracycline	23 (82.1)	5 (62.5)
Ampicillin	22 (78.6)	5 (62.5)
Nalidixic Acid	15 (53.6)	4 (50.0)
Cefuroxime	5 (17.9)	2 (25.0)
Ceftazidime	2 (7.1)	2 (25.0)

Results

Characteristics of farms and retail outlets sampled

Both farms were medium scale raising over 6000 layers and broilers. Day old chicks and feed mills were bought from other farms in the country. The farms produced mainly eggs and dressed broilers purposefully for restaurants and on festive occasions to cooperate bodies. Both farms maintained regular vaccination and drug administration programs. The farmers also sold live birds to consumers and retailers. All outlets sold imported whole chicken meat and chicken parts from Brazil, USA, and Belgium. Four of these were into wholesale and retail of chicken and other frozen meat to individuals and restaurant operators. These outlets had commercial-size freezers on site and received high patronage. The other 4 outlets were however mostly retailers. At the request of the consumer, chicken meat was cut and bagged.

*Antibiotic resistance among *Escherichia coli* isolates*

In all 75 samples, including 50 fecal samples and 25 chicken samples were analysed. *Escherichia coli* was isolated from 28 (56.2%) of the fecal samples and 8 (32%) of the chicken samples.

As shown in Table 1, isolates were fairly susceptible to cefuroxime, ceftazidime, and cefotaxime since the resistance was less than 25%. Resistance to ciprofloxacin, a fluoroquinolone, ranged from 12.5% to 53.6% and cefoxitin was from 35.7% to 50.0%. The isolates showed resistance to broad-spectrum antibiotics, with the highest being tetracycline (62.5%-82.1%) followed by ampicillin (62.5%-78.6%) and chloramphenicol (37.5%-46.4%). The resistance to gentamicin was up to 39.3% and that of nalidixic acid and

Table 2. Multidrug resistance exhibited by *Escherichia coli* isolates.

SAMPLE	NO. OF ISOLATES	MDR (%)
Feces	28	16 (57.1)
Chicken	8	5 (62.5)

co-trimoxazole ranged from 50.0% to 53.6% and 37.5% to 67.9%, respectively. All *E. coli* isolated from chicken samples were susceptible to gentamicin and cefotaxime but fairly susceptible to ceftazidime and cefuroxime with resistance of 7.1% and 17.9%, respectively.

Fecal isolates were highly resistant to tetracycline (82.1%) and ampicillin (78.6%) compared to those of chicken samples with resistance of 62.5% apiece, ($P = .000$). As shown in Table 2, multidrug resistance of 57.1% and 62.5% was recorded among isolates recovered from feces and chicken samples, respectively.

Discussion

Over the years, poultry environment and products have been documented to be carriers of antibiotic resistant bacteria.²⁴⁻²⁷ These resistant bacteria can be transferred to humans through hand contamination or consumption of contaminated products.^{1,13,28} The public health implication of antibiotic resistant bacteria circulating among humans, animals and the environment can never be overemphasized.^{24,27} Persons infected by these resistant bacteria may be at risk of severe illness or death as these bacteria becomes difficult to treat.

A review of studies conducted in Ghana on AMR published between 1975 and 2015, found that, of 60 studies that included laboratory detection of AMR, a whopping 86.7% were in isolates obtained from human samples, with only 5%, 3%, and 2% from environmental, animal and food samples respectively.² Interestingly the review reported that resistance to *E. coli* was the highest at 65% followed by *Klebsiella* and *Pseudomonas*. Clearly there is a paucity of data on AMR in food animals. In our study, the prevalence of *E. coli* in poultry feces and poultry product (chicken) was 56.0% and 32%, respectively. The prevalence of *E. coli* in chicken in this study is comparable to the 33.0% reported in Nepal²⁹ and 31.1% reported in Taif-Saudi Arabia³⁰ but lower than the 14.29% in Nigeria by Aniokette et al.²⁸ In Ghana, Guetaba,⁹ Rasmussen et al,²⁶ and Adzitey et al⁵ reported a much higher prevalence when they examined chicken for contamination and detected a prevalence of 46.98%, 64.29%, and 80.0%, respectively. This variation in prevalence could be attributed to differences in sampling methods and culture techniques. *Escherichia coli* in chicken is of significant public health importance not only due to its role as an indicator for fecal contamination² but also because it has been implicated in many foodborne infections.^{1,5,30} *Escherichia coli* was also detected in 56.0% of the sampled feces, a rate that is lower than 94.5% reported by

