*Original Research Article*

Prevalence and Resistance Patterns of *E. coli* and *S. aureus* in Poultry Processing Environments: Public Health Implications

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ABSTRACT

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| **Aim:** Antimicrobial resistance represents a critical, multifaceted global challenge involving human, animal, and environmental sectors. The potential horizontal transfer of resistance genes across these domains, particularly in food production systems such as poultry processing, poses a significant risk to food safety. Contamination of chicken meat with resistant bacteria not only threatens public health but also complicates treatment options for foodborne infections. These factors necessitate robust and integrated surveillance systems to effectively monitor and address evolving resistance patterns in both food products and the broader environment. This study investigated antimicrobial resistance patterns in *Escherichia coli* and *Staphylococcus aureus* isolated from broiler meat and water samples collected from retail broiler shops in Chennai, Tamil Nadu, India.**Methodology:** A total of 100 samples (50 chicken meat and 50 water) were collected. Bacterial isolation was performed using standard microbiological methods, and species identification was confirmed by PCR. The antibiotic resistance profiles of the isolates were assessed against commonly used antibiotics**Results:** The prevalence of *E. coli* was 26% in chicken meat and 24% in water samples, while *S. aureus* was detected in 30% of chicken meat and 18% of water samples. *E. coli* isolates exhibited high resistance to tetracycline, amoxicillin, and co-trimoxazole, whereas *S. aureus* isolates demonstrated complete resistance to tetracycline, doxycycline, co-trimoxazole, methicillin, and enrofloxacin. Nevertheless, over 75% of *E. coli* and 50% of *S. aureus* isolates remained susceptible to gentamicin and amikacin. Notably, all *S. aureus* and more than 90% of *E. coli* isolates displayed multidrug resistance, with multiple antibiotic resistance (MAR) indices exceeding 0.2.**Conclusion:** This study highlights a concerning level of antimicrobial resistance among *E. coli* and *S. aureus* in chicken meat and water sample, underscoring the critical need for stringent antibiotic stewardship, comprehensive surveillance, and effective control measures to curb the spread of resistant pathogens via the food chain. |

*Keywords: Antimicrobial resistance, Broiler, water, Escherichia coli, Staphylococcus aureus*

1. INTRODUCTION

Antimicrobial resistance (AMR) is a rapidly escalating global threat, responsible for an estimated 1.27 million deaths worldwide in 2019 due to bacterial AMR. As per WHO report, AMR could lead to an additional one trillion US dollar in healthcare expenditure by 2050. The leading bacterial pathogens contributing to AMR-related mortality include *Escherichia coli*, followed by *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Streptococcus pneumoniae*, *Acinetobacter* *baumannii*, and *Pseudomonas aeruginosa* (Murray *et al.*, 2022).

*E. coli*, a commensal bacterium of the gastrointestinal tract, plays a significant role in the health of both animals and humans. It is commonly employed as an indicator organism for detecting faecal contamination in food products (Rafiq *et al*., 2024). *S. aureus* is normally present in the skin and nasal mucosa of human and animals. The colonization of bacteria in the skin poses significant threat for the possible contamination of meat hence transmission to the human.

The Indian poultry industry is rapidly expanding, with broiler and egg production increasing annually by 5–6%, compared to 1.5–2% for agricultural crops (Basic Animal Husbandry Statistics, 2022). Antimicrobials are extensively used in poultry for growth promotion, prophylaxis, and therapeutic purposes, resulting in intense selection pressure that promotes the emergence resistance bacteria within the gut microbiota. This not only compromises animal health but also poses significant risks to human health through the food chain. Moreover, wastewaters from abattoirs, often released untreated into the environment, act as reservoirs and dissemination pathways for antimicrobial resistance (AMR) genes, facilitating their spread among environmental microbial communities (Rhouma *et al*., 2021; van den Bogaard *et al*., 2001).

