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Prevalence and antimicrobial resistance of *Escherichia coli* from frozen chicken meat

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Abstract

The emergence of antimicrobial-resistant bacteria, such as *Escherichia coli*, poses a growing threat to human health, veterinary medicine, and food hygiene. Therefore, the aim of this research was screening of *E. coli* and identifying antibiotic resistance profiles in isolates obtained from frozen chicken meat samples collected from super shops in Sylhet, Bangladesh. The study was conducted in 2023 with 40 chicken samples. Gram-negative *E. coli* isolates were identified based on their green metallic sheen on selective media and confirmed through biochemical examination. Of the 40 samples, 38 (95% prevalence) samples tested positive for *E. coli*. Antibiotic resistance profiles were determined using the disk diffusion method against 16 antibiotics from 13 antimicrobial classes. Resistance rates were particularly high against ampicillin, ciprofloxacin, nalidixic acid, oxytetracycline, and cephalexin, with 100% of multidrug-resistant (MDR) isolates. Six isolates (15.79%) were resistant to all thirteen antibiotic classes tested, while more than 70% of the MDR *E. coli* were resistant to 9-13 antibiotic classes. Antibiotics such as chloramphenicol (44.74%) and aztreonam (39.47%) showed a higher rate of susceptibility. The findings of this research may contribute to a better understanding of antimicrobial resistance in *E. coli* from frozen chicken meat samples.

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Introduction

The consumption of animal products has increased due to factors such as increased population growth, urbanization, globalization, rising per capita income, and shifting consumer preferences towards higher-protein diets (Dharma *et al*., 2013). Among animal products, meat is a significant dietary item for humans because of its high nutritional content and delectableness. Globally, both poultry meat production and consumption are rising (Bilgili, 2002), and the advantages of chicken meat includes low-calorie content, low saturated fat content, low collagen content, and easy digestibility (Marangoni *et al*., 2015). Bangladesh has a thriving chicken industry, which is essential for boosting agricultural growth and the availability of protein and nutrients (Rahman *et al*., 2015). The poultry industry supplies quality protein to the people of Bangladesh at some of the lowest prices globally (Islam *et al*., 2014) and contributes 22-27 % of the country's total meat supply (Department of Livestock Services, 2022). Although harmful microorganisms are absent from the muscles of healthy animals, meat tissues can become contaminated with microorganisms at different points in the value chain (Ibrahim *et al*., 2019). Consuming contaminated meat and meat products is thought to be one of the main causes of the high frequency of zoonotic and food-borne illnesses in humans.

Gastrointestinal complications caused by the consumption of specific foods or beverages are referred to as food-borne illnesses or food poisoning. Each year, food-borne disease affects over one-third of the world's population in affluent countries (Tanveer *et al*., 2017). There are around sixteen types of bacteria, three virus families, twenty-two parasites, and three protozoa among the microbial pathogens that can be transmitted from animals to humans through food (Thapa *et al*., 2020). Among bacterial food-borne agents, *Escherichia coli,* a gram-negative, facultative anaerobic, rod-shaped bacterium from the genus of *Escherichia*, poses a potential infection risks to humans. Chicken meat can become contaminated by it due to improper handling, cleaning, dressing, and unhygienic meat-selling practices. Consequently, pathogenic *E. coli* can be transmitted to humans either directly during food preparation or via consumption of undercooked or raw meat products (Addis and Sisay, 2015).

Undoubtedly, antibiotics have revolutionized the way for treating many infectious diseases, which in turn reduce morbidity and mortality rates (Huemer *et al*., 2020). However, the unwise use of antibiotics in food animal production for both treatment and growth enhancement purposes led the emergence of antimicrobial resistance (AMR) (Agyare *et al*., 2019), which is now a significant public health concern in both human and veterinary medicine globally (Ferri *et al*., 2017; Palma *et al*., 2020). AMR against first-line antibiotics in pathogens like *E. coli* has rendered these treatments ineffective (Cosgrove, 2006).

