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Original Article

Prevalence and antimicrobial resistance of *Salmonella* serotypes isolated from retail chicken meat and giblets in Iran

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Abstract

Introduction: *Salmonella* is one of the major foodborne pathogens responsible for outbreaks of foodborne illness in humans worldwide.
Methodology: A total of 560 samples of chicken meat and giblets were collected from retail markets for *Salmonella* identification, serotyping, and antimicrobial resistance testing.
Results: *Salmonella* was detected in 19.8% of samples. Among the five serotypes identified, *S.* Thompson was the predominant type (48.7%). High antimicrobial resistance rates were observed to nalidixic acid (92.8%), tetracycline (81%), trimethoprim (68.4%), sulfamethoxazole / trimethoprim (61.2%), streptomycin (56.7%), and kanamycin (36.9%). Although resistance to chloramphenicol (3.6%), amoxicillin-clavulanic acid (5.4%), and ampicillin (11.7%) was detected, none of the isolates were resistant to ceftazidime, ceftriaxone, cefotaxime, ciprofloxacin, colistin, gentamicin, nor imipenem.
Conclusions: Restrictions on the irrational use of antibiotics in humans and animals are suggested for the reduction of resistant strains.

Key words: *Salmonella*; chicken meat and giblets; serotypes; antimicrobial resistance.

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Introduction

Salmonella is one of the major foodborne pathogens worldwide [1,2] and is responsible for outbreaks of foodborne illness in humans via cross-contamination and consumption of undercooked meats [3,4]. It has been estimated that *Salmonella* has caused approximately one million cases of foodborne illness and 378 deaths per year in the United States [5]. *Salmonella* spp. is also the most commonly reported cause of foodborne outbreaks (39.2%) in the European Union (EU), with 2,201 outbreaks in 2007, of which 142 occurred in France [6]. High percentages (approximately 70%–80%) of foodborne bacterial outbreaks in China were caused by *Salmonella* [7]. Although other food products, such as eggs and red meats, can be contaminated with *Salmonella* spp., poultry products have been recognized to be the main source of this pathogen in humans [8]. Contamination of poultry meat can occur at different stages, including production, slaughter, processing, handling, and storage [9]. Antimicrobial agents are widely use in veterinary medicine, not only for prevention and treatment of diseases, but also as growth-promoting substances. However, the widespread use of antibiotics promotes the development of antimicrobial-resistant bacteria, potentially worldwide [10]. The prevalence of *Salmonella* serotypes and their antimicrobial resistance in chicken meat and giblets have been reported in many investigations around the world [11-20]. Despite considerable of progress in human public health, *Salmonella* still remains a significant foodborne pathogen in the food chain. Based on the importance of this pathogen in human public health, this study was carried out to determine the prevalence and antimicrobial resistance of *Salmonella* strains isolated from retail chicken meat and giblets in Alborz Province, Iran.

Methodology

A total of 560 samples of retail chicken meat (200) and giblets, which included liver (120), gizzard (120), and heart (120), were purchased from retail markets in Alborz Province between October 2013 and March 2014. All samples were transferred under cold conditions (using icepacks) to the laboratory for microbial examination. For *Salmonella* identification, 25g of each meat sample was homogenized for 2 minutes with 225 mL of buffered peptone water

(Merck, Darmstadt, Germany) and then incubated for 24 hours at 37°C. After incubation, 0.1 mL of the broth was transferred into 10 mL of selenite cystine broth (Merck) and incubated at 42°C for 24 hours. The enrichment samples were then subcultured on to Salmonella-Shigella agar and brilliant green agar (both Merck) and incubated for 24 hours at 37°C. Presumptive *Salmonella* isolates were identified by using triple sugar iron agar, lysine iron agar, and urea agar (all Merck) at 37°C.

Serotyping

Salmonella isolates were further serotyped by direct agglutination method using antisera against O and H antigens (BioRad, Marnes-la-Coquette, France) and the Kauffman-White classification schema [21].

