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ORIGINAL ARTICLE

The emergence of carbapenem-resistance and New Delhi metallo-β-lactamase-1 (*bla*$_{NDM-1}$) among *Salmonella* spp. in Kerman, Iran

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ABSTRACT

Background and Objectives: *Salmonella* species (spp) are the most prevalent zoonotic pathogens that cause outbreaks of gastroenteritis worldwide. Therefore evaluation of the profile of antibiotic resistance, virulence factors, and plasmid replicon types in these bacteria is necessary to control and prevent the spread of potentially pathogenic and drug-resistant strains.
Materials and Methods: This study was performed on 39 *Salmonella* spp. The antibacterial susceptibility of isolates to various antibiotic agents was determined using disk diffusion test. β-lactamases (*bla*) including ESBLs, AmpC, MBLs, and virulence genes were detected by PCR methods. Plasmid incompatibility groups among the isolates were identified using PCR-based replicon typing (PBRT).
Results: The most prevalent virulent gene was *phoP/Q* (84.6%). *slyA*, *sopB*, and *stn* were identified in 79.4% (n=31), 69.2% (n=27), and 2.5% (n=1) of the isolates, respectively. The antibiotic susceptibility testing showed that 30.7% of the isolates were ESBL-producing. *bla*$_{TEM}$ (41%; n=16) was the most frequent β-lactamase gene among the isolates followed by *bla*$_{NDM-1}$ (15.4%; n=6), *bla*$_{DHA}$ (7.7%; n=3), and *bla*$_{CTX-M}$ (1.5%; n=1). Six different plasmid replicon types, including IncP (n=9; 23%), IncFIC (n=3; 7.70%), IncY (n=3; 7.70%), IncI1-Iγ (n=2; 5.12%), IncFIIAs (n=1; 2.56%), and IncN (n=1; 2.56%) were observed among the isolates.
Conclusion: Our study showed the emergence of carbapenem-resistant and *bla*$_{NDM-1}$ among *Salmonella* spp. for the first time in Kerman, Iran. Since *Salmonella* spp. plays an important role in the transmission of resistance genes in livestock and humans in the food chains, so more stringent control policies are recommended to prevent the circulation of drug-resistant and potentially pathogenic strains from animals to humans.

Keywords: *Salmonella*; Beta-lactamase genes; Virulence factors; New Delhi metallo-beta-lactamase-1 (NDM-1)

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INTRODUCTION

Non-typhoidal *Salmonella* species (spp) such as *Salmonella enterica* subsp. *enterica* serotype Typhimurium (*S*. Typhimurium) and *Salmonella enterica* subsp. *enterica* serotype Enteritidis (*S*. Enteritidis) are members of the *Enterobacteriaceae* family (1). These bacteria are the most prevalent zoonotic pathogens that cause outbreaks of gastroenteritis in the world and can act as a reservoir for various genes encoding antimicrobial resistance (1). The extensive use of antibiotics in the agriculture systems for the prevention, therapeutic, and control of infectious diseases has contributed to the development of drug-resistant in non-typhoidal *Salmonella* spp. and recently, multidrug-resistant (MDR) *Salmonella* spp. has been reported in many countries worldwide (2, 3). The emergence of MDR *Salmonella* reduces treatment options for invasive infections in both humans and animals, also the spreading of MDR strains of *Salmonella* spp. is an important global health issue. In different countries, resistance to streptomycin, ampicillin, nalidixic acid, and tetracycline in non-typhoidal *Salmonella* serovar was reported (2-5). MDR isolates of *Salmonella* spp. can act as a reservoir and subsequent horizontal spreading of antibiotic-resistance genes to non-resistant bacteria. Beta-lactamase enzymes such as extended-spectrum β-lactamases (ESBLs), AmpC-β-lactamases, and carbapenemases such as Metallo-β-lactamases (MBLs) can hydrolyze various β-lactam antibiotics from the fourth generation of cephalosporins to carbapenems (6-8). Gram-negative bacilli with ESBLs, AmpC, and MBLs genes, in addition to resistance to beta-lactam antibiotics, are usually resistant to the wide range of antibiotics such as aminoglycosides, fluoroquinolones, and are a serious threat to public health. New Delhi metallo-β-lactamase (bla_{NDM}) is a MBL and for the first time was reported in *Klebsiella pneumoniae* and then reported worldwide among other Gram-negative bacilli (9). NDM enzyme can hydrolyze a wide range of β-lactam antibiotics especially carbapenems as a last line of antibiotics for the treatment of extended-spectrum β-lactamases (ESBLs) and AmpC-β-lactamases-producing Gram-negative bacteria (10). NDM-producing of *Salmonella* spp. has not been reported in Iran but recently reported in India, China, and Australia (11-14). Antibiotic resistance genes and virulence factors usually are located on plasmids that these mobile genetic elements have important role in genetic diversity, dissemination of virulence and resistance genes among various bacteria via horizontally transfer (15). PCR-based replicon typing (PBRT) is a method for plasmid identification and typing in *Enterobacteriaceae* for determination of phylogenetic relatedness (15). In this study we determined the profile of antibiotic resistance, virulence, ESBLs, AmpC, and MBLs genes as well as plasmid replicon types among 39 *Salmonella* spp. isolates in Kerman, Iran.