Furthermore, *E. coli* can transfer antibiotic resistance mechanisms not only among its different strains but also to other bacterial species (Rasheed *et al*., 2014). There is a limited amount of research available in assessing the contamination of frozen chicken meat with antibiotic resistant bacteria in super shops (Parvin *et al*., 2020). Moreover, people's preferences to go shopping in super shops are increasing all over Bangladesh, and the safety of using frozen chicken from super shops needs to be assessed. Therefore, the present study aimed to determine the prevalence of *E. coli* and assess their multidrug-resistant (MDR) pattern in frozen chicken meat samples from super shops in Sylhet, Bangladesh.

Materials and methods

Sample collection and processing

Meat samples were collected from six outlets of two popular super shop brands in Sylhet metropolitan city, Bangladesh. The samples included drumstick, wing, and breast pieces, in both frozen and non-frozen conditions. A total of forty samples were taken from these super shops in separate sterile zipper bags, labelled, kept cool, and transported for immediate processing. Samples (25 g of each) processing was done as described earlier (Parvin *et al*., 2020).

Detection of E. coli

After pre-enrichment in buffered peptone water (BPW), 1 mL of each diluted meat sample was incubated overnight at 37°C in nutrient broth. Samples were then streaked onto Eosin Methylene Blue (EMB) agar media followed by incubation at 37°C for 18–24 hours. Colonies displaying a metallic sheen on EMB agar were identified as *E. coli*. These colonies were subsequently sub-cultured on MacConkey agar for further confirmation. After that, a single colony was sub-cultured again on EMB agar to obtain pure culture.

Identification of E. coli

Further identification of *E. coli* isolates was performed using a series of biochemical techniques, such as Gram staining, catalase, oxidase, indole, methyl red, Voges–Proskauer tests, and triple sugar iron test. Biochemically confirmed *E. coli* isolates were then used for further analysis.

Antimicrobial susceptibility testing of E. coli

Each isolate was tested for susceptibility to sixteen commercially available antibiotic discs (Oxoid, UK) spanning thirteen antimicrobial classes using the disk diffusion test. The antibiotics tested included: ampicillin (10 µg), amoxicilin/calvulanic acid (30 µg), cephalexin (30 µg), gentamicin (10 µg), streptomycin (10 µg), chloramphenicol (30 µg), azithromycin (15 µg), cefotaxime (30 µg), imipenem (10 µg), cefoxitin (30 µg), doxycycline (30 µg), ciprofloxacin (5 µg), nalidixic acid (30 µg), aztreonam (30 µg), oxytetracycline (30 µg), and colistin sulphate (10 µg). Antimicrobial susceptibility was determined in accordance with the guidelines of Clinical and Laboratory Standards Institute (CLSI, 2021). The prevalence of multidrug-resistant (MDR) *E. coli* was determined after interpreting the susceptibility and resistant status of each antibiotic tested. Multidrug-resistant isolates were identified as those demonstrating resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Data analysis

All data were collected, and a normal frequency distribution was used to measure prevalence and multidrug resistance. The standard zone of inhibition was measured to determine whether each isolate was resistant or sensitive.

Results

Sample collection

During 2023, we aseptically collected forty chicken meat samples (both frozen and non-frozen) from six outlets of two popular super shops in Sylhet city, Bangladesh. All available outlets of these two super shops in Sylhet city were included in the study. Thirty samples (frozen) were collected from brand one, while the remaining ten samples (non-frozen) were from brand two. The chicken meat samples included ten breasts, fifteen drumsticks, and fifteen wings (Table 1).

Table 1. Prevalence and distribution of *E. coli*

Variables	No. of samples	No. of samples positive	Prevalence (%)
Brands			
Brand 1	30	29	96.67
Brand 2	10	9	90
Meat Types			
Wing	15	14	93.33
Breast	10	10	100
Drumstick	15	14	93.33
Outlets			
Outlet 1	5	5	100
Outlet 2	5	5	100
Outlet 3	5	5	100
Outlet 4	5	5	100
Outlet 5	10	9	90
Outlet 6	10	9	90
Total	40	38	95

Prevalence and distribution of E. coli in chicken meat samples

Out of the forty samples assessed, thirty-eight chicken meat samples were found to be contaminated with *E. coli*. Isolates were identified as *E. coli* based on characteristic green metallic sheen colonies on EMB agar, dark pinkish colonies on MacConkey agar, Gram staining results, and a series of biochemical tests. The prevalence of *E. coli* was notably high (95%) (Table 1). Among the brands considered, the highest prevalence was observed in brand 1 (96.67%), having a larger sample size. In addition, the highest prevalence of *E. coli* was observed in breast samples (100%), and the prevalence was 93.33% in both wings and drumsticks.