Given the scale of poultry production and antimicrobial use, there is an urgent need for integrated management strategies, including improved waste treatment, stricter regulation of antibiotic use, and robust surveillance systems to monitor and control the dissemination of AMR. Against this background, the present study was undertaken to assess the prevalence and resistance profile of *E. coli* and *S. aureus* in chicken meat and slaughter house water samples.

2. material and methods

**2.1 Sampling**

Sample collection was carried out from March to May 2024 across all zones of Chennai district, Tamil Nadu, India. A total of 100 samples were collected from retail broiler outlets, comprising 50 chicken meat samples and 50 water samples from broiler processing areas. All samples were transported to the laboratory under chilled conditions and stored at 4°C until further analysis.

**2.2 Isolation of organisms**

The samples were inoculated into Nutrient Broth and Brain Heart Infusion (BHI) Broth and incubated aerobically at overnight 37°C. For the selective isolation of *E. col*i, cultures from nutrient broth were streaked onto MacConkey Agar and Eosin Methylene Blue (EMB) Agar and incubated overnight at 37°C. A loopful of culture from BHI Broth was streaked onto Mannitol Salt Agar for the selective isolation of *Staphylococcus* sp. Presumptive identification of the isolates was based on colony morphology, Gram staining, and standard biochemical tests (Kalaiselvi *et al*, 2018)

**2.3 Molecular confirmation of *E. coli* and *S. aureus***

Genotypic confirmation of *E. coli* and *S. aureus* was performed by PCR targeting the 16S rRNA gene and the nuc gene, respectively. Genomic DNA was extracted from bacterial cultures by heat lysis, following the procedure described by Arora et al. (2006), with slight modifications. The extracted DNA was used as a template for PCR amplification. The oligonucleotide primers used were given in table 1 and PCR conditions were given in table 2

**Table 1 Oligonucleotide primers**

|  |  |  |
| --- | --- | --- |
| **Gene** | **Oligonucleotide sequence** | **Reference** |
| 16sRNA | Forward- 5’-GACCTCGGTTTAGTTCACAGA-3’Reverse - 5’-CACACGCTGACGCTGACCA-3’ | Fanjip *et al*, 2022 |
| Nuc gene | Forward - 5’-GCGATTGATGGTGATACGGTT-3’Reverse- 5’-AGCCAAGCCTTGACGAACTAAAGC-3’ | Brakstad *et al*., 1993 |

**Table 2 PCR Cyclic conditions**

|  |  |  |
| --- | --- | --- |
| **Cycle step** | **16s rRNA** | **nuc gene** |
| **Temperature** | **Time** | **Cycle** | **Temperature** | **Time** | **Cycle** |
| Initial Denaturation | 95°C | 3 min | 1 | 94°C | 4 min | 1 |
| Denaturation | 94°C | 45 sec | 30 | 95°C | 30 sec | 34 |
| Annealing | 58°C | 45 sec | 57°C | 30 sec |
| Extension | 72°C | 1 min | 72°C | 1 min |
| Final Extension | 72°C | 3 min | 1 | 72°C | 5 min | 1 |

PCR products were subjected to agarose gel electrophoresis at 100 V for 30 minutes. The gels were stained with ethidium bromide, visualized under a UV transilluminator, and photographed using the Bio-Rad Gel Doc 1000 gel documentation system (Bio-Rad, USA) (Fanjip *et al*., 2022)

**2.4 Antibiotic Sensitivity testing (ABST)**

Antibiotics sensitivity testing of the isolated organisms was performed using the Kirby-Bauer disc diffusion method, as described by Hudzicki (2009). A total of ten antibiotics representing various classes (Table 3) purchased from M/s Himedia Laboratories, Mumbai were used in this study.

