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Study of *salmonella* infection in broiler turkey carcasses in some provinces in Iran; molecular detection and antimicrobial susceptibility pattern

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ABSTRACT

Salmonella, as one of the most important zoonotic foodborne pathogens, is a major global threat to the health of human communities and animals and its impact is economically significant in the world. This study aimed to investigate Salmonella infection and bacterial susceptibility test on possible isolates in some broiler turkey flocks in Iran. For this purpose, diagnostic samples were collected at slaughterhouse from 15 different broiler turkey flocks at the ages of 16 to 18 weeks, from different geographical regions of the country. In this regard, 15 complete intestinal samples were taken from each flock and a total of 225 intestinal samples were collected and sent to the laboratory for culture, isolation and identification of Salmonella. From every 5 intestinal samples different parts were pooled and cultured in selenite F medium and transferred to Salmonella Shigella agar (SS) and MacConkey agar (MAC) media after 24 hours, then suspected Salmonella colonies were transferred to the differential medias. Salmonella positive colonies were used for serogroup identification and all isolates were identified being from D serogroup. The PCR were performed for identification of Salmonella spp and 7 Salmonella isolates were confirmed. Then the PCR with specific primers for the identification of *Salmonella* Enteritidis was performed on 5 isolates and 4 isolates were identified as *Salmonella* Enteritidis. In addition, antimicrobial susceptibility test was performed on 7 isolates and it was found that all of them were completely sensitive to Fosfomycin and the highest resistance was observed in Colistin.

1. Introduction

Salmonella is considered as one of the most important foodborne zoonose pathogens present among humans and animals which causes many gastrointestinal infections (Kurtz et al., 2017).

Salmonella species are rod-shaped, gram-negative, anaerobic bacteria belonging to the Enterobacteriaceae family (Davin-Regli et al., 2019). One of the most common Salmonella serotypes in human infections is *Salmonella* Typhimurium and *Salmonella* Enteritidis, which are commonly transmitted to humans through the consumption of contaminated poultry products including meat and egg (Rostagno et al., 2006; Su et al., 2018).

Statistical studies on salmonella infection epidemies in humans considered poultry, especially turkyes as one of the three main sources of Salmonella food contamination (Greig and Ravel, 2009; Tietjen and Fung, 1995). High consumption of poultry products as

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cheap protein sources has made them the most important source of Salmonella infection in humans (Acar et al., 2017; Campioni et al., 2012).

Due to the contaminations occuring in the slaughterhouse during storage and marketing of the poultry meat, checking the presence of salmonella is of particular importance in poultry flocks (Authority et al., 2020).

The occurence of Salmonella contamination in food in addition to the importance of public health, from an economic point of view is associated with great economic losses due to the trade ban in contaminated poultry products (Sodagari et al., 2020; Soumet et al., 1999).

Therefore, according to the high reports of Salmonella infections in poultry flocks in Iran and grownig industry of turkey breeding in the country and the lack of sufficient information about salmonella infection in turkey flocks in Iran. The purpose of this study is to investigate salmonella infection and antimicrobial susceptibility tests on possible isolates in some broiler turkey flocks in the country (Akbarian et al., 2012).

2. Materials and Methods

2.1. Sampling

In this study, a total of 225 whole intestine samples were collected from 15 different broiler turkey flocks from five provinces of the country including Qom, Hamedan, Markazi, Lorestan, and Esfahan which were sent to a slaughterhouse in Qom province (in the center of Iran). In this way, after recording the data of each flock, 15 complete intestinal samples were randomly taken from each flock. The age of the poults were 16-18 weeks old at the time of slaughter and weighed an average of 16 kg and all were from white Holland breed. Sampling was performed immediately after slaughter then the samples were sent to the laboratory separately in the sterile plastic bags packed with dry ice for culture and isolation of Salmonella.

2.2. Bacteriological culture and identification

Different parts of every 5 intestinal samples (including duodenum, cecum, colon and cecal tonsils) were pooled. The samples were cultured in 250 cc of selenite F broth.Then after 24 hours of incubation at 37 ° C, they were transferred to selected culture media of Salmonella Shigella agar and MacConkey agar and placed in incubator at 37 ° C for 24 hours, then suspected Salmonella colonies, colorless tiny and slightly raised colonies with smooth edge, were cultured in differential TSI and Urea medias for another 24 hours at 37°C, afterwards Salmonella positive colonies were cultured in SIM medium to determine bacterial motility (Waltman and Mallinson, 1995).

