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Prevalence of ESBLs and Integrons in Clinical Isolates of *Salmonella spp*. From Four Hospitals of Tehran

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Background: *Salmonella*e have become increasingly resistant to antimicrobial agents, partly as a result of genes carried on integrons.
Objectives: Here we describe the antibiotic susceptibility pattern, ESBL production and the prevalence of integrons genes among clinical isolates of *Salmonella spp*.
Materials and Methods: This descriptive study was done on 110 isolates collected from four hospitals in Tehran during 2012-2013 and identified by routine biochemical tests. Then, disk diffusion method was used for testing the antibiotic susceptibility. ESBL phenotype was confirmed by Combined Disk. The existence of integron classes was investigated by PCR assay through the amplification of integrase genes.
Results: Maximal resistance in *Salmonella* isolates was noticed against trimethoprim–sulfamethoxazole (63/6%) and nalidixic-acid (47/3%). All of isolates were susceptible to imipenem and ciprofloxacin. Four (3.6%) isolates showed ESBLs phenotype. Thirty six of *Salmonella* isolates have integron but there was not detected class 3 of integrons among isolates.
Conclusions: The present study shows the high prevalence of MDR among *Salmonella* isolates and so alarms the importance of continued monitoring of drug resistance in clinical settings.

Keywords:Salmonella; integron; Antibiotic Resistance; ESBLs

1. Background

*Salmonella*e are zoonotic enterobacteria that infect humans and animals. Human diseases ranging from diarrhea to systemic typhoid fever (1). The increasingly number of infections with antimicrobial drug-resistant *Salmonella*, including the extended-spectrum β-lactamase (ESBL)-producing *Salmonella* and fluoroquinolones-resistant *Salmonella* strains, merits special attention (2-4). The spread of antimicrobial resistance potential in *Salmonella* is mainly attributed to integrons. Integrons are genetic elements that recognize and capture mobile gene cassettes, which usually encode antimicrobial drug resistance determinants (5). Three classes of integrons have been characterized are involved in antibiotic resistance: classes 1, 2 and 3. Integrons are common in *Salmonella*, particularly in *S. enterica* and make an important contribution to the extent of antimicrobial resistance of the species (6-9). A basic role in the spread of antibiotic resistance in *Salmonella* has been ascribed to class 1 and 2 of integrons (10-13).

2. Objectives

The objectives of our study were to determine the antibiotic susceptibility pattern, ESBL production and the prevalence of integrons genes among clinical isolates of *Salmonella* species.

3. Materials and Methods

3.1. Bacterial Isolates and Identification

A total of 110 *Salmonella* isolates were collected from four hospitals in Tehran between 2012 -2013. The following characteristics were used to confirm *Salmonella* isolates: gram-negative bacilli, catalase positive and oxidase negative, motility positive, lactose negative and production of H2S gas.

3.2. Antibiotic Susceptibility Testing

The antibiotic susceptibility of integron positive isolates, was determined by disk diffusion method on Mueller-Hinton agar plates (Merck, Darmstadt, Germany) as recommended by the Clinical Laboratory Standards Institute (CLSI) (14). The disks containing the following antibiotics were used (Mast, UK): cefotaxime (30 μg), ceftriaxone (30 μg), ceftazidime (30 μg), imipenem (10 μg), aztreonam (30 μg), ciprofloxacin (5 μg), trimethoprim-

sulfamethoxazole (25 μg), tetracycline (30 μg), ofloxacin (5 μg), ampicillin (25 μg), chloramphenicol (30 μg), nalidixic acid (30 μg), cefoxitin (30 μg), tobramycin (10 μg), amikacin (30 μg), gentamicin (10 μg). *E. coli* ATCC 25922 was used as a control for antimicrobial susceptibility test.

3.3. ESBL Confirmation by Combination Disk Method

The isolates showing reduced susceptibility to ceftazidime or cefotaxime were tested for ESBLs production by the combination disk method according to CLSI guidelines (CLSI). Combination disk method was performed using four disks: cefotaxime (CTX) (30 μg), cefotaxime (30 μg) + clavulanic acid (10 μg), ceftazidime (CAZ) (30 μg), and ceftazidime (30 μg) + clavulanic acid (10 μg). A 5 mm increase in a zone diameter for antimicrobial agent tested in combination with clavulanic acid versus its zone when tested alone was considered as a ESBLs positive. Quality control for the production of ESBL was performed using *E. coli* ATCC 25922 as negative control. Minimum inhibitory concentration (MIC) of ceftazidime and cefotaxime was determined for ESBLs isolates by the E-test (AB Biodisk, Solna, Sweden) according to the guidelines of CLSI.