Antibiotic resistance among E. coli isolates

Antibiotic resistance was detected against all antimicrobial agents evaluated by disk diffusion test (Fig. 1). The overall individual resistance patterns for tested antibiotics are shown in Table 2. The antimicrobial susceptibility test revealed that the highest percentage of resistance was found in the case of ampicillin (97.37%), nalidixic acid (94.77%), and ciprofloxacin (94.77%). On the contrary, the lowest resistance was observed for imipenem (36.84%) followed by chloramphenicol (44.74%), aztreonam (52.63%), and gentamicin (63.16%). An equal resistance percentage (89.47%) was observed for both cephalexin and oxytetracycline (Table 2).

Table 2. Antibiotic susceptibility profile of isolated *E. coli*

Antimicrobial agents	No. of isolates (%)		
	Sensitive	Intermediate	Resistant
Ampicillin	1(2.63)	0(0.00)	37(97.37)
Amoxicilin/Calvulanic acid	5(13.16)	6(15.79)	27(71.05)
Cephalexin	1(2.63)	3(2.63)	34(89.47)
Gentamicin	13(34.21)	1(2.63)	24(63.16)
Streptomycin	1(2.63)	5(13.16)	32(84.21)
Chloramphenicol	17(44.74)	4(10.53)	17(44.74)
Azithromycin	9(23.68)	0(0.00)	29(76.32)
Cefotaxime	8(21.05)	3(7.89)	27(71.05)
Imipenem	9(23.68)	15(39.47)	14(36.84)
Cefoxitin	4(10.53)	1(2.63)	33(86.84)
Doxycycline	4(10.53)	6(15.79)	28(73.68)
Ciprofloxacin	0(0.00)	2(5.26)	36(94.77)
Nalidixic Acid	1(2.63)	1(2.63)	36(94.77)
Aztreonam	15(39.47)	3(7.89)	20(52.63)
Oxytetracycline	4(10.53)	0(0.00)	34(89.47)
Colistin sulphate	1(2.63)	10(26.32)	27(71.05)

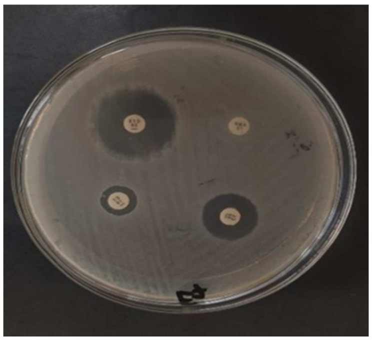

Fig. 1. Antibiotic susceptibility testing of *E. coli* by disk diffusion method

Table 3. Multidrug-resistant *E. coli* from different samples

No. of antibiotic classes	No. of resistant isolates			
	Wing	Breast	Drumstick	Total (%)
3	1	0	0	1(2.63)
6	1	0	0	1(2.63)
7	2	1	1	4(10.53)
8	1	1	2	4(10.53)
9	1	2	1	4(10.53)
10	4	3	4	11(28.95)
11	2	0	3	5(13.16)
12	0	0	2	2(5.26)
13	2	3	1	6(15.79)
Total	14	10	14	38

Multidrug resistance pattern of E. coli

All 38 isolates were resistant to antibiotics from three or more antimicrobial classes (Table 3), indicating that all were multidrug-resistant (MDR). We used sixteen antibiotics from thirteen antimicrobial classes, including non-extended spectrum cephalosporins, extended-spectrum cephalosporins, cephamycins, fluoroquinolones, penicillins, penicillins with β-lactamase inhibitors, carbapenems, polymyxins, monobactams, aminoglycosides, tetracyclines, phenicols and macrolides. Notably, 6 isolates (15.79%) were resistant to all thirteen classes, while 5 isolates (13.16%) exhibited resistance to eleven antimicrobial classes. Resistance to nine antimicrobial classes was observed in 4 isolates (10.53%). The same percentage of isolates showed resistance to seven and eight antibiotic classes. Moreover, the highest number of isolates (11) showed resistant properties to ten classes. Only 2 isolates (5.26%) were resistant to twelve antimicrobial classes (Table 3). On the brand-wise distribution of MDR *E. coli*, the highest percentage