2.3. Determination of Serogroups

Salmonella positive colonies were used for serogroup identification. First, the presence of Salmonella was confirmed by using polyvalent antiserum(Bahar Afshan Company, Iran) through agglutination reaction on the slide surface, then A, B, C, D antiseras were used to determine the salmonella serogroups (Wattiau et al., 2011).

2.4. DNA extraction

DNA extraction of isolated Salmonella was performed using a commercial DNA extraction kit (Sinapure DNA kit, Sinaclon, Iran).

2.5. PCR assay

Two pairs of primers (Sina clone, Iran) from different gene sequences used to amplify the DNA fragments expected to detect Salmonella spp and *Salmonella* Enteritidis serotype including ST11-ST15 primers specific for Salmonella species and sef167-sef478 primers specific for *Salmonella* Enteritidis (Aabo et al., 1993; Woodward and Kirwan, 1996). (Table 1).

The PCR was performed in a reaction containing 50 mmol l^{-1} Kcl, 1/5 mmol l^{-1} $Mgcl_2$, 10 mmol l^{-1}Tris- Hcl, 100 μmol l^{-1} dNTP, 1U Taq polymerase (Sina clone, Iran) ,0·6 μmol l^{-1} primers (Sinaclon, Iran) and 0·1% Gelatin.

The amplification cycles were performed automatically in a thermocycler (Applied Biosystems, USA) as follows: 35 cycles of 30 s for denaturation at 94°C, 90 s for annealing at 56°C and 30 s for primer extension at 72°C followed by a terminal extension at 72°C for 10 min. Then the PCR products were electrophoresed on the 1.7% agarose gel with100 volts for 1 hour. Photography was

performed under UV light by gel doc system (Vilber Lourmat, France).

Table 1. Primer sequences were used for the PCR reaction

Target sequence	Primer sets	Length	sequence 5´···· 3´	Amplification region (bp)	Refrences
Random sequence	ST11	25	AGCCAACCATTGCTAAATTGGCGCA	429	15 & 16
	ST15	24	GGTAGAAATTCCCAGCGGGTACTG		
sefA gene	Sef167	20	AGGTTCAGGCAGCGGTTACT	312	15 & 16
	Sef478	20	GGGACATTTAGCGTTTCTTG		

2.6. Antimicrobial susceptibility test

Antibiotic susceptibility pattern in confirmed Salmonella isolates was determined by qualitative disk diffusion method, according to Kirby-Bauer standard for antibiogram testing, in Müller-Hinton agar medium(Bauer et al., 1966; Jorgensen and Turnidge, 2015).

The antibiotic discs (Padtan Teb Co-Iran) used in this study and their potential concentrations in micrograms include Danofloxacin (DFX; 10 µg), Enrofloxacin (ENR; 5 µg), Lincomycin-Spectinomycin (LS; 15/200 µg), Oxytetracyclin (T; 30 µg), Doxycyclin (DOX; 30 µg), Florfenicol (FF; 30 µg), Sulfamethaxazole+Trimethoprim (SXT; 23/75 + 25/1 µg), Colistin (CL; 10 µg), Neomycin (N; 30 µg) and Fosfomycin (FOS; 50 µg). The results were reported as sensitive (+3), semi-sensitive (+2) and resistant (+1) using the manufacturer's zone of inhibition interpretation chart (Padtan Teb Co-Iran).

2.7. Statistical analysis

The data obtained from intestinal samples were statistically analyzed by SPSS software version 24. Binomial test (P-values <0.05) was used to evaluate the difference between the results of Salmonella samples among 15 broiler turkey flocks.

3. Results

In 5 out of 15 broiler turkey flocks bred in different geographical regions of the country, 7 Salmonella (motile) isolates were obtained. The prevalence rate of infection was evaluated 15.5% (Table 2). A significant difference (P <0.05) was found in the detection of Salmonella isolates in intestinal samples. In serogroup determining, it was found that all isolates belonged to D serogroup. The PCR test was performed for 5 Salmonella isolates using one isolate as the indicator of each flock by which Salmonella genus was confirmed in all of the tested isolates and *Salmonella* Enteritidis in 4 of them (Fig. 1 and 2). The results of antimicrobial susceptibility test indicated all isolates being completely sensitive to Fosfomycin with the highest resistance seen in Colistin. A resistance to two to three antibiotics was also observed in some isolates (Tables 3 and 4).