Sung et al¹⁹ in Nepal. The persistence and continued proliferation of *E. coli* in poultry feces increases the probability of contaminating eggs and poultry carcasses.⁵

High resistance to broad-spectrum antibiotics, the highest being tetracycline (62.5%–82.1%), ampicillin (62.5%–78.6%), and chloramphenicol (37.5%–46.4%) have been reported in Ghana.^{1,5} Adzitey et al⁵ reported that *E. coli* were highly resistant to tetracycline (73.33%), and ampicillin (71.67%). Agyare et al¹ also detected resistance to tetracycline (81%), ampicillin (36%), and chloramphenicol (22%). However, resistance to gentamicin (8%) and nalidixic acid (25%) and ciprofloxacin (42%) detected by Agyare et al¹ was lower than the 39.3%, 53.6%, and 53.6%, respectively reported in this study.

The detected MDR *E. coli* (57.1%–62.5%) is similar to the 62.6% detected by Johnson et al¹⁴ but lower to the 68.33% reported in Ghana by Adzitey et al.⁵ Varying levels of MDR *E. coli* have been reported.^{14,26,28} This could be attributable to farm specific antibiotic use, national policies on use of antimicrobials in farm animals or choice of antibiotics for susceptibility tests. Excessive use of antibiotics in poultry for growth promotion, treatment and prophylaxis may have contributed to the resistance. In Ghana, studies by Boamah et al² showed that antibiotic classes such as tetracyclines (24.17%), aminoglycosides (17.87%), penicillin (16.51%), and fluoroquinolones (10.55%) were the most widely used antibiotics for both prophylaxis and treatment. The high resistance to cotrimoxazole (Sulphur-Trimethoprim) may be due to the use of zinc and copper-supplemented animal feed for growth promotion and disease prevention.^{1,4,31,32} This supplement also promotes resistance to tetracycline, which was high among isolates. Many studies have shown that tetracycline resistance can persist for a long period after stopping drug use. This underscores the difficulty in controlling antibiotic resistance in meat products.⁴ The high level of *E. coli* resistance in fecal matter is of public health concern because cross-contamination often occurs during slaughter and processing. This calls for close surveillance and strict adherence to Hazard Analysis and Critical Control Points (HACCP) principles in the chicken production chain to prevent the spread of antimicrobial-resistant *E. coli* strains through the food chain. Our study has limitations that should be noted. We collected samples from 2 medium-scale farms and 8 popular retail stores that allowed for the study to be conducted. To generalize our report, data from more retail shops and farms will be needed. Also the study did not consider the detection of resistance genes in bacterial isolates.

Conclusion

In this study, the prevalence of *E. coli* detected in poultry feces and poultry product (chicken) was 56.0% and 32%, respectively. Isolates showed high resistance to tetracycline, ampicillin, cotrimoxazole, and chloramphenicol were fairly susceptible to cefuroxime, ceftazidime, and cefotaxime. This varying degree of resistance shown by *E. coli* supports the assertion that the poultry environment is a potential reservoir for

antibiotic-resistant genes that can spread from the animals to the human population using poultry litter for farming purposes. Future studies should focus on detection of resistance genes in bacterial isolates from chicken, poultry environment, and humans to demonstrate the need for one health consideration in the usage of antibiotics in the poultry industry.

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Author Contributions

GIM and VYA designed the study. GIM, VYA and EKV wrote the protocol and the first draft. PSA and DLB did the sample collection, VYA, PSA and DLV performed the bacteria isolation, identification, and antimicrobial susceptibility tests. GIM, VYA, EKV, PSA and DLB performed the data analysis. GIM, VYA, EKV, SAMJ and KKA reviewed the manuscript. All authors read and approved the final manuscript.

Significance Statement

A review of 60 studies on AMR in Ghana in 2020, reported that the majority (86.7%) were from human samples, with 5%, 3%, and 2% from environmental, animal, and food samples respectively. This study, therefore, addresses the paucity in AMR data from animal and food samples in Ghana. Our results provide evidence of the scale and scope of antimicrobial resistance within the food animal chain. Detection of resistant pathogens in retailed chicken signifies a failure or lack of implementation of Hazard Analysis and Critical Control Point (HACCP) principles in the chicken production chain. Surveillance of antimicrobial use is recommended.

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