

Antimicrobial Resistance and Extended Spectrum β -Lactamases of *Escherichia coli* isolates from Raw Meat in Greater Accra Region, Ghana

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Abstract

Background Typically, raw meat can be contaminated with antimicrobial resistant pathogens at unhygienic slaughter and sale points. Consumption of meat contaminated with antimicrobial resistant *E. coli* is associated with grave health care consequences. The aim of this study was to determine the microbial quality of raw meat, the antimicrobial susceptibility and Extended Spectrum Beta Lactamase (ESBL) production in *E. coli* isolates from raw meat.

Methods We collected 205 meat samples from beef (n=81), chevon (n=108) and mutton (n=16) at three slaughter sites in the Greater Accra Region of Ghana. We performed Total Plate Counts (TPC) and Total Coliform Counts (TCC) and isolated *E. coli* using standard bacteriological methods. Presumptive isolates were confirmed by matrix-assisted laser desorption ionization-time of flight mass spectrometry (MALDI-TOF-MS). We assessed the susceptibility to 11 antibiotics using the Kirby Bauer disk diffusion method and assessed ESBL production using the combination disk test. PCR amplification of associated resistant genes *bla* TEM, *bla* SHV, and *bla* CTX-M was performed for presumptive ESBL isolates.

Results Total Plate Counts exceeded the acceptable limit of 5.0 log CFU/ cm² in 60.5% (124/205) of raw meat samples. Total Coliform Counts in 70.7% (145/205) of samples were in excess of the acceptable limit of 2.5 log CFU/cm². *E. coli* was detected in about half of raw meat samples (48%), ranging from 9.5% -79.0% among the slaughter sites. Isolates were susceptible to meropenem (100%), ceftriaxone (99%), cefotaxime (98%), chloramphenicol (97%), gentamycin (97%), ciprofloxacin (92%) and amikacin (92%), but resistant to ampicillin (57%), tetracycline (45%), sulfamethoxazole-trimethoprim (21%) and cefuroxime (17%). Multi-drug resistance (MDR) was identified in 22% of the isolates. We detected the *bla* TEM gene in 4% (4/98) of *E. coli* isolates in this study.

Conclusion The levels of microbial contamination of raw meat in this study were unacceptable. Meat handlers and consumers are at risk of foodborne infections from *E. coli* including ESBL producing *E. coli* that are resistant to most antibiotics in use. We recommend an enhanced surveillance for antimicrobial resistance in food products for the early detection of emerging resistant bacteria species in the food chain.

Background

The emergence of antibiotic resistance in bacteria commonly found in food animals has garnered attention globally for its potential contribution to human colonization with antibiotic resistant bacteria. Antibiotics are used in food animals for therapeutic and non-therapeutic purposes. The continuous use of antibiotics in food animal production is cited as a major determinant for carriage of antibiotic resistant bacteria in food animals (1). A proportion of antimicrobial classes are used in both animals and humans, creating the need to monitor the spread of resistant bacteria from animals to humans at all stages of the transmission pathway (2).

Food products of animal origin contaminated with drug resistant bacteria may provide a direct route for human colonization (3). Meat is an important part of the Ghanaian diet and is often sourced from open markets and cold stores (4). Although selective pressure due to antibiotic use in primary production is considered as a major source of antibiotic resistant bacteria in livestock products, sanitary conditions at slaughter, sale and processing points may affect the pattern and intensity of spread along the food chain (5).

Escherichia coli is a common colonizer of the intestinal tract of humans and animals. It is frequently isolated as the causative organism in urinary tract infections, neonatal meningitis and bacteremia in humans (6). The presence of pathogenic strains of *E. coli* in meat and dairy products has been associated with foodborne disease outbreaks in humans (7).

Extended spectrum beta lactamase (ESBL), an enzyme produced by some gram negative bacteria, pose a peculiar challenge for treatment as they are resistant to penicillins, cephalosporins and monobactams (8). ESBL-producing bacteria such as *E. coli* have been reported in patients with urinary tract infections worldwide (9). The contribution of the food chain to the occurrence of ESBL bacteria in humans is widely debated with fecal-oral and nosocomial transmissions largely influencing its emergence and spread in humans(10). Monitoring the trend of spread of ESBL positive *E. coli* through the food chain is necessary in settings where humans have routine contact with livestock and their products, because of the increased risk of spread of resistance genes in these interactions.

