Isolation and Identification of Salmonella spp. from Broiler Chicken Meat in Sri Lanka and their Antibiotic Resistance

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ABSTRACT

Purpose: Salmonella infections continue to be a global problem with millions of humans and animal cases occurring annually. Broiler chicken plays a significant role causing Salmonella infections in Sri Lanka. Consumption of food contaminated with antimicrobial resistant Salmonella aggravates the problem. This study isolated, identified, and serotype the Salmonella spp. from broiler chicken meat in Sri Lanka and examined their antimicrobial susceptibility to be used in establishment of control measures.

Research Method: Isolation of Salmonella species from broiler chicken meat was done by conventional method of isolation followed by polymerase chain reaction (PCR) confirmation. All PCR confirmed isolates of Salmonella were serotype and then, isolates were tested for antibiotic susceptibility using disc diffusion assay followed by the detection of antibiotic resistance genes using PCR.

Findings: Broiler chicken meat in Sri Lanka is contaminated with Salmonella spp. at the prevalence of 11.6% and 8.9% of them carried hns and invA specific genes. Isolates were serotyped as Salmonella Typhimurium (47.8%), Salmonella Enteritidis (26.1%) and non typable (26.1%). Three isolates were resistant to ampicillin. Intermediate resistance was shown to three antibiotics and all the isolates were sensitive to nine antibiotics. Majority (56.5%) of Salmonella were sensitive to all the tested antibiotics. Prevalence of resistant genes for tetracycline, sulfonamides and aminoglycosides were within 4%-26%. None of the isolates except one (4%) carried chloramphenicol resistance genes.

Originality / Value: Steps must be taken to minimize contamination of broiler chicken meat with Salmonella spp in Sri Lanka. Although, there is a low prevalence of antibiotic resistant isolates, its mere presence in broiler chicken is a warning signal of possibility of emergence of multidrug resistant strains.

Keywords: Salmonella, Isolation, PCR, Serotyping, Antibiotics, Sensitivity

INTRODUCTION

Salmonella is an important food borne pathogen which is the second most reason for the gastroenteritis after the Campylobacter spp. and distributed worldwide (Lamas et al., 2018). Nearly 100 million Salmonellosis cases have been reported annually worldwide, resulting 160,000 deaths every year. In 2015, around 100 000 confirmed cases of humans salmonellosis were reported in the European Union causing 126 deaths (Majowicz et al., 2010; EFSA, 2016). Aggravating the problem, consumption of food contaminated with a strain of Salmonella that is resistant to antimicrobials may lead to an infection in humans that cannot be successfully treated with antibacterial drugs (Kulasooriya et al., 2019). The major route of transmission of

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Salmonella from animals to humans is through contaminated food or foodstuffs such as eggs, egg products, poultry meat and dairy products (EFSA, 2015).

Salmonella is a facultative anaerobe belongs to the family Enterobacteriaceae, which consists of two species (S. enterica and S. bongori). It is reported that exceeding 2600 serovars of Salmonella are causing gastroenteritis in both human and animals (Bhowmick et al., 2011; Issenhuth-Jeanjean et al., 2014; EFSA 2015; Ryan et al., 2017). S. enterica is further subdivided into six subspecies, namely; subspp. enterica, salamae, arizonae, diarizonae, houtenae and indica. S. enterica subspecies enterica consist of more than 1500 clinically important serovars. Salmonella Typhimurium and Salmonella Enteritidis are major such two serovars and they have been isolated predominantly from poultry (Lamas et al., 2018).

Understanding the characteristics of different strains of Salmonella is important for assessment of prevalence, survival and risk to human health. In this regard, phenotypic characteristics such as serotyping have been used for epidemiological investigation of Salmonella. Multiple typing methods are available including phenotypic biotyping, serotyping, phage-typing, antibiotic susceptibility testing, mass spectrometry and other nucleic acid based molecular techniques that can discriminate microorganisms up to the strain level based on phenotypic traits (Bhowmick et al., 2012; Karatug et al., 2018). Since poultry and poultry products are one of the major routes of transmission of Salmonella to humans, it is important to identify and serotype Salmonella strains isolated from poultry meat for diagnosis, treatment and also for the epidemiological surveillance of salmonellosis (Turki et al., 2014).