4. Discussion

Studies on various protein sources have shown that poultry meat, especially turkey is the main source of Salmonella contamination in the world. Therefore, monitoring the presence of Salmonella infection beside rapid and accurate detection of the infection by the new methods such as PCR, helps to prevent the spread of this infection (Acar et al., 2017; Vinayaka et al., 2019).

The aim of this study was isolation and detection of Salmonella from broiler turkey flocks bred in different regions of the country and to investigate bacterial susceptibility of possible isolates. Salmonella was identified in 5 out of 15 broiler turkey flocks (33.3%) and 7 isolates were obtained. This indicates a significant infection with a prevalence of %15.5 in the studied turkey flocks.

Salmonella paratyphoid infections in poultry are usually associated with colonization in the intestines. Therefore, sampling of intestinal tissues with their contents is more accurate for isolation of Salmonella comparing to the other sampling methods.

The results of a similar study on chickens and turkeys showed that Salmonella isolated from intestinal specimens accounted for 78% and 70% of positive cases, respectively. These findings confirm the preference of sampling from intestinal tissues with their contents to the

other methods such as cloacal swabs and fecal samples (Faddoul and Fellows, 1966).

Table 2. Herd data and Salmonella isolation results

Herd number	Geographica location of the herd	Average weight of herd (Kg)	Number of birds	Number of isolates	Serotype / serum group
1	Markazi	12.5	1300	0	
2	Lorestan	17	6000	1	SE/D
3	Lorestan	16.5	16000	0	
4	Markazi	15	20000	2	SE/D
5	Markazi	13.5	5000	1	SE/D
6	Hamedan	22	11000	0	
7	Hamedan	18.5	3000	1	D
8	Hamedan	20	7000	0	
9	Kashan	13.5	10000	0	
10	Qom	11	14000	0	
11	Qom	19.5	8000	0	
12	Markazi	21	2000	0	
13	Markazi	13.5	6000	0	
14	Lorestan	13.5	3000	0	
15	Lorestan	14	8000	2	SE/D

SE: Salmonella Enteritidis

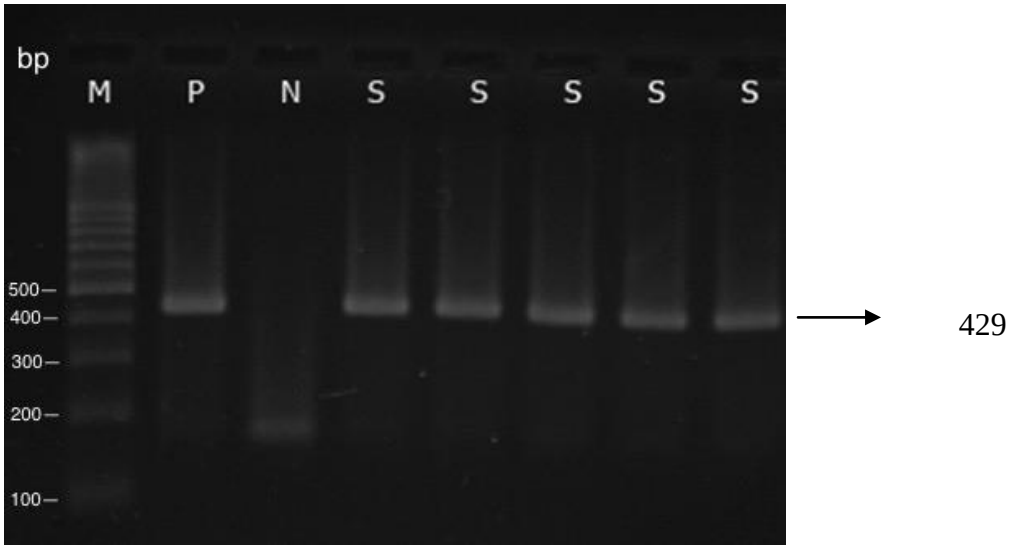

Fig 1. PCR analysis. The 429bp specific fragment from ST11/ST15 primers: Lane M: 100 bp DNA ladder molecularweight marker (Thermo Scientific O'GeneRuler 100 bp DNA Ladder) Lane P: positive control(Salmonella, laboratory approved isolate). Lane N: negative control (Sterile distilled water). Lanes S: *Salmonella* spp. positive samples

