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On-site slaughter and dressing in retail chicken outlets: Escherichia coli contamination and antimicrobial resistance on food-contact surfaces in Dhaka Division, Bangladesh

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Abstract

Background

The retail poultry-processing environment is a critical reservoir for multidrug-resistant (MDR) *Escherichia coli*, posing significant zoonotic and public health risks. In Bangladesh, there is limited regulatory oversight on antibiotic application in poultry production, leading to frequent and indiscriminate use which allows resistant bacteria to persist on processing surfaces and potentially enter the human food chain, posing significant public health risks. This study assessed the prevalence, antimicrobial resistance (AMR) profiles, and genetic determinants of *E. coli* isolated from retail chicken outlets in Dhaka Division.

Methods

A total of 180 swab samples were collected from knives, cutting tables, and dressing machines across six outlets in Savar, Sreepur, and Dhamrai upazilas from July to October 2025. *E. coli* was isolated using selective culture, biochemically confirmed via VITEK®2, and molecularly validated by PCR targeting the malB gene. Susceptibility to twelve antimicrobial agents was determined by disc diffusion and interpreted against CLSI criteria, and isolates were screened by PCR for blaOXA-1, sul1 and sul3.

Results

Of 180 samples, 90 (50%) yielded *E. coli*, with knives showing the highest contamination (57.9%), followed by tables (47.6%) and dressing machines (45.0%). Resistance was most frequent to ampicillin (96%), trimethoprim (96%), azithromycin (93%), nalidixic acid (89%) and tetracycline (89%); meropenem remained active against 93% of isolates. The Spearman correlation analysis revealed strong positive associations among β-lactam antibiotics, moderate correlations between β-lactams and aminoglycosides, and negative correlation between ciprofloxacin and meropenem, suggesting both co-selection and independent resistance mechanisms among the studied isolates. Multiple Antibiotic Resistance (MAR) indices ranged from 0.00 to 0.83, with 93.3% exceeding 0.2, indicating significant selective pressure from antimicrobial exposure. Molecular analysis revealed sul-3 (26.7%) and blaOXA-1 (23.3%) as predominant resistance genes, with 13.3% of isolates harboring multiple determinants.

Conclusion

E. coli was present on half of all food-contact surfaces at comparable frequency across implements, and most isolates were resistant to multiple antimicrobial classes. Contamination was distributed across the dressing station rather than concentrated on any single implement, so hygiene measures targeting one

item are unlikely to suffice. The gap between phenotypic resistance and the genes screened indicates further determinants warranting genomic investigation.

INTRODUCTION

Antimicrobial resistance (AMR) constitutes one of the most pressing threats to global health security. The Global Research on Antimicrobial Resistance (GRAM) project estimated 4.95 million deaths associated with bacterial AMR in 2019, of which 1.27 million were directly attributable to resistance (1). Updated analyses forecast 1.91 million attributable and 8.22 million associated deaths annually by 2050, with South Asia projected to bear the heaviest all-age mortality burden (2). *Escherichia coli* contributes disproportionately to this crisis, ranking first among all pathogens for resistance-associated mortality; third-generation cephalosporin-resistant and fluoroquinolone-resistant *E. coli* alone each accounted for 50,000–100,000 attributable deaths in 2019 (1). Accordingly, the WHO 2024 Bacterial Priority Pathogens List retains third-generation cephalosporin-resistant Enterobacterales as a standalone critical-priority category, underscoring the acute burden borne by low- and middle-income countries (LMICs) (3).

Poultry production occupies a critical node in the emergence and circulation of resistant *E. coli*. As one of the fastest-growing livestock sectors globally, poultry supplies an accessible, affordable protein source (4). In Bangladesh, the sector is economically vital, supporting rural livelihoods and national dietary demand (5), yet it sustains substantial losses from infectious disease (6). Antimicrobials are used extensively - therapeutically and at sub-therapeutic doses for prophylaxis, growth promotion, and risk mitigation (7). Crucially, these drugs are widely available without veterinary prescription, and sociodemographic surveys confirm frequent unguided use by producers (7, 8). Such sustained selective pressure enriches resistant commensal populations that readily mobilize along the food chain (9, 10).

E. coli is an ideal sentinel for tracking this mobilization. As a normal inhabitant of the avian gut and a routine contaminant of retail poultry meat (11), it also causes diverse human intestinal and extraintestinal pathologies (12, 13). While most poultry-derived strains are commensal, some belong to lineages of direct clinical relevance, and even opportunistic strains serve as critical reservoirs for transferable resistance determinants (13). Sulphonamide resistance genes (sul1, sul3) and the class D β-lactamase gene blaOXA-1 are frequently carried on mobile genetic elements, and integron-associated gene cassettes in animal faecal *E. coli* escalate in frequency with proximity to human activity (14). These characteristics make *E. coli* a practical indicator for AMR surveillance across animal, human, and environmental compartments - a One Health framing increasingly applied to poultry-associated resistance (10, 15).

In Bangladesh, the evidence base on poultry-associated resistant *E. coli* has expanded rapidly, yet it remains unevenly distributed across the value chain. A systematic review (2010–2021) documented consistently high resistance among poultry and poultry-environment isolates nationally (16). Subsequent studies have characterized *E. coli* from broiler cloacal swabs (17), farmworkers and sewage (18), farm environments (19), live-bird markets (20, 21), and retail meat (22–24). Antimicrobial use on commercial

farms has been directly linked to on-farm resistance patterns (25), and recent genomic surveillance in Dhaka has revealed pervasive multidrug resistance in retail poultry *E. coli* (26), with ESBL-producing strains isolated in 70% of meat and 79.7% of sewage samples (27). Collectively, this body of work has concentrated heavily on birds, carcasses, meat, and wastewater.

Food-contact surfaces within the retail processing environment, however, constitute a distinct and critically under-characterized node. In Bangladesh, most chicken is purchased live and dressed on-site; thus, slaughter, defeathering, evisceration, and portioning occur at the retail point itself. Knives, cutting tables, and mechanical dressing machines are used repeatedly across successive carcasses, frequently with limited intermediate cleaning or disinfection. Such surfaces accumulate organic residue, support persistent microbial biofilms, and act as potent matrices for cross-contamination to carcasses and handlers (28, 29). Because retail processing represents the final point at which contamination can be amplified before meat reaches the household, equipment surfaces are both a plausible reservoir for resistant *E. coli* and a highly tractable target for hygiene intervention. To our knowledge, the prevalence, resistance phenotypes, and resistance-gene content of *E. coli* on processing equipment in Bangladeshi retail chicken outlets have not been systematically described. This gap fundamentally limits the evidence available to guide stewardship and sanitation measures at the point of greatest consumer relevance.

This study was therefore designed to characterize *E. coli* contamination of poultry-processing equipment surfaces in retail chicken outlets in Dhaka Division, Bangladesh. Specifically, we sought to determine the prevalence of *E. coli* on knives, cutting tables, and dressing machines across sampling areas; characterize phenotypic antimicrobial susceptibility against a panel of clinically and veterinary relevant agents and quantify multiple antibiotic resistance (MAR) indices; determine the occurrence and co-occurrence of the resistance genes blaOXA-1, sul1, and sul3; and assess the concordance between resistance phenotypes and the genetic determinants examined. By targeting the equipment interface rather than birds or meat, this study evaluates whether retail processing surfaces function as active reservoirs and transmission vectors for antimicrobial-resistant *E. coli* within the poultry value chain.

MATERIALS & METHOD

2.1 Sampling area and period of study

A total of 180 samples were collected from six retail poultry processing outlets across three upazilas - Savar, Sreepur, and Dhamrai in Dhaka Division, Bangladesh, from July to October 2025. Two outlets were sampled per upazila to capture within-area variation in hygiene and operational practice. A total of 180 environmental swabs were collected across the six outlets, comprising 57 knife samples, 63 cutting-table samples, and 60 dressing-machine samples. These three surface types were selected to represent the principal points of potential cross-contamination in retail poultry processing. Outlets were selected on the basis of accessibility, willingness of proprietors to participate, and representativeness of typical small- to medium-scale retail chicken processing in peri-urban Dhaka.

