High prevalence of clonally related multiple resistant Salmonella *Infantis carrying class 1 integrons in broiler farms*

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Summary
The poultry industry in Iran is the main supplier of protein in the food chain. In the present study, we showed the importance of the possible dissemination of clonally related multiple drug resistant (MDR) *Salmonella* Infantis in broiler farms in Iran. In total, 156 fecal samples belonging to 23 poultry farms in Razavi Khorasan province, northeast of Iran, were examined for the presence of *Salmonella* serovars. Molecular serotypes and serogroups, class 1 and 2 integron types, colistin resistance genes (*mcr-1* and *mcr-2*) and antimicrobial susceptibility patterns were determined on the recovered *Salmonella* isolates. Based on PCR analysis, 30 recovered *Salmonella* isolates were identified as *S.* Infantis (23 isolates; 76.6%), *S.* Enteritidis (six isolates; 20%), and one isolate (3.3%) was not serotyped by the applied method. Class 1 integrons were detected in 22 isolates (95.6%) and class 2 integrons were not detected in any of the isolates. Although colistin resistance was prevalent in disc diffusion test, *mcr-1* and *mcr-2* genes were not detected. All class 1 integrons carried the cassette *aadA1* gene. All *Salmonella* isolates were resistant to colistin and amoxicillin/clavulanic acid and MDR patterns were observed in most (96.6%) isolates. This study revealed a high prevalence rate of *S.* Infantis and the presence of class 1 integrons in broiler farms. The presence of the same integron cassettes in the sequenced isolates suggests that strains are clonally related. Stringent monitoring programs are required to prevent the spreading of MDR *Salmonella* serovars into food chain via poultry products.

Introduction

Antimicrobial resistance has become a worldwide public health problem that has a direct impact on food safety (Shabana *et al.* 2019). The use of antimicrobials has been beneficial for the producers to control and treat *Salmonella* in the food industry (Threlfall 2002). However, the overuse of antimicrobial agents, especially in the poultry farms, leads to the emergence and spread of antibiotic-resistant strains (Threlfall 2002, Irani *et al.* 2018).

In recent years, *Salmonella* species were recognized as a serious and problematic foodborne pathogen in poultry farms on a global level (Antunes *et al.* 2016). In these farms, antibiotics are used therapeutically, prophylactically or for growth promotion purposes (Mehdi *et al.* 2018). The extensive use of these antibiotics has led to a significant increase in the distribution of multidrug-resistant (MDR) *Salmonella* strains in foods. Likewise, these MDR strains can be transmitted to humans through the food chain, or direct contact with poultry and their houses (Marshall and Levy 2011).

Salmonellosis is one of the most important bacterial foodborne diseases in both developed and developing countries such as Iran (Aziz *et al.* 2018). Human salmonellosis is commonly associated with

the consumption of contaminated poultry and its products. In most cases, the disease is caused by eating raw or undercooked poultry, eggs or egg products (El-Prince *et al.* 2019). Nowadays, high rates of MDR *Salmonella* strains are represented as a major threat to public health in Iran (Nirmala *et al.* 2018).

In many countries, *Salmonella* Infantis has been mentioned as a cause of food-borne zoonotic pathogen among serovars of *Salmonella enterica* (Merino *et al.* 2003, Zhao *et al.* 2017, Borowiak *et al.* 2018, Wajid *et al.* 2019). In Iran, many studies show that *Salmonella* Infantis serovar is an important public health issue and has become a serious problem for the medical and veterinary communities (Firoozeh *et al.* 2011, Firoozeh *et al.* 2014, Peighambari *et al.* 2018). Poultry, especially broilers, are known as one of the main reservoirs for *S.* Infantis in Iran (Rahmani *et al.* 2013).

Today, *S.* Infantis like other *Salmonella* serovars is becoming resistant to key antimicrobials such as the fluoroquinolones and broad-spectrum β-lactams (Gupta *et al.* 2019). Antibiotic resistance genes, play a major role in the evolution of MDR *Salmonella* strains that can be located on chromosomes, plasmids, transposons or integrons (Almeida *et al.* 2018). The distribution of MDR *Salmonella* strains is mainly related to integrons (Kaushik *et al.* 2018). Integrons are genetic elements that are able to capture antibiotic resistance genes and spread them among sensitive strains, so they have a fundamental role in the emergence of MDR *Salmonella* strains (Gillings *et al.* 2008, Kaushik *et al.* 2018). Integrons consist of two major types including chromosomal integrons and mobile integrons (MIs). MIs are divided into five classes (Class 1 to 5) and class 1 integron has been the most commonly reported class in MDR *Salmonella* strains (Gillings *et al.* 2008, Kaushik *et al.* 2018, Hossain *et al.* 2019).