In Ghana, it has been found that, resistance in enterobacteriaceae to commonly used antimicrobial agents is widespread (11). Research evidence on microbial contamination of meat and their antibiotic resistance patterns is limited and thus, it is difficult to assess the risk posed to human health through the food chain. Yet, such evidence is critical for guiding future antimicrobial therapy. The aim of this study was to determine the level of microbial contamination of raw meat, presence of *E. coli* and their resistance to commonly prescribed antibiotics for human infections. This study also detected the occurrence of ESBL production among *E. coli* isolates recovered from raw meat.

Materials And Methods

Study sites and sample collection

In this study, we collected raw meat samples from three slaughter sites in the Greater Accra region of Ghana. The sites consisted of a public slaughterhouse (PS), a slaughter slab (SS) and a privately-owned slaughterhouse (PO). Livestock such as cattle, sheep and goats were slaughtered daily at all three sites. Butchers operating at these slaughter sites obtained their livestock mainly from the three northern regions of Ghana. All three sites were points of meat sale and had the basic modules of production, which included a slaughter floor and lairage/holding pen. All parts of carcasses are sold as meat in large cuts and small cuts at these sites. The daily throughputs of livestock slaughtered at these sites ranged from 30-150 for large stock (cattle) and 20-300 for small stock (sheep and goats). Livestock at each site were selected randomly on predetermined sampling days. A total of 205 surface swab samples were collected from beef (n=81), chevon (n=108) and mutton (n=16) at the three slaughter sites (PS=63, SS=79, PO=63). Sample collection was done immediately after carcass dressing with a sterile cotton swab and a sterile template of size 100 cm² for cattle and 25 cm² for goat and sheep carcasses. For each beef carcass, surface swabs were collected from the thigh, brisket and flank and pooled into a single tube containing 10ml of Buffered Peptone Water (BPW). Swabs from goat and sheep carcasses was obtained from thigh, brisket and mid-loin (12). All samples were transported to the laboratory within 2 hours of sample collection for processing.

Enumeration of bacteria

Total plate counts and total coliform counts were performed for all samples using the pour plate method. Serial dilutions of ten-fold units of each sample were plated on Plate Count Agar (Oxoid) and incubated for 48 hours at 30°C. Following incubation, plates with colonies ranging from 30-300 were counted and expressed in CFU/cm². The total coliform count was performed in the same way using Brilliance *E. coli*/ Coliform selective agar (Oxoid). The results of both counts were interpreted using guidelines set by the Ghana Standards Authority and the European Commission Regulation on the microbial criteria for food stuffs (13,14). Limits to total coliform counts were not specified in these documents hence limits set for Enterobacteriaceae counts in both documents were adopted for total coliform counts.

Identification of *E. coli* in meat

To isolate *E. coli*, samples were pre-enriched in Brain Heart Infusion (BHI) at 37°C for a period of 24 hours. Ten (10µl) microliters of each sample were then plated on MacConkey agar and incubated overnight at 37°C. We identified *E. coli* by colonial morphology and confirmed the isolates using Matrix Assisted Laser Desorption/Ionization -Time of Flight Mass Spectrometry (MALDI-TOF MS). Colonies from fresh overnight cultures were spotted on the MALDI-TOF MS target plate. One (1) µl of formic acid was then added and allowed to dry for 15 min. One (1) µl of matrix preparation was placed on each sample and left to dry for a further 15 minutes. MALDI-TOF MS was then conducted and ionization peaks (spectra) generated were matched against the integrated reference library of the MALDI system for confirmation of species of bacteria.

Antimicrobial susceptibility testing

Antimicrobial susceptibility testing was done by the Kirby Bauer disk diffusion method using the following antimicrobial agents (Oxoid Ltd., Hampshire, UK): ampicillin (10ug), tetracycline (30ug), cefuroxime (30ug), cefotaxime (5ug), ceftriaxone (30ug), amikacin (30ug), gentamycin (10ug), chloramphenicol (30ug), ciprofloxacin (5ug), sulphamethoxazole trimethoprim (25ug) and meropenem (10ug). The procedure consisted of the preparation of a standard inoculum (0.5 McFarland) and inoculation on Mueller Hinton (MH) agar plates for 18 hours at 35°C (15). Interpretation of zone sizes was done using the EUCAST guideline (16). *E. coli* ATCC 25922 was used to monitor the performance of the test. In this study, we defined multidrug resistance (MDR) as resistance to three or more classes of antimicrobial agents.

Phenotypic and genotypic detection of ESBL production

The combination disk method was used to identify ESBL producing *E. coli*. Briefly, ceftazidime disks and ceftazidime combined with clavulanic acid disks were positioned 30 mm apart on the inoculated plates, and incubated overnight at a temperature of 37°C. Isolates were classified as ESBL positive if the difference between the inhibition zone diameter of ceftazidime combined with clavulanic acid and diameter of the inhibition zone for the ceftazidime-only disk was ≥5mm (17). Cefotaxime disks and cefotaxime combined with clavulanic acid disks were used concurrently with ceftazidime for confirmation of ESBL production. DNA was extracted from presumptive colonies for the genotypic detection of ESBL production.

