Persistence of Salmonella and Campylobacter in Chicken Meat under the Different Chlorination Techniques in Poultry Processing Plants in Sri Lanka

Gayani Weerasooriya * , H.M.T Dulakshi , P.S de Alwis , Sandun Bandara , K.R.P.S Premarathne , Nayanajith Dissanayake , N. Liyanagunawardena , M.I Wijemuni , M.A.R Priyantha

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Article

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Gayani Weerasooriya *, H.M.T. Dulakshi, P.S. de Alwis, Sandun Bandara, K.R.P.S. Premarathne, Nayanajith Dissanayake, N. Liyanagunawardena, M.I. Wijemuni and M.A.R Priyantha

Bacteriology division, Veterinary Research Institute, P.O. Box 28, Peradeniya Sri Lanka
* Correspondence: gayaniw13@gmail.com

Abstract: The persistence of *Salmonella* and *Campylobacter* in chicken meat is a considerable public health risk and a future challenge. This study aimed to determine the prevalence of *Salmonella* and *Campylobacter* in poultry processing lines where different chlorination techniques were used. The samples were collected from four types of processing plants considering the chlorine concentration used in the chill tank, which ranged from 2 ppm to 50 ppm. *Salmonella* and *Campylobacter* were isolated from carcass washings, neck skin, and, caecal samples. Subsequently, an antimicrobial susceptibility test was performed for the isolates. The results revealed the overall prevalence of *Salmonella* and *Campylobacter* was 78.25%, and 64% respectively. Positive percentages of *Salmonella* and *Campylobacter* were high in carcasses compared to neck skin and caeca. Carcass *Campylobacter* counts at higher chlorine concentrations ranging from 20 – 50 ppm was significantly low ($p<0.001$). The highest resistance was observed for tetracycline (63.8%) in *Salmonella*, while it was for gentamicin (87.8%) in *Campylobacter*. The prevalence of multi-drug resistant percentage was higher in *Campylobacter* (46.3%) compared to *Salmonella* (4.25%). Despite the chemical decontamination, the persistence of *Salmonella* and *Campylobacter* with a higher prevalence of multidrug-resistant *Campylobacter* on post-spin chill carcasses is a significant public health threat that has to be addressed urgently.

Keywords: *Campylobacter; Salmonella*; chlorine; chicken meat; MDR

1. Introduction

Foodborne infections are a major public health issue with a significant socioeconomic impact on the globe [1]. World Health Organization (WHO) has reported that contaminated food resulted in 600 million cases of illness and 420,000 deaths worldwide [2]. *Campylobacter* and *Salmonella* species are identified as leading causes of acute bacterial gastroenteritis in humans [3]. According to the Food Net Surveillance Data System in the United States reported that 34.6% and 37.9% of observed bacterial gastroenteritis cases were caused by *Campylobacter* and *Salmonella spp* respectively [4]. As a result of colonization in the chicken gut, consumption of raw or undercooked chicken meat and by-products may cause infections in humans [5,6] Although possible interventions implemented and practiced in the poultry industry to limit the concept of 'hygienic farm to fork' practices, a significant portion of chicken meat were found as contaminate with foodborne pathogens. To strengthen Good Management Practises (GMP) in the production chain, the USDA Food Safety and Inspection Service (FSIS) has introduced performance standards; Hazard Analysis, and Critical Control Point (HACCP), for *Campylobacter* and *Salmonella* safeguarding public health by ensuring food safety throughout the food chain [7]. According to the surveillance data, depending on geographical location, the chicken meat contamination percentages varied between 10 – 100% and the post-production poultry meat products were contaminated with *Campylobacte*r (Suzuki and Yamamoto, 2009). In Australia, the presence of *Campylobacter* and *Salmonella* on raw chicken meat was estimated at 84.3% and 22.1%,

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respectively [3], while *Salmonella* recovery percentages in chicken meat were reported as 35% to 50% in Asia with limited information is found for *Campylobacter* [8,9].

Chicken meat is the most consumed meat commodity in Sri Lanka [10], The meat industry still plays an important role in the livestock sub-sector of Sri Lanka and chicken meat contributes about 70% and chicken is the only meat exported [10], The per capita availability of chicken meat is 10.3kg in 2021[11], The poultry meat production in 2020 was reported as 216160 MT and chicken meat-based value-added production was 11,220.40 MT in the year 2020 [12]. Notably, Sri Lanka opened the export market for poultry meat in 2020, and 128.58 MT was exported to Middle East and South Asian countries [12]. In Sri Lanka, broiler meat is mostly produced in large-scale or semi-automated processing plants with 20,000 -30,000 birds/day processing capacity, where operating under GMP, and HACCP standard methods and certification. Using chlorinated water in the inside-outside carcass washers and the spin chill tank are the most common chemical decontamination practices implemented to mitigate bacterial contamination during processing in Sri Lanka. However, the prevalence of food-borne pathogens in chicken meat is considerably high in Sri Lanka according to the few studies done [13]. Although the exact reason is not known differences in chemical decontamination techniques in the chill tank and high bacterial load entering into the processing line may be identified as possible reasons in the country [14]. In contrast, the reduction of human infection with high heat, longer cooking time, and using high concentrations of spices in Sri Lankan traditional cooking practices may be a reason to reduce the of human infections, misdiagnosis, and underreporting could be the main reason for documented fewer outbreaks compared to developed countries. Notably, due to the drastic changes in lifestyle and cooking patterns in society, during the last few decades, the risk of consuming contaminated pre-cooked products has increased. Further, the increased tourism and chicken meat exportation in Sri Lanka have emphasized the importance of quality poultry meat production. Therefore it is required to understand the level of food-borne pathogen contamination in chicken meat in Sri Lanka. Although prevalence studies have been done in the retail market and a few selected processing plants [13,15], there is no evidence of comprehensive studies done including the majority of large-scale processing plants in Sri Lanka. The first objective of this study was to determine the prevalence of *Salmonella* and *Campylobacter* in the poultry processing line.