Antimicrobial susceptibility test

The antimicrobial susceptibility of *Salmonella* isolates was determined by the disk diffusion method on Mueller-Hinton agar (Britania, Buenos Aires, Argentina), performed according to the Clinical and Laboratory Standards Institute guidelines [22]. The following antibiotics were used: amoxicillin-clavulanic-acid (30µg), ampicillin (10µg), ceftazidime (30µg), ceftriaxone (30µg), cefotaxime (30µg), chloramphenicol (30µg), ciprofloxacin (5µg), colistin (10µg), gentamicin (10µg), kanamycin (30µg), imipenem (10µg), nalidixic acid (30µg), streptomycin (10µg), sulfamethoxazole / trimethoprim (1.25/23.75 µg), tetracycline (30µg), and trimethoprim (5µg). After incubation at 35°C for 24 hours, the zone of inhibition around each disk was measured and evaluated according to the Clinical and Laboratory Standards Institute guidelines [22]. *Escherichia coli* ATCC25922 was used as a quality control strain.

Results and Discussion

Of 560 examined samples, 111 (19.8%) were contaminated with *Salmonella* spp. Table 1 shows the prevalence of *Salmonella* spp. isolated from examined samples. The incidence of *Salmonella* spp. in chicken products obtained by other authors varied between 0 and 100% [23,24]. In this study, the overall prevalence of *Salmonella* in chicken meat and giblets was 19.8% (111/560).The contamination rate of chicken meat samples (29%) is in agreement with results reported in Belgium [25], the United Kingdom [26], Iran [27],China [28], Turkey [29], Iraq [30], and the Russian Federation [31], but lower than results found in Iran [20,32] and many other countries, such as China, Mexico, and Poland [16,18,33-35]. The incidence of *Salmonella* spp. was found to be 21.6% in chicken liver samples, 14.1% in heart samples, and 8.3% in gizzard samples. In a previous study in Iran, the incidence of *Salmonella* spp. contamination in chicken liver, heart, and gizzard was found to be 18%, 6%, and 4%, respectively [36], which was lower than that found in the present study. However, contamination levels higher than those in this study were reported from Egypt and Ethiopia at incidence rates of 40% and 48%, respectively, in chicken livers, and 34.5% and 23.7%, respectively, in chicken hearts [37,11]. The reasons for the higher incidence of *Salmonella* contamination in chicken meat than in giblets in the present study can be due to the defeathering process, which may spread microorganisms between carcasses [37]. All previous studies showed that *Salmonella* was widely distributed in retail meat globally, which increased salmonellosis following consumption of contaminated meat or cross-contamination in households. This risk may be higher if chicken meat or giblets are consumed undercooked [38,25]. Data on the prevalence of *Salmonella* in different studies were difficult to compare because the observed prevalence may be biased by diversity in sampling methods, sampling seasons, and techniques [28]. However, variations observed between the reported *Salmonella* prevalence in previous investigations around the world may be due to several factors, including the initial salmonellosis in live birds, sanitation within the slaughterhouse, possible contamination during poultry processing steps (*e.g.*, the amount of cross-contamination of chicken carcasses by contact with intestinal tracts during slaughter or processing), and differences among isolation methods applied to detect *Salmonella* [36]. Among the 111 positive isolates found in the present study, five different serotypes (*S.* Thompson, *S.* Enteritidis, *S.* Typhimurium, *S.* Newport, and *S.* Hadar) were completely identified in 107 samples. *S.* Thompson (48.7%) was the serotype most frequently detected, followed by *S.* Enteritidis (22.5%), *S.* Typhimurium (12.6%), *S.* Newport (7.2%), and *S.* Hadar (5.4%). Four isolates (3.6%) were not completely serotyped (Table 2). *S.* Thompson was the predominant serotype in uncooked chicken and beef in Tehran. It is one of the most important *Salmonella* serotypes that can cause outbreaks and infection in poultry [32]. In the United States, the Centers for Disease Control and Prevention (CDC) has reported that *S.* Enteritidis, *S.* Typhimurium, and *S.* Newport are the most prevalent serotypes reported by public health laboratories [39].