Bacterial DNA extraction consisted of placing pure colonies of overnight growth in 1ml of distilled water. The mixture was boiled for 10 minutes and centrifuged for 5 minutes, at 1000 rotations per minute. Using the supernatant as a DNA template, PCR was done to detect ESBL resistance genes (*bla*_{TEM}, *bla*_{SHV}, and *bla*_{CTX-M}).

The final volume of 25µl contained 2µl DNA template, 10mM of each primer (Table 1) (18), PCR grade water (10µl) and multiplex PCR master mix (13µl) (QIAGEN). Multiplex PCR was carried out in a thermal cycler with the following cycling conditions: 95°C for 5 minutes, 35 cycles of 95°C for 30 seconds, 60°C for 30 seconds, 72°C for 2 minutes, and a final extension lap at 72°C for 10 minutes.

Statistical analysis

Descriptive statistics were performed on carriage rates of *E. coli* and their resistance patterns. The Chi-square test was used to assess differences in the proportion of *E. coli* detected in raw meat between the three slaughter sites. A Kruskal-Wallis test was performed to determine if there were significant differences in microbial counts in raw meat among the slaughter sites. Statistical analyses were performed at 95% confidence level using STATA version 15.0.

Results

Microbial contamination of raw meat

Overall, 124 (60.5%) samples from raw meat in this study exceeded the maximum limit of 5.0 log CFU/cm², for total aerobic counts for cattle, sheep and goat carcasses set by the Ghana Standards Authority and the European Commission Regulation (EC) on the microbial criteria for food stuffs. Total Plate Counts for all 205 samples ranged from 2.86 log CFU/cm² to 7.26 log CFU/cm² with a median of 5.28 log CFU/cm² (Table 2). Majority of the samples exceeding the maximum limit were from PS (93.7%, 59/63) and SS (77.2%, 61/79). Of the 69 meat samples obtained from PO, 21.7 % exceeded the maximum limit.

More than half of all samples (70.7%) had coliform counts exceeding the acceptable limit of 2.5 log CFU/cm². Total coliform counts ranged from 0.11 log CFU/cm² to 5.76 log CFU/cm² with a median of 3.12 log CFU/cm² (Table 2). The proportion of samples exceeding the maximum limit for coliform counts was 98.4 % at PS, 97.5% at SS and 11.1 % at PO. The TPC and TCC distribution among the three slaughter sites is shown in Figures 1 and 2.

The Kruskal-Wallis test showed that there was a statistically significant difference in total plate counts between the three slaughter sites, $\chi^2(2) = 73.81$, $p = 0.0001$. The distribution of coliforms in samples from the three sites were also found to differ significantly, $\chi^2(2) = 110.94$, $p = 0.0001$.

Contamination of raw meat with *E. coli*

E. coli was detected in 48% (98/205) of all samples. The proportion of meat contaminated with *E. coli* was 79% (50/63) at PS, 53% (42/79) at SS and 9.5% (6/63) at PO (Table 3). The rate of *E. coli* contamination was 62% (50/81) in samples obtained from beef, 41% (44/108) for chevon and 25% (4/16) for mutton. The results of the chi-square test showed that there was a significant difference in the proportions of *E. coli* contaminated meat among the slaughter sites ($p < 0.05$).

Antimicrobial susceptibility and detection of ESBL production

E. coli isolates recovered were frequently resistant to ampicillin (57%), tetracycline (44%), cefuroxime (21%), and sulphamethoxazole /Trimethoprim (17%) (Table 4). High rates of susceptibility were observed for ceftriaxone (99%), cefotaxime (98%), chloramphenicol (97%), gentamycin (97%), ciprofloxacin (92%) and amikacin (92%) (Table 4). All isolates were susceptible to meropenem. Of the 98 *E. coli* isolates tested, 84% (82/98) showed resistance to at least one out of the eleven antimicrobial agents. In all, 22% (22/98) of the isolates were multidrug resistant. The highest frequency of MDR was found among isolates from beef (n=13) (Table 4). Among the multidrug resistant *E. coli*, concurrent resistance to penicillin, tetracycline and sulphonamide/trimethoprim (32%), was most common (Figure 3). Other patterns observed were resistance to penicillin, tetracycline, SXT, and fluoroquinolone class of antibiotics (14%), penicillin, tetracycline and cephalosporin class of antibiotics (14%), penicillin, tetracycline and amphenicols (10%) and penicillin, aminoglycoside, SXT, tetracycline and cephalosporin (10%). Most of the MDR patterns observed in the *E. coli* isolates (95%) showed resistance to penicillin and tetracycline class of antibiotics in addition to other antimicrobial classes. Out of the 98 *E. coli* isolates recovered from raw meat, 14 isolates were presumed to be ESBL producers based on the results of the combination disk test. Out of these, four (4) *E. coli* isolates were found to have the bla_{TEM} gene. These isolates were multidrug resistant with most of them (3/4), showing phenotypic resistance to ampicillin, amikacin, tetracycline, SXT and cefuroxime. CTX-M and SHV genes were not detected in the isolates screened.

