

Research Article

Antibiotic Resistance Profile of *E. coli* Isolates from Lettuce, Poultry Manure, Irrigation Water, and Soil in Kumasi, Ghana

Abigail Abena Anokyewaa Appau  and Linda Aurelia Ofori 

Department of Theoretical and Applied Biology, Kwame Nkrumah University of Science Technology, Kumasi, Ghana

Correspondence should be addressed to Linda Aurelia Ofori; laandoh.cos@knust.edu.gh

Received 26 June 2023; Revised 10 January 2024; Accepted 18 January 2024; Published 27 January 2024

Academic Editor: Todd R. Callaway

Copyright © 2024 Abigail Abena Anokyewaa Appau and Linda Aurelia Ofori. This is an open access article distributed under the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original work is properly cited.

Inputs such as irrigation water and poultry manure used in lettuce cultivation have been found to be associated with antibiotic-resistant pathogens. The study assessed the antibiotic resistance profile of *Escherichia coli* isolated from lettuce, poultry manure, irrigation water, and soil in Kumasi. One hundred and fifty-six samples of lettuce, irrigation water, soil, and manure were collected from three farms over a seven-week cultivation period (seedlings to harvest stage). *E. coli* were enumerated using standard methods. 98% of the samples were positive for *E. coli*. Geometric means for lettuce, irrigation water, and soil ranged from 2.0×10^5 to 1.67×10^7 MPN/100 ml while that of manure ranged from 2.0×10^5 to 1.31×10^7 MPN/100 ml. Generally, the microbial load of all parameters on all farms across the weeks was significant and exceeded World Health Organization (WHO) and International Commission on Microbiological Specifications for Foods (ICMSF) standard recommendations for food. Using the Kirby Bauer method, antibiotic sensitivity testing was performed against 225 biochemically confirmed *E. coli* with twelve antibiotics. Relatively high resistance was recorded for some members of the beta-lactam class: meropenem: 94.2%, ampicillin: 91.9%, cefuroxime: 95.1%, ceftriaxone: 94.7%, and cefotaxime: 94.2%. Eighty of the isolates were screened for extended spectrum beta lactamase (ESBL) production using cefotaxime (CTX) and cefotaxime/clavulanic acid (CTX/CLA) discs and three showed positive: one each from poultry manure, irrigation water, and soil. Polymerase chain reaction (PCR) confirmed the presence of *bla*_{CTX-M} gene. The occurrence of antibiotic-resistant *E. coli* in vegetables and their production environment is alarming and poses serious health threats to the general public. The presence of *bla*_{CTX-M} gene in *E. coli* from a vegetable production site recorded for the first time in Ghana requires enforcement by regulatory bodies on the inappropriate use of antibiotics in the country.

1. Introduction

Antimicrobial resistance (AMR) is the potential of a microbe (usually a bacteria) to resist the effects of a medicine that was previously used to treat that microbe. Disease-causing organisms: pathogens naturally develop resistance but the abuse of antimicrobial substances increases the frequency of the emergence of AMR and further spread resistance genes in the microbes. With regards to this, medicines that were previously used to treat infections effectively become less effective or totally ineffective [1].

This results in prolonged infections and increased mortality as well as production losses. An outbreak of a multiresistant strain *E. coli* 0157: H7 in 1991 caused hospitalization of 71 people out of whom 2 died in New York

[2]. Similarly, in Canada, outbreaks of *E. coli* 0157: H7 strain and *Campylobacter jejuni* in drinking water from contaminated underground water sources also resulted in 2750 cases, leaving 7 out of the 65 hospitalized dead [3]. According to the Food and Agriculture Organization of the United Nations [1], about 700 thousand people die each year as a result of antimicrobial resistance and, if the issue of AMR is not solved, this could increase to 10 million in the coming years.

Currently, AMR is a major global threat to both human and animal health and it is influenced by the excessive and inappropriate use of antibiotics [4]. Antibiotics have been used not only in human medicine but also in animal production (especially poultry farming) since the introduction of penicillin in the early twentieth century. Formerly, it was

used for therapeutic purposes (prophylaxis) to treat sick animals and later as feed additives to promote growth at low concentrations [1, 5]. This will promote the development of resistant types of microorganisms by selection pressure [6]. The increasing human population and the high demand for animal products and eggs across the globe will escalate the use of antibiotics.

About 90 percent of these antibiotics may be excreted unmetabolized in water and animal waste and can thereafter spread into the environment. Subsequently, microbes associated with poultry may develop AMR genes through constant exposure to antimicrobial residues and further spread of resistance through transfer of resistance genes to other microorganisms [5].

The frequent use of antibiotics in poultry is likely to favor some pathogenic bacteria in the poultry (chicken) or in soils where antibiotics-contaminated poultry manure is being applied. Studies by Kumar et al. [7] and Van and Blaney [8] recorded some classes of commonly used antibiotics excreted in poultry manure. Consumption of antibiotics-contaminated foods will increase antibiotic resistance, which may further lead to food poisoning or allergies. According to Danilova et al. [9], chicken manure contributes highly to antibiotic-resistant organisms in soil compared to other livestock. There has been an increased use of poultry manure in vegetable production.

An effective and cheap way of disposing waste accumulated in the poultry industry is manure. In Ashanti region and most periurban areas in Ghana, poultry manure is used due to its high levels of nitrogen and other trace elements in its composition. Nevertheless, studies by [10] revealed that poultry manure is also contaminated with pathogenic microorganisms which may or may not confer antimicrobial resistance and residues of antibiotics.

In urban areas such as Accra and Kumasi, most vegetable farmers resort to wastewater for irrigation. The waste water could comprise of urine, feces, wastewater from kitchens, laundries, leaking septic tanks, and industrial waste [11]. Helminth infections, diarrhea, and other foodborne diseases have been associated with wastewater use for irrigation although its disease transmission is unclear [12]. Previously, possible antibiotic residues have also been found in irrigation water used in Kumasi for vegetable farming [13].

According to Amponsah-Doku et al. [14], vegetables grown in urban areas such as lettuce are mostly contaminated with highly pathogenic microorganisms which are multidrug resistant. This poses serious health threats to the consumer and affects food security at large. Attaining information on the profile of antibiotic resistance of the organisms associated will aid public health interventions.

This study aimed at assessing the microbial load of *E. coli* on lettuce, irrigation water, poultry manure, and soil across the lettuce growth period and also assessed the resistance profile of isolates against some selected antibiotics.

2. Materials and Methods

2.1. Study Area and Sample Size. The study involved a longitudinal research design carried out from November 2020 to August 2021. Antibiotic resistance profiles of *E. coli* on three lettuce farms at Kotei-Deduako, Boadi, and KNUST, all located in Kumasi, Ghana, were studied. The study areas have coordinates 6°40'N 1°37'W. Kumasi is one of the largest cities in Ghana with a population of 5,400,000 [15]. The average temperature of the three study sites is about 26°C with an average rainfall of 1314.7 mm and relative humidity (RH) of 73%.