Campylobacter infection can result in a systemic disease requiring the use of antimicrobials [16]. Erythromycin is considered the first-line treatment, and fluoroquinolones are also frequently used due to their broad-spectrum activity against enteric pathogens [17]. Recently, multidrug-resistant *Campylobacter* strains have been detected in poultry and several other sources of meat in the world [18–20]. Although the antimicrobial resistance of *Campylobacter* has been reported previously in the limited studies [13], a comprehensive study has not been done yet, in Sri Lanka. In addition, the antimicrobial resistance and MDR in *Salmonella* are well studied, the antimicrobial resistance of *Salmonella* and *Campylobacter* chicken meat isolates for the common antimicrobials was not revealed in the same study. Further *Salmonella* and *Campylobacter* can be colonized in the intestine together, it is important to understand the antimicrobial resistant pattern in the two organisms. Therefore the second objective of the study was to determine the prevalence of *Salmonella* and *Campylobacter* in large-scale processing plants operating with different chemical decontamination methods and the antimicrobial susceptibility of *Campylobacter* and *Salmonella* isolated from chicken meat.

2. Materials and Methods

2.1. Experimental Design

Ten large-scale processing plants were selected through a survey, where the processing capacity was between 15,000 to 30,000 birds per day. All the processing plants except one used chlorine as the sanitizer to wash the carcass in the spin chiller. According to the different chlorine concentrations used in the chill tank, three different types of processing plants were identified (A, B, C). Type A; Chlorine at 3-5 ppm (n=3), Type B; Chlorine at 20-30 ppm (n=3), and Type Chlorine at 40-50 ppm (n=3). Additionally, Type D; Other (Megabite Sulphate) 200 ppm (n=1). All the processing plants

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except in Type D, used chlorinated portable water (2-3 ppm) in carcass washing at the evisceration. Caecal samples, Neck skin samples, and whole carcass washing were collected from three different processing locations to isolate *Salmonella* and *Campylobacter*. Caecal samples were collected at the evisceration section before reaching the inside-outside carcass washer, the neck skin samples were collected after the inside-outside carcass washer, and the whole carcass rinse was collected at the post-spin chill stage. Chlorine concentrations and the weight of the carcasses were recorded for each sampling step.

2.2. Enumeration of Salmonella and Campylobacter

Individual carcasses from the post-pin chill were placed in 15in X 12in sterile polythene bags and washed with massaging for 2 min in 200 mL of sterile distilled water. The rinse was collected into 50 mL of sterile microcentrifuge tube. The average weight was recorded for all 10 carcasses. Ten neck skin samples were collected from the evisceration line after the inside outside carcass washing and placed individually in sterile 5in X 6in polythene bags. Subsequently, 10 caecal samples were collected and placed in the sterile bags separately. All the samples were stored in ice until transport to the laboratory.

Salmonella and *Campylobacter* were isolated according to the method described by Chousalkar et al., 2019 and ISO 6579-1:2017 guidelines [21,22]. Two hundred micro litter of the distilled water wash were spread plated onto a modified charcoal-cefoperazone-deoxycholate agar (mCCDA) (Thermo Scientific, Oxoid) plates and incubated at 42 °C with 10% CO_2 for 48 hours to assess direct *Campylobacter spp*. counts. From the initial 50 mL distilled water wash, 10 mL was added to 90 mL of 1% buffered peptone water (BPW; Thermo Scientific, Oxoid) incubated 18-20 hr at 37 °C. Ten grams of neck skin samples and 10g of caecal contents were homogenized in 90 mL of 1% buffered peptone water, 200µL of rinsate was spread plated onto mCCDA and the rest was incubated at 42 °C with 10% CO_2 for 48 hr to access direct *Campylobacter* counts. The rest of the rinsate was incubated 18-20 hr at 37 °C for *Salmonella* isolation. Ten grams of the caecal content was taken into the sterile polythene bag and 90 mL of 1% buffered peptone water was added and homogenized and streaked on mCCDA and incubated at 42 °C with 10% CO_2 for 48 hr, to detect the positivity of the *Campylobacter*. Subsequently, the rest of the rinsate was incubated for 18-20 hr at 37 °C for *Salmonella* isolation.