The present study was conducted to investigate the prevalence, distribution, antimicrobial resistance patterns and recognition of class 1 and 2 integrons among *Salmonella* serovars from broiler chicken farms in Khorasan Razavi province, Iran.

Materials and methods

Isolation and identification of *Salmonella*

A total of 156 fecal samples were collected from 23 poultry farms in Khorasan Razavi province, Iran, from September 2013 to October 2013. All samples were transferred to Selenite F broth (Merck Co., Germany) and incubated at 37 °C for 16 h. A loopful of the enriched samples were cultured on MacConkey agar (Merck Co., Germany) and XLD agar (Merck Co., Germany) and incubated at 37 °C for 24-48 h. Suspected colonies were cultured into the TSI agar (Merck Co., Germany) and incubated at 37 °C for 24 h. Finally, the lactose-negative and H_2S positive isolates were examined using standard biochemical tests (Markey *et al.* 2013).

Salmonella serogrouping

Salmonella serogroups were determined by slide agglutination using antisera against O antigen according to the manufacturer's instructions (Bahar Afshan, Iran).

Molecular detection of Salmonella serovars

The DNA of *Salmonella* isolates was extracted by using the boiling method as described previously (Badouei *et al.* 2015). Then, the polymerase chain reaction (PCR) was performed to confirm the biochemical identification of the isolates at the genus level using S139 and S141 primers which target *invA* gene (Zahraei Salehi *et al.* 2013). A multiplex-PCR assay for molecular detection of five important *Salmonella* serovars including *S.* Infantis, *S.* Heidelberg, *S.* Gallinarum, *S.* Enteritidis, and *S.* Kentucky was performed on all confirmed isolates as described previously (Kardos *et al.* 2007, Zhu *et al.* 2015). When *Salmonella* serovars was not identified in the multiplex-PCR, a two-step nested PCR approach was used for molecular identification of *S.* Infantis (Kardos *et al.* 2007). Twenty-five µl final reaction volume including 3 µl DNA extract, 0.3 µM of each primer (Table I), 12.5 µl 2x Taq DNA Polymerase Master Mix RED (Ampliqon, Denmark) and distilled water up to volume of reaction was used in all PCR reactions. The PCR conditions were adjusted on the basis of cited references for each assay (Kardos *et al.* 2007, Zhu *et al.* 2015). For positive controls, *S.* Infantis (Collection isolate, University of Tehran), and *S.* Enteritidis (ATCC: 13076) were used.

Detection of class 1 and 2 integrons and colistin resistance genes

The presence of gene cassettes containing class 1 and 2 integrons were detected by two PCR assays (Table I). All *Salmonella* isolates were screened for the detection of most prevalent plasmid-mediated colistin resistance genes (*mcr-1* and *mcr-2*) using two PCR assays with specific primers (Table I). This reaction was conducted in 25 µl volumes based on the protocol described by Barbieri and colleagues (Barbieri *et al.* 2017). All PCR products were electrophoresed on 2% agarose gel at 100 V for 1 h with ethidium bromide and visualized by GelDoc 1000 (Vilber Lourmat, France).