Under favorable conditions, lettuce takes an average of 6 to 8 weeks to mature and be ready for harvest. In this study, the average time for which all lettuce took to mature was 7 weeks. In the first two weeks of the growth process, the lettuce seedlings remain on a seedling bed. During this period, there is no manure application since the manure has high ammonia and can cause the seedlings to die. After the seedling stage, the seedlings are transplanted onto larger beds and spaced out to make room for growth. Manure is applied from the 3rd week upwards to support plant growth. This sampling procedure was employed to study the microbial load of the samples across the lettuce growth period.

Forty-two samples of lettuce, irrigation water, soil, and 30 samples of poultry manure were collected. For the purpose of replication, two samples of lettuce, soil, and water were collected from week 1 to week 7 for all three farms, whereas 2 samples of manure were collected from week 3 to week 7 from all three farms. This is because manure was only applied after the seedlings were transplanted to the main planting beds (week 2).

No ethical clearance was sought for this research because no human or animal participants were used.

2.2. Sample Collection. Prior to sample collection, farmers gave their consent to use their farms for the study. On sample collection day, two samples of lettuce, poultry manure, irrigation water, and soil were collected from all three farms. All farms had an average size of about 80 km × 40 km. Lettuce, manure, and soil samples were picked with sterile gloves and were put into well-labelled sterile ziplock bags. Lettuce samples were selected randomly from the designated bed (the particular batch of lettuce which were being studied over the 7-week period), whereas soil samples (loamy soil) with a depth of 2 cm on the same bed were collected. About 20 grams of poultry manure which was ready to be applied on the beds were also taken for analysis. Water samples were collected with sterile plastic bottles by dipping it some few centimeters (2–5 centimeters) below the water surface to avoid collecting debris on the surface. To prevent contamination, all samples were picked with different gloves. All samples were preserved in their raw state and transported on ice in a cool box within two hours to the laboratory for analysis.

2.3. Isolation and Identification of *E. coli*. 10 g of lettuce, soil, and manure were weighed into well-labelled sterile zip lock bags and 90 ml of peptone water (Oxoid, UK) was added and pulsed using a pulsifier (PUL 100E; Stuart Scientific Co. Ltd., UK) for 30 s. One millimeter (1 ml) of the resultant stock solution was inoculated in 5 ml of MacConkey broth (Oxoid, UK) and incubated for 24 ± 2 hours at 44°C . 1 ml of the overnight positive culture was transferred into test tubes containing 5 ml of Tryptone Soy Broth (Oxoid, UK) and incubated for 24 ± 2 hours at 44°C . A few drops of Kovac's Indole Reagent (LOBA CHEMIE PVT. Ltd., India) were added to each positive tube. All tubes that retained the red color ring following simple agitation were selected, and a loop full streaked on Eosin Methylene Blue Agar (EMBA) (Oxoid, UK) and incubated for 24 ± 2 hours at 37°C . Colonies with a shiny green metallic appearance were confirmed as *E. coli* and stored in Brain Heart Infusion (Oxoid, UK) with 20% glycerol.

2.4. Antibiotic Sensitivity Testing of *E. coli*. The Kirby Bauer disc diffusion method was used with 12 antibiotic discs from Biomark Laboratories (ISO certification: ISO 9001-2015 and ISO 13485-2016) while following the Clinical and Laboratory Standard Institute guidelines ("M100-S11, Performance Standards for Antimicrobial Susceptibility Testing," 2019). The antibiotics tested were AMP: ampicillin-10 μg , TET: tetracycline-10 μg , COT: cotrimoxazole-25 μg , CRX: cefuroxime-30 μg , VAN: vancomycin-30 μg , CHL: chloramphenicol-10 μg , CTR: ceftriaxone-30 μg , CTX: cefotaxime 30 μg , CIP: ciprofloxacin-5 μg , and MEM: meropenem-10 μg . Isolates were thawed from the freezer and streaked on Muller-Hinton agar for incubation at 37°C for 24 hours. Using a sterile swap, 2 to 3 distinct colonies were picked to make a suspension at 0.5 McFarland in normal saline. This was spread on Muller-Hinton agar, and the disc placed on it with the aid of forceps. The samples were incubated at 37°C for 18 to 24 hours. Zone diameters of each of the antibiotic discs were measured, and the results were interpreted with the CLSI breakpoints.

2.5. ESBL Screening. *E. coli* isolates were screened for ESBL using a combination disc test (CDT) with cefotaxime in combination with clavulanic acid and interpreted using CLSI standards recommendations. A 0.5 McFarland standard of the isolates was prepared using fresh pure cultures. Samples were spread on a plate containing Muller-Hinton agar (Oxoid, UK) with the aid of a sterile cotton swab. The antibiotic discs: CTX: cefotaxime (30 μg) and CTX/CLA: cefotaxime/clavulanic acid (10 μg) (all Oxoid, UK), were placed on the plate while ensuring that there were enough spaces between the individual discs to enhance accurate measurements of the zones of inhibition. The plates were incubated at $35 \pm 2^\circ\text{C}$ for 18 to 24 hours.

2.6. DNA Extraction. DNA was extracted using the boiling method. *E. coli* isolates were cultured on blood agar and a single colony grown on nutrient agar (Oxoid, UK). Two

distinct colonies were placed in an Eppendorf tube containing 1 ml of phosphate-buffered saline (PBS) (Sigma Aldrich Inc., USA), vortexed and centrifuged at 8000 rpm for 3 min. The supernatant was discarded after which 100 μL of nuclease-free water was added. The mixture was vortex again and boiled (using a heating block at 95°C) for 5 minutes. It was later centrifuged at 14000 rpm for 3 min. 100 μL of the supernatant, which is the bacteria DNA, was pipetted into a new Eppendorf tube and the pellet was discarded.

2.7. PCR Detection of *bla*_{CTX-M} Gene. The primer used for the detection of the *bla*_{CTX-M} gene was described by Bauernfeind et al. [16]. Sequences for forward and reverse reactions were 5'-GCGATGTGCAGCACCAGTAA-3' and 5'-GGTTGAGGCTGGGTGAAGTA-3', respectively, with an expected band size of 500 to 600 bp. The optimized PCR conditions used included an initial denaturation step at 94°C for 3 min, followed by 35 cycles of denaturation at 94°C for 45 s. Annealing was done at 62°C for 45 s, extension at 72°C for 30 s, and a final extension at 72°C for 10 minutes with 4°C for holding. The amplified products were loaded in a 1.5% agarose gel with a 100 bp molecular weight DNA gene marker as a ladder. Electrophoresis was done at 100 amp for 40 minutes. Ethidium bromide (1 $\mu\text{g}/\text{mL}$) was used to stain the amplified DNA fragments for visualization under UV light illumination. Both positive (CTX-M-positive *E. coli*) and negative (nuclease-free water) controls were employed in carrying out these procedures.

2.8. Data Analysis. The percentages of antibiotic sensitivities were generated using Microsoft Excel whereas the geometric means, ranges, and standard deviation (Tables 1 and 2) were also analyzed with SPSS version 23. ANOVA was also used to generate associations between the weeks and parameters using R code/software.