2.2 Sample collection and preparation

From each outlet, samples were collected from three frequently contacted processing surfaces (knives, cutting tables, and dressing machines), which are considered critical points for microbial cross-contamination. Surface sampling was performed using sterile cotton swabs pre-moistened with phosphate-buffered saline (PBS; HiMedia Laboratories Pvt. Ltd., Mumbai, India) were used to sample approximately 100 cm^2 of each accessible surface whenever possible. For irregular surfaces such as knives, the entire blade surface was thoroughly swabbed using a rotating motion to maximize microbial recovery. Each sampling procedure required approximately 20–30 seconds per surface. After collection, swabs were immediately placed into sterile zipper-seal bags and transported to the laboratory under cold-chain conditions. Each swab was immersed in 10 mL sterile phosphate-buffered saline (PBS) and vortexed vigorously for approximately 60 seconds to facilitate the release and homogenization of attached microorganisms before enrichment. Subsequently, 1 mL of the suspension was inoculated into 9 mL nutrient broth (NB; HiMedia Laboratories Pvt. Ltd., Mumbai, India) and incubated at 37°C for 18–24 h for bacterial enrichment prior to isolation.

2.3 Bacterial isolation and identification

Isolation and identification of *Escherichia coli* were carried out following the guidelines of the International Organization for Standardization (ISO) (30). The samples were inoculated into nutrient broth and incubated at 37°C for 24 h to facilitate bacterial growth. Presumptive *Escherichia coli* isolates were initially identified based on their characteristic colony morphology, including colony size, shape, color, and margin characteristics following standard bacteriological procedures. Colonies grown on eosin methylene blue (EMB) agar (HiMedia Laboratories Pvt. Ltd., Mumbai, India) exhibited the characteristic metallic green sheen with dark centers, a characteristic feature of lactose-fermenting *E. coli*. Presumptive isolates were further confirmed using the VITEK®2 automated identification system (bioMérieux, USA), which determines bacterial identity based on biochemical profiling. Molecular confirmation was subsequently performed by PCR (31).

2.4 Molecular detection

Genomic DNA from presumptive *E. coli* isolates was extracted using the conventional boiling method (32). Briefly, isolates were grown on nutrient agar and incubated at 37°C overnight. A few well-isolated colonies were collected and suspended in nuclease-free water. The suspension was boiled at 99°C for 15 min, immediately cooled on ice, and centrifuged at 10,000 rpm for 10 min to remove cell debris. The resulting supernatant served as the DNA template for PCR. Polymerase chain reaction (PCR) was employed to confirm the identity of *Escherichia coli* isolates and to detect selected antimicrobial resistance genes. Species confirmation was performed by amplification of the *malB* gene, a highly conserved and species-specific genetic marker widely used for the reliable molecular identification of *E. coli*. In addition, PCR assays were conducted to detect the antimicrobial resistance genes *oxa-1*, *sul1*, and *sul3*, which are associated with β-lactam and sulfonamide resistance. The presence of these genes was used to evaluate the molecular basis of antimicrobial resistance among the recovered isolates (33).

Each 20 µL reaction contained 10 µL of 2× Master Mix (Promega, USA), 1 µL of each primer, 3 µL of template DNA, and 5 µL of nuclease-free water. Amplification was carried out under the following conditions: initial denaturation at 95°C for 5 min, followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 55–60°C for 30 s (depending on primer pair), and extension at 72°C for 45 s, with a final extension at 72°C for 5 min. PCR products were separated on 1.5% agarose gels containing ethidium bromide (0.5 µg/mL). A 100 bp DNA ladder (Promega, USA) was used to determine fragment sizes. Electrophoresis was performed at 90 V for ~ 1.5 h, and bands were visualized and documented using a gel documentation system (VILBER LOURMAT Doc Print Ltd, USA). *E. coli* ATCC 25922 was used as a positive control, and nuclease-free water served as a negative control. Supplementary Table (S1) has been including the primer sequences, expected amplicon sizes, and PCR conditions used for amplification of the malB gene for *E. coli* confirmation and the antimicrobial resistance genes oxa-1, sul-1, and sul-3.

2.5 Antimicrobial susceptibility test (AST)

Antimicrobial susceptibility of *E. coli* isolates was determined using the Kirby–Bauer disk diffusion method according to the Clinical and Laboratory Standards Institute (CLSI) Performance Standards for Antimicrobial Susceptibility Testing (CLSI M100, 35th edition) (34). Briefly, bacterial suspensions were prepared in sterile normal saline and adjusted to the turbidity of a 0.5 McFarland standard by visual comparison against a commercially prepared McFarland standard under adequate lighting conditions. The 0.5 McFarland standard corresponds to an approximate bacterial density of 1.5×10^8 CFU/mL. The standardized suspension was subsequently diluted 1:100 to achieve a final inoculum concentration of approximately 10^6 CFU/mL for antimicrobial susceptibility testing, following CLSI guidelines. The isolates were tested against twelve antibiotics (Oxoid, UK) representing nine major classes: tetracyclines (Tetracycline, 10 µg); quinolones/fluoroquinolones (Nalidixic acid, 30 µg; Ciprofloxacin, 5 µg); carbapenems (Meropenem, 10 µg); penicillins (Ampicillin, 10 µg); cephalosporins (Cefotaxime, 10 µg; Cefoxitin, 30 µg; Cefuroxime, 30 µg; Ceftriaxone, 30 µg); macrolides (Azithromycin, 15 µg); aminoglycosides (Gentamicin, 10 µg); and folate pathway inhibitors (Trimethoprim, 5 µg). A standardized bacterial suspension was uniformly inoculated onto Mueller–Hinton agar (MHA; HiMedia Laboratories Pvt. Ltd., Mumbai, India) plates using a sterile swab. Antibiotic disks were then aseptically placed on the agar surface, and the plates were incubated aerobically at 37°C for 18–24 hours. Following incubation, the diameters of the inhibition zones surrounding each disk were measured and interpreted according to Clinical and Laboratory Standards Institute (CLSI) guidelines. After incubation, inhibition zones were measured using an automated zone reader (Interscience Scan 4000) and interpreted according to CLSI M100 (35th edition) breakpoints where validated interpretive criteria were available. For azithromycin, CLSI M100 does not provide validated breakpoints for *E. coli* isolated from environmental, food, or veterinary sources. Therefore, azithromycin susceptibility results were included for descriptive purposes only and should be interpreted with caution. *E. coli* ATCC 25922 was included as a quality control strain. All twelve antibiotics were tested against each isolate.

2.6 Classification of Multidrug-Resistant (MDR), Extensively Drug-Resistant (XDR), and Multiple Antibiotic Resistance (MAR) Index Profiles

Isolates resistant to at least one agent in three or more antimicrobial classes were classified as multidrug-resistant (MDR); those susceptible to agents in only one or two classes as extensively drug-resistant (XDR); and those resistant to all tested classes as pandrug-resistant (PDR), following the international expert definitions of (35). The Multiple Antibiotic Resistance (MAR) index for each isolate was calculated as the ratio of the number of antibiotics to which the isolate was resistant to the total number of antibiotics tested (36, 37):

MAR index = a / b

where a is the number of antibiotics to which the isolate was resistant and b is the total number tested (b = 12). MAR values exceeding 0.2 were interpreted as indicative of origin from environments with sustained antimicrobial-selection pressure (36).

2.7 Statistical analysis

Data were curated in Microsoft Excel (version 2021) and analysed in R version 4.4.1 (38) within RStudio (version 2025.05.0 + 496). Prevalence estimates with 95% confidence intervals were calculated using the Wilson score method implemented in the binom package. Between-surface and between-upazila differences in *E. coli* prevalence were compared using Pearson's χ^2 test. Antimicrobial resistance patterns were visualised as bar plots and clustered heatmaps generated with ggplot2, ggpubr, and pheatmap. Pairwise associations between antibiotic-resistance phenotypes were assessed by Spearman rank correlation and visualised with ggcorrplot. Relationships among sample sources, antibiotic classes, and resistance phenotypes were displayed with ggalluvial. Data preparation was performed using dplyr and tidyr. All figures were exported at publication-quality resolution (≥ 300 dpi). Statistical significance was defined as $p < 0.05$.

