









## Research Article

# Prevalence and Antimicrobial Resistance of *Escherichia coli* in Chicken Meat and Edible Poultry Organs Collected from Retail Shops and Supermarkets of North Western Province in Sri Lanka

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*Escherichia coli* is a commensal bacterium that lives in human and animal intestines. Shiga toxin-producing strains of *E. coli* STECs are responsible for most food-related *E. coli* infections. Pathogenic *E. coli* transmits to human bodies due to the consumption of contaminated, raw, or undercooked food. This study was conducted to identify the prevalence of *E. coli* contamination in edible poultry meat and meat organs in the North Western Province of Sri Lanka. A total of 250 samples consisting of chicken meat ( $n = 144$ ) and edible organs ( $n = 106$ ) were collected from retail shops ( $n = 181$ ) and supermarkets ( $n = 69$ ), in both Kurunegala and Puttlam districts. The prevalence of *E. coli* from 250 chicken meat samples was 66.80% (167/250); *E. coli* prevalence at retail shops (66.85%) was higher than that at supermarkets (66.67%) and was not statistically significant. *E. coli* prevalence in chicken meat and edible organs was 65.73% and 69.16%, respectively. Molecular confirmation for the positive samples was done through polymerase chain reaction (PCR) using previously designed primers. An antibiotic susceptibility test was performed according to CLSI using nine antibiotics: ampicillin, amoxicillin, chloramphenicol, ceftazidime, ciprofloxacin, cephalixin, erythromycin, gentamicin, and tetracycline. Most isolates were resistant to erythromycin (80.84%) and amoxicillin (76.05%), while the least resistance was observed for gentamicin (4.79%). This study indicates the potential public health risk associated with chicken sold at retail and supermarket levels in the North Western Province of Sri Lanka.

## 1. Introduction

*Escherichia coli* (*E. coli*) is a commensal microorganism that is associated with the gut of warm-blooded animals and humans [1]. *E. coli* is a member of the family

Enterobacteriaceae and a commonly identified faecal coliform. Although not all *E. coli* are considered pathogenic, some strains of *E. coli* have the ability to acquire genes encoding virulence factors and be pathogenic to humans [2]. Pathogenic *E. coli* can cause multiple complications to

affected humans, such as prolonged diarrheal disease and vomiting, which may further progress into adverse medical conditions such as traveller's diarrhoea and haemolytic uremic syndrome (HUS) [3]. Pathogenic *E. coli* are commonly identified as verotoxigenic *E. coli* (VTEC)- or Shiga toxin (ST)-producing *E. coli* (STEC) [2].

Chicken meat and edible chicken organs are the most commonly consumed meat source in Sri Lanka [4]. The highest poultry production and poultry meat production in Sri Lanka has been reported from the North Western Province [5], where this research was carried out. Chicken meat and its edible organs are considered a rich source of *E. coli*. The commonly identified *E. coli* contamination pathway in humans is known as the faecal oral route [6]. Contamination by the faecal oral route can be minimized by establishing proper hygienic practices and effective surveillance systems, and should be launched for large-scale poultry meat production systems [7]. Pathogenic *E. coli* is considered a major health concern worldwide [8]. Although a significant *E. coli* outbreak has not been reported from Sri Lanka, the risk level of causing diarrheal illnesses among the public cannot be neglected.

Antibiotics play a vital role in human and veterinary clinical medicine for the prophylaxis and treatment of numerous bacterial infections, thereby reducing morbidity and mortality in a considerable fraction [9]. However, the misuse of antimicrobial agents during clinical medicine, animal husbandry, and agriculture has generated a selective pressure toward generating multiple drug-resistant microbial populations in the world. Multiple antibiotic resistance (MAR) indexing is a method that is widely used to track the bacterial source. MAR indexing is a cost-effective method that is calculated as the ratio between the number of antibiotics that the organism is resistant to and the total number of antibiotics that the organism is exposed to. If the MAR index values are greater than 0.2, it indicates a high risk of contamination by multidrug-resistant bacteria, where antibiotics are often used [10, 11]. The magnitude of the antimicrobial resistance problem is potentially higher in developing countries, where the level of infectious diseases is high and this corresponds to a higher use of broad-spectrum antibiotics [12].

This research was conducted to identify the level of *E. coli* contamination of chicken meat and edible organs collected from supermarkets and retail shops in the North Western Province and to determine the level of antimicrobial resistance among the collected *E. coli* bacterial samples.

