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Class1-3 integrons and antimicrobial resistance profile in *Salmonella* spp. isolated from broiler chicken in Western Iran

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ABSTRACT: *Salmonella* spp. are major etiologies of food-borne infections that are disseminated primarily through poultry to human. Nowadays, the high rate of antimicrobial resistance and the emergence of multi-drug resistant (MDR) strains, worse the threat imposed to the public health. Integrons are attributed as important contributors to MDR isolates. The present research aimed to identify the frequency of integrons 1-3 and the antimicrobial resistance patterns in *Salmonella* spp. isolated from broiler chicken in Western Iran. A total of 500 fecal samples were screened for *Salmonella* phenotypically. The isolates were confirmed genotypically and the frequency of integrons 1-3 was evaluated molecularly among the isolates. Besides, the antimicrobial resistance of the isolates was determined through the agar disk diffusion method. In general, 67 (13.4%) isolates of *Salmonella* spp. were recovered phenotypically, all of which were confirmed molecularly. The incidence of class 1, 2, and 3 integrons was 40.29% (27 isolates), 28.35% (19 isolates), and 11.94% (eight isolates), respectively. Coexistence of integrons was also detected in 26.86% of the isolates including class 1+2 (13 isolates, 40.62%), class 1+3 (2 isolates, 6.25%), and class 1+2+3 (3 isolates, 9.37%). No statistical association was detected between the frequencies of *Salmonella* spp. or *Salmonella*-bearing integron isolates with age, season, and location. The most frequent antimicrobial resistance was exhibited to ampicillin, nalidixic acid, trimethoprim-sulphamethoxazole, and tetracycline; while ciprofloxacin, gentamicin, and ceftazidime were the most effective drugs. 35.82% of the isolates were MDR, all of which harbored at least one class of integrons. Statistical assessment represented an association between the prevalence of integrons and tetracycline, chloramphenicol, streptomycin, and ceftazidime resistance rates. An alarming rate of integrons and MDR frequency among poultry-originated *Salmonella* spp. in the studied region demands the constant stewardship and prudent prescription and use of antibiotics to prevent human infections and preserve the effectiveness of those antibiotics in treating human salmonellosis.

Keywords: *Salmonella*, broiler, integrons 1-3, antimicrobial resistance, Western Iran

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INTRODUCTION

Poultry is incriminated as a leading reservoir of non-typhoid *Salmonella* spp. (Parry et al., 2008). This healthy carrier state may lead to food-borne infections following direct or indirect contact (egg and carcass contamination) (Hugas and Beloeil, 2014). Some previous studies have reported the prevalence of *Salmonella* spp. in broiler in different districts of Iran; like 22.5% in Shiraz (Ansari-Lari et al., 2014), 26% in Mazandaran province (Seifi et al., 2016), 1.8% in Sanandaj (Doulatyabi et al., 2016), 19% in Lorestan province (Haeri and Ahmadi, 2019), and 19.2% in Mashhad (Peighambari et al., 2019). Likewise, the frequency of *Salmonella* spp. isolated from pediatric diarrhea in Tehran, the capital of Iran, was reported as 7% (Farahani et al., 2018).

Although antibiotic therapy is crucial in controlling and treating infectious diseases, recent indiscriminative application of antibiotics has been linked as a major contributor to the emergence of substantial antibiotic resistance in bacterial agents (Gong et al., 2013). The antimicrobial agents which are frequently prescribed in Iranian poultry industry include fosfomycin, enrofloxacin, florfenicol, linco-spectin, colistin, trimethoprim-sulphamethoxazole, tetracycline, tilmicosin, tiamulin, streptomycin, doxycycline, gentamycin, amoxicillin, and bacitracin. In addition to the zoonotic importance of the infection, emergence and dispersion of multi-drug resistant (MDR) *Salmonella* spp. constitutes a pre-eminent health issue in both public and veterinary sectors worldwide (Shan and Huang, 2010). Integrons are attributed as ambulatory DNA elements to the dissemination of antimicrobial resistance genes in *Enterobacteriaceae* including *Salmonella* spp. Their capability of acquisition and distribution of resistance determinants facilitates the development and propagation of subsequent MDR strains (Correa et al., 2014). The presence of two conserved segments, separated by a variable region supports them to harbour one or several cassettes in the latter. These gene cassettes are mobile and mostly encode antimicrobial resistance proteins (Labbate et al., 2009). Insertion of these elements in plasmids enables the spread of resistance among bacteria (Su et al., 2004). Despite the retrieval of several classes of integrons from GenBank, only some of them are sustained in Gram-negative bacteria. Class 1, the most ubiquitous category, is associated with encoding over 130 resistance gene cassettes. However, six cassettes and more limited diversity are appraised in class 2 and 3, respectively (Cambray et al., 2010; Correa et al., 2014; Mazel et al., 2006; Rahmani et al., 2013).