2.8 Ethics and consent to participate

To ensure adherence to ethical standards and safeguard the welfare of all participants and biological materials, this study was conducted in full compliance with the ARRIVE guidelines, and permission for specimen collection was obtained from the institution’s ethical review committee. The research protocol was reviewed and approved by the Gono Bishwabidyalay Ethical Review Committee (GERC), Dhaka, Bangladesh (Approval No. GERC/2025/107; Approval Date: 10th July 2025). The study was conducted in accordance with institutional ethical guidelines and relevant national regulations. All procedures, including sample collection, handling, and laboratory analysis, were performed with strict precautions to minimize risk and maintain confidentiality. Samples were collected from six retail market outlets, and we obtained informed consent from the owners of the outlets. Throughout the study, the research team

remained committed to ethical conduct, emphasizing transparency, accountability, and respect for responsible research practices.

RESULTS

3.1 Prevalence of *E. coli* infections in retail poultry processing surfaces (dressing machine, knife and table)

Of 180 environmental swabs collected from six retail poultry outlets, 90 (50.0%; 95% CI: 42.6–57.4%) yielded *E. coli* on cultural and biochemical confirmation.

3.1.1 Prevalence by surface type

E. coli recovery differed numerically by surface, with the highest prevalence on knives (33/57; 57.9%; 95% CI: 44.1–70.9%), followed by cutting tables (30/63; 47.6%; 95% CI: 34.9–60.6%) and dressing machines (27/60; 45.0%; 95% CI: 32.1–58.4%) (Table 1). The differences did not reach statistical significance ($p = 0.339$, Pearson's χ^2 test), indicating that all three surface types contributed comparably to environmental *E. coli* contamination in the sampled outlets.

Table 1
Prevalence of *E. coli* recovered from different poultry-processing surfaces.

Source	Sample Tested	Positive (%)	Negative (%)	95%CI
Dressing machine	60	27 (45%)	33 (55%)	32.1–58.4%
knife	57	33 (57.9%)	24 (42.1%)	44.1–70.9%
table	63	30 (47.6%)	33 (52.4%)	34.9–60.6%

3.1.2 Location-wise Prevalence of *E. coli*

Prevalence varied across upazilas: Sreepur (21/39; 53.8%; 95% CI: 37.2–69.9%), Savar (54/105; 51.4%; 95% CI: 41.5–61.3%), and Dhamrai (15/36; 41.7%; 95% CI: 25.5–59.2%) (Fig. 1). The overlapping confidence intervals indicate no clear spatial gradient at the resolution of this sample.

Figure 1: Geographic distribution of sampling locations and prevalence of *E. coli* in retail poultry-processing outlets located in Savar, Sreepur, and Dhamrai, Dhaka Division, Bangladesh. The map was generated using QGIS software (version 3.36.1), with district boundary shapefiles obtained from the GADM database (https://gadm.org).

3.2 Antimicrobial resistance profile of *E. coli* isolates

All 90 confirmed *E. coli* isolates were tested against 12 antibiotics representing nine mechanistic classes.

3.2.1 Distribution of resistance across sources and classes

The alluvial representation (Fig. 2) shows that isolates from all three surface types carried resistance to multiple antibiotic classes, most frequently to penicillins, tetracyclines, quinolones, macrolides, folate-pathway inhibitors, and cephalosporins. Resistance was infrequent for carbapenems and moderate for aminoglycosides, consistent with lower reported use of these agents in Bangladeshi poultry production.

Figure 2

Alluvial plot showing the Antimicrobial Resistance Flow in *E. coli* Isolates across antibiotic classes and sample sources.

3.2.2 Antibiotic-specific resistance

Resistance frequencies (Fig. 4) were highest for ampicillin (96%) and trimethoprim (96%), followed by azithromycin (93%), nalidixic acid (89%), tetracycline (89%), ciprofloxacin (86%), cefotaxime (82%), and gentamicin (64%). Intermediate resistance was recorded for cefoxitin (54%) and ceftriaxone (50%). Meropenem remained effective, with only 7% of isolates classified as resistant (93% susceptible)

3.2.3 Hierarchical clustering of resistance profiles

Hierarchical clustering of resistance phenotypes (Fig. 4) grouped isolates from knives and dressing machines more closely than either grouped with table isolates, and grouped β-lactam antibiotics (cefuroxime, cefotaxime, ceftriaxone, cefoxitin) into a single class-level cluster. The class-level clustering is consistent with shared resistance determinants across β-lactam agents, though the present data do not distinguish between clonal spread, plasmid-mediated co-transfer, and independent selection.

3.3 Pairwise correlation of resistance phenotypes

Spearman correlations between resistance phenotypes (Fig. 5) identified three notable patterns. First, β-lactam agents (cefuroxime, ceftriaxone, cefoxitin, cefotaxime) showed strong positive intra-class associations ($r = 0.39–0.67$), consistent with shared resistance mechanisms. Second, gentamicin correlated moderately with several β-lactams ($r = 0.32–0.44$), a pattern compatible with co-selection on shared mobile genetic elements, though this was not directly tested. Third, ciprofloxacin and meropenem were strongly negatively correlated ($r = -0.72$), and tetracycline–nalidixic acid correlations were weak ($|r| < 0.2$), together indicating that fluoroquinolone, carbapenem, and tetracycline resistance track independently of the β-lactam cluster in this dataset.

3.4 Multiple Antibiotic Resistance (MAR) index

MAR indices ranged from 0.00 to 0.83 (Fig. 6). The most frequent values were 0.67 and 0.75 (each 21/90; 23.3%), followed by 0.58 and 0.83 (each 15/90; 16.7%). Six isolates (6.7%) had a MAR index of

0.00, indicating susceptibility to all 12 agents; the remaining 84 isolates (93.3%) exceeded the 0.2 threshold of (36), which is conventionally interpreted as indicative of origin from environments with high antimicrobial-selection pressure. Fifteen isolates (16.7%) carried the maximum observed index (0.83; resistant to 10/12 agents) (Table 2).

Table 2
Multiple Antibiotic Resistance (MAR) Index of *E. coli* isolates

Number of Isolates	Antibiotics	Resistant Antibiotics	MAR Index	MAR Index
15	12	10	0.833	High resistance
21	12	9	0.75	High resistance
21	12	8	0.667	High resistance
15	12	7	0.583	Moderate resistance
6	12	6	0.5	Moderate resistance
3	12	5	0.417	Moderate resistance
3	12	4	0.333	Moderate resistance
6	12	0	0.00	Fully susceptible

3.5 Molecular detection of resistance genes

All 90 *E. coli* isolates were screened by conventional PCR for sul1, sul3, and blaOXA-1.

sul3 was the most frequently detected gene (24/90; 26.7%), followed by blaOXA-1 (21/90; 23.3%) and sul1 (12/90; 13.3%) (Fig. 7). Gene carriage differed by source (Table 3): sul3 was most frequent in dressing-machine isolates (12/27; 44.4%), blaOXA-1 in table isolates (12/30; 40.0%), and sul1 was absent from knife isolates (0/33; 0%).

Co-occurrence analysis showed that 45/90 isolates (50.0%) carried none of the three tested genes, 33/90 (36.7%) carried one gene, and 12/90 (13.3%) carried two genes; no isolate carried all three (Fig. 8). Notably, the phenotypic MDR frequency (93.3% with MAR > 0.2) substantially exceeded the proportion of isolates carrying any of the three tested genes (50.0%), indicating that resistance in this collection is driven by determinants beyond the targeted panel - plausibly other β-lactamases (e.g., blaTEM, blaCTX-M), efflux pumps, and chromosomal mutations conferring fluoroquinolone resistance - that were not screened here.

Table 3
Distribution of antimicrobial resistance genes among *E. coli* isolates recovered from different sample sources.