MATERIALS AND METHODS

Bacterial isolates. This study was conducted on 39 *Salmonella* spp. that were stored at -70°C in tryptic soy broth (TSB, CONDA, Co, Spian) supplemented with 15% glycerol. These isolates were collected from fecal samples of chicken. All bacterial isolates were re-identified using standard biochemical identification tests including growth in xylose lysine deoxycholate (XLD), MacConkey agar, triple sugar iron agar (TSI), salmonella-shigella agar, urea medium IMViC (indole, methyl-red-Voges-Proskauer, citrate), o-nitrophenyl-β-D-galactopyranoside (ONPG), lysine decarboxylase, malonate tests. All isolates were confirmed as *Salmonella* spp. using PCR method. All culture mediums and biochemical reagents for identification of isolates in this study were purchased from CONDA (CONDA, Co, Spain) or HiMedia (HiMedia, Co, India). Primers sequences that were used for identification of *Salmonella* spp. are presented in Table 1. Four genes including *invA* for *Salmonella* spp. *fliC* for *S*. Typhimorium, *sdfI* for *S*. Enteritidis, and *fliB* for *S*. Infantis of were used in PCR assays to confirm the identity of *Salmonella* spp. (1-4).

Antimicrobial susceptibility of isolates. The susceptibility of isolates to antibacterial agents including amoxicillin (AMX; 30µg), cefotaxime (CTX; 30µg), ceftazidime (CAZ; 30µg), cefepime (FEP; 30µg), imipenem (IPM; 10µg), meropenem (MEM; 10µg), cefoxitin (FOX; 30µg), aztreonam (AZT; 30µg), ceftriaxone (CRO; 30µg), chloramphenicol (CHL; 30µg), ciprofloxacin (CIP; 10µg), tetracycline (TET; 30µg), amikacin (AMK; 30µg), and gentamicin (GEN; 10µg) was determined using Kirby-Bauer disk diffusion test as instructed by Clinical and Laboratory Standards Institute (16). The antibiotic disks obtained from PADTAN TEB Co, Iran and *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were used as standard strains in antimicrobial susceptibil-

Table 1. The primer sequences were used in this study.