Considering the elevating antimicrobial resistance frequency in *Salmonella* spp., it is of great magnitude to determine the route of resistance dissemination, vertical or horizontal, in the evolution of MDR strains. Surveillance programs for the presence of mobile genetic contributors of resistance genes in *Salmonella* spp. have been underscored regarding the mentioned issue (Goldstein et al., 2001; Mahero et al., 2013; Asgharpour et al., 2014; Correa et al., 2014; Lu et al., 2014; Trongjit et al., 2017; Doosti Irani et al., 2018). Hence, the aim of the present survey was to evaluate the frequency of class 1, 2, and 3 integrons and the distribution of phenotypic antimicrobial resistance among *Salmonella* spp. derived from a selection of broiler chicken in the west of Iran.

MATERIALS AND METHODS

Collection of samples

A total of 500 fecal samples were collected aseptically from apparently healthy broilers from March to September 2019 based on random cluster sampling method in Kurdistan province, in the west of Iran. Each poultry house of a farm was divided at least to four sections and an approximately 2-3 gram of fresh fecal sample from two birds were gathered from each part. The average number of houses in each farm and the density of chickens in each house were three and 5000, respectively. Besides, the biosecurity measures in all farms were implemented based on the principles of OIE biosecurity procedures in poultry production (OIE, 2019). Samples were then chilled until delivery to the laboratory within maximum three to five hours. Demographic information regarding the samples is presented in Table 1.

Isolation of *Salmonella* spp.

The initial step for *Salmonella* spp. isolation from each sample was undertaken by hemogenizing 10 gram stool in 90 mL Buffered peptone water (BPW, Merck, Germany) and incubation for 18 to 24 hours at 37 °C, followed by inoculation of 0.1 mL of the pre-enrichment medium into 10 mL Rappaport Vassiliadis enrichment (RV, Merck, Germany) broth and incubation at 41.5-42 °C for 15-18 hours. Next, a loopful of the previous medium was streaked onto xylose lysine deoxycholate (XLD, Merck, Germany) that were incubated for 24 hours at 37 °C. An individual red colony with black center on XLD, presumptive to *Salmonella* spp. was further subcultured on MacConkey (MAC, Merck, Germany) agar and identified based on Gram staining and biochemical reactions including Urea, Tryptic Sugar Iron (TSI), Indole, Methyl Red, Voges Proskauer, and Simmons Citrate (IMViC) reactions.

Table 1. Characterization of the samples in the present study

City	Farm code	Season of sampling	Age (days-old)	No. of sampling	No. of *Salmonella* isolates	No. of *Salmonella* isolates harboring integrons	Class of integron	No. of MDR* isolates	Antimicrobial resistance profile
Sanandaj	A	spring	20	25	2	1	Int1	1	AMP, NAL, CAZ, STR, TET, CHL
		summer	12	25	2	1	Int1 + Int2	1	AMP, NAL, CAZ, STR, TET, CHL
Sanandaj	B	spring	27	25	1	0	-	0	-
		summer	33	25	1	1	Int1	1	AMP, NAL, TET, CHL
Saggez	C	spring	45	25	7	3	Int2	2	AMP, NAL, STR, SXT, TET, CHL
							Int1 + Int2		AMP, NAL, STR, SXT, TET, CHL
							Int1 + Int2		AMP. STR
		summer	17	25	9	5	Int1	3	AMP, NAL
							Int1		AMP, NAL
							Int2		AMP, NAL, CAZ, STR, SXT, TET
							Int1 + Int2		AMP, NAL, SXT, TET, CHL
							Int1 + Int2 + Int3		AMP, NAL, CAZ, TET, CHL, GEN
Saggez	D	spring	52	25	0	0	-	0	-
		summer	43	25	0	0	-	0	-
Marivan	E	spring	49	25	4	2	Int1	2	AMP, NAL, STR, SXT, TET, CHL
							Int1 + Int2 + Int3		AMP, NAL, CAZ, STR, TET, CHL
		summer	25	25	6	4	Int 1	3	AMP, NAL, STR, SXT, TET, CHL
							Int2		AMP, CHL
							Int1 + Int2		AMP, NAL, STR, SXT, TET, CHL
							Int1 + Int3		AMP, CAZ, STR, TET, CHL
Marivan	F	spring	20	25	4	3	Int1	2	AMP,TET
							Int1 + Int2		AMP, NAL, STR, SXT, TET, CHL
							Int1 + Int2		AMP, NAL, STR, SXT, TET, CHL
		summer	24	25	5	2	Int1	1	AMP, TET
							Int1 + Int2		AMP, NAL, STR, SXT, TET, CHL