The gastroenteritis occurred due to nontyphoidal Salmonella serovars is usually self-limiting. But, application of antimicrobial therapy is required when it invades the other parts of the body. Emergence of antimicrobial resistance among nontyphoidal Salmonella serovars to medically important antibiotics has been documented during the last two decades. However, the resistance varied among the serotypes and to different antibiotics. It is reported that S. enterica Typhimurium demonstrate advanced resistance to frequently used antibiotics than S. Enteritidis, among all the Salmonella serotypes (Barilli et al., 2018).

Due to lack of funding and technical facilities, there is poor understanding about the causes of food-borne infections such as Salmonellosis in Sri Lanka. However, according to DAPH, 2015, broiler chicken meat production as well as consumption in the country show a rapid increase during the past few years. It is also reported that the contribution of broiler chicken meat and egg production to the livestock sector of Sri Lanka is around 70%. Previous studies conducted in Sri Lanka have found contamination of poultry with Salmonella, Campylobacter and E. coli (Kamalika et al., 2008; Dissanayake et al., 2008; Kottawatta et al., 2017). Furthermore, resistance to commonly used antimicrobials was found in many of the bacterial isolates taken during these studies.

Lack of molecular level identification, not performing serotyping and lack of molecular level investigations on antibiotic resistance of Salmonella were the major precincts of the earlier studies. Thus this study was formulated to isolate, identify, serotype and to detect the vulnerability to commonly used antibiotics along with the detection of antibiotic resistance genes corresponding to phenotypic resistance in Salmonella spp. found in the broiler meat locally produced in Sri Lanka.

**MATERIALS AND METHODS**

Isolation of Salmonella species from broiler chicken meat was done by conventional method of isolation followed by polymerase chain reaction (PCR) confirmation.

**Collection of samples**

260 broiler chicken meat samples were randomly obtained from different locations of the country from 2012 August to 2013 August. All the samples were immediately stored in ice and
transported to the laboratory and analyzed as soon as possible following the collection. Laboratory investigations to isolate followed by identification of *Salmonella* was performed at the Livestock laboratory of the Faculty of Agricultural Sciences of the Sabaragamuwa University of Sri Lanka and molecular characterization of the isolates was performed at the Laboratory of UNESCO MIRCEN for Marine Biotechnology, Division of Infectious Biology, Nitte University Centre of Science Education and Research, Mangalore.

**Isolation of Salmonella by conventional method**

Conventional method recommended by FDA Bacteriological Analytical Manual was used to isolate *Salmonella* from broiler chicken meat samples (Andrews and Hammack, 2011). Briefly, 225 ml lactose broth was used to homogenize a 25g portion of the meat sample for 2 minutes using a stomacher (Bag mixer®400, Interscience International, France). This mixture was incubated for 24 hours at 37°C as the pre-enrichment step. Then 1 mL each of pre-enriched sample was added to 10 mL each of selinite cystine broth and tetrathionate broth (TTB), while 10 mL of Rappaport-Vassiliadis broth was used to add 0.1 mL of the pre-enriched sample. The inoculated selinite cystine and tetrathionate broths were incubated at 37°C for 24 hours, while Rappaport Vassiliadis broth was incubated at 43°C in a water bath for 24 hours. Loop full each from these broths was streaked on the selective media of Hektoen Enteric Agar and Bismuth sulphite Agar together with Xylose-Lysine-Desoxycholate agar and incubated at 37°C for 24 hours.

**Biochemical Identification**

Identification of *Salmonella* spp was performed through a series of biochemical tests using colonies grown in selective media. Minimum of five typical colonies from each of the selective agars were used for the study. Indole test, triple sugar iron test, Simmons citrate test, urease test, methyl red and Voges-Proskauer test (MR-VP) were used as biochemical tests. To preserve the biochemically confirmed isolates, 40% glycerol was used. Then the isolates were stored at -80°C to be used in further analyses.

**Confirmation of Isolates by PCR**

*Salmonella* isolates were further confirmed by PCR, targeting *Salmonella* specific invasion gene *invA* (284 bp) (Jones et al., 1993) and the gene encoding a DNA binding protein *hns* (152 bp) (Rahn et al., 1992). N-cetyl-N,N,N-trimethylammonium bromide (CTAB) method was used for DNA extraction (Ausubel et al., 1992). According to the protocol described by Bhowmick et al., (2011), a thermal cycler (BioRad, PTC-200, CA, USA) was used to carry out PCR reactions (Table 01). Agarose gel (2%) was used to resolve the PCR products and stained with ethidium bromide (0.5µg/ml) followed by photographed and analyzed using gel documentation system (Gel Doc™ EZ Gel Documentation System, BioRad, USA).

**Serotyping of Salmonella**

All PCR confirmed isolates (23) of *Salmonella* were serotyped at the Reference Centre for *Salmonella* and *E. coli* at the Central Research Institute, Himachal Pradesh, India.

**Detection of Antibiotic Sensitivity Using Disc Diffusion Method**

Following isolation and identification, *Salmonella* isolates were tested for antibiotic susceptibility by means of the disc diffusion assay as explained by Bauer et al., (1966). Antibiotic discs such as Nitrofurantoin (30 μg) (NIT), cefotaxime (300 μg) (CTX), nalidixic acid (30 μg) (NA), piperacillin (10/100) (PIT), chloramphenicol (30 μg) (C), co-trimoxazole (25 μg) (COT), ciprofloxacin (5 μg) (CIP), tetracycline (30 μg) (TE), meropenem (10 μg) (MRP), kanamycin (30 μg) (K) and Gentamycin (10 μg) (GEN) were used for the antibiogram test according to manufacturer’s (Indian HiMedia Laboratories Pvt Ltd) guidelines. In order to prepare a lawn, a *Salmonella* culture grown-up for 10-12 h in 5 ml Mueller-Hinton broth attuned to 0.5 McFarland (Indian HiMedia Laboratories Pvt Ltd) was poured on well-dried Mueller–Hinton agar (Indian HiMedia Laboratories Pvt Ltd).
The antibiotic discs were placed on the surface of the medium after gently air drying in a laminar flow and incubated for 16-18 hours at 37 °C until a clear zone is obtained and interpretation of the results were done as described by Clinical and Laboratory Standards Institute (CLSI), USA guidelines. As the control strain, *E. coli* ATCC 22592 was used.

Presence of antimicrobial resistance genes

In order to detect the genes responsible for antimicrobial resistance in the 23 isolates of *Salmonella*, specific genes ensuing resistance to common antibacterials were tested by PCR using their respective primers. The genes *tetA*, *tetB*, *tetC*, *tetD*, *tetE*, and *tetG* were used to detect the resistance for tetracyclines while *sul1*, *sul2*, and *sul3* were used for sulfonamides. Resistance to chloramphenicol was checked using *cat1*, *cat2*, *cmlA*, *cmlB* and *floR* genes and *aph (3)Ia, aac (3)Ia* and *aac6* were used for aminoglycosides. DNA extraction was carried out as described earlier following the protocol by Ausubel *et al.*, (1992). A thermal cycler (Bio-Rad, PTC-200, Hercules, CA) was used to carry-out reactions and PCR conditions and primer sequences were maintained as described in previous studies (Ma *et al.*, 2007) (Table 02).

| Gene | Gene description | Primer sequences (5’-3’) | Product size (bp) | Reference |
|------|------------------|--------------------------|------------------|-----------|
| Hns  | Histone like nucleoid structuring gene | F-TACCAAGCTAAACGCGCAGCT<br>R-TGATCAGGAATCTTCCAGTTGC | 152 | Jones *et al.*, 1993 |
| invA | Gene encoding the invasion-associated protein | F-GTGAATATCGCCACGTCCGGGCAA<br>R-TCATCGCACCCTCAAAGGAACC | 284 | Rahn *et al.*, 1992 |

The antibiotic discs were placed on the surface of the medium after gently air drying in a laminar flow and incubated for 16-18 hours at 37 °C until a clear zone is obtained and interpretation of the results were done as described by Clinical and Laboratory Standards Institute (CLSI), USA guidelines. As the control strain, *E. coli* ATCC 22592 was used.

Out of 260 broiler chicken meat samples, 30 isolates (11.6%) were identified as *Salmonella* by means of conventional methods. Out of that 30 isolates, 23 isolates (89%) were confirmed as *Salmonella* by PCR.

Though there is a paucity of literature on isolation of *Salmonella* from broiler chicken meat in Sri Lanka, few studies have shown *Salmonella* as a common organism isolated from different food commodities in the country. Kamalika *et al.*, (2008) found that the prevalence of *Salmonella* in captured shrimps and cultured shrimps in Sri Lanka was 14.4% and 11.1%, respectively. Ariyawansa *et al.*, (2016) investigated the quality of the fish in western province of Sri Lanka and revealed that 5.6% of the fish samples were contaminated with *Salmonella*. It indicates the higher prevalence of *Salmonella* spp. in broiler chicken compared to that of fish. Their study also showed that 50% of harbor basin water samples and 20% ice samples were heavily contaminated with *Salmonella* spp.

High prevalence (40.6%) of *Salmonella* in broiler chicken meat from vendor shops in Sri Lanka was discovered by Thilakaratne *et al.*, (2012). A higher presence of *Salmonella* has been found in broiler chicken meat than that was found in the present study. Using conventional method, Kulasooriya *et al.*, (2019) found that the contamination level with *Salmonella* spp. were 10% and 17% in chilled raw broiler chicken meat and frozen broiler chicken meat respectively. These findings are also in agreement with the results of the present study.

RESULTS AND DISCUSSION

Isolation, identification, confirmation and serotyping of *Salmonella* from broiler chicken meat samples

Isolation of *Salmonella* species from broiler chicken meat was done by conventional method of isolation followed by polymerase chain reaction (PCR) confirmation. Then the isolates were subjected to serotyping.
Though the prevalence of Salmonella in broiler chicken in Sri Lanka was found as 11.6% by conventional methods in the present study, higher prevalence have been reported in other countries, such as 36.5% in Belgium (Uyttendaele et al., 1999), 35.8% in Spain (Dominguez et al., 2002), 35.5% in Malaysia (Rusul et al., 1996), 34% in Turkey (Yildirim et al., 2011) and 39.5% in Greece (Zdragas et al., 2012). Further, a higher incidence (88.5%) of Salmonella has been discovered in broiler chicken meat in Malaysia (Nidaullah et al., 2017). In South Africa too, Salmonella has been identified as the most prevalent pathogen in broiler chicken meat (Magwedere et al., 2015). It has been reported that the prevalence of Salmonella in poultry meat in Thailand was 84% (Bodhidatta et al., 2013; Chotinun et al., 2014). The reasons for the above different observations could be various factors related to the handling process and meat processing activities in different countries. Similar to the present findings, 17.91% prevalence of Salmonella in broiler chicken meat was reported in Iran (Jalali et al., 2008). The lower contamination level found in our present study could be a result of quality improvements achieved in meat processing activities in the country in the recent past.

Many studies have demonstrated the higher prevalence of Salmonella in other livestock

### Table 02: Primers used for the detection of presence of antibiotic resistance genes.

| Resistance gene | Nucleotide sequence | Product size (bp) | Annealing temperature (°C) | Code of antibiotic | Reference |
|-----------------|---------------------|-------------------|-----------------------------|--------------------|-----------|
| tetA            | F TTGGCATTCTGCATTTCACTC | 494 | 55 | TET | Ma et al., (2007) |
|                 | R GTATAGGCTGGAAGGCTCTC |     |     |     |           |
| tetB            | F CAGTTCCTGGTGATCATTA | 571 | 55 | TET | Ma et al., (2007) |
|                 | R GCTTGAATACGTGACTTAA |     |     |     |           |
| tetC            | F ATGTGTCGTCATCTACGCC | 418 | 55 | TET | Ma et al., (2007) |
|                 | R AGCAACAGAATCGGGAAAC |     |     |     |           |
| tetD            | F GCTCGTGATCTCATCTGTC | 546 | 55 | TET | Ma et al., (2007) |
|                 | R AGCTGTCAGGTTGGTAAAA |     |     |     |           |
| tetE            | F TATTAACGGCGTGGGATTC | 544 | 55 | TET | Ma et al., (2007) |
|                 | R AGCTGTCAGGTTGGTAAAA |     |     |     |           |
| tetG            | F GCTCGTGATCTCATCTGTC | 550 | 55 | TET | Ma et al., (2007) |
|                 | R CAAAGCCCTTTGCTTTTAG |     |     |     |           |
| Sul1            | F TTTCCGACCCAGGCCTTAT | 793 | 55 | COT | Ma et al., (2007) |
|                 | R GTGCCGAGTGATCTGGCCCA |     |     |     |           |
| Sul2            | F CCTGTTCAGGCGACACAGA | 667 | 55 | COT | Ma et al., (2007) |
|                 | R GAAGCGAGCGGGAATTCAT |     |     |     |           |
| Sul3            | F ATGACGCAAGATTGTTGGAATCGTA | 792 | 55 | COT | Ma et al., (2007) |
|                 | R CTAACCTAAGGGCTTTG |     |     |     |           |
| cat1            | F AACCGAGCCGTCGCTGAT | 549 | 55 | CHL | Zhao et al., (2001) |
|                 | R CCTGCAACTCATCGGATAC |     |     |     |           |
| cat2            | F AAGGCGATGACACCTGAA | 547 | 55 | CHL | Ma et al., (2007) |
|                 | R ATCCCAAATGCGATGTAAAG |     |     |     |           |
| cat3            | F ATCCGGCTCGATTACCTAT | 310 | 55 | CHL | Ma et al., (2007) |
|                 | R ATTCGCCCTCTTGTGATATT |     |     |     |           |
| cmlA            | F GCCCTGCTCTTTAGCTGAT | 662 | 55 | CHL | Ma et al., (2007) |
|                 | R GCGACACCAATACCGACTGC |     |     |     |           |
| cmlB            | F ACTCGGCAATGACATGTACT | 840 | 55 | CHL | Ma et al., (2007) |
|                 | R ACGGACTGCGGAATCCATAG |     |     |     |           |
| floR            | F ATGGACCACACACGCCCG | 198 | 55 | CHL | Ma et al., (2007) |
|                 | R AGACGACTGCGGCTTCT |     |     |     |           |
| aac (3)11a      | F CGGCCGCTAGAATGACATTTC | 439 | 55 | GEN | Ma et al., (2007) |
|                 | R AAAAGGCACACCCACCTCCT |     |     |     |           |
| aph(3)11a       | F TCTGAAACATGGCAAAGGTAG | 582 | 55 | GEN | Ma et al., (2007) |
|                 | R AGCCGTATTCTGAAATAGG |     |     |     |           |
| aac6            | F TTGAGCGCTGAGATATAGA | 476 | 55 | GEN | Ma et al., (2007) |
|                 | R CTCCTTCTGCCAAATCTTT |     |     |     |           |

Tetracyclines (tetA, tetB, tetC, tetD, tetE, and tetG), sulfonamides (sul1, sul2, and sul3), chloramphenicol (cat1, cat2, and cat3, cmlA, cmlB, floR) and aminoglycosides (aph (3)11a, aac (3)11a and aac6).
species as well. Farzan et al., (2010) reported 31.5% prevalence of *Salmonella* in swine. Pork and beef also play a great role in causing salmonellosis apart from poultry meat (Litrup et al., 2010; Osman et al., 2014; Abatcha et al., 2018).

Two different pairs of primers were used to confirm isolates as *Salmonella*, targeting *hns* (DNA binding protein encoding gene) and *invA* genes. The isolates were confirmed as *Salmonella* only when they are positive for both *hns* (152 bp) (Figure 1A) and *invA* (284 bp) (Figure 1B) genes.

Though the conventional identification of *Salmonella* is an important tool for speciation of the isolates, molecular confirmation is necessary to complete the identification process. A study to compare the conventional isolation methods vs molecular identification procedure to detect *Salmonella* in broiler chicken meat has revealed that prevalence of *Salmonella* in meat samples was 12% in molecular method whereas it was 22% in the conventional method (Ibrahim et al., 2014). Use of PCR by targeting the sequences of *hns* and *invA* genes for rapid detection of *Salmonella* has been proven earlier too (El-Sebay et al., 2017). As a unique sequence to this genus available in fragment of *invA* gene, it has been verified as a precise PCR target (Rahn et al., 1992). The current study has shown the presence of *invA* gene in all the isolates and the finding is in agreement with other studies that detected *invA* in all the isolates (100%) obtained from chicken samples (Abd El Tawwab et al., 2013; Cossi et al., 2013; Karatug et al., 2018). A protein responsible for invasion into the host cells is located in the inner membrane of the *Salmonella* bacterium and *invA* gene encodes that protein. As it is highly specific to the bacterium, that gene can be used to detect all *Salmonella* species with more accuracy (Shanmugasamy et al., 2011; Karmi 2013). Hence, *invA* has been now proved as an international standard specific gene for the identification of genus *Salmonella*. The oligonucleotide sequence of *hns* gene used here was designed by Jones et al., (1993) from the regions where the *S*. Typhimutium nucleotide sequences mismatch with the *hns* gene in other members of the Enterobacteriaceae to have specific primer for *Salmonella*. This study also used these two sets of genes for confirming the *Salmonella* isolates as they have the ability to detect *Salmonella* with high accuracy.

![Figure 01: PCR amplification of hns gene (A) and invA gene (B) of Salmonella isolates.](image)

![Table 03: Serotyping of Salmonella isolates.](image)
All the PCR confirmed isolates were serotyped and 11 out of 23 (47.8%) were identified as Salmonella Typhimurium and 6 isolates (26.1%) were identified as Salmonella Enteritidis. The remaining six (26.1%) PCR confirmed isolates were not serotyped. The results of serotyping of Salmonella isolates are given in Table 3.

Weerasooriya et al., (2008) found that the most common serovar of Salmonella found in broiler chicken meat in Sri Lanka was S. Typhimurium while Wijemanne et al., (2008) revealed that S. Enteritidis as the most common serovar in poultry breeder farms in Sri Lanka. Kottawatta et al., (2014) reported 9% prevalence of Salmonella in broiler chicken in Sri Lanka with S. Typhimurium as the common serovar. The present study also found that 11 isolates out of 23 were S. Typhimurium and only 6 out of 23 isolates were S. Enteritidis. Hence, the current study has also shown that the prominent Salmonella serovar in Sri Lanka is S. Typhimurium which is in accordance with many of the previous studies.

Previous studies have obtained different outcomes on prevalence of Salmonella serovars in poultry meat in many other countries as well. Mir et al., (2015) reported that Salmonella Enteritidis was the foremost serotype followed by Salmonella Typhimurium. Parvej et al., (2016) also reported the higher prevalence of Salmonella Enteritidis in Bangladesh. Those findings were in contrast with the current study as Salmonella Typhimurium was found as the most prominent serotype in Sri Lanka. In parallel to the present findings, Abdellah et al., (2009) reported the predominance (40%) of S. Typhimurium in poultry samples in Morocco. El-Aziz (2013) found that the prevalence of S. Typhimurium in chicken meat was 44% in Egypt. Findings of Moawad et al., (2017) isolating Salmonella from chicken and beef are also in line with the current study, which showed that S. Typhimurium is the most common Salmonella serovar in broiler chicken meat. A study in Spain has shown that the S. Typhimurium being the most prevalent (Lamas et al., 2016). High prevalence of both S. Typhimurium and S. Enteritidis has been discovered from USA meat industry as well (Andino and Hanning, 2015). It is also in agreement with the current study as it also showed the presence of S. Enteritidis as the second most common organism found in broiler chicken meat in Sri Lanka.

Detection of antibiotic sensitivity using disc diffusion method

Antibiotic sensitivity testing revealed that three (13.5%) isolates (S5, S6 and S18) were resistance to ampicillin and five (21.7%) isolates (S2, S10, S12, S21, S22) have intermediate resistance to ampicillin. Seven (30.5%) isolates (S6, S10, S11, S12, S20, S21, S22) showed intermediate resistance for nitrofurantoin (NIT) while all the other isolates were sensitive to the same antibiotic. Only one (4.3%) isolate (S20) exhibited intermediate resistance to cefotaxime (CTX) while all the other isolates were sensitive to that. All the isolates were sensitive to NA, C, PIT, COT, CIP, TE, MRP, K and G. The positive massage obtained by the results of this study was that 56.5% (13/23) isolates were sensitive to all the tested antibiotics (Table 04).

Resistance genes for tetracyclines tetA, tetB, tetC, tetD, tetE and tetG were present in few isolates. 26% (6), 13% (3), 4% (1), of the isolates carried tetA, tetB, tetD genes, respectively, and other tetracycline genes such as tetC, tetE and tetG were present in the 17% (4) of the isolates for each gene. While one of the resistance genes (sul3) for sulfonamides was absent in all the 23 isolates other two resistance genes for sulfonamides (sul1 and sul2) were present only in one of the isolates (4%). All the resistance genes (cat1, cat2, cmlA, cmlB, floR) checked for chloramphenicol were absent in the isolates except cat3 which was present in one isolate (S6; 4%). aac (3) 11a, one of the genes for aminoglycoside resistance was present in one isolate (4%), 11a aph (3) and aac6 were harbored by two isolates (9% for each gene) (Table 05).

Resistance to antibiotics is a major burning public health problem in the world. Illnesses that were once easily treatable with antibiotics are becoming more difficult to cure due to the emergence of resistance to present generation
drugs. Rapid annual development of antibiotic resistance in nontyphoidal *Salmonella* serovars has become a significant problem (Angelo et al., 2016; Davidson et al., 2018).