Table I. *List of primers which were used in this study.*

Target	Primer	Sequence (5´ to 3´)	Size (bp)	Reference
S. enterica	S139	GTGAAATTATCGCCACTGTCGGGCAA	218	Zahraei Salehi *et al.* 2013
	S141	TCATCGCACCGTCAAAGGAACC		
S. Infantis	558f	AACAACGACAGCTTATGCCG	Variable	Kardos *et al.* 2007
	878f	TTGCTTCAGCAGATGCTAAG		
	1275r	CCACCTGCGCCAACGCT		
S. Heidelberg	heli-F	ACAGCCCGCTGTTTAATGGTG	782	Zhu *et al.* 2015
	heli-R	CGCGTAATCGAGTAGTTGCC		
S. Gallinarum biotype Gallinarum	steB-F	TGTCGACTGGGACCCGCCCGCCCGC	636	Zhu *et al.* 2015
	steB-R	CCATCTTGTAGCGCACCAT		
S. Gallinarum	rhs-F	TCGTTTACGGCATTACACAAGTA	402	Zhu *et al.* 2015
	rhs-R	CAAACCCAGAGCCAATCTTATCT		
S. Enteritidis	sdf-F	TGTGTTTTATCTGATGCAAGAG	293	Zhu *et al.* 2015
	sdf-R	CGTTCTTCTGGTACTTCAGATGAC		
S. Kentucky	gly- F	TTCCAATTGAAACGAGTGCGG	170	Zhu *et al.* 2015
	gly-R	ACTAACCGCTTGGGTTGTTGCTGT		
Class 1 integron	5´-CS	GGCATCCAAGCAGCAAG	Variable	Firoozeh *et al.* 2019
	3´- CS	AAGCAGACTTGACCTGA		
Class 2 integron	hep74	CGGGATCCCGGACGGCATGCACGATTTGTA	Variable	Firoozeh *et al.* 2019
	hep51	GATGCCATCGCAAGTACGAG		
mcr-1	CLR5-F	CGGTCAGTCCGTTTGTTC	309	Barbieri *et al.* 2017
	CLR5-R	CTTGGTCGGTCTGTAGGG		
mcr-2	MCR2-IF	TGTTGCTTGTGCCGATTGGA	567	Barbieri *et al.* 2017
	MCR2-IR	AGATGGTATTGTTGGTTGCTG		

Sequencing of integron class 1

PCR products of class 1 integron of *Salmonella* strains belonging to eight geographically separated farms were sequenced by Sanger dideoxy sequencing (Seoul, South Korea) using the amplification primers. The sequences were compared and analyzed by Chromas Pro version 1.7.5 Technelysium as well as online BLAST software (http://www.ncbi.nlm.nih.gov/BLAST/) and Integron Database INTEGRALL (http://integrall.bio.ua.pt/). To confirm the results of detection of class 1 integrons, all positive isolates (possess class 1 integrons) were compared to available whole genome sequencing data.

Antimicrobial susceptibility test

The susceptibility of 30 *Salmonella* isolates to a panel of 27 antimicrobial agents was determined by the agar disc diffusion method and the interpretation of results was carried out according to the Clinical and Laboratory Standards Institute (CLSI) guidelines (Jorgensen *et al.* 2007, Reller *et al.* 2009, CLSI 2018). The antimicrobial agents tested and their concentrations (µg) were: amoxicillin/clavulanic acid (AMC; 20/10 µg), amoxicillin (AMX; 10 µg), cefixime (CFM; 5 µg), ceftriaxone (CRO; 30 µg), cefazolin (CFZ; 30 µg), chloramphenicol (CHL; 30 µg), chlortetracycline (CTC; 30 µg), ciprofloxacin (CIP; 5 µg), colistin (CST; 10 µg), difloxacin (DIFL; 10 µg), doxycycline (DOX; 30 µg), enrofloxacin (ENR; 5 µg), florfenicol (FLOR; 30 µg), flumequine (FLU; 30 µg), fosfomycin (FOF; 200 µg), furazolidone (FZD; 100 µg), gentamicin (GEN; 10 µg), kanamycin (KAN; 30 µg), linco-spectin (LP; 15/200 µg), nalidixic acid (NAL; 30 µg), neomycin (NEO; 30 µg), nitrofurantoin (NIT; 300 µg), norfloxacin (NOR; 10 µg), oxytetracycline (OTC; 30 µg), streptomycin (STR; 10 µg), tetracycline (TET; 30 µg), and trimethoprim/sulfamethoxazole (SXT; 1.25/23.75 µg). Each *Salmonella* isolate which were resistant to at least one antibiotic in three or more antimicrobial classes were designated as MDR isolates.

Results

Prevalence of *Salmonella* spp. and serovars

In total, out of 156 fecal samples tested, 30 (19.2%) *Salmonella* isolates were recovered. In 23 broiler farms in Khorasan Razavi province *Salmonella*

serovars were detected in nine farms (39.1%). The Kauffman-White group serotyping showed that 23 *Salmonella* isolates (76.6%) belonged to serogroup C (*S.* Infantis), six isolates (20%) belonged to serogroup D (*S.* Enteritidis), and one isolate (3.3%) belonged to serogroups other than A-D. Based on PCR analysis, among the 30 *Salmonella* isolates, 23 isolates were identified as *S.* Infantis (76.6%) which belonged to six different farms, and six isolates were identified as *S.* Enteritidis (20%) which belonged to five different farms, and one isolate (3.3%) was not serotyped by PCR.

