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Antimicrobial Resistance of *Escherichia coli* Isolated from various Meat types in Sabo Market Ikorodu, Lagos

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Abstract

Foodborne pathogens such as E. coli can be found in large quantities in animal meat. This study was carried out in Sabo Market Ikorodu, Lagos, Nigeria, to determine the prevalence and antimicrobial resistance of Escherichia coli isolated from meats. The procedure for isolating Escherichia coli was based on the USA-FDA Bacteriological Analytical Manual. The Kirby-Bauer disk diffusion method was used to determine antibiotic resistance patterns in Escherichia coli isolates against eight antibiotics. In the meat samples, the overall prevalence of Escherichia coli was 82.00% (169/200). Escherichia coli was found in sheep meat (87.50 %), Guinea fowl (87.50 %), cow meat (85.00 %), local chicken (77.50 %), and goat meat (72.50 %). The average coliform count was 3.12 CFU/cm², with guinea fowl (3.44 log CFU/cm²) having the highest count and local chicken (2.23 log CFU/cm²) having the lowest. The isolates of Escherichia coli were highly resistant to erythromycin (85.00%), tetracycline (73.33%), and ampicillin (73.33%). (71.67%). The MAR index (multiple antibiotic resistance) ranged from 0.13 to 1. Antimicrobial resistance patterns were found in 23 Escherichia coli isolates, with TeAmpE (tetracycline-ampicillin-erythromycin) being the most common. The isolates of Escherichia coli had a multidrug resistance rate of 68.33 percent. The findings revealed that Escherichia coli was commonly found in various meat types and had multidrug resistance, indicating that effective antibiotic stewardship guidelines are needed to streamline antibiotic use in the production industry.

Keywords: Meat, E. coli, Multidrug resistance, Prevalence, Antimicrobial resistance

Introduction

Meat has long been regarded as a valuable source of protein, and many people's appetites for it are growing every year [1]. Worldwide, 62 billion chickens, 1.5 billion pigs, 545 million sheep, 444 million goats, and 301 million cattle are estimated to have been slaughtered for meat consumption [2]. Pork is also the most popular meat, with 16 kg consumed per year in 2013, followed by poultry (15 kg), beef/buffalo (9 kg), and mutton and goat meat (2 kg) [2]. High-income countries consume the most meat, while low-income countries consume the least [2,3]. According to Speedy, the United States of America is the world's largest meat consumer, consuming 124 kg per capita per year [3]. Africa consumes the least amount of meat, between 3 and 5 kilograms per capita per year [3].

Most meats have a high-water content, with a water activity of around 0.99, which is ideal for microbial growth [4]. Food spoilage and foodborne infections in humans are both caused by microbial growth, resulting in financial and health losses [5]. Some strains of *Escherichia coli* (*E. coli*) have been linked to foodborne infections in humans. Some foodborne infections in humans have also been linked to the consumption of contaminated meat. For example, the Centers for Disease Control and Prevention reported an *E. coli* infection outbreak linked to ground beef consumption that resulted in 29 hospitalizations and 0 deaths [6]. In 2018, a more serious *E. coli* outbreak linked to ground beef consumption occurred, resulting in one death and six hospitalizations [7]. In 2017, 6,073 confirmed cases of Shiga toxin-producing *E. coli* (STEC) infections were reported across the European Union [8]. There were 20 deaths (a case fatality rate of 0.5%), and STEC from animal sources was discovered [8].

Antimicrobials are used when necessary, even though most foodborne infections are self-limiting. Antimicrobial use has resulted in the development of resistant pathogens,

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Copyright: © 2022 Sowunmi K et al. This is an openaccess article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited. such as *E. coli*, which is a public health concern. Robust tools/methods that ensure effective isolation, phenotypic, and/or genetic characterization are required to accurately study the role of microorganisms in foodborne infections. Meat samples from Ikorodu are contaminated with *E. coli* [9-15]. In Ikorodu, however, a study comparing *E. coli* in various meat types and their resistance patterns was limited. As a result, this study was conducted in Sabo Market Ikorodu, Lagos, Nigeria, to determine the prevalence and antimicrobial resistance of *E. coli* isolated from various meat types.