Table 04: Sensitivity of *Salmonella* isolates to different antibiotics.

| Isolate number | NIT | NA | C | AMP | PIT | COT | CIP | TE | CTX | MRP | K | GEN |
|----------------|-----|----|---|-----|-----|-----|-----|----|-----|-----|---|-----|
| 1S             | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 2S             | S   | S  | S | I   | S   | S   | S   | S  | S   | S   | S | S   |
| 3S             | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 4S             | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 5S             | S   | S  | S | R   | S   | S   | S   | S  | S   | S   | S | S   |
| 6S             | I   | S  | S | R   | S   | S   | S   | S  | S   | S   | S | S   |
| 7S             | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 8S             | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 9S             | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 10S            | I   | S  | S | I   | S   | S   | S   | S  | S   | S   | S | S   |
| 11S            | I   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 12S            | I   | S  | S | I   | S   | S   | S   | S  | S   | S   | S | S   |
| 13S            | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 14S            | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 15S            | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 16S            | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 17S            | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 18S            | S   | S  | S | R   | S   | S   | S   | S  | S   | S   | S | S   |
| 19S            | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 20S            | I   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |
| 21S            | I   | S  | S | I   | S   | S   | S   | S  | S   | S   | S | S   |
| 22S            | I   | S  | S | I   | S   | S   | S   | S  | S   | S   | S | S   |
| 23S            | S   | S  | S | S   | S   | S   | S   | S  | S   | S   | S | S   |

*S* indicates sensitivity; *I* indicates intermediate sensitivity and *R* indicates resistance to antibiotic nitrofurantoin (NIT), cefotaxime (CTX), nalidixic acid (NA), chloramphenicol (C), Ampicillin (AMP), piperacillin (PIT), co-trimozole (COT), ciprofloxacin (CIP), tetracycline (TE), meropenem (MRP), Kanamycin (K) and Gentamycin (GEN)

Table 05: Presence of antibiotic resistance genes in *Salmonella* isolates.

| Resistant genes | S1 | S2 | S3 | S4 | S5 | S6 | S7 | S8 | S9 | S10 | S11 | S12 | S13 | S14 | S15 | S16 | S17 | S18 | S19 | S20 | S21 | S22 | S23 |
|-----------------|----|----|----|----|----|----|----|----|----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|-----|
| tetA            | -  | -  | -  | +  | +  | -  | -  | -  | -  | -    | +    | -    | -    | -    | +    | -    | -    | -    | -    | +    | +    | +    |
| tetB            | -  | -  | -  | +  | +  | -  | -  | -  | -  | -    | +    | -    | -    | -    | +    | -    | -    | -    | -    | -    | -    | -    |
| tetC            | -  | -  | -  | -  | -  | +  | -  | -  | -  | -    | -    | +    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| tetD            | +  | +  | +  | +  | +  | +  | -  | -  | -  | -    | +    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| tetE            | +  | -  | +  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | +    | -    | -    | -    | -    | -    | -    | -    |
| tetG            | +  | -  | -  | +  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| sul1            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | +    | +    | +    | +    | +    | +    | +    | +    |
| sul2            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| sul3            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| cat1            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| cat2            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| cat3            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| cmlA            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| cmlB            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| floR            | +  | -  | -  | -  | -  | -  | -  | -  | -  | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    | -    |
| aac(3)11a       | +  | +  | +  | +  | +  | +  | +  | +  | +  | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| aac(3)11a       | +  | +  | +  | +  | +  | +  | +  | +  | +  | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |
| aac6            | +  | +  | +  | +  | +  | +  | +  | +  | +  | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    | +    |