Then, 100 µL of the incubated BPW rinsates (Carcass wash, Neck skin, and Caeca) were transferred into 10 mL Rappaport Vassiliadis soya peptone broth (RVS, ThermoScientific, Oxoid) and incubated 18-20 hr at 42 °C for selective growth of *Salmonella enterica* serovars. A loop-full of the RVS broth was streaked onto xylose lysine deoxycholate agar (XLD; Thermo Scientific, Oxoid) plates. Suspected *Salmonella* colonies were subcultured onto Colombia sheep blood agar (Thermo Scientific Oxoid) and performed biochemical tests; TSI, Urease, Citrate, and SIM for confirmation. In *Campylobacter* confirmation gram stain smears were prepared to identify the specific spiral morphology of the bacteria. For *Campylobacter spp*. the limit of detection was 10 CFU/mL of rinsate.

2.3. Minimum inhibitory concentration for Campylobacter and Salmonella

As both *Salmonella* and *Campylobacter* can be colonized at the same time in the chicken gut, commonly used antimicrobials in animal surveillance studies for *Campylobacter* were selected for the *Campylobacter* MIC study and also to perform *Salmonella* MIC. Micro broth dilution test was performed on the MIC for four clinically relevant antibiotics (Sigma-Aldrich): gentamicin, ciprofloxacin nalidixic acid, and tetracycline as described by the Clinical and Laboratory Standards Institute (CLSI) and European Committee on Antimicrobial Susceptibility Testing (EUCAST) [21,22], with some modifications. Briefly, the inoculum was prepared from the overnight growth forty one (41) *Campylobcater* chicken meat isolates on sheep blood agar. The inoculum concentrations were prepared to 0.5 McFarland Standard by suspension in sterile distilled water. Ninety microliters (90 µL) of Nutrient Broth No 2 (Thermo Scientific Oxoid) was added into columns 2-12 of 96 well microtiter plates 180 µL of prepared antimicrobials were added into the first column and the two-fold dilutions were done to obtain, gentamicin (0.064–0.312 µg/ml), ciprofloxacin (0.076–16 µg/mL),

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nalidixic acid (0.125–256 µg/mL), and tetracycline (0.076–16µg/mL). Subsequently, 10µL of the prepared inoculum was added into all the wells and incubated at 42 °C with 10% CO_2 for 20 hr.

The same method was used to determine MIC for chicken meat isolate *Salmonella* for the same antimicrobial panel used for *Campylobacter*. Forty seven (n=47) *Salmonella* isolates from the whole carcass washing used in MIC assay only changing the antimicrobial concentrations; gentamicin (0.064–0.312 µg/mL), ciprofloxacin (0.076–16 µg/mL), nalidixic acid (0.125–256 µg/mL), and tetracycline (0.076–16µg/mL). The inoculums were prepared in the same method to 0.5 McFarland Standard by suspension in sterile distilled water, and 10µl of the prepared inoculum was added into all the wells except the growth control and incubated at 37 °C for 20 hr.

Plates were read against a dark background. The MIC was the lowest concentration of antimicrobials that inhibited bacterial growth. The strains were classified as susceptible or non-susceptible (including intermediate strains) according to the breakpoints described either in the CLSI standards or epidemiological MIC cut-off (ECOFF) values of EUCAST breakpoints [21,23]. ATCC 29213 *Staphylococcus aureus* was used as a quality control strain.

2.4. Statistical Analysis

The data obtained for the microbiological counts and the percentages were compared for significance differences ($p<0.05$) by using two tests (nonparametric) and One-way analysis of Variance (ANOVA) and the Fishers exact test, using GraphPad Prism 8 (San Diego, CA, USA). All the data were normally distributed

3. Results

This section may be divided by subheadings. It should provide a concise and precise description of the experimental results, their interpretation, as well as the experimental conclusions that can be drawn.

3.1. Enumeration of Campylobacter and Salmonella

In total 150 whole chicken carcass rinsate, 100 neck skin samples, and 100 caeca samples were collected. The overall prevalence of *Salmonella* was 78.25%, while *Campylobacter* was 64%. There was no significant difference between the positive percentages and sample types for both *Salmonella* and *Campylobacter* (Table 1).

Table 1. Prevalence of *Salmonella* and *Campylobacter* in poultry processing plants.

Sample type	Sample number	*Salmonella* Positive Percentage (%)	*Campylobacter* positive Percentage (%)
Carcass wash	150	80.66 (121/150)	68.66 (103/150)
Neck Skin	100	73 (73/100)	57 (57/100)
Caeca	100	79 (79/100)	63 (63/100)

Salmonella and *Campylobacter* positive percentages were separately calculated to understand the effect of different chlorination techniques on cross-contamination. The neck skin samples were collected after washing with potable water (2-3 ppm of chlorine) except plant type D (without chlorination), while the chicken carcasses were undergone chemical sanitization in the chill tank, either with chlorine (A, B,C,) or other sanitizers(D).

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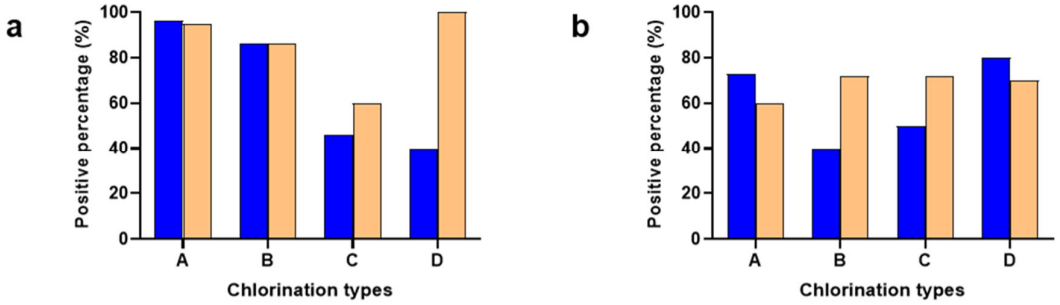

Figure 1. Positive percentages of Neck Skin (Blue) and Whole carcass (Orange) for *Salmonella* (a) and *Campylobacter* (b), in different chlorination types;3-5 ppm (A). 20-30 ppm (B), 40-50 ppm (C) and Megabite sulphate (D).

The carcass contamination was significantly higher compared to the neck skin in both *Salmonella* ($p<0.001$) and *Campylobacter* ($p< .0.05$) in the chill tank irrespective of the plant type, where different chlorination techniques were used. Especially Type D used Megabite sulphate in the chill tank and detected significantly higher ($p< 0.001$) *Salmonella* contamination (100%), compared to the neck skin samples (40%). A significant difference was not observed between the types of chlorination either in positive percentages of neck skin or whole carcasses.

Campylobacter count was determined to understand the effect of chemical decontamination in the chill tank (Figure 2). The *Campylobacter* counts in caecal samples were determined to understand the *Campylobacter* load entering the processing line and it was 1.2 $X10^{10}$ CFU/mL.

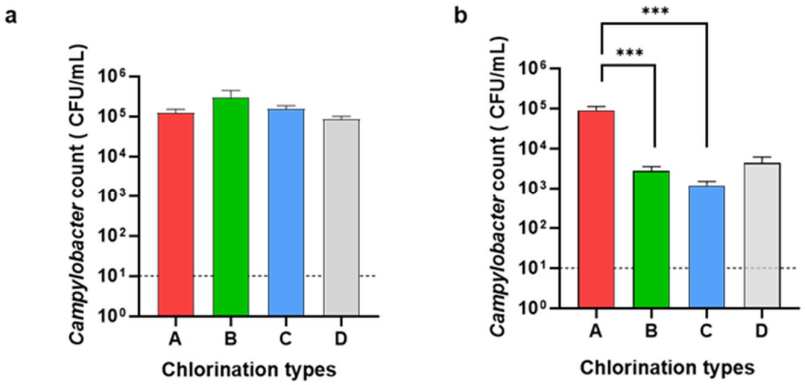

Figure 2. *Campylobacter* counts of Neck Skin (a) and Whole carcass (b) in different chlorination types;-5ppm (A). 20ppm (B), 40ppm (C), and Megabite sulphate (D). *= significant difference between treatment groups.

While the *Campylobacter* load on the neck skin was almost similar in all four types; A, B, C, and D, the *Campylobacter* load on the whole carcasses was different. A significant difference ($p<0.001$) was observed in the *Campylobacter* load in the carcasses when using 20-30ppm (2.8 $X10^3$ CFU/mL) and 40 -50ppm (1.3$X10^3$ CFU/mL) of chlorine compared to using 3-5ppm (9 $X10^4$ CFU/mL) of chlorine in the chill tank. There was no significant difference between the counts on the whole carcass when using either 20-30 ppm or 40-50 ppm of chlorine in the chill tank. Although the usage of Megabite sulfate reduced the level of *Campylobacter* (4.3$X10^3$ CFU/mL), it was not significant. When comparing the *Campylobacter* reduction in the whole carcasses in the chill tank compared to neck skin, an average 2 log reduction was observed, where 20-30 ppm and 40-50 ppm chlorine were used in the chill tank.

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In Type D (Megabite sulphate) only 1 log reduction was observed in whole carcasses compared to neck skin.

3.2. Minimum Inhibitory Concentration for Campylobacter and Salmonella

Micro broth dilution test was done to understand the antimicrobial resistance of Campylobacter and Salmonella. Four antimicrobials; gentamicin, ciprofloxacin, nalidixic acid, and tetracycline of four different types of antimicrobials, which are commonly used in the poultry industry and can be used in AMR surveillance for both Campylobacter and Salmonella, were used for the MIC test. The antimicrobial resistance profile and MIC results are described in Table 2.