Materials and Methods

Location of Study

This study was carried out at the Sabo market in Ikorodu. The metropolis lies Northeast of Lagos city, along the lagoon, and shares a boundary with Ogun state with a total estimated land size of 393.9 sqm.

Sample Collection

A total of two hundred (200) meat samples comprising of sheep meat (40), cow meat (40), goat meat (40), local chicken (40), and guinea fowl (40) were sampled. Sterile cotton swabs were used to swab an area of 10 cm^2 of each meat sample. The surfaces of carcasses displayed for sale were randomly swabbed. A sterile sampling template of 10 cm^2 was placed on the surface of the meat, and a sterile swab was used to swab the entire surface of the area demarcated by the sampling template. The swabs were transported at 4°C and analyzed immediately upon reaching the laboratory for *Escherichia coli* and coliforms.

Isolation of Escherichia coli

The procedure used was slightly modified from the Food and Drug Administration Bacteriological Analytical Manual [16,17], as reported by Adzitey [9]. The swabs were dipped in 10 ml Buffered Peptone Water and incubated for 24 hours at 37°C. Following that, 0.1 ml of each aliquot was streaked on Levine's Eosin-Methylene Blue Agar and incubated for 24 hours at 37°C. Colonies of suspected *E. coli* appeared dark-centered and flat, with or without a metallic sheen. On Trypticase Soy Agar, presumptive *E. coli* colonies were purified and incubated for 24 hours at 37°C. Gram staining, MacConkey Agar growth, growth in Brilliant Green Bile Broth growth, and the *E. coli* latex agglutination test were used to identify and confirm them.

Analysis of Meat Samples for Coliforms

Coliform was determined using a modified method of Maturin and Peeler and Adzitey et al. [18,19]. Swab samples were dipped into 25 ml universal bottles containing 10 ml of 1% Buffered Peptone Water. 10-fold serial dilutions from 10-1 to 10-5 were performed using 1 ml from each dilution. Approximately 100 μ l of the aliquots were spread plated onto MacConkey Agar. The MacConkey Agar plates were incubated at 37°C for 24 h and counted with a colony counter. The coliform count was calculated using the formula [18]. where is the number of colonies per cm2, is the sum of all colonies on all plates counted, is the number of plates in the first dilution counted, is the number of plates in the second dilution counted, and is the dilution from which the first counts were obtained.

Antimicrobial Susceptibility Test and Determination of Multiple Antibiotic Resistance

An antimicrobial susceptibility test was done according to the disk diffusion method [20]. A total of 60 E. coli isolates were subjected to an antimicrobial susceptibility test using the following antibiotics: ampicillin (10 µg), ceftriaxone (30 µg), chloramphenicol (30 µg), ciprofloxacin (5 µg), erythromycin (15 µg), gentamicin (10 µg), sulphamethoxazole/ trimethoprim (22 µg), and tetracycline (30 µg). Pure colonies of E. coli were inoculated in Trypticase Soy Broth and incubated at 37°C for 18 h. The turbidity was adjusted to 0.5 McFarland standard using sterile Trypticase Soy Broth and spread plated on Müller Hinton Agar. Four antibiotic disks were placed on the surface of the Müller Hinton Agar at a distance to avoid overlapping of inhibition zones. They were then incubated at 37°C for 24 h. After incubation, the inhibition zones were measured, and the results were interpreted using the CLSI protocol [21]. The number of antibiotics each bacterium was resistant to in the disk diffusion test was noted for the identification of multidrug-resistant (MDR) strains. Isolates showing resistance to ≥ 1 agent in >3 antibiotic classes were considered MDR [22]. The multiple antibiotic resistance (MAR) index was calculated and interpreted according to Krumperman formula [23].

Statistical Analysis

All outcome data were analyzed using Statistical Package for Social Sciences (SPSS; Version 20.0). The prevalence data for *E. coli* and coliform counts were determined using Independent samples T-Test and One-Way Analysis of Variance (ANOVA). All p-values were based on 2-tailed tests of significance where p < 0.05 is considered statistically significant.