## 2. Materials and Methods

**2.1. Sampling Procedure.** A total of 250 chicken meat ( $n = 144$ ) and edible chicken organs ( $n = 106$ ) were collected from retail shops ( $n = 181$ ) and supermarkets ( $n = 69$ ) from 25 divisional secretariat divisions of the North Western Province of Sri Lanka during the year 2018. A 100 g portion of chicken meat samples, preferably drumstick, chicken thigh or leg pieces, and approximately 100 g of edible chicken organs such as liver, gizzard, and heart, were collected during the sampling process. One sample of chicken meat and/or edible poultry organs was collected from a

single shop based on its availability. The sample size for this study was calculated using the following equation described in Naing et al., [13]:

$$n = \frac{Z^2 P(1 - P)}{d^2} \quad (1)$$

where  $n$  indicates the sample size; and  $Z$ ,  $P$ , and  $d$  indicate  $Z$  statistic for a level of confidence (95% for this study), the expected prevalence or proportion (0.8 for this study, according to the pilot study conducted by Anwarama et al., [14]), and precision (0.05 at 95% confidence level), respectively.

Samples for this study were collected by using the random sampling method. Ten random retail shops and supermarkets were selected for the sample collection from each divisional secretariat division, to collect a total of 250 poultry samples.

**2.2. Bacterial Isolation and Identification.** All samples were collected aseptically and transported isothermally using refrigerated conditions to the laboratory within 4-5 hours of collection. Sampling methods were collected using Bacteriological Analytical Manual standard methods (1998); 10.0 g of the sample was mixed with 90.0 mL of peptone water (HiMedia, Mumbai, India); and the mixture was vortexed for homogenization. Serial dilutions were prepared up to  $10^{-3}$  by mixing 1 mL from the original dilution with 9 mL of MacConkey broth (HiMedia, Mumbai, India) in culture tubes. A Durham tube was added to the bottom of the culture tube for the observation of positive gas production. Serial dilutions were then incubated at 37°C for 24 hours. The level of contamination was categorized based on the Bacteriological Analytical Manual (BAM) table standards to understand the risk level of chicken meat and edible organ consumption. Samples with positive *E. coli* growth were cultured on MacConkey agar (HiMedia, Mumbai, India) plates and incubated at 37°C for 24 hours. Colony morphology and standard biochemical tests were conducted to identify the presumptive colonies of *E. coli*.

**2.3. Molecular Biological Identification.** *E. coli* colonies that were positive on biochemical tests were then subjected to molecular biological identification by polymerase chain reaction (PCR). The genomic *E. coli* DNA was extracted using the boiling method as described by Al Gallas et al. [15] with a slight modification. An Eppendorf tube containing one millilitre of an overnight *E. coli* bacterial culture in peptone water broth (HiMedia, Mumbai, India) was first centrifuged to harvest a bacterial pellet. The resulted bacterial pellet was then washed in 1 mL of distilled water to eliminate the residual media and possible inhibitors present. The bacterial pellet was resuspended in 500  $\mu$ L of sterile water and boiled at 100°C for 10–12 minutes to perform cell lysis to allow nucleic acids to be released to the lysate. The resulted lysate was centrifuged, and a 100  $\mu$ L sample of the supernatant (an aliquot) of each bacterial culture was stored at  $-20^\circ\text{C}$  as a template DNA stock [15]. Quantification of the

bacterial DNA concentration was obtained using a Nanodrop spectrophotometer (Thermo Fisher Scientific, USA) at the wavelength of 260 nm for double standard DNA. After measuring the absorbance of DNA sample through a spectrophotometer at 260 nm and 320 nm, the DNA quantity was measured using an equation mentioned in Ref. [16].

**2.4. PCR Amplification.** PCR amplification was done by using two oligonucleotide primers lacZ3 and YaiO, according to the protocol described in Ref. [17]. The multiplex PCR assay was performed as follows: each 25  $\mu$ L of reaction mixture contained 2.5 mM deoxynucleoside triphosphates, 10 pmol of each oligonucleotide primers, 2.5 mM of  $Mg^{2+}$ , 2.5  $\mu$ L of 10X Taq reaction buffer (50 mM Tris-HCl, pH 7.9, 50 mM KCl, 0.1 mM EDTA, 1 mM DTT, 0.5 mM PMSF, 50% glycerol), 0.2  $\mu$ L of Taq DNA polymerase (AmpONE™ Taq DNA polymerase), and 1  $\mu$ L of template DNA. Samples were amplified for 35 cycles, with each cycle consisting of 30 s at 95°C for denaturation, 30 s at 58°C for primer annealing, and 30 s at 72°C for strand elongation. PCR products were visualized following electrophoresis (80 V, 120 mA current, 40 min) through 1.5% agarose gels (W/V) stained with ethidium bromide, and the amplicons were identified by a 100-bp ladder (Vivantis, Malaysia) based only on the size of the amplified product.

**2.5. Antimicrobial Susceptibility Test.** Antimicrobial susceptibilities of the isolates to nine common antimicrobial agents were determined by the Kirby–Bauer disc diffusion method for *E. coli* samples using Mueller Hinton agar (HiMedia, Mumbai, India) by the guidelines of the Clinical Laboratory Standards Institute [18]. Isolates were inoculated onto nutrient agar and incubated at 37°C for 24 hours. A single colony was picked from the nutrient agar plate, and it was suspended in 0.9% saline water and adjusted to give a reading of 0.5 McFarland turbidity standard. A 0.1 mL volume of the 0.5 McFarland suspension was swabbed evenly in at least three directions on Mueller Hinton agar plates. Each plate was left to dry, and the antimicrobial discs for each antimicrobial were placed at a specific place on the agar plates. A sterilized filter paper disc dipped in sterilized distilled water was placed on the centre of each Petri dish as the control disc. Petri plates were placed with antimicrobial discs, and the control was incubated lid side up at 37°C for 24 hours. *E. coli* ATCC 25922 was used as quality control organism in antimicrobial susceptibility determination.

The tested antibiotics (HiMedia, Mumbai, India) were ampicillin (AMP; 25  $\mu$ g/mL), amoxicillin (AMX; 30  $\mu$ g/mL), chloramphenicol (C; 30  $\mu$ g/mL), ceftazidime/clavulanic acid (CAC; 30/10  $\mu$ g/mL), ciprofloxacin (CIP; 30  $\mu$ g/mL), cephalexin (CN; 30  $\mu$ g/mL), erythromycin (E; 15  $\mu$ g/mL), gentamicin (GEN; 30  $\mu$ g/mL), and tetracycline (TE; 30  $\mu$ g/mL). The diameter of the inhibition zone was used as a measurement of the effectiveness of antibiotics against each sample. Isolates were classified as resistant, intermediate, or sensitive based on the Clinical and Laboratory Standards Institute guidelines (CLSI M100-ED30: 2020 Performance

Standards for Antimicrobial Susceptibility Testing, 30th Edition (2020)).

**2.6. Statistical Analysis.** Obtained data related to *E. coli* presence in chicken meat and edible chicken organs collected from retail shops and supermarkets were transferred to Microsoft Excel spreadsheet (Microsoft Corp., Redmond, WA) for the analysis of *E. coli* prevalence. Prevalence levels were calculated based on *E. coli* presence in the collected samples using Microsoft Excel software, and the obtained prevalence data were subjected to a two-proportion Z test using SPSS 16.0 software (SPSS Inc., Chicago, IL). *P*-values were calculated, and statistical analysis was used to find any significant difference of *E. coli* prevalence levels between retail shops and supermarkets of North Western Province, Sri Lanka. The *P*-value less than 0.05 was considered statistically significant.

### 3. Results

Among the 250 collected samples, 167 (66.80%) chicken meat and edible organ samples were contaminated with *E. coli*, and isolated *E. coli* were confirmed by PCR identification (Figure 1). The prevalence of *E. coli* in retail shops and in supermarkets was calculated as 66.85% and 66.67%, respectively. Statistical analysis at 95% confidence level shows the MPN value for *E. coli* contamination is not significant between the supermarkets and retail shops. According to the results of MPN values for *E. coli* prevalence in supermarket chains, 17.39% of the positive samples had a lower contamination level, and 9.66% of the samples showed an intermediate level of contamination. The majority (39.62%) of the positive samples collected from supermarkets showed a higher *E. coli* concentration. Comparatively, *E. coli* contamination in retail shops showed a similar pattern of prevalence, with 24.75% of positive samples showing a lower contamination level, 3.32% showing an intermediate, and 38.78% of positive samples collected from retail shops showing a higher *E. coli* contamination.

