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## RESEARCH ARTICLE

### Distribution of Class 1 and 2 Integrons Among *Salmonella Enterica* Serovars Isolated from Iranian Patients

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#### Abstract:

#### Background:

*Salmonella enterica* has become increasingly resistant to antimicrobial agents, partly as a result of genes carried by integrons.

#### Objective:

The aim of the present study was to investigate the prevalence of class 1 and 2 integrons and resistance to antimicrobial agents in clinical isolates of *S. enterica*.

#### Methods:

This study included all *Salmonella* isolates, recovered from patients with salmonellosis, admitted to Medical Children Hospital, Tehran, Iran during 2015-2016. Bacterial isolates were identified using standard biochemical and agglutination tests. Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute guidelines. The presence of class 1 and 2 integrons was investigated by Polymerase Chain Reaction (PCR) assay, using specific primers.

#### Results:

A total of 138 *Salmonella* strains were isolated and included in this study. Integrons were detected in 45 (32%) isolates. Class 1 and 2 integrons were detected in 24 (17.3%) and 21 (15.2%) isolates, respectively. All integron-positive isolates showed multidrug-resistant phenotypes. Resistance to more than three antimicrobial agents was observed in integron-positive isolates.

#### Conclusions:

Our findings showed that integrons were widely distributed among *S. enterica* isolates in Tehran. Class 1 integrons are more prevalent than class 2 integrons in *Salmonella* isolates, and there is an association with MDR patterns. Therefore, these integrons are more likely to be involved in the distribution of resistant phenotypes in *Salmonella* strains.

**Keywords:** Antibiotic resistance, Integrons, Multidrug-Resistant (MDR), *Salmonella enterica*, Antimicrobial agents, Phenotypes.

#### Article History

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## 1. INTRODUCTION

Gastroenteritis and diarrheal diseases are among the most important health problems worldwide, especially in developing countries [1]. *Salmonella* remains one of the most common causes of acute gastroenteritis and food-borne infections

around the world [2]. Different species of this bacterium are frequently encountered in different countries, including Iran [3]. Antibiotic resistance is common among these serovars, and a high prevalence of multidrug-resistance (MDR) isolates has also been reported [4].

There are many mechanisms of antibiotic resistance through which bacteria exhibit resistance to antibiotics. Acquisition of genetic elements is one of the major mechanisms, which frequently occurs in both Gram-positive and Gram-

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**Table 1. Oligonucleotides used in the study.**

Primers	(5'→3') Sequence	Amplicon size (bp)	References
Int1	F:5'-GGGTCAAGGATCTGGATTTCG-3'	483	[11]
	R:5'-ACATGCGTGTAATCATCGTCG -3'		
Int2	F:5'-GCAAATGAAGTGCAACGC-3'	466	[11]
	R:5' -ACACGCTTGCTAACGATG -3'		

**Table 2. Antimicrobial susceptibility of *Salmonella enterica* isolates from different serogroups.**

Antibiotics (µg)*	Group A	Group B	Group C	Group D	Total
TE (30)	1	9	24	21	55
S (10)	0	9	16	19	44
SXT (1.25+23.75)	0	4	12	2	18
AMP (10)	0	1	4	4	9
GM (10)	0	6	8	17	31

\*Concentration of disks; TE, tetracycline; S, streptomycin; SXT, trimethoprim-sulfamethoxazole; AMP, ampicillin; GM, gentamicin. Numbers within boxes display number of resistant isolates against different antibiotics.

negative bacteria. Integrons are DNA elements with major involvement in the spread of antibiotic-resistant genes through horizontal or vertical gene transfer mechanisms [5]. Although at least six classes of integrons have been reported, there is limited data about these integrons classes, and only classes 1, 2, and 3 are well-studied [6].

Integrons are very common in *S. enterica* serovars and seem to be significantly involved in the advent of antibiotic-resistant phenotypes in the strains [7]. The presence of integrons in the strains increases resistance to fluoroquinolones, which are very effective in the treatment of salmonellosis [8]. In addition to fluoroquinolone resistance, resistance to cephalosporins has been also associated with integron cassettes. It is clear that *veb-1* gene, encoding resistance to cephalosporins, is a part of integron cassettes. Fluoroquinolones and cephalosporins are two main classes of antibiotics, frequently used to treat *Salmonella* infections. The prevalence of resistant genes in association with integron cassettes can be a new challenge in the treatment of salmonellosis and its rate is increasing; therefore, it is necessary to determine different classes of integrons in the strains [9].