3.4. Detection of Integrons

Bacterial DNA were harvested by conventional boiling method (15). Two or three colonies of overnight culture of the bacteria on nutrient agar (Merck, Germany) were transferred into a 1.5 mL centrifugal tube with PBS and centrifuged it at 12,000 rpm for 10 minutes. After removal of the supernatant, the sediment was suspended in 200 μL of distilled water. The tube was placed in a boiling-water bath at 95 for 10 minutes and supernatant was used as template DNA. Determination of integron classes was performed by multiplex PCR using the primers described in Table 1. Polymerase chain reactions were performed in a 25 μL volume. Amplification reactions were performed in a total volume of 25 μL of reaction mixture containing 5 μL of 10 × PCR buffer, 2.5 mM MgCl2, 200 μM dNTP, 1.25 units of Taq polymerase, 10 pmol of each primer and 1 μL of sample DNA. PCR was performed on a DNA Engine Dyad, Peltier Thermal Cycler (Bio-Rad, Hercules, CA). PCR condition was showed in Table 1. *Acinetobacter baumannii* TMU1, TUM2 and TMU3 were used as positive control for class 1, 2 and 3 of integrons, respectively. PCR products were electrophoresed in 1.5% agarose, stained by Gel Red dye.

4. Results

4.1. Bacterial Isolates and Identification

A total of 110 *Salmonella* isolates were collected. They were mostly isolated from the stool culture (105 samples), and blood culture (5 samples) and then, identified at the level genus by biochemical tests.

4.2. Antibiotic Susceptibility Testing and ESBL Confirmation

Analysis of the antimicrobial susceptibility profile of the isolates showed that all were susceptible to imipenem and ciprofloxacin. Of 110 isolates, 63.6 % of the isolates were resistant to trimethoprim-sulfamethoxazole, 47.3 % were resistant to nalidixic acid, 6.4 % were resistant to ceftriaxone and ceftazidime, and 2.7 % were resistant to cefotaxime (Table 2). Of the 110 *Salmonella* isolates, 16 (14.5%) were susceptible to all antimicrobials tested and 39 (35.5%) were multidrug-resistant and showed resistance to more than two antimicrobial families.

Combined disc test was performed for 7 isolates. Four isolates of *Salmonella* showed ESBL phenotype.

Table 1. Primers, PCR Conditions, and Respective References

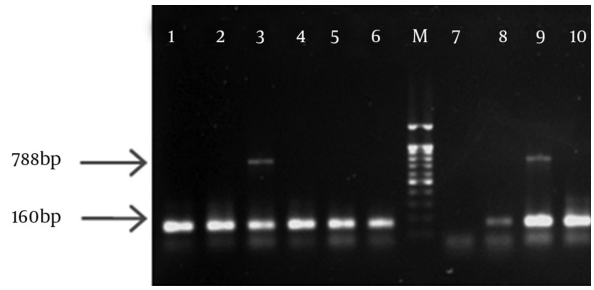
Primers	Nucleotide Sequence (5´to3´)	Amplicon, bp	PCR Condition			References
			Denaturin	Annealin	Extension	
			94, 30 s	55, 30 s	72, 3 s	
Int1		160				(16)
F	CAGTGGACATAAGCCTGTTC					
R	CCCGAGGCATAGACTGTA					
Int2		788				(16)
F	CACGGATATGCGACAAAAAGGT					
R	GTAGCAAACGAGTGACGAAATG					
Int3						
F	GCCTCCGGCAGCGACTTTCAG	979				(17)
R	ACGGATCTGCCAAACCTGAC					

Table 2. Percentage of isolates Susceptible, Moderately Susceptible or Resistance to Each Antibiotic [a,b]

Antibiotic	Susceptible	Intermediate	Resistant
NA	52 (47.3)	6 (5.5)	52 (47.3)
SXT	38 (34.5)	2 (1.8)	70 (63.6)
OFX	105 (95.5)	3 (2.7)	2 (1.8)
AMP	83 (75.5)	0 (0)	27 (24.5)
CHL	80 (72.7)	0 (0)	30 (27.3)
CIP	110 (100)	0 (0)	0 (0)
IPM	110 (100)	0 (0)	0 (0)
T	68 (61.8)	5 (4.5)	37 (33.6)
CRO	103 (93.6)	0 (0)	7 (6.4)
CTX	107 (97.3)	0 (0)	3 (2.7)
CAZ	103 (93.6)	0 (0)	7 (6.4)
ATM	104 (94.5)	0 (0)	6 (5.5)
FOX	104 (94.5)	0 (0)	6 (5.5)
AK	108 (98.1)	0 (0)	2 (1.81)
GM	109 (99.1)	0 (0)	1 (0.9)
TN	109 (99.1)	0 (0)	1 (0.9)

[a] Abbreviations: NA, nalidixic acid; SXT, trimethoprim-sulfamethoxazole; OFX, ofloxacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; IPM, imipenem; T, tetracycline; CRO, ceftriaxon; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; FOX, cefoxitin; AK, amikacin; GM, gentamicin.
[b] Data are presented as No. (%).

Figure 1. Amplification of Integrase



Lane M, 100 bp Plus Blue DNA ladder (GeneON); Lanes 3, 9: Integrase 1 (160 bp) and integrase 2 (788 bp) amplicons; Lane 7; integrin Negative isolate; Lanes 1, 2, 4-6, 8, 10; integron class 1 positive isolates.

4.3. Detection of Integrons

Of 110 isolates, 36 (32.7 %) *Salmonella* isolates exhibited either a class 1 integron or class 1 and 2 integron (Figure 1). However integron class 3 was not found and integron class 2 was not found alone among integron-positive isolates. Primers *Int*1F and *Int*1R were used to amplify a 160 bp fragment of the *intI*1 gene for the class1 integrