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RESEARCH ARTICLE

Food Microbiology

Decadal trends in antimicrobial susceptibility of *Escherichia coli* and *Salmonella* spp. in chicken from small-scale farm shops in Kandy district, Sri Lanka

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Abstract: *E. coli* and *Salmonella* spp. are two important foodborne pathogens that rapidly develop antimicrobial resistance (AMR) and frequently contaminate animal products, particularly chicken meat. In Sri Lankan small scale poultry operations, substandard hygienic practices are common. The current study reports and compares two consecutive studies conducted in 2010-2011 and 2020-2021, which investigated AMR *E. coli* and *Salmonella* spp. contamination in chicken meat sold at small-scale farm shops in the Kandy district, Sri Lanka. The first study included 72 chicken meat samples collected from 10 out of the 20 divisional secretariat divisions (DSDs), while the second study included 37 meat samples from the same DSDs, collected 10 years later. *Salmonella* spp. and *E. coli* were isolated and identified, and AMR profiles were obtained using disc diffusion assay and CLSI guidelines. The first study reported 100% *E. coli* contamination in chicken meat, but a decade later, the percentage had significantly decreased to 70.3%. In both studies, *E. coli* isolates demonstrated resistance to more antimicrobials than *Salmonella* isolates, and the proportion of multidrug-resistant (MDR) *E. coli* isolates (defined as resistance to five or more antimicrobials) was higher than that of the *Salmonella* spp. isolates. Significantly lower MDR *E. coli* isolates were detected in the recent study. However, tetracycline resistance among *E. coli* isolates was higher compared to the previous study. Despite these improvements, the contamination level of chicken meat in small-scale retail shops remains concerning. Therefore, it is crucial to promote prudent antimicrobial use and ensure proper food processing practices at all levels of production in Sri Lanka.

Keywords: Antimicrobials, chicken, *E. coli*, meat, resistance *Salmonella* spp.

INTRODUCTION

Food safety is a growing public health concern worldwide, owing to the rising numbers of foodborne illness (FI) cases and the demand to meet food standards to ensure consumer safety (Gizaw, 2019). Foodborne illnesses cause significant morbidity and mortality, posing a noteworthy influence in terms of the cost of medical care, resulting in a substantial loss of productive hours for the nation (Newman *et al*., 2015a; 2015b). Even in the United States, where the food supply is considered one of the safest in the world, 48 million cases of FI occur each year (Huffaker & Hartmann, 2021). However, these numbers are considered an underestimation, because milder cases are often undiagnosed and thus unreported (Todd, 2014). The scenario with FI is even worse in developing countries, because no proper surveillance system is in place and thus reported cases might only be the tip of the iceberg (Grace, 2015). Among FI caused by known agents, bacteria continue to be a major cause of foodborne diseases worldwide (Bintsis, 2017). As a result qualitative or quantitative assessments of bacteria have been a priority in meeting food safety standards.

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Escherichia coli (*E. coli*) and *Salmonella* spp. are major foodborne pathogens that have been prioritized in all food safety standards (Abebe *et al.*, 2020). According to the Center for Disease Control and Prevention (CDC), pathogenic *E. coli* is one of the top five pathogens responsible for domestically acquired FI resulting in hospitalization, while *Salmonella* spp. (non-typhoidal) is among the top five pathogens resulting both hospitalization and death (CDC, 2011). Both these pathogens share some common features such as the source of infection, pathogenicity, and development of resistance against antimicrobials. *E. coli* and *Salmonella* spp. are found colonized in intestinal tracts of a variety of wild and domestic animals, especially farm animals. Therefore contaminated food of animal origin has been identified as the main source of infection (Heredia & García, 2018). Upon infection, these pathogens often cause mild to moderate self-limiting enteritis unless it is complicated or progresses into an invasive condition which may be fatal (Croxen *et al.*, 2013; Marchello *et al.*, 2022). Moreover, due to the intensive use of antimicrobials in farming, these pathogens demonstrate an increased rate of acquired resistance against commonly used antimicrobial agents (Frye & Jackson, 2013). Additionally, these pathogens carry multidrug resistant determinants that can be disseminated via horizontal and vertical transfer (Card *et al*., 2017; Li *et al*., 2021). Further, contaminated poultry meat has identified to be an important source of transmitting these pathogens (Heredia & García, 2018).

Chicken meat is the most consumed meat variety in Sri Lanka and the per capita consumption has significantly increased during the past few decades (Alahakoon *et al*., 2016). The popularity of chicken meat is primarily due to the absence of cultural or religious obstacles, availability in retail markets and relatively low cost. Previous studies conducted in Sri Lanka have demonstrated substantial contamination of chicken eggs and meat with *E. coli* and *Salmonella* spp. (Kalupahana *et al*., 2017; Kulasooriya *et al*., 2019).

Different scales of poultry operations function within Sri Lanka and their management practices are diverse. Even though large-scale companies release quality-certified branded chicken meat to the market, a considerable fraction of meat consumers prefer purchasing fresh chicken meat from small-scale retail shops. Generally, the chicken sold in these small-scale farm shops is processed in facilities not designed as processing plants, where slaughtering and evisceration are done manually and mostly on-site. Like in many other developing countries, hygienic practices in these small scale retail meat shops are often below the standards and function with minimum resources. Therefore, the potential of meat getting contaminated with foodborne pathogens is relatively high. Meat contamination with bacteria found in animal guts and the presence of AMR strains can be used to assess operational hygiene at both the production and processing levels. It was hypothesized that substandard processing practices in small scale farm shops would increase contamination of chicken meat with foodborne *E. coli* and *Salmonella* spp.. Additionally, these bacteria might exhibit resistance to antimicrobials commonly used in veterinary practice. Therefore, two investigations were done 10 years apart to reveal the level of *E. coli* and *Salmonella* spp. contamination in chicken meat sold at small scale shops in the Kandy district, Sri Lanka and the antimicrobial susceptibility of these contaminants.

MATERIALS AND METHODS

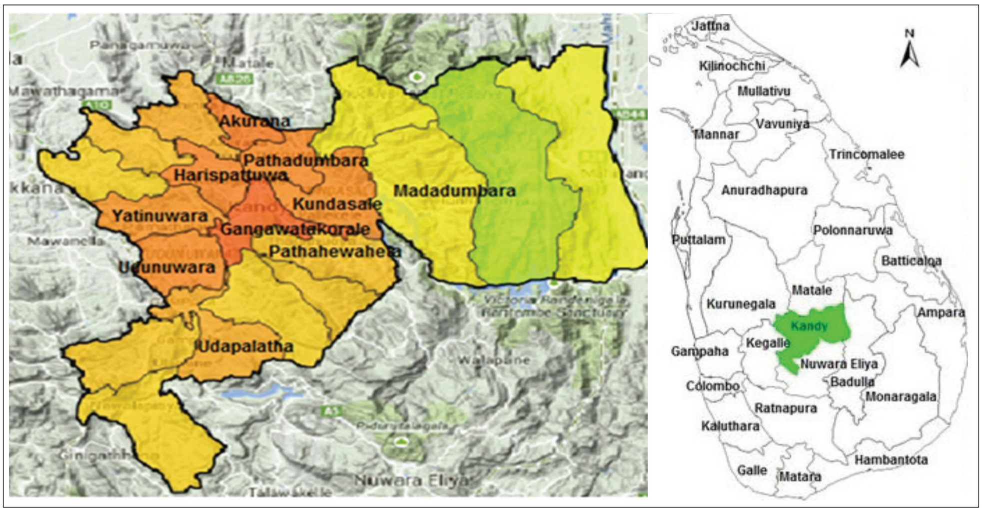
Study area and sampling design

The two investigations were done in 2010-2011 and 2020-2021 in Kandy district, one of the most densely populated districts of Sri Lanka. The Kandy district consists of 20 divisional secretariat divisions (DSDs). To address the variation in population density among DSDs (the number of meat retail shops varies accordingly) and to increase the precision of the study, the DSDs of Kandy district were considered as strata.

Only the small scale 'farm shops' were sampled to avoid selection bias, and to maintain uniformity among sampling sites. In Sri Lanka, a 'farm shop' is typically a small scale retail shop where fresh or frozen and thawed poultry carcasses are sold by keeping them in display units at 10-15°C. Most of these shops sell only poultry products such as whole carcasses, cuts, poultry visceral organs, eggs, and the value-added products such as sausages, meatballs, etc. However, some farm shops may also sell food products from other animals (dried fish, canned fish, etc.) and food products of non-animal origin. These farm shops are often found in the centre of a town and, besides, they can be found scattered in certain streets in larger towns.

As reliable data on the number of farm shops in each DSD was unavailable, the sample size was calculated based on an estimated 150 farm shops in the study area. Assumptions included a 90% prevalence of *E. coli* contamination in chicken meat, a 95% confidence interval, and a 5% margin of error. This resulted in a required sample size of 73 farm shops for investigation.

It was assumed that population densityis proportional to the farm shops available in each DSD. The top five DSDs based on the population were selected purposefully and the remaining 5 were picked randomly using Minitab software. In the 2010-2011 investigation, chicken meat samples were collected from 72 small-scale farm shops across 10 DSDs (Figure 1). In the 2020-2021 investigation, efforts were made to collect samples from the same geographical locations. However, only 37 farm shops could be sampled due to permanent shop closures, COVID-19 restrictions, and financial limitations.



(The colours used in the map do not indicate any scale or a variable)

Figure 1: Divisional Secretariat Divisions (DSDs) of Kandy district of Sri Lanka sampled during two studies

Sample collection

In Sri Lanka, traditionally, the central market/main stall complex is located adjacent to the central bus stand. Therefore, sampling was performed in the main population centre of each DSD starting from the farm shop proximal to the central bus stand and continued along the main street until the required sample size was fulfilled. The number of farm shops sampled in the main centre of each DSD is shown in Table 1.

Table 1: Number of farm shops sampled from the main population centre of the selected DSDs during each investigation

Selected divisional secretariat divisions	Main population centre	Number of farm shops sampled	
		2010-2011 investigation	2020-2021 investigation
Gangawatakorale	Kandy	20	5
Udapalatha	Gampola	13	5
Akurana	Akurana	9	5
Harispattuwa	Katugastota	9	5
Pasbagakorale	Nawalapitiya	6	5
Yatinuwara	Pilimathalawa	3	3
Udunuwara	Gelioya	3	*
Pathahewaheta	Thalathuoya	3	3
Madadumbara	Theldeniya	3	3
Kundasale	Kundasale	3	3
Total		72	37

*No sampling was done

At each farm shop, a chicken leg was purchased as the sample assuming that it is the part which has the highest possibility of contamination during carcasses handling and manual evisceration. The collected samples were transported to the Food Microbiology Laboratory of the Department of Veterinary Public Health and Pharmacology, University of Peradeniya and stored at 4°C until the isolation and identification of foodborne bacteria were done.

Isolation, identification, and confirmation of *E. coli* and *Salmonella*

The qualitative identification protocols described by SLS 516: part 3: 1982 were followed, with certain modifications for isolation and identification of *E. coli*. If summarized, 25 g of meat were weighed, blended and homogenized with 225 mL of buffered peptone water (BPW) (Oxoid, CM0509). The homogenate was incubated for 18 - 24 h at 36 °C ± 1°C in a screw-capped bottle. A loop full (10 µg) of this enriched homogenate was taken and streaked on a MacConkey (MAC) agar (Oxoid, CM0007) plate and incubated at 36°C ± 1°C for 18-24 h. A presumptive *E. coli* colony (red, non-mucoid colonies) was picked and cultured on an Eosin-methylene blue (EMB) agar (Oxoid, CM0069) plate, incubating for 36°C ± 1°C for 18 - 24 h. A typical *E. coli* colony (purple colour colonies with a green metallic sheen) from an EMB plate was sub-cultured on nutrient agar (Oxoid, CM0003), incubating at 37°C for 18 - 24 h. Confirmation was done by subjecting the isolates to Grams staining and a panel of biochemical tests (Catalase test, Triple Sugar Iron reaction, Urease test, Citrate test, motility test, and Kovacs' Indole test).

For isolation and identification of *Salmonella,* methods described in ISO 6579 were followed. In brief, from the sample, 25 g of meat were weighed and blended using stomacher for 2 minutes to homogenize with 225 ml of BPW (Oxoid, CM0509). The homogenate was incubated 16 – 20 hours at 36 °C ± 1°C in a screw-capped bottle. From this pre-enrichment 0.1 mL was mixed with 10 mL of Rappaport-Vassiliadis (RV) broth (Oxoid, CM0669) and incubated at 41.5°C ± 0.5°C for18 - 24 h. A loop full (10 µg) of this selective enrichment was taken and streaked on a Xylose Lysine Deoxycholate (XLD) agar (Oxoid, CM0469) plate, which was then incubated at 36°C ± 1°C for 18 - 24 h. The plate was observed for the presence of typical *Salmonella* spp.colonies (red colonies with black centers) and such colonies were sub-cultured on nutrient agar (Oxoid, CM0003) at 37°C for 18 - 24 h. Confirmation of recovered *Salmonella* spp. was done by Grams staining, biochemical testing (Catalase test, Triple Sugar Iron reaction, Urease test, Citrate test, and motility test) and performing an agglutination test with polyvalent *Salmonella* antiserum.

Antimicrobial susceptibility testing

E. coli and *Salmonella* spp. isolates were subjected to disk diffusion assay to identify their antimicrobial susceptibility profiles. Guidelines and clinical breakpoints given by the Clinical Laboratory Standard Institute, 2008, were used to conduct the disk diffusion assay and to interpret the results. However, in situations where the particular antimicrobial was not listed in the publication, breakpoints of the closest antimicrobial within the same group were used for interpretation. In each investigation the panel of antimicrobials was selected based on availability as well as considering whether the antimicrobials are repetitively used in poultry medicine and in humans. *E. coli* ATCC 25922 was used as the quality control strain.

The antimicrobial panel of both studies included ampicillin (10 µg), ciprofloxacin (5 µg), chloramphenicol (30 µg), gentamicin (10 µg), streptomycin (10 µg), and tetracycline (30 µg). However, amoxicillin (10 µg), cephalothin (30 µg), enrofloxacin (5 µg), and neomycin (10 µg) were only available for the 2010-2011 investigation, whereas amikacin (30 µg), amoxicillin-clavulanic acid (30 µg), cefotaxime (30 µg), ceftazidine (30 µg), nalidixic acid (30 µg) and trimethoprim-sulphonamide (25 µg) were only available for the 2020-2021 investigation. Multidrug resistance was defined as resistance to five or more antimicrobials from different classes tested in the study.

Data management

Microsoft Excel spreadsheets were used to manage data and GraphPad was used to generate figures. Two sample proportion analyses were conducted on Minitab statistical software where $p \leq 0.05$ was considered significant.

RESULTS AND DISCUSSION

Contamination of meat with *Salmonella* spp. and *E. coli*

The study conducted in 2010-2011 detected *E. coli* in all (100%) meat samples tested and *Salmonella* spp. in 34/72 (47.2%) of the meat samples tested (Figure 2a and 2b). However, in 2020-2021 investigation, *E. coli* contamination was 70.3% (26/37) and *Salmonella* spp. contamination was 59.45% (22/37) (Figure 2c and 2d).

Statistical comparison between the proportions revealed that the *E. coli* contamination observed in the 2020-2021 investigation is significantly lower ($p = 0.00$) than that of the 2010-2011 study, and the difference observed for *Salmonella* spp. contamination is not statistically significant.

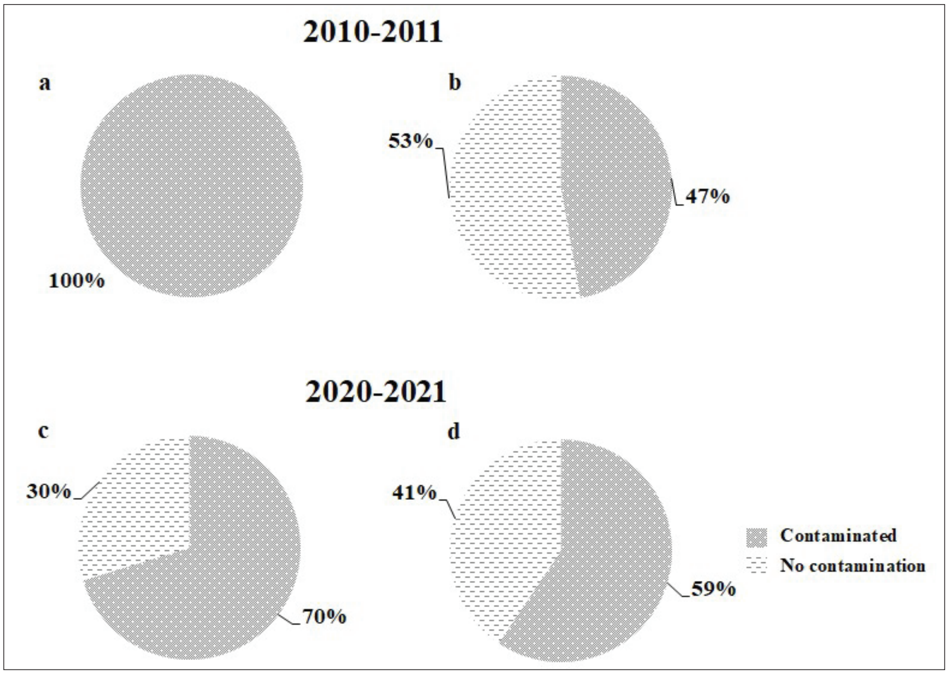


Figure 2: a) *E. coli* contamination in chicken meat detected in 2010 - 2011, b) *Salmonella* spp. contamination in chicken meat detected in 2010 - 2011, c) *E. coli* contamination in chicken meat detected in 2020 - 2021, and d) *Salmonella* spp. contamination in chicken meat detected in 2020 - 2021.

Table 2: *E. coli* and *Salmonella* contamination in meat by DSD

Selected Divisional Secretariat Divisions	Main town	2010-2011				2020-2021			
		E. coli contamination		*Salmonella* contamination		*E. coli* contamination		*Salmonella* contamination	
		Proportion	Percentage	Proportion	Percentage	Proportion	Percentage	Proportion	Percentage
Gangawatakorale	Kandy	20/20	100.0	7/20	35.0	3/5	60.0	3/5	60.0
Udapalatha	Gampola	13/13	100.0	8/13	61.5	3/5	60.0	2/5	40.0
Akurana	Akurana	9/9	100.0	4/9	44.4	3/5	60.0	2/5	40.0
Harispattuwa	Katugastota	9/9	100.0	3/9	33.3	4/5	80.0	3/5	60.0
Pasbagakorale	Nawalapitiya	6/6	100.0	5/6	83.3	4/5	80.0	2/5	40.0
Yatinuwara	Pilimathalawa	3/3	100.0	0/3	0.0	3/3	100.0	2/3	66.6
Udunuwara	Gelioya	3/3	100.0	3/3	100.0				
Pathahewaheta	Thalathuoya	3/3	100.0	0/3	0.0	3/3	100.0	2/3	66.6
Madadumbara	Theldeniya	3/3	100.0	2/3	66.7	1/3	33.3	3/3	100.0
Kundasale	Kundasale	3/3	100.0	2/3	66.7	2/3	66.6	3/3	100.0

Table 2 shows the contamination of meat samples by DSD in each investigation. Despite the fact that the data were collected a decade apart, meat sold in all of the farm shops investigated is tainted with *E. coli* and *Salmonella* spp.

Antimicrobial susceptibility profiles of the isolates

E. coli isolates of the investigation conducted in 2010-2011 showed a markedly higher level of resistance against the panel of antimicrobials tested than those of *Salmonella* spp. isolated from the same investigation. *E. coli* isolates reported the highest percentage of resistance (97.2%) against neomycin and the least percentage of resistance (8.3%) resistance against chloramphenicol (Figure 3). The percentages of *E. coli* isolates exhibited resistance against ampicillin, amoxicillin, cephalothin, streptomycin, and tetracycline (30 µg) are 90.3%, 83.3%, 80.5%, 77.8%, and 61.1% respectively.

E. coli isolated during the 2010-2011 investigation also demonstrated higher levels of resistance to the panel of antimicrobials tested than *Salmonella* spp. isolated during the same period. The highest percentage of resistance (69.2%) was observed in *E. coli* against ampicillin and tetracycline. Furthermore, 61.5% of the *E. coli* isolates tested were resistance to the trimethoprim-sulphonamide combination.

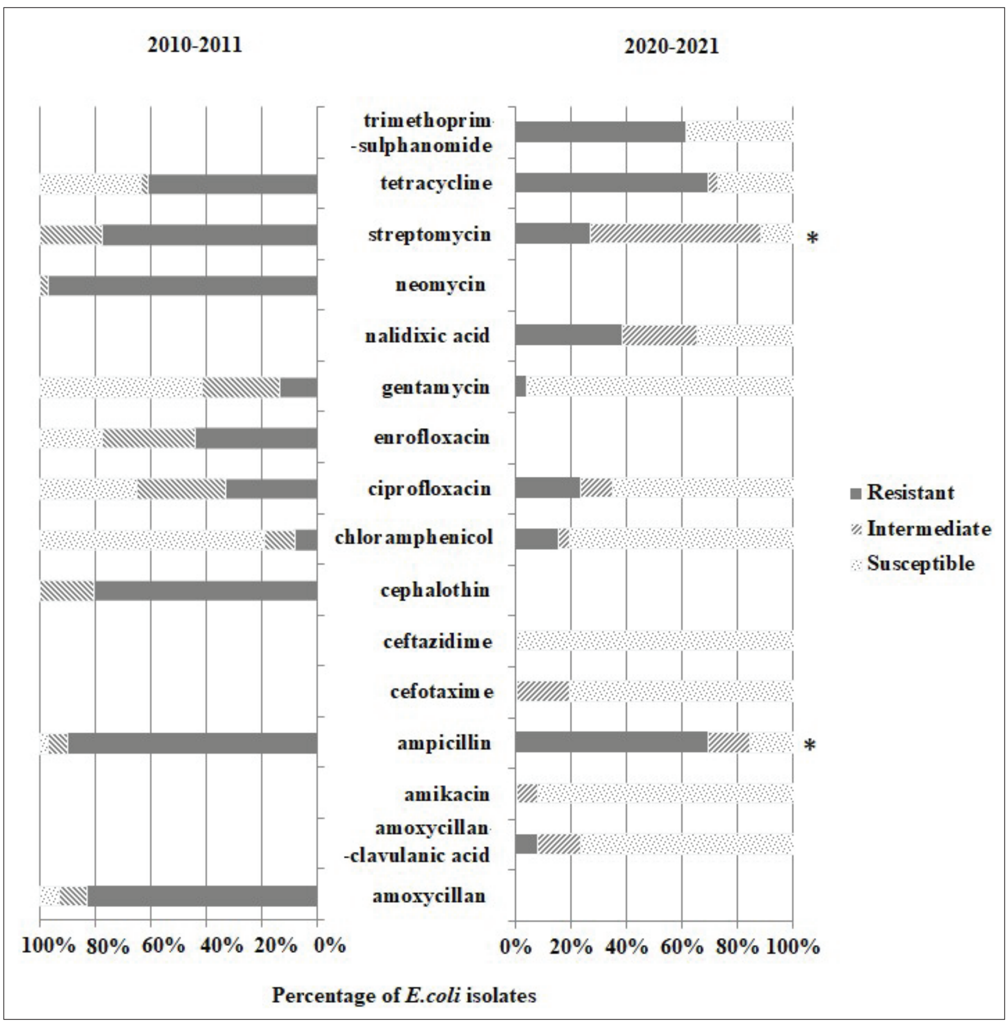


Figure 3: Antimicrobial resistance observed in chicken meat contaminant *E. coli* from the 2010-2011 study and the 2020-2021 study.

When resistance to antimicrobials common to both investigations was considered, *E. coli* isolates from the 2020-2021 study exhibited a significantly lower proportion of resistance to ampicillin (p = 0.01) and streptomycin (p = 0.00) than *E. coli* isolates from the previous study.

More than 40% of the *Salmonella* spp. isolates from the 2010-2011 study were resistant to neomycin (55.8%), streptomycin (35.3%), ampicillin (41.2%), and amoxicillin (41.2%) (Figure 4). Furthermore, only a small percentage of *Salmonella* spp. isolates from the same study were resistant to tetracycline (20.6%), enrofloxacin (20.6%), cephalothin (11.8%), gentamycin (8.8%), chloramphenicol (5.9%), and ciprofloxacin (2.9 %).

In the 2020-2021 investigation, 54.5% of the *Salmonella* spp. isolates tested positive for tetracycline resistance, while 40.9% and 27.2% tested positive for ampicillin and nalidixic acid resistance, respectively. Antimicrobials such as amikacin, amoxicillin-clavulanic acid, cefotaxime, ceftazidine, chloramphenicol, gentamycin, and trimethoprim-sulphonamide were effective against more than 90% of the *Salmonella* spp. isolated during this study.

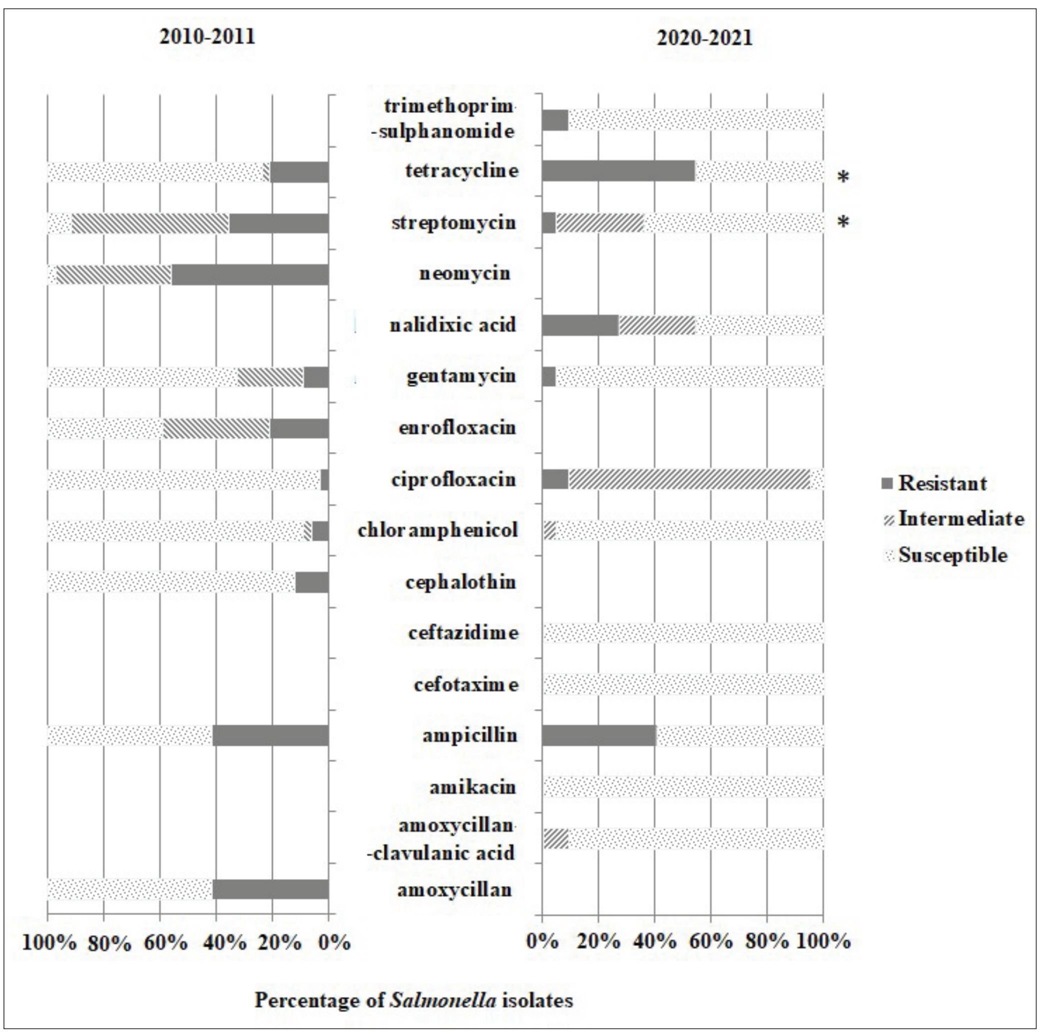


Figure 4: Antimicrobial resistance observed in chicken meat contaminant *Salmonella* spp. isolates from the 2010-2011 study and the 2020-2021 study.

Table 3: Percentage of *E. coli* and *Salmonella* spp. isolates showing antimicrobial resistance by divisional secretariat divisions in study 2010-2011

Divisional Secretariat Division	Percentage (%)																			
	AMP		AML		CEP		NEO		GEN		STR		TET		ENR		CIP		CHL	
	E. coli	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*
Gangawatakorale	100	42.8	85	42.8	85	28.6	95	57.1	5	14.3	55	57.1	50	0	35	0	25	0	15	0
Udapalatha	92.3	25	92.3	25	84.6	0	92.3	50	0	12.5	92.3	50	61.5	25	30.8	25	30.8	0	7.7	0
Akurana	88.9	75	88.9	75	77.8	50	100	75	55.5	25	77.8	25	44.4	25	66.7	50	55.5	25	44.4	0
Harispathuwa	100	0	88.9	0	77.8	0	100	33.3	11.1	0	88.9	0	88.9	0	88.8	0	55.5	0	11.1	0
Pasbagakorale	100	20	100	20	100	0	100	6	16.7	0	83.3	40	83.3	20	50	20	50	0	0	40
Pathahewaheta	100	0	100	0	100	0	100	0	66.7	0	100	0	100	0	0	0	0	0	0	0
Madadumbara	33.3	100	33.3	100	66.7	0	100	0	0	0	100	0	33.3	100	33.3	0	33.3	0	0	0
Kundasale	66.7	50	66.7	50	66.7	0	100	50	0	0	100	0	66.7	0	0	50	0	0	0	0
Yatinuwara	100	0	100	0	100	0	100	0	0	0	100	0	66.7	0	33.3	0	33.3	0	0	0
Udunuwara	33.3	66.7	33.3	66.7	33.3	0	100	100	0	0	33.3	0	33.3	33.3	0	33.3	0	0	0	0

Table 4: Percentage of *E. coli* and *Salmonella* spp. showing antimicrobial resistance by divisional secretariat divisions in study 2020-2021

Divisional Secretariat Division	Percentage (%)																							
	AMP		GEN		STR		TET		CIP		CHL		AMC		AK		CTX		CAZ		NA		SXT	
	E. coli	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*	*E. coli*	*Salm.*
Gangawatakorale	33.3	100	0	0	66.7	0	66.7	0	33.3	0	0	0	0	0	0	0	0	0	0	0	33.3	33.3	33.3	33.3
Udapalatha	66.7	50	0	0	33.3	0	100	50	0	0	0	0	0	0	0	0	0	0	0	0	33.3	0	66.7	0
Akurana	100	100	0	50	0	50	66.7	100	33.3	50	0	0	0	0	0	0	0	0	0	0	66.7	100	100	0
Harispathuwa	100	0	0	0	0	0	75	100	0	0	25	0	25	0	0	0	0	0	0	0	0	0	25	0
Pasbagakorale	25	0	0	0	0	0	50	0	0	0	0	0	0	0	0	0	0	0	0	0	50	0	50	0
Pathahewaheta	66.7	0	0	0	66.7	0	100	100	66.7	0	33.3	0	0	0	0	0	0	0	0	0	66.7	0	100	0
Madadumbara	100	66.7	100	0	100	0	100	33.3	100	0	100	0	0	0	0	0	0	0	0	0	100	66.7	100	0
Kundasale	100	0	0	0	0	0	50	100	0	0	0	0	50	0	0	0	0	0	0	0	0	0	50	0
Yatinuwara	66.7	50	0	0	33.3	0	33.3	0	33.3	50	33.3	0	0	0	0	0	0	0	0	0	33.3	50	66.7	50
Udunuwara																								

The proportion of *Salmonella* spp. isolates recovered with tetracycline resistance during the 2020-2021 study is significantly higher ($p = 0.01$) than in the previous study. Furthermore, the proportion of *Salmonella* spp. isolated in the 2020-2021 study with streptomycin resistance was significantly lower ($p = 0.01$) than in the previous study.

Geographic areas with the highest number of resistant isolates were identified based on the assumption that these farm shops receive birds for slaughter or carcasses for sale from the same DSD. Tables 3 and 4 show the percentage of *E. coli* and *Salmonella* spp. isolates that showed resistance to the selected panel of antimicrobials by DSDs in the 2010-2011 study and the 2020-2021 study, respectively.

In the 2010-2011 study, more than 50% of the *E. coli* isolates from the Akurana, Harispattuwa, and Pasbagekorale DSDs were resistant to 8/10 of the antimicrobials tested. Furthermore, over 50% of the *E. coli* isolates from Pathahewaheta and Kundasale were resistant to 7/10 antimicrobials tested, while more than 50% of the *E. coli* isolates from the Gangawatakorale, Udapalatha, and Yatinuwara DSDs were resistant to 6/10 antimicrobials in the panel. *Salmonella* spp. isolates from the same study were mostly susceptible to the majority of antimicrobials tested, however more than half of the *Salmonella* spp. isolates from Akurana DSDs were reported to be resistant to 5/10 antimicrobials used in the study.

During the 2020-2021 investigation, more than half of the *E. coli* isolated from the Madadumbara and Pathahewaheta DSDs were resistant to 8/12 and 6/12 antimicrobials tested, respectively. Furthermore, more than half of the *Salmonella* spp. isolated from the Akurana DSD were resistant to 6/12 of the antimicrobials tested.

Multidrug resistance (MDR)

Fifty-six *E. coli* isolates (77.8%) from the 2010-2011 investigation met the MDR criteria (see Figure 5).

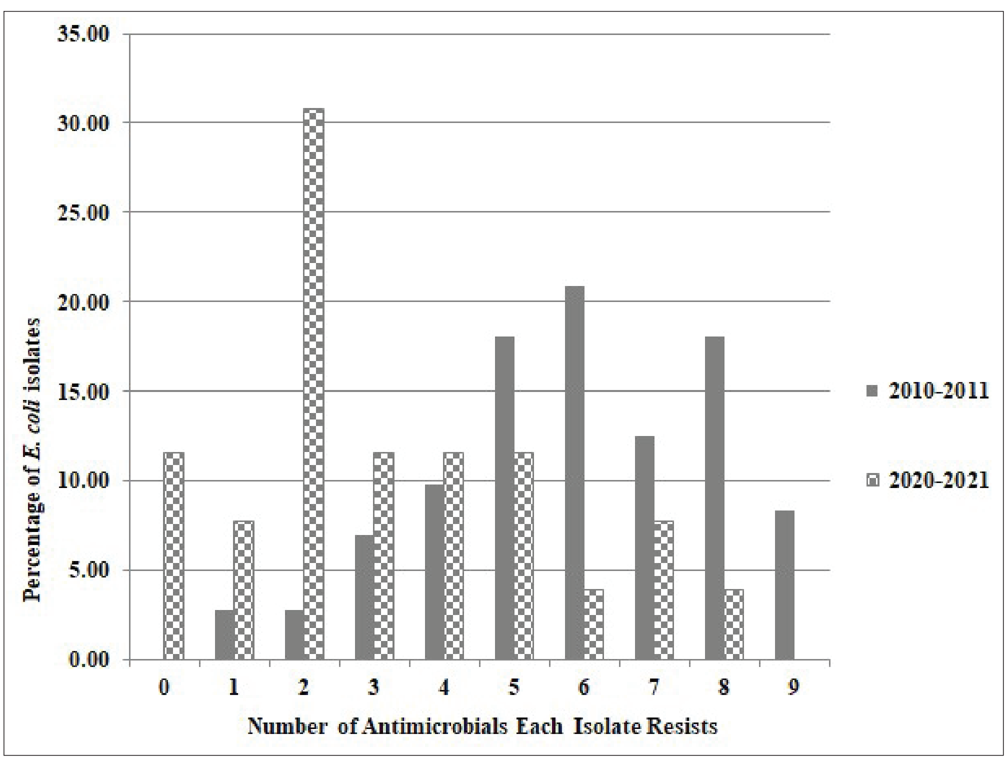


Figure 5: Numbers of chicken meat contaminant *E. coli* with multiple drug resistance in the 2010-2011 study and the 2020-2021 study.

In particular, 13 isolates (18%) were resistant to five antimicrobials, while 15 (20.8%) were resistant to all six antimicrobials tested in this study. Resistance to seven and eight antimicrobials was found in nine (12.5%) and 13 (18%) *E. coli* isolates, respectively. Furthermore, six *E. coli* isolates (8.3%) were resistant to 9/10 antimicrobials in the panel. Only five (14.7%) of the *Salmonella* spp. isolates studied in 2010 - 2011 had MDR profiles. *Salmonella* spp. isolates were twice as susceptible to all antimicrobials tested (10 isolates, 29.4%).

Only seven *E. coli* isolates (26.9%) from the 2020-2021 investigation were identified as MDR isolates (Figure 5). One (3.8%) was resistant to 8/12 antimicrobials tested, while two (7.7%) were resistant to 7/12 antimicrobials in the panel. Another *E. coli* isolate (3.8%) was resistant to 6/12 antimicrobials tested, while the remainder (11.5%) was resistant to 5/12 antimicrobials tested. Only one *Salmonella* spp. isolate (4.5%) from Akurana DSD showed MRD during the same investigation, and this isolate was resistant to 5/12 antimicrobials tested in the study.

When the two studies are compared, there is a significant reduction ($p = 0.00$) in the proportions of MDR *E. coli* isolates in the 2020-2021 study compared to the previous one. Even though the proportion of MDR *Salmonella* spp. detected in the 2020-2021 study was lower than in the previous study, the difference was not statistically significant.

Food-borne illnesses in Sri Lanka have historically received less research attention compared to other infectious diseases (Sandipani *et al.*, 2020). There is either no or very limited epidemiological data on animal-origin foodborne pathogens and their antimicrobial resistance profiles. In such a context, this study reveals the potential risk of acquiring AMR *E. coli* and *Salmonella* spp. through consumption of contaminated chicken meat sold at small scale farm shops, as well as trends in AMR profiles following a decade.

The number of farm shops operating in each population centre in Kandy district was greater than we anticipated; however, the operational hygiene of all farm shops is substandard as the following unsanitary practices were observed: the same person issuing all available animal products with bare hands, repetitive freezing and thawing of meat, the lack of a functional cooler, fly access to meat, and visually unclean meat. All of these unsanitary practices have been shown to promote foodborne bacteria contamination, multiplication, and spread. For instance, Todd *et al.* (2009) clearly demonstrated how workers contributed to the spread of *E. coli* and *Salmonella* spp. through unsanitary meat handling. According to Mohammed *et al.* (2021), repeated freezing and thawing increases microbial counts in chicken meat (Mohammed *et al*., 2021). Casanova *et al*. (2021) demonstrated the importance of maintaining a cold chain for poultry meat in order to reduce the multiplication of foodborne bacteria. According to Barreiro *et al.* (2013), flies can transmit foodborne pathogens along with their associated toxin or resistance, and bacteria in the *enterobacteriacea* group are the most common pathogen transmitted by flies.

Substandard slaughtering and handling hygiene in meat retail shops has been reported in many developing countries, including India (Balakrishnan *et al.*, 2018; Vaidya *et al.*, 2016), Pakistan (Mallhi *et al.*, 2019; Zakki *et al*., 2017), Bangladesh (Uddin *et al.*, 2019) and Nepal (Bantawa *et al*., 2018; Khanal & Poudel, 2017). One major reason for this poor operational hygiene is the willingness of consumers with low to medium incomes to buy these meats despite their sanitary status. These customers appear to prefer low-cost fresh meat over expensive branded frozen meat. As a result, retailers believe that it is unnecessary to invest in additional labour and money to upgrade their outlets because it has no effect on demand. Other possible explanations include lack of resources, lack of awareness on about foodborne pathogens, and false sense of security about the cooking method etc., and so on. Therefore, identifying these issues and enacting the necessary regulations to ensure food safety is critical at this time (Kurugala, 2022)

According to the current study, *E. coli* contamination of chicken meat sold in these stores has decreased significantly over the past 10 years. However, because the sample size in the second investigation is smaller than in the first, it should be interpreted with caution. According to recent research, *E. coli* contamination of chicken meat was 4.8% in Nepal (Shrestha *et al.*, 2017), 43.5% in Pakistan (Zainab *et al*., 2022), 85% in Bangladesh (Islam *et al.*, 2018), and 91% in India (Hussain *et al.*, 2017). *E. coli* is a marker of faecal contamination (Ekici & Dümen, 2019), and therefore cross contamination during evisceration or handling, or due to fly sitting, may have contributed to the high *E. coli* contamination. *E. coli* detection in meat is of little value, and most food standards recommend enumeration of *E. coli* to ensure that the numbers are within acceptable limits. However, contamination of chicken meat with AMR/MDR *E. coli* or *E. coli* pathotypes (diarrheogenic *E. coli*, ExPEC or APEC that becomes ExPEC later in life) has always been a serious concern (Díaz-Jiménez *et al*., 2021; Rahman *et al*., 2020; Seo & Lee, 2018).