The aim of the current study was to investigate the prevalence of integrons and resistance to antimicrobial agents in clinical isolates of *S. enterica*, isolated from patients admitted to the Medical Children Hospital of Tehran, Iran.

## 2. MATERIALS AND METHODS

### 2.1. Bacterial Isolates

This study included all recovered *Salmonella* isolates from patients with *Salmonella* infections, who were admitted to the Children Medical Center of Tehran, Iran during 2015-2016. The isolates were identified by standard biochemical and agglutination tests using commercial antisera (Staten Serum Institute, Copenhagen, Denmark), according to the manufacturer's instructions.

Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute

(CLSI) guidelines and Kirby-Bauer disc diffusion method. The tested antibiotics included cotrimoxazole (1.25/23.75 µg), gentamicin (10 µg), ampicillin (10 µg), streptomycin (10 µg), and tetracycline (30 µg).

### 2.2. DNA Extraction

DNA extraction was carried out using a previously described method, with some modifications [10]. Briefly, overnight bacterial cultures were separately suspended in 250 µL of lysis buffer (10 mM tris-HCl, 0.1 M NaCl, 1 mM EDTA, and 5% [v/v] Triton X100; pH, 8.0) and boiled at 100°C for three minutes. The suspensions were centrifuged at 10 000 rpm for 10 minutes, and the supernatants were transferred to new sterile microtubes. Then, 250 µL of ethanol 95% was added to each microtube and kept at -20°C for 30 minutes. The solutions were centrifuged at 14 000 rpm for 20 minutes, and then, the sediments were dissolved in 100 µL of sterile distilled water and stored at -20°C. DNA concentration was measured using Thermo Scientific NanoDrop spectrophotometers.

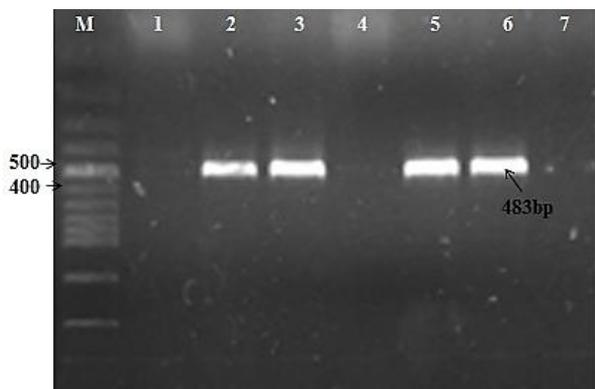
### 2.3. Polymerase Chain Reaction (PCR) Assay

The PCR assay was carried out for the reaction mixture in a final volume of 25 µL, containing 12.5 µL of 2X PCR Master Mix (CinnaGen, Iran), 10 pmol of forward and reverse primers (Bioneer, Korea) (Table 1), and 50 ng DNA. The PCR conditions were as follows: initial denaturation at 94°C for five minutes; 30 cycles of 94°C for 50 seconds; annealing at 60°C and 72°C for one minute; and a final extension at 72°C for 10 minutes using a thermocycler (Eppendorf Thermal Cycler, Germany). The PCR products were electrophoresed on 1% (W/V) Agarose gel (Sigma-Aldrich, USA) and visualized under UV light after staining with a DNA Safe Stain (CinnaGen, Iran).

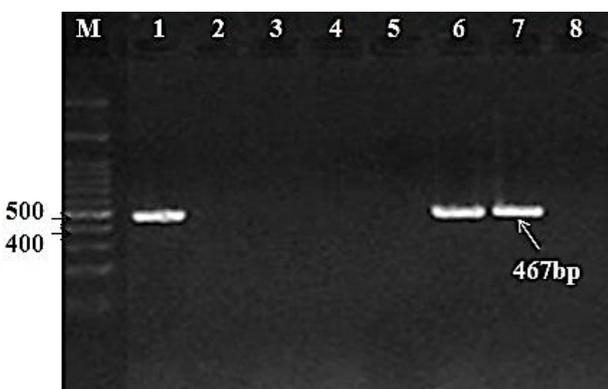
## 3. RESULTS

Among 138 *Salmonella* isolates, 1, 18, 41, and 78 isolates belonged to serogroups A, B, C, and D, respectively. The results of the antimicrobial susceptibility test, based on the serogroup categories, are presented in Table (2). As shown in

this Table, 55 isolates were resistant to tetracycline, while only nine isolates had resistant phenotypes to ampicillin.



**Fig. (1).** Agarose gel electrophoresis of Int1 amplicons. Lane M, 100-bp DNA ladder; lanes 1-5, *Salmonella enterica* isolates; lane 6, positive control; lane 7, negative control.



**Fig. (2).** Agarose gel electrophoresis of Int2 amplicons. Lane M, 100-bp DNA ladder; lane 1, positive control; lanes 2-7, *Salmonella enterica* isolates; lane 8, negative control.

A total of 138 isolates were screened for *int1* and *int2* genes. Integron classes 1 and 2 were only detected in 45 isolates (class 1: 24 isolates (17.3%); class 2: 21 isolates (15.2%)). Figs. (1 and 2) demonstrate the amplicons of class 1 and 2 integrons, respectively.

#### 4. DISCUSSION

Different levels of antimicrobial resistance to several antibiotics are common among bacteria worldwide [12]. Integrons are genetic elements associated with resistance to antimicrobial agents. They are categorized into more than six classes, based on the *intI* gene sequence [13]. Transferability of integrons, especially class 1 integrons, has been found to be common among bacteria through the horizontal gene transfer mechanism [14]; our findings are consistent with the literature.

In the present study, the prevalence of antibiotic resistance among *Salmonella* isolates was determined. The results showed that most isolates were resistant and susceptible to tetracycline and ampicillin, respectively. In accordance with our findings, Jackson *et al.* showed that *S. typhimurium* isolates are resistant to tetracycline, ampicillin, and cotrimoxazole [15]. In a pre-

vious study, we revealed that *Salmonella* isolates had high levels of resistance to doxycycline, tetracycline, and streptomycin [16]. Our findings, in line with previous studies, indicate that resistance to tetracycline is common among isolates, and this agent seems to be ineffective in the treatment of *Salmonella* infections.

In this study, resistance to antimicrobial agents varied among *Salmonella* serogroups; this finding is predictable due to the presence of different strains in these groups. These serogroups were formerly classified as *Salmonella* species. Also, different patterns of antibiotic resistance have been reported among *Salmonella* species by other researchers [17, 18].

In the present study, we characterized two classes of integrons, and 45 out of 138 isolates harbored class 1 (17.3%) and class 2 (15.2%) integrons. In a previous study, Asgharpour *et al.* showed that 36% of *S. infantis* isolates were positive for class 1 integrons, and all these isolates were also resistant to nalidixic acid, tetracycline, and streptomycin [19]. In comparison with our study, the higher prevalence of class 1 integrons in the mentioned study shows that application of antibiotics in the poultry industry may increase antibiotic resistance [20]. In another study, 45% of *S. enterica* serotypes, including Mbandaka, Brandenburg, Agona, Alachua, and Panama serotypes, harbored class 1 integron genes [21].

Up to 17.39% of isolates harboring class 1 integrons were resistant to ampicillin (2.89%), gentamicin and trimethoprim / sulfamethoxazole (5.79%), tetracycline (10.14%), and streptomycin (7.97%), whereas resistance to these antibiotics in integron-negative isolates was 14.49%, 7.97%, 7.24%, 26.08%, and 22.46%, respectively. The low level of ampicillin resistance among integron-negative isolates suggests that resistance to this antibiotic is not associated with integrons and is commonly associated with TEM  $\beta$ -lactamases, as described in the literature [22].

The prevalence of class 2 integrons was found to be 15.21% among the studied isolates, indicating the low prevalence of this class of integrons. In studies by other researchers, the prevalence of class 2 integrons was also lower than our study [23, 24]. In this regard, in a study by Jin *et al.*, class 2 integrons were not detected even among 834 *Salmonella* isolates [25].

#### CONCLUSION

According to our findings, the prevalence of antibiotic resistance is high among *Salmonella* isolates, and screening for integrons, as likely indicators of antibiotic resistance, may be useful in detecting *Salmonella* organisms with antibiotic-resistance phenotypes. Although our study showed that integrons are associated with antibiotic-resistance phenotypes, further studies are needed to determine the exact relationship between classes of integrons and resistance to different antibiotics.

#### ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved in Ethical Committee No.33 in

Baqiyatallah University of Medical Sciences, Tehran.

## HUMAN AND ANIMAL RIGHTS

No animals/humans were used for studies that are the basis of this research.

## CONSENT FOR PUBLICATION

Informed consent was obtained from all the participants.

## CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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## REFERENCES

- [1] Ranjbar R, Salimkhani E, Sadeghifard N, *et al.* An outbreak of gastroenteritis of unknown origin in Tehran, July 2003. *Pak J Biol Sci* 2007; 10(7): 1138-40. [http://dx.doi.org/10.3923/pjbs.2007.1138.1140] [PMID: 19070067]
- [2] Ranjbar R, Ahmadi M, Memariani M. Multiple-locus variable-number tandem repeat analysis (MLVA) for genotyping of *Salmonella enterica* subspecies enterica serotype Infantis isolated from human sources. *Microb Pathog* 2016; 100: 299-304. [http://dx.doi.org/10.1016/j.micpath.2016.10.012] [PMID: 27765620]
- [3] Mulvey MR, Soule G, Boyd D, Demczuk W, Ahmed R. Characterization of the first extended-spectrum beta-lactamase-producing *Salmonella* isolate identified in Canada. *J Clin Microbiol* 2003; 41(1): 460-2. [http://dx.doi.org/10.1128/JCM.41.1.460-462.2003] [PMID: 12517894]
- [4] Wang Y, Cao C, Alali WQ, *et al.* Distribution and antimicrobial susceptibility of foodborne *Salmonella Serovars* in eight provinces in China from 2007 to 2012 (Except 2009). *Foodborne Pathog Dis* 2017; 14(7): 393-9. [http://dx.doi.org/10.1089/fpd.2016.2237] [PMID: 28375673]
- [5] Mazel D. Integrons: agents of bacterial evolution. *Nat Rev Microbiol* 2006; 4(8): 608-20. [http://dx.doi.org/10.1038/nrmicro1462] [PMID: 16845431]
- [6] Ploy M-C, Lambert T, Couty J-P, Denis F. Integrons: an antibiotic resistance gene capture and expression system. *Clin Chem Lab Med* 2000; 38(6): 483-7. [http://dx.doi.org/10.1515/CCLM.2000.070] [PMID: 10987194]
- [7] Lopes GV, Michael GB, Cardoso M, Schwarz S. Antimicrobial resistance and class I integron-associated gene cassettes in *Salmonella enterica* serovar Typhimurium isolated from pigs at slaughter and abattoir environment. *Vet Microbiol* 2016; 194: 84-92. [http://dx.doi.org/10.1016/j.vetmic.2016.04.020] [PMID: 27142182]
- [8] Vo AT, van Duijkeren E, Gaastra W, Fluit AC. Antimicrobial resistance, class I integrons, and genomic island 1 in *Salmonella* isolates from Vietnam. *PLoS One* 2010; 5(2): e9440. [http://dx.doi.org/10.1371/journal.pone.0009440] [PMID: 20195474]
- [9] Naghoni A, Ranjbar R, Tabaraie B, *et al.* High prevalence of integron-mediated resistance in clinical isolates of *Salmonella enterica*. *Jpn J Infect Dis* 2010; 63(6): 417-21. [PMID: 21099092]
- [10] Hassanzadeh S, Pourmand MR, Afshar D, Dehbashi S, Mashhadi R. TENT: A rapid DNA extraction method of *Staphylococcus aureus*. *Iran J Public Health* 2016; 45(8): 1093-5. [PMID: 27928541]
- [11] Mazel D, Dychinco B, Webb VA, Davies J. Antibiotic resistance in the ECOR collection: integrons and identification of a novel aad gene. *Antimicrob Agents Chemother* 2000; 44(6): 1568-74. [http://dx.doi.org/10.1128/AAC.44.6.1568-1574.2000] [PMID: 10817710]
- [12] Hendriksen RS, Mevius DJ, Schroeter A, *et al.* Occurrence of antimicrobial resistance among bacterial pathogens 2008.
