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

T.S.P. Jayaweera[1*], H.A.D. Ruwandeepika[1], V.K. Deekshit[3], J.K. Vidanarachchi[2], S.P. Kodithuwakku[2], I. Karunasagar[3] and H.W. Cyril[2]

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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

[1] *Department of Livestock Production, Faculty of Agricultural Sciences, Sabaragamuwa University of Sri Lanka, Belihuloya, Sri Lanka.*

sanjeewaprasadj@yahoo.com

[2] *Department of Animal Science, Faculty of Agriculture, University of Peradeniya, Peradeniya, Sri Lanka.*

[3] *UNESCO Microbial Resources Center for Biotechnology, Faculty of Biological Sciences, Nitte University Center for Science Education and Research, Nitte (Deemed to be University), Deralakatte, Mangalore, India.*

ORCID http://orcid.org/0000-0002-8722-0529

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; subsp. *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^{R}400, Interscience International, France). This mixture was incubated for 24 hours at 37^{o}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 selenite cystine and tetrathionate broths were incubated at 37^{o}C for 24 hours, while Rappaport Vassiliadis broth was incubated at 43^{o}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^{o}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-trimoxozole (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).

Table 01: Primers used for the confirmation of *Salmonella* isolates

Gene	Gene description	Primer sequences (5'-3')	Product size (bp)	Reference
Hns	Histone like nucleoid structuring gene	F-TACCAAAGCTAAACGCGCAGCT R-TGATCAGGAAATCTTCCAGTTGC	152	Jones *et al.,* 1993
invA	Gene encoding the invasion-associated protein	F-GTGAAATTATCGCCACGTTCGGGCAA R-TCATCGCACCGTCAAAGGAACC	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.

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*, *cat3*, *cmlA*, *cmlB* and *floR* genes and *aph* (*3)11a*, *aac (3)11a* 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).

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.

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 Thilakarathne *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.

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	TTGGCATTCTGCATTCACTC	494	55	TET	Ma *et al.*, (2007)
	R	GTATAGCTTGCCGGAAGTCG				
tetB	F	CAGTGCTGTTGTGTCATTAA	571	55	TET	Ma *et al.*, (2007)
	R	GCTTGGAATACTGAGTGTAA				
tetC	F	CTTGAGAGCCTTCAACCCAG	418	55	TET	Ma *et al.*, (2007)
	R	ATGGTCGTCATCTACCTGCC				
tetD	F	GCTCGGTGGTATCTCTGCTC	546	55	TET	Ma *et al.*, (2007)
	R	AGCAACAGAATCGGGAACAC				
tetE	F	TATTAACGGGCTGGCATTTC	544	55	TET	Ma *et al.*, (2007)
	R	AGCTGTCAGGTGGGTCAAAC				
tetG	F	GCTCGGTGGTATCTCTGCTC	550	55	TET	Ma *et al.*, (2007)
	R	CAAAGCCCCTTGCTTGTTAC				
Sul1	F	TTTCCTGACCCTGCGCTCTAT	793	55	COT	Ma *et al.*, (2007)
	R	GTGCGGACGTAGTCAGCGCCA				
Sul2	F	CCTGTTTCGTCCGACACAGA	667	55	COT	Ma *et al.*, (2007)
	R	GAAGCGCAGCCGCAATTCAT				
Sul3	F	ATGAGCAAGATTTTTGGAATCGTAA	792	55	COT	Ma *et al.*, (2007)
	R	CTAACCTAGGGCTTTGGTATTT				
cat1	F	AACCAGACCGTTCAGCTGGAT	549	55	CHL	Zhao *et al.*, (2001)
	R	CCTGCCACTCATCGCAGTAC				
cat2	F	AACGGCATGAACCTGAA	547	55	CHL	Ma *et al.*, (2007)
	R	ATCCCAATGGCATCGTAAAG				
cat3	F	ATCGGCATCGGTTACCATGT	310	55	CHL	Ma *et al.*, (2007)
	R	ATCCCCTTCTTGCTGATATT				
cmlA	F	GGCCTCGCTCTTACGTCATC	662	55	CHL	Ma *et al.*, (2007)
	R	GCGACACCAATACCCACTAGC				
cmlB	F	ACTCGGCATGGACATGTACT	840	55	CHL	Ma *et al.*, (2007)
	R	ACGGACTGCGGAATCCATAG				
floR	F	ATGACCACCACACGCCCCG	198	55	CHL	Ma *et al.*, (2007)
	R	AGACGACTGGCGACTTCTTCG				
aac (3)11a	F	CGGCCTGCTGAATCAGTTTC	439	55	GEN	Ma *et al.*, (2007)
	R	AAAGCCCACGACACCTTCTC				
aph(3)11a	F	TCTGAAACATGGCAAAGGTAG	582	55	GEN	Ma *et al.*, (2007)
	R	AGCCGTTTCTGTAATGAAGGA				
aac6	F	TTGGACGCTGAGATATATGA	476	55	GEN	Ma *et al.*, (2007)
	R	GCTCCTTTTCCAGAATACTT				