**Table 3 List of antibiotic discs**

|  |  |
| --- | --- |
| **Antibiotic Class** | **Name of the Antibiotic** |
| β-lactam antibiotics | Amoxycillin |
| Amoxycillin-clavulanic acid |
| Cephalexin |
| Cefotaxime |
| Tetracycline | Tetracycline |
| Doxycycline |
| Aminoglycosides | Gentamicin |
| Amikacin |
| Fluoroquinolones | Enrofloxacin |
| Sulphonamide-trimethoprim | Co-trimoxazole |

Zones of inhibition were measured in millimeters using a Vernier caliper, and the results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, 2020) guidelines, categorizing isolates as susceptible (S), intermediate (I), or resistant (R).

The multiple antibiotic resistance (MAR) index for each isolate was calculated as the ratio of the number of antibiotics was resistance to the total number of antibiotics tested (Abdalla et al, 2021).

**2.5 Statistical Analysis**

The prevalence of bacterial isolates and their antibiotic susceptibility patterns were analyzed using descriptive statistics in Microsoft Excel. The multiple antibiotic resistance (MAR) indices of E. coli and S. aureus isolated from two different sources—chicken meat and water—were analyzed using one-way ANOVA, followed by Duncan’s post hoc test, in IBM SPSS (Statistical Package for the Social Sciences) software, version 2.0 for Windows. A p-value of <0.05 was considered statistically significant.

3. results and discussion

**3.1 Prevalence of *E. coli* and *S. aureus***

The isolates were initially identified based on colony morphology on selective media, Gram staining, and biochemical tests including Indole, Methyl Red, Voges-Proskauer, Citrate Utilization, Catalase, and Oxidase tests. Molecular confirmation was performed by PCR (Figs. 1 and 2).



**Fig. 1 PCR amplification of *16S rRNA* gene**

(Lane 1: 100 bp DNA ladder; Lane 2 & 8: negative control; Lane 3-7: positive for *16S rRNA gene)*



**Fig. 2 PCR amplification of *nuc* gene**

(Lane 1: 100 bp DNA ladder; Lane 2: negative control; Lane 3-8: positive for *nuc* gene)

In this present study, the prevalence of *E. coli* in broiler chicken meat was 26%, comparable to reports from Romania (30%), Nepal (35%), and Ethiopia (37%) (Bratfelan *et al*., 2023; Bantawa *et al*., 2019; Messele *et a*l., 2017). Higher prevalence rates have been documented in the United Arab Emirates (65.73%), Sri Lanka (79.68%), and Spain (91%) (Habib *et al*., 2023; Ranasinghe *et al*., 2022; Garcia-Bejar *et al.*, 2021), with notably elevated rates of 89.09% and 100% reported in Guwahati and Hyderabad, India, respectively (Deka and Ahmed, 2022; Kumar *et al*., 2020). In this study, *E. coli* was detected in 24% of water samples from slaughterhouses, a prevalence lower than the 50% reported in Ebonyi State, Nigeria (Ugbo *et al*., 2023), but higher than the 12% reported by Amir *et al*. (2017) in Pakistan.

Poor hygiene practices during carcass evisceration and inadequate water sanitation are likely contributors to contamination. These findings underscore the need for strict adherence to sanitary protocols in poultry processing facilities to mitigate the risk of *E. coli* transmission through the food chain.

The prevalence of *S. aureus* in chicken meat in this study was 30%, which is lower than reported rates in Indonesia (58.3%), Cambodia (42.1%), the Republic of Serbia (100%), Bangladesh (54.9%), and Nepal (68%) (Wardhana *et al*., 2021; Rortana *et al*., 2021; Lika *et* *al*., 2021; Parvin *et al*., 2021; Bantawa *et al*., 2019). Within India, higher prevalence rates have been observed in Chennai (66.6%), Punjab (46.5%), and Hyderabad (84–100%) (Ruban *et al*., 2018; Herve *et al*., 2017; Kumar *et al*., 2020), although some studies reported lower rates in Chennai (16.6%) and Punjab (21.8%) (Meti *et* *al*., 2002; Zehra *et* *al*., 2019). These variations highlight regional differences and the influence of local processing and hygiene practices on *S. aureus* contamination in poultry.