Table 1. Prevalence of *Salmonella* isolated from chicken meat and giblets

Sample type	Number of samples		
	Examined	**Positive**	**%**
Chicken meat	200	58	29
Liver	120	26	21.6
Heart	120	17	14.1
Gizzard	120	10	8.3
Total	**560**	**111**	**19.8**

Table 2. Distribution of *Salmonella* serotypes in chicken meat and giblets

Serotype	Number of positive samples				
	Chicken meat	**Liver**	**Heart**	**Gizzard**	**Total**
S. Thompson	35 (31.6%)	8 (7.2%)	6 (5.4%)	5 (4.5%)	54 (48.7%)
S. Enteritidis	12 (10.8%)	7 (6.3%)	4 (3.6%)	2 (1.8%)	25 (22.5%)
S. Typhimuruim	3 (2.7%)	7 (6.3%)	2 (1.8%)	2 (1.8%)	14 (12.6%)
S. Newport	0 (0.0%)	3 (2.7%)	5 (4.5%)	0 (0.0%)	8 (7.2%)
S. Hadar	6 (5.4%)	0 (0.0%)	0 (0.0%)	0 (0.0%)	6 (5.4%)
NCS*	2 (1.8%)	1 (0.9%)	0 (0.0%)	1 (0.9%)	4 (3.6%)
Total	**58 (52.3%)**	**26 (23.4%)**	**17 (15.3%)**	**10 (9%)**	**111 (100%)**

*Not completely serotyped

Table 3. Prevalence of antimicrobial resistance of *Salmonella* serotypes isolated from retail chicken meat and giblets

Serotypes	No. of isolates	Antimicrobials n (%)															
		AMC	AMP	CTZ	CRO	CTX	CHL	CIP	COL	GEN	IMI	KAN	NAL	STR	SXT	TET	TMP
S. Thompson	54	3 (5.5)	7 (12.9)	0 (0)	0 (0)	0 (0)	2 (3.7)	0 (0)	0 (0)	0 (0)	0 (0)	17 (31.5)	52 (94.4)	32 (59.2)	39 (72.2)	49 (90.7)	41 (75.9)
S. Enteritidis	25	0 (0)	2 (8)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	11 (44)	22 (88)	14 (56)	13 (52)	12 (48)	17 (68)
*S.*Typhimurium	14	0 (0)	2 (14.2)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	5 (35.7)	12 (85.7)	8 (57.1)	9 (64.2)	12 (85.7)	11 (78.5)
S. Newport	8	2 (25)	1 (12.5)	0 (0)	0 (0)	0 (0)	1 (12.5)	0 (0)	0 (0)	0 (0)	0 (0)	3 (37.5)	7 (87.5)	4 (50)	0 (0)	8 (100)	1 (12.5)
S. Hadar	6	0 (0)	1 (16.6)	0 (0)	0 (0)	0 (0)	1 (16.6)	0 (0)	0 (0)	0 (0)	0 (0)	4 (66.7)	6 (100)	3 (50)	5 (83.3)	6 (100)	4 (66.6)
NCS	4	1 (25)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	1 (25)	4 (100)	2 (50)	2 (50)	3 (75)	2 (50)
Total	**111**	**6 (5.4)**	**13 (11.7)**	**0 (0)**	**0 (0)**	**0 (0)**	**4 (3.6)**	**0 (0)**	**0 (0)**	**0 (0)**	**0 (0)**	**41 (36.9)**	**103 (92.8)**	**63 (56.7)**	**68 (61.2)**	**90 (81)**	**76 (68.4)**