3. Results

3.1. Microbial Analysis. The geometric means for *E. coli* load on lettuce, manure, soil, and irrigation water for all the farms ranged from 2.00×10^5 to 1.67×10^7 MPN/100 ml (Table 1). On the KNUST farm, soil recorded the highest microbial load of 6.57×10^6 MPN/100 ml, whereas irrigation water recorded the lowest of 3.25×10^6 MPN/100 ml. On the contrary, the highest microbial load was recorded for irrigation water (4.68×10^6 MPN/100 ml) and the lowest (1.83×10^6 MPN/100 ml) for lettuce. Lettuce, however, recorded the highest microbial load at Boadi (5.62×10^6 MPN/100 ml) whereas manure recorded the lowest (2.97×10^6 MPN/100 ml) (Table 1).

Total microbial load of lettuce differed significantly over the weeks. Specifically, weeks 4 and 6 as well as weeks 2 and 7 had similar microbial loads but differed significantly from the microbial load in weeks 1, 3, and 5. However, the lowest microbial contamination was recorded in week 5 (1.24×10^6 MPN/100 ml), and the highest in week 3 (1.06×10^7 MPN/100 ml) (Table 2).

TABLE 1: Bacterial counts, geometric means, and ranges of *E. coli* isolates from lettuce, manure, soil, and irrigation water on all farms ($n = 156$).

Sampling site	Parameter	Geometric mean (MPN/100 ml) (\log_{10} SD)	Range
KNUST	Lettuce	5.90×10^6 (± 0.65)	$2.00 \times 10^5 - 1.67 \times 10^7$
	Manure	3.954×10^6 (± 0.74)	$2.00 \times 10^5 - 1.31 \times 10^7$
	Soil	6.57×10^6 (± 0.69)	$3.00 \times 10^5 - 1.31 \times 10^7$
	Irrigation water	3.25×10^6 (± 0.57)	$3.00 \times 10^5 - 1.24 \times 10^7$
Deduako	Lettuce	1.83×10^6 (± 0.49)	$2.00 \times 10^5 - 5.7 \times 10^6$
	Manure	3.95×10^6 (± 0.71)	$2.00 \times 10^5 - 1.67 \times 10^7$
	Soil	4.15×10^6 (± 0.75)	$2.00 \times 10^5 - 1.31 \times 10^7$
	Irrigation water	4.68×10^6 (± 0.71)	$3.00 \times 10^5 - 1.31 \times 10^7$
Boadi	Lettuce	5.62×10^6 (± 0.56)	$5.55 \times 10^5 - 1.42 \times 10^7$
	Manure	2.97×10^6 (± 0.51)	$2.80 \times 10^5 - 5.7 \times 10^6$
	Soil	4.93×10^6 (± 0.60)	$4.00 \times 10^5 - 1.28 \times 10^7$
	Irrigation water	4.32×10^6 (± 0.69)	$2.80 \times 10^5 - 1.67 \times 10^7$

TABLE 2: Mean microbial load of lettuce per week ($n = 42$).

Week	Mean of microbial load (MPN/100 ml)
1	3.18×10^{5a}
2	4.56×10^{6e}
3	1.06×10^{7c}
4	5.23×10^{6b}
5	1.24×10^{6d}
6	5.35×10^{6b}
7	4.10×10^{6e}

Means in a row with different superscripts are significantly different ($p < 0.05$).

3.2. *Antibiotic Sensitivity.* A total of 225 biochemically confirmed *E. coli* isolates (60: lettuce, 41: manure, 61: water, and 63: soil).

The highest resistance was recorded for cefuroxime (95.1%), cefotaxime (94.7%), ciprofloxacin (91.5%), and meropenem (94.2%) whereas the lowest was recorded for chloramphenicol (89.4%) (Table 3).

3.2.1. *Percentage Resistance of E. coli Isolates from Lettuce, Manure, Soil, and Irrigation Water.* *E. coli* isolated from lettuce leaves showed high resistance to ceftriaxone (95%), cefuroxime (93.3%), and vancomycin (93.1%) (Table 4).

Isolates from manure also showed resistance to cefuroxime (90.3%), vancomycin (87.8%), ceftriaxone (85.4%), meropenem (85.4%), and cefotaxime (85.4%) (Table 5).

Isolates from soil also were resistant to vancomycin (98.4%), cefotaxime (98.4%), chloramphenicol (96.9%), and meropenem (98.4%) (Table 6).

The isolates showed high resistance to all antibiotics, notably cefuroxime, vancomycin, cefotaxime, and meropenem all recorded 98.4% resistance except for chloramphenicol 90.1%. Highest susceptibility was also recorded for chloramphenicol as well 9.9% (Table 7).

TABLE 3: Antimicrobial sensitivity of *E. coli* isolates ($n = 225$).

Antibiotic	% Resistant	% Susceptible
Ampicillin	91.1	8.1
Tetracyclin	90.6	9.4
Cotrimoxazole	90.2	9.8
Cefuroxime	95.1	4.9
Vancomycin	95.1	4.9
Chloramphenicol	89.4	10.6
Ceftriaxone	94.7	5.3
Cefotaxime	94.2	5.8
Ciprofloxacin	91.5	8.5
Meropenem	94.2	5.8

TABLE 4: Antimicrobial sensitivity of *E. coli* isolates from lettuce ($n = 60$).

Antibiotic	% Resistant	% Susceptible
Ampicillin	90	10
Tetracycline	90	10
Cotrimoxazole	88.3	11.7
Cefuroxime	93.3	6.7
Vancomycin	93.3	6.7
Chloramphenicol	86.6	13.4
Ceftriaxone	95	5
Cefotaxime	91.7	8.3
Ciprofloxacin	90	10
Meropenem	91.7	8.3

3.3. *Antibiotic Sensitivity Profiles.* Four percent (4%) of the *E. coli* isolates were susceptible to all 12 antibiotics used, whereas the rest (96%) were resistant to at least 7 antibiotics making them multidrug-resistant pathogens. Twenty-seven resistance patterns were derived from the 225 isolates with AMPTETCOTCRXVANCHLCTRCTXCIPMEM being the most occurring resistant pattern; 212 out of 225 *E. coli* isolates were resistant to cefotaxime which is an important factor for screening for pathogens that produce ESBL (Table 8).

TABLE 5: Antimicrobial sensitivity of *E. coli* isolates from manure ($n = 41$).

Antibiotic	% Resistant	% Susceptible
Ampicillin	82.9	17.1
Tetracycline	82.9	17.1
Cotrimoxazole	82.9	17.1
Cefuroxime	90.3	9.7
Vancomycin	87.8	12.2
Chloramphenicol	80.5	19.5
Ceftriaxone	85.4	14.6
Cefuroxime	85.4	14.6
Ciprofloxacin	83	17
Meropenem	85.4	14.6

TABLE 6: Antimicrobial sensitivity of *E. coli* isolates from soil ($n = 63$).

Antibiotic	% Resistant	% Susceptible
Ampicillin	95.3	4.7
Tetracycline	95.2	4.8
Cotrimoxazole	92.1	7.9
Cefuroxime	96.8	3.2
Vancomycin	98.4	1.6
Chloramphenicol	96.9	3.1
Ceftriaxone	96.8	3.2
Cefotaxime	98.4	1.6
Ciprofloxacin	95.2	4.8
Meropenem	98.4	1.6

TABLE 7: Antimicrobial sensitivity of *E. coli* isolates from irrigation water ($n = 61$).