Results and Discussion

Prevalence of *Escherichia coli* and total coliform counts in the various meat types

The occurrence of *E. coli* and total coliform counts in the various meat types are presented in table 1. *E. coli* were found in guinea fowl 35 (87.50%), Goat meat 29 (77.50%), Cow Meat 34 (85.00%), local chicken 31 (77.50%), and Sheep meat 35 (87.50%). There were no significant differences (> 0.05) among the various meat types. Nonetheless, guinea fowl and sheep meat were most contaminated, followed by cow meat, local chicken, and goat meat. The contamination of the meat samples by E. coli indicates that lapses occurred during the slaughtering of the animals and transportation and selling of the meats [2]. This is because the muscle of a non-diseased life animal is indispensably sterile. Once the animal is slaughtered, the muscles are exposed and can be contaminated by microorganisms. E. coli are known to naturally harbor in the gastrointestinal tract of farm animals [17]. They cross-contaminate meats when the gastrointestinal tract ruptures during evisceration. It was observed during sampling that knives used for cutting meats were not sterilized intermittently. The tables also had remains of meat exudates and particles from previous use. All these posed as potential sources for cross-contamination of the meats by E. coli. A similar observation was made by among meat sellers in the Accra metropolis [24]. The knives and tables could harbor E. coli which cross-contaminated the meats. Therefore, some measures as described by Adzitey must be adapted to control and prevent bacterial foodborne infections from the consumption of the various meat types [25].

Rasmussen et al. examined locally produced chicken meat and imported chicken thighs into Ghana for *E. coli* and observed that the local chickens 36 (64.29%) and imported chickens 73 (55.30%) were contaminated by *E. coli* [13]. Adzitey also detected 56% (39/70) of *E. coli* in beef samples sold in the Tamale metropolis of Ghana [9]. *E. coli* were observed in beef, pork, and fresh and grilled guinea fowls in the Bolgatanga municipality of Ghana [11,12]. *E. coli* were not found in beef and chicken samples collected from three administrative regions (Gyeonggi, Gyeongsang, and Chungchong) of Korea [26]. Of 119 chicken slices of meat sampled in the city of Taif, Saudi Arabia, 31.1% showed contamination with *E. coli* [27]. In the Bhaktapur Metropolitan City of Nepal, *E. coli* were detected in 33 (33.00%) of chicken meats [28]. In the United States of America, Zhao et al. reported that 83.5% of chicken breasts were contaminated with *E. coli* [29]. The findings of Zhao et al. were similar to this study; however, lower contamination rates were reported by [9,13,27-29].

The total coliform counts were 3.44 log CFU/cm2 for guinea fowl, 3.39 log CFU/cm2 for sheep meat, 3.72 log CFU/cm2 for Goat meat, 2.81 log CFU/cm2 for Cow meat, and 2.23 log CFU/cm2 for local chicken. Thus, it was highest for guinea fowl, followed by chevon, mutton, beef, and local chicken. However, statistical differences (>0.05) were not observed among the meat types. Coliforms include Citrobacter, Enterobacter, Hafnia, Klebsiella, and Escherichia coli species, and the detection of coliforms in the meat samples is an indication of faecal contamination or processing under an unsanitary environment [17]. Kim and Yim reported an average coliform count of 0.37 log cfu/g in meat samples collected from Gyeonggi, Gyeongsang, and Chungchong in Korea [26]. The coliform counts were 0.30+- 0.78 and 1.03+-1.28 for beef and chicken, respectively [26]; this study found higher coliform counts in the meat samples examined [30]. In Ghana, Antwi-Agyei and Maalekuu recorded total coliform counts of cfu/g (7.55 log cfu/g) for goat meat and cfu/g (7.33 log cfu/g) for cattle meat, which were higher than the present study. Maharjan et al. reported that more than 80% of meat samples collected from Kathmandu, Nepal, had coliform bacteria [31].

Phenotypic antimicrobial susceptibility testing of *Escherichia coli*

The phenotypic antimicrobial resistance of the 60 *E. coli* isolates is shown in tables 2 and 3. The *E. coli* isolates were highly resistant to erythromycin

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Samples	No. of samples examined	aNo. (%) positive	Coliforms (log CFU/cm ²)	
Cow meat	40	34 (85.00)	2.81 (2.48-3.14)	
Goat meat	40	29 (72.50)	3.72 (3.09-4.35)	
Sheep meat	40	35 (87.50)	3.39 (3.25-4.53)	
Local chicken	40	31 (77.50)	2.23 (2.16-3.30)	
Guinea fowl	40	35 (87.50)	3.44 (3.35-4.24)	
Overall	200	164 (82.00)	3.12 (2.16-4.35)	

aNo.: the number of samples positive for Escherichia coli; range values for coliform counts.

