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*Original Article*

# Exploring Multidrug Resistant and Possible Extensively Drug Resistant Extended-Spectrum B-Lactamase-Producing *Escherichia coli* Isolated from Frozen Chicken Meat in Bangladesh

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**Abstract:** Multidrug resistant extended-spectrum β-lactamase (ESBL)-producing *Escherichia coli* is considered a serious concern to public health worldwide including Bangladesh, and chicken meat is recognized as an important reservoir of ESBL-Ec dissemination to humans. Therefore, this study aimed to determine the prevalence, and phenotypic and genotypic antimicrobial resistance pattern of ESBL-producing *Escherichia coli* (ESBL-Ec) in frozen chicken meat. A total of 113 frozen chicken meat samples were purchased from 40 outlets of 9 branded supershops in five megacities in Bangladesh. Isolation and identification of *Escherichia coli* were done based on cultural, biochemical properties and PCR assay. The resistance pattern was determined by disk diffusion method. ESBL-encoding genes were determined by multiplex PCR. The results showed that 76.1% samples were positive for *Escherichia coli*, of which 86% were ESBL producers. All the isolates were multidrug-resistant (MDR). Resistance to 9–11 and 12–13 antimicrobial classes was observed in 38.4% and 17.4% isolates, respectively while only 11.6% were resistant to 3–5 classes. The possible extensively drug resistance (pXDR) was found in 2.3% isolates. The high single resistance was observed for oxytetracycline (93%) and amoxicillin (91.9%), followed by ampicillin (89.5%), trimethoprim-sulphamethoxazole and pefloxacin (88.4%), and tetracycline (84.9%). Most importantly, 89.6% of isolates were resistant to carbapenems. All the isolates were positive for *bla*TEM gene. However, the *bla*SHV and *bla*CTX-M-2 genes were identified in two ESBL-non producer isolates. None of the isolates were carried *bla*CTX-M-1 gene. This study provided evidence of wide dissemination of MDR and existence of pXDR ESBL-Ec in frozen chicken meat in Bangladesh. Our data clearly indicated that frozen chicken meat is, at the present time, the most significant known food source of ESBL-Ec to which peoples are regularly exposed.

**Keywords:** *Escherichia coli*; antimicrobial resistance; ESBL; MDR; frozen chicken meat; Bangladesh

## 1. Introduction

*Escherichia coli* (*E. coli*), a member of the *Enterobacteriaceae* family, is a normal inhabitant of the gut of poultry, and a frequent microbial contaminant of retail poultry meat [1]. *E. coli* is also known as one of the most important foodborne pathogens in humans [2]. Chicken meat is frequently contaminated by *E. coli* during handling, improper dressing, cleaning, and unhygienic practices of selling meat. Contaminated chicken meat have been considered as a potential source for transmission of *E. coli*, either by direct contact or consumption of contaminated meat products,

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leading to the colonization in the intestinal tract and eventually to severe infection in humans, which may cause public health hazards [2].

Over the past decades, antibiotic resistance trends have been increasing at a faster rate among chicken isolates of *E. coli* than human clinical isolates [3]. Commensal *E. coli* were determined as an important reservoir of antimicrobial resistance genes that may spread to pathogenic strains [4]. One of the most common resistance mechanisms reported in the members of the family *Enterobacteriaceae* is the production of β-lactamase enzymes that hydrolyze β-lactam antibiotics [5]. Extended-spectrum β-lactamases (ESBLs), variants of β-lactamases, a heterogeneous group of enzymes, are encoded by genes, which efficiently hydrolyze third and fourth generation cephalosporins and monobactams (e.g. aztreonam) but are inhibited by β-lactamase inhibitors such as clavulanic acid and tazobactam [6]. *E. coli* that produces ESBL has been of particular concern because of their implications for human and food animal health worldwide [7]. The emergence of ESBLs, is considered as an important cause of transferable multidrug resistant superbugs, particularly *E. coli*. Furthermore, ESBL-producing *E. coli* often exhibit co-resistance to multiple classes of antimicrobials mainly fluoroquinolones, sulfonamides, aminoglycosides, chloramphenicol, trimethoprim and tetracyclines, which may increase the risk of poor clinical outcomes due to lack of effective treatment options [7].

The major genes responsible for ESBL production include TEM genes (*bla*TEM), SHV genes (*bla*SHV), and CTX-M genes (*bla*CTX-M). The CTX-M type ESBL-producing *E. coli* is the most dominant globally [8]. In Bangladesh, *bla*CTX-M-1 (94.4%) and *bla*TEM (50%–91.3%) ESBL producing *E. coli* have been reported in droppings of chickens [9-11]. Chickens are considered as a potential reservoir of ESBL-producing *E. coli* [12]. Chicken meat contaminated with ESBL-producing bacteria is thought to be one of the potential risk factors for wide dissemination of ESBL-producing bacteria in humans [13].

The prevalence of multidrug resistant superbugs and ESBL-producing bacteria is increasing in humans as well as animals. The multidrug-resistant (MDR) and extensively drug-resistant (XDR) bacteria have been well-defined by European Centre for Disease Control, and Centers for Disease Control and Prevention, Atlanta [14]. MDR is defined as acquired non-susceptibility to at least one agent in three or more antimicrobial categories and XDR is defined as non-susceptibility to at least one agent in all but two or fewer antimicrobial categories (i.e., bacterial isolates remain susceptible to only one or two antimicrobial categories).

Nowadays, likewise other countries, the lifestyle, preference and demands of consumers in Bangladesh are changing rapidly. With the current shopping practice, supershops have become a necessity as they offer a unique shopping experience with all essential commodities under one roof. Consumers, especially, city dwellers are increasingly becoming more aware of the conveniences and their own lifestyles, they in many cases prefer to go to supershops rather than to wet markets around to buy their everyday stuffs including frozen chicken meat. City dwellers tend to buy frozen chicken meat along with other frozen and ready-to-cook food stuffs as these frozen items need minimal processing for cooking, and thus, they can save their time [15]. However, microbiological safety of these frozen chicken meat has become an important concern in the context of public health hazards as two studies report bacterial contamination in frozen chicken meat in Dhaka city of Bangladesh [16,17]. Both studies were restricted to three to five supershops of Dhaka city only. Furthermore, none of these two reports investigated the multidrug resistance pattern of ESBL-producing *E. coli*. Therefore, a comprehensive study is required to have a greater scenario of *E. coli* contamination along with resistance pattern in frozen chicken meat sold in various supershops located in all the megacities of Bangladesh. The present study determined the (i) prevalence and distribution of MDR and possible XDR ESBL-producing *E. coli* in frozen chicken meat sold in various supershops located in five megacities of Bangladesh and (ii) phenotypic as well as genotypic antimicrobial resistance pattern of ESBL-*E. coli* in frozen chicken meat, which have not yet been investigated in Bangladesh.

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## 2. Materials and Methods

### *2.1. Sample collection*

A cross-sectional survey was conducted in 40 supershop outlets of nine brands available in five megacities (Dhaka, Sylhet, Mymensingh, Chattogram and Rajshahi) of Bangladesh (Figure 1) during April to December 2019. A total of 113 frozen chicken meat samples (82 broiler chicken meat, 31 cockerel chicken meat) were purchased from these outlets. On availability, meat samples included whole chicken or chopped chicken comprising breast, drumstick, leg, and wings muscle. Samples were placed in separate sterile plastic bags, labelled, kept in ice box, and transported to the laboratory and processed as soon as possible. Alongside, data on brand name, source of chicken, processing and packaging of meat, and special labels (green chickens/organic) were collected.