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

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

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 Enterobacterieceae 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.

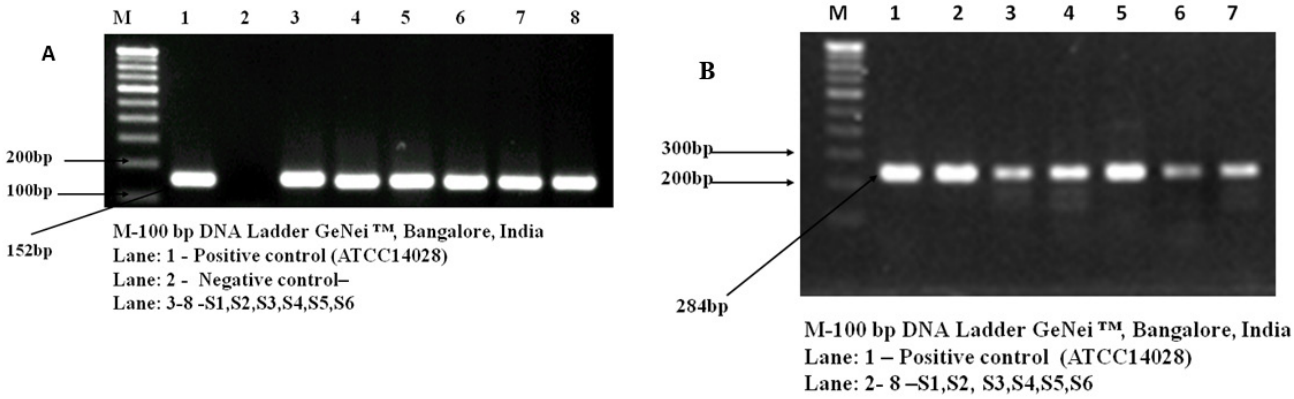

Figure 01: PCR amplification of *hns* gene (A) and *invA* gene (B) of *Salmonella* isolates.

Table 03: Serotyping of *Salmonella* isolates.

Serotypes	Isolate number
Salmonella Typhimurium	S1, S2, S4, S6, S8, S9, S11, S16, S19, S21, S23
Salmonella Enteritidis	S3, S10, S15, S17, S20, S22
Non typable Salmonella spp.	S5, S7, S12, S13, S14, S18

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 prominant *Salmonella* serovar in Sri Lanka is *S*. Typhimurium which is inaccordance with many of the 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).

Presence of antimicrobial resistant genes

Resistant 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	I	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	*Salmonella* isolates																						
	S1	S2	S3	S4	S5	S6	S7	S8	S9	S 10	S 11	S 12	S 13	S 14	S 15	S 16	S 17	S 18	S 19	S 20	S 21	S 22	S 23
tetA	-	-	-	+	+	+	-	-	-	-	-	+	-	-	-	+	-	-	-	-	-	+	-
tetB	-	-	-	-	+	-	+	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-
tetC	-	-	-	+	-	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	+	-
tetD	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
tetE	-	-	-	-	-	+	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	+	+
tetG	+	-	-	+	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sul1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+
Sul2	-	-	-	-	-	-	-	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-
Sul3	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cat1	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cat2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cat3	-	-	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cmlA	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
cmlB	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
floR	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
aac (3)11a	-	-	-	+	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
aph(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 (co-trimozole) 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 chioramphenicol. 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 ciproflioxacin 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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