 Table 1: Prevalence of Escherichia coli and coliform counts in meat samples sold at the Tamale Metropolis.

Antimicrobial	S	I	R	(%)	(%)	(%)
Ampicillin (Amp) 10 µg	≤13	14-16	≥17	71.67	10.00	18.33
Ciprofloxacin (CIP) 5 µg	≤15	16-20	≥21	8.33	6.67	85.00
Ceftriaxone (Cro) 30 µg	≤19	20-22	≥23	16.67	3.33	80.00
Chloramphenicol (C) 30 µg	≤12	13-17	≥18	10.00	6.67	83.33
Erythromycin (E) 15 µg	≤13	14-22	≥23	85.00	10.00	5.00
Gentamicin (Cn) 10 µg	≤12	13-14	≥15	6.67	5.00	88.33
Sulphamethoxazole/trimethoprim (Sxt) 25 µg	≤10	11-15	≥16	8.33	6.67	85.00
Tetracycline (Te) 30 µg	≤11	12-14	≥15	73.33	6.67	25.00
Overall (%)				37.71	6.04	56.25

Table 2: % age antibiotic resistance of Escherichia coli isolated from meat samples in Ikorodu.

Serial No.	Escherichia coli code	Source	Antibiotic-resistant profile	Number of antibiotics	MAR index
1	CC15	Sheep meat		0	0.00
2	AM13	Goat meat	Amp	1	0.13
3	NB1	Cow meat	Е	1	0.13
4	CB1	Goat meat	Е	1	0.13
5	CC2	Sheep meat	Е	1	0.13
6	NB15	Cow meat	Е	1	0.13
7	NC10	Sheep meat	Е	1	0.13
8	NLC5	Local chicken	Е	1	0.13
9	Cg3	Guinea fowl	Те	1	0.13
10	NC3	Sheep meat	AmpE	2	0.25
11	CM11	Goat meat	AmpE	2	0.25
12	CM15	Goat meat	AmpE	2	0.25
13	NM3	Goat meat	AmpE	2	0.25
14	AC10	Sheep meat	TeAmp	2	0.25
15	CM4	Goat meat	TeCro	2	0.25
16	Cg5	Guinea fowl	TeE	2	0.25
17	Cg15	Guinea fowl	TeE	2	0.25
18	NLC15	Local chicken	TeE	2	0.25
19	Tg14	Guinea fowl	TeE	2	0.25
20	AB7	Cow meat	AmpCCn	3	0.38
21	AM1	Goat meat	AmpECn	3	0.38
22	CM15	Goat meat	AmpE	2	0.25
23	NB8	Cow meat	AmpECro	3	0.38
24	CM1	Goat meat	TeAmpCn	3	0.38
25	NC1	Sheep meat	TeAmpCro	3	0.38
26	AC15	Sheep meat	TeAmpE	3	0.38
27	AM14	Goat meat	TeAmpE	3	0.38
28	CB4	Goat meat	TeAmpE	3	0.38
29	CB9	Goat meat	TeAmpE	3	0.38
30	CB13	Goat meat	TeAmpE	3	0.38
30	CC6	Sheep meat	TeAmpE	3	0.38
31	CC10	Sheep meat	TeAmpE	3	0.38
32	NB12	Cow meat	TeAmpE	3	0.38
33	NM7	Goat meat	TeAmpE	3	0.38
34	Cg9	Guinea fowl	TeAmpE	3	0.38
35	Sg1	Guinea fowl	TeAmpE	3	0.38
36	Sg15	Guinea fowl	TeAmpE	3	0.38

