Journal of Poultry Sciences and Avian Diseases

Journal homepage: www.jpsad.com

Frequency of *Salmonella* Infection among Backyard and Commercial Duck Flocks in Iran, Serotyping and Drug Resistance Profile of *Salmonella* Isolated from Ducks

Javad Javidnezha , Seyed Mostafa Peighambi*[1] , Jamshid Razmyar[1] , Abbas Barin[2]

[1] Department of Avian Diseases, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran
[2] Department of Clinical Sciences, Faculty of Veterinary Medicine, University of Tehran, Tehran, Iran

*** Corresponding author email address**: mpeigham@ut.ac.ir

A r t i c l e I n f o

Article type:
Original Research

How to cite this article:
Javidnezhad, J., Peighambari, S. M., Razmyar, J., & Barin, A. (2025). Frequency of *Salmonella* Infection among Backyard and Commercial Duck Flocks in Iran, Serotyping and Drug Resistance Profile of *Salmonella* Isolated from Ducks. *Journal of Poultry Sciences and Avian Diseases, 3*(1), 1-13.
http://dx.doi.org/10.61838/kman.jpsad.3.1.4

A B S T R A C T

Salmonella is one of the most important food-borne infections with worldwide distribution that infects humans and a wide range of animals. Poultry and poultry products are considered major sources of *Salmonella* infections for humans. Like chickens, ducks can play an important role in the transmission of *Salmonella* bacteria to humans. The aim of this study was to obtain more knowledge about the frequency of *Salmonella* infection in ducks in Iran, to determine the serovar and the antimicrobial resistance pattern of *Salmonella* isolates. In this study, fecal samples were collected from four provinces of Iran. Each six fecal samples were pooled and 352 samples was obtained in total. In order to isolate *Salmonella*, all samples were cultured according to the previously described standard techniques. From a total of 352 stool samples, 20 *Salmonella* isolates (17.6%) were isolated and serotyped by using slide agglutination test and then were subjected to polymerase chain reaction with *Salmonella* genus-specific primers and species-specific primers for serovars Enteritidis, Typhimurium and Infantis. The disc diffusion method was used to determine the sensitivity of isolates to 21 antimicrobial agents. Serotyping identified 15 serovars Enteritidis and three serovar Typhimurium. Two isolates remained unknown. No isolate was resistant to ceftazidime, ceftriaxone, lincospectin, fosfomycin, and colistin. The resistance to other agents was variable. There were 17 resistance patterns to 21 antimicrobial agents. Among the resistant isolates, the occurrence of multiple resistance was very significant, so that they showed resistance to at least 1 and at most 11 drugs. By comparing the findings of this study and other investigation in this field, it was shown that, like chicken flocks, *Salmonella* are circulating in duck flocks of Iran.

Keywords: *Salmonellosis, Antimicrobial susceptibility, Salmonella Enteritis, Salmonella Typhimurium, Salmonella Infantis, Duck, Iran*

Article history:
Received 17 July 2025
Revised 27 September 2025
Accepted 04 October 2025
Published online 01 January 2025

1 Introduction

In 2022, the first and second most reported zoonosis in humans were campylobacteriosis and salmonellosis, respectively (1). The genus *Salmonella* belongs to the *Enterobacteriaceae* family. The members of these gram-negative bacilli are facultatively aerobic and anaerobic, and with the exception of two serovars, *Salmonella* Pullorum and *Salmonella* Gallinarum, all other serovars have peri-trich flagella and are motile (2). Members of this genus can grow in the temperature range of 5 to 45 °C, but the optimum growth occurs at 37 °C (2). Bacteria of this genus are classified into two species, enterica and bongori. Enterica includes six sub-species and it is considered an important species because pathogenic *Salmonella* for humans and warm-blooded animals are under species I (enterica). To date, more than 2600 *Salmonella* serovars have been identified, and most of those serovars are classified under type I (2). The main way of *Salmonella* transmission is through the digestive tract, but its transmission through the mucous membranes of theeye conjunctiva or the upper respiratory tract has also been reported (2). *Salmonella* and in particular *S.* Enteritidis remained the most frequently reported causative agent for foodborne outbreaks (2). This genus is considered as an important factor in foodborne diseases, which is mostly transmitted through the consumption of meat, milk and eggs and is considered an important indicator for the water and food safety of a country (2). Many researches on the prevalence of *Salmonella* have shown that the most important and common food sources of *Salmonella* infection in humans are poultry and poultry products (3, 4). Gastroenteritis, which is accompanied by fever, abdominal cramps and diarrhea, is the most common clinical form of salmonellosis in humans (1). Causing a high economic loss on one hand and the occurrence of food poisoning on the other hand are considered the reasons for the importance of *Salmonella* for individuals and societies (1).

In addition to horizontal transmission, *Salmonella* serovars are also transmitted by vertical transmission, which can lead to the contamination of day-old chickens as well as the contamination of other birds. In processing plants, the slaughtering and packaging steps may also spread the *Salmonella* infection and pose a major risk for consumers. Penetration into the shell and transmission through the shell is also possible for *Salmonella*, and hence, it can also contaminate the produced eggs (5).

Breeding ducks for the purpose of producing meat and eggs dates back to several hundred years ago (6). The connection between duck and *Salmonella* has been known for many years and it is referred to as a cause-and-effect situation because it has been indicated that the consumption of duck eggs is very likely to be associated with stomach upset (6). This possibility is mainly due to the presence of *Salmonella* Typhimurium, which is transmitted to the egg by a clinically healthy duck (6). Meanwhile, *Salmonella* food poisoning due to consumption of duck meat is a rare event, which is probably due to its cooking methods and the dietary habits of the not so large population that consumes duck meat (6).