Table 2. Minimum Inhibitory concentration and Antimicrobial susceptibility of Campylobacter .

ANTIMICROBIAL AGENT	MINIMUM INHIBITORY CONCENTRATION (MIC) n (%)															RESISTANT %
	0.076	0.312	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	124	256	
GEN*		0	0	0	0	1 (2.4)	3 (7.3)	1 (2.4)	1 (2.4)	1 (2.4)	4 (9.8)	30 (73.2)				87.8
CIP	2 (4.9)	1 (2.4)	1 (2.40)	1 (2.4)	1 (2.4)	3 (7.3)	1 (2.4)	3 (7.3)	6 (14.6)	8 (19.5)	14 (34.1)					68.3
NAL				0	0	0 (2.4)	1 (2.4)	1 (2.4)	2 (4.9)	3 (7.3)	4 (9.8)	27 (65.9)	2 (4.9)	1 (2.4)	0	7.31
TET	0	0	0	0	3 (7.3)	1 (2.4)	0 0	2 (4.9)	4 (9.8)	15 (36.6)	16 (39)					39

Gentamicin (GEN), Ciprofloxacin (CIP), Nalidixic acid (NAL), and Tetracycline (TET). Reference values are based on *Campylobcater jejuni/coli* breakpoints from CLSIM45,3rd Ed. * Reference values are based on *Campylobacter jejuni/coli* epidemiological MIC cut-off (ECOFF) values (EUCAST breakpoints (https://mic.eucast.org/). Continuous lines indicate CLSI/CAST breakpoints. Shaded areas indicate the tested concentrations.

In *Campylobacter*, according to the MIC results the highest resistant percentages were observed for gentamicin and ciprofloxacin which were 87.8% and 68.3% respectively. The lowest resistance was observed in nalidixic acid (7.31%). Only two isolates were susceptible to all types of antimicrobials used in the study. Out of 41 isolates, 19 isolates (46.3%) were detected as resistant to three types of antimicrobials (Figure 4). There out of 46.3%, 43.9 % showed multidrug resistant by being resistant to gentamicin, ciprofloxacin, and, tetracycline. Only 1 isolate (2.42%) was resistant to all four types of antimicrobials.

Table 2. Minimum Inhibitory concentration and Antimicrobial susceptibility of Salmonella .

ANTIMICROBIAL AGENT	MINIMUM INHIBITORY CONCENTRATION (MIC) N (%)														RESISTANT %
	0.063	0.125	0.25	0.5	1	2	4	8	16	32	64	128	256	512 1028	
GEN*		0	4 (8.5)	7 (14.8)	14 (29.7)	6 (12.7)	9 (19.1)	3 (2.4)	3 (6.3)			2 (4.2)			8.5
CIP		27 (57.4)	6 (12.7)	7 (14.8)	2 (4.2)	3 (2.4)	0	0	0	2 (4.2)	0				14.9
NAL		0	0	0	0	6	4	14	6	7	7	3			

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					(12.7)	(8.5)	(29.7)	(12.7)	(14.8)	(14.8)	(6.3)		36.2
TET		0	2	2	1	6	6	16	13	0	0	1	
				(4.2)	(2.1)	(12.7)	(12.7)	(34)	(27.6)			(2.1)	63.8

Gentamicin (GEN), Ciprofloxacin (CIP), Nalidixic acid (NAL), and Tetracycline (TET). Reference values are based on Enterobacterales breakpoints from CLSIM100, 33rd Ed.

In *Salmonella*, the highest resistance was observed for tetracycline (63.8%) and nalidixic acid (36.2 %).The lowest resistance was observed in gentamycin (8.5%). Out of 47 isolates, only one (2.12%) isolate was resistant for all four types of antimicrobials, while two isolates (4.25%) were resistant to three types of antimicrobials (Gentamicin, Ciprofloxacin , Nalidixic acid). The MDR of *Salmonella* for the selected antimicrobials in the present study was 4.25%.