Gene	Primer sequence (5′-3′)	PCR product size (bp)	Annealing TM (°C)	Reference
invA	F: GTGAAATTATCGCCACGTTCGGGCAA	285	60	(1)
	R: TCATCGCACCGTCAAAGGAACC			
fliC	F: CGGTGTTGCCCAGGTTGGTAAT	559	60	(2)
	R: ACTCTTGCTGGCGGTGCGACTT			
sdfI	F: TGTGTTTTATCTGATGCAAGAGG	304	56	(3)
	R: TGAACTACGTTCGTTCTTCTGG			
fljB	F: AACAACGACAGCTTATGCCG	734	56	(4)
	R: CCACCTGCGCCAACGCT			
slyA	F: GCCAAAACTGAAGCTACAGGTG	700	60	(5)
	R: CGGCAGGTCAGCGTGTCGTGC'			
spvC	F: ACTCCTTGCACAACCAAATGCGGA	424	60	(6)
	R: TGTCTTCTGCATTTCGCCACCATCA			
sopB	F: GATGTGATTAATGAAGAAATGCC	1170	60	(7)
	R: GCAAACCATAAAAACTACACTCA			
Stn	F: TTAGGTTGATGCTTATGATGGACACCC	617	60	(9)
	R: CGTGATGAATAAAGATACTCATAGG			
phoP/Q	F: ATGCAAAGCCCGACCATGACG	299	60	(10)
	R: GTATCGACCACCACGATGGTT			
bla_{SHV}	F: TTAACTCCCTGTTAGCCA	768	61	(11)
	R: GATTTGCTGATTTCGCCC			
bla_{TEM}	F: AAAATTCTTGAAGACG	1080	61	
	R: TTACCAATGCTTAATCA			
bla_{CTX-M}	F: CGATGTGCAGTACCAGTAA	585	59	
	R: TTAGTGACCAGAATCAGCGG			
bla_{NDM}	F: GAATTCGCCCCATATTTTTGC	977	61	
	R: AACGCCTCTGTCACATCGAAAT			
bla_{IMP}	F: GGAATAGAGTGGCTTAAYTCTC	232	58	
	R: GGTTTAAYAAAACAACCACC			
bla_{VIM}	F: ATGTTAAAAGTTATTAGTAGT	801	53	
	R: CTACTCGGCGACTGAGCGAT			
pAmpC genes	F: GCTGCTCAAGGAGCACAGGAT	520	63 (Multiplex PCR)	(18)
	R: CACATTGACATAGGTGTGGTGC			
	F: TGGCCAGAACTGACAGGCAAA	462		
	R: TTTCTCCTGAACGTGGCTGGC			
	F: AACTTTCACAGGTGTGCTGGGT	405		
	R: CCGTACGCATACTGGCTTTGC			
	F: AACAGCCTCAGCAGCCGGTTA	346		
	R: TTCGCCGCAATCATCCCTAGC			
	F: TCGGTAAAGCCGATGTTGCGG	302		
	R: CTTCCACTGCGGCTGCCAGTT			
	F: AACATGGGGTATCAGGGAGATG	190		
	R: CAAAGCGCGTAACCGGATTGG			

ity tests. Combined disk method with clavulanic acid (CLA) including CAZ, CPD, and CTX (30µg) with/without 10µg CA was used for detection of extended-spectrum β-lactamases (ESBLs) producing isolates as confirmatory test (16). *Klebsiella pneumoniae* ATCC 700603 was used as positive control in confirmatory test for detection of ESBL-producing isolates.

DNA extraction. The total genomic DNA was extracted by boiling method. Briefly, 400 µL of the culture of presumptive *Salmonella* were placed in 1.5 mL tubes and centrifuged for 2 min at 12,000 rpm, then the supernatant was discarded and 500 µL of DNase and RNase free water were added to resuspend the culture of *Salmonella*. The suspension boiled for 10 min and transferred immediately into ice for 10 min. Then, another centrifugation was performed at 12,000 rpm for 5 min and the obtained supernatant was used as template DNA (17).

Detection of virulence genes. The virulence genes including *spvC, slyA*, *sopB, phoP/Q*, and *stn* were detected by PCR methods. The sequences, annealing temperature of the virulence primers are presented in Table 1.

Detection of ESBLs, pAmpC, MBLs genes. ESBLs and MBLs genes including bla_{TEM}, bla_{SHV}, bla_{CTX-M}, bla_{VIM}, bla_{IMP}, and bla_{NDM} were identified using PCR technique. Plasmid-mediated *ampC* genes (pAmpC); bla_{FOX}, bla_{DHA}, bla_{CMY}, bla_{ACT}, bla_{CIT}, bla_{ACC}, were detected by multiplex PCR described previously by Pérez-Pérez et al. (18). PCR products for bla_{NDM} were bidirected sequenced at the Macrogen, Inc, South Korean Public. The sequence, annealing temperature, and PCR product sizes of beta-lactamase genes are presented in Table 1. *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* strains harboring some ESBLs, AmpC, and MBLs genes that isolated in our previous studies were used as positive controls in PCR (19-21). PCR reactions in sections 4.2 and 5.2 were done using Taq DNA Polymerase 2× Master Mix RED (Ampliqon, Co, Denmark) according to manufacturer's protocol.