Antibiotic	% Resistant	% Susceptible
Ampicillin	93.5	6.5
Tetracycline	91.8	8.2
Cotrimoxazole	95.1	4.9
Cefuroxime	98.4	1.6
Vancomycin	98.4	1.6
Chloramphenicol	90.1	9.9
Ceftriaxone	98.3	1.7
Cefotaxime	98.4	1.6
Ciprofloxacin	95.1	4.9
Meropenem	98.4	1.6

3.4. ESBL Screening and Confirmation. Three (3.75%) out of 80 isolates that were screened using CTX and CTX/CLA antibiotic disc had a difference greater than 5 mm, implying possible ESBL production. All three isolates: 1 each from manure, irrigation water, and soil expressed the $\text{bla}_{\text{CTX-M}}$ gene after PCR. Below is a figure representing the amplified product of the $\text{bla}_{\text{CTX-M}}$ genes (Figure 1).

4. Discussion

4.1. Microbial Analysis. Microbial contaminations occur during production processes, harvest, and even handling before they reach the consumer with chances of the presence of pathogenic microorganisms. Fresh leafy vegetables have been linked to the spread of foodborne illnesses all over the

world. The presence of higher bacteria loads of *E. coli* on vegetable food items is a sign of fecal contamination and poor sanitation with higher chances of zoonotic pathogens being present [17]. As an indication of fecal contamination, the levels of *E. coli* in vegetables and their production environment are highly relevant.

The microbial loads on lettuce, irrigation water, manure, and soil in this present study confirm studies previously done by Keraita et al. [13], Hassan et al. [18], Holvoet et al. [19], Amponsah-Doku et al. [14], and Amoah et al. [12] on high loads of bacteria on lettuce and the production environment. In the current study, all parameters had microbial loads ranging from 2.0×10^5 to 1.67×10^7 MPN/100 ml which is approximately 5.30 to 7.22 Log MPN/100 ml (Table 1). Contrary to this study, Holvoet et al. [19] recorded relatively low *E. coli* counts in soil, irrigation water, and lettuce samples (0.7 to 3.2, 0 to 3.6, and 0.7 to 2.0 log CFU/ml, respectively). Similarly, a study by Antwi-Agyei et al. [20] recorded *E. coli* counts between 0.64 to 3.84 log CFU/g on fresh produce (lettuce) in Accra, Ghana. In Ethiopia, Weldezigina and Muleta [21] recorded counts between 5.0 and 8.36 log CFU/g of Enterobacteriaceae from irrigation water sources and fresh vegetables. Reports from Drechsel et al. [22] on fresh poultry litter used for cultivating vegetables in Kumasi, Ghana, indicated that they contained fecal coliforms between 3.6×10^4 and 1.1×10^7 CFU/g which is higher than *E. coli* counts recorded in the current study.

Statistically, there was a significant difference (Table 2) in microbial loads of lettuce across the seven weeks ($p < 0.05$). Weeks 1, 3, and 5 were significantly different from all other weeks ($p < 0.05$). Similarly, weeks 2 and 7 were statistically the same as weeks 4 and 6 ($p > 0.05$). Generally, microbial loads differed significantly over time (weeks). Variations in microbial loads on lettuce across the weeks could be attributed to the source of irrigation water and soil as well as manure.

Microbes can be transferred to lettuce through the vascular bundles and during irrigation via splashes [23]. Although soil is a natural habitat for many microorganisms, the loads of sources of irrigation water and manure contribute greatly to the levels of microorganisms present. On the other hand, microbial loads in manure could also be attributed to changes in storage conditions of manure at the farm and source of manure, poultry farm-specific, and the type of bedding materials used in the poultry farm [24]. Fluctuations in microbial loads in irrigation water could be attributed to variations in the composition of the irrigation water. Irrigation water could contain more sewage at one point and less at another point since it is not a stagnant water body [25].

4.2. Antibiotic Sensitivity. Food pathogens such as *E. coli* have developed resistance to several antibiotics which is now a global health issue. The usage of antibiotics in agricultural techniques has imparted greatly to antibiotic resistance development. Antibiotics are the basic treatments given for human and animal infections and could trigger some of the bacteria to develop resistance to the antibiotics which were

TABLE 8: Common resistance patterns of *E. coli* ($n=225$).

Resistance profile	Number of isolates	%
AMPCOTCRXVANCTRCTXCIPMEM	1	0.44
AMPCR XVANCHLCTRCTXCIPMEM	3	1.33
AMPCR XVANCTRCTXCIPMEM	1	0.44
AMPTETCOTCRXCHLCIPMEM	1	0.44
AMPTETCOTCRXCHLCTXCIP	1	0.44
AMPTETCOTCRXVANCHLCTRCIPMEM	2	0.89
AMPTETCOTCRXVANCHLCTRCTXCIP	2	0.89
AMPTETCOTCRXVANCHLCTRCTXCIPMEM	174	77.33
AMPTETCOTCRXVANCHLCTRCTXMEN	4	1.78
AMPTETCOTCRXVANCHLCTXMEN	1	0.44
AMPTETCOTCRXVANCTRCTXCIPMEM	7	3.11
AMPTETCOTCRXVANCTRCTXMEN	2	0.89
AMPTETCOTVANCHLCTRCTXCIPMEM	1	0.44
AMPTETCOTVANCTRCTXCIPMEM	1	0.44
AMPTETCRXVANCHLCTRCTXCIPMEM	2	0.89
COTCRXVANCHLCTRCTXCIPMEM	1	0.44
COTCRXVANCTRCTXCIPMEM	1	0.44
CRXVANCHLCTRCIPMEM	1	0.44
CRXVANCHLCTRCTXCIPMEM	2	0.89
CRXVANCTRCTXCIPMEM	1	0.44
None	9	4

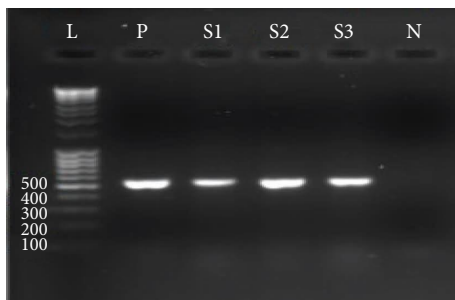


FIGURE 1: Gel electrophoresis amplification of bla_{CTX-M} genes in *E. coli* isolates (586 bp). L: DNA ladder (100 bp), P: positive control, S1: *E. coli* isolated from manure, S2: *E. coli* isolated from water, and S3: *E. coli* isolated from soil.

used to manage them. Maka et al. [26] confirmed that single usage of an antibiotic may aid the development of antibiotic resistance to the same antibiotic class, a different class, or possibly other antimicrobial substances. These pathogens may subsequently enter water bodies and finally be used in irrigating vegetables for consumption. Vegetables eventually get contaminated with resistant pathogens and affect the whole food chain.