Salmonella control will require the adoption of a surveillance program for regular sampling and appropriate control measures. In this regard, the first step is to establish surveillance, determine the status of infection and the underlying factors of infection (7).

The aims of this study were to determine the frequency of *Salmonella* infection among backyard and commercial duck flocks in Iran, serotyping and drug resistance profile of *Salmonella* isolated form ducks.

2 Materials and Methods

2.1 Sampling and bacteriological procedures

This study was conducted from August 2022 to August 2023 in four Iranian provinces of Tehran, Golestan, Mazandaran and Guilan, where the duck population was higher than that of in other provinces. During this study, a total of 2112 fresh stool samples were taken, each six samples pooled, and a total of 352 pooled samples were obtained. The share of each province of total and pooled samples were as follows: Tehran, 978 (163 pooled) samples and the other three northern provinces, each 378 (63 pooled) samples. In Tehran province, samples were taken from 53 commercial duck breeding farms, in which, in each flock, a house was divided into six areas and samples were taken from all six areas and the whole house was considered as one sample and the rest of 110 samples of this province were provided from domestic ducks in different areas of the province. In Tehran province, due to the importance and commercial production of ducks in Varamin city, all samples were provided from flocks located in Varamin area. It has been estimated that Varamin area has a population of two million ducks. Breeding flocks of Varamin area are the major sources of ducklings for northern provinces. In three norther provinces, samples from domestic ducks were taken from different cities of Golestan (Kordkoi, Gorgan, Kalaleh, Agh-Ghala, Bandar-Gaz, Bandar-Turkman), Mazandaran (Sari, Behshahr, Jouybar, Babol, Amol, Chalus) and Guilan (Rudsar, Langrood, Amlash,

Lahijan, Bandar Anzali, Rasht). After sampling and pooling, samples were transferred to the university laboratory in a closed container and cold condition in less than 24 hours.

All fecal samples were cultured for the isolation and identification of *Salmonella* according to standard procedures that have been previously described (8). Briefly, the selective enrichment of samples in selenite F at 41°C for 24 h was followed by sub-cultivation on *Salmonella-Shigella* (SS) and MacConkey agar plates at 37°C for 24 h. Then, the suspect colonies were selected, isolated and further characterized by biochemical identification. Positive samples were kept at -70°C freezer for future use.

2.2 *Serogroup determination*

The serogroup of each *Salmonella* isolate was determined by slide agglutination test using antisera against O antigen (Poly A-I & Vi) according to the instructions of manufacturer (Difco, USA).

2.3 *Molecular identification of Salmonella isolates by PCR*

In this study, *invA* gene specific primers were used to confirm the *Salmonella* genus (Table 1). Also, in order to identify serovars Enteritidis, Typhimurium and Infantis, three pairs of specific primers sdf-1, *fli*-C and fli-B were used respectively (Table 1). The characteristics of the primers used to detect the genus *Salmonella* (9) and serovars Typhimurium (10), Enteritidis (11) and Infantis (12) are shown in Table 1. All primers used in PCR reaction were provided from Pishgam Biotech Co. (Tehran, Iran). Other materials were purchased from Yekta Tajhiz Azma Co (Tehran, Iran).

Table 1. Characteristics of the primers used to detect *Salmonella* genus and serovars Enteritidis, Typhimurium and Infantis

Bacteria	Target gene	Nucleotide sequence (5'-3')	Amplicon size (bp)	Reference
Salmonella genus	*invA*	F: GTG AAA TTA TCG CCA CGT TCG GGC AA R: TCA TCG CAC CGT CAA AGG AAC C	284	9
Salmonella Enteritidis	*sdf*-1	F: TGT GTT TTA TCT GAT GCA AGA GG R: CGT TCT TCT GGT ACT TAC GAT GAC	293	11
Salmonella Typhimurium	*fliC*	F: CCC CGC TTA CAG GTG GAC TAC R: AGC GGG TTT TCG GTG GTT GT	433	10
Salmonella Infantis	*fliB*	F: TTG CTT CAG CAG ATG CTA AG R: CCA CCT GCG CCA ACG CT	413	12

To extract bacterial DNA, one ml pure overnight culture of each *Salmonella* isolate grown at 37 °C for 16 h was transferred to a clean 1.5 ml microtube and centrifuged for five min at 10000 x *g*. The supernatants were carefully removed and discarded. The pellet was re-suspended in 300 µl sterile double distilled water by vertexing, incubated for 15 min at 100 °C, chilled on ice immediately, and centrifuged again for five min at 14000 x *g* in 4 °C. The supernatant was removed and used as template DNA. The concentration of DNA was determined by Biophotometer (Eppendorff, Germany) and adjusted to approximately 200 ng for each PCR reaction. The supernatant was stored at -20 °C for further use.