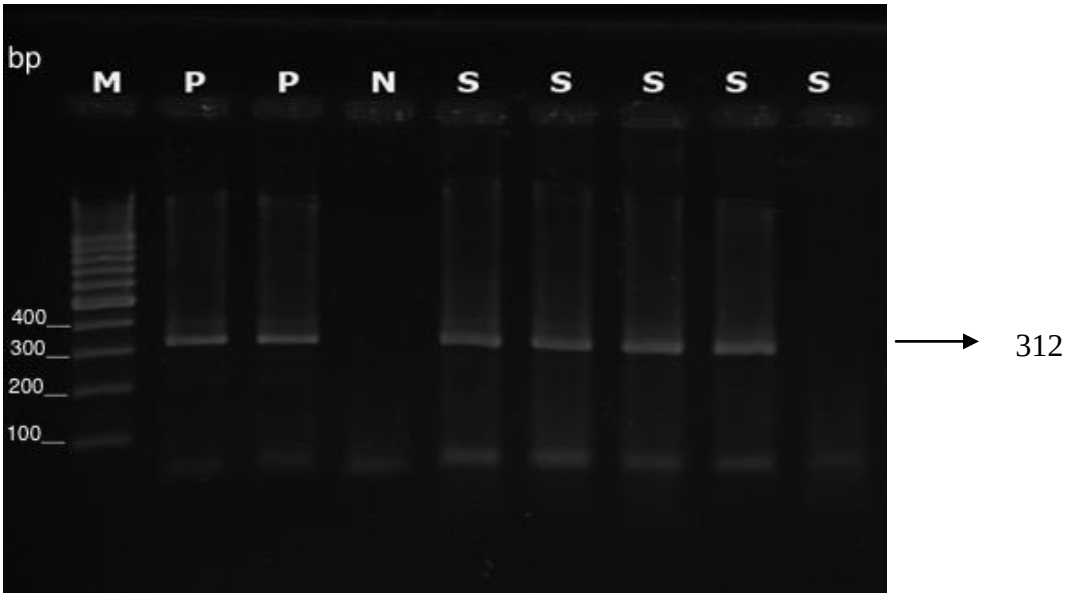

Fig 2. PCR analysis. The 312bp specific fragment from Sef167/Sef478 primers: Lane M: 100 bp DNA ladder molecular weight marker (Thermo Scientific O'GeneRuler 100 bp DNA Ladder) Lanes P: positive control (Salmonella, laboratory approved isolate). Lane N: negative control (Sterile distilled water). Lanes S: *Salmonella* Enteritidis positive samples

Table 3. Antimicrobial susceptibility patern of 7 Salmonella isolates obtained from 5 positive herds

Number and geographical location of the herd	Number of isolates	SXT	DFX	ENR	N	CL	FF	LS	FOS	T	DOX
Lorestan,2	1	MS	MS	MS	MS	R	MS	MS	S	MS	MS
Markazi,4	2	MS	MS	MS	MS	MS	S	S	S	MS	MS
Markazi,5	1	R	MS	R	MS	MS	MS	S	S	MS	MS
Hamedan,7	1	S	R	S	MS	R	S	MS	S	MS	R
Lorestan,15	2	MS	R	R	MS	R	MS	MS	S	MS	MS

(R, resistant; S, sensitive; MS, medium sensitivity)

FOS, Fosfomycin; LS, Lincomycin-Spectinomycin; FF, Florfenicol; CL, Colistin; N, Neomycin; ENR, Enrofloxacin; DFX, Danofloxacin; SXT, Sulfamethaxazole-Trimethoprim; T, Oxytetracyclin; DOX, Doxycyclin

Table 4. Sensitivity or resistance of Salmonella isolates to 10 antibiotic compounds