Globally, bacteria phenotypes with similar multidrug resistance characteristics have been observed on fresh vegetable [27–29]. The current study confirmed the presence of antibiotic-resistant pathogens on vegetables, especially lettuce grown with contaminated irrigation water or wastewater and poultry manure on contaminated soils and gives some baseline information to compare with previous studies although direct comparisons are usually hindered by different antibiotics and methods used. 9 out of the 225 isolates tested, which is approximately 4%, showed complete susceptibility to all 12 antimicrobials used. The remaining 216 (96%) isolates

showed resistance to not less than 3 antibiotic classes rendering them multidrug resistant. All *E. coli* isolated from lettuce showed relatively high resistance to all 10 antibiotics which were used with AMPTETCOTCRXVANCHLCTRCTXCIPMEM being the most common resistance pattern (73.83%). The presence of antibiotic-resistant *E. coli* on lettuce samples confirms studies previously done by Österblad et al. [29] and Schwaiger et al. [30].

The study recorded extremely high resistance to some members of the beta-lactam class such as ampicillin: 91.1%, cefuroxime: 95.1%, ceftriaxone: 94.7%, cefotaxime: 94.2%, and meropenem: 94.2% which contradicts other studies. A typical example is seen in studies by Holvet et al. [19], where only 1 out of 473 isolates showed resistance to cefotaxime and 7% (including isolates from lettuce and its growing environment) to ampicillin. In Finland, Österblad et al. [29] similarly recorded no resistance for cefotaxime and imipenem. Previous studies by Schwaiger et al. [30] in Germany recorded full susceptibility to two beta-lactams: cefotaxime and imipenem. However, a recent study by Adzitey [31] in Tamale, Ghana, recorded high resistance for ampicillin: 86.67% but 40.0% resistance for ceftriaxone on ready-to-eat lettuce fields [31].

The presence of multidrug-resistant pathogens on lettuce could be due to the transmission of multidrug-resistant pathogens present in irrigation water, poultry manure, and soil during irrigation or land application of manure. Emerging pathogens resistant to penicillin have been reported decades ago and have been attributed to two enzymes: penicillinase and amidase. These enzymes hydrolyze penicillin and render its antibiotic activity negligible. However, Eriksson-Grennberg et al. [32] reported that the inoculum size used in antibiotic sensitivity tests had a significant impact on the minimum inhibitory concentration since more cells release more enzymes [32].

Resistance levels to 2nd generation (cefuroxime) and 3rd generation (ceftriaxone, cefotaxime) cephalosporins on both lettuce and its production environment: irrigation water, poultry manure, and soil were significantly high. This is in agreement with findings from Li et al. [33] in China and Donkor et al. [34] in Ghana on cefuroxime and cefotaxime resistance in Enterobacteriaceae isolates from livestock (food animals). However, Rasheed et al. [35] reported 8% ($n = 99$) resistance to cefotaxime from food sources in contrast to this study [35].

Resistance to carbapenems has increased over the past decade, especially in strains of Enterobacteriaceae, and has become a global health concern. Reports from Yao et al. [36] and Xu et al. [37] confirmed the presence of carbapenem-resistant *E. coli* [36, 37]. The emergence and prevalence of carbapenem-resistant *E. coli* have also been identified in some provinces in China. Similar to this study, Liang et al. [38] reported 100% resistance to imipenem, meropenem, cefotaxime, cefepime, and ampicillin in *E. coli* isolates from hospital sources: feces, urine, and sputum [38]. In India, Murugan et al. [39] recorded 23.30% resistance to meropenem from the feces of calves [39]. Ohene Larbi et al. [40] recently recorded 100% meropenem resistance in *E. coli* isolates ($n = 43$), from pig sources.

Contrary to studies by Adzitey [31], Rasheed [35], and Österblad et al. [29] who recorded low resistance to ciprofloxacin in fresh vegetables, this study recorded very high resistance to quinolones (ciprofloxacin) 91.5%. Although very few studies have been done about the movement of antibiotic residues from soil to plants, studies by Lilienberg et al. [41] revealed that 90 to 100% of ciprofloxacin was adsorbed onto lettuce [41]. Quinolone resistance is mostly facilitated by plasmid-mediated quinolone resistance, chromosomal mutations in DNA gyrase, and topoisomerase.

Contrary to Adzitey [31] who recorded 72.2% resistance to tetracycline, 90.6% was also recorded for tetracycline on *E. coli* isolates in the current study. In Lebanon, Faour-Klingbeil et al. [42] recorded 42% tetracycline resistance in *E. coli* isolates from fresh vegetables. However, *E. coli* isolates from poultry recorded resistance of 92.7%, 95.7%, and 87.9% from Adzitey [43], Larbi et al. [44], and Li et al., [33]. Studies from Sekyere [45] in Ashanti Region showed that about 60% ($n = 108$) of farms used tetracycline in pig farming. Tetracycline has been used in animal husbandry over the years for prophylaxis.

Vancomycin resistance has usually been associated with pathogens from hospital sources; however, this study recorded 95.1% which is quite significant. Studies by Gastmeier et al. [46] on vancomycin resistance development in enterococci in German hospitals reported that there were large variations in resistance to vancomycin due to situations in the four states. Cotrimoxazole as classified by the Food and Drugs Authority (FDA) of Ghana in 2015 is an over-the-counter-antibiotic. Similar to this study, Newman et al. [47] recorded 73% resistance to cotrimoxazole in Enterobacteriaceae. Adeleke and Omafuvbe [48] also recorded 60% resistance to cotrimoxazole in *E. coli* isolates from poultry feces. Donkor et al. [34] similarly recorded high resistance to cotrimoxazole in poultry.

Antibiotic resistance genes (ARGs) living on mobile genetic components or elements are found in abundance in manure. Bacteria harboring ARGs and antimicrobial substances together with their metabolites enter the soil during manure application. Upon entry into the soil, the ARGs are more likely to undergo horizontal transmission with the soil bacteria, which is a procedure facilitated by manure. The presence of antibiotic residues in food poses serious health risks due to recent reports of increased antimicrobial resistance. Food pathogens have developed various resistance patterns to antibiotics over the years. There could be occurrences of similarities or differences in the resistance patterns of isolates from the same or different food sources. Reports from Adzitey [49] show that the bacteria species involved, the type of production system employed in animal production, geographical location, sampling period, sampling technique, and the degree of antibiotic usage can greatly influence differences in antibiotic resistance profiles.

E. coli is one of the regular inhabitants of the gastrointestinal tract of both animals and humans [6]. While the majority of *E. coli* strains are not harmful, some can lead to severe diseases in animals, including septicemia, lung infections, gastrointestinal infections, and urinary tract infections [50]. Third-generation cephalosporins, fluoroquinolones, and sulfamethoxazole are among the common antibiotics used for such infections [51]. Resistance to such antibiotics by *E. coli* is a public health issue which may have a negative influence on the country's healthcare systems and a large financial burden, prolonged hospital stays, and the necessity for more costly and intense care treatments can lower output or productivity [6]. A more alarming situation in the healthcare system is the prognosis for patients in who are immunocompromised or in critical conditions has changed due to emerging patterns of bacterial drug resistance. Doctors and other healthcare givers are now faced with more and more difficulties in giving their patients appropriate antibiotic regimens while preventing the development of additional drug resistance [52].