Amplification reactions for *Salmonella* genus and three serovars confirmation were carried out in a 25 µl reaction volume containing 12.5 µl of 2x Mastermix (*Taq* 2x Red Master Mix, Ampliqon, Denmark), 0.5 µl each of forward and reverse primers (10 pmol/µl), 2 µl of DNA template, and 9.5 µL nuclease-free water. Negative controls (dH2O instead of template DNA) were included in all PCR reaction sets. Amplifications were programmed in a thermocycler (SensoQuest, Germany) as described below. For *Salmonella* genus, 95 °C for one min followed by 38 cycles of 95 °C for 30 sec, 64 °C for 30 sec, 72 °C for 30 sec, and a final extension at 72 °C for 4 min was used (9). For serovar Enteritidis, program was as follows: 95 °C for 2 min followed by 30 cycles of 95 °C for 60 sec, 57 °C for 60 sec, 72 °C for 2 min, and a final extension at 72 °C for 5 min (11). For serovar Typhimurium, 94 °C for 5 min followed by 34 cycles of 94 °C for 60 sec, 58 °C for 60 sec, 72 °C for 90 sec, and a final extension at 72 °C for 10 min was programmed in thermocycler (10) and for serovar Infantis, 95 °C for one min followed by 35 cycles of 95 °C for 60 sec, 56 °C for 15 sec, 72 °C for 60 sec, and a final extension at 72 °C for 270 sec was applied (12). The amplified products were detected by gel electrophoresis in 1% agarose gel at 70 V for 80 min in 1 x TAE buffer.

2.4 *Drug susceptibility test*

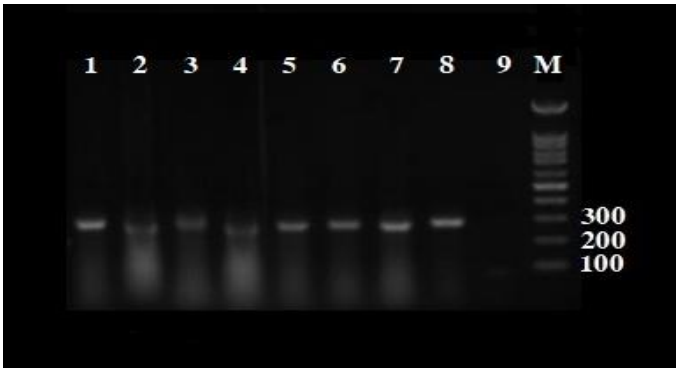
The susceptibility of the *Salmonella* isolates to a panel of antimicrobial agents was determined by the agar disk diffusion method and the interpretation of results was carried

out according to the National Committee for Clinical Laboratory Standards guidelines (13). The antimicrobial agents that were tested and their concentrations (µg) were: ciprofloxacin (5), difloxacin (10), ofloxacin (5), norfloxacin (10), enrofloxacin (5), levofloxacin (5), nalidixic acid (30), flumequine (30), ceftazidime (30), ceftriaxone (30), cefixime (5), ampicillin (10), Co-amoxiclav (30), neomycin (30), streptomycin (10), gentamicin (10), lincospectin (15/200), florfenicol (30), colistin (10), trimethoprim-sulfamethoxazole (1.25/23.75), and fosfomycin (200 µg). All antibacterial disks were provided from Padtan Teb Co (Tehran, Iran). The ATCC reference strains *Escherichia coli* ATCC 25922, *Pseudomonas aeruginosa*, ATCC 27853, and *E. coli* ATCC 35218 were used for quality control purposes. In this study, the *Salmonella* isolates with intermediate susceptibility classification were considered not to be resistant to that drug and the multi-resistance was defined as resistance to more than one drug.

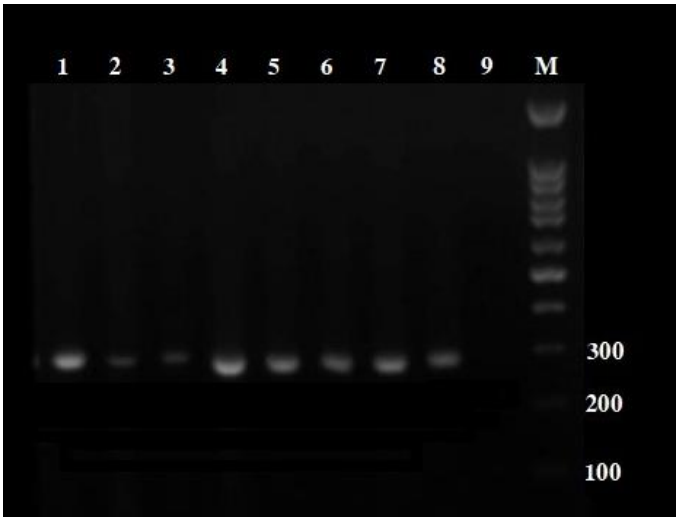
3 Results

Bacteriological, biochemical tests and genus-specific PCR confirmed the isolation and identification of 20 (17.6%) *Salmonella* isolates (Figure 1-A). The frequency of *Salmonella* isolates in provinces of Tehran, Golestan, Guilan and Mazandaran were 10, 5, 3, and 2, respectively. Serovar-specific PCR revealed that out of 20 isolated *Salmonella*, 15 and 3 isolates were identified as Serovars Enteritidis and Typhimurium, respectively (Figure 1-B and C). No isolates were identified as *Salmonella* Infantis. Two remaining *Salmonella* isolates were not positive in any of three serovar-specific PCR and agglutination tests performed with a number of available antisera against *Salmonella* O antigen and, therefore, remained unknown.