We observed a comparatively lower prevalence of *S. aureus* in water samples (18%) compared to *E. coli*. Although this prevalence is lower, it remains a significant concern because the water is used for washing carcasses, knives, tables, and hand and potentially serving as a source of contamination for the meat during processing.

**3.2 Antibiotic sensitivity of the isolates**

**Table 4. Antibiotic susceptibility of *E. coli* isolates**

|  |  |  |
| --- | --- | --- |
| **Antibiotic****(Concentration in µg/disc)** | **Chicken Meat (n=13)** | **Water Sample (n=12)** |
| **Sensitive** | **Intermediate** | **Resistant** | **Sensitive** | **Intermediate**  | **Resistant** |
| **%** | **%** | **%** | **%** | **%** | **%** |
| AMX (30) | 7.69 | 7.69 | 84.62 | 16.67 | 0 | 83.33 |
| AMC (30/10) | 53.85 | 23.08 | 23.08 | 16.67 | 25 | 58.33 |
| CTX (30) | 69.23 | 0 | 30.77 | 50 | 25 | 25 |
| CN (30) | 0 | 30.77 | 69.23 | 0 | 16.67 | 83.33 |
| TET (30) | 7.69 | 0 | 92.31 | 16.67 | 0 | 83.33 |
| DOX (30) | 38.46 | 53.85 | 7.69 | 25 | 25 | 50 |
| COT (30) | 23.08 | 0 | 76.92 | 16.67 | 0 | 83.33 |
| AK (25) | 69.23 | 7.69 | 23.08 | 83.33 | 8.33 | 8.33 |
| GEN (30) | 61.54 | 15.38 | 23.08 | 58.33 | 16.67 | 25 |
| EX (10) | 30.77 | 7.69 | 61.54 | 16.67 | 16.67 | 66.67 |

(AMX-amoxicillin; AMC-Amoxicillin-clavulanic acid; CTX- Cefotaxime; CN-Cephalexin; TET-Tetracycline; DOX-Doxycycline; COT- Co-trimoxazole; AK-Amikacin; GEN-Gentamicin; EX-Enrofloxacin)

**Table 5. Antibiotic susceptibility *of S. aureus* isolates**

|  |  |  |
| --- | --- | --- |
| **Antibiotic****(Concentration in µg/disc)** | **Chicken Meat (n=15)** | **Water Sample (n=9)** |
| **Sensitive** | **Intermediate**  | **Resistant** | **Sensitive** | **Intermediate**  | **Resistant** |
| % | % | % | % | % | % |
| AMX (30) | 0 | 6.67 | 93.33 | 22.22 | 0 | 77.77 |
| AMC (30/10) | 40 | 0 | 60 | 55.56 | 0 | 44.44 |
| CTX (30) | 73.33 | 20 | 6.67 | 88.89 | 11.11 | 0 |
| MET (5) | 0 | 0 | 100 | 0 | 0 | 100 |
| TET (30) | 0 | 0 | 100 | 0 | 0 | 100 |
| DOX(30) | 0 | 0 | 100 | 11.11 | 0 | 88.89 |
| COT(30) | 0 | 0 | 100 | 44.44 | 0 | 55.56 |
| AK (25) | 60 | 0 | 40 | 88.89 | 0 | 11.11 |
| GEN (30) | 40 | 20 | 40 | 66.67 | 11.11 | 22.22 |
| EX (10) | 0 | 0 | 100 | 22.22 | 22.22 | 55.56 |

(AMX-amoxicillin; AMC-Amoxicillin-clavulanic acid; CTX- Cefotaxime; MET-Methicillin; TET-Tetracycline; DOX-Doxycycline; COT- Co-trimoxazole; AK-Amikacin; GEN-Gentamicin; EX-Enrofloxacin)

The antimicrobial susceptibility pattern of *E. coli* and *S. aureus* isolates from meat, cloacal and water samples against antibacterial agents of different classes were found to be highly variable which are shown in the Table 4 and 5

In this study, *E. coli* isolates showed the highest resistance to tetracycline (92.31%), followed by amoxicillin, co-trimoxazole, and cephalexin. This aligns with global findings, with similar tetracycline resistance reported in Nepal (93%), Romania (80%), and Bangladesh (87%) (Bantawa *et al*., 2019; Bratfelan *et al*., 2023; Alam *et al*., 2023). Studies in India also reported rates exceeding 80% in samples from New Delhi and Guwahati (Rawat *et al*., 2024; Deka & Ahmed, 2022). In contrast, lower resistance was found in *E. coli* from slaughterhouse water in Nigeria (Ugbo *et al*., 2023). The widespread use of tetracycline in poultry for growth promotion and disease prevention likely drives this resistance. These findings raise concerns about the potential for resistant strains to enter the food chain through contamination at slaughterhouses.

Resistance to amoxicillin was observed in 84.62% of *E. coli* isolates from chicken meat and 83.33% from water samples. Bantawa *et al.* (2019) reported 100% resistance in *E. coli* from chicken meat in Eastern Nepal, while Saad *et al.* (2019) found a lower rate of 69.65% in Bhaktapur Metropolitan City. Notably, *E. coli* sensitivity improved when amoxicillin was combined with clavulanic acid, indicating that resistance may be due to β-lactamase production, which hydrolyzes the β-lactam ring of these antibiotics.

High resistance to co-trimoxazole was observed in *E. coli* isolates from chicken meat (76.92%) and water samples (83.33%). These levels are higher than those reported in Romania for sulfamethoxazole (73.33%) and trimethoprim (50%) among *E. coli* isolates from chicken meat (Bratfelan *et al*., 2023) and in Nigeria, where 65.5% resistance was reported in isolates from slaughterhouse wastewater (Ugbo *et al*., 2023).

Resistance to cephalexin among *E. coli* isolates ranged from 69.23% to 83.3%, significantly higher than resistance to cefotaxime (25–30.77%). Ugbo *et al.* (2023) reported a 61.8% resistance rate in *E. coli* from slaughterhouse water in Nigeria, notably higher than the 25% observed in our study.

Resistance to enrofloxacin in *E. coli* isolates (61.5–66.7%) was comparable to ciprofloxacin resistance (56.7%) reported among *E. coli* isolates from Chicken Meat in Romania (Bratfelan *et al.,* 2023). Similarly, Habib *et al.* (2023) found high ciprofloxacin resistance (89%) in *E. coli* from chicken meat in the UAE, and Rawat *et al.* (2024) reported 65% resistance to nalidixic acid in New Delhi. The high resistance to fluoroquinolones likely reflects their widespread use for prophylaxis in poultry through drinking water.

In this study, 60–70% of *E. coli* isolates were sensitive to gentamicin and amikacin. Similarly, Bratfelan *et al.* (2023) reported 40% resistance in *E. coli* from chicken meat in Romania. In contrast, Rawat *et al*. (2024) found very low resistance (3–5%) in New Delhi, likely due to minimal use of these antibiotics as growth promoters in poultry.

Unlike *E. coli*, *S. aureus* isolates from chicken meat and water showed complete resistance to tetracycline and methicillin. Globally, tetracycline resistance in *S. aureus* from chicken meat varies widely, ranging from 38.8% to 88.2% across countries such as Serbia, South Africa, Turkey, and Korea (Lika *et al*., 2021; Jaja *et al*., 2020; Can *et al*., 2017; Kim *et al*., 2020). In India, a lower resistance rate of 45.1% was reported in Punjab (Zehra *et al*., 2019).

In the present study, *S. aureus* isolates from chicken meat (100%) and water (88.9%) exhibited higher resistance to doxycycline compared to *E. coli*. In contrast, studies from Oklahoma, Korea, and Punjab, India, reported lower resistance levels (20.7–42.6%) (Abdalrahman *et al*., 2015; Kim *et al*., 2020; Bantawa *et al*., 2017).