4.3. ESBL Confirmation and Genotyping. Globally, CTX-M genes have been found in *E. coli* and *Salmonella* isolates from hospital sources, humans and their excreta, animals, and animal farms [35, 53]. In most regions of Africa [50] and Ghana [54], *E. coli* isolates from livestock showing ESBL resistance commonly express the CTX-M gene. Studies in the UK recorded the gene in a water body [55] and from a chicken meat [56]. Similarly, in Iran, the CTX-M gene was identified in 79.8% of *E. coli* isolates from urban wastewater [57]. Additionally, the results of another investigation in Iran indicate that surface waters and wastewater are important reservoirs of *E. coli* with pathogenic characteristics, high levels of antibiotic resistance, and ESBL genes carriers [58].

The presence of CTX-M gene in *E. coli* from the soil at a vegetable production site has also been reported in France [59]. In the Netherlands, cases of CTX-M on *E. coli* from humans and poultry have similarly been recorded [53]. The current study for the first time in Ghana, reports the isolation presence of CTX-M gene in *E. coli* isolates from the

vegetable production environment. The presence of these genes from various sources could be attributed to poultry manure and irrigation water. *E. coli* from poultry has been associated with CTX-M genes [40] and could potentially transfer them to soil or through their excreta. These genes could also come from the irrigation water since they contain hospital sewage and waste from pharmaceutical sources.

ESBL-producing bacteria have been concomitantly associated with multidrug resistance, thus conferring resistance to more than two or more classes of antibiotics [60]. The occurrence of ESBL-producing *E. coli* in vegetable production environment shows a potential transmission reservoir for livestock infections and human infections [61]. In the current study, ESBL bacteria can be transmitted directly when contaminated vegetables are ingested by humans and livestock. This will result in persistent, difficult-to-treat infections, and increase mortality.

5. Conclusion

The microbial loads of *E. coli* on lettuce grown in Kumasi and its production environment (irrigation water, manure, and soil) are quite alarming. All loads recorded exceed the ICMSF [62] and WHO [63] acceptable limits (10^3) for bacterial loads on production to consumer chain. Lettuce contamination during its production period is greatly influenced by its production environment, most especially the source of irrigation water and the use of poultry manure as a nitrogen source.

The study also recorded relatively high resistance to some antibiotic classes of medical importance such as beta-lactams and quinolones. This indicates the extent to which antibiotics are being abused not only in animal husbandry but in our everyday lives. The study unequivocally highlights the gut pathogen; *E. coli* as an essential reservoir for the ESBL gene, CTX-M, which for the first time in Ghana is being isolated from a vegetable production environment.

The occurrence of antibiotic-resistant *E. coli* in vegetables and their production environment is alarming and poses serious health threats to the general public. The presence of bla_{CTX-M} gene in *E. coli* from a vegetable production site recorded for the first time in Ghana requires enforcement by regulatory bodies on the inappropriate use of antibiotics in the country. Antibiotics use in livestock should be regulated together with ensuring the use of and safe clean water for irrigation to ensure safe farming and healthy agricultural produce.

5.1. Limitations. Due to financial constraints, the study was unable to use highly sensitive techniques such as sequencing to study other ESBL genes such bla_{SHV} and bla_{TEM} which are of clinical significance as well as identification of plasmids which play an important role in resistance transmission. Another setback includes unwillingness of lettuce farmers to permit sample collections from their farms with the notion that, their businesses may be terminated after analysis of their produce.

Data Availability

The data used in the study are available within the manuscript.

Disclosure

The abstract of this manuscript has been presented in a poster in the poster presentation at KNUST Kumasi, Book of Abstracts, WACCBIP Research Conference July 2022, and Annual Microbiology Society Conference 2023.

Conflicts of Interest

The authors declare that they have no conflicts of interest.

Acknowledgments

The authors thank Eric Acheampong (TAB, KNUST) and Denis Fosu (KCCR) for the technical support. Farmers in the Oforikrom municipality who gave their consent for the studies to be conducted are also appreciated. This was self-funded.

References

- [1] Fao, "Antimicrobial resistance in food and agriculture amr in the food and agriculture sectors food chain crisis emergency prevention system information sheets," 2017, <http://www.fao.org/antimicrobial-resistance>.
- [2] Z. Chen and X. Jiang, "Microbiological safety of chicken litter or chicken litter-based organic fertilizers: a review," *Agriculture*, vol. 4, no. 1, pp. 1–29, 2014.
- [3] M. B. Jenkins, D. M. Endale, H. H. Schomberg, P. G. Hartel, and M. L. Cabrera, "17 β -estradiol and testosterone in drainage and runoff from poultry litter applications to tilled and no-till crop land under irrigation," *Journal of Environmental Management*, vol. 90, no. 8, pp. 2659–2664, 2009.
- [4] L. M. Mancilla-Becerra, T. Lías-Macias, C. L. Ramírez-Jiménez, and J. B. León, "Multidrug-resistant bacterial foodborne pathogens: impact on human health and economy," *Pathogenic Bacteria [Working Title]*, Intech open, London, UK, 2019.
- [5] S. Diaz-Sanchez, S. Moscoso, F. Solís De Los Santos, A. Andino, and I. Hanning, "Antibiotic use in poultry: a driving force for organic poultry production," *Food Protection Trends*, vol. 35, no. 6, pp. 440–447, 2015.
- [6] A. M. Almansour, M. A. Alhadlaq, K. O. Alzahrani, L. E. Mukhtar, A. L. Alharbi, and S. M. Alajel, "The silent threat: antimicrobial-resistant pathogens in food-producing animals and their impact on public health," *Microorganisms*, vol. 11, no. 9, p. 2127, 2023.
- [7] K. Kumar, A. Thompson, A. K. Singh, Y. Chander, and S. C. Gupta, "Enzyme-linked immunosorbent assay for ultratrace determination of antibiotics in aqueous samples," *Journal of Environmental Quality*, vol. 33, no. 2, p. 797, 2004.
- [8] A. Van Epps and L. Blaney, "Antibiotic residues in animal waste: occurrence and degradation in conventional agricultural waste management practices," *Current Pollution Reports*, vol. 2, no. 3, pp. 135–155, 2016.
- [9] N. V. Danilova, P. Y. Galitskaya, and S. Y. Selivanovskaya, "Antibiotic resistance of microorganisms in agricultural soils