Discussion

The study findings show that raw meat, ready for sale at the selected study sites in most cases (> 60%) had microbial loads exceeding the acceptable limit and thus, were of poor quality. The majority of slaughtering in Ghana is conducted at slaughterhouses and slabs with sub-standard conditions of slaughter and minimal oversight from food safety authorities (19). The absence of hygiene standards at points of slaughter has been linked to increased microbial contamination of carcasses (20). High coliform and plate counts observed at the Public Slaughterhouse (PS) and Slaughter Slab (SS) may stem from the introduction of contaminants from floor surfaces, hides and faeces of livestock in the absence of standard operating procedures that ensure sanitary standards are met in the slaughter environment. Contamination of meat in such situations may also be exacerbated by inadequate slaughter infrastructure that ensures that work areas are properly segregated (21). The consequence for human health lies in the potential exposure of consumers to disease causing organisms and carriage of drug resistant bacteria (22).

E. coli is commonly used as an indicator organism for assessing food and water hygiene (23). Although *E. coli* was detected in close to half of raw meat sampled, wide-ranging proportions of *E. coli* contamination was observed at the slaughter sites (9.5–79%). Previous studies on *E. coli* in beef from retail joints in Northern Ghana similarly reported wide-ranging values of 0-100% (24). While high *E. coli* contamination rates often indicate poor slaughter hygiene, the presence of other environmental contaminants in heavily contaminated settings may reduce recovery rates of *E. coli*. Slaughter slabs have less adequate service modules in comparison to slaughterhouses in Ghana. Privately owned slaughterhouses often have better slaughter infrastructure and compliance to hygienic practices in an effort to maintain customer satisfaction. This phenomenon may account for the low levels of *E. coli* contamination (9.5%) observed for the Privately Owned Slaughterhouse (PO).

The highest rate of resistance in *E. coli* was observed for ampicillin (57%). This follows a commonly reported pattern of β lactam antibiotic resistance rates of more than 50% in foods of animal origin (25, 26). While these observations were attributed to the common use of ampicillin and penicillin derivatives in food animals, the scale of resistance is largely dependent on the food animal type and route of administration (27). Sub-therapeutic doses of β lactams administered through feed and water in poultry and the pig industry may yield higher rates of resistance than in cattle where parenteral administration is common. The levels of resistance to tetracycline (44%) and SXT (17%) observed in this study have similarly been reported in *E. coli* from beef in Ghana (44% and 18%) (28). Tetracyclines are commonly used for therapy in humans and livestock and for growth promotion in intensive farming systems through feed (29). High transferability of tetracycline resistance determinants in gut bacteria of livestock in such settings may contribute to the observed rates of tetracycline resistance in *E. coli* isolated from meat, meat products and the environment (30).

We documented lower rates of resistance of *E. coli* to cefuroxime in comparison to isolates recovered from clinical specimens in Ghana (31). In comparison to its use in humans, second generation cephalosporins are less commonly used in food animals and are broadly approved for the treatment of mastitis in dairy cattle (32). Among SXT resistant *E. coli* isolates of animal origin, ampicillin and tetracycline have been identified in previous studies as common co-transferred resistant phenotypes (33). The MDR rate of 22% in *E. coli* isolates was similar to studies carried out by Saud et al on raw buffalo meat while significantly higher rates were observed for chicken in the same study (34). These findings can be attributed to the routine use of antibiotics in poultry feed and less common use of oral antibiotics in large animals. The detection of the *TEM* gene in four *E. coli* isolates is comparable to studies by Sheikh et al that found a lower incidence of the *TEM* gene in ground beef as compared to other meat types (35). Several factors may account for the low proportion of ESBL producing *E. coli* recovered in this study. Studies conducted by Aldeyab et al posited that community incidence of ESBLs was significantly linked to amoxicillin-clavulanic acid use while hospital incidence was linked to fluoroquinolone use in health care settings (36). ESBL production in enterobacteriaceae recovered from foods of animal origin may reflect the burden of resistance to certain classes of antimicrobial drugs in gut bacteria in different food animals. The presence of ESBL-producing *E. coli* in raw meat is a major concern for food safety as these enzymes are transferred easily amongst Enterobacteriaceae capable of causing infections in humans. The consequences thereof for human health include prolonged hospital stays, increased mortality and morbidity and increased costs (37) .