- [13] Domingues S, da Silva GJ, Nielsen KM. Integrons: Vehicles and pathways for horizontal dissemination in bacteria. *Mob Genet Elements* 2012; 2(5): 211-23. [http://dx.doi.org/10.4161/mge.22967] [PMID: 23550063]
- [14] van Essen-Zandbergen A, Smith H, Veldman K, Mevius D. Occurrence and characteristics of class 1, 2 and 3 integrons in *Escherichia coli*, *Salmonella* and *Campylobacter spp.* in the Netherlands. *J Antimicrob Chemother* 2007; 59(4): 746-50. [http://dx.doi.org/10.1093/jac/dkl549] [PMID: 17307772]
- [15] Onyuka JH, Kakai R, Onyango DM, Arama PF, Gichuki J. Prevalence and antimicrobial susceptibility patterns of enteric bacteria isolated from water and fish in lake victoria basin of western 2011.
- [16] Ranjbar R, Giammanco GM, Farshad S, Owlia P, Aleo A, Mammina C. Serotypes, antibiotic resistance, and class I integrons in *Salmonella* isolates from pediatric cases of enteritis in Tehran, Iran. *Foodborne Pathog Dis* 2011; 8(4): 547-53. [http://dx.doi.org/10.1089/fpd.2010.0736] [PMID: 21204690]
- [17] Busani L, Graziani C, Battisti A, *et al.* Antibiotic resistance in *Salmonella enterica* serotypes typhimurium, enteritidis and infantis from human infections, foodstuffs and farm animals in Italy. *Epidemiol Infect* 2004; 132(2): 245-51. [http://dx.doi.org/10.1017/S0950268803001936] [PMID: 15061499]
- [18] Tassios PT, Markogiannakis A, Vatopoulos AC, *et al.* Molecular epidemiology of antibiotic resistance of *Salmonella enteritidis* during a 7-year period in Greece. *J Clin Microbiol* 1997; 35(6): 1316-21. [PMID: 9163436]
- [19] Asgharpour F, Rajabnia R, Ferdosi Shahandashti E, Marashi MA, Khalilian M, Moulana Z. Investigation of class I integron in *Salmonella infantis* and its association with drug resistance. *Jundishapur J Microbiol* 2014; 7(5): e10019. [http://dx.doi.org/10.5812/jjm.10019] [PMID: 25147710]
- [20] Bada-Alamedji R, Fofana A, Seydi M, Akakpo AJ. Antimicrobial resistance of *Salmonella* isolated from poultry carcasses in Dakar (Senegal). *Braz J Microbiol* 2006; 37(4): 510-5. [http://dx.doi.org/10.1590/S1517-83822006000400020] [PMID: 16845431]
- [21] Ribeiro VB, Lincopan N, Landgraf M, Franco BD, Destro MT. Characterization of class I integrons and antibiotic resistance genes in multidrug-resistant *Salmonella enterica* isolates from foodstuff and related sources. *Braz J Microbiol* 2011; 42(2): 685-92. [http://dx.doi.org/10.1590/S1517-83822011000200033] [PMID: 24031680]
- [22] Ebner P, Garner K, Mathew A. Class I integrons in various *Salmonella enterica* serovars isolated from animals and identification of genomic island SG11 in *Salmonella enterica* var. Meleagridis. *J Antimicrob Chemother* 2004; 53(6): 1004-9. [http://dx.doi.org/10.1093/jac/dkh192] [PMID: 15117931]
- [23] Antunes P, Machado J, Peixe L. Characterization of antimicrobial resistance and class 1 and 2 integrons in *Salmonella enterica* isolates from different sources in Portugal. *J Antimicrob Chemother* 2006; 58(2): 297-304. [http://dx.doi.org/10.1093/jac/dkl242] [PMID: 16782741]
- [24] Peirano G, Agerso Y, Aarestrup FM, dos Reis EMF, dos Prazeres Rodrigues D. Occurrence of integrons and antimicrobial resistance genes among *Salmonella enterica* from Brazil. *J Antimicrob Chemother* 2006; 58(2): 305-9. [http://dx.doi.org/10.1093/jac/dkl248] [PMID: 16782743]
- [25] Jin Y, Ling JM. Prevalence of integrons in antibiotic-resistant *Salmonella spp.* in Hong Kong. *Jpn J Infect Dis* 2009; 62(6): 432-9. [PMID: 19934534]