- in Russia," *IOP Conference Series: Earth and Environmental Science*, vol. 107, no. 1, Article ID 012054, 2018.
- [10] M. K. Aworh, J. K. P. Kwaga, R. S. Hendriksen, E. C. Okolocha, and S. Thakur, "Genetic relatedness of multidrug resistant *Escherichia coli* isolated from humans, chickens and poultry environments," *Antimicrobial Resistance and Infection Control*, vol. 10, no. 1, 13 pages, 2021.
 - [11] G. Danso, P. Drechsel, E. Obuobie, G. Forkuor, and G. Kranjac-Berisavljevic, "Urban vegetable farming sites, crops and cropping practices. Irrigated urban vegetable production in Ghana: characteristics, benefits and risk mitigation," pp. 7–27, 2014, <https://www.iwmi.cgiar.org/publications/other-publication-types/books-monographs/iwmi-jointly-published/irrigated-urban-vegetable-production-ghana/>.
 - [12] P. Amoah, P. Drechsel, and R. C. Abaidoo, "Irrigated urban vegetable production in Ghana: sources of pathogen contamination and health risk elimination," *Irrigation and Drainage*, vol. 54, no. S1, pp. 49–61, 2005.
 - [13] B. Keraita, P. Drechsel, and P. Amoah, "Influence of urban wastewater on stream water quality and agriculture in and around Kumasi, Ghana," *Environment and Urbanization*, vol. 15, no. 2, pp. 171–178, 2003.
 - [14] F. Amponsah-Doku, K. Obiri-Danso, R. C. Abaidoo, L. A. Andoh, P. Drechsel, and F. Kondrasen, "Bacterial contamination of lettuce and associated risk factors at production sites, markets and street food restaurants in urban and peri-urban Kumasi, Ghana," *Scientific Research and Essays*, vol. 5, no. 2, pp. 217–223, 2010.
 - [15] Ghana Statistical Service, "Ghana 2021 population and housing census," vol. 1, 2021 Preliminary Report.
 - [16] A. Bauernfeind, I. Stemplinger, R. Jungwirth, S. Ernst, and J. M. Casellas, "Sequences of beta-lactamase genes encoding CTX-M-1 (MEN-1) and CTX-M-2 and relationship of their amino acid sequences with those of other beta-lactamases," *Antimicrobial Agents and Chemotherapy*, vol. 40, no. 2, pp. 509–513, 1996.
 - [17] C. Alimentarius, *Code of Hygienic Practice For Fresh Fruits and Vegetables (Cac/Rcp 53-2003, With Annex For Sprout Production)*, WHO, Geneva, Switzerland, 2010.
 - [18] S. A. Hassan, A. D. Altalhi, Y. A. Gherbawy, and B. A. El-Deeb, "Bacterial load of fresh vegetables and their resistance to the currently used antibiotics in Saudi Arabia," *Foodborne Pathogens and Disease*, vol. 8, no. 9, pp. 1011–1018, 2011.
 - [19] K. Holvoet, I. Samplers, B. Callens, J. Dewulf, and M. Uyttendaele, "Moderate prevalence of antimicrobial resistance in *Escherichia coli* isolates from lettuce, irrigation water, and soil," *Applied and Environmental Microbiology*, vol. 79, no. 21, pp. 6677–6683, 2013.
 - [20] P. Antwi-agyei, S. Cairncross, A. Peasey et al., "A farm to fork risk assessment for the use of wastewater in agriculture in Accra, Ghana," *PLoS One*, vol. 10, pp. 1–19, 2015.
 - [21] D. Weldezzgina and D. Muleta, "Bacteriological contaminants of some fresh vegetables irrigated with awetu river in jimma town, southwestern Ethiopia," *Advances in Biology*, vol. 2016, Article ID 1526764, 11 pages, 2016.
 - [22] P. Drechsel, U. J. Blumenthal, and B. Keraita, "Balancing health and livelihoods. Adjusting wastewater irrigation guidelines for resource-poor countries," *Urban Agriculture Magazine*, vol. 8, no. December, pp. 7–9, 2002.
 - [23] G. Berg, L. Eberl, and A. Hartmann, "The rhizosphere as a reservoir for opportunistic human pathogenic bacteria," *Environmental Microbiology*, vol. 7, no. 11, pp. 1673–1685, 2005.
 - [24] K. R. Sistani, G. E. Brink, S. L. McGowen, D. E. Rowe, and J. L. Oldham, "Characterization of broiler cake and broiler litter, the by-products of two management practices," *Bio-resource Technology*, vol. 90, no. 1, pp. 27–32, 2003.
 - [25] G. A. Cornish, E. Mensah, and P. Ghesquire, "Water quality an pery-urban irrigation. A assessment of surface water quality for irrigation and its implications for human health in the peri-urban zone of Kumasi, Ghana," vol. 44, HR Wallingford Ltd, Oxfordshire, UK, 1999, Project Report.
 - [26] N. Maka Instytut, Z. Publicznego, and M. Popowska, "Antimicrobial resistance of salmonella spp. isolated from food," *Roczniki Państwowego Zakładu Higieny*, vol. 67, 2016.
 - [27] S. Boehme, G. Werner, I. Klare, R. Reissbrodt, and W. Witte, "Occurrence of antibiotic-resistant enterobacteria in agricultural foodstuffs," *Molecular Nutrition and Food Research*, vol. 48, no. 7, pp. 522–531, 2004.
 - [28] J. Hamilton-Miller and S. Shah, "Identity and antibiotic susceptibility of enterobacterial flora of salad vegetables," *International Journal of Antimicrobial Agents*, vol. 18, no. 1, pp. 81–83, 2001.
 - [29] M. Österblad, O. Pensala, M. Peterzéns, H. Helenius, and P. Huovinen, "Antimicrobial susceptibility of Enterobacteriaceae isolated from vegetables," *Journal of Antimicrobial Chemotherapy*, vol. 43, no. 4, pp. 503–509, 1999.
 - [30] K. Schwaiger, K. Helmke, C. S. Hölzel, and J. Bauer, "Antibiotic resistance in bacteria isolated from vegetables with regards to the marketing stage (farm vs. supermarket)," *International Journal of Food Microbiology*, vol. 148, no. 3, pp. 191–196, 2011.
 - [31] F. Adzitey, "Antibiotic resistance of escherichia coli and salmonella enterica isolated from cabbage and lettuce samples in tamale metropolis of Ghana," *International Journal of Flow Control*, vol. 5, no. 1, p. 7, 2018.
 - [32] K. G. Eriksson-grennberg, H. G. Boman, J. A. T. Jansson, and S. Thorén, "Resistance of *Escherichia coli* to penicillins I. Genetic study of some ampicillin-resistant mutants," *Journal of Bacteriology*, vol. 90, no. 1, pp. 54–62, 1965.
 - [33] J. Li, Y. Ma, C. Hu et al., "Dissemination of cefotaxime-M-producing *Escherichia coli* isolates in poultry farms, but not swine farms, in China," *Foodborne Pathogens and Disease*, vol. 7, no. 11, pp. 1387–1392, 2010.
 - [34] E. S. Donkor, M. J. Newman, and D. Yeboah-Manu, "Epidemiological aspects of nonhuman antibiotic usage and resistance: Implications for the control of antibiotic resistance in Ghana," *Tropical Medicine and International Health*, vol. 17, no. 4, pp. 462–468, 2012.
 - [35] M. U. Rasheed, N. Thajuddin, P. Ahamed, Z. Teklemariam, and K. Jamil, "Antimicrobial drug resistance in strains of *Escherichia coli* isolated from food sources," *Revista Do Instituto de Medicina Tropical de Sao Paulo*, vol. 56, no. 4, pp. 341–346, 2014.
 - [36] X. Yao, Y. Doi, L. Zeng, L. Lv, and J. H. Liu, "Carbapenem-resistant and colistin-resistant *Escherichia coli*-producing NDM-9 and MCR-1," *The Lancet Infectious Diseases*, vol. 16, no. 3, pp. 288–289, 2016.
 - [37] Y. Xu, B. Gu, M. Huang et al., "Epidemiology of carbapenem resistant Enterobacteriaceae (CRE) during 2000–2012 in asia," *Journal of Thoracic Disease*, vol. 7, no. 3, pp. 376–385, 2015.
 - [38] W. J. Liang, H. Y. Liu, G. C. Duan et al., "Emergence and mechanism of carbapenem-resistant *Escherichia coli* in Henan, China, 2014," *Journal of Infection and Public Health*, vol. 11, no. 3, pp. 347–351, 2018.
 - [39] M. S. Murugan, D. K. Sinha, O. R. Vinodh Kumar et al., "Epidemiology of carbapenem-resistant *Escherichia coli* and