A



B



C

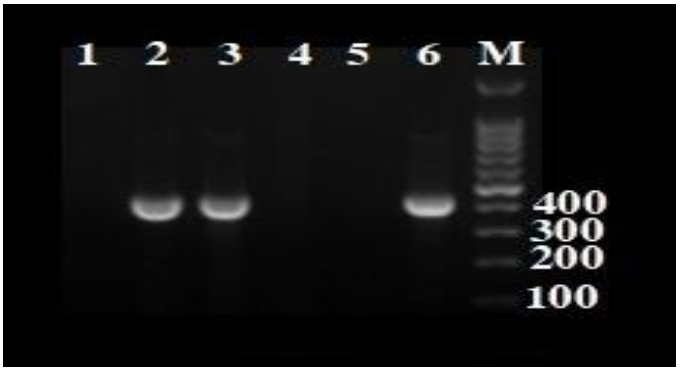


Figure 1. Gel electrophoresis of PCR products for amplification of *invA* (for *Salmonella* genus), *sdf-1* (for *Salmonella* Enteritidis), and *fliC* (for *Salmonella* Typhimurium). A: M, 100 bp ladder; columns 1-7, positive *Salmonella* samples; columns 8 and 9, positive and negative controls, respectively. B: M, 100 bp ladder; columns 1-7, positive *Salmonella* Enteritidis samples; columns 8 and 9, positive and negative controls, respectively. C: M, 100 bp ladder; columns 2 and 3, positive *Salmonella* Typhimurium samples; columns 5 and 6, negative and positive controls, respectively.

In antimicrobial susceptibility test, no isolate was resistant to ceftazidime, ceftriaxone, lincospectin, fosfomycin, and colistin (Table 2). The rate of resistance to Co-amoxiclav, nalidixic acid, flumequine, and streptomycin was above 50%, in which, resistance to Co-amoxiclav ranked first with 95%. The resistance to the rest of the agents was below 25% (Table 2). There was a high variation in the resistance pattern of among 20 *Salmonella* isolates of this study, so that 17 resistance patterns to 21 antimicrobial agents were found among isolates (Table 3). Among the resistant isolates, the occurrence of multiple resistances were very significant, so that they showed resistance to at least 1 and at most 11 drugs (Table 4).

Table 2. Antimicrobial susceptibility profile of 20 *Salmonella* isolates to 21 antimicrobial drugs

No. (%) of susceptible isolates	No. (%) of intermediate susceptible isolates	No. (%) of resistant isolates	Drugs
16 (80)	2 (10)	2 (10)	Ciprofloxacin (CIP)
6 (30)	10 (50)	4 (20)	Difloxacin (DFX)
7 (35)	9 (45)	4 (20)	Enrofloxacin (NFX)
17 (85)	0 (0)	3 (15)	Levofloxacin (LEV)
17 (85)	0 (0)	3 (15)	Norfloxacin (NOR)
17 (85)	0 (0)	3 (15)	Ofloxacin (OFX)
6 (30)	1 (5)	13 (65)	Nalidixic acid (NA)
7 (35)	2 (10)	11 (55)	Flumequine (FM)
9 (45)	9 (45)	2 (10)	Cefixime (CFM)
16 (80)	4 (20)	0 (0)	Ceftazidime (CAZ)
11 (55)	9 (45)	0 (0)	Ceftriaxone (CRO)
0 (0)	1 (5)	19 (95)	Co-Amoxiclav (AMC)
6 (40)	11 (55)	3 (15)	Ampicillin (AM)
9 (45)	1 (5)	10 (50)	Streptomycin (S)
15 (75)	0 (0)	5 (25)	Gentamicin (GM)
8 (40)	10 (50)	2 (10)	Neomycin (N)
18 (90)	2 (10)	0 (0)	Linco-spectin (LP)
7 (35)	11 (55)	2 (10)	Florfenicol (FF)
20 (100)	0 (0)	0 (0)	Fosfomycin (FOS)
3 (15)	17 (85)	0 (0)	Colistin (CL)
19 (95)	0 (0)	1 (5)	Trimethoprim- Sulfamethoxazole (SXT)

Table 3. Drug resistance patterns among 20 Salmonella isolates.

Pattern #	Resistant to[a]	No. (%) of isolate
1	AM, FM, NA	10 (50)
2	AMC, FM, NA	10 (50)
3	AMC, S	7 (35)
4	AMC, FM, NA, S	6 (30)
5	AMC	3 (15)
6	AM, AMC, GM, S	3 (15)
7	AMC, FM, NA, NFX	3 (15)
8	AMC, FM, GM, NA, S	3 (15)
9	AMC, FF	2 (10)
10	AMC, CIP, DFX, FM, LEV, NA, NFX, NOR, S	2 (10)
11	N, NA	Each pattern included only one isolate (5%)
12	AMC, N, NA, S	
13	AMC, CFM, CIP, DF, FM, NA, LEV, NFX, NOR, S	
14	AM, AMC, FF, FM, GM, NA, S, SXT	
15	AMC, DFX, FM, GM, LEV, NA, NFX, OFX, S	
16	AMC, FM, DFX, NA	
17	AM, AMC, GM, NFX, NOR, S	

Table 4. Multi-drug resistance[a] among 20 *Salmonella* isolates of this study.

No. of antimicrobial drugs used	No (%) of resistant isolates
≥ 1	20 (100)
> 2	17 (85)
> 3	14 (70)
> 4	11 (55)
> 5	6 (30)
> 6	5 (25)
> 7	5 (25)
> 8	4 (20)
> 9	3 (15)
> 10	2 (10)
> 11	1 (5)

4 Discussion

In this study, the frequency of *Salmonella* infection among backyard and commercial duck flocks in Iran, the serotypes and drug resistance patterns of isolated *Salmonella* were evaluated. There is a paucity of information on duck infections in Iran especially on *Salmonella*. The duck flock contamination rate in this study was found to be 17.6%. The contamination of duck carcasses and eggs to *Salmonella* may lead to infection of human populations and, therefore, investigating the extent of contamination and isolation of *Salmonella* from ducks are of important public health concerns. It is noteworthy to mention that a duck can be infected with *Salmonella* without any apparent symptoms (14).