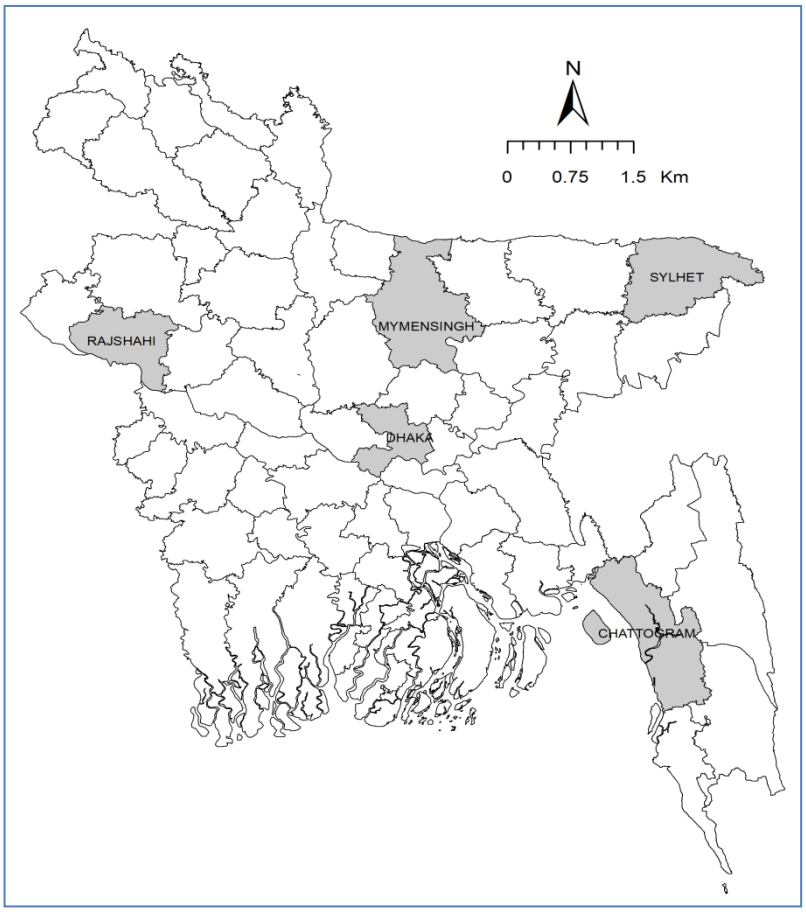


**Figure 1.** Map showing sampling sites in five megacities of Bangladesh.

### *2.2. Enrichment and identification of E. coli*

The preparation of the meat samples was based on the European standard ISO-16654:2001 [18]. About 25 g of the meat samples were chopped into very small fine pieces using sterile scissors and scalpel, mixed with 225 mL buffered peptone water, homogenized for two minutes with gentle shaking and enriched overnight (18-24 h) at 37°C. After pre-enrichment, 1 mL of diluted meat samples was taken using a sterile pipette and transferred into test-tube containing the nutrient broth and incubated overnight at 37 °C. Then a loopful of this overnight culture was streaked onto Eosin Methylene Blue agar and incubated at 37 °C for 18–24 h. One presumptive *E. coli* colony having a dark blue colour with a characteristic metallic sheen from each selective agar plate was then subcultured to obtain a pure culture and identification was performed using standard microbiological and biochemical procedures including Gram staining, catalase, oxidase, indole,

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methyl red, Voges-Proskauer tests, and sugar fermentation test using triple sugar iron agar. Positive isolates were stored in nutrient broth containing 50% (v/v) glycerol at –20°C for further study.

*2.3. Molecular detection of E. coli*

Bacterial DNA was extracted by boiling of 1 mL of overnight culture as described earlier [19]. The DNA concentration was measured by NanoDrop One (Thermo Fisher Scientific, USA). PCR was performed for the confirmation of *E. coli* using *16S rRNA* gene specific primers as described earlier [20]. The sequence of forward primer was 5'-GACCTCGGTTTAGTTCACAGA-3 and of reverse primer 5'- CACACGCTGACGCTGACCA-3'. Amplification reactions were done in a 25 µL volume containing 12.5 µL PCR Master Mix (Thermo Scientific, USA), 1.5 µL (15 pmol) of each forward and reverse primer, template DNA 0.5 µL (50 ng) and nuclease-free water 9.0 µL. The PCR was run under the following conditions in Veriti™ 96-Well Thermal Cycler (Thermo Fisher Scientific Inc., USA): an initial denaturation at 95 °C for 5 min followed by 35 cycles of amplification, denaturation for 1 min at 94 °C, annealing at 58 °C for 1 min, extension for 1 min at 72 °C and final extension at 72 °C for 7 min. After amplification, PCR product was subjected to electrophoresis on 1.5% agarose gel containing ethidium bromide (5 µg/mL). The resulting band of PCR product was examined under UV-transilluminator and documented.

*2.4. Antimicrobial susceptibility testing*

Antimicrobial susceptibility was determined by disk diffusion assay with 38 antimicrobials belonged to16 antimicrobial classes. Following antimicrobial discs (Biomaxima, Poland; Oxoid, UK) were procured and used for the testing:

A) Non-extended spectrum cephalosporins including:
   - First generation cephalosporins: Cephalexin (30 µg), Cefradine (30 µg)
   - Second generation cephalosporins: Cefuroxime (30 µg), Cefaclor (30 µg)
B) Extended-spectrum cephalosporins including:
   - Third generation cephalosporins: Cefotaxime (30 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefixime (5 µg)
   - Fourth generation cephalosporins: Cefepime (30 µg)
C) Cephamycins: Cefoxitin (30 µg)
D) Fluoroquinolones: Nalidixic acid (30 µg), Ciprofloxacin (5 µg), Levofloxacin (5 µg), Norfloxacin (10 µg), Ofloxacin (5 µg), Gatifloxacin (5 µg), Pefloxacin (5 µg)
E) Penicillins: Ampicillin (10 µg), Amoxycillin (10 µg),
F) Penicillins + β-lactamase inhibitors: Amoxicillin-clavulanic acid (30 µg)
G) Antipseudomonal penicillins + β-lactamase inhibitors: Pipercillin-tazobactam (110 µg)
H) Carbapenems: Imipenem (10 µg), Meropenem (10 µg)
I) Polymyxins: Colistin (10 µg), Polymyxin B (300 units)
J) Monobactams: Aztreonam (30 µg)
K) Aminoglycosides: Gentamicin (10 µg), Amikacin (30 µg), Streptomycin (10 µg), Neomycin (30 µg), Tobramycin (10 µg)
L) Tetracyclines: Tetracycline (30 µg), Oxytetracycline (30 µg), Doxycycline (10 µg)
M) Folate pathway inhibitors: Trimethoprim-Sulfamethoxazole (25 µg)
N) Glycylcyclines: Tigecycline (15 µg)
O) Phenicols: Chloramphenicol (30 µg)
P) Macrolides: Azithromycin (15 µg)

After preparation of each bacterial suspension, the turbidity was adjusted equivalent to 0.5 McFarland standard and then inoculated onto Mueller-Hinton agar in duplicate. After overnight incubation at 37 °C, diameter of the clear zone of inhibition around each antimicrobial disc was measured in millimeters. These results were interpreted as per guidelines of the Clinical and Laboratory Standards Institute (CLSI) [21]. The isolates were classified as susceptible, intermediate and resistant. Isolates resistant to ≥ 1 agent in three or more antimicrobial classes were classed as

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multidrug-resistant (MDR) and isolates resistant to ≥ 1 agent in all but ≤ 2 antimicrobial classes were categorized as extensively drug resistant (XDR) [14].

*2.5. Detection of ESBL-producing E. coli*

ESBL-producing *E. coli* was detected by a double disc synergy technique, in which an amoxicillin/clavulanic acid disc (amoxicillin 20 µg and clavulanic acid 10 µg) was placed in the centre of a plate and cefotaxime (30 µg), ceftazidime (30 µg) and ceftriaxone (30 µg) discs were placed 30 mm (centre to centre) apart from the amoxicillin/clavulanic acid disc. The enhancement of the zone of inhibition of any one of the three discs towards the disc containing clavulanic acid suggested the presence of an extended-spectrum β-lactamases [22]. The isolates that produced zone of inhibition ≥ 22 mm for ceftazidime, ≥ 27 mm for cefotaxime, ≥ 25 mm for ceftriaxone, were considered as potential ESBL producers as recommended by CLSI [21].