- first report of blaVIM carbapenemases gene in calves from India," *Epidemiology and Infection*, vol. 147, p. e159, 2019.
- [40] R. Ohene Larbi, L. A. Ofori, A. A. Sylverken, M. Ayim-Akonor, and K. Obiri-Danso, "Antimicrobial resistance of *Escherichia coli* from broilers, pigs, and cattle in the greater Kumasi metropolis, Ghana," *International Journal of Microbiology*, vol. 2021, Article ID 5158185, 7 pages, 2021.
 - [41] M. Lillenberg, S. Litvin, L. Nei, M. Roasto, and K. Sepp, "Enrofloxacin and ciprofloxacin uptake by plants from soil," *Agronomy Research*, vol. 8, no. 1, pp. 807–814, 2010.
 - [42] D. Faour-Klingbeil, V. Kuri, S. Fadlallah, and G. M. Matar, "Prevalence of antimicrobial-resistant *Escherichia coli* from raw vegetables in Lebanon," *The Journal of Infection in Developing Countries*, vol. 10, no. 04, pp. 354–362, 2016.
 - [43] F. Adzitey, "Animal and meat production in Ghana-an overview," *Journal of World's Poultry Research*, vol. 3, no. 31, pp. 1–4, 2013.
 - [44] R. Ohene Larbi, L. A. Ofori, A. A. Sylverken, M. Ayim-Akonor, and K. Obiri-Danso, "Antimicrobial Resistance of *Escherichia coli* from broilers, pigs, and cattle in the greater Kumasi metropolis, Ghana," *International Journal of Microbiology*, vol. 2021, pp. 2021–7, Article ID 5158185.
 - [45] J. Osei Sekyere, "Antibiotic types and handling practices in disease management among pig farms in Ashanti region, Ghana," *Journal of Veterinary Medicine*, vol. 2014, pp. 1–8, 2011.
 - [46] P. Gastmeier, C. Schröder, M. Behnke, E. Meyer, and C. Geffers, "Dramatic increase in vancomycin-resistant enterococci in Germany," *Journal of Antimicrobial Chemotherapy*, vol. 69, no. 6, pp. 1660–1664, 2014.
 - [47] M. Newman, E. Frimpong, E. S. Donkor, J. A. Opintan, and A. Asamoah-Adu, *Resistance to antimicrobial drugs in Ghana*, vol. 4, pp. 215–220, 2011.
 - [48] B. Omafuvbe and E. Adeleke, "Antibiotic resistance of aerobic mesophilic bacteria isolated from poultry faeces," *Research Journal of Microbiology*, vol. 6, no. 4, pp. 356–365, 2011.
 - [49] F. Adzitey, "Prevalence of *Escherichia coli* and *Salmonella* spp. in beef samples sold at tamale metropolis, Ghana," *International Journal of Meat Science*, vol. 5, no. 1, pp. 8–13, 2014.
 - [50] C. A. Alonso, G. B. Michael, J. Li et al., "Analysis of blaSHV-12-carrying *Escherichia coli* clones and plasmids from human, animal and food sources," *Journal of Antimicrobial Chemotherapy*, vol. 72, no. 6, pp. 1589–1596, 2017.
 - [51] J. Soltani, B. Poorabbas, N. Miri, and J. Mardaneh, "Health care associated infections, antibiotic resistance and clinical outcome: a surveillance study from Sanandaj, Iran," *World Journal of Clinical Cases*, vol. 4, no. 3, p. 63, 2016.
 - [52] B. Poorabbas, J. Mardaneh, Z. Rezaei et al., "Nosocomial Infections: multicenter surveillance of antimicrobial resistance profile of *Staphylococcus aureus* and Gram negative rods isolated from blood and other sterile body fluids in Iran," *Iranian Journal of Microbiology*, vol. 7, no. 3, pp. 127–135, 2015.
 - [53] H. Hasman, D. Mevius, K. Veldman, I. Olesen, and F. M. Aarestrup, " β -Lactamases among extended-spectrum β -lactamase (ESBL)-resistant *Salmonella* from poultry, poultry products and human patients in The Netherlands," *Journal of Antimicrobial Chemotherapy*, vol. 56, no. 1, pp. 115–121, 2005.
 - [54] M. M. Rasmussen, J. A. Opintan, N. Frimodt-Møller, and B. Styrisshave, "Beta-lactamase producing *Escherichia coli* isolates in imported and locally produced chicken meat from Ghana," *PLoS One*, vol. 10, no. 10, Article ID e0139706, 2015.
 - [55] G. C. A. Amos, P. M. Hawkey, W. H. Gaze, and E. M. Wellington, "Waste water effluent contributes to the dissemination of CTX-M-15 in the natural environment," *Journal of Antimicrobial Chemotherapy*, vol. 69, no. 7, pp. 1785–1791, 2014.
 - [56] H. Dhanji, N. M. Murphy, M. Doumith et al., "Cephalosporin resistance mechanisms in *Escherichia coli* isolated from raw chicken imported into the UK," *Journal of Antimicrobial Chemotherapy*, vol. 65, no. 12, pp. 2534–2537, 2010.
 - [57] M. Afsharnia, B. Naraghi, J. Mardaneh, M. Kianmehr, and H. Biglari, "The data of *Escherichia coli*," *Data in Brief*, vol. 21, pp. 763–766, 2018.
 - [58] B. Naraghi, M. Afsharnia, J. Mardaneh, M. Kianmehr, H. Biglari, and J. Bazeli, "Pathogenesis traits and antimicrobial resistance pattern in *Escherichia coli* isolates recovered from sewage," *Crescent Journal of Medical and Biological Sciences*, vol. 7, no. 4, pp. 488–496, 2020.
 - [59] A. Hartmann, A. Locatelli, L. Amoureux et al., "Occurrence of CTX-M producing *Escherichia coli* in soils, cattle, and farm environment in France (Burgundy region)," *Frontiers in Microbiology*, vol. 3, no. MAR, pp. 83–87, 2012.
 - [60] W. H. Organization, *Global Antimicrobial Resistance and Use Surveillance System (Glass) Report 2021*, World Health Organization, Geneva, Switzerland, 2021.
 - [61] C. W. Akenten, N. A. Khan, J. Mbwana et al., "Carriage of ESBL-producing *Klebsiella pneumoniae* and *Escherichia coli* among children in rural Ghana: a cross-sectional study," *Antimicrobial Resistance and Infection Control*, vol. 12, no. 1, p. 60, 2023.
 - [62] Icmsf, "Microorganisms in foods. 2. Sampling for microbiological analysis: principles and specific applications," *Journal of AOAC International*, vol. 58, no. 6, p. 1308, 1986.
 - [63] Who, "Surface decontamination of fruits and vegetables eaten raw: a review," World Health Organization, Geneva, Switzerland, WHO/FSF/FOS/98.2, 1998.