Bijar	G	spring	17	25	1	1	Int1 + Int2	1	AMP, NAL, CAZ, STR, SXT, TET, CHL
		summer	34	25	3	1	Int1 + Int2 + Int3	1	AMP, NAL, CAZ, STR, SXT, TET, CHL, GM
Bijar	H	spring	25	25	11	3	Int1 + Int2	3	AMP, NAL, STR, SXT, CHL
							Int1 + Int2		AMP, NAL, STR, SXT, TET, CHL
							Int1 + Int3		AMP, NAL, CZA, STR, SXT
		summer	36	25	3	2	Int3	1	AMP, STR, TET, CHL
							Int3		AMP, CAZ
Baneh	I	spring	14	25	7	4	Int1	2	AMP, NAL, SXT, TET, CHL
							Int3		AMP, CAZ
							Int1 + Int2		AMP, CAZ, SXT, TET, CHL
							Int1 + Int2		AMP, NAL, CAZ, STR, SXT, TET
		summer	36	25	1	0	-	0	-
Baneh	G	spring	32	25	0	0	-	0	-
		summer	50	25	0	0	-	0	-

Resistance to ≥3 classes of antibiotics

Table 2. Details of primer sequences and thermal conditions used in the present study.

Gene	Primer Sequence (5′→3′)	PCR thermal condition					Product Size (bp)	Reference
		Initial denaturation	Denaturation	Annealing	Extension	Final Extension		
invA	GTGAAATTATCGCCACGTTCGGGCAA TCATCGCACCGTCAAAGGGAACC	94 °C for 5 min	94 °C for 1 min	53 °C for 2 min	72 °C for 3 min	72 °C for 7 min	284	Rahn et al., (1992)
			35 cycles					
IntI	GGTGTGGCGGGCTTCGTG GCATCCTCGGTTTTCTGG		94 °C for 30 S	50 °C for 30 S	72 °C for 30 S	72 °C for 5 min	164	Koeleman et al., (2001)
IntII	CTAGAATAGGCTGTATAGGCAGA GAGTGACGAAATGTATGACAAG			52 °C for 1 min			233	Goldstein et al., (2001)
IntIII	CAGTCTTTCCTCAAACAAGTG TACATCCTACAGACCGAGAAA			47 °C for 1 min			600	
			35 cycles					

Molecular characterization of *Salmonella* spp.

Total DNA of each isolate was extracted by boiling method of its overnight culture in Luria Bertani (LB, Merck, Germany) broth. The isolates were molecularly delineated as *Salmonella* spp. in an *invA*-based polymerase chain reaction (PCR) in accordance with the primers and thermal protocol introduced by Rahn et al. (1992) (Table 2). The master mix used in this study included 12.5 μL of 2X ready-to-use PCR master mix (CinnaGen, Iran), 50 ng (2 μL) of template DNA, and 0.7 μL of each primer in a final volume of 25 μL. The positive and negative controls used in the reaction were *Salmonella* Typhimurium ATCC 1730 and DNA-free master mix, respectively.

Molecular detection of integrons

All of the *Salmonella* spp. isolates were screened for class 1-3 integrons in three separate reactions in accordance with the method prescribed elsewhere (Goldstein et al., 2001; Koeleman et al., 2001). The primer sequences and PCR protocols are represented in Table 2. The final PCR master mix consisted of 12.5 μL of 2X ready-to-use PCR master mix (CinnaGen, Iran), 50 ng (2 μL) of template DNA, 0.4 μL of each primer in a total volume of 25 μL. Positive and negative controls were used in all reactions.

Antimicrobial susceptibility testing

The antimicrobial sensitivity profile of the isolates was designated using the agar disk diffusion method, following the Clinical and Laboratory Standards Institute (CLSI, 2012) guidelines. The antibiotic disks included ampicillin (AMP, 10 μg), nalidixic acid (NAL, 30 μg), streptomycin (STR, 10 μg), trimethoprim-sulphamethoxazole (SXT, 25 μg), tetracycline (TET, 30 μg), chloramphenicol (CHL, 30 μg), ceftazidime (CAZ, 10 μg), gentamicin (GEN, 5 μg), and ciprofloxacin (CPR, 5 μg). *E. coli* ATCC 25922 was used for quality control.