Our isolates showed high susceptibility rates to meropenem, ceftriaxone, chloramphenicol, and gentamycin. Third generation cephalosporins and phenicols are not commonly used in livestock in Ghana, thus their efficacy has been maintained (38). Preserving the efficacy of these drugs is a necessary measure as 3rd generation cephalosporins fall in the category of critically needed antimicrobials used to treat life-threatening infections caused by *E. coli* in humans.

While this study provides evidence of the presence of drug resistant bacteria in raw meat, its interpretation is limited to known determinants of resistance in the primary production chain, as environmental determinants were not assessed.

Conclusions

Our study found that more than half of meat samples had unacceptable levels of microbial contamination. Contamination with *E. coli* was found in 48% of meat samples with multidrug resistance observed in 22% of isolates. Meat handlers in such environments and consumers are at risk of foodborne infections from *E. coli* including ESBL producing *E. coli* that are resistant at various levels to most antibiotics in use. This calls for further investigation of the sources of meat contamination and an assessment of hygiene standards in slaughterhouses in the Greater Accra Region to improve meat quality. Enhanced surveillance in food products of animal origin is required to monitor trends in resistance patterns and promptly detect emerging resistant bacteria.

Abbreviations

AMR

Antimicrobial resistance

ATCC

American type culture collection

CFU

Colony forming unit

EUCAST

European Committee on Antimicrobial Susceptibility Testing

ESBL

Extended spectrum beta lactamase

TPC

Total Plate count

TCC

Total coliform count

Declarations

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Authors' contributions

ED and BE were involved in the design, acquisition of data, statistical analysis, interpretation of data and drafting of the manuscript. BE, AEA and ADA supervised the project work. EK and BBK contributed to data analysis and interpretation. All authors contributed to the writing of the manuscript and approved the final manuscript.

Competing interests

The authors declare that they have no competing interests.

Availability of data and materials

All essential data and materials have been provided in the manuscript.

Consent for publication

Not applicable

Ethics approval and consent to participate

This study formed part of a thesis for a Master of Philosophy (MPhil) degree in Applied Epidemiology and Disease Control, and was approved by the Department of Epidemiology and Disease Control at the School of Public Health, University of Ghana. Sample collection was carried out with consent from butchers and livestock owners present at slaughter after permission was obtained from slaughterhouse authorities.

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Tables

Table 1. Oligonucleotide primers used to detect ESBL genes in *E. coli* isolates

Genes	Primer sequences	Expected amplicon size(bp)
TEM-1	Forward: 5'-GAGACAATAACCCTGGTAAAT-3' Reverse: 5'-AGAAGTAAGTTGGCAGCAGTG-3'	459
CTX-M	Forward: 5'-GAAGGTCATCAAGAAGGTGCG-3' Reverse: 5'-GCATTGCCACGCTTTTCATAG-3'	560
SHV	Forward: 5'-GTCAGCGAAAAACACCTTGCC' Reverse: 5'-GTCTTATCGGCGATAAACAG-3'	383

Table 2. Microbiological quality of raw meat samples from selected slaughterhouses in the Greater Accra region of Ghana

Slaughterhouse	Median count in log CFU/cm ² (range)	
	Total Plate Count	Total Coliform Count
PS	5.33 (4.92-5.65)	3.12 (2.42-3.92)
SS	6.15 (3.15-6.87)	4.05 (1.52-5.76)
PO	3.57 (2.86-7.26)	0.84 (0.11-5.54)
Total (all sites)	5.28 (2.86-7.26)	3.12 (0.11-5.76)

PS- Public slaughterhouse, SS- Slaughter slab, PO—Privately-owned slaughter facility

Table 3. Distribution of *E. coli* isolated from meat at slaughterhouses

	PS	SS	PO	Total
No. of samples	(n=63)	(n=79)	(n=63)	(N=205)
No. of <i>E. coli</i> isolates	50 (79%)	42 (53%)	6 (9.5%)	98 (48%)
Beef	42 (84%)	8 (19%)	-	50 (51%)
Mutton	-	3 (7%)	1 (17%)	4 (4%)
Chevon	8 (16%)	31 (74%)	5 (83%)	44 (45%)