Furthermore, 28.30% of the collected *E. coli*-positive chicken meat samples had lower contamination between 3 and 240 MPN values, and 3.20% had an intermediate contamination level of 240–1100 MPN values; 34.23% of the positive chicken meat samples had higher contamination, which is higher than 1100 MPN/g. According to the overall prevalence records of this research, the highest *E. coli* contamination was recorded from the chicken meat samples collected from retail shops. Based on the MPN value, edible chicken organs collected from retail shops showed higher contamination (84%) compared to the meat and organ samples collected from supermarkets. Overall prevalence data for chicken meat and edible poultry organs from retail shops and supermarkets are indicated in Tables 1 and 2.

The majority of the isolates were resistant to at least one or more antibiotics (Table 3).

Antimicrobial resistance patterns of 166 *E. coli* isolates showed high rates of resistance to erythromycin (80.84%) and amoxicillin (76.05%). However, the lowest rate of resistance

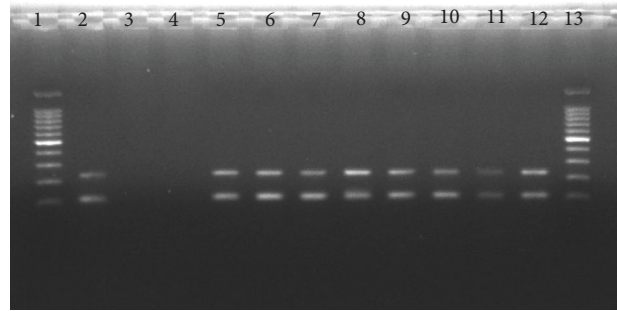


FIGURE 1: Multiplex PCR image of bacterial DNA extracted from isolated *E. coli* colonies of chicken meat and edible meat organs collected from retail shops and supermarkets in the North Western Province using LacZ3 and YaiO primers. Lanes; 1, 100-bp molecular marker (Vivantis, Malaysia); 2, *Escherichia coli*-positive control; 3, *Escherichia coli*-negative control; 4, technical negative control; 5–12, *E. coli* DNA isolated from chicken meat and edible organs from retail shops and supermarkets; 13, 100-bp molecular marker (Vivantis, Malaysia).

TABLE 1: Prevalence of *E. coli* in chicken meat and edible organs sold at retail shops and supermarkets in the Kurunegala district.

Source	Sample type	Sample size	Positive number (%)	Negative number (%)
Retail	Meat	109	72 (39.78%)	37 (20.44%)
	Edible organs	72	49 (27.07%)	23 (12.71%)
Supermarket	Meat	35	23 (33.33%)	12 (17.39%)
	Edible organs	34	23 (33.33%)	11 (15.94%)
	Total	250	167 (66.80%)	83 (33.20%)

TABLE 2: Level of *E. coli* contamination in chicken meat and edible organs sold at retail shops and supermarkets in the Kurunegala district.

Source	Sample type	Sample size	Low MPN (%)	Intermediate MPN (%)	High MPN (%)
Retail	Meat	109	8.45	2.82	88.73
	Edible organs	72	0.00	16.00	84.00
Supermarket	Meat	35	17.39	34.78	47.83
	Edible organs	34	26.09	43.48	30.43
	Total	250			

was observed for gentamicin (4.79%) and cephalixin (25.75%). The MAR index of *Escherichia coli* ranges from 0.1 to 0.8. The current study indicates 82.63% multiple antimicrobial resistance of *E. coli*, indicating resistance to at least two or more antimicrobial agents. Only 17.37% of the isolates were resistant to two or fewer antimicrobial agents. The highest MAR value of 0.8 is denoted by several patterns of combined antimicrobial agents: AMP, AMX, CAC, CN, E, GEN, TE; AMP, AMX, C, CAC, CIP, E, TE; AMP, AMX, CAC, CIP, CN, E, TE; AMP, AMX, C, CIP, CN, E, TE and AMP, AMX, C, CIP, E, GEN, TE. Among these patterns, the most frequent pattern of 0.8 MAR value is AMP, AMX, C, CIP, CN, E, TE. The least significant MAR value 0.1 is indicated by E (3/167), TE (3/167), CIP, CAC, CN, and AMX (1/167 for each). This study reveals the presence of multidrug resistance in *E. coli* isolates obtained from chicken meat and edible poultry organs (Figure 2). The graphical interpretation of MAR of the collected *E. coli* isolates is indicated in Figure 3.

#### 4. Discussion

Faecal contamination of fresh produce is a significant public hazard in the majority of developing countries [19]. Chicken meat and edible chicken organs are the most popular meat

types consumed among Sri Lankans [4, 20]. They are mostly consumed fully cooked or sometimes half-cooked (in salads), processed (sausage, luncheon meats, and hot dogs), cured (bacon and ham), and as further processed meat products (nuggets, meat fingers, drumsticks) based on the application [21]. The primary objective of this study was to identify the *E. coli* prevalence in chicken meat and edible organs available in the North Western Province, Sri Lanka. This study indicates an alarming risk of high *E. coli* prevalence in poultry meat and edible organs collected from retail shops and supermarkets, and multiple antimicrobial resistance in *E. coli* isolated from the North Western Province. These results are significant as this province has the highest poultry and poultry meat production in Sri Lanka [4].

Although *E. coli* prevalence in edible chicken organs shows a greater value when compared to *E. coli* prevalence in chicken meat, the difference between *E. coli* prevalence in chicken meat and edible organs is not statistically significant. Maintenance of poor sanitation is the major cause of high prevalence of gut microflora such as *E. coli* in food commodities. It was clear that meat handlers, especially at retail levels were not maintaining an adequate levels of hand washing. A majority of the meat handlers were not using

TABLE 3: MAR indexes and resistant antibiotics by antimicrobial resistance profiles of collected *E. coli* samples.

MAR index	Frequency	Resistant antibiotics
0.8	2	AMP, AMX, CAC, CN, E, GEN, TE
0.8	3	AMP, AMX, C, CAC, CIP, E, TE
0.8	2	AMP, AMX, CAC, CIP, CN, E, TE
0.8	8	AMP, AMX, C, CIP, CN, E, TE
0.8	1	AMP, AMX, C, CIP, E, GEN, TE
0.7	1	AMP, AMX, C, CAC, E, TE
0.7	12	AMP, AMX, C, CIP, E, TE
0.7	1	AMP, AMX, CAC, CIP, E, TE
0.7	5	AMP, AMX, CIP, CN, E, TE
0.7	1	AMP, AMX, CIP, E, GEN, TE
0.7	1	AMP, AMX, C, CAC, CIP, E
0.7	1	AMP, AMX, C, CAC, CIP, CN
0.7	1	AMP, AMX, C, E, GEN, TE
0.7	1	AMX, AMP, C, E, CIP, TE
0.7	1	CN, CAC, C, CIP, E, TE
0.6	8	AMP, AMX, C, E, TE
0.6	4	AMP, AMX, CAC, E, TE
0.6	1	AMP, AMX, CIP, E, GEN
0.6	6	AMP, AMX, CIP, E, TE
0.6	6	AMP, AMX, CN, E, TE
0.6	2	AMP, AMX, CIP, C, E
0.6	2	AMP, AMX, CIP, CN, E
0.6	2	AMP, CIP, CN, E, TE
0.6	1	AMP, CAC, CIP, E, TE
0.6	1	AMP, C, CIP, E, GEN
0.6	1	AMX, CIP, CN, E, TE
0.6	1	AMP, CAC, CN, E, TE
0.6	1	AMX, AMP, CAC, CIP, E
0.6	1	AMP, C, CIP, E, TE
0.4	1	AMX, CAC, CN, E
0.4	8	AMP, AMX, E, TE
0.4	2	AMP, AMX, C, E
0.4	9	AMP, AMX, CIP, E
0.4	1	AMP, AMX, CN, E
0.4	1	AMP, AMX, CAC, TE
0.4	1	AMP, AMX, CN, E
0.4	5	AMP, AMX, CIP, TE
0.4	1	C, CIP, CN, TE
0.4	1	C, CN, E, TE
0.3	11	AMP, AMX, E
0.3	3	AMP, AMX, CIP
0.3	1	AMX, CAC, CN
0.3	1	AMX, CAC, CIP
0.3	6	CIP, E, TE
0.3	1	AMX, CN, E
0.3	1	AMX, E, TE
0.3	1	CAC, E, TE
0.3	1	AMP, CAC, E
0.3	1	C, CIP, TE
0.3	1	AMP, AMX, TE
0.3	1	AMX, CN, CAC
0.3	1	CN, E, TE
0.2	5	E, TE
0.2	1	C, E
0.2	1	C, TE
0.2	4	CIP, E
0.2	1	CAC, CN
0.2	1	AMX, TE
0.2	1	AMP, CIP

TABLE 3: Continued.

MAR index	Frequency	Resistant antibiotics
0.2	1	AMX, E
0.1	3	TE
0.1	1	CIP
0.1	1	CAC
0.1	1	CN
0.1	3	E
0.1	1	AMX

gloves or meat handlers to handover meat to the customers. Moreover, they were using bear hands to handle meat, and instead of washing their hands, the common practice they had was to wipe their hands with a piece of cloth. Meat handling and selling shops did not maintain adequate cold chains, especially in retail shops. Although cold chains were maintained properly and were using meat handlers or gloves to handle meat in regard to supermarkets, *E. coli* prevalence in samples collected from supermarkets was still considerably high. However, the sanitation of meat sold in both retail and supermarkets was unclear after the slaughtering step until the vendor level in Sri Lanka. High *E. coli* contamination in the Sri Lankan scenario from both retail shops and supermarkets may be due to improper food handling [22], poor hygienic practices [22, 23], and not maintaining refrigeration or appropriate cold chains [6].

The establishment of good hygienic practices in food handling, maintenance of continuous cold chains for raw material storage at retail levels, and the application of adequate heat treatments to inactivate live pathogens, as well as harmful toxins while cooking are effective remedies against high *E. coli* prevalence [24]. Ruminants such as cattle and sheep act as active carriers of pathogenic Shiga toxin-producing *E. coli* (STEC); faecal contamination of water bodies and raw food items by these animals' faeces can lead to a potential *E. coli* risk to the general public [25, 26]. The need for various treatment options to the affected public includes the use of antimicrobial drugs and the high cost of hospitalization. These issues significantly affect the medical sector of a country.

When *E. coli* prevalence data are compared with literature findings, it is clear that the prevalence data can be affected due to numerous reasons. Although *E. coli* is considered a heat-sensitive bacterium that is destroyed with proper cooking methods, enterotoxigenic *E. coli* (ETEC), enterotoxins, and verotoxins produced by *E. coli* O157:H7 can remain in food commodities causing food-borne diseases, a potential public health risk [27, 28]. Moreover, the survival of *E. coli* in raw or undercooked food products such as salads or hamburgers can lead to diarrheal diseases and related complications since they are directly consumed without preliminary heat treatment [29]. Although the presence of *E. coli* in food commodities does not necessarily emphasize the risk of *E. coli* leading to an outbreak, the public health risk of contaminated food items cannot be readily ignored until the pathogenicity of the present *E. coli* is completely ruled out [30]. Outbreaks related to generic

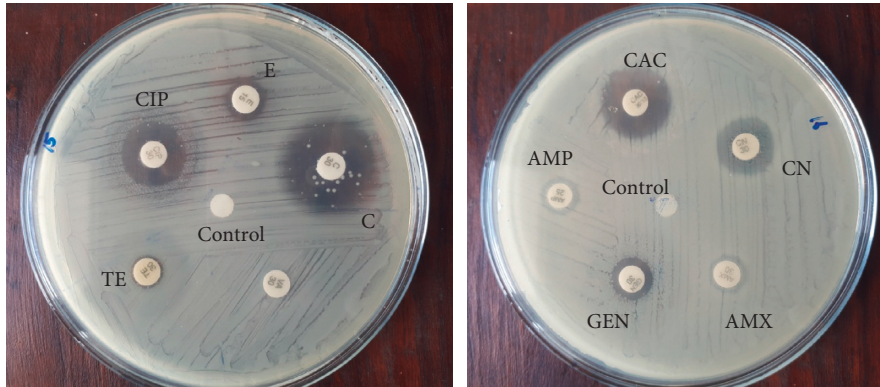


FIGURE 2: *E. coli* samples showing multiple antimicrobial resistance (MAR).

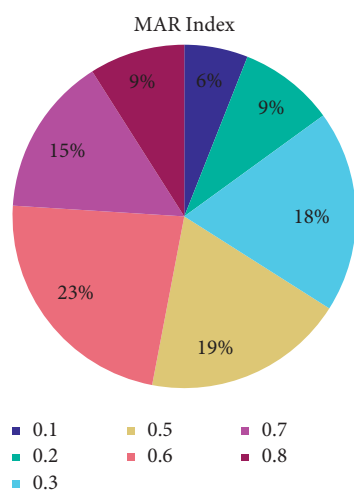


FIGURE 3: Graphical representation for multiple antimicrobial resistance in *E. coli*.

*E. coli* contamination have also been reported worldwide apart from pathogenic *E. coli* strains [31].

The results of the current research study show a similar pattern of *E. coli* significance in raw meat with similar studies conducted worldwide. For example, prevalence study conducted using raw meat collected from retail shops in the United States indicated 72% of *E. coli* contamination [32] and a second study in Washington D.C. indicated 70.7% [33] prevalence rate. Similar studies conducted in the Czech Republic and Eastern Turkey using poultry meat indicate *E. coli* contaminations of 63% [34] and 70% [35], respectively.

The prevalence percentages described in the current study show similar patterns to the *E. coli* contaminations in the South Asian studies. Neighbouring countries to Sri Lanka such as India and Bangladesh have conducted similar studies with similar ranges of prevalence scores. An Indian study suggests 77% of *E. coli* prevalence [36], while two studies conducted in Bangladesh suggest the overall *E. coli* contamination values of 63.5% in broiler and layer chicken meat [37], and 76.1% prevalence using frozen chicken meat [38].

However, the prevalence of generic *E. coli* values varies between the ranges of very high to very low based on the

differences of countries, reagents, and the associated sample type. For example, a study conducted in Mexico City indicates a very high *E. coli* prevalence of 85% in ready-to-eat (RTE) salads [39]. Conversely, a study associated with *E. coli* contamination in beef and lamb samples denotes the prevalence as 17.8% and 16.7%, respectively [40].

The MAR value for *E. coli* in the current study elaborates a greater value of 82.63%. This indicates a considerable adverse effect in common antibiotic usage. The high prevalence of erythromycin and amoxicillin resistance in *E. coli* isolates from poultry meat shows the frequent usage of common antibiotics. Antibiotics are used in farm animals and in veterinary medicine as growth promoters [41] to increase feed efficacy, to decrease waste production [42], and to prevent diseases [43]. The highest frequency of resistant pattern (12/167) in all MAR indices is denoted by AMP, AMX, C, CIP, E, TE, with a MAR value of 0.7. The highest MAR frequency of 0.8 was showed by the pattern of AMP, AMX, C, CIP, CN, E, and TE (8/167), indicating possible drug interactions between the above resistant antimicrobial agents.

A MAR value greater than 0.2 indicates a high contamination risk where frequent antibiotics were used. Frequent and unmonitored application of antibiotics gives rise to multidrug resistance in associated bacteria, acquiring antibiotic-resistant genes. On the other hand, antimicrobial residues could affect the persistent normal microflora of host animals, which eventually grow and replicate into antibiotic-resistant bacteria [44]. *E. coli* especially has the ability to transfer resistant genes to other bacteria once antimicrobial resistance is acquired [45]. Multidrug-resistant bacteria may transfer antimicrobial resistant genes to other bacteria in human intestinal microflora resulting resistance acquired zoonoses [46]. *E. coli* shows a specific mechanism of MAR through the production of extended-spectrum  $\beta$ -lactamases (ESBL) [9, 47], which makes *E. coli* infections even more difficult to treat with fluoroquinolones, aminoglycosides, and trimethoprim-sulfamethoxazole. ESBL-producing *E. coli* are capable of inactivating such antibiotics due to the resistant mechanisms present [48]. The global burden of MAR has reached unacceptable levels due to the wide consumption of inexpensive and outdated antimicrobial agents [12, 49]. If necessary actions have not been taken in



the near future, this usage pattern will show an increasing trend. The weight of the MAR problem is considerably high in low-income countries, where inexpensive antimicrobials are often used to treat a wide range of nosocomial diseases when compared to many other developed countries [50].