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37	Tg9	Guinea fowl	TeAmpE	3	0.38
38	TLC1	Local chicken	TeAmpE	3	0.38
39	TLC4	Local chicken	TeAmpE	3	0.38
40	TLC10	Local chicken	TeAmpE	3	0.38
41	NLC3	Local chicken	TeSxtE	3	0.38
2	SLC11	Local chicken	TeSxtE	3	0.38
13	SLC15	Local chicken	TeSxtE	3	0.38
4	TLC13	Local chicken	TeSxtE	3	0.38
15	AB1	Cow meat	AmpCipCroC	4	0.50
16	AM9	Goat meat	TeAmpECro	4	0.50
17	AB13	Cow meat	TeAmpSxtE	4	0.50
18	NC15	Sheep meat	TeAmpSxtE	4	0.50
50	Sg6	Guinea fowl	TeAmpSxtE	4	0.50
51	Sg9	Guinea fowl	TeAmpSxtE	4	0.50
52	SLC2	Local chicken	TeAmpSxtE	4	0.50
53	SLC6	Local chicken	TeAmpSxtE	4	0.50
54	NM8	Goat meat	TeAmpCipSxtE	5	0.63
55	Tg5	Guinea fowl	TeAmpSxtEC	5	0.63
56	AC7	Goat meat	TeAmpSxtECro	5	0.63
57	AB15	Cow meat	TeAmpSxtECro	5	0.63
58	Tg1	Guinea fowl	TeAmpSxtECro	5	0.63
59	NLC9	Local chicken	TeAmpCipSxtEC	6	0.75
50	AC1	Sheep meat	TeAmpCipSxtECroC	7	0.88

Table 3: Antibiotic resistance profile and multiple antibiotic resistance index of individual Escherichia coli isolated from meat samples in Sabo Market, Ikorodu.

(85.00%), tetracycline (73.33%), and ampicillin (71.67%) but susceptible to gentamicin (88.33%), ciprofloxacin (85.00%), sulphamethoxazole/ trimethoprim (85.00%), chloramphenicol (83.33%), and ceftriaxone (80.00%). Intermediate resistance was observed for all the antibiotics examined, and it ranged from 3 to 10%. The E. coli of meat origin being resistant to antimicrobials can be linked to their use in animal production. Residues from these antimicrobials can also be deposited in meats which can be transferred into humans when consumed. The overall consequence is humans not responding to antimicrobial treatments due to the presence of resistant strains or residues in them. In Ghana, antibiotics are mainly used as prophylactics and treatment of sick animals, rather than as growth promoters. Ekli et al. reported that antimicrobials including ciprofloxacin (32.0%), sulphamethoxazole/trimethoprim (17.1%), gentamicin (1.8%), ceftriaxone (0.9%), chloramphenicol (0.9%), and tetracycline (0.9%) were used by farmers in Wa, municipality of Ghana, as prophylactics or to treat animal diseases [1]. They also indicated that the farmers (73.2%) did not observe withdrawal periods when they administer, or antimicrobials are administered to their animals before sales or slaughter. These prone bacteria of these animals develop resistance to antimicrobials and deposition of antimicrobial residues in their muscle tissues.

Adzitey observed that E. coli isolated from cow meat in Ghana were resistant to tetracycline (44.44%), erythromycin (68.89%), and chloramphenicol (44.44%), but susceptible to ciprofloxacin (95.56%), sulphamethoxazole/trimethoprim (82.22%), and gentamicin (75.56%) [10]. Resistance to tetracycline and erythromycin but not chloramphenicol was higher in the present study compared with Adzitey [10]. Similarly, high susceptibility to ciprofloxacin and gentamicin was found in both studies [13]. Also, Rasmussen et al. reported that E. coli from locally produced chicken meats were resistant to tetracycline (88.9%) and ampicillin (69.4%), while those from imported chicken meats were resistant to tetracycline (57.5%) and ampicillin (61.6%). Resistance to ampicillin in locally produced chicken meat was similar to the current study but not the rest. Saud et al. found that E. coli isolated from chicken meats in Bhaktapur Metropolitan City, Nepal, were resistant to gentamicin (24.2%) and tetracycline (60.6%), which contradicts this study [28]. E. coli from chicken meats in Indonesia were resistant to tetracycline (79.24%) and chloramphenicol (9.43%), which were similar to this study. Altalhi et al. observed that E. coli isolated from retail raw chicken meat in Taif, Saudi Arabia, were resistant to ampicillin (78.4%), chloramphenicol (32.4%), and gentamicin (24.3%) [27,32]. Resistance to ampicillin was similar to this study but lower for chloramphenicol and gentamicin. Martínez-Vázquez et al. reported that E. coli from retail meats in Tamaulipas, Mexico, were resistant to ampicillin

(92%) and tetracycline (75%), which were comparable to this study [33].