Plasmid replicon typing of isolates. Plasmid replicon typing of the *Salmonella* spp. isolates were determined using PCR-based replicon typing (PBRT) described previously by Carattoli et al. (14). The PBRT method is performed with 18 pairs of primers sequence recognizing L/M, N, P, W, T, A/C, K, B/O, X, Y, F, FIA, FIB, FIC, HI1, HI2, I1-Iγ, and FIIA in five multiplex and three simplex-PCR reactions. *Klebsiella pneumoniae* strains harboring some plasmid replicon types were used as positive controls in PBRT method, these bacteria were identified in our previous studies (21).

PCR product evaluation. All PCR products were electrophoresed in a 1.5% agarose with 0.5× TBE (Tris/Borate/EDTA) buffer and were visualized using Gel Documentation UV light system after stained using Green Viewer dye (Parstous, Co, Iran).

RESULTS

***Salmonella* spp.** Among 39 *Salmonella* spp. isolates, 21 isolates (53.8%) were *S.* Enteritidis, 14 isolates (35.9%) were *S.* Infantis, and 4 isolates (10.2%) were *S.* Typhimurium.

Antimicrobial susceptibility results. The frequency of resistance to antibiotic agents was 82% to AMK, 76.9% to CIP, 58.9% to TET, 25.6% to CHL, 25.6% to IPM, 25.6% to AMX, 23.7% to AZT, 23.7% to CAZ, 12.8% to CTX, 12.8% to GEN, 10.25% to FOX, 10.25% to CRO, 10.25% to AMX/CLA, and 5.1% to FEP. All of isolates were sensitive to MEM and among cephalosporin-resistant isolates 30.7% of them were ESBL-producing.

Prevalence of virulence genes. The prevalence of virulence genes including *phoP/Q*, *slyA*, *sopB*, and *stn* were 84.6% (n=33), 79.4% (n=31), 69.2% (n=27) and 2.5% (n=1), respectively and all isolates were negative for *spvC* gene. Simultaneous presence of virulence gene was: *phoP/Q-slyA-sopB-stn* (2.5%; n=1), *phoP/Q-slyA-sopB* (59%; n=23), and *phoP/Q-slyA* (15.3%, n=6).

Prevalence of ESBLs, AmpC, and Carbapenemase genes (*bla* genes). The bla_{TEM} (n=16; 41%), was the most frequent β-lactamase gene among the isolates, and bla_{CTX-M} and bla_{DHA} were detected in 1.5% (n=1) and 7.7% (n=3) of isolates, respectively. Among IPM resistant isolates bla_{NDM-1} was detected in 15.4% (n=6) of the isolates. Coexistence of bla_{TEM} with bla_{DHA}, and bla_{TEM} with bla_{NDM-1} were identified in 5.1% (n=2) and 10.2% (n=4) of the isolates, respectively. The bla_{IMP} and bla_{VIM} were not detected in our study. Sequencing of bla_{NDM} amplicon in PCR method

confirmed that *Salmonella enterica* subsp. *enterica* serovar Enteritidis (*Salmonella* Enteritidis) was positive for bla_{NDM-1}. Some complete sequences of bla_{NDM-1} were submitted to GenBank with accession numbers MW075220, ON715003, and OR786309.

Prevalence of plasmid replicon types. Overall, 6 different plasmid replicon types including [IncP (n=9; 23%), IncFIC (n=3; 7.70%), IncY (n=3; 7.70%), IncI1-Iγ (n=2; 5.12%), IncFIIAs (n=1; 2.56%) and IncN (n=1; 2.56%)] were identified. In our study, there was not any significant relation among presence of Inc plasmids with antibiotic resistance pattern, beta-lactamases, and virulence genes. However, all bla_{DHA} positive isolates were positive for IncFIC replicon type and Inc plasmids were not detected in 58.9% (n=23) of the isolates. Distribution of beta-lactamase, virulence genes, and Inc plasmid replicon types among *Salmonella* isolates were shown in Table 2.