*2.6. Detection of ESBL-encoding genes*

The ESBL-encoding genes (*bla*TEM, *bla*SHV, *bla*CTX-M-1 and *bla*CTX-M-2) were detected by multiplex PCR using specific primers as described in Table 1 [23]. Amplification reactions were set in a 25 µL volume containing 12.5 µL PCR Master Mix (Thermo Scientific, USA), 1.0 µL (10 pmol) of each of the forward and reverse primers, DNA 1 µL and nuclease-free water 3.5 µL. The multiplex PCR conditions used were as follows: initial denaturation at 95 °C for 5 min, followed by 25 cycles of denaturation at 95 °C for 30 s, annealing at 60 °C for 1 min, and extension at 72 °C for 1 min, with a final extension at 72 °C for 10 min. Appropriate positive and negative controls (sterile phosphate buffer saline) were included in each PCR run. The PCR products were visualized by electrophoresis on a 1.5% agarose gel containing ethidium bromide. The DNA bands were photographed using a UV-transilluminator.

**Table 1.** Oligonucleotide primers used for the detection of ESBL-encoding genes

| Gene | Name of primers | Sequence 5′→ 3′ | Amplified product (bp) |
|---|---|---|---|
| *bla*TEM | TEM-410F | GGTCGCCGCATACACTATTCTC | 372 |
| | TEM-781R | TTTATCCGCCTCCATCCAGTC | |
| *bla*SHV | SHV-287F | CCAGCAGGATCTGGTGGACTAC | 231 |
| | SHV-517R | CCGGGAAGCGCCTCAT | |
| *bla*CTX-M-1 | ctxm1-115F | GAATTAGAGCGGCAGTCGGG | 588 |
| | ctxm1-702R | CACAACCCAGGAAGCAGGC | |
| *bla*CTX-M-2 | ctxm2-39F | GATGGCGACGCTACCCC | 107 |
| | ctxm2-145R | CAAGCCGACCTCCCGAAC | |

*2.7. Statistical analysis*

Descriptive statistics were used to compute the prevalence of *E. coli* and resistance percentage. Z-test for proportions was done to find out the significant difference in the frequencies of *E. coli* and their resistance percentage between supershops, sampling area, chicken types, production types and meat types etc. If any of the expected cell frequencies was less than five, Fisher's exact tests were used. The level of significance was set at $p < 0.05$. The SPSS version 22.0 software (SPSS, IBM, Somers, NY, USA) was used for the analyses.

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## 3. Results

### *3.1. Source of chicken, processing and packaging of frozen chicken meat*

The findings of questionnaire survey, conducted in 40 outlets of 9 branded supershops of 5 megacities in Bangladesh, revealed that supershops of all brands had purchased chickens from their own contact farm (Table 2). All the outlets of brands 4, 6, 8 and 9, and majority outlets of brands 1 to 3 had their chicken meat processed outside the supershops. Regarding packaging of meat, it was observed that 100% outlets of brands 6, 8 and 9, and the majority of brands 1 to 3 packaged their chicken meat inside the shops. However, all outlets of brand 5 processed and packaged chicken meat inside the shop, in contrast, all outlets of brand 7 did it outside the shop.

**Table 2.** Demographic information of 9 branded supershops in 5 megacities

| Name of Supershops (N) | Source of chicken (%) | Processing of chicken | | Packaging of chicken | |
|---|---|---|---|---|---|
| | | Inside shop N (%) | Outside shop N (%) | Inside shop N (%) | Outside shop N (%) |
| Brand 1 (7) | Contact farm (100) | 1(14.3) | 6(85.7) | 6(85.7) | 1(14.3) |
| Brand 2 (15) | Contact farm (100) | 2(13.3) | 13(86.7) | 10(66.7) | 5(33.3) |
| Brand 3 (10) | Contact farm (100) | 2(20.0) | 8(80.0) | 8(80.0) | 2(20.0) |
| Brand 4 (3) | Contact farm (100) | 0 | 3(100.0) | 2(66.7) | 1(33.3) |
| Brand 5 (1) | Contact farm (100) | 1(100.0) | 0 | 1(100.0) | 0 |
| Brand 6 (1) | Contact farm (100) | 0 | 1(100.0) | 1(100.0) | 0 |
| Brand 7 (1) | Contact farm (100) | 0 | 1(100.0) | 0 | 1(100.0) |
| Brand 8 (1) | Contact farm (100) | 0 | 1(100.0) | 1(100.0) | 0 |
| Brand 9 (1) | Contact farm (100) | 0 | 1(100.0) | 1(100.0) | 0 |

N= No. of outlets

### *3.2. Prevalence and distribution of ESBL –producing and ESBL -non producing E. coli*

The overall prevalence of *E. coli* was 76.1% (86/113) in frozen chicken meat samples, and it was varied from 33.3% to 100% among 9 different brands (Table 3). All *E. coli* isolates were confirmed by PCR as they generated 585 bp fragment size on amplification (Figure 2). Out of 86 *E. coli* isolates, 74 (86%) were ESBL –producing *E. coli* (ESBL-Ec) and 14% (12/86) was ESBL -non producing *E. coli* (Non-ESBL-Ec) (Table 3). None of the *E. coli* isolates were recovered from one brand (brand 9). The prevalence of ESBL-Ec and Non-ESBL-Ec varied significantly from brand to brand. The prevalence of ESBL-Ec in frozen chicken meat of different brands varied from 50% to 100%, while prevalence of Non-ESBL-Ec varied from 30% to 100% (Table 3).

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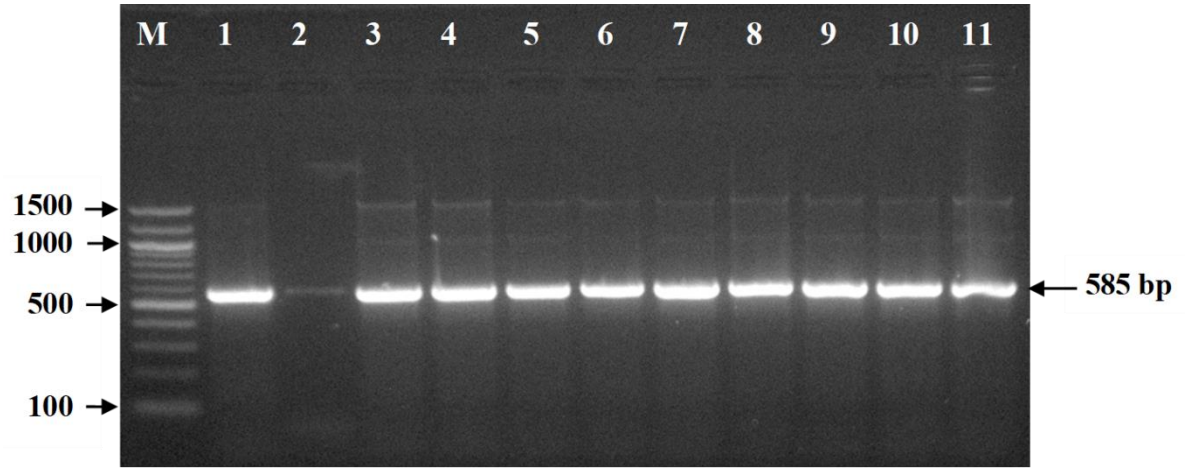


**Figure 2.** PCR amplified product of 585 bp from *16S rRNA* gene of *E. coli* following 1.5% agarose gel electrophoresis and ethidium bromide staining.
Legends: M = DNA marker (100 bp), Lane 1 = Positive control of *E. coli*, Lane 2 = Negative control, Lane 3-11 = PCR product of tested *E. coli* isolates.