Few studies have been done in Iran on the prevalence of *Salmonella* in ducks. In a study of duck intestinal content, 291 samples were collected from meat shops located in Varamin city. The amount of *Salmonella* infection was reported in 84 cases (28.9%) (15). During a study on wild mallard ducks (*Anas platyrhynchos*) in the suburbs of Semnan city, out of 247 freshly collected feces samples, 18 cases (7.29%) were positive for *Salmonella* (16).

Several studies on *Salmonella* infections in ducks have been reported from other countries. In Taiwan, out of 2000 cloacal swab samples from ducks, in 100 different flocks, the overall infection rate was reported as 4.6% (17). In Vietnam, by examining the internal organs of ducks, the contamination rate varied from 6.3% to 14.3% in different regions of country, while, the incidence rate in dead embryos varied from 3.2% to 31.7% (18). In a survey conducted in Egypt, 40 samples of clinically sick duck feces and 120 wasted ducks were obtained to determine the frequency of different pathogens. The most common bacterial agent was *Salmonella* with a frequency rate of 3.3% (19). In a Malaysian study in Penang area in 2009, the prevalence and resistance of *Salmonella* isolated from ducks

were estimated and among 531 collected samples, 125 (23.5%) *Salmonella* isolates were recovered (20). The prevalence of *Salmonella* in 159 South Korean duck flocks was studied and it was found that *Salmonella* was common among flocks, so that 69 flocks (43.4%) were *Salmonella*-infected (21). In another South Korean study, 400 samples of cecal content were taken from duck farms in order to estimate the prevalence and level of antimicrobial resistance of *Salmonella* isolates. The results showed that 83 isolates (20.75%) were *Salmonella* (22). In a study in Bangladesh, 120 fecal samples were taken from 12 small to medium breeding farms and 28 native duck flocks using cloacal swabs. In this study, 32 samples (26.67%) were infected with *Salmonella*. The prevalence rate was 36% in native flocks and 40.2% in breeding farms (23). In another Egyptian research, 100 samples of ducklings suffering from diarrhea and 50 samples from the litter were collected. The prevalence rate of *Salmonella* in the pooled samples of liver, spleen, cecum and gall bladder was 7% and the rate of litter contamination was 6% (24). In a recent study in China, 180 *Salmonella* isolates (25.7%) were isolated from a total of 701 samples taken from all stages of the duck production chain (25).

The prevalence of various *Salmonella* serovars in ducks of Tehran, Golestan, Mazandaran and Guilan provinces was variable. In this research, 20 *Salmonella* isolates were isolated from duck flocks, and the most isolated serovar was *Salmonella* Enteritidis, which accounted for 15 isolates (75%) of the total isolates. While in Jamali et al.'s study in 2014, 84 isolates (28.86%) were isolated from a total of 291 samples of duck feces, of which 56 isolates were *Salmonella* Thompson (59.6%). and only 8 isolates (8.5%) were *Salmonella* Enteritidis (15). This change in the predominant *Salmonella* serovar in ducks can be a warning for its transmission to humans through duck eggs and meat because *Salmonella* Enteritidis is the most transmitted *Salmonella* from infected eggs to humans among different *Salmonella* serovars all over the world (with the exception of Australia). It is noteworthy to mention that this in turn can be considered a warning for more cases of human infection (26).

During a study in the United States backs to sixty's, in a period of 10 years, 491 *Salmonella* isolates were isolated from 7029 duck carcasses, of which 457 (93%) were *Salmonella* Typhimurium. The diversity of *Salmonella* serovars in this study was less than 10 different serovars (27). In 2011, in India, two serovars of *Salmonella* Typhimurium and *Salmonella* Enteritidis were isolated by examining duck eggs (28). In Belgium, during a 32-month study period, 95 *Salmonella* isolates were recovered from 100 duck flocks, which included 11 different *Salmonella* serovars such as Indiana (42.1%) and Regent (36.8%), Typhimurium (1.1%) and Enteritidis (1.1%) (29). In South Korea, 51 isolates (61.45%) of *Salmonella* Typhimurium were found among 400 samples collected from the cecal contents of ducks which was the most dominant serovars identified (22). In a study conducted in England, the contamination rate of 1.4% was reported from 145 combined samples of eggs and in which serovar Typhimurium was the predominant serovar (30). In Shandong Province of China, 49 *Salmonella* isolates were detected among 2,342 samples collected from four duck farms and the serovar Enteritidis was found to be the most dominant serovar (20 out of 49) (31). However, in a recent from China, the most dominant serovar was reported to be serovar Typhimurium (25). Studies completed in Vietnam, have also reported *Salmonella* Typhimurium as the most common serovar in Vietnamese ducks (32, 33). *Salmonella* serovar Potsdam also has been found in studies conducted in Taiwan (17) China (34) and has been reported as the most prevalent *Salmonella* serovar among many samples provided from duck flocks. In Thailand, during a survey, on *Salmonella* contamination of egg shells and contents, 23 different serovars were identified among 133 *Salmonella* isolates and four dominant serovars of Typhimurium (5.5%), Cerro (4.1%), Tennessee (2.8%), and Amsterdam (2.1%) were confirmed (35). *Salmonella* serovar Kentucky also has been reported by Abou Zeid et al. as the most dominant *Salmonella* serovar in *Salmonella* isolates recovered from litter and pooled samples of liver, spleen, cecum, and gall bladder (24). In general, studies throughout the world shows the dominance of *S.* Enteritidis and *S.* Typhimurium among duck populations.