The prevalence of MRSA in the present study was 100%, which is considerably higher than the rates reported from chicken meat in Jalandhar, Punjab, India (57.5%) (Herve and Kumar, 2017) and Bangladesh (37.1%) (Parvin *et al*., 2021). The exceptionally high prevalence observed here is of significant concern, as chicken meat may serve as a critical reservoir for MRSA transmission to humans, posing substantial public health risks.

Resistance to amoxicillin observed among *S. aureus* isolates from chicken meat (93.33%) and water samples (77.77%) are consistent with the resistance reported in Nepal (100%), the Republic of Serbia (100%), and Bangladesh (87%) among *S. aureus* isolates from chicken meat (Bantawa *et al*., 2019; Lika *et al*., 2021; Parvin *et al*., 2021).

We observed low resistance to cefotaxime in *S. aureus* from chicken meat (6.67%). Similar findings were reported in Eastern Nepal (13%) (Bantawa *et al*., 2019) and by Herve and Kumar (2017), who found 15% resistance in chicken meat isolates from Punjab, India.

In this study, *S. aureus* isolates from chicken meat showed higher resistance to aminoglycosides amikacin and gentamicin (40%) than *E. coli* isolates (23.08%). Similar rates were reported by Herve and Kumar (2017), who found 35% resistance to amikacin and 60% to gentamicin in *S. aureus* from chicken meat in Jalandhar, Punjab.

*S. aureus* isolates from chicken meat in this study showed complete resistance (100%) to enrofloxacin, exceeding previously reported resistance to fluoroquinolones such as ciprofloxacin and levofloxacin (10–12.5%) among *S. aureus* isolates from chicken meat in Punjab, India (Herve and Kumar, 2017). However, higher ciprofloxacin resistance rates (33.9–63.2%) among *S. aureus* isolates from chicken meat have been reported in Turkey, Korea, and Punjab (Can *et al*., 2017; Kim *et al*., 2020; Zehra *et al*., 2019).

The elevated resistance to fluoroquinolones observed in this study may be attributed to their extensive prophylactic use in poultry production, particularly via administration through drinking water, a common practice that facilitates widespread exposure and selection pressure.

In this study, *S. aureus* isolates from chicken meat showed 100% resistance to co-trimoxazole, while nearly 50% of water isolates were resistant. These rates are markedly higher than those reported in South Africa (16.2%), Serbia (38.2%), Korea (0.8%) (Jaja *et* *al*., 2020; Lika *et al*., 2021; Kim *et al*., 2020), and Punjab, India (16.0%) (Zehra *et al*., 2019).

**3.3 Multiple antibiotic resistance**

The Multiple Antibiotic Resistance (MAR) index is a quantitative measure used to assess the level of resistance exhibited by bacterial isolates against multiple antimicrobial agents. An MAR value greater than 0.2 is typically indicative of isolates originating from high-risk environments with frequent antibiotic exposure, such as hospital or intensive farming operations. This index is widely used as an epidemiological tool to compare resistance patterns across different bacterial species, environmental sources, and geographic regions, helping to identify potential hotspots for antimicrobial resistance dissemination.