**Table 3.** Prevalence of ESBL-Ec and Non-ESBL-Ec isolated from frozen chicken meat in different supershops

| Name of supershops | Total no. of samples | No. of *E. coli* positive isolates (%) | ESBL-Ec No. (%) | Non-ESBL-Ec No. (%) |
|---|---|---|---|---|
| Brand 1 | 23 | 21 (91.3) | 21 (100.0)[a] | 0 |
| Brand 2 | 40 | 30 (75.0) | 21 (70.0)[b] | 9 (30.0)[b] |
| Brand 3 | 28 | 24 (85.8) | 24 (100.0)[a] | 0 |
| Brand 4 | 8 | 3 (37.5) | 2 (66.7)[b] | 1 (33.3)[b] |
| Brand 5 | 2 | 2 (100.0) | 2 (100.0)[a,b] | 0 |
| Brand 6 | 2 | 2 (100.0) | 1 (50.0)[b] | 1 (50.0)[b] |
| Brand 7 | 5 | 3 (60.0) | 3 (100.0)[a,b] | 0 |
| Brand 8 | 3 | 1 (33.3) | 0 | 1 (100.0)[a] |
| Brand 9 | 2 | 0 | - | - |
| **Total** | **113** | **86 (76.1)** | **74 (86.0)** | **12 (14.0)** |

ESBL-Ec = ESBL-producing *E. coli*; Non-ESBL-Ec = ESBL- non producing *E. coli*; [a,b]Values in the same column with different superscripts differ significantly ($p < 0.05$).

As shown in Table 4, the highest prevalence of ESBL-Ec was recorded in both Chattogram and Mymensingh divisions (100.0%), followed by Dhaka (92.3%) division, which was significantly higher than those in Rajshahi division (33.3%). On the other hand, the highest prevalence of Non-ESBL-Ec was in Sylhet (100.0%) division and lowest in Dhaka division (7.7%). Moreover, in broiler and cockerel chickens, similar prevalence of ESBL-Ec (87.3% and 82.6%, respectively) and Non-ESBL-Ec (12.7% and 17.4%, respectively) were observed (Table 4). We did not find any significant differences in prevalence of both ESBL-Ec and Non-ESBL-Ec between organic and non-organic chickens. Considering the types of meat sample, though the highest isolation rate of ESBL-Ec was found in leg muscle (100%) there was no significant differences between different types of meat sample. The isolation rate of Non-ESBL-Ec was highest in breast muscle (18.2%) and lowest in drumstick (9.1%) (Table 4).

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**Table 4.** Distribution of ESBL-Ec and Non-ESBL-Ec isolated from frozen chicken meat

| Variables (N) | No. of *E. coli* positive isolates (%) | ESBL-Ec No. (%) | Non-ESBL-Ec No. (%) |
|---|---|---|---|
| **Divisions** | | | |
| Dhaka (82) | 65 (79.3) | 60 (92.3)[a] | 5 (7.7)[a] |
| Chattogram (10) | 10 (100.0) | 10 (100.0)[a] | 0 |
| Sylhet (11) | 5 (45.5) | 0 | 5 (100.0)[b] |
| Mymensingh (5) | 3 (60.0) | 3 (100.0)[a,b] | 0 |
| Rajshahi (5) | 3 (60.0) | 1 (33.3)[b] | 2 (66.7)[b] |
| **Chicken types** | | | |
| Broiler (82) | 63 (76.8) | 55 (87.3)[a] | 8 (12.7)[a] |
| Cockerel (31) | 23 (74.2) | 19 (82.6)[a] | 4 (17.4)[a] |
| **Production types** | | | |
| Organic (10) | 5 (50.0) | 4 (80.0)[a] | 1 (20.0)[a] |
| Non organic (103) | 81 (78.6) | 70 (86.4)[a] | 11 (13.6)[a] |
| **Meat sample types** | | | |
| Breast (27) | 22 (81.5) | 18 (81.8)[a] | 4 (18.2)[a] |
| Drumstick (30) | 22 (73.3) | 20 (90.9)[a] | 2 (9.1)[a] |
| Leg (3) | 3 (100.0) | 3 (100.0)[a] | 0 |
| Wing (19) | 16 (84.2) | 14 (87.5)[a] | 2 (12.5)[a] |
| Whole chicken pool sample (34) | 23 (67.6) | 19 (82.6)[a] | 4 (17.4)[a] |
| **Total (113)** | **86 (76.1)** | **74 (86.0)** | **12 (14.0)** |

N = No. of samples; ESBL-Ec = ESBL-producing *E. coli*; Non-ESBL-Ec = ESBL- non producing *E. coli*; [a,b]Values in the same column with different superscripts differ significantly ($p < 0.05$).

*3.3. Distribution of possible extensively drug resistant (pXDR) E. coli*

Notably, in this study, 2.3% (2/86) *E. coli* isolates were pXDR (resistant to 13 out of 16 antimicrobial classes). The pXDR *E. coli* isolates were only susceptible to polymyxins, monobactams and glycylcyclines antimicrobial classes. One pXDR *E. coli* isolate was recovered from broiler meat of brand 5 in Dhaka division and another one from cockerel meat of brand 7 in Mymensingh division. Both pXDR isolates were originated from non-organically produced chickens.

*3.4. Distribution of multidrug resistant E. coli*

Of the 86 *E. coli* isolates tested, all the isolates were multidrug-resistant (MDR). In our study we used 16 antimicrobial classes. The overall distributions of MDR *E. coli* are shown in Figure 3a-e. It was observed that 38.4% isolates were resistant to 9–11 antimicrobial classes, 32.6% to 6–8 classes, and 11.6% to 3–5 classes. Notably, 17.4% isolates were resistant to 12–13 antimicrobial classes. Multidrug resistant *E. coli* was widespread among different brands, and all isolates from brand 6 and brand 8 showed higher rate of resistance to 6–8 and 9–11 antimicrobial classes, respectively (Figure 3a). Regarding division-wise distribution of MDR *E. coli*, the highest percentage of isolates, resistant to 6–8 and 12–13 antimicrobial classes, was observed in Rajshahi and Mymensingh divisions, respectively (Figure 3b). Considering chicken types, it was revealed that 43.5% isolates from cockerel chicken meat and 42.9% isolates from broiler chicken meat were resistant to 6–8 and 9–11 antimicrobial classes, respectively (Figure 3c). Production type-wise MDR pattern results revealed

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that 40% isolates from organically produced chickens were resistant to 9–11 and 12–13 antimicrobial classes, respectively, while 38.3% isolates from non-organically produced chickens were resistant to 9–11 antimicrobial classes (Figure 3d). Looking at the meat sample types-wise distribution, 50% of the isolates, recovered from breast and wing muscle, was resistant to 9–11 antimicrobial classes (Figure 3e).