Antimicrobial susceptibility evaluation of *Salmonella* isolates form poultry sources has been the subject of many investigations in recent years. In Arak city, in research by Ezzatpanah et al. (2013), 75 *Salmonella* isolates were obtained from chicken abattoirs. All of isolates were sensitive to enrofloxacin, gentamicin and ceftriaxone and all were resistant to nalidixic acid (36). In another study, 8 *Salmonella* isolates were obtained from 20 broiler flocks in Guilan province and it was found that all isolates were resistant to sulfamethoxazole + trimethoprim, streptomycin and nalidixic acid and all were sensitive to gentamicin, ceftriaxone and chloramphenicol (37). During a study in Golestan province, after isolating 25 *Salmonella* isolates from broiler flocks, 100% of these isolates were sensitive to ceftriaxone and cefixime and also were resistant to flumequine and nalidixic acid (38). In a large study that was conducted in Mazandaran and Guilan provinces, 32 resistance patterns were found in

Salmonella isolates from poultry (39). They reported no resistance to levofloxacin and ofloxacin, while in our study, 15% of the isolates were resistant to these two antibiotics. In the present study, 17 resistance patterns to 21 antimicrobial drugs were observed among 20 *Salmonella* isolates. Similar multi-drug resistance (MDR) patterns were found among ducks of different flocks. Resistance to ampicillin, flumequine and nalidixic acid among 10 isolates; or resistance to nalidixic acid, flumequine and Co-amoxiclav among 10 isolates, or resistance to amoxiclav and streptomycin among 7 isolates or resistance to streptomycin, flumequine, nalidixic acid and Co-amoxiclav among 6 isolates were noticeable.

In the current study, the highest resistance rate among isolates was observed against Co-amoxiclav (95%), nalidixic acid (65%), flumequine (55%) and streptomycin (50%). Considering that amoxiclav is not widely used in veterinary medicine, this level of high resistance to Co-amoxiclav may have negative impact on its usefulness for the treatment of human salmonellosis. On the other hand, flumequine and streptomycin are widely used in veterinary medicine and these results demonstrated significantly increased resistance compared to the past as described in the scientific literature. These facts prompt the veterinarians to replace these antimicrobial agents with other compounds such as probiotics and prebiotics. All investigated isolates were sensitive to fosfomycin, lincospectin, ceftazidime, ceftriaxone and colistin. Although no resistance was observed against agents such as colistin and ceftriaxone, the number of isolates with intermediate sensitivity can be a warning for the not so far future because it is clear that *Salmonella* is able to gain resistance against these antibiotics. In this study, it was shown that the lowest level of drug resistance was against ceftazidime, sulfamethoxazole + trimethoprim, ciprofloxacin, ofloxacin, norfloxacin, levofloxacin and gentamicin. Of course, it was noted that the number of isolates with intermediate sensitivity against ampicillin and third-generation cephalosporins such as ceftriaxone and ceftazidime was on the rise, so that it may be suggested that in the coming years, these antibiotics may not be so effective in the treatment of salmonellosis. Among the third-generation fluoroquinolones, the level of resistance to enrofloxacin was higher than resistance to other members of fluoroquinolones that were tested. The widespread use of enrofloxacin in veterinary medicine compared to other members of this family may be the reason for the occurrence of this high resistance. Also, the number of isolates with intermediate sensitivity to enrofloxacin was relatively high that may affect the clinical efficacy of this drug in the coming years. The transfer of resistance genes to human isolates, especially in the case of antibiotics of the fluoroquinolone and cephalosporin families, which are important antimicrobial agents for the treatment of human salmonellosis, is the most important issue in antimicrobial susceptibility evaluation of bacterial isolates. All of 20 *Salmonella* isolates of this study were resistant to more than one antimicrobial agent, so that they showed resistance to at least one and at most 11 agents at the same time. One of the important challenges of treating infections caused by microorganisms in humans and animals is the emergence of multi-drug resistances among bacterial isolates in Iran and around the world (40-42).

Comparison of the current study and other studies conducted in different parts of the country, as well as considering or ignoring different species of birds, it can be concluded that the resistance patterns among *Salmonella* isolates, despite having similarities, may demonstrate great diversity so that it can be different not only between different countries but also from one province to another in one country. For this reason, it is suggested is that targeted studies be conducted by the control and prevention centers of food-borne diseases in order to estimate the association between the prevalence of resistance in birds and cases of human disease throughout the country. A regular program for the periodic monitoring of *Salmonella* infection in the flocks should be established and also restrictions on the use of antimicrobial agents in animals that are raised for human consumption should be regulated and implemented.

Acknowledgements

None.

Conflict of Interest

The authors declared no conflicts of interest.

Author Contributions

JJ drafted the manuscript. SMP, JR and AB critically reviewed and revised it. All authors have read and approved the final manuscript and agreed to the published version of the manuscript.

Data Availability Statement

Data are available from the first author upon reasonable request.

Ethical Considerations

All ethical principles were fully observed in this study in accordance with the guidelines of the Ethical Committee of the Faculty of Veterinary Medicine, University of Tehran. The research protocol was reviewed and approved under the ethical approval code 7508007-6-47/ 2021.

Funding

This research was funded by a grant (7508007-6-47) from the University of Tehran Research Council.

Editorial Transparency Statement

Although Professor Jamshid Razmyar is a member of the editorial board of this journal, he had no involvement in the peer review or editorial decision-making process for this manuscript. The review and editorial handling were conducted independently by other qualified editors to ensure the integrity and impartiality of the publication process.