Tetracycline (tetA, tetB, tetC, tetD, tetE and tetG), Sulfonamides (sul1, sul2, and sul3), Chloramphenicol (cat1, cat2, and cat3, cmlA, cmlB, floR) and Aminoglycosides (aph (3)11a, aac (3)11a and aac6)
In a similar study aimed at examining the prevalence and antimicrobial resistance of *Salmonella* isolates from broiler chickens, pigs and their associated meat products revealed that the multidrug resistance was 34% in Thailand and 52% in Cambodia. In accordance with the findings of the present study, the majority of the Thai isolates were also resistant to ampicillin (72.4%). However, most Cambodian isolates were resistant to sulfamethoxazole (71%) (Trongjit *et al.*, 2017) giving different results from the present study where there was no resistant showed to the cotrimazole, the same group antibiotic with sulfamethoxazole. Obtaining similar results to the present study, Xia *et al.*, (2009) in the USA showed that most of the *Salmonella* isolates in broiler chicken meat were susceptible to 15 commonly used antibiotics. In agreement with the present study, Moawad *et al.*, (2017) in Egypt found that *S. enterica* isolated from chicken meat showed higher resistant to ampicillin but all were vulnerable to chloramphenicol as well as ciprofloxacin. A Study from Spain revealed that 60% of the total *Salmonella* isolates were resistant to minimum of one antibiotic and 20% were resistant to more than one antibiotic. Showing the same results as in the present study, all *Salmonella* spp. were susceptible to gentamicin, cefotaxime, kanamycin, ciprofloxacin and trimethoprim (cotrimazole) in that study too. However, in contrast to the present study, a high level of resistance has been observed in that study against nalidixic acid (Lamas *et al.*, 2016). Furthermore, in a study conducted on prevalence and antimicrobial resistance profiles of *Salmonella* serotypes isolated from broiler chicken meat in Republic of Korea also revealed that the isolates were often resistant to different antibiotics including 85% to nalidixic acid (Kim *et al.*, 2012). The above published data is also in contrast with the findings of the present study as all the tested isolates were sensitive to nalidixic acid in this study. Im *et al.*, (2015) revealed that *Salmonella* isolates displayed resistance to ampicillin, tetracycline, gentamicin and nalidixic acid. However, in the current study, isolates displayed 13.5% resistance only to ampicillin and all the isolates were sensitive to nalidixic acid, tetracyclines and gentamicin. Prevalence and antimicrobial resistance patterns of *Salmonella* isolated from poultry farms in the United States were carried out by Velasquez *et al.*, (2018) and resistance to gentamycin was not observed while resistance to chloramphenicol was observed at a low level. The present study showed that *Salmonella* isolated from Sri Lanka were sensitive to both gentamycin and chloramphenicol. It is an interesting finding that local *Salmonella* have not yet developed resistance against chloramphenicol, which is one of the limited number of antibiotics that can be used to treat typhoid fever in human.

Despite to the increasing incidence of ciprofloxacin resistant *Salmonella* in some countries (Threlfall *et al.*, 2002; Medalla *et al.*, 2013) several studies have found that there is a decreasing tendency of developing resistance in *Salmonella* against few antibiotics and it is in agreement with the findings of the current study. For instance, Davidson *et al.*, (2018) have found no isolate resistant to ciprofloxacin among total of 242 *Salmonella* isolates. In other studies too, monitoring *Salmonella* isolates have shown that there was no resistance to ciprofloxacin and nalidixic acid (Cummings *et al.*, 2013; Davidson *et al.*, 2018). A study conducted on *Salmonella enterica* isolated from 4976 clinical samples observed parallel findings that showed a tendency for gradual declining of resistance for gentamicin, trimethoprim as well as neomycin (Valenzuela *et al.*, 2017). Mąka *et al.*, (2015) also showed that *Salmonella* spp. isolated from non meat food items were fully sensitive to many commonly used antibiotics, but some were resistant to chloramphenicol. Though it is reported that S. Enteritidis is relatively more susceptible to commonly used antibiotics than S. Typhimurium (Barilli *et al.*, 2018), it was not clearly shown in the present study. Out of the three isolates that displayed resistance against ampicillin, only one isolate (S6) belongs to S. Typhimurium serotype whereas other two isolates were non-serotyped.

The results of the present study have shown that most of the *Salmonella* isolates exhibited a high level of sensitivity to most of the tested antibiotics. Although that message is gratifying, antimicrobial susceptibility must be assessed continuously and conduct more extensive work to identify the whole picture on antibiotic resistance in *Salmonella* in the livestock sector in the country in order to make a general conclusion.
CONCLUSIONS

The present study concluded the presence of *Salmonella* spp. in broiler chicken meat in Sri Lanka during the investigation period and the *S. Typhimurium* is the most common organism followed by *S. Enteritidis*. Further, it can be concluded that there is a low prevalence of antibiotic resistance among the isolates, nevertheless, the detection of intermediate resistance to antimicrobial agents in many isolates could predict the possibility of developing and spread of multidrug resistance strains in the future.

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Conflicts of interest

The authors declare that there is no conflict of interests.

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