PS- Public slaughterhouse, SS- Slaughter slab, PO—Privately-owned slaughter facility

Table 4. Antimicrobial resistance profile of *E. coli* isolates from raw meat at the selected slaughter sites in the Greater Accra region of Ghana

Antimicrobial agent	PS n=50 (%)	SS n=42, (%)	PO n=6 (%)	Total N=98, (%)
Ampicillin	23 (46)	33 (79)	0(0)	56 (57)
Tetracycline	26 (52)	18 (43)	0(0)	44 (45)
Cefuroxime	8 (16)	13 (30)	0(0)	21 (21)
Sulphamethoxazole/Trimethoprim	10 (20)	7 (16)	0(0)	17 (17)
Amikacin	2 (4)	6 (14)	0(0)	8 (8)
Ciprofloxacin	4 (8)	3 (7)	1(16)	8 (8)
Cefotaxime	1(2)	1(2)	0(0)	2 (2.1)
Gentamycin	2(4)	1(2)	0(0)	3 (3.1)
Chloramphenicol	2(4)	1(2)	0(0)	3 (3.1)
Ceftriaxone	0(0)	1(2)	0(0)	1 (1)
Meropenem	0(0)	0(0)	0(0)	0 (0)

n= Number of *E. coli* isolates

PS- Public slaughterhouse, SS- Slaughter slab, PO—Privately-owned slaughter facility