References

1. Authority EFS. The European Union One Health 2022 Zoonoses Report. EFSA Journal. 2023;21(12). [PMID: 38089471] [PMCID: PMC10714251] [DOI]
2. Gast RK, Porter Jr RE. Salmonella infections. Diseases of poultry. 2020:717-53. [DOI]
3. Amavisit P, Browning G, Lightfoot D, Church S, Anderson G, Whithear K, et al. Rapid PCR detection of Salmonella in horse faecal samples. Veterinary microbiology. 2001;79(1):63-74. [PMID: 11230929] [DOI]
4. Hennessy TW, Cheng LH, Kassenborg H, Ahuja SD, Mohle-Boetani J, Marcus R, et al. Egg consumption is the principal risk factor for sporadic Salmonella serotype Heidelberg infections: a case-control study in FoodNet sites. Clinical infectious diseases. 2004;38(Supplement_3):S237-S43. [PMID: 15095195] [DOI]
5. Berghaus R, Mathis D, Bramwell R, Macklin K, Wilson J, Wineland M, et al. Multilevel analysis of environmental salmonella prevalences and management practices on 49 broiler breeder farms in four South-Eastern States, USA. Zoonoses and public health. 2012;59(5):365-74. [PMID: 22650982] [DOI]
6. Shivaprasad H, Barrow PA. 11 Salmonella Infections in Ducks. Salmonella in domestic animals. 2013:221. [DOI]
7. WOAH. Terrestrial Manual. Salmonellosis, Chapter 3.10.7. World Organization of Animal Health. 2022.
8. Waltman WD GR, Mallinson ET. Salmonellosis. American Association of Avian Pathologists (AAAP). 1998:4-13.
9. Malorny B, Hoorfar J, Hugas M, Heuvelink A, Fach P, Ellerbroek L, et al. Interlaboratory diagnostic accuracy of a Salmonella specific PCR-based method. International journal of food microbiology. 2003;89(2-3):241-9. [PMID: 14623390] [DOI]
10. Arkali A, Çetinkaya B. Molecular identification and antibiotic resistance profiling of Salmonella species isolated from chickens in eastern Turkey. BMC veterinary research. 2020;16(1):205. [PMID: 32560721] [PMCID: PMC7304202] [DOI]
11. Agron PG, Walker RL, Kinde H, Sawyer SJ, Hayes DC, Wollard J, et al. Identification by subtractive hybridization of sequences specific for Salmonella enterica serovar Enteritidis. Applied and environmental microbiology. 2001;67(11):4984-91. [PMID: 11679316] [PMCID: PMC93261] [DOI]
12. Kardos G, Farkas T, Antal M, Nógrády N, Kiss I. Novel PCR assay for identification of Salmonella enterica serovar Infantis. Letters in applied microbiology. 2007;45(4):421-5. [PMID: 17897386] [DOI]
13. Humphries R, Ambler J, Mitchell S, Castanheira M, Dingle T, Hindler J, et al. on behalf of the CLSI Methods Development and Standardization Working Group of the Subcommittee on Antimicrobial Susceptibility Testing. 2018. CLSI Methods Development and Standardization Working Group best practices for evaluation of antimicrobial susceptibility tests. J Clin Microbiol. 2018;56:01934. [PMID: 29367292] [PMCID: PMC5869819] [DOI]
14. Henry RR. Salmonella infection in ducks. 2000.
15. Jamali H, Radmehr B, Ismail S. Prevalence and antimicrobial resistance of Listeria, Salmonella, and Yersinia species isolates in ducks and geese. Poultry Science. 2014;93(4):1023-30. [PMID: 24706981] [DOI]
16. Staji H, Rezaei S, Rassouli M, Namroodi S. Prevalence and genetic characteristics of Salmonella strains in wild Mallard ducks (Anas platyrhynchos) in Semnan suburb, Iran. Bulgarian Journal of Veterinary Medicine. 2017;20(4). [DOI]
17. Tsai H-J, Hsiang P-H. The prevalence and antimicrobial susceptibilities of Salmonella and Campylobacter in ducks in Taiwan. Journal of Veterinary Medical Science. 2005;67(1):7-12. [PMID: 15699587] [DOI]
18. Hanh TT, Thanh NT, Thoa HQ, Thi LT, Thuan LM, Ly NT. Prevalence of Salmonella spp. in poultry in Vietnam. Annals of the New York Academy of Sciences. 2006;1081(1):266-8. [PMID: 17135523] [DOI]
19. Asawy A, ABD EL-LATIF M. Some bacteriological and serological studies on enteritis in ducks. Assiut Veterinary Medical Journal. 2010;56(125):1-11. [DOI]
20. Adzitey F, Rusul G, Huda N. Prevalence and antibiotic resistance of Salmonella serovars in ducks, duck rearing and processing environments in Penang, Malaysia. Food Research International. 2012;45(2):947-52. [DOI]
21. Cha S-Y, Kang M, Yoon R-H, Park C-K, Moon O-K, Jang H-K. Prevalence and antimicrobial susceptibility of Salmonella isolates in Pekin ducks from South Korea. Comparative immunology, microbiology and infectious diseases. 2013;36(5):473-9. [PMID: 23618792] [DOI]
22. Kim H, Lee J, Jang Y, Chang B, Kim A, Choe N. Prevalence and antimicrobial resistance of Salmonella spp. and Escherichia coli isolated from ducks in Korea. Korean Journal of Veterinary Research. 2016;56(2):91-5. [DOI]
23. Dey R, Khan M, Nazir K, Islam M, Belal S. Epidemiological investigation on duck salmonellosis in some selected areas of Bangladesh. Bangladesh Journal of Veterinary Medicine. 2016;14(2). [DOI]
24. Abou Zeid MA, Nasef SA, Ali G, Hegazy A. A field study on biochemical changes associated with salmonella infection in ducklings. Journal of World's Poultry Research. 2020;10(2s):250-62. [DOI]
25. Kang X, Wang M, Meng C, Li A, Jiao X, Pan Z. Prevalence and whole-genome sequencing analysis of Salmonella reveal its spread along the duck production chain. Poultry Science. 2022;101(9):101993. [PMID: 35839552] [PMCID: PMC9289855] [DOI]
26. Chousalkar K, Gole VC. Salmonellosis acquired from poultry. Current Opinion in Infectious Diseases. 2016;29(5):514-9. [PMID: 27434307] [DOI]
27. Price JI, Dougherty 3rd E, Bruner D. Salmonella infections in white pekin duck. A short summary of the years 1950-60. Avian Diseases. 1962:145-7. [DOI]
28. Harsha H, Reshmi R, Varghese R, Divya PS, Rahiman KM, Hatha AM. Prevalence and antibiotic resistance of Salmonella from the eggs of commercial samples. Journal of Microbiology and Infectious Diseases. 2011;1(03):93-100. [DOI]