(33.33%) of isolates resistant to ten and eleven antimicrobial classes was found in non-frozen meat from brand 2. Additionally, frozen chicken samples having *E. coli* from brand 1 indicated the highest resistance to ten antibiotic classes (Fig. 2). Considering the meat sample types, the highest percentages of isolates (28.57%) from both wings and drumsticks were resistant to ten classes of antibiotics. Similarly, the highest number of isolates (30%) from breasts was resistant to both thirteen and ten antibiotic classes (Fig. 3).

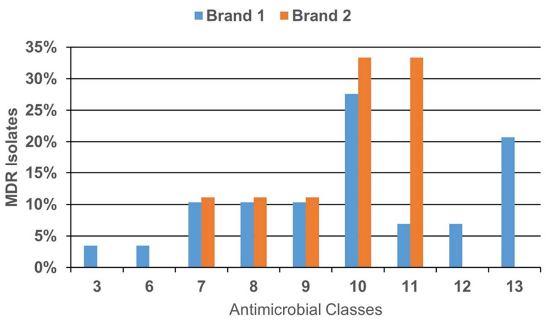

Fig. 2. Brand-specific distribution of multidrug-resistant *E. coli*

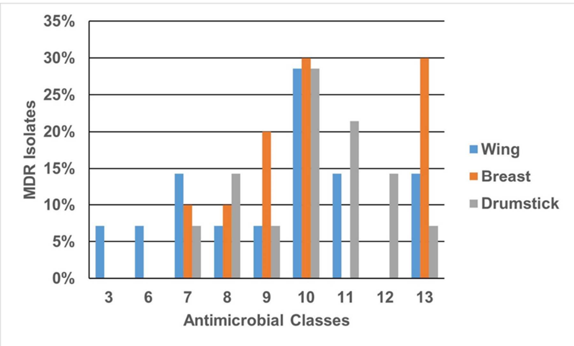

Fig. 3. Distribution of multidrug-resistant *E. coli* by meat types

Phenotypic resistance pattern of isolates

The phenotypic resistance patterns among the isolated *E. coli* are depicted in Fig. 4. Six isolated *E. coli* were resistant to all tested antibiotics, showing the most common resistance pattern that included all 16 antibiotics. Another resistance pattern consisting of 14 antimicrobial agents (excluding doxycycline and imipenem) was observed in two isolates. Similarly, a resistance pattern containing 13 antibiotics was identified in two isolates.

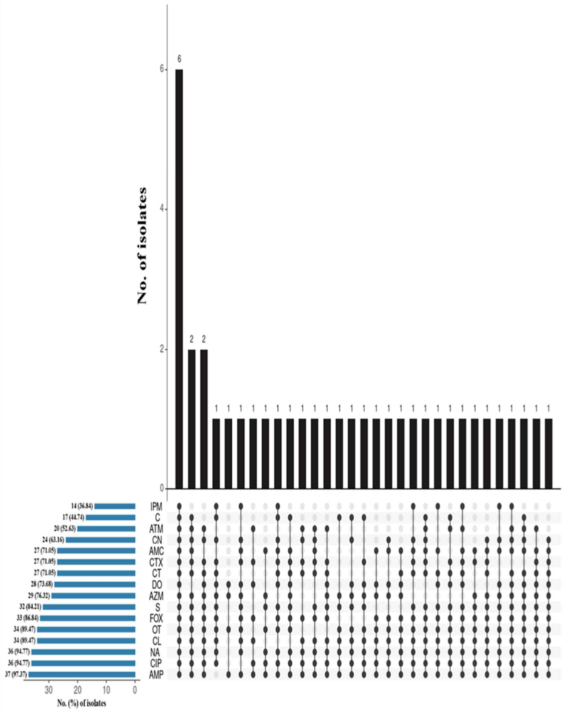

Fig. 4. The phenotypic resistance pattern of *E. coli* isolates summarized by an UpSet plot. Abbreviations: AMP: ampicillin; AMC: amoxicilin/calvulanic acid; CL: cephalexin; CN: gentamicin; S: streptomycin; C: chloramphenicol; AZM: azithromycin; CTX: cefotaxime; IPM: imipenem; FOX: cefoxitin; DO: doxycycline; CIP: ciprofloxacin; NA: nalidixic acid; ATM: aztreonam; OT: oxytetracycline; CT: colistin sulphate