AMC: amoxicillin-clavulanic acid; AMP: ampicillin; CTZ: ceftazidime; CRO: ceftriaxone; CTX: cephotaxime; CHL: chloramphenicol; CIP: ciprofloxacin; COL: colistin; GEN: gentamicin; IMI: imipenem; KAN: kanamycin; NAL: nalidixic acid; STR: streptomicin; SXT: sulfamethoxazole/trimethoprim; TET: tetracycline; TMP: trimethoprim; NC: not completely serotyped

All these serotypes are common isolates from chicken meat. *Salmonella* serotypes detected in this study are in agreement with CDC report. Previous studies have reported some of the serotypes that were identified in this study [13,14,16,20,32,36]. This distribution of *Salmonella* serotypes could vary from country to country. Among the *Salmonella* serotypes, *S.* Enteritidisand, *S.* Typhimuriumare the predominant serotypes found in human salmonellosis in many developed countries, including Japan, Australia, New Zealand, and many countries in Europe [40]. There are reports of human salmonellosis caused by consumption of poultry meat contaminated by *S.* Typhimurium [41]. Although *S.* Typhimurium is the most common agent of human foodborne disease, in the last few decades, *S.* Enteritidis has become more common [42].

The increase in the prevalence of resistant microorganisms is an important problem for the treatment and prevention of infectious diseases in both

Table 4. Multiple antimicrobial resistance patterns of *Salmonella* serotypes isolated from chicken meat and giblets

Serotype	Antibiotic resistance profile (resistance to two or more)	Number of multi-resistant isolates
***S.* Thompson**	NAL, TET, TMP, SXT, STR, KAN, AMP	1
	NAL, TET, TMP, SXT, STR, KAN	3
	NAL, TET, TMP, SXT, STR, AMC	1
	NAL, TET, STR, KAN, TMP	5
	NAL, TET, STR, KAN, SXT	2
	NAL, TET, STR, CIP	1
	NAL, TET, TMP	3
	NAL, TET, SXT	1
	NAL, TET	4
	TET, STR	2
	TET, TMP	3
***S.* Enteritidis**	NAL, TET, TMP, SXT, STR	2
	NAL,TET, STR, KAN	1
	NAL, TET, TMP, SXT	3
	NAL,TET, STR, KAN	2
	NAL, TET, STR	3
	NAL, TET, AMP	1
	NAL, TMP	4
	NAL, AMP	1
***S.* Typhimurium**	NAL, TET, TMP, SXT	2
	NAL, TET, TMP, STR	1
	NAL, TET, SXT, KAN	1
	NAL, TET, TMP	1
	NAL, TET, KAN	1
	TET, TMP, AMP	1
	NAL, TET, AMP	1
***S.* Newport**	NAL, TET, STR, KAN	2
	NAL, TET, STR, TMP	1
	NAL, TET, STR	1
	NAL, TET, KAN, CHL	1
	NAL, TET, AMC	2
	TET, AMP	1
***S.* Hadar**	NAL, TET, TMP, SXT, STR, KAN	3
	NAL, TET, TMP,SXT	1
	NAL, TET, KAN, AMP	1
	NAL, TET, SXT, CHL	1
NCS	NAL, TET, TMP, SXT, KAN	1
	NAL, TET, TMP, STR	1
	NAL, TET, AMC	1
	NAL, STR, SXT	1

AMC: amoxicillin-clavulanic acid; AMP: ampicillin; CTZ: ceftazidime; CRO: ceftriaxone; CTX: cephotaxime; CHL: chloramphenicol; CIP: ciprofloxacin; COL: colistin; GEN: gentamicin; IMI: imipenem; KAN: kanamycin; NAL: nalidixic acid; STR: streptomicin; SXT: sulfamethoxazole/trimethoprim; TET: tetracycline; TMP: trimethoprim; NC: not completely serotyped humans and animals [2]. Antimicrobial resistance in *Salmonella* serotypes has been a global problem, with rates increasing from between 20% and 30% in the early 1990s to as high as 70% in some countries at the turn of the century [43,44]. The results of antimicrobial resistance tests of 111 *Salmonella* isolates to 16 antimicrobials are shown in Table 3.