DISCUSSION

This study aimed to determine the antibiotic resistance profile, virulence factors, beta-lactamases genes (*bla* genes), and plasmid replicon types among 39 *Salmonella* spp. in Kerman, Iran. Zoonotic pathogens such as *Salmonella* spp. are frequently transmitted through chickens, eggs, and other animal products to humans and public heaths (22). Various *Salmonella* spp. can cause illness in humans, but *S.* Enteritidis is particularly significant as a leading cause of foodborne salmonellosis. In Iran, eggs and chicken meat are the common sources of *Salmonella* spp. (23). In our study, among the 39 *Salmonella* spp., 53.8% were *S.* Enteritidis, 35.9% were *S.* Infantis, and 10.2% were *S.* Typhimurium, which were more than the frequency of *Salmonella* spp. isolated from chickens in eastern Turkey and Canada (24, 25). Differences in *S.* Enteritidis contamination rates can be

Table 2. Distribution of *bla* and virulence genes, and Inc plasmid replicon types among *Salmonella* spp.

Isolates (n)	*bla* gene	Virulence genes	Plasmid replicon types
S. Infantis (4)	-	*slyD, sopB, phoP/Q*	IncP
S. Infantis (3)	-	*slyD, sopB, phoP/Q*	-
S. Infantis (2)	bla_{TEM}	*slyD, sopB, phoP/Q*	IncP
S. Infantis (1)	-	*slyD, sopB, phoP/Q*	IncI1-Iγ, IncP
S. Infantis (1)	-	*slyD, sopB, phoP/Q*	IncY
S. Infantis (1)	-	*slyD, sopB, phoP/Q*	IncN
S. Infantis (1)	bla_{TEM}	*slyD, sopB, phoP/Q*	-
S. Infantis (1)	bla_{TEM}	*slyD, phoP/Q*	-
S. Enteritidis (1)	bla_{TEM}	*slyD, sopB, phoP/Q, stn*	IncFIIAs
S. Enteritidis (2)	bla_{TEM}	*slyD, sopB, phoP/Q*	-
S. Enteritidis (3)	-	*slyD, sopB, phoP/Q*	-
S. Enteritidis (3)	bla_{NDM} , blq_{TEM}	*slyD, sopB, phoP/Q*	-
S. Enteritidis (1)	-	*slyD, sopB, phoP/Q*	IncP
S. Enteritidis (1)	bla_{NDM}	*slyD, sopB, phoP/Q*	- IncI1-
S. Enteritidis (1)	bla_{NDM} , bla_{TEM}	*slyD, sopB, phoP/Q*	Iy
S. Enteritidis (1)	bla_{TEM}	*slyD, phoP/Q*	IncP
S. Enteritidis (1)	*bla*	*slyD, phoP/Q*	-
S. Enteritidis (1)	bla^{NDM}_{DHA}	*sopB*	IncY, IncFIC
S. Enteritidis (1)	-	*phoP/Q*	-
S. Enteritidis (1)	bla_{TEM}, bla_{DHA}	-	IncY, IncFIC
S. Enteritidis (1)	bla_{CTX-M}	-	-
S. Enteritidis (1)	bla_{TEM} , bla_{DHA}	-	IncFIC
S. Enteritidis (2)	-	-	-
S. Typhimurium (1)	bla_{TEM}	*slyD, sopB, phoP/Q*	-
S. Typhimurium (1)	-	*slyD, sopB, phoP/Q*	-
S. Typhimurium (1)	bla_{TEM}	*slyD, phoP/Q*	-
S. Typhimurium (1)	-	*sopB*	-