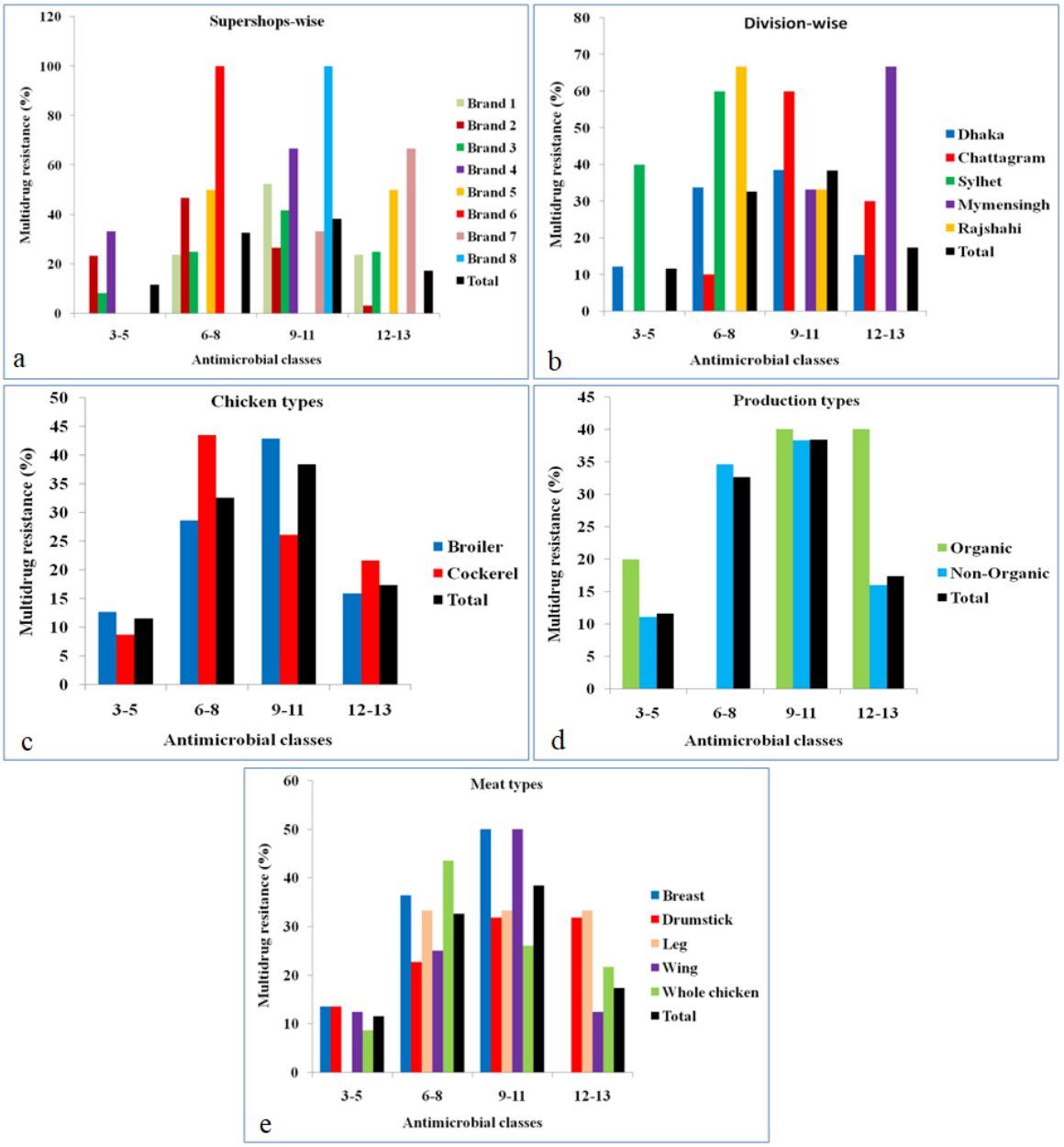


**Figure 3a-e**. Antimicrobial-class wise distribution of multidrug resistance pattern of *E. coli* isolated from frozen chicken meat

It is noted that among the 86 *E. coli* isolates, all isolates were resistant to at least four, and up to twenty-eight antimicrobials (Table 5). The frequency of resistance to 19–23 antimicrobials was observed in 22 (25.6%) isolates, while only 11 (12.8%) isolates were resistant to 4–8 antimicrobials. The percentage of resistance to 9–13 and 14–18 antimicrobials were same (22.1%). Notably, 15 (17.4%) isolates were resistant to 24–28 antimicrobials. Most importantly, resistance to 3 or fewer antimicrobials was not observed in any of the isolates tested. Brand-wise resistance to antimicrobials revealed that the highest resistance to 19–23 antimicrobials was observed in 42.9% isolates from brand 1. Two (66.7%) isolates from brand 7, one (50%) from brand 5, and 7 (29.2%) from brand 3

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were resistant to 24–28 antimicrobials (Table 5). Significant differences were observed in the resistance percentages to antimicrobial agents between brands.

**Table 5.** Supershops-wise distribution of resistant *E. coli* isolated from frozen chicken meat

| Name of Supershops (N) | No. (%) of isolates resistant to antimicrobial agents | | | | |
|---|---|---|---|---|---|
| | **4-8** | **9-13** | **14-18** | **19-23** | **24-28** |
| Brand 1 (21) | 0 | 3 (14.3)$^{a}$ | 7 (33.3)$^{a}$ | 9 (42.9)$^{a}$ | 2 (9.5)$^{a}$ |
| Brand 2 (30) | 8 (26.7)$^{a}$ | 9 (30.0)$^{ab}$ | 5 (16.7)$^{b}$ | 5 (16.7)$^{b}$ | 3 (10.0)$^{a}$ |
| Brand 3 (24) | 2 (8.3)$^{b}$ | 5 (20.8)$^{ab}$ | 3 (12.5)$^{b}$ | 7 (29.2)$^{ab}$ | 7 (29.2)$^{b}$ |
| Brand 4 (3) | 1 (33.3)$^{a}$ | 0 | 1 (33.3)$^{a}$ | 1 (33.3)$^{a}$ | 0 |
| Brand 5 (2) | 0 | 1 (50.0)$^{ab}$ | 0 | 0 | 1 (50.0)$^{c}$ |
| Brand 6 (2) | 0 | 0 | 2 (100.0)$^{c}$ | 0 | 0 |
| Brand 7 (3) | 0 | 0 | 1 (33.3)$^{a}$ | 0 | 2 (66.7)$^{c}$ |
| Brand 8 (1) | 0 | 1 (100.0)$^{b}$ | 0 | 0 | 0 |
| **Total** | **11 (12.8)** | **19 (22.1)** | **19 (22.1)** | **22 (25.6)** | **15 (17.4)** |

N = No. of *E. coli* isolates; $^{a,b,c}$Values in the same column with different superscripts differ significantly ($p < 0.05$).

Overall, the highest single resistance in *E. coli* was detected against oxytetracycline (93%) and amoxicillin (91.9%). In addition, resistances to ampicillin (89.5%), trimethoprim-sulphamethoxazole and pefloxacin (88.4%), tetracycline (84.9%), cefepime (72.1%), and piperacillin-tazobactam (70.9%) were also very high in *E. coli* isolates (Table 6). Among all the antibiotics, resistance to aztreonam was observed to be the lowest (1.2%) followed by ceftriaxone and tigecycline (2.3%) (Table 6).