Sample Source	Gene	Prevalence (%)
Dressing Machine	*oxa-1*	11.1
	sul-1	22.2
	sul-3	44.4
Knife	*oxa-1*	18.2
	sul-1	0.0
	sul-3	18.2
Table	*oxa-1*	40
	sul-1	20
	sul-3	20

DISCUSSION

Retail poultry-processing surfaces represent a critical control point for bacterial dissemination within the food chain (28). This study assessed the prevalence and antimicrobial resistance profiles of *Escherichia coli* on knives, cutting tables, and dressing machines in retail poultry outlets across Dhaka Division, Bangladesh. Of 180 environmental swabs, 50.0% (95% CI: 42.6–57.4%) yielded *E. coli*. While prevalence was numerically highest on knives (33/57, 57.9%), followed by tables (30/63, 47.6%) and dressing machines (27/60, 45.0%), these differences were not statistically significant ($p = 0.339$). The higher contamination of knives is biologically plausible given their repeated direct incision into contaminated tissue and the propensity for biofilm formation on micro-serrated blades, yet the overlapping confidence intervals mandate that these surface-level differences be treated as descriptive rather than evidence of differential risk. Similarly, the observed variation across sampling locations (Sreepur 53.8%, Savar 51.4%, Dhamrai 41.7%) is consistent with heterogeneity in local sanitation practices and upstream supply-chain contamination (25), but cannot be disentangled from sampling variation at this sample size. Overall, the 50% prevalence aligns with widespread *E. coli* contamination documented on retail poultry and processing surfaces in Bangladesh (20, 23, 24) and analogous international settings (25, 29).

Isolates exhibited extensive phenotypic resistance, predominantly against ampicillin (96%), trimethoprim (96%), azithromycin (93%), nalidixic acid (89%), and tetracycline (89%). This profile directly mirrors the antimicrobials most widely and inexpensively available in Bangladeshi poultry production. The high azithromycin resistance should be interpreted cautiously, however, as standardized disc-diffusion breakpoints for macrolides in *E. coli* are not well established; thus, the reported prevalence may reflect interpretive criteria rather than clinically meaningful resistance. Strong positive correlations among β-

lactams (cefuroxime, ceftriaxone, cefoxitin, cefotaxime) are expected where shared β-lactamases confer overlapping activity, and moderate correlations with gentamicin suggest the physical linkage of resistance determinants on common mobile elements (18, 21). Conversely, the apparent negative correlation between ciprofloxacin and meropenem resistance is likely a sparse-data artifact driven by the low prevalence of carbapenem resistance (7%), rather than a biological antagonism, as their resistance mechanisms - chromosomal gyrA/parC mutations and efflux for fluoroquinolones, versus acquired carbapenemases for meropenem - are mechanistically independent (39). The retained susceptibility to meropenem is an informative finding, contrasting with escalating carbapenem resistance recently reported from Bangladeshi retail chicken meat and live-bird-market sewage (27). Ultimately, 93.3% of isolates exceeded the multiple antibiotic resistance (MAR) index threshold of 0.2 associated with high-risk contamination sources (36), corroborating earlier Bangladeshi reports of pervasive MDR in poultry ecosystems (19, 23, 24).

Molecular screening identified sul3 (26.7%), blaOXA-1 (23.3%), and sul1 (13.3%), with notable distribution heterogeneity: sul3 was most frequent in dressing-machine isolates (44.4%), blaOXA-1 in table isolates (40.0%), and sul1 was absent from all knife isolates. Because sul1 typically resides in the 3' conserved segment of class 1 integrons while sul3 associates with distinct genetic contexts, this distribution may reflect differential mobilization of resistance cassettes across micro-niches; however, the sample size precludes definitive inference. Co-detection of two genes occurred in 13.3% of isolates, consistent with co-selection pressures but insufficient, without conjugation assays or genomic context, to confirm horizontal transfer.

The most critical molecular finding is the profound discordance between phenotypic MDR (93.3%) and the carriage of targeted resistance genes. The screened panel fails to account for the dominant resistance phenotypes: blaOXA-1 encodes a narrow-spectrum oxacillinase active against penicillins and inhibitor combinations but lacks extended-spectrum cephalosporinase activity, and thus cannot explain the observed cephalosporin resistance; similarly, sul1 and sul3 confer sulphonamide resistance but do not account for the trimethoprim resistance (96%), which is typically dfrA-mediated. Resistance in this collection is therefore driven predominantly by determinants outside the screened panel - plausibly blaTEM and blaCTX-M (the predominant ESBL determinants in Bangladeshi animal and clinical *E. coli* (16, 40), dfrA cassettes, and chromosomal mutations. Although the surface prevalence (50%) is lower than the 70–79.7% ESBL-producing *E. coli* prevalence reported in Bangladeshi retail meat and market sewage (27) - as expected when sampling inanimate fomites rather than tissue or effluent - the comparably high MDR frequencies demonstrate that resistant *E. coli* is broadly disseminated across the retail poultry environment, and processing surfaces actively reflect this upstream resistome.

Several limitations constrain these conclusions. The cross-sectional design captures point-prevalence contamination and cannot demonstrate microbial persistence; thus, "reservoir" is used descriptively rather than ecologically. The three-gene panel represents a minimal fraction of the resistome and notably omitted sul2, a highly prevalent sulphonamide determinant in *E. coli*; furthermore, the absence of plasmid characterization, integron cassette mapping, or sequencing leaves the resistome architecture

largely undefined. Finally, sampling was restricted to three upazilas and six outlets, limiting geographic generalizability, and the lack of concurrent carcass or worker isolates precludes source attribution.

From a One Health perspective, the contamination of knives, cutting tables, and dressing machines with MDR *E. coli* constitutes a direct and plausible route for resistant pathogens to enter the human food chain, particularly given the manual handling and minimal processing typical of poultry preparation in Bangladesh. These findings underscore the compounding effects of upstream antimicrobial use (16) and deficient retail hygiene (8). Practical interventions - routine equipment disinfection, worker hygiene training, and enforced prudent antimicrobial use - are achievable without major infrastructural investment. Future research employing whole-genome sequencing of surface isolates is essential to resolve the resistome architecture left undefined by targeted panels and to assess zoonotic spillover by comparing these strains with concurrent clinical isolates from the same geographic regions (41).

CONCLUSION

This study demonstrates that poultry-processing equipment surfaces in retail chicken outlets of Dhaka Division serve as important reservoirs of antimicrobial-resistant *Escherichia coli*. The observed contamination rate, high frequency of multidrug resistance, elevated MAR indices, and detection of β-lactam and sulfonamide resistance genes indicate that retail processing environments may contribute to the persistence and dissemination of antimicrobial resistance along the poultry food chain. These findings are consistent with reports from Bangladesh and other countries that identify poultry production and retail systems as critical interfaces for the transmission of resistant bacteria within a One Health context. Future interventions should be aligned with internationally recognized frameworks, including Codex Alimentarius guidelines on antimicrobial stewardship and surveillance of foodborne antimicrobial resistance, to strengthen food safety and public health protection.

Abbreviations

AMR (Antimicrobial Resistance), MDR (Multidrug Resistance), XDR (Extensively Drug-Resistant), PDR (Pan Drug-Resistant), MAR (Multiple Antibiotic Resistance), AST (Antimicrobial Susceptibility Test), PCR (Polymerase Chain Reaction), DNA (Deoxyribonucleic Acid), PBS (Phosphate-Buffered Saline), NB (Nutrient Broth), EMB (Eosin–Methylene Blue), CSPs (Cold-Shock Proteins), LHR (Locus of Heat Resistance), CFU (Colony-Forming Units), CI (Confidence Interval), mRNA (Messenger Ribonucleic Acid), CLSI (Clinical and Laboratory Standards Institute), ISO (International Organization for Standardization), R (Resistant), I (Intermediate), S (Sensitive), bp (Base Pair).

Declarations

Data availability statement

All data supporting this article are available from the corresponding author upon reasonable request.

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Authors' contribution

All authors have thoroughly reviewed and approved the final manuscript, and no qualified individuals have been excluded from authorship. The individual contributions of each author are detailed below:

Md. Rihad.: Conceptualization, Methodology, Microbiological investigation, Data curation, Writing - original draft, Validation.

Nure Hamim Mohammod Ammar.: Conceptualization, Methodology, Microbiological investigation, Data curation, Data analysis and interpretation, Writing - original draft, Validation.

Md. Niamul Shahadat.: Conceptualization, Methodology, Writing - critical review & editing, supervision, Validation.

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Uttam Kumar Roy.: Conceptualization, Methodology, Microbiological investigation, Data curation, Data analysis and interpretation, Writing - original draft, Validation.