Row	Drug	Sensitive number (%)	Medium sensitivity number(%)	Resistant number (%)
1	Fosfomycin	7 (100)	0(0)	0 (0)
2	Lincomycin-Spectinomycin	3 (42.85)	4 (57.14)	0 (0)
3	Florfenicol	3 (42.85)	4 (57.14)	0 (0)
4	Colistin	0 (0)	3 (42.85)	1 (14.28)
5	Neomycin	0 (0)	7 (100)	0 (0)
6	Enrofloxacin	1 (14.28)	3 (42.85)	3 (42.85)
7	Danofloxacin	0 (0)	4 (57.14)	3 (42.85)
8	Sulfamethoxazole and Trimethoprim	1 (14.28)	5 (71.42)	1 (14.28)
9	Oxytetracycline	0 (0)	7 (100)	0 (0)
10	Doxycycline	0 (0)	6 (85.71)	1 (14.28)

Examination of serogroup and motility of the isolates revealed all isolates as motile salmonellae belonging to D serogroup.

Identification of *Salmonella* Enteritidis in 4 out of 5 isolates indicates this serotype as the most common serotype in broiler turkey flocks. Moreover, identification of *Salmonella* Enteritidis is of particular importance because of being one of the main causes of Salmonella infections occuring in humans (Greig and Ravel, 2009).

In one study in Shanghai on Salmonella contamination of protein sources such as beef, pork, mutton and chicken, *Salmonella* Enteritis and *Salmonella* Typhimurium were respectively the most prevalent serotypes involved in contaminations which is consistent with the results of the present study (Ni et al., 2017).

Another similar study in broiler turkeys flocks showed that *Salmonella* Worthington, *Salmonella* Anatum and *Salmonella* Agonal have the highest frequency among isolates which is not corresponded to the results of the present study, indicating the variant prevalence of dominant serotypes relying on geographical origins (Sanad et al., 2016).

Also, in the study of an outbreak of human Salmonella in Europe between 2011 and 2013 *Salmonella* Stanley was found as the causative agent of the epidemy and turkey as the main source in transmition and initiation of the epidemy. This study shows the importance of turkey's role in transmitting salmonella infections to the human communities (Kinross et al., 2014).

In antimicrobial susceptibility test of the current study, 100% sensitivity for Fosfomycin was seen in all isolates with a resistance to Colistin seen in majority of them. In addition, in some isolates resistance to two or three antibiotics were observed.

In the study conducted in Taiwan, the most common serovars isolated from turkyes at different ages were *Salmonella* Albany, *Salmonella* Schwarzengrund and *Salmonella* Hadar, respectively. This finding is inconsistent with the findings of our study and shows that the prevalent serotype in each country or geographical region can differ with the other regions and constant monitoring of the infection in turkey flocks is of major concern. Also in susceptibility profile, the highest resistance was observed in Florfenicol, Oxytetracycline, and Colistin. The result of the antimicrobial susceptibility test of this study regarding Colistin is consistent with the present study in which approximately 57% of isolates were resistant to it (Yeh et al., 2018).

Another study on breeder turkey flocks in Morocco, reported the prevalence of Salmonella was 35% and *S.* Kentucky and *S.* Parkroyal serotypes being the most prevalent serotypes. Multiple drug resistance was observed in 80.64% of species and the highest resistance was seen for Tetracycline. Despite the high prevalence of salmonella infection in this study which is consistent with the current study antimicrobial susceptibility patterns look different. This inconsistent findings could be due to differences in the pattern of antibiotics used in turkey flocks for their bacterial treatments or differences in serotypes and strains of isolates related to differences in geographical regions or the research limitations (El Allaoui et al., 2017).

Conclusion

The results of this study indicate that there is a significant incidence of Salmonella infection in some broiler turkey flocks of Iran and the predominant serotype is *Salmonella* Enteritidis. However, because the number of sampled flocks was limited; therefore, it is difficult to make a right conclusion about the frequency and epidemiology of the infection for the all broiler tukey flocks in the country. Due to the importance of paratyphoid Salmonella, especially *Salmonella* Enteritidis as one of the most important foodborne pathogens in humans, this level of contamination and antibiotic resistance is very important in terms of public health. For this purpose, extensive studies and monitoring of turkey flocks and their products contamination to Salmonella in other regions of the country are recommended along with the more accurate and precise implementation of biosecurity measures.

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