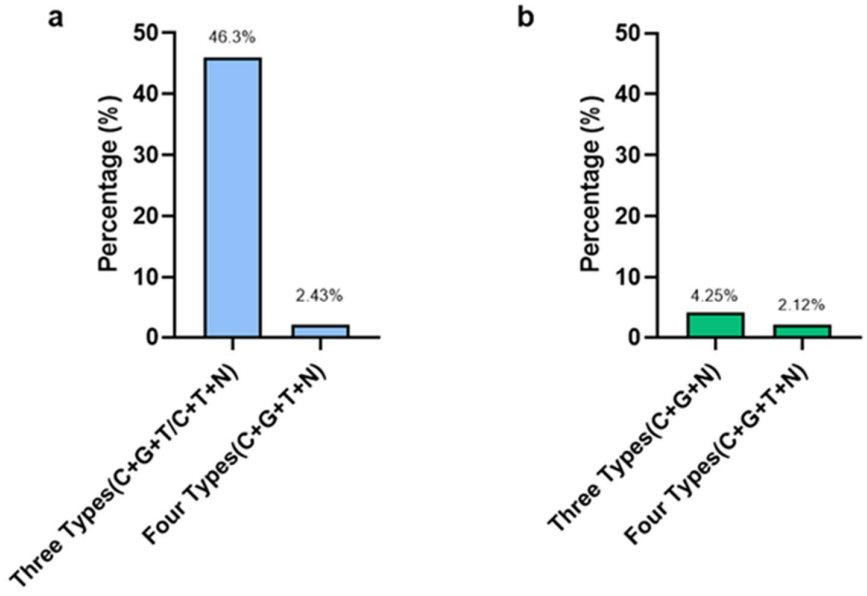

Figure 3. Prevalence of multidrug-resistant *Campylobacter (a) and Salmonella (b)* in whole chicken carcasses. Gentamicin (G), Ciprofloxacin (C), Nalidixic acid (N), and Tetracycline (T).

In the present study, *Campylobacter* showed higher resistance to gentamicin and ciprofloxacin than *Salmonella*. And *Salmonella* showed higher resistance for nalidixic acid and tetracycline than *Campylobacter*. The resistant percentage for three types of antimicrobials for the selected antimicrobials was ten times higher in *Campylobacter* (46.3%) compared to *Salmonella* (4.25%).

4. Discussion

Consumption of chicken meat contaminated with food-borne pathogens such as *Salmonella* and *Campylobacter* remains a significant public health risk worldwide. Despite the many interventions implemented in the processing plants to minimize the bacterial load, the persistence of foodborne pathogens in chicken meat is a continuous burden in the industry. Although the European Union banned to use of chlorine in poultry processing [26] as a bacterial reduction method, chlorinated water both in carcass washing and in post-spin chillers I Sri Lanka and many other countries in the world [21,27]. Due to the variation in chorine concentrations used in the chill tanks, in the poultry processing plants, the risk of the persistence of foodborne pathogens in the production chain may be significant. In the present study, mainly three types of poultry processing establishments were

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identified in which a range from 2 ppm to 50 ppm chlorine concentrations were utilized. While one processing plant used megabyte sulphate instead of chlorine. As this is the first comprehensive study done in Sri Lanka to determine the *Salmonella* and *Campylobacter* contamination in the poultry processing line, while determining the prevalence of food-borne pathogens, we tried to understand the differences in bacterial contamination levels in different setups.

The overall prevalence of *Salmonella* was 78.5%, while that of *Campylobacter* was 64%. The present prevalence levels of both pathogens are significantly higher than the studies done earlier in Sri Lanka [15]. Due to the financial crisis followed by COVID-19 in Sri Lanka the increased production cost, specifically for feed materials, affected badly in the poultry industry, hence financial allocation for the improvement of the infrastructure and the biosecurity facilities were limited. This can be the main reason for the observed higher prevalence of *Salmonella* and *Campylobacter* in the present study. Further, the results stand with a study done to understand the weather correlation in *Campylobacter* prevalence in Sri Lanka [28]. Notably, *Salmonella* contamination was significantly higher ($p<0.05$) compared to *Campylobacter* positive percentages in caeca, neck skin, and whole carcass. This could be due to the difference in colonization and their stress response mechanisms and the survivability of *Salmonella* is greater than *Campylobacter* even after the chlorine treatment [29,57]. Studies have shown that the persistence of *Salmonella* in chicken meat is higher compared to *Campylobacter* [30]. Not like *Salmonella, Campylobacter* is a very fastidious organism that can't survive in the environment easily [31]. The prevalence of *Campylobacter* in caeca was 63% which is very similar to a previous study conducted in broiler flocks which, revealed a 67.6% positive percentage in caecal contents [13]. However, the overall prevalence of *Salmonella* is lower than the previous study done in Malaysia [32], while *Salmonella* prevalence in cecal samples was significantly higher in the present study compared to the study done in India [33]. This could be due to high infection pressure and the differences in isolation techniques.

Inside-outside carcass washers are used to minimize bacterial contamination at the end of the evisceration process. All the processing plants selected in the present study used portable water with 2-3 ppm chlorine, except type D, where used water without chlorination. The results revealed that even after the washing, the *campylobacter* and *Salmonella c*ontamination rates of neck skin samples was higher than the previous studies reported as 27.4% *Campylobcater* contamination in semi-automated poultry processing plants [13] and 21.4 % *Salmonella* positive percentage respectively [34]. Although exact reason is not known, increased bacterial load entering into the line and differences in evisceration techniques practiced in different countries may be identified as possible explanation for high prevalence of foodborne pathogen. Majority of the processing plants in Sri Lanka, use the manual evisceration technique, while few of the plants use automated evisceration. The increased carcass contamination could be a result of viscera rupture during evisceration, inevitably leading to the contamination of equipment, working surfaces, and water, hence facilitates the cross-contamination of either *Campylobacter* or *Salmonella* free carcasses during processing [35].The high contamination rate of neck skin in the present study highlighted the inefficiency of using carcass washers to reduce cross-contamination [36]. Although the inside-outside carcass washers remove fecal materials and tissue debris, it has been shown the limited effectiveness in reducing bacterial levels in poultry carcasses [37]. After inside-outside carcass washers, the carcasses enter the chill tank where the bacterial load is reduced by washing, chemical decontamination, and chilling. The whole carcass contamination with *Salmonella* and *Campylobacter* in the present study was 80.66 % and 68.66% respectively, which was significantly higher than the in a previous study conducted in Sri Lanka, reported as 10% and 32% respectively [15]. This can be due to the increased colonization in broiler flocks and also the difference in sample size and isolation techniques. Our finding stands with a study conducted using chicken meat from retail shops where a 59% prevalence in *Campylobacter* was observed [13]. A positive correlation has been observed between the contamination of carcasses and the high positivity rates for *Campylobacter* of flocks at the farm level [38]. Therefore the farm intervention to reduce colonization is also very important in reducing *Campylobacter* chicken meat contamination [38]. The detected high *Campylobacter* cecal colonization ($1.2 X10^{10}$ CFU/mL) in the present study ensured the entering of high loads of *Campylobacter* into the processing line, which can