Several studies in the literature suggest the presence of MAR and a relatively similar level of MAR levels to support the current research findings in *E. coli* isolated from poultry meat. Reference [37] shows the presence of 78.06% of MDR in Bangladesh, with ampicillin, erythromycin, and tetracycline resistance (98.95%, 89.5%, and 85.3%, respectively). Amoxicillin and ampicillin resistance among humans is found in most of the cases, and it is due to the presence of plasmid-encoded  $\beta$ -lactamases, such as TEM-1, TEM-2, or SHV-1, where they can hydrolyse and inactivate amoxicillin and ampicillin drugs [51]. Previous studies suggest antimicrobial resistance by extended-spectrum cephalosporin (ESC)-resistant *E. coli* showing coresistance to ampicillin, amoxicillin/clavulanic acid, chloramphenicol, and tetracycline [52]. Another study suggests that nearly 75% of ampicillin-resistant *E. coli* were also resistant to tetracycline [53]. Higher resistance to amoxicillin [54], ampicillin [54], tetracycline [55], and chloramphenicol has also been reported from *E. coli* specimens isolated from poultry samples, due to increased usage of the above drugs in commercial poultry [56]. High resistance to ciprofloxacin around the world may be due to the increased use of quinolones in poultry farms, and this result supports the possible reasons for the high resistance to ciprofloxacin in human specimens to be due to the colonization of ciprofloxacin-resistant *E. coli* in the human gut [56]. Ciprofloxacin resistance of the family Enterobacteriaceae has become a growing concern in clinical settings around the world [54]. Antimicrobial resistance to gentamicin may be due to the use of apramycin, a commonly used veterinary medicine that is structurally similar to gentamicin. These apramycin-resistant *E. coli* may be resistant to gentamicin as well [56]. Resistance to cephalosporin antibiotics is mainly associated with extensive usage of cephalosporins (cephalexin, ceftazidime) in clinical practice [57]. *E. coli* isolated from faecal-contaminated water bodies show significantly higher resistance to erythromycin [54], which indicates a high risk of erythromycin-resistant *E. coli* infections after the consumption of contaminated water from such water bodies. However, previous studies suggest that the level of resistance observed by *E. coli* isolates from human samples indicates comparatively low resistance compared to the *E. coli* isolates collected from poultry and animal samples [56, 58].

Although the usage of antibiotics as growth promoters in animal feed has been banned in many countries around the world (EU since 2016), some countries still use common antimicrobial agents to promote growth in farm animals [59]. Many of the commonly used antibiotics are freely accessible in several countries and can be purchased over the counter without a prescription [60]. The convenience of obtaining antimicrobial drugs without a prescription over

the counter makes it easy for the general public to misuse antimicrobial drugs to a greater extent.

As MDR problem is an alarming issue that needs immediate effective approaches to prevent further generation of MDR genes, several initiatives could be taken to reduce the acceleration of the AMR problem. The usage of narrow-spectrum antibiotics [61], prudent use of antibiotics in veterinary medicine and animal feed [62], use of antimicrobial agents in a rotation pattern [63], use of combined antimicrobial therapy in clinical medicine [63], administration of proper veterinary practices to avoid the usage of antimicrobials [61], and antibiotic regulatory approaches and monitoring systems [62] are few such actions to be launched immediately. The prevention of misusing antibiotics by the general public and enabling antimicrobial surveillance programmes, especially in nosocomial infections, are mandatory actions that need to be taken to reduce the spread of antibiotic-resistant bacteria [44]. Multiple antimicrobial resistance in *E. coli* isolated from poultry meat and edible poultry organs may be due to the linkage of resistant genes in their plasmids [30, 63]. Further studies are required to identify the genetic basis of coresistant phenotypes.

## 5. Conclusion

Findings from this research project emphasize the need for adequate heat treatment prior to consuming raw chicken meat and edible chicken organs available in the Sri Lankan market and the importance of prudent antibiotic usage during veterinary medicine and farm animals to stop the further spread of the present unacceptable levels of antimicrobial resistance further.

A limitation of this study was that it has only focussed on the prevalence and antimicrobial resistance in generic *E. coli*, and the pathogenicity of the collected *E. coli* was not tested. As the North Western Province has the highest poultry production in Sri Lanka and operates a large number of commercial poultry farms compared to other provinces, there is a possibility that the current study results may have slightly exaggerated the prevalence and MAR values in comparison with the wider poultry farming sector in Sri Lanka.

*5.1. Further Approaches.* Pathogenicity of the collected *E. coli* isolates needed to be tested for pathogenicity identification. PCR confirmed the samples needed to be tested for phylogenetic analysis. The prevalence of pathogenic *E. coli* strains should also be tested for other types of meat as well as fresh produce associated with the wider food industry in Sri Lanka.

## Data Availability

The data collected to support this study are included within the article.

## Disclosure

A small part of this manuscript has been published as conference abstracts in “7th Conference on Sri Lanka-Japan Collaborative Research-SLJCR 2019,” also funded by National Research Council grant no. 17-008.

## Conflicts of Interest

The authors declare that there are no conflicts of interest in this study.

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