Figures

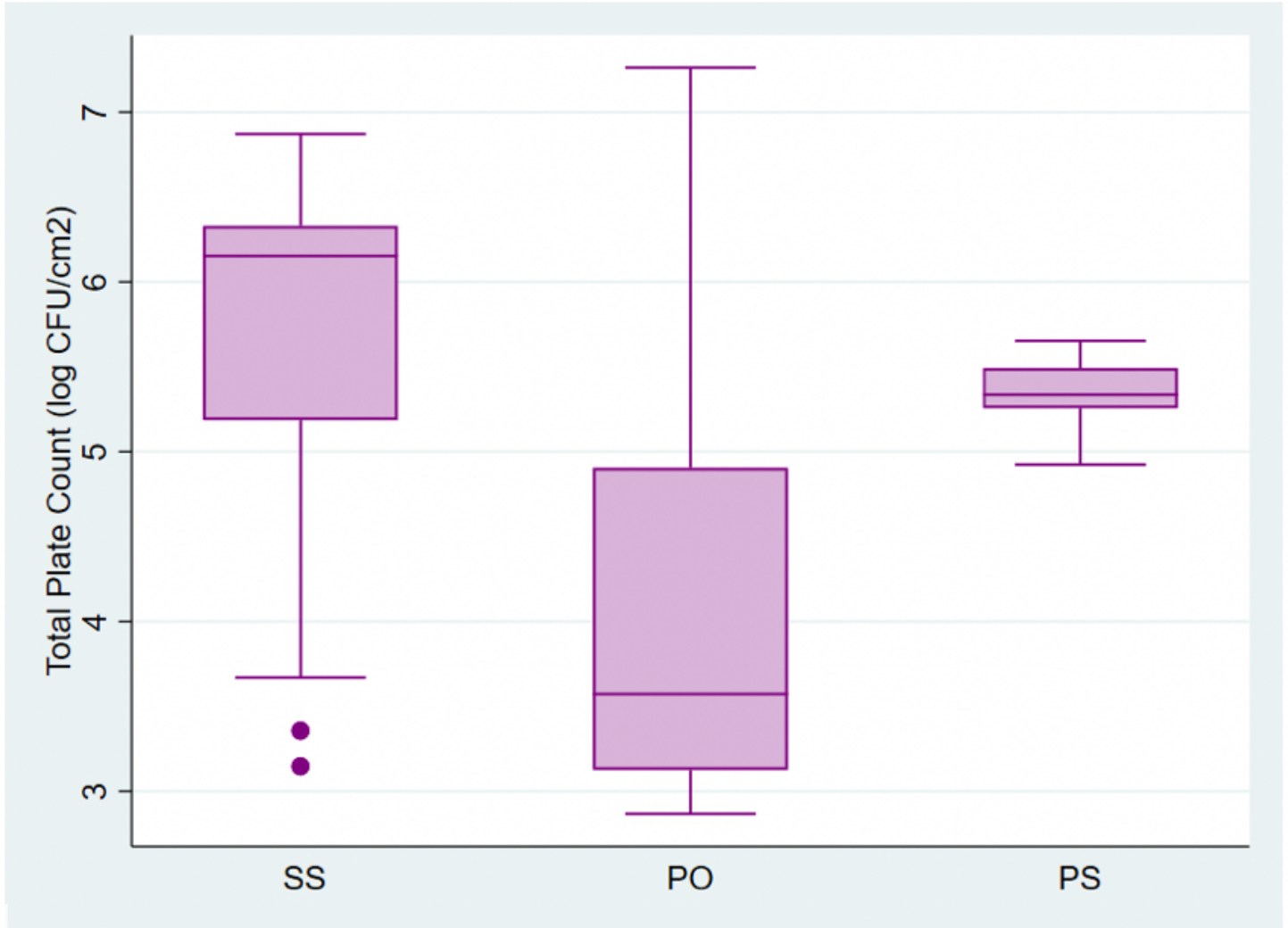


Figure 1

Distribution of Total Plate Counts among the three slaughter sites. PS- Public slaughterhouse, SS- Slaughter slab, PO—Privately-owned slaughter facility

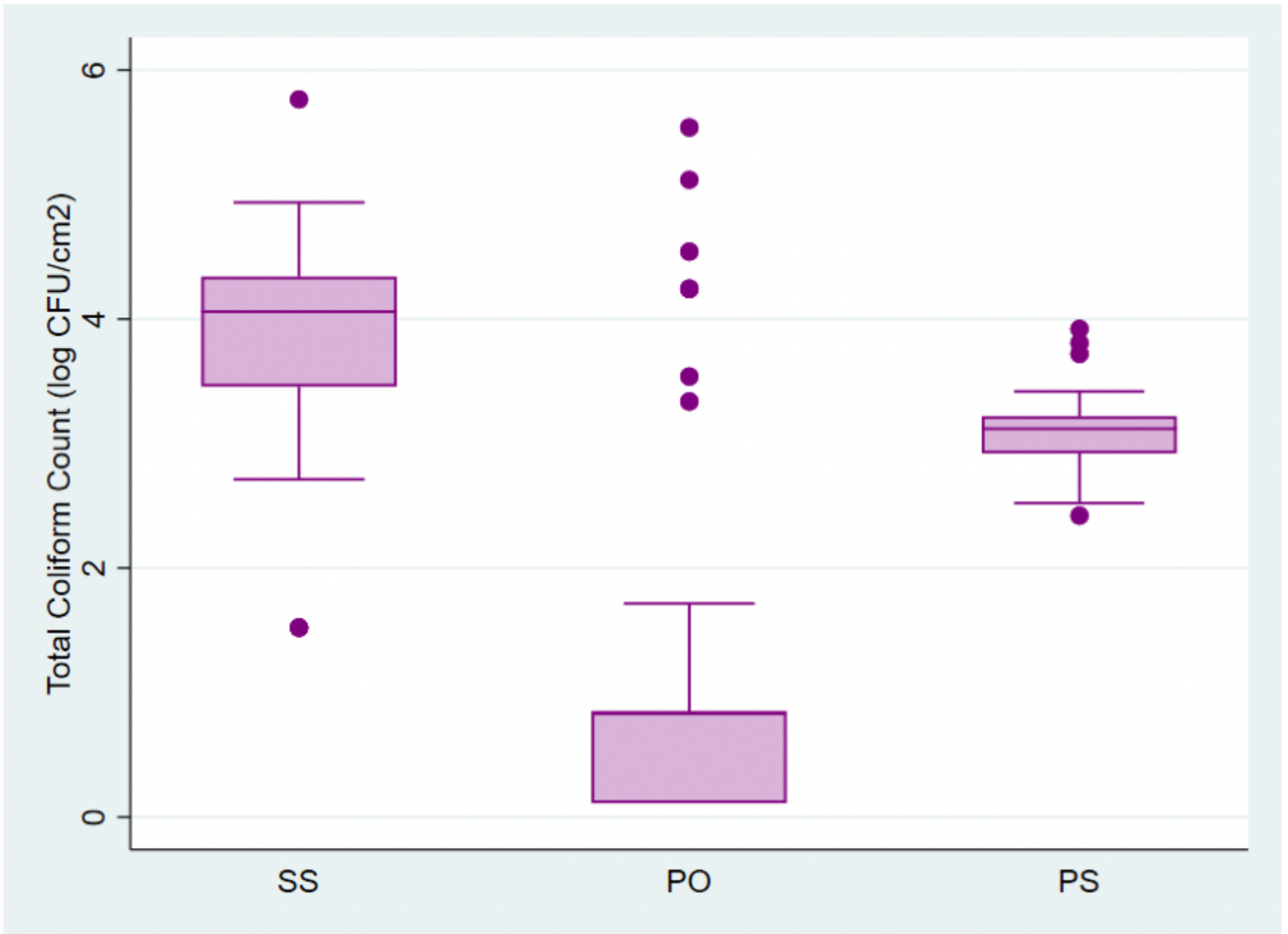


Figure 2

Distribution of Total Coliform Counts among the three slaughter sites. PS- Public slaughterhouse, SS- Slaughter slab, PO—Privately-owned slaughter facility