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increase the risk of carcass contamination [39,40]. Furthermore, previous studies demonstrate that even though the flock prevalence of *Salmonella* is low, the cross-contamination during processing leaves the plant with significantly higher numbers of contaminated carcasses [41] *Salmonella* contaminated carcasses cross-contaminate uncontaminated carcasses when they are chilled and rinsed in spill chiller. [42]. The percentages of whole carcass contaminations were significantly higher compared to the neck skin in both *Salmonella* ($p<0.001$) and *Campylobacter* ($p< .0.05$) irrespective of the chlorine concentrations used in the chill tanks. This can be due to cross-contamination of the chill tank water by entering contaminated carcasses into the chill tank. These findings were supported by the fact that the number of positive carcasses increased significantly ($P < 0.05$) after evisceration [43]. Even after using 20 ppm or 40 ppm of chlorine in the chill tank, *Salmonella*-contaminated carcasses percentage was not reduced. This can be due to increased organic matter contents, (such as residual fecal material, blood, skin, or feathers) which reduces the availability of free chlorine in the solution [44]. A previous study has shown that there is no significant difference in using either water or chlorine in a chill tank to reduce either *Campylobacter* or *Salmonella* [45]. Interestingly, *Salmonella* positivity in whole carcasses (100%) was significantly higher ($p<0.05$), compared to the neck skin samples (40%), where Megabite sulphate was used in the chill tank. This finding suggests the lower effectiveness of Megabite sulphate in reducing the bacterial level. The effect of a disinfectant always depends on the type of active ingredient, concentration, and time of exposure [45] . Anyway, further studies have to be done to understand the effect of Megabite sulphate in reducing the bacterial load in the carcass. Similarly, the carcass contamination with *Campylobacter* was also high either 20-30 ppm or 40-50ppm of chlorine used in the chill tank and it was 72% in both setups. Although the European Union has banned the use of chlorine in food processing, WHO has recommended 50-70 ppm with 0.4-4 ppm free available chlorine (FAC) for use in chiller water. The finding of the present highlights, that even with the 50 ppm chlorine the prevalence of *Campylobacter* is significant. A laboratory experiment showed that *Campylobacter* needed at least 128 ppm of chlorine for inactivation. Although chlorine is considered the fast oxidative agent which damages both cell membrane and the cytoplasm [47], the lower efficacy has been reported compared to other sanitizers [44]. Further the adaptive stress response mechanism of *Campylobacter*, enhances the survivability under chemical stress [48].In the present study, we tried to understand the reduction of *Campylobacter* load in the whole carcasses by using sanitizers in carcass washing. *Campylobacter* load in the neck skin was almost similar in all four types of processing establishments ; A, B, C, and D with an average of 10^5 CFU/mL, which could be due to the same intervention of carcass washing; using portable water for inside –outside carcass washers. As expected *Campylobacter* load was significantly low ($p<0.001$) in the whole carcass, where 20 ppm (2.8×10^3 CFU/mL) and 40 ppm (1.3×10^3 CFU/mL) chlorine was used compared to 3-5 ppm chlorine in the chill tank. *Campylobacter* needs higher chlorine concentrations to inactivate [49].Further, an average 2 log reduction of *Campylobacter* count was observed in the whole carcass compared to the neck skin after the chemical decontamination of the carcasses in the chill tank. A risk assessment study has shown, that by reducing *Campylobacter* load on raw poultry by 2 log units, human campylobacteriosis could be reduced by 30 folds [43,50]. Reduction of *Campylobacter* load in chicken meat is very important as the *Campylobacter* infection dose is very low and ingestion of 500 - 1000 cells can cause human infection [14].Anyway the sub-lethal injury and formation of viable but non-culturable (VBNC) form of *Campylobacter* in exposure to sanitizer, could reduce detectable count while the persistence in the food chain is significant [48,49,57]. Importantly, expression of virulence gens in sub-lethally injured *Salmonella* and *Campylobacter* in exposure to chlorine have been demonstrated in a previous study [49]. Therefore entering of these foodborne pathogens into the food chain are a public health risk.