The multiple antibiotic (MAR) index ranged from 0.13 (resistant to one antibiotic) to 1.0 (resistant to eight antibiotics) (Table 3). Bacteria have originated from a high-risk source of contamination where several antibiotics or growth promoters are used while showing bacteria from the source with less antibiotic use [34,35]. A completely resistant isolate has a MAR index of 1.0. The E. coli isolates were resistant to one (13.33%), two (16.67%), three (41.67%), four (13.33%), and five (8.33%) antimicrobials. Resistance to zero, six, seven, and eight antimicrobials was 1.67% each. The E. coli isolates also exhibited twenty-three (23) different resistance patterns. The resistance pattern TeAmpE (tetracycline-ampicillin-erythromycin) was the most common and was exhibited by sixteen isolates. Most of the E. coli isolates exhibited a MAR index of ≥0.25 reflecting a greater resistance to the group of antimicrobial agents studied. This means that there is greater antimicrobial use in production on the farms the animals were reared, which needs the attention of all relevant stakeholders in Ghana. Furthermore, E. coli isolates of meat origin with a MAR index of 0.4 and above are associated with human faecal contamination, while a MAR index of less than 0.4 is associated with nonhuman faecal contamination [36]. Based on this assumption, 26.7% of the samples were human faecal contamination and the rest were not. It has been reported that meat sellers at Sabo markets do not adhere to strict hygiene in the sale of meat, and this could contribute to faecal contamination (Adzitey et al. [37]). Similarly, Adzitey showed that E. coli isolated from beef in Techiman exhibited twenty-five (25) resistance patterns, and the MAR index ranged from 0.11 to 0.78. Adzitev also found that majority of E. coli isolates were resistant to three antimicrobials (14 isolates), followed by four antimicrobials (13 isolates) [10]. In addition, three and one isolates were resistant to 5 and 7 antimicrobials, respectively.

Multidrug resistance (MDR), that is, resistance to 3 or more different classes of antimicrobials, was observed in 41 (68.33%) of the isolates. Multidrug-resistant *E. coli* can be transferred from one carcass to the other and finally consumed by humans. Multidrug resistance is a cause for concern because it limits therapeutic options available for animal and human treatment. *E. coli* isolates of meat origin exhibiting multidrug resistance with susceptible ones serve as sources of resistant genes and increase the chances for the transfer of resistance genes to those that are sensitive. In Nigeria, Kehinde et al. reported that 4.8% of *E. coli* from meat sources were multidrug-resistant to cefuroxime-chloramphenicol-ampicillin [15]. Altalhi et al. found that *E. coli* of raw chicken meat were resistant to at least one antimicrobials [27]. They also found that 86.5% were resistant to at least three

antimicrobials. Saud et al. observed 52.5% multidrug resistance in *E. coli* isolates of meat origin (chicken and buffalo meat) [28]. In addition, they found overall multidrug resistance of 69.81%, and resistance to zero, one, two, three, four, five, and six antibiotics was 13.21%, 16.98%, 33.96%, 15.09%, 20.75%, 0.00%, and 0.00%, respectively [30]. In Tamaulipas, Mexico, Martínez-Vázquez et al. detected that 92.4% of *E. coli* obtained from retail meats exhibited multi-resistance [33].

Conclusion

Overall, 164 (84.00%) of the meat samples were positive for *Escherichia coli*, and the overall total coliform counts were 4.22 log CFU/cm². Contamination of the meat samples by *Escherichia coli* and coliforms did not differ significantly (> 0.05) from each other. Phenotypic characterization revealed a high resistance to ampicillin, erythromycin, and tetracycline but susceptibility to ceftriaxone, chloramphenicol, ciprofloxacin, gentamicin, and sulfamethoxazole/trimethoprim. The high resistance of the *Escherichia coli* isolates of meat origin to the various antibiotics observed requires that farmers should use fewer antibiotics in animal production. They should rely on good management practices to prevent the occurrence of diseases that will necessitate the use of antibiotics. Further research will involve the use of molecular characterization to determine resistant genes, virulence, and whole-genome sequencing.

Data Availability

All datasets on which the conclusions of the manuscript rely are presented in the paper.

Conflicts of Interest

The authors declare no competing interests.

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