Class 1 and 2 integrons and colistin resistance genes

The class 1 integron was detected in 22/23 (95.6%) *S.* Infantis isolates. Among the seven *S.* Enteritidis isolates, class 1 integrons were identified only in one isolate (14.2%). The Class 2 integrons were not detected in any of the obtained *Salmonella* isolates. Also, the *mcr-1* and *mcr-2* genes were not detected in any of the 30 *Salmonella* isolates.

Sequencing analysis

Analysis of DNA sequencing results revealed that all sequenced isolates harbored an integron class 1 carrying one gene cassette including *aadA1* gene.

Phenotypic antimicrobial resistance

Out of 30 *Salmonella* isolates, all (100%) of *S.* Infantis and *S.* Enteritidis isolates were susceptible to cefixime, gentamicin, ceftriaxone, norfloxacin, and fosfomycin. Also, 96.6% of isolates were susceptible to amoxicillin and ciprofloxacin. All isolates (100%) were resistant to colistin and amoxicillin/clavulanic acid and 93.3% of isolates were resistant to nitrofurantoin and oxytetracycline. MDR patterns were observed in 29 isolates (96.6%). The details of phenotypic resistance to antimicrobials have been presented in Table II and Table III.

Discussion

Poultry are one of the most important carriers of *Salmonella*, which carry the bacterium asymptomatically and shed it to the environment through their feces (Akbarian *et al.* 2010, Jajere 2019). This bacterium can survive for a long time in the environment and may be transmitted to human through the consumption of contaminated avian meat and egg products (VT Nair *et al.* 2018); salmonellosis is one of the most common foodborne diseases in humans worldwide.

In many countries, poultry and its products are the main reservoirs for human salmonellosis. Our study showed that prevalence of *Salmonella* spp. were increased dramatically in poultry farms during 2013-2014 in Iran; *S.* Infantis had the most significant role in the contamination of broiler farms. Similarly, other studies from different regions of Iran have

Table II. *Resistance (number and percentage) of recovered* Salmonella *isolates from broilers in Khorasan Razavi province, Iran.*

Antimicrobial agent	S. Infantis (n = 23)	S. Enteritidis (n = 6)	Other serovars (n = 1)	Total (n = 30)
β- Lactams antibiotics:				
Penam penicillins:				
Amoxicillin/ clavulanic acid	23 (100.0)	6 (100.0)	1 (100.0)	30 (100.0)
Amoxicillin	0 (0.0)	1 (16.6)	0 (0.0)	1 (3.3)
Cephalosporins:				
First generation:				
Cefazolin	3 (13.0)	0 (0.0)	1 (100.0)	4 (13.3)
Third generation:				
Cefixime	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Ceftriaxone	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Polymyxins:				
Colistin	23 (100.0)	6 (100.0)	1 (100.0)	30 (100.0)
Aminoglysides:				
Gentamicin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Kanamycin	13 (56.5)	0 (0.0)	1 (100.0)	14 (46.6)
Neomycin	14 (60.8)	0 (0.0)	1 (100.0)	15 (50.0)
Streptomycin	13 (56.5)	0 (0.0)	1 (100.0)	14 (46.6)
Phenicols:				
Chloramphenicol	6 (26.0)	2 (33.3)	0 (0.0)	8 (26.6)
Florfenicol	7 (30.4)	1 (16.6)	0 (0.0)	8 (26.6)
Tetracyclines:				
Tetracycline	22 (95.6)	2 (33.3)	0 (0.0)	24 (80.0)
Chlortetracycline	23 (100.0)	0 (0.0)	1 (100.0)	24 (80.0)
Doxycycline	23 (100.0)	3 (50.0)	0 (0.0)	26 (86.6)
Oxytetracycline	23 (100.0)	5 (83.3)	0 (0.0)	28 (93.3)
Sulfonamides:				
Trimethoprim/ sulfamethoxazole	20 (86.9)	0 (0.0)	1 (100.0)	21 (70.0)
Quinolones:				
Nalidixic acid	23 (100.0)	3 (50.0)	0 (0.0)	26 (86.6)
Fluoroquinolones:				
Ciprofloxacin	1 (4.3)	0 (0.0)	0 (0.0)	1 (3.3)
Difloxacin	0 (0.0)	0 (0.0)	1 (100.0)	1 (3.3)
Enrofloxacin	0 (0.0)	0 (0.0)	1 (100.0)	1 (3.3)
Flumequine	15 (65.2)	0 (0.0)	1 (100.0)	16 (53.3)
Norfloxacin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Others:				
Fosfomycin	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Furazolidone	22 (95.6)	0 (0.0)	1 (100.0)	23 (76.6)
Linco-spectin	23 (100.0)	0 (0.0)	1 (100.0)	24 (80.0)
Nitrofurantoin	23 (100.0)	5 (83.3)	0 (0.0)	28 (93.3)
Total	23 (51.5)	6 (20.9)	1 (48.1)	30 (45.2)