Statistical analysis

The statistical associations of the frequency of *Salmonella* spp. and integron determinants with the variables of age, season, city, and antimicrobial resistance patterns were analyzed in SPSS software (version 21.0, Chicago, IL) using Chi-square test. A P value of ≤ 0.05 was considered to be statistically significant.

RESULTS

In general, 67 (13.4%) *Salmonella* spp. isolates were detected phenotypically, all of which were confirmed molecularly, producing the expected 285 bp amplicon (Figure 1). The production of 164 bp (Figure 2), 233 bp (Figure 3), and 600 bp (Figure 4) amplicons were representative of the presence of classes 1, 2, and 3 integrons, respectively. The total frequency of integrons among the isolates was 49.25% (33 isolates). In details, the frequency of class 1, 2, and 3 were 40.29% (27 isolates), 28.35% (19 isolates), and 11.94% (eight isolates), respectively. The coexistence of the integrons among the isolates, from the most to the least, were related to class 1+2 (13 isolates, 40.62%), class 1+2+3 (3 isolates, 9.37%), and class 1+3 (2 isolates, 6.25%). While no isolate was detected with the coexistence of class 2+3 integrons. As shown in Table 1, no statistical relationship was observed between the frequency of *Salmonella* spp. with age (P = 0.225), season (P = 0.392), and city (P = 0.107). The same results were obtained for the frequency of integron-harboring *Salmonella* spp. with age (P = 0.666), season (P = 0.724), and city (P = 0.314).

Figure 1. Agarose gel electrophoresis of PCR products with *invA* primers (285 bp). M: 100 bp DNA ladder (CinnaGen, Iran), NC: negative control, PC: positive control, Lanes 1–8: field samples.

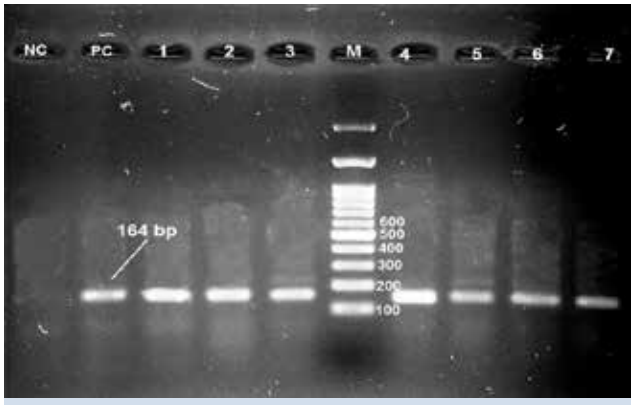

Figure 2. Agarose gel electrophoresis of PCR products with *Int1* primers (164 bp). M: 100 bp DNA ladder (CinnaGen, Iran), NC: negative control, PC: positive control, Lanes 1–7: field samples.

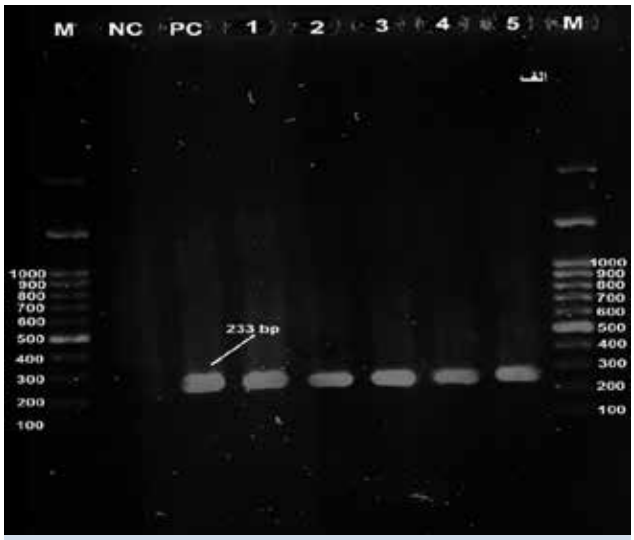

Figure 3. Agarose gel electrophoresis of PCR products with *IntII* primers (233 bp). M: 100 bp DNA ladder (CinnaGen, Iran), NC: negative control, PC: positive control, Lanes 1–5: field samples