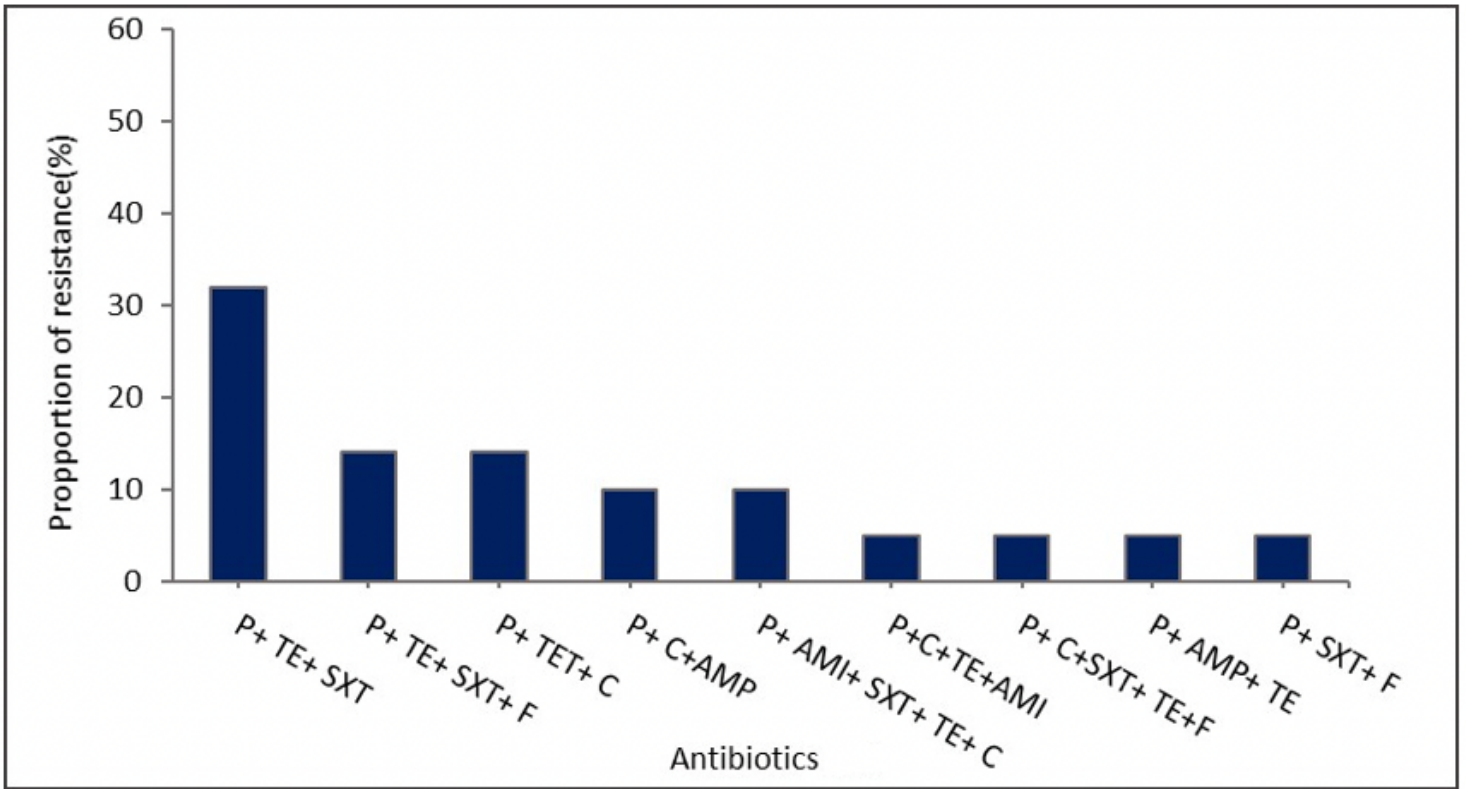


Figure 3

Pattern of multidrug resistant E. coli isolates. P=Penicillin, TE= Tetracycline, SXT=Sulphamethoxazole/Trimethoprim, AMI= Aminoglycoside, C=Cephalosporin, F=Quinolones/Fluoroquinolones, AMP=Amphenicol