attributed to variations in health control and management programs in different countries (26). In this study, the prevalence of resistance to amikacin and ciprofloxacin were considerably high. This results is consistent with previous studies on chicken meat conducted in Egypt and Iraq (27, 28). The reporting of resistance to antibiotic agents in *Salmonella* isolates from fecal samples of chicken farms suggests the contamination and spread of resistant strains in the environment, posing a potential risk to both animals, humans, and public heaths. Different studies on chicken meat in China reported the rate of resistance to ceftazidime resistance was 7.14% and amikacin was 8.13% which were much lower than our results (17, 29). The difference in resistance profiles in various studies may be attributed to geographic locations, local antimicrobial usage strategy, farms management, and the inappropriate use or overuse of antimicrobial agents, genetic exchanges, and chromosomal mutations. In Gram-negative bacteria, resistance to β-lactam antibiotics is typically attributed to the production of carbapenemase (MBLs), AmpC, and ESBLs as well as the overactivity of efflux pumps or loss of specific outer membrane porins (30). In our study, resistance to third generation cephalosporins including cefotaxime and ceftazidime can be explained by the presence of extended-spectrum β-lactamases (ESBL) enzymes. In this study, the antibiotic susceptibility test showed that 30.7% of the isolates were identified as ESBL producing isolates. The most frequency β-lactamase gene in the present study was bla_{TEM} with prevalence 41%. In recent years, ESBLs have been expanded among *Salmonella* spp. in different countries (31, 32). In a study in China, the prevalence of ESBLs positive isolates among *Salmonella* spp. in raw retail chicken carcasses 10.8% was reported which was lower than our results (33). The spread of ESBL-producing bacteria can cause resistance to multiple types of antibiotics, making them more difficult to treat, because ESBL-producing isolates usually are resistant to other classes of antibiotics including aminoglycosides and fluoroquinolones. Additionally, the contamination of the environment with these bacteria can increase the risk of infection for humans and animals. Therefore, it is important to properly use antibiotics and implement measures to control the spread of ESBL-producing bacteria. Our findings, as well as previous reports, suggest that isolates producing carbapenemase are often resistant to various antibiotics, including aminoglycosides, cephalosporins, monobactams, fluoroquinolones, and tetracycline, as resistance genes are frequently located on the same plasmids (30). In this study, we reported bla_{NDM-1} genes and resistance to IPM for the first time in Kerman, Iran. Recently, between 2015-2018, we reported the bla_{NDM-1} in *K. pneumoniae* and *Pseudomonas aeruginosa* among different clinical samples from hospital settings in Kerman, Iran (19, 20). Reporting the bla_{NDM-1} among various Gram-negative bacteria in our region shows that Kerman has become an endemic region for bla_{NDM-1} in Iran. Therefore, the transfer of bla_{NDM-1} and other resistance genes among various bacteria may occurred through hospital or agriculture wastewater, although more research is needed. The rate of carbapenem resistance in *Salmonella* spp. is much lower compared to other *Enterobacteriaceae* species (33). The first carbapenemase-producing *S. enterica* strains isolated from livestock pig and poultry farms in Germany and that were positive for bla_{VIM} (34). In a study in Egypt, *S.* Enteritidis isolates that were positive for bla_{NDM} were reported (35). The bla_{NDM-1} gene was also found in *S.* Stanley in China, *S.* Senftenberg among clinical samples in the UK, India, the USA (36-39). The presence of the bla_{NDM-1} and the spread of MDR isolates among *Salmonella* spp. represents a significant concern for public health as these bacteria serve as a reservoir for resistance genes. Additionally, they can contribute to the transmission of resistance genes within communities and farms through the food chains, also, the emergence of MDR *Salmonella* strains results in a limitation in treatment options for invasive infections in both humans and animals.

The presence of virulence factors and antibiotic-resistance genes in bacteria can contribute to their increased pathogenicity. *Salmonella* virulence factors are typically located within *Salmonella* pathogenicity islands (SPIs). Although the *phop/Q* and *slyA* genes are not located in the pathogenicity islands of *Salmonella*, they play a serious role in the pathogenicity of this bacterium. In the present study, the *phoP/Q* gene was the dominant virulence gene (84.6%), followed by *slyA*, *sopB*, and *stn* with frequencies of 79.4%, 69.2%, and 2.5%. In contrast to our study, a previous report from China by Hai et al. found that over 90% of *Salmonella* spp. isolates harbored the *stn* gene (40). Also, Wang et al. from China reported a high incidence of *sopB* (100%), and *spvC* (93.8%) among *Salmonella* serovars isolated from 120 re-