In both studies reported here, *E. coli* isolates had a higher level of AMR than *Salmonella* spp. isolates. Tetracycline resistance has increased significantly over the years, and in both studies, more than 60% of the isolates were ampicillin resistant. Differences in the antimicrobial panel used in the two studies made a fair comparison difficult, however the observed trends are consistent with a previous study conducted in Sri Lanka (Kulasooriya *et al*., 2019). Even studies from other South Asian regions report markedly high resistance in *E. coli* for tetracycline (Hussain *et al*., 2017; Islam *et al.*, 2018; Zainab *et al*., 2022) and ampicillin (Islam *et al.*, 2018; Shrestha *et al*., 2017). The first study disclosed 77.8 % MDR *E. coli* isolates as contaminants, while the second study revealed it as 26.9%. This significant reduction in MDR could be attributed to the difference of the antimicrobial disks used in each study. If the same antimicrobial panel was tested in each of these studies, the results could have been different. Despite the fact that the criterion for defining MDR has been different in this study, 100% MDR *E. coli* have been found in recent investigations in Bangladesh (Parvin *et al*., 2020) and Pakistan (Zainab *et al*., 2022). Further, the most recent percentages of MDR *E. coli* in contaminated chicken meat in India and Nepal have been 80% and 68%, respectively (Hussain *et al.*, 2017; Shrestha *et al*., 2017). This highlights the potential risk of acquiring MDR *E. coli* from chicken meat. Therefore, urgent action is required, particularly in developing countries, to reduce contamination and acquisition of foodborne MDR *E. coli* via chicken meat.

The first study found 47.2% *Salmonella* spp. contamination in chicken meat, while the second study found a statistically insignificant increase (59.45%) in the *Salmonella* spp. contamination. Both studies showed higher *Salmonella* spp. contamination than values previously reported in Sri Lanka (8.9% and 11.6%) Jayaweera *et al.* (2020). According to Jayaweera *et al.* (2021), the majority of these contaminated chicken meats harbour a number of virulence related pathogenicity islands. Given that the prevalence of *Salmonella* spp. in broiler birds in Sri Lanka is 9% (Kottawatta *et al*., 2014), spilled gut content may not be the only source of contamination. According to Alwis *et al*. (2014), meat contact surfaces and utensils in Sri Lankan retail shops are highly contaminated with *Salmonella* spp.. Because unsanitary conditions promote *Salmonella* spp. colonization and biofilm formation (Merino *et al*., 2019), not only careless evisceration but also operational hygiene appear to have a significant impact on *Salmonella* contamination of meat. *Salmonella* contamination in chicken meat has been reported to be 70%, 40%, 33%, and 26.2% in Bangladesh (Islam *et al.*, 2018), India (Sharma *et al*., 2019), Pakistan (Samad *et al*., 2018), and Nepal (Shrestha *et al*., 2017), respectively.

Salmonella spp. isolates recovered from both studies were susceptible to the majority of antimicrobials tested, which is consistent with Jayaweera *et al.,* (2020). However, 58% of the *Salmonella* spp. in the first study were resistant to neomycin, while 54% of the *Salmonella* spp. in the second study were resistant to tetracycline. According to recent regional reports, tetracycline resistance among *Salmonella* spp. isolates recovered from chicken meat is 85.7% in Bangladesh and 100% in India (Islam *et al.*, 2018; Sharma *et al*., 2019). *Salmonella* spp. isolates with MDR profiles were 14.7% in 2010-2011 and 4.5% in 2020-2021 studies, respectively. These MDR *Salmonella* spp. percentages are lower than the MDR *Salmonella* spp. percentages isolated from chicken meat in Nepal (85.2%) and India (100%) (Sharma *et al*., 2019; Shrestha *et al*., 2017). Nonetheless, it should be noted that the criteria used to define MDR differ among studies.

The AMR *E. coli* and *Salmonella* spp. isolates from these two studies did not show any prominent spatial clustering. The spatial variation in AMR profiles is attributed to the level of antimicrobials used within a region, the number of farms located in each region, the degree of environmental contamination with resistant bacteria, the extent of husbandry/other practices favouring resistance dissemination and socio-economic factors, etc. (Galvin *et al*., 2013).

The findings of this study underscore the significant risk of harvest and post-harvest cross-contamination of chicken meat sold at small-scale farm shops in Kandy district, as well as the persistent risk of consumers being exposed to AMR *E. coli* and *Salmonella* spp.. This situation highlights the urgent need for interventions to limit the emergence and spread of MDR bacteria in animal-origin food products in Sri Lanka. Establishing a national surveillance system to monitor antimicrobial usage in food animals and detect emerging antimicrobial resistance in bacteria from these animals is crucial. Additionally, promoting antimicrobial stewardship among veterinarians is essential.

It is also important to mandate the registration of meat retail shops with relevant authorities to ensure traceability in the event of a FI outbreak. Implementing a regular quality monitoring and grading system for meat retail shops is necessary. Moreover, awareness programmes targeting both retailers and consumers should be conducted to reduce cross-contamination and the spread of AMR foodborne pathogens.

CONCLUSION

Chicken meat sold in small-scale shops in Kandy district is frequently contaminated with *E. coli* and *Salmonella* spp.. Although *E. coli* contamination has decreased over the past decade, *Salmonella* spp. colonization in meat has increased. Antimicrobial resistance and multidrug resistance are prevalent among *E. coli* isolates contaminating chicken meat, with a higher frequency compared to *Salmonella* spp.. Resistance to tetracycline is commonly observed in both *E. coli* and *Salmonella* spp. found in chicken meat. These findings highlight the urgent need to implement measures to reduce bacterial contamination in chicken meat and to address the rise of antimicrobial resistance in farming environments.

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