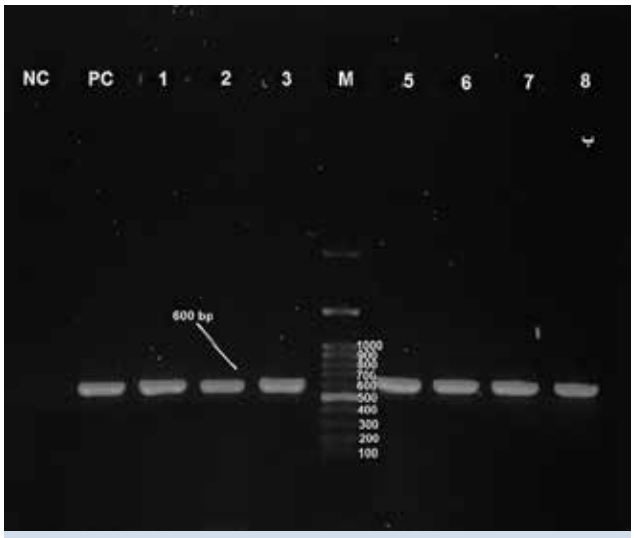

Figure 4. Agarose gel electrophoresis of PCR products with *IntIII* primers (600 bp). M: 100 bp DNA ladder (CinnaGen, Iran), NC: negative control, PC: positive control, Lanes 1–8: field samples

Phenotypic antimicrobial assessment represented the most resistance against AMP (100%), NAL (73.13%, 49 isolates), SXT (58.20%, 39 isolates), and TET (43.28%, 29 isolates). In comparison, CPR (100%), GEN (92.53%, 62 isolates), CAZ (73.13%, 49 isolates), and TET (55.22%, 37 isolates) represented the most antimicrobial susceptibility among the isolates (Table 3). None of the isolates was sensitive to all challenged antibiotics. Besides, 24 (35.82%) isolates were nominated as MDR due to their resistance to ≥ 3 classes of antibiotics. Moreover, all of the MDR isolates harbored at least one of the studied classes of integrons. The frequency of antimicrobial resistance profile of MDR isolates regarding the presence of integrons and the antimicrobial resistance in relation to integron classes is depicted in Tables 1 and 4, respectively. Additionally, statistical association was revealed between the frequency of integrons and resistance against TET (P = 0.001), CHL (P = 0.048), CAZ (P = 0.045), and STR (0.045). However, analyzing the statistical relationship between the frequency of individual integrons with resistance to the studied antibiotics depicted a merely association between integron 1 with TET (P = 0.035).

Table 3. The frequency of antibiotic resistance among *Salmonella* spp. isolated in the current study

Antibiotic code	Sensitive % (n)	Intermediate % (n)	Resistance % (n)
AMP	0	0	100% (67)
NAL	17.91% (12)	8.95% (6)	73.13 (49)
STR	52.23% (35)	0	47.76% (32)
SXT	34.32% (23)	7.46% (5)	58.2% (39)
TET	55.22% (37)	1.49% (1)	43.28% (29)
CHL	49.25% (33)	16.41% (11)	34.32% (23)
CAZ	73.13% (49)	2.98% (2)	23.88% (16)
GEN	92.53% (62)	0	23.88% (5)
CRP	100% (67)	0	0

Table 4. The frequency of antimicrobial resistance in relation to individual integrons

Antibiotic code	Class of integron	Sensitive % (n)	Intermediate % (n)	Resistance % (n)
AMP	1	0	0	100% (26)
	2	0	0	100% (19)
	3	0	0	100% (8)
NAL	1	19.23% (5)	7.69% (2)	73.07% (19)
	2	15.78% (3)	0	84.21% (16)
	3	50% (4)	0	50% (4)
STR	1	30.76% (8)	0	69.23% (18)
	2	21.05% (4)	0	78.94% (15)
	3	37.5% (3)	0	62.5% (5)
SXT	1	41.66% (10)	0	61.53% (16)
	2	26.31% (5)	5.26% (1)	68.42% (13)
	3	75% (6)	12.5% (1)	12.5% (1)
TET	1	19.23% (5)	3.84% (1)	76.92% (20)
	2	15.78% (3)	0	84.21% (16)
	3	37.5% (3)	0	62.5% (5)
CHL	1	26.92% (7)	15.38% (4)	57.69% (15)
	2	15.78% (3)	15.78% (3)	68.42% (13)
	3	37.5% (3)	0	62.5% (5)
CAZ	1	61.53% (16)	0	38.46% (10)
	2	57.89% (11)	0	42.10% (8)
	3	12.5% (1)	12.5% (1)	75% (6)
GEN	1	92.30% (24)	0	7.69% (2)
	2	89.47% (17)	0	10.52% (2)
	3	75% (6)	0	25% (2)
CPR	1	100% (26)	0	0
	2	100% (19)	0	0
	3	100% (8)	0	0