29. Flament A, Soubbotina A, Mainil J, Marlier D. Prevalence of Salmonella serotypes in male mule ducks in Belgium. Veterinary Record. 2012;170(12):311-. [PMID: 22266688] [DOI]
30. Owen M, Jorgensen F, Willis C, McLauchlin J, Elviss N, Aird H, et al. The occurrence of Salmonella spp. in duck eggs on sale at retail or from catering in England. Letters in applied microbiology. 2016;63(5):335-9. [PMID: 27561893] [DOI]
31. Yang J, Ju Z, Yang Y, Zhao X, Jiang Z, Sun S. Serotype, antimicrobial susceptibility and genotype profiles of Salmonella isolated from duck farms and a slaughterhouse in Shandong province, China. BMC microbiology. 2019;19:1-12. [PMID: 31477003] [PMCID: PMC6720067] [DOI]
32. Tran TP, Ly TLK, Nguyen TT, Akiba M, Ogasawara N, Shinoda D, et al. Prevalence of Salmonella spp. in pigs, chickens and ducks in the Mekong Delta, Vietnam. Journal of Veterinary Medical Science. 2004;66(8):1011-4. [PMID: 15353859] [DOI]
33. Binh DX, Lan DTM, Lan PTP, Minh NN. PREVALENCE AND VIRULENCE GENE OF Salmonella STRAINS ISOLATED FROM DUCKS IN NORTH VIETNAM. Suranaree Journal of Science & Technology. 2020;27(1).
34. Su Y-C, Yu C-Y, Lin J-L, Lai J-M, Chen S-W, Tu P-C, et al. Emergence of Salmonella enterica serovar Potsdam as a major serovar in waterfowl hatcheries and chicken eggs. Avian Diseases. 2011;55(2):217-22. [PMID: 21793436] [DOI]
35. Saitanu K, Jerngklinchan J, Koowatananukul C. Incidence of salmonellae in duck eggs in Thailand. Southeast Asian Journal of Tropical Medicine and Public Health. 1994;25:328-.
36. Ezatpanah E, Moradi Bidhendi S, Khaki P, Ghaderi R, Seyedan Jasbi E, Moghtadaee Far S. Isolation, serotyping and antibiotic-resistance pattern of isolated Salmonella from chicken of Arak. Iranian Veterinary Journal. 2013;9(2):88-96.
37. Asadpour Y, Mohammadi M, Pourbakhsh S, Rasa M. Isolation, serotyping and antibiotic resistance of Salmonella isolated from chicken carcasses in Guilan province. Iranian veterinary journal. 2014;9(4):5-13.
38. Peighambari, Morshed, Rima, Baziar, Sharifi, Aryan, et al. Salmonellosis in broiler flocks of Golestan province: Frequency, serogroups and drug resistance patterns of Salmonella isolates. Updates in veterinary microbiology. 2018;1(1):70-8.
39. Peighambari SM, Morshed R, Shojadoost B, Nikpiran H, Haghbinn Nazarpak H, Khakpour M, et al. Survey of non-typhoid Salmonella infections among some broiler flocks of Mazandaran and Gilan provinces, 2010-2015. Iranian Journal of Veterinary Clinical Sciences. 2019;12(2).
40. M'ikanatha NM, Sandt CH, Localio AR, Tewari D, Rankin SC, Whichard JM, et al. Multidrug-resistant Salmonella isolates from retail chicken meat compared with human clinical isolates. Foodborne pathogens and disease. 2010;7(8):929-34. [PMID: 20443729] [DOI]
41. Gieraltowski L, Higa J, Peralta V, Green A, Schwensohn C, Rosen H, et al. National outbreak of multidrug resistant Salmonella Heidelberg infections linked to a single poultry company. PloS one. 2016;11(9):e0162369. [PMID: 27631492] [PMCID: PMC5025200] [DOI]
42. Trongjit S, Angkititrakul S, Tuttle RE, Poungseree J, Padungtod P, Chuanchuen R. Prevalence and antimicrobial resistance in Salmonella enterica isolated from broiler chickens, pigs and meat products in Thailand–Cambodia border provinces. Microbiology and immunology. 2017;61(1):23-33. [PMID: 28042666] [DOI]