Discussion

E. coli belongs to the groups of the seven species, which are named by the WHO as of key concern for antimicrobial resistance (Ibrahim *et al.*, 2016). Globally, chicken meat is considered a potential reservoir of MDR *E. coli*, responsible for growing human health-related problems (Trkov *et al.*, 2014). This research reports the detailed data on the antimicrobial resistance pattern and prevalence of MDR *E. coli* in meat samples from two well-known supermarkets in Sylhet city, Bangladesh. The detection process was conducted by using phenotypic and biochemical techniques. Distinct parts of chicken like wings, drumsticks in both frozen and non-frozen conditions, breast pieces were screened for *E. coli*. While exploring the presence of *E. coli*, it was found that the prevalence was notably high (95%). The prevalence detected is higher than the earlier research (45.5%) in frozen chicken meat from the Sylhet region (Parvin *et al*., 2020). Similarly, Rahman *et al.* (2020) reported 63.5% prevalence of *E. coli* in chicken samples from Sylhet. Our reported prevalence is also comparatively higher than prevalence in raw chicken samples (49-53%) in Bangladesh reported earlier (Faruque *et al*., 2019; Rahman *et al*., 2017). The reason behind such variations in prevalence of *E. coli* in several reports is unknown, but factors like differences in sampling methods and sample collection time may contribute to variation in prevalences. Moreover, there is no study yet involving the number of samples we considered from super shops.

The disk diffusion method was employed to assess the antibiotic susceptibility against 16 antibiotics. The test revealed that the isolates were resistant to all classes of antibiotics evaluated. The highest percentage of resistance was observed against penicillins (97.37%). Grave *et al.* (2010) found similar (98.9%) resistance to ampicillin in *E. coli* isolates.

Furthermore, higher resistance (94.77%) to fluoroquinolones (nalidixic acid and ciprofloxacin) was detected which is following Mandal *et al.* (2022). The most often used antibiotics in chicken industry of Bangladesh is fluoroquinolones particularly ciprofloxacin (Islam *et al.*, 2016). Thus, high resistance to fluoroquinolones may be the outcome of indiscriminate use of ciprofloxacin. High antibiotic resistance (89.47%) was also found in first generation cephalosporins and tetracyclines (e.g., oxytetracycline). Similarly, Parvin *et al.* (2020) detected high resistance to oxytetracycline as it is also vastly used in veterinary practices. Resistance to imipenem (36.84%) was the lowest among the tested antibiotics as it is not used in poultry. Among all the antibiotics tested, chloramphenicol (44.74%) and aztreonam (39.47%) showed a higher rate of susceptibility.

In the present research, 100% of the *E. coli* were multidrug-resistant. These suggest that the highest prevalence of MDR *E. coli* is a matter of concern in our country, where super shops keep better hygiene than other retail shops. We found that six isolates (15.79%) were resistant to all thirteen antibiotic classes tested. In addition, more than 70% of the MDR *E. coli* were resistant to 9-13 antibiotic classes which indicate the risk of MDR increasing with time passing and becoming a dangerous threat to human health and treatments. Similarly, Parvin *et al.* (2020) observed that a great percentage of isolates from frozen chicken meat were resistant to 9-13 classes of antibiotics. We also found that antibiotic resistance varied between brands, and by meat types. The data gathered in this research could function as a baseline study involving chicken meat samples from super shops to detect AMR in *E. coli*. However, further investigations with more samples are needed to get a complete understanding of AMR in *E. coli* from frozen chicken meat.

Conclusion

The high prevalence of MDR isolates in chicken meat samples from super shops offers new insights into the AMR in *E. coli*. The scenario we get is alarming. Keeping this in mind, we must use antibiotics properly as well as developing new antibiotics for combating MDR. Additionally, we need to cut the route to transmit AMR through good food handling and cooking practices.

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