**Table 6.** Antimicrobial susceptibility pattern of ESBL-Ec and Non-ESBL–Ec isolated from frozen chicken meat

| Antimicrobial category | No. of isolates (%) | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| | Overall (n = 86) | | | ESBL-Ec (n = 74) | | | Non-ESBL-Ec (n = 12) | | |
| | **R** | **I** | **S** | **R** | **I** | **S** | **R** | **I** | **S** |
| **Fluoroquinolones** | | | | | | | | | |
| Ciprofloxacin | 38 (44.2) | 20 (23.3) | 28 (32.6) | 35 (47.3)$^{a}$ | 17 (23.0) | 22 (29.7) | 3 (25.0)$^{b}$ | 3 (25.0) | 6 (50.0) |
| Nalidixic acid | 53 (61.6) | 15 (17.4) | 18 (20.9) | 46 (62.2)$^{a}$ | 14 (18.9) | 14 (18.9) | 7 (58.3)$^{a}$ | 1 (8.3) | 4 (33.3) |
| Levofloxacin | 29 (33.7) | 21 (24.4) | 36 (41.9) | 27 (36.5)$^{a}$ | 17 (23.0) | 30 (40.5) | 2 (16.7)$^{b}$ | 4 (33.3) | 6 (50.0) |
| Norfloxacin | 37 (43.0) | 11 (12.8) | 38 (44.2) | 33 (44.6)$^{a}$ | 9 (12.2) | 32 (43.2) | 4 (33.3)$^{a}$ | 2 (16.7) | 6 (50.0) |
| Gatifloxacin | 43 (50.0) | 14 (16.3) | 29 (33.7) | 38 (51.4)$^{a}$ | 11 (14.9) | 25 (33.8) | 5 (41.7)$^{a}$ | 3 (25.0) | 4 (33.3) |
| Pefloxacin | 76 (88.4) | 0 | 10 (11.6) | 65 (87.8)$^{a}$ | 0 | 9 (12.2) | 11 (91.7)$^{a}$ | 0 | 1 (8.3) |
| Ofloxacin | 49 (57.0) | 9 (10.5) | 28 (32.6) | 43 (58.1)$^{a}$ | 9 (12.2) | 22 (29.7) | 6 (50.0)$^{a}$ | 0 | 6 (50.0) |
| **Non-extended spectrum cephalosporins** | | | | | | | | | |
| **1st generation** | | | | | | | | | |
| Cefalexin | 40 (46.5) | 0 | 46 (53.5) | 39 (52.7)$^{a}$ | 0 | 35 (47.3) | 1 (8.3)$^{b}$ | 0 | 11 (91.7) |
| Cephradine | 43 (50.0) | 0 | 43 (50.0) | 41 (55.4)$^{a}$ | 0 | 33 (44.6) | 2 (16.7)$^{b}$ | 0 | 10 (83.3) |
| **2nd generation** | | | | | | | | | |
| Cefuroxime | 37 (43.0) | 4 (4.7) | 45 (52.3) | 36 (48.6)$^{a}$ | 4 (5.4) | 34 (45.9) | 1 (8.3)$^{a}$ | 0 | 11 (91.7) |
| Cefaclor | 13 (15.1) | 38 (44.2) | 35 (40.7) | 12 (16.2)$^{a}$ | 31 (41.9) | 31 (41.9) | 1 (8.3)$^{a}$ | 7 (58.3) | 4 (33.3) |
| **Extended-spectrum cephalosporins** | | | | | | | | | |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| **3rd generation** | | | | | | | | | |
| Cefixime | 0 | 4 (4.7) | 82 (95.3) | 0 | 2 (2.7) | 72 (97.3) | 0 | 2 (16.7) | 10 (83.3) |
| Ceftazidime | 25 (29.1) | 3 (3.5) | 58 (67.4) | 25 (33.8) | 3 (4.1) | 46 (62.2) | 0 | 0 | 12 (100) |
| Ceftriaxone | 2 (2.3) | 9 (10.5) | 75 (87.2) | 2 (2.7) | 9 (12.2) | 63 (85.1) | 0 | 0 | 12 (100) |
| Cefotaxime | 46 (53.5) | 12 (14.0) | 28 (32.6) | 46 (62.2) | 12 (16.2) | 16 (21.6) | 0 | 0 | 12 (100) |
| **4th generation** | | | | | | | | | |
| Cefepime | 62 (72.1) | 13 (15.1) | 11 (12.8) | 60 (81.1)[a] | 10 (13.5) | 4 (5.4) | 2 (16.7)[b] | 3 (25.0) | 7 (58.3) |
| **Cephamycins** | | | | | | | | | |
| Cefoxitin | 42 (48.8) | 8 (9.3) | 36 (41.9) | 41 (55.4)[a] | 7 (9.5) | 26 (35.1) | 1 (8.3)[a] | 1 (8.3) | 10 (83.3) |
| **Penicillins** | | | | | | | | | |
| Ampicillin | 77 (89.5) | 5 (5.8) | 4 (4.7) | 66 (89.2)[a] | 5 (6.8) | 3 (4.1) | 11 (91.7)[a] | 0 | 1 (8.3) |
| Amoxicillin | 79 (91.9) | 0 | 7 (8.1) | 68 (91.9)[a] | 0 | 6 (8.1) | 11 (91.7)[a] | 0 | 1 (8.3) |
| **Penicillins + β-lactamase inhibitors** | | | | | | | | | |
| Amoxicillin-clavulanic acid | 36 (41.9) | 28 (32.6) | 22 (25.6) | 31 (41.9)[a] | 25 (33.8) | 18 (24.3) | 5 (41.7)[a] | 3 (25.0) | 4 (33.3) |
| **Antipseudomonal penicillins + β-lactamase inhibitors** | | | | | | | | | |
| Piperacillin-tazobactam | 61 (70.9) | 23 (26.7) | 2 (2.3) | 54 (73.0)[a] | 18 (24.3) | 2 (2.7) | 7 (58.3)[a] | 5 (41.7) | 0 |
| **Carbapenems** | | | | | | | | | |
| Imipenem | 41 (47.7) | 19 (22.1) | 26 (30.2) | 40 (54.1)[a] | 15 (20.3) | 19 (25.7) | 1 (8.3)[b] | 4 (33.3) | 7 (58.3) |
| Meropenem | 36 (41.9) | 14 (16.3) | 36 (41.9) | 36 (48.6) | 13 (17.6) | 25 (33.8) | 0 | 1 (8.3) | 11 (91.7) |
| **Polymyxins** | | | | | | | | | |
| Colistin | 9 (10.5) | 0 | 77 (89.5) | 9 (12.2) | 0 | 65 (87.8) | 0 | 0 | 12 (100) |
| Polymyxin B | 6 (7.0) | 0 | 80 (93.0) | 6 (8.1) | 0 | 68 (91.9) | 0 | 0 | 12 (100) |
| **Monobactams** | | | | | | | | | |
| Aztreonam | 1 (1.2) | 5 (5.8) | 80 (93.0) | 1 (1.4) | 5 (6.8) | 68 (91.9) | 0 | 0 | 12 (100) |
| **Aminoglycosides** | | | | | | | | | |
| Gentamicin | 7 (8.1) | 7 (8.1) | 72 (83.7) | 6 (8.1)[a] | 6 (8.1) | 62 (83.8) | 1 (8.3)[a] | 1 (8.3) | 10 (83.3) |
| Tobramycin | 7 (8.1) | 13 (15.1) | 66 (76.7) | 5 (6.8)[a] | 13 (17.6) | 56 (75.7) | 2 (16.7)[b] | 0 | 10 (83.3) |
| Amikacin | 15 (17.4) | 43 (50.0) | 28 (32.6) | 14 (18.9)[a] | 38 (51.4) | 22 (29.7) | 1 (8.3)[b] | 5 (41.7) | 6 (50.0) |
| Streptomycin | 50 (58.1) | 17 (19.8) | 19 (22.1) | 47 (63.5)[a] | 17 (23.0) | 10 (13.5) | 3 (25.0)[b] | 0 | 9 (75.0) |
| Neomycin | 24 (27.9) | 41 (47.7) | 21 (24.4) | 22 (29.7)[a] | 39 (52.7) | 13 (17.6) | 2 (16.7)[a] | 2 (16.7) | 8 (66.7) |
| **Tetracyclines** | | | | | | | | | |
| Tetracycline | 73 (84.9) | 5 (5.8) | 8 (9.3) | 62 (83.8)[a] | 5 (6.8) | 7 (9.5) | 11 (91.7)[a] | 0 | 1 (8.3) |
| Oxytetracycline | 80 (93.0) | 0 | 6 (7.0) | 68 (91.9)[a] | 0 | 6 (8.1) | 12 (100.0)[a] | 0 | 0 |
| Doxycycline | 59 (68.6) | 20 (23.3) | 7 (8.1) | 52 (70.3)[a] | 16 (21.6) | 6 (8.1) | 7 (58.3)[a] | 4 (33.3) | 1 (8.3) |
| **Folate pathway inhibitors** | | | | | | | | | |
| Trimethoprim-sulpham ethoxazole | 76 (88.4) | 0 | 10 (11.6) | 66 (89.2)[a] | 0 | 8 (10.8) | 10 (83.3)[a] | 0 | 2 (16.7) |
| **Glycylcyclines** | | | | | | | | | |
| Tigecycline | 2 (2.3) | 15 (17.4) | 69 (80.2) | 1 (1.4)[a] | 13 (17.6) | 60 (81.1) | 1 (8.3)[b] | 2 (16.7) | 9 (75.0) |
| **Phenicols** | | | | | | | | | |