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Table III. *Resistance patterns of* Salmonella *serovars isolated from broilers in Khorasan Razavi province, Iran.*

Resistance patterns[a]	*S.* Infantis	*S.* Enteritidis	Other serovars	Total
LP-C-TE-CP-K-NA-FR-FM300-SXT-N-CL-D-FM30-S-AMC-FF-CTE-T	1	-[b]	-	1
LP-C-TE-K-NA-FR-FM300-SXT-N-CL-D-FM30-S-AMC-FF-CTE-T	2	-	-	2
LP-C-TE-K-NA-FR-FM300-SXT-N-CL-D-S-AMC-FF-CTE-T	1	-	-	1
LP-TE-NA-FR-FM300-CL-D-FM30-AMC-CTE-T	3	-	-	3
LP-TE-NA-FR-FM300-CL-D-SXT-AMC-CTE-T	4	-	-	4
CL-AMC	-	1	-	1
TE-FM300-CL-D-AMC-T	-	1	-	1
LP-C-TE-NFX-K-NA-FR-FM300-SXT-N-CL-D-FM30-S-AMC-DF-CTE-T	-	-	1	1
LP-TE-K-NA-FR-FM300-SXT-N-CL-D-S-AMC-CTE-T	2	-	-	2
LP,-TE-K-NA-FR-FM300-SXT-N-CL-D-FM30-S-AMC-CTE-T	5	-	-	5
LP-TE-K-NA-FR-FM300-SXT-N-CL-D-FM30-S-AMC-CTE-T	1	-	-	1
LP-TE-K-NA-FR-FM300-SXT-N-CL-D-FF-S-AMC-CTE-T	1	-	-	1
C-NA-CL-AMC-FF-T-AMX	-	1	-	1
LP-TE-NA-FR-FM300-SXT-N-CL-D-FM30-AMC-CTE-T	1	-	-	1
NA-FM300-CL-AMC	-	1	-	1
LP-C-TE-NA-FR-FM300-SXT-CL-D-FM30-AMC-FF-CTE-T	1	-	-	1
FM300-CL-AMC-T	-	1	-	1
LP-C-NA-FR-FM300-SXT-CL-D-FM30-AMC-FF-CTE-T	1	-	-	1
FM300-CL-D-AMC-T	-	1	-	1
Total	23	6	1	30

[a]Antimicrobial agent tested were Amoxicillin/clavulanic acid (AMC), Amoxicillin (AMX), Cefazolin (CEZ), Chloramphenicol (C), Chlortetracycline (CTE), Ciprofloxacin (CP), Colistin (CL), Difloxacin (DF), Doxycycline (D), Enrofloxacin (NFX), Florfenicol (FF), Flumequine (FM30), Furazolidone (FR), Kanamycin (K), Linco-spectin (LP), Nalidixic acid (NA), Neomycin (N), Nitrofurantoin (FM300), Oxytetracycline (T), Streptomycin (S), Tetracycline (TE), Trimethoprim/sulfamethoxazole (SXT).
[b]No resistance pattern detected.