Due to the high prevalence of *Campylobacter* and *Salmonella* contamination in chill chicken carcasses, it is timely important to understand the antimicrobial susceptibility of isolates. Due to the high possibility of getting co-infection of *Campylobacter* and *Salmonella,* development of resistance to common antimicrobials used in both infections is a risk. Very few studies have been conducted to determine the AMR status in *Campylobacter*, and comprehensive data were not available in Sri Lanka. The results of the preset study revealed that gentamicin has the highest level of resistance (which was

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87.8%), which was higher than in the previous studies reported for gentamicin 10% [13]. The ciprofloxacin resistance was 68.3% and it was lower than the previously reported 80%. This would be very important information to consider in human campylobacteriosis treatments in Si Lanka. Fluoro quinolones are considered the second-line treatment against human campylobacteriosis [17]. Therefore the risk in effectiveness in antimicrobial treatments in future. The observed resistance to ciprofloxacin can be due to the tremendous use of enrofloxacin in the poultry industry as ciprofloxacin is structurally related to enrofloxacin and it shares the same resistant mechanism [51]. Interestingly the observed resistance to nalidixic acid was very low (7.31%) compared to the previous study which was reported as 80% [13]. A study conducted in Brazil revealed that 90.7% and 81.5% of the strains, respectively, were resistant to both ciprofloxacin and nalidixic acid [52]. Again the tetracycline resistance percentage; 39% was lower than the previously reported. This can be due to the strain variation, sample number, and differences in antimicrobial usage. Therefore, patterns and practices of antimicrobial usage in food animals can determine the development of antimicrobial resistance in foodborne pathogens such as *Campylobacter*. As human campylobacteriosis is highly travel associated infection, travellers to Asia have been shown to carry resistant *Campylobacter* reflecting the above situation [53].

Interestingly, only two isolates were susceptible to all kinds of antimicrobials, which stands with the results of previous studies [52]. Importantly, from the *Campylobacter* isolates 43.9% were resistant to three types of antimicrobials; tetracycline, ciprofloxacin, and gentamycin. The present study showed the multi-drug resistant *Campylobacter* in the post spin chill carcasses of the processing plants, which was higher than the previous studies [52]. The persistence of MDR *Campylobacter* in poultry processing lines in Sri Lanka would be an alarming situation for future antimicrobial usage in both livestock and human medicine. Emerging resistance to the antimicrobial agents of choice for treating *Campylobacter* infections is becoming a serious threat to public health worldwide. From the Salmonella isolates in the present study, higher resistance was observed for tetracycline (63.8%) and Nalidixic acid (36.2 %). This can be due to the greater usage of tetracycline and quinolone drugs in the poultry industry in Sri Lanka. Similar results were observed in a previous study conducted using *Salmonella* [54]. Also, the resistant rates and the pattern are in agreement with a previous study in Iran, which revealed that the majority of the *Salmonella* isolates were resistant to nalidixic acid, tetracycline, and streptomycin [55]. The resistance patterns associated with this pathogen include important therapeutic antimicrobial classes used in human medicine, such as tetracycline and fluoroquinolones in this study and this association represents a public health concern. The observed higher AMR percentage in *Campylobacter* than *Salmonella* could be due to continuous exposure of *Campylobacter* population into the antimicrobial treatments than *Salmonella* by being a commensal in the chicken gut. And also due to induced antimicrobial resistance in *Campylobacter* in exposure to chlorine [56,57]. The present study emphasizes the importance of *Campylobacter* as a foodborne pathogen, which could be a more serious public health issue than *Salmonella* in the future.

5. Conclusions

The present study showed that, despite the chemical decontamination techniques, the persistence of *Salmonella* and *Campylobacter* in poultry processing lines is significant. This study highlighted the ineffectiveness of chlorine usage strategies in reducing the contamination, with the high pathogen load entering into the processing plants, in Sri Lanka. Considering the new lifestyles and changes in cooking patterns in Sri Lanka, the possibility of consuming partially cooked chicken is Therefore, it is important to introduce an effective chemical decontamination strategy to minimize cross-contamination. Emerging of multidrug-resistant *Campylobacter* and *Salmonella* be a future challenge in treating infections in humans. It is important to have 'farm-to-fork' effort to reduce *Salmonella* and *Campylobacter* contamination in chickens and control the development of antimicrobial resistance. Consumers should continue to be encouraged to follow proper food safety practices when handling raw chicken, including proper storage, handling, and cooking temperature. Further persistent of MDR *Campylobacter* and *Salmonella* and observed higher resistance to fluoroquinolones insists that treatment options for serious infections with these zoonotic bacteria can be reduced.

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Notably, Sri Lanka is an attractive tourist destination and the poultry industry is moving into the export market. Therefore, having a national food borne pathogens surveillance program and developing a national antimicrobial stewardship program to minimize the misuse of antimicrobials in the poultry industry is also essential.

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