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| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| Chloramphenicol | 27 (31.4) | 0 | 59 (68.6) | 21 (28.4)[a] | 0 | 53 (71.6) | 6 (50.0)[b] | 0 | 6 (50.0) |
| **Macrolids** | | | | | | | | | |
| Azithromycin | 30 (34.9) | 0 | 56 (65.1) | 29 (39.2)[a] | 0 | 45 (60.8) | 1 (8.3)[b] | 0 | 11 (91.7) |

n = number of isolates, R = Resistant, I = Intermediate, S = Susceptible; ESBL-Ec = ESBL-producing *E. coli*; Non-ESBL-Ec = ESBL- non producing *E. coli*; [a,b]Values in the same row with different superscripts differ significantly ($p < 0.05$).

The variation in the resistance pattern of ESBL-Ec (n = 74) and non-ESBL-Ec (n = 12) isolates was determined (Table 6). Resistances to oxytetracycline and amoxicillin (91.9%), ampicillin and trimethoprim-sulphamethoxazole (89.2%), pefloxacin (87.8%), cefepime (81.1%), piperacillin-tazobactam (73.0%) and doxycycline (70.3%) were found to be higher in ESBL-Ec isolates, while, resistances to oxytetracycline (100.0%), tetracycline, pefloxacin, ampicillin and amoxicillin (91.7%) and trimethoprim-sulphamethoxazole (83.3%) were observed to be higher in non-ESBL-Ec isolates. No significant differences were observed among these antimicrobial agents between ESBL-Ec and Non-ESBL-Ec except cefepime, streptomycin and chloramphenicol. It is important to note that 77 (89.5%) isolates showed resistance to carbapenems, the antimicrobials used in human medicine, of which, 76 isolates were ESBL-Ec. The resistance to imipenem was 47.7%, and to meropenem was 41.9%.

### *3.5. Genotypes of ESBL-Ec and Non-ESBL-Ec*

The findings of ESBL genes, *bla*TEM, *bla*SHV, *bla*CTX-M-1 and *bla*CTX-M-2 genes are presented in Table 7 and Figure 4. All the isolates were positive for *bla*TEM gene. One isolate of non-ESBL-Ec was positive for *bla*SHV gene and another one isolate of non-ESBL-Ec was positive for *bla*CTX-M-2 gene. None of the tested isolates harbored *bla*CTX-M-1 gene.

**Table 7.** Prevalence of ESBL-Ec and Non-ESBL-Ec genotypes isolated from frozen chicken meat

| Genotypes | ESBL-Ec (n = 74) | Non-ESBL-Ec (n = 12) | Total (n = 86) |
|---|---|---|---|
| *bla*TEM | 74 (100.0) | 12 (100.0) | 86 (100.0) |
| *bla*SHV | 0 | 1 (8.3) | 1 (1.2) |
| *bla*CTX-M-1 | 0 | 0 | 0 |
| *bla*CTX-M-2 | 0 | 1 (8.3) | 1 (1.2) |

ESBL-Ec = ESBL-producing *E. coli*; Non-ESBL-Ec = ESBL- non producing *E. coli*

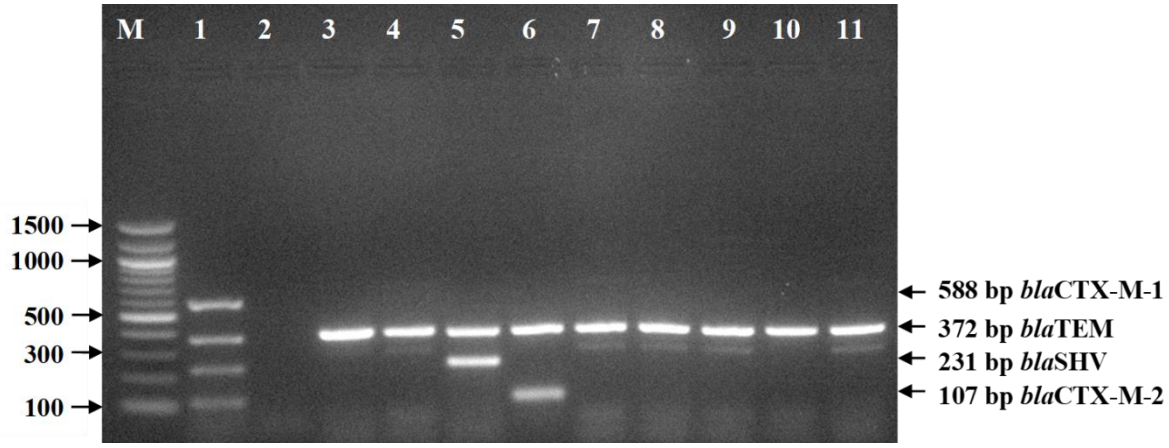


**Figure 4.** ESBL-encoding genes of *E. coli* isolates from frozen chicken meat by multiplex PCR following 1.5% agarose gel electrophoresis and ethidium bromide staining.
**Legends:** M = DNA marker (100 bp), Lane 1 = Positive control, Lane 2 = Negative control, Lane 3-11 = Positive for *bla*TEM gene; Lane 5 = Positive for *bla*SHV gene; Lane 6 = Positive for *bla*CTX-M-2 gene.

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## 4. Discussion

*E. coli* is a common enteric pathogen, specific strains of which can cause human and animal disease. It is one of the groups of seven species that the World Health Organization (WHO) has highlighted as of key AMR concern and serves as a sentinel organism for the assessment of development of antimicrobial resistance [24]. The emergence and spread of ESBL-Ec linked to chickens and other farm animals has been of particular concern [12].

The present study reports the first comprehensive findings on the extent and distribution of ESBL-Ec and their antimicrobial resistance pattern including resistance genes in frozen chicken meat collected from almost all branded supershops located in five megacities of Bangladesh. This study showed the high prevalence (76.1%) of *E. coli* in frozen chicken meat compared with 49–53% prevalence in raw chicken meat as reported earlier in Bangladesh [25,26], 66.3% in India [27], 47.1% in Nepal [28] and 50.5% in Korea [29], and this may be a potential hazard to the consumers. The prevalence of *E. coli* may be attributed to several factors including source of meat, sample number, isolation methods, possible cross contamination during slaughtering, slaughterhouse sanitation as well as personal hygiene, and other practices through to the food chain.

One of the main findings in this study was the high prevalence (86.0%) of ESBL-Ec in frozen chicken meat. These results corroborate the findings of similar studies conducted in Japan, in which, the authors reported that 65–77% frozen chicken meat were contaminated with ESBL-Ec [30,31]. The present study observed that the prevalence of ESBL-Ec in frozen chicken meat varied from brand to brand, which might be due to variation in processing, packaging and personnel's hygienic practices in different supershops. It is expected that different brands follow different types of management and thus, there are different risks regarding the prevalence of ESBL-Ec. The contamination may also occur during transportation of chicken meat from farm to supershops or during the steps involved in slaughtering, defeathering, plucking and chilling of the chicken meat [2]. The distribution of ESBL-Ec has been found to be varied from division to division, with Chattogram, Mymensingh and Dhaka divisions having the highest prevalence and the least in Rajshahi division. The highest distribution of Non-ESBL-Ec was observed in Sylhet division of Bangladesh. An earlier study has shown that 30% ESBL-Ec was detected from droppings of domestic chickens in Rajshahi division of Bangladesh [32]. In the present study, a considerably high percentage of ESBL-Ec was recovered from different types of meat samples. The pathogenic *E. coli* are usually absent in the muscle tissue and body fluids of healthy living chickens, but can be entered into the meat during slaughtering or at the time of processing and packaging from the gastrointestinal tract [33]. This high prevalence is very alarming, and requires risk assessments and pertinent risk management to keep down the occurrence and spread of ESBL-Ec. This result also indicates that the contamination of frozen chicken meat with ESBL-Ec in Bangladesh is more frequent, which may rapidly raise the risk of human being infected.