**Table 6 Multidrug Resistance status of *E. coli* isolates**

| **Isolate No.** | **Antibiotics resistant** | **No. of antibiotics resistant** | **MAR index** |
| --- | --- | --- | --- |
| **Chicken Meat** |
| C2 | AMX, AMC, CN, TET, COT | 5 | 0.5 |
| C3 | AMX, AMC, CTX, CN, TET, DOX, AK, EX, COT | 9 | 0.9 |
| C8 | AMX, AMC, CN, TET, GEN, AK, EX, COT | 8 | 0.8 |
| C9 | AMX, TET, COT | 3 | 0.3 |
| C10 | AMX, CTX, CN, TET, EX, COT | 6 | 0.6 |
| C12 | AMX, TET, COT | 3 | 0.3 |
| C13 | AMX, CTX, CN, TET, GEN, AK, EX, COT | 8 | 0.8 |
| C15 | AMX, CTX, CN, TET, EX, COT | 6 | 0.6 |
| C19 | AMX, TET, EX, COT | 4 | 0.4 |
| C23 | AMX, CN, TE,GEN, EX, COT | 6 | 0.6 |
| C26 | CN, TET | 2 | 0.2 |
| C28 | AMX, CN, TE, EX | 4 | 0.4 |
| **Water Sample** |  |  |
| W1 | CN, TET, DOX, EX, COT | 5 | 0.5 |
| W3 | AMX, AMC, CN, TET, DOX, EX, COT | 7 | 0.7 |
| W6 | AMX, AMC, CN, TET, AK, EX, COT | 7 | 0.7 |
| W13 | AMX, AMC, CN, GEN, COT | 5 | 0.5 |
| W14 | AMX, CTX, CN, TET, COT | 5 | 0.5 |
| W17 | AMX, AMC, CN, TET, GEN, EX, COT | 7 | 0.7 |
| W18 | AMX, AMC, CN | 3 | 0.3 |
| W19 | AMX, CN, TET, DOX, GEN, EX, COT | 7 | 0.7 |
| W21 | AMX, TET, EX, COT | 4 | 0.4 |
| W24 | AMX, AMC, CN, TET, DOX, EX, COT | 7 | 0.7 |
| W28 | AMX, AMC, CTX, CN, TET, DOX | 6 | 0.6 |
| W30 |  CTX, TET, DOX, EX, COT | 5 | 0.5 |

(AMX-amoxicillin; AMC-Amoxicillin-clavulanic acid; CTX- Cefotaxime; CN-Cephalexin; TET-Tetracycline; DOX-Doxycycline; GEN-Gentamicin; AK-Amikacin; EX-Enrofloxacin; COT- Co-trimoxazole)

**Table 7 Multidrug Resistance Status of *S. aureus* isolates**

|  |  |  |  |
| --- | --- | --- | --- |
| **Isolate No.** | **Antibiotics resistant pattern** | **No. of antibiotics resistant** | **MAR index** |
| **Chicken Meat** |
| C6 | AMX, MET,TET, DOX, AK, GEN, EX, COT | 8 | 0.8 |
| C8 | AMX, AMC, MET,TET, DOX, AK, EX, COT | 8 | 0.8 |
| C9 | AMX, MET,TET, DOX, AK, GEN, EX, COT | 8 | 0.8 |
| C15 | AMX, AMC, MET,TET, DOX, AK, GEN, EX, COT | 9 | 0.9 |
| C16 | AMX, AMC, MET,TET, DOX, AK, GEN, EX, COT | 9 | 0.9 |
| C18 | AMX, MET,TET, DOX, AK, GEN, EX, COT | 8 | 0.8 |
| C21 | AMX, AMC, MET,TET, DOX, GEN, EX, COT | 8 | 0.8 |
| C26 | AMC, MET,TET, DOX, EX, COT | 6 | 0.6 |
| C27 | AMX, AMC, MET,TET, DOX, EX, COT | 7 | 0.7 |
| C32 | AMX, CTX, MET,TET, DOX, EX, COT | 7 | 0.7 |
| C36 | AMX, MET,TET, DOX, EX, COT | 6 | 0.6 |
| C39 | AMX, AMC, MET,TET, DOX, EX, COT | 7 | 0.7 |
| C45 | AMX, MET,TET, DOX, EX, COT | 6 | 0.6 |
| C46 | AMX, AMC, MET,TET, DOX, EX, COT | **7** | 0.7 |
| C49 | AMX, AMC, MET,TET, DOX, EX, COT | **7** | 0.7 |
| **Water Sample** |
| W14 | MET,TET, DOX, GEN, COT | 5 | 0.5 |
| W28 | AMX, MET,TET | 3 | 0.3 |
| W33 | AMX, AMC, MET,TET, DOX,EX, COT | 7 | 0.7 |
| W36 | AMX, AMC, MET,TET, DOX, GEN | 6 | 0.6 |
| W37 | AMC, MET,TET, DOX | 4 | 0.4 |
| W39 | AMX, MET,TET, DOX, EX, COT | 6 | 0.6 |
| W45 | AMX, MET,TET, DOX, EX, COT | 6 | 0.6 |
| W46 | AMX, AMC, MET,TET, DOX, AK, EX, COT | 8 | 0.8 |
| W50 | MET,TET, DOX, EX | 4 | 0.4 |