DISCUSSION

Salmonella spp. is a major etiological agent of food-borne infection and poultry is characterized as a main source of human contamination. Induction of subclinical carriers under stressful conditions leads to shedding bacteria in feces. This may contaminate carcasses following evisceration in poor sanitation practices (Mannion et al., 2012). It is claimed that the frequency rate of less than 5% *Salmonella* contamination in a farm may constitute 50-100% of carcass contamination in a retail outlet (Barrow, 2000). The 13.4% occurrence of *Salmonella* in the present research was lower than the frequency reported from Turkey (41.30%) (Carli et al., 2001), Basrah province of Iraq (19%) (Al–Abadi and Al-Mayah, 2012), Shiraz (southern Iran) (22.50%) (Ansari-Lari et al., 2014), Mazandaran province (North of Iran) (26%) (Seifi et al., 2016), Lorestan province of Iran (19%) (Haeri and Ahmadi, 2019), and Mashhad (Northeast of Iran) (19.2%) (Peighambari et al., 2019). In contrast, some studies corroborated lower recovery of *Salmonella* in broiler including 2.11% and 1.80% in Tehran and Sanandaj cities of Iran, respectively (Morshed et al., 2010; Doulatyabi et al., 2016). Plausible explanations for diverse frequencies of infection among different studies may be due to several causes such as geographical location, sampling season and methodology, culture media, diet, management measures particularly at early rearing stages, hygienic status, and biosecurity measures at farms (Zhao et al., 2017). For instance, inappropriate fencing of warehouses in farms may confer easier traffic of insidious animals and *Salmonella* vectors, which may increase the oral attainment of infection (Pui et al., 2011). Although cold seasons (fall and winter) were not included in the present study, the approximate recovery of *Salmonella* spp. in the two studied seasons were higher than the frequency reported from Spain (Lamas et al., 2016). Haeri and Ahmadi (2019) also found a similar distribution. Further, as the immunity status is extended at advanced ages, the most distribution of the bacteria in the present study was in lower ages, which was in line with the results from northeastern Algeria. The authors stated that the samples collected at

the age of 15–30 days were more contaminated with *Salmonella* spp. than those collected at 45–60 days (Djeffal et al., 2017). Moreover, Haeri and Ahmadi (2019) represented the statistical association between the frequency of *Salmonella* spp. with age in broiler; the lower the age, the more frequency of contamination was observed.

The frequent use/abuse of antimicrobial agents in animal husbandry is a serious menace threatening the effectiveness of chemotherapy through emergence and spread of antimicrobial resistance among both commensal and pathogenic bacteria. Exchange of genetic materials not only may lead to cross-resistance among the microbial community but also may confer genes prerequisite for bacterial survival in stressful conditions (Lamas et al., 2016). The massive consumption of different classes of antibiotics in poultry husbandry in Iran, including macrolides, fosfomycin, quinolones and fluoroquinolones, lincosamides, tiamulin, polymyxins, sulfonamides, penicillins, aminoglycosides, bacitracin, tetracyclines, and florfenicol, is worrying. Based on the empirical experience of the authors, antimicrobial agents are occasionally used without a veterinarians' prescription or antibiogram testing in the studied region. A recent increasing trend of resistance to common antimicrobial agents has been delineated in *Salmonella* spp. (Antunes et al., 2004). Discordant with the results obtained in the present study, 100% of *Salmonella* isolated from poultry in a former study in Babol, a city in the north of Iran, were susceptible to NAL, TET, and STR (Asgharpour et al., 2014). In agreement with the present study, high rates of resistance to NAL have been reported in *Salmonella* spp. in some studies from Iran and other countries (Yan et al., 2010; Rahmani et al., 2013). TET, a commonly used antibiotic in poultry husbandry in Iran, represented 43.28% resistance. An increasing trend of resistance against this antimicrobial agent has been recently reported in Iran (Mirzaie at al., 2010; Morshed et al., 2010; Asgharpour et al., 2018). High rates of resistance to TET and AMP are also consistent with the findings reported elsewhere (Wannaprasat et al., 2011; Trongjit et al., 2017, Zhao et al., 2017). CHL, a prohibited antibiotic in the poultry industry in Iran, represented 34.32% resistance, which may be related to co-selection and co-resistance (Trongjit et al., 2017). A relatively high rate of sensitivity was revealed to CAZ (73.13%), which was lower than the report published in Thailand (Trongjit et al., 2017). Third-generation cephalosporins are choice drugs for the treatment of human invasive salmonellosis (Wilke et al., 2005). Prolonged and widespread application of trimethoprim in veterinary practices leads to approximately high rates of resistance to it. The resistance rate against the latter in the present study was higher than the one reported by Okamoto et al., (2009). Susceptibility to CPR was represented in 100% of the isolates, which was in contrast with the 35.9% resistance reported from China (Zhao et al., 2017). The identified profound and regulated use of antibiotics, particularly not at their inhibitory concentrations, is the clue for lowering the resistance rates. This is related to omit the selection pressure for the maintenance of resistance genes (Lamas et al., 2016).