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Ethics and consent to participate

To ensure adherence to ethical standards and safeguard the welfare of all participants and biological materials, this study was conducted in full compliance with the ARRIVE guidelines, and permission for specimen collection was obtained from the institution's ethical review committee. The research protocol was reviewed and approved by the Gono Bishwabidyalay Ethical Review Committee (GERC), Dhaka, Bangladesh (Approval No. GERC/2025/107; Approval Date: 10th July 2025). The study was conducted in accordance with institutional ethical guidelines and relevant national regulations. All procedures,

including sample collection, handling, and laboratory analysis, were performed with strict precautions to minimize risk and maintain confidentiality. Samples were collected from six retail market outlets, and we obtained informed consent from the owners of the outlets. Throughout the study, the research team remained committed to ethical conduct, emphasizing transparency, accountability, and respect for responsible research practices.

Consent for publication

Not- Applicable.

Clinical Trial: Not applicable

Conflict of interest

The authors declare no potential conflicts of interest related to this publication.

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Figures

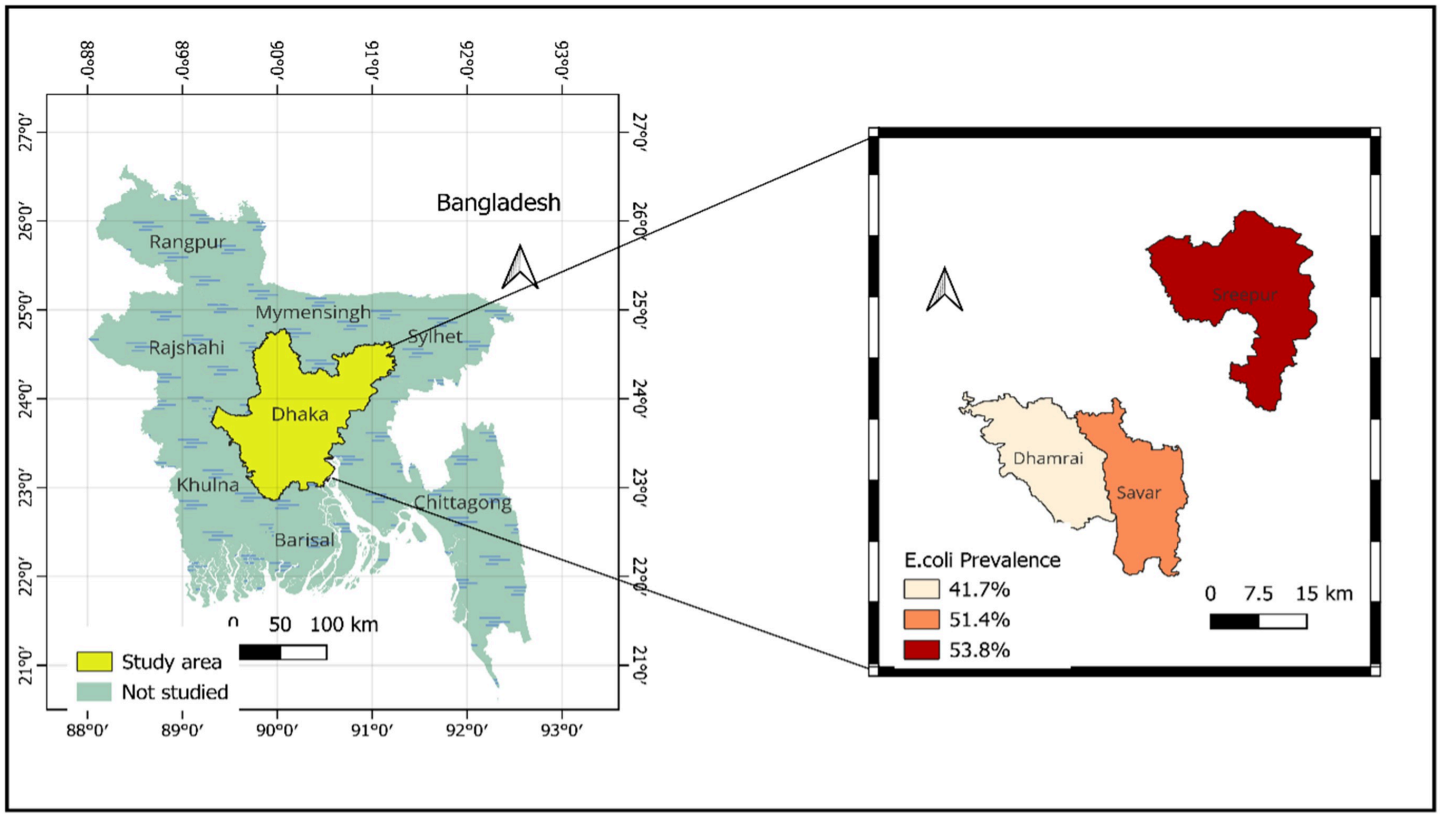


Figure 1

Geographic distribution of sampling locations and prevalence of *E. coli* in retail poultry-processing outlets located in Savar, Sreepur, and Dhamrai, Dhaka Division, Bangladesh. The map was generated using QGIS software (version 3.36.1), with district boundary shapefiles obtained from the GADM database (https://gadm.org).

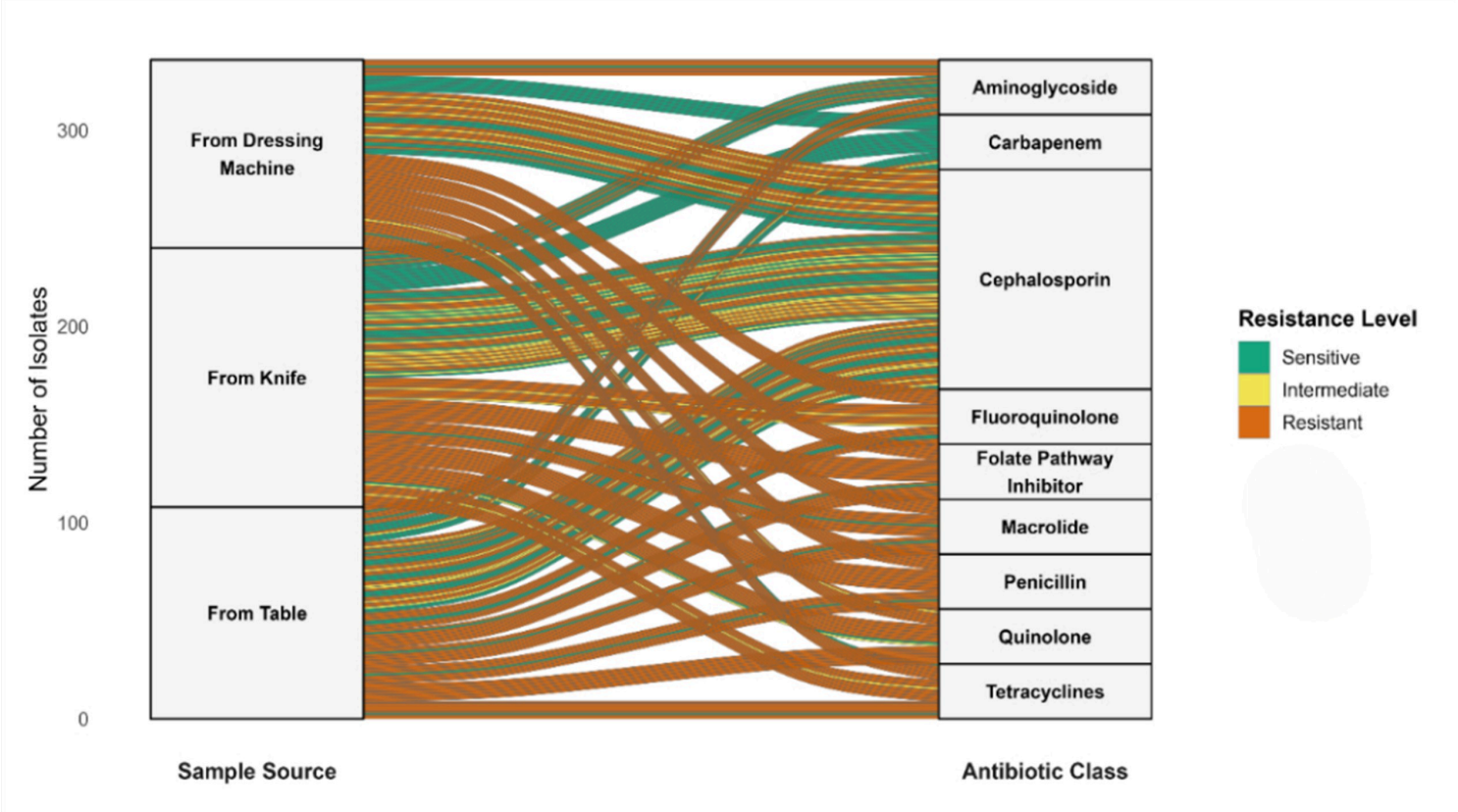


Figure 2

Alluvial plot showing the Antimicrobial Resistance Flow in *E. coli* Isolates across antibiotic classes and sample sources.