reported that *S.* Infantis had the highest frequency of contamination in broiler farms in the same time frame (Fallah *et al.* 2013, Rahmani *et al.* 2013). In a study conducted in northern provinces of Iran, Rahmani and colleagues showed that out of 36 *Salmonella* isolates, 75% (n = 27) and 25% (n = 9) were identified as *S.* Infantis and *S.* Enteritidis, respectively (Rahmani *et al.* 2013). Fallah and colleagues reported that out of 44 *Salmonella* isolates, 79.5% (n = 34) were identified as *S.* Infantis; the remaining 18.2% (n = 8) and 2.3% (one strain) belonged to serogroup D and serogroup C, respectively (Fallah *et al.* 2013). However, in other studies conducted by Ezzat Panah and colleagues and Asad Poor and colleagues in Iran, it was showed that *S.* Enteritidis had the highest rate (45.3% and 75%, respectively) in broiler farms (Ezatpanah *et al.* 2013, Asadpour *et al.* 2014). Different results have been obtained in other countries; in Colombia, Canada, and Spain, *S.* Paratyphi B variant Java (76%), *Salmonella* Hadar (40.4%), and *S.* Enteritidis (79.6%) were the most prevalent serovars, respectively (Carramiñana *et al.* 2004, Donado-Godoy *et al.* 2012, Mainali *et al.* 2014). These differences with our study may be related to several factors such as geographical locations, sample selection criteria and hygiene level of broiler farms (Firouzabadi *et al.* 2020).

Integrons are genetic elements that contain a site to integrate a segment of DNA that could be disseminated the antimicrobial-resistant genes using a mobile genetic element (MGE) such as plasmids and transposons among *Salmonella* spp. The class 1 integron has been the most extensively reported class in the dissemination of resistance genes in *Salmonella* spp. (Kaushik *et al.* 2018). Interestingly, class 1 integrons were detected in most of our *S.* Infantis isolates and class 2 integrons were not detected in any of the studied isolates. These results are in accordance with a nother study conducted in Iran (Rahmani *et al.* 2013). The class 1 integron seems to be an important player in dissemination of resistant factors among *S.* Infantis strains in the broiler farms in Iran. Importantly, the carriage of the same cassette (*aadA1*) within the class 1 integron in eight isolates from different farms strongly suggests the presence of the clonally related *S.* Infantis in poultry farms in northeast of Iran.

Based on phenotypic antimicrobial susceptibility examination of *Salmonella* isolates in the present study, all *S.* Infantis isolates were resistant to colistin, amoxicillin/clavulanic acid, chlortetracycline, doxycycline, oxytetracycline, nalidixic acid, linco-spectin, and nitrofurantoin. Also, all of them were susceptible to amoxicillin, cefixime,

ceftriaxone, gentamicin, difloxacin, enrofloxacin, norfloxacin, and fosfomycin. Besides, MDR patterns were observed in all of *S.* Infantis isolates (100%). In Rahmani and colleagues (Rahmani *et al.* 2013), and Asadpour and colleagues (Asadpour *et al.* 2014) studies, most of *S.* Infantis strains were resistant to tetracycline, spectinomycin, streptomycin, sulfamethoxazole, nalidixic acid, and nitrofurantoin; also they observed MDR patterns in 92% of *S.* Infantis isolates which are similar to our findings. In total, our results and also studies of Ezatpanah and colleagues (Ezatpanah *et al.* 2013), Asadpour and colleagues (Asadpour *et al.* 2014), Chung and colleagues (Chung *et al.* 2003), and Carramiñana and colleagues (Carramiñana *et al.* 2004), showed that *Salmonella* isolates are highly sensitive to gentamicin and highly resistant to tetracycline. Interestingly, in this study, despite observing a high level of phenotypic resistance to colistin, none of the isolates carried the studied resistance genes, *mcr-1* and *mcr-2*. It seems that screening for other types of *mcr* genes should be considered for future studies on *Salmonella* strains in Iran.

This study revealed a high prevalence rate of *S.* Infantis and a strong association between MDR patterns and the presence of class 1 integrons in broiler farms by 2013-2014 in Khorasan Razavi Province, Iran. Colistin resistance is a major concern because it is the latest treatment of bacterial infection caused by gram-negative bacteria with MDR and carbapenem resistance in humans. All integrons carried the same gene cassette, which indicates that they were clonally related strains which spreaded via a possible common source. The results of the present research highlight the uncontrolled use of antibiotics in broiler farms that may cause the emergence of MDR *Salmonella* strains in broiler products. Therefore, there are an emerging need for systematic monitoring and characterizing MDR *Salmonella* serovars in poultry industry in order to prevent the spread to food chain and humans.

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