Meanwhile, the emergence of MDR *Salmonella* spp. is a matter of global concern. It is proved that MRD *Salmonella* is associated with particular serotypes including *S.* Indiana, *S.* Typhimurium, and *S.* Enteritidis (Clemente et al., 2014). Hence, it is highly recommended to determine the serotype of the isolates in upcoming studies. Approximately high rate of MDR *Salmonella* spp. in the current research concurs with that stated previously from Iran (Asgharpour et al., 2018), China (Zhao et al., 2017), and Egypt (Abdel Aziz et al., 2018). Integrons, particularly class I, have been recognized as important contributors of MDR isolates (Idrees et al., 2011). The mechanism of MDR in integron-harboring isolates is due to diminished sensitivity to antimicrobial agents, either their respective genes are incorporated or not in integron cassettes (Malek et al., 2015). Because of their ability to propagate resistance gene cassettes through acquisition and shearing modes and to integrate in mobile genetic elements, dispersion of antimicrobial resistance genes may be facilitated. They capture one or more mobile gene cassettes to form cassette arrays which can readily be lengthened by incorporation of new cassettes, shortened by excision of one or more cassettes, or reshuffled to create new orders. All these reactions are mediated by integrases which catalyze recombination between two primary or a primary and a secondary recombination sites and allows the rapid formation and expression of new combinations of genes in response to selection pressures. This acquired resistance can be transferred among bacteria via horizontal gene transfer by conjugation and transformation (Mazel 2006; van Hoek et al., 2011). The consequence is limited options in treating infectious diseases (Recchia and Hall, 1995). Resistance genes, related to various classes of antimicrobial genes, including beta-lactams, sulfonamides, macrolides, aminoglycosides, trimethoprim, chloramphenicols, and