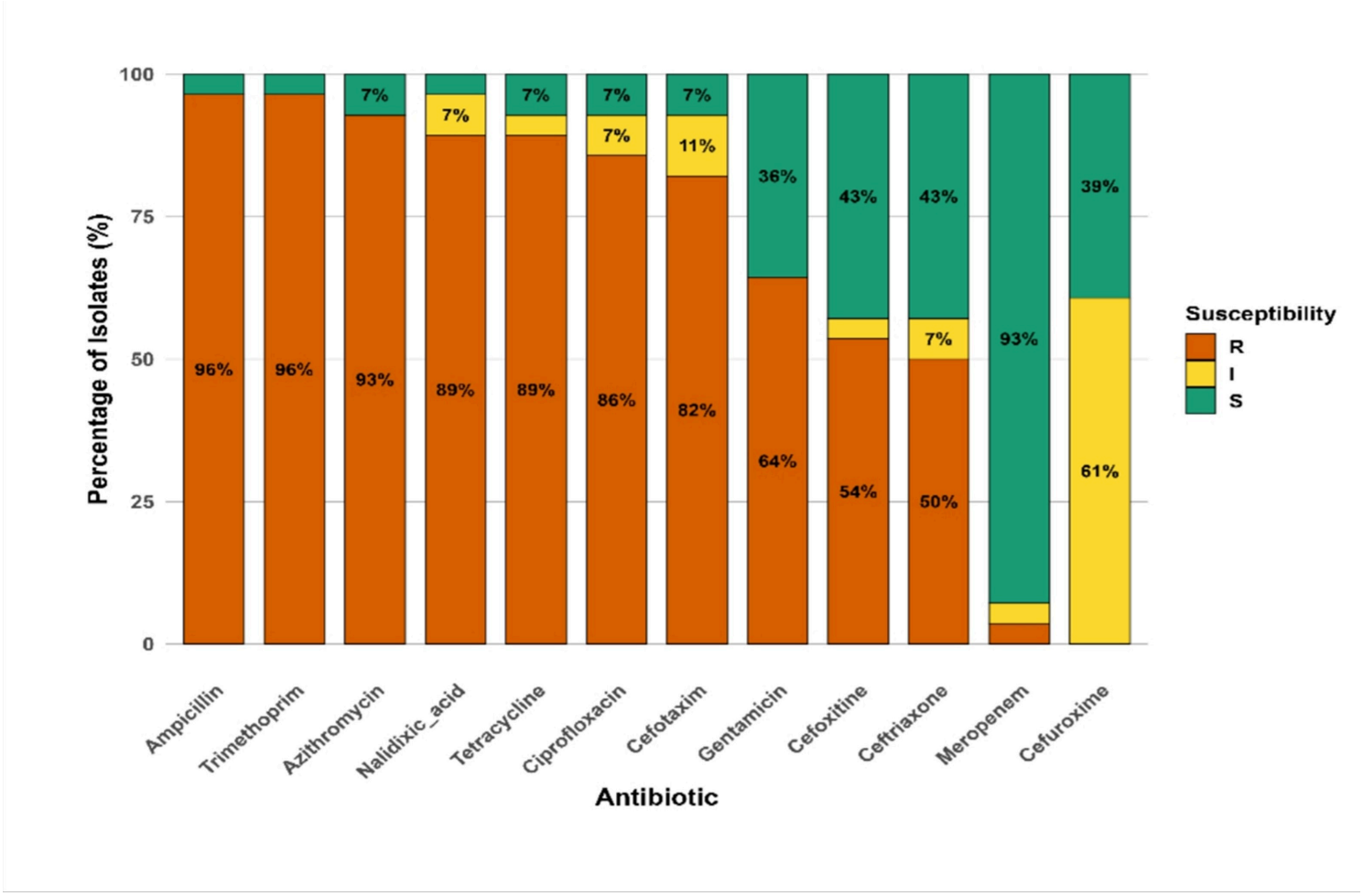


Figure 3

Percentage distribution of resistant (R), intermediate (I), and sensitive (S) *E. coli*isolates against tested antibiotics.

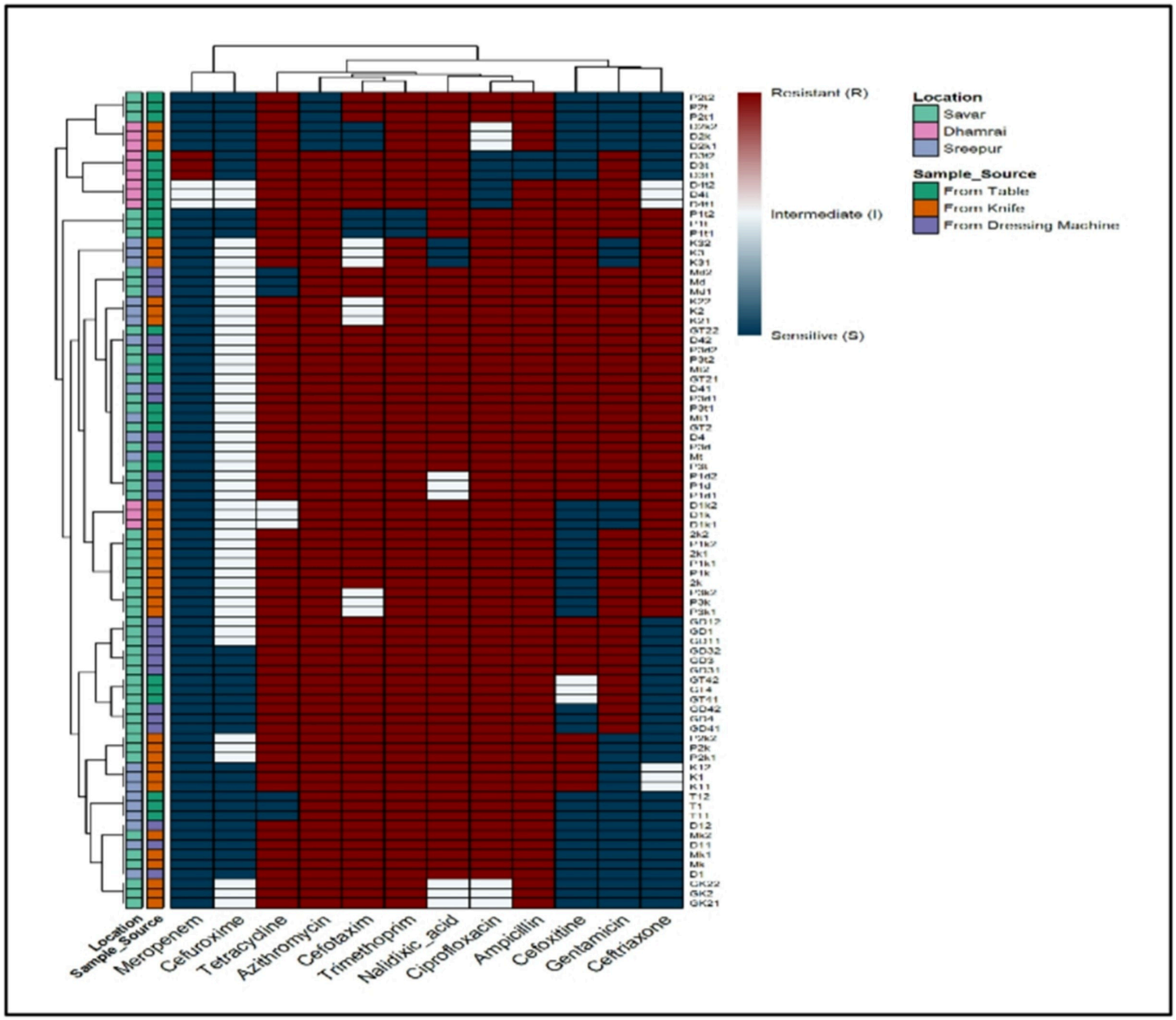


Figure 4

Hierarchical Clustered Antibiotic Resistance heatmap representing antimicrobial resistance patterns of *E. coli* isolates.