In this study, high antimicrobial resistance rates were found against nalidixic acid (92.8%), tetracycline (81%), trimethoprim (68.4%), sulfamethoxazole / trimethoprim (61.2%), streptomycin (56.7%), and kanamycin (36.9%). As in the present study, resistance to the above antibiotics have also been frequently reported in a number of other investigations on poultry products in Iran and other countries [12,18-20,28,32,36,45-48]. The *Salmonella* resistance rates to nalidixic acid (92.8%) and tetracycline (81%) found in this study were higher than resistance rates to other antimicrobials because of the overuse of these antimicrobials for treatment and growth promotion in different fields. These findings are comparable with those reported in Iran (nalidixic acid, 90.6%; tetracycline, 71.6%) [20], but higher than those reported in Turkey (nalidixic acid, 62.5%; tetracycline, 6.2%) [13] and China (nalidixic acid, 47%; tetracycline, 50%) [28]. Also, low antimicrobial resistance rates were observed in the present study to chloramphenicol (3.6%), amoxicillin-clavulanicacid (5.4%), and ampicillin (11.7%); these rates were slightly higher than those found by Soltan Dallal *et al*. [32]. Although a few *Salmonella* isolates showed resistance to chloramphenicol, amoxicillin-clavulanic acid, and ampicillin, the isolates were still largely susceptible to these antimicrobials. Luckily, no isolates were identified that were resistant to ceftazidime, ceftriaxone, cephotaxime, ciprofloxacin, colistin, gentamicin, and imipenem. This maybe explained by the limited availability and high cost of these antimicrobials, which would decrease their frequent utilization in veterinary or public health practices in Iran. These antimicrobials can be used effectively to treat *Salmonella* infections. Despite increasing resistance to commonly used antibiotics in animal and human medicine globally, the numbers of multidrug-resistant *Salmonella* isolates continues to increase [2,11]. The percentage of multidrug-resistant *Salmonella* strains observed in this study (62.2%) is higher than that reported in Italy (2.3%) [49] and Iran (23.5%) [20], although lower than that found in Morocco (75.43%) [17], Portugal (75%) [50], Turkey (100%) [13,16,29], Spain (100%) [2], Brazil (100%) [51], Nepal (100%) [52], the United States (92%) [53], Mexico (85.4%) [34], and China (80%) [54]. In the present study, all *S.* Hadar isolates showed multidrug resistance to nalidixic acid and tetracycline (Table 4).

These findings confirm that in Iran, poultry is a major reservoir of multidrug-resistant *Salmonella*, and suggest that it is difficult to achieve successful antimicrobial therapy for human salmonellosis caused by strains of poultry origin. Resistance to two or more antibiotics was found in 26 isolates of *S.* Thompson, followed by 17, 8, 8, 6, and 4 isolates of *S.* Enteritdis, *S.* Typhimurium, *S.* Newport, *S.* Hadar, and not completely serotyped serotypes, respectively (Table 4). The high prevalence of antimicrobial resistance identified in the present study can be explained by the widespread use of common antimicrobials as prophylactics, growth promoter agents, or in veterinary medicine. Furthermore, unlimited access to these agents without a prescription as well as low rates of antibiotic sensitivity tests for the selection of suitable drugs in Iran may be additional reasons for the high level of resistance.

Conclusions

The results of this study suggest that improving sanitation in poultry slaughterhouses could decrease *Salmonella* contamination and, as a result, human disease. Increasingly antibiotic-resistant strains constitute a public health hazard through transmission of these strains to humans via food products. Therefore, conducting antibiotic sensitivity tests and establishing a regular monitoring system for identification of resistance prevalence in food is necessary to reduce the spread of resistant strains.

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