rifampin may be transported by integrons (Peters et al., 2001). The most common gene cassettes and arrays and the corresponding antimicrobial resistances which have been frequently reported in class 1 integrons are *aadB* (resistance to dibekacin, gentamicin, kanamycin, sisomicin and tobramycin) (Shaw et al., 1993), *dfrA7* (resistance to trimethoprim) (Roberts et al., 2012), *aadA1a* and *aadA2* (resistance to spectinomycin, streptomycin, and kanamycin) (Ramirez and Tolmasky, 2010), $bla_{\text{CARB-2}}$ (resistance to penicillins including carbenicillin) (Matthew and Sykes, 1977), *dfrA1–gcuC* (resistance to trimethoprim) (Roberts et al., 2012), *dfrA1–aadA1a* (resistance to trimethoprim, spectinomycin and streptomycin) (Ramirez and Tolmasky, 2010; Roberts et al., 2012), *dfrA17–aadA5* (resistance to trimethoprim, spectinomycin and streptomycin) (Ramirez and Tolmasky, 2010; Roberts et al., 2012), *oxa10/aadA1* (resistance to ampicillin, cefaclor and spectinomycin) (Tennstedt et al., 2003), *gca-L/M/P* and *catB* (resistance to chloramphenicol) (Tennstedt et al., 2003), *dfrA12–gcuF–aadA2* (resistance to trimethoprim, spectinomycin and streptomycin) (Sandvang et al., 1997), $floR_{st}$ (resistance to florfenicol) (Ramirez and Tolmasky, 2010; Roberts et al., 2012), and *tetR* and *tetA* (resistance to tetracyclines) (Briggs et al., 1999; Sandvang et al., 1997). Some of the most prevalent gene cassettes and arrays integrated in class 2 integron are *aadA* and *sat-1* (resistance to aminoglycosides and sulfonamides) (Hansson et al., 2002), and *ere(A)* (resistance to erythromycin and rifampin) (Tribuddharat et al., 1999). Class 3 integron has been reported to harbor the bla_{IMP} gene cassette that confers resistance to broad-spectrum β-lactams including carbapenems (Carattoli 2001). The overall prevalence of integrons were 49.25%, with the highest frequency related to class 1 (40.29%), followed by class 2 (28.35%) and 3 (11.94%), herein. In a former study carried out in the southwest of Iran, the prevalence of class 1 to 3 integrons in *Salmonella* spp. isolated from broiler chicks were 50%, 28%, and 48%, respectively (Doosti Irani et al., 2018). Moreover, 82% of the *Salmonella* spp. isolated from poultry in Cairo, Egypt, harbored class 1 integron, with the total absence of class 2 among the isolates (Abdel-Maksoud et al., 2015). Most of the reports regarding the frequency of integrons in *Salmonella* spp. originated from poultry are related to class 1, ranging from 0 to 100% (Okamoto et al., 2009; Asgharpour et al., 2014; Lu et al., 2014; Halawa et al., 2016; Zhao et al., 2017; Abdel Aziz et al., 2018; Shabana et al., 2019). Our results were in contrast with those that claimed class 3 integron is not harbored in *Salmonella enterica* serovars Enteritidis, Typhimurium, and Infantis (Eshraghi et al., 2010; Ranjbar et al., 2011). Nevertheless, the presence of the aforementioned class in other *Enterobacteriaceae*, retrieved from random French hospital effluent samples, may reflect its role in antimicrobial resistance (Barraud et al., 2013). Higher frequency of integrons reported in some studies may imply the abundant use of antimicrobials in those study regions (Trongjit et al., 2017). The statistical association between the presence of integrons with resistance genes in *Salmonella* spp. in literature points toward their role in distribution of resistance genes (Abdel Aziz et al., 2018). However, amplification and sequencing of cassette regions should be performed in order to genetically confirm this association.

Besides, the discrepancy about selective and non-selective pressure of antibiotics in dispersion of integrons has been demonstrated, as environmental pollution with quaternary compounds may influence the dissemination of integron 1 (Gaze et al., 2005). Phenols, formaldehyde and aldehydes, chlorhexidine, sodium hydroxide, quaternary ammoniums, and iodophors are frequently used as disinfectants in Iranian poultry farms. Although the antimicrobial patterns of *Salmonella* isolates harbouring different classes or a combination of different classes of integrons revealed varieties, it was less than the diversities observed in other studies (Macedo-Viñas et al., 2009; Dessie et al., 2013; Rahmani et al., 2013; Asgharpour et al., 2018). This may be due to the fact that prescribing antibiotics in the studied region is relatively based on implementing empirical therapy rather than surveillance analysis. This may limit the usage of different antimicrobial classes in the region. Further, the relationship between the presence of integrons with MDR *Salmonella* was 100%, as a confirmation with previous investigations (Molla et al., 2007; Lu et al., 2014). This renders a scientific basis to guide the prudent clinical use of antibiotics (Lu et al., 2014). A high frequency of penta-resistance ACSSuT (ampicillin, chloramphenicol, streptomycin, sulfonamides, and tetracycline) phenotype was manifested among MDR *Salmonella* isolates, which is in agreement with other studies (Mahero et al., 2013; Lu et al., 2014). Besides, 15 other different antimicrobial resistance profiles were detected among the isolates. The patterns NAL-SXT-STR-TET-CHL and NAL-CAZ-STR-SXT-TET-CHL have also been reported in *Salmonella* Infantis isolated from chicken in Iran (Asgharpour et al., 2018). To the best of our knowledge, other patterns

were not found in similar studies; this does not mean their specificity to the isolates of this research, but rather relates to assessing the other antimicrobial agents' resistance in various studies.

In brief, a relatively high distribution of integrons was identified among *Salmonella* spp. isolated from broiler chicken in the west of Iran. In addition, antimicrobial and multi-drug resistance was detected in approximately high rates. This emphasizes the continuous surveillance measures and cautious application of antimicrobial agents in poultry husbandry in order to reduce the emergence of resistant strains and to prevent food-borne diseases caused by them.

CONFLICT OF INTEREST

None declared.

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