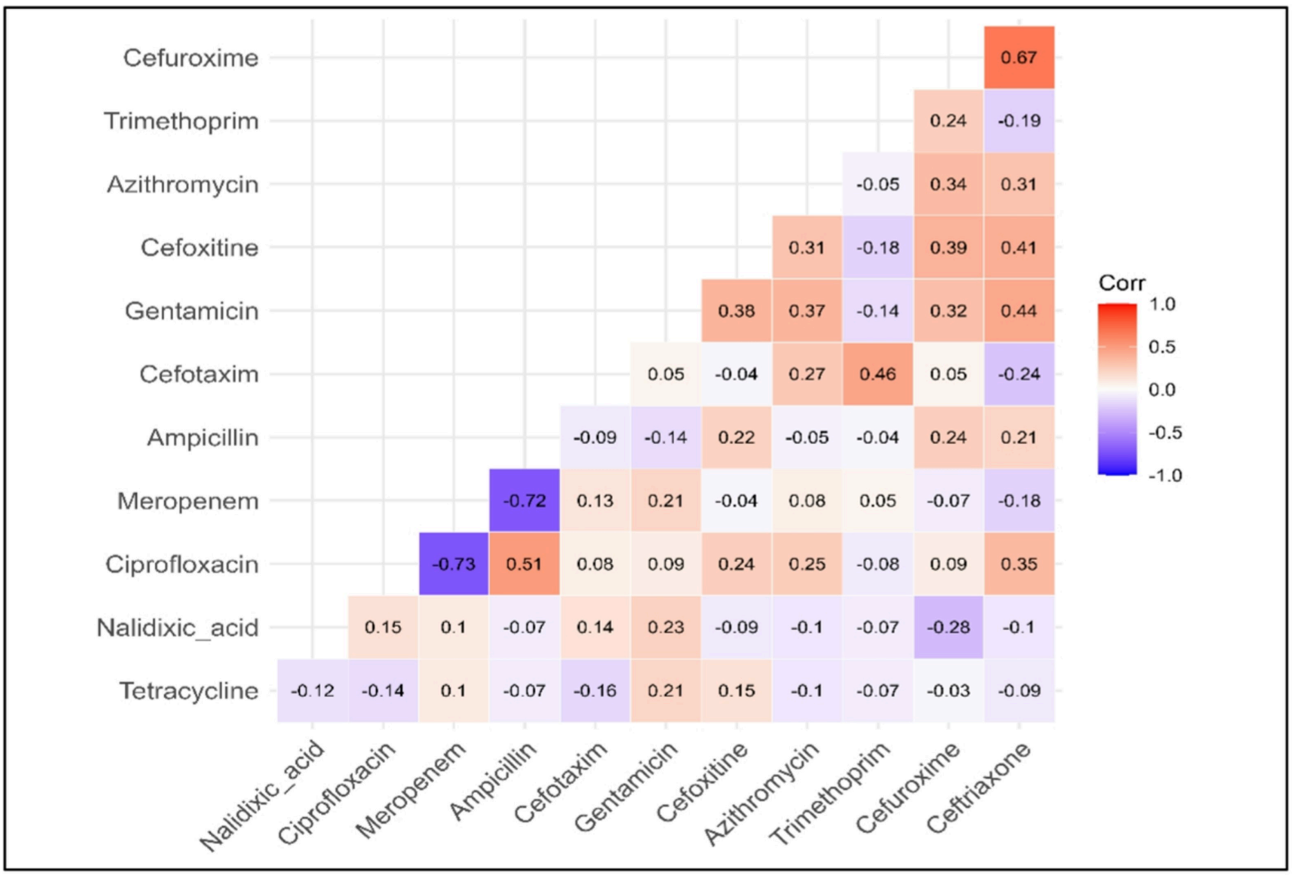


Figure 5

Spearman correlation plot of antimicrobial agents, where 1.0 indicates a strong positive correlation and –1.0 indicates a strong negative correlation.

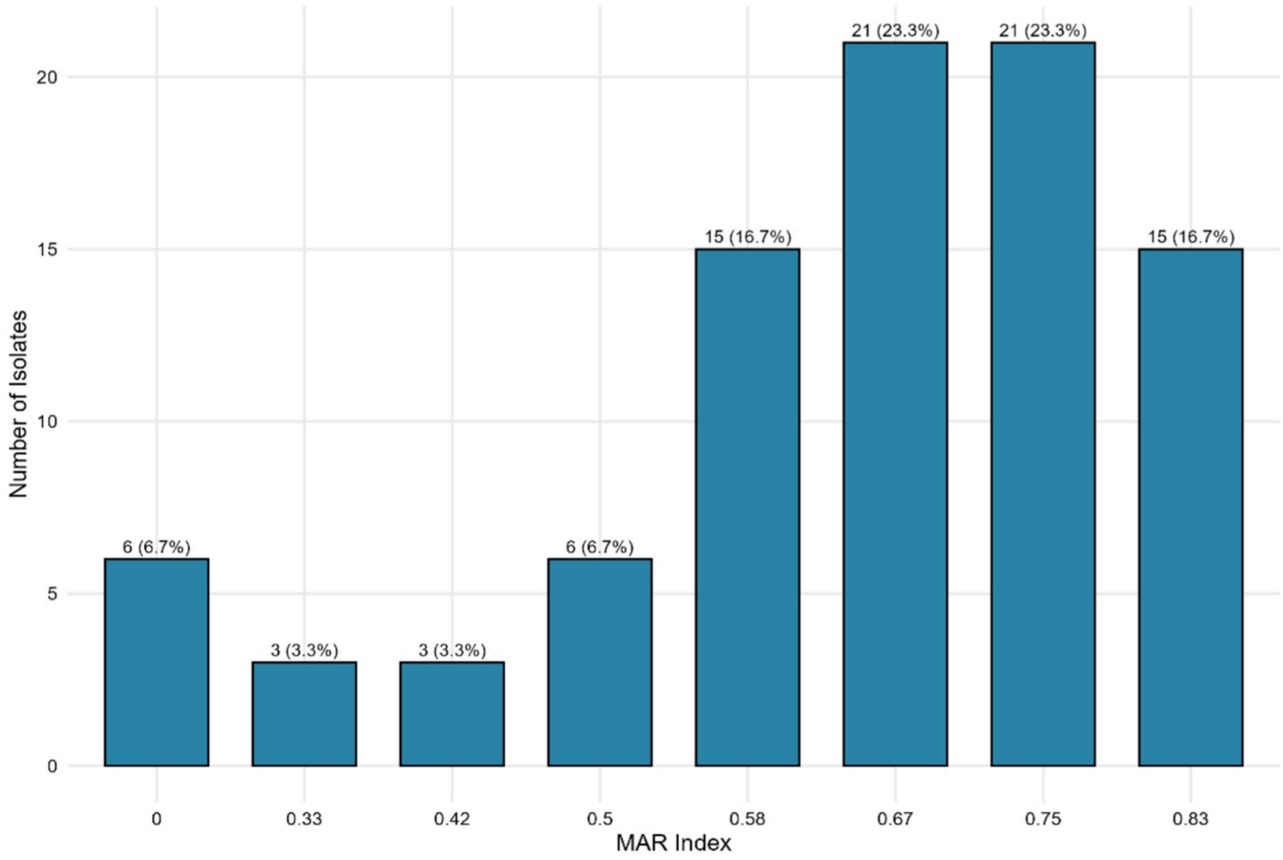


Figure 6

Bar diagram between Multiple Antibiotic Resistance Index (MAR Index) and number of *E. coli* isolates with number and frequency