tail meat samples (41). In studies in Iran and Nigeria the prevalence rates of *spvC* gene were 50.8%, 59.1%, and 45.8%, respectively (26, 28). According to a study by Takaichi et al. conducted in Indonesia, the prevalence rates of *slyA, phoP/Q,* and *sopB* were reported as 60%, 86%, and 88%, respectively (42). Interestingly, the *spvC* gene was not identified in the study, which is consistent with current research. *SopB,* a virulence factor that plays a crucial role in the internalization of bacteria into host cells, has been identified as an important factor in salmonellosis. A Brazilian study on chicken carcasses the frequency of *sopB* was similar to the frequency of this gene in our study (43).

Virulence and *bla* genes have been identified on Inc plasmid replicon types. An Inc type refers to a specific incompatibility group of plasmids that cannot co-exist stably with other plasmids belonging to different Inc types in the same bacterial strain (44). Although bla_{NDM-1} carrying plasmids can transfer between different species of enterobacteria, there are restrictions on their transfer, and the characteristics of these plasmids can vary depending on the bacterial species. Understanding the mechanisms of plasmid transfer and the characteristics of them is important for developing strategies to control the spread of antibiotic resistance (45). Hadzjabdich et al. conducted a study on the experimental transfer and microevolution of the IncA/C2 plasmid carrying bla_{NDM-1} in *S. enterica* serovar Corvallis that was present in broiler flocks. The study concluded that this plasmid has the potential to spread widely across different hosts (46). Ingti et al. showed that bla_{DHA} is associated with K, FIA, L/M, FIB, HI1, B/O & I1 Inc types but in this study the isolates were positive for bla_{DHA} harbored IncFIC and IncY replicon types (47). Previous studies showed the relationship between bla_{NDM-1} gene and several incompatible plasmid groups such as FIIK, FII, FIB, FIC, L and Inc M (36, 48). In the current study, all isolates positive for bla_{NDM-1} were found to be negative for the Inc plasmid groups. It is possible that our PBRT method could not detect some replicon types, therefore, more sensitive and accurate methods such as real-time PCR, whole genome sequencing, and DNA-DNA hybridization should be considered for identification of other plasmid replicon types. However, some studies have indicated that the bla_{NDM-1} gene may be associated with several incompatibility plasmid groups, including IncA/C, in *Salmonella* isolates (49). Additionally, Kocsis et al. highlighted the significance of the IncR plasmid that carries bla_{NDM-1} (50). In the current study IncP was the most prevalent plasmid replicon type because IncP plasmid is a wide-host-range replicon type can almost transfer among all Gram-negative bacteria (51). In the study by Zurfluh, IncI1 type was the dominant Inc plasmid type among bla_{CTX-M} positive *Enterobacteriaceae* isolated from avian samples such as chicken farms (52). Previous studies have identified various incompatibility plasmids in *Salmonella* spp. which are associated with virulence genes. IncFII and IncFI groups have been shown to contribute to intracellular replication, resistance to complement, bacterial adhesion, and host cell invasion through the *spvRABCD, pef, rck,* and *rsk* genes in *S. enterica* serovar Typhimurium (53). This diversity in the prevalence of plasmid replicon types in different *Salmonella* spp. shows the high potential of this bacterium in storing and transferring drug resistance and virulence genes.

CONCLUSION

This study is the first report on the presence of the bla_{NDM-1} gene among *Salmonella* spp. from broiler chicken in Kerman, Iran. Based on our results, the detection of six plasmid replicon types among *Salmonella* isolates indicated their ability as a reservoir to preserve and spread virulence and drug-resistance genes. In addition, we did not find a significant relationship between the presence of plasmid replicon types with virulence and *bla* genes. This result suggests that other replicon types may be involved in the spread of virulence and resistance genes as well as bla_{NDM-1} in this bacterium. Finally, it is important to implement measures that promote the appropriate use of antibiotics in both human, veterinary medicine, and agriculture, also the development of alternative strategies for the prevention and treatment of infectious diseases such as vaccines, phage therapy, and surveillance of antibiotic resistance in bacterial populations are important.

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