It is of particular concern that all the isolates of *E. coli* in this study were MDR, of which a substantial percentage of isolates showed resistance to 9 to 13 classes of antimicrobials, which is in line with previous observation among *E. coli* recovered from retail chicken meat in Korea [29], but differed from some other reports [26,28]. The highest percentage of isolates from Rajshahi and Mymensingh divisions expressed MDR, which was in disagreement with the previous reports in Bangladesh, in which, 10–35% of *E. coli* isolates in retail chicken meat from Mymensingh and Dhaka divisions showed MDR [34,35]. Of note, the current study also observed that 2.3% of *E. coli* isolates were pXDR. An earlier report from Japan detected extensive MDR *E. coli* in 70% of frozen chicken meat samples [31]. The high rates of MDR and existence of pXDR in this study imply that this can reflect the frequent use or misuse of antimicrobials in poultry production as therapy and growth promoting agents in Bangladesh, creates a selection pressure, and thus contributing to the emergence and spread of MDR bacteria in poultry production systems. Other plausible explanation is that the high prevalence of MDR *E. coli* may be attributed to the possible cross contamination during slaughtering, cutting, and further processing [2]. These observations support the possibility that chicken meat might be one of the potential sources of MDR *E. coli* infections causing possible

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transmission of resistant bacteria to consumers, and it suggests that continued surveillance is important.

Increasing rates of antimicrobial resistance in both ESBL-Ec and Non-ESBL-Ec are a growing public health problem that needs to be monitored continuously. Our study indicated that all isolates of *E. coli* exhibited absolute resistance (100%) to at least four antimicrobial agents. Of note, 17.4% isolates of *E. coli* showed resistance to more than 24 antimicrobials. A high percentage of antimicrobial resistant *E. coli* from frozen chicken meat was also reported by several investigators [28,31]. In the current study, oxytetracycline resistance was the most frequently observed antimicrobial resistance in both ESBL-Ec and Non-ESBL-Ec, which is consistent with several other studies in frozen chicken meat [28,36]. The finding is not surprising because, since its approval in 1948, oxytetracycline has been widely used in veterinary practices, is probably a consequence of this [37].

A very high degree of resistance was also observed for amoxicillin, ampicillin and trimethoprim-sulphamethoxazole in both ESBL-Ec and Non-ESBL-Ec. Similar resistivity pattern was observed in Bangladesh [26,35], Japan [33], Korea [30] and Vietnam [24]. This may be attributed to the long term and indiscriminate use of these antimicrobial agents in poultry production in Bangladesh [9]. As fluoroquinolones and cephalosporins are the drugs of choice for the treatment of bacterial infection in humans, *E. coli* resistant to these antimicrobials could represent a big challenge to animals and human therapeutics interventions, and symbol a relevant public health implication [38]. Unfortunately, this study demonstrated that the prevalence of fluoroquinolones (mainly pefloxacin) resistance in both ESBL-Ec and Non-ESBL-Ec was also very high. This result may imply the more frequent use of fluoroquinolones in poultry production in Bangladesh. Moreover, more than 80% isolates of ESBL-Ec showed resistance to cefepime, a fourth-generation cephalosporin antimicrobials, which is higher than a previous observation in retail chicken meat (4.8%) in Korea [29]. Cephalosporins resistance is a matter of concern because cefepime is not used in veterinary practices in Bangladesh, and it is worrisome to find these phenotypes in chicken meat. The rate of resistance to multiple antimicrobials among ESBL-Ec isolates is usually common due to carrying of multi-resistance genes and plasmids [39]. These plasmids can also carry genes for co-resistance to multiple classes of antimicrobials including fluoroquinolones, sulfonamides, aminoglycosides, chloramphenicol, trimethoprim and tetracyclines [7]. Surprisingly, remarkably high resistance prevalence was found against carbapenems (last-line therapeutics to treat multidrug resistant superbugs), mainly imipenem and meropenem, though carbapenems is not used in poultry practices in Bangladesh. There was no clear explanation for these high levels of resistance but it might be due to co-selection and/or cross-resistance generated by other antimicrobials [40].

On the other hand, a relatively low resistance rate to aztreonam, ceftriaxone and tigecycline were observed, probably because these antimicrobials are not used in poultry practices in Bangladesh, resulting in a lack of selective pressure by these antimicrobials in poultry production. It also supports the contention that antimicrobial resistance, induced once, is difficult to eliminate, because of associated resistance to other related antimicrobials [41]. Therefore, resistance to these antimicrobials should be carefully monitored.

Among the prevalent ESBL-Ec from chicken meat, *bla*TEM, *bla*SHV, and *bla*CTX-M (*bla*CTX-M-1 and *bla*CTX-M-2) are considered to be most diverse. The ESBL genes are usually located on plasmids, which could promote the dissemination of ESBL genes in Gram-negative bacteria [12]. The most prevalent ESBL encoding gene in the current study is *bla*TEM, which is consistent with the similar study conducted in Vietnam [23]. Interestingly, *bla*SHV and *bla*CTX-M-2 were detected in two Non-ESBL-Ec isolates. No *bla*CTX-M-1 was detected in this study. These findings are inconsistent with earlier studies in Bangladesh, where more than 50% of *E. coli* isolates from droppings of chickens, harbored *bla*TEM gene and 94.4% carried *bla*CTX-M-1 gene [10,32]. It may be hypothesized that frozen chicken meat which are sold to the consumers could potentially act as a major source of gut colonization by avian strains of *E. coli* that carry *bla*TEM ESBL genes.

It would be worthy if we could sample more outlets of supershops. However, frozen chicken meat samples were purchased from 40 outlets of almost all the renowned branded supershops located in five megacities of Bangladesh, thus the data represent the scenario of whole Bangladesh.

This study seems to indicate the current status of contamination with ESBL-Ec in frozen chicken meat. It would be very important to investigate horizontal gene transfer such as exchanges of plasmid or mobile genetic elements carrying genes for ESBLs between bacteria isolated from chicken meat.

## 5. Conclusions

The presence of ESBL-producing *E. coli* in frozen chicken meat in Bangladesh poses a risk to human health. Our data clearly indicate that frozen chicken meat is, at the present time, the most important known food source of ESBL-producing *E. coli* to which peoples are regularly exposed. Additionally, all *E. coli* isolates were multidrug resistant. The presence of antimicrobial resistance along with resistance genes enhances the potential risk of transfer to humans. The abundance of MDR and existence of pXDR ESBL-producing *E. coli* in frozen chicken meat warrants the importance of immediate steps to ensure good production and processing practices in Bangladesh. Continuous monitoring and public health efforts targeting food safety management are warranted to proactively manage risks associated with the presence and spread of these antimicrobial resistant *E. coli* in frozen chicken meat consumed by humans.

**Author Contributions:** M.S.P. conceived the study design, literature search, acquisition of data, statistical analysis and drafting of the manuscript. M.T.I. involved in conceptualizing, supervising, designing and coordinating the study, interpreting the data and critical revision of the manuscript. E.H.C. and M.T.R. participated in supervision, study design and revision of the manuscript. S.T and M.Y.A. greatly contributed in performing the experiments and analyzing the data.

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