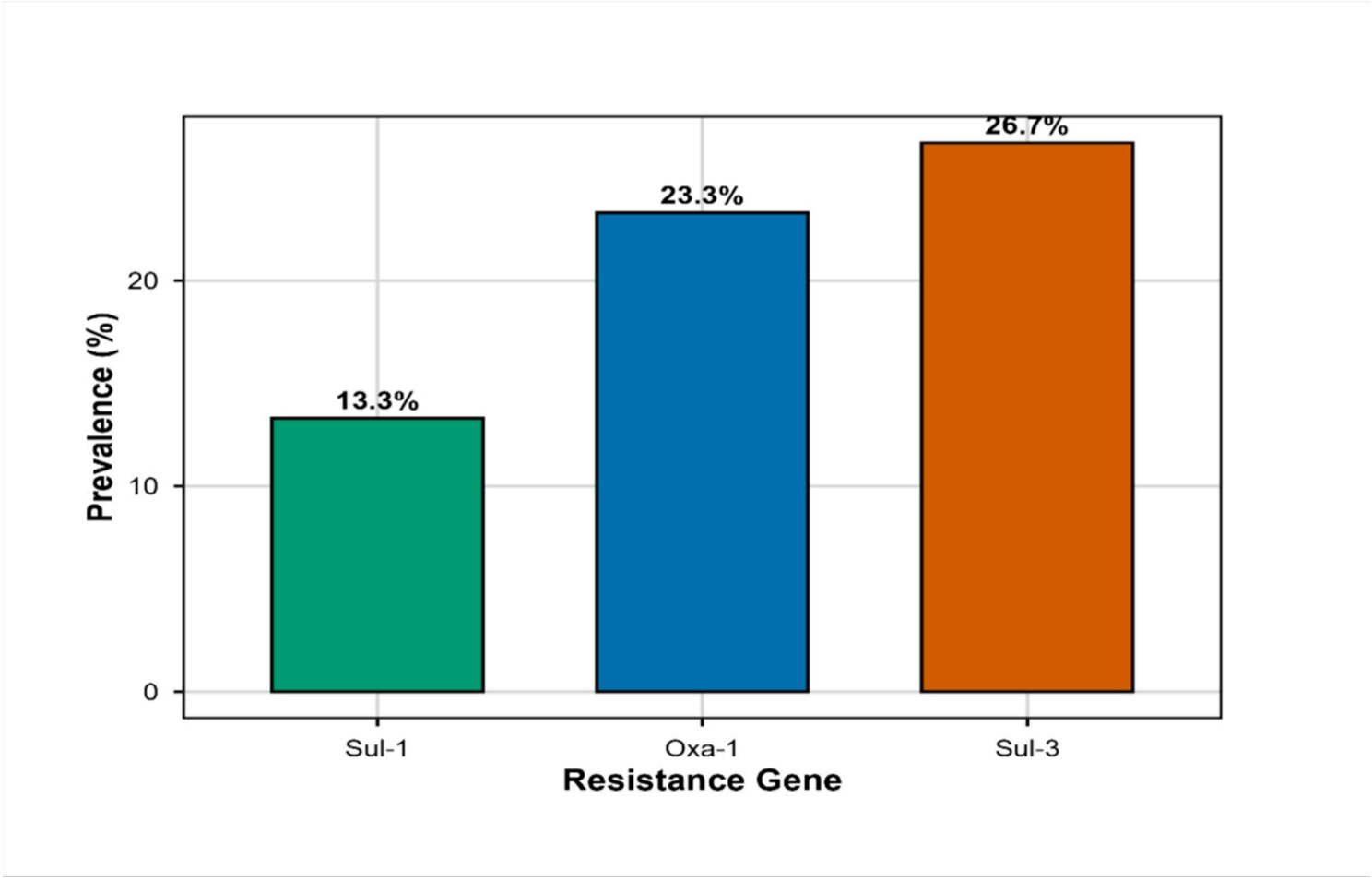


Figure 7

Prevalence of antimicrobial resistance genes (oxa-1, sul-1, and sul-3) detected among *E. coli* isolates.

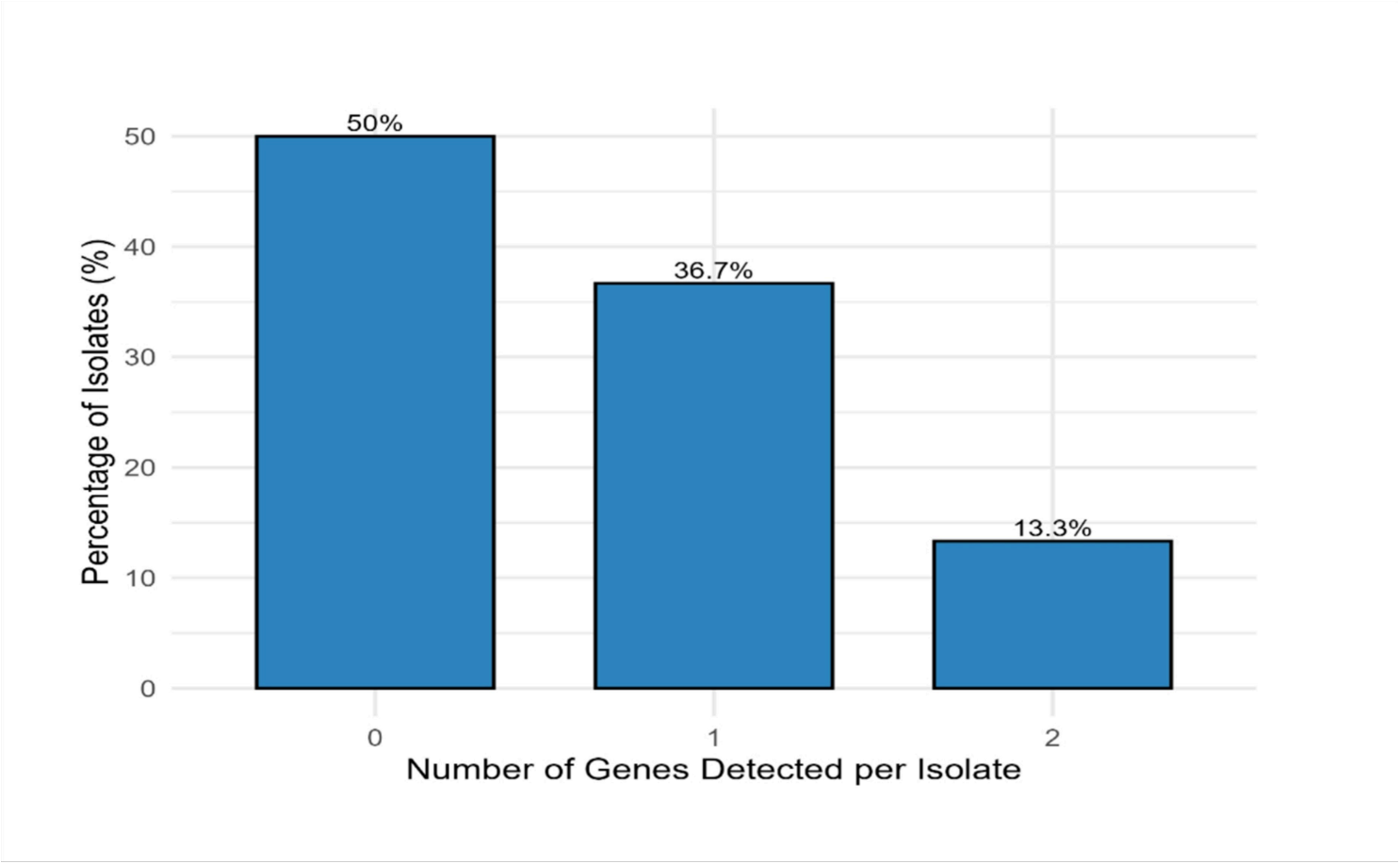


Figure 8

Co-occurrence pattern of antimicrobial resistance genes (oxa-1, sul-1, and sul-3) among *E. coli* isolates.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- SupplementaryFile.docx
- SupplementaryfileOxa1.jpg
- Supplementaryfilesul1.jpg
- SupplementaryfileSul3.jpg