(AMX-amoxicillin; AMC-Amoxicillin-clavulanic acid; CTX- Cefotaxime; MET-Methicillin; TET-Tetracycline; DOX-Doxycycline; GEN-Gentamicin; AK-Amikacin; EX-Enrofloxacin; COT- Co-trimoxazole)



Fig. 3 Resistance index of the isolates

The multidrug resistance pattern of the *E. coli* and *S. aureus* from chicken meat and water sample are given in Table 3 and 4 and Fig. 3.

In this study, 84.6% of *E. coli* isolates from chicken meat and 100% from water samples exhibited an MAR index >0.2, consistent with Khanom *et al.* (2025), who reported 84.7% MAR in *E. coli* from chicken meat in Bangladesh. Similarly, 100% multidrug resistance with MAR >0.2 has been reported in *E. coli* isolates from chicken meat in Bangladesh (Rafiq et al., 2024), Eastern China (Afayibo *et al*., 2022), and South Africa (Jaja *et al*., 2020). In this study, all *S. aureus* isolates were multidrug-resistant with an MAR index >0.2. Similar findings of 100% multidrug resistance in *S. aureus* from chicken meat have been reported in South Africa (Jaja *et al.,* 2020), Serbia (Lika *et al.,* 2021), and Bangladesh (Parvin *et al.,* 2021).

The highest MAR index observed was 0.9, detected in 7.69% of *E. coli* isolates from chicken meat and in 13.33% of *S. aureus* isolates from the same source. Phenotypic antibiotic resistance patterns varied considerably among the isolates. The MAR index of *S. aureus* isolates from chicken meat differed significantly (*P*=0.05) from that of *E. coli* isolates and *S. aureus* isolates from water samples. However, no significant difference was observed between *S. aureus* isolates from water samples and *E. coli* isolates from both sources. The emergence of multidrug resistance in commensal and foodborne pathogens is driven by high selection pressure and horizontal gene transfer, both of which increase with antibiotic use.

4. Conclusion

The findings of the present study underscore the prevalence of multidrug-resistant *E. coli* and *S. aureus* in chicken meat and water samples. The high occurrence of these resistant isolates highlights the critical role of antibiotic overuse in the poultry industry as a driving force behind antimicrobial resistance. The presence of multidrug-resistant commensals such as *E. coli* in poultry meat is a significant public health concern, as it can be transmitted to humans through the food chain and serve as a reservoir for resistance genes that can be transferred to other bacteria within the human microbiota. This trend poses serious public health concerns, including increased disease severity, treatment failures, and elevated healthcare costs. Addressing this pressing issue requires the implementation of stringent antimicrobial stewardship programs to regulate antibiotic usage in poultry production. Regular surveillance of antimicrobial resistance patterns is also essential to monitor emerging trends and inform effective intervention strategies. Furthermore, promoting awareness among farmers and stakeholders about the responsible use of antibiotics, alongside the exploration of alternative disease control measures, is vital for mitigating the spread of multidrug-resistant pathogens.

Consent (where ever applicable)

This study did not involve human participants; therefore, informed consent was not required.

Ethical approval (where ever applicable)

This study did not involve the use of animals; therefore, ethical approval was not required.

Disclaimer (Artificial intelligence)

Author(s) hereby declare that NO generative AI technologies such as Large Language Models (ChatGPT, COPILOT, etc.) and text-to-image generators have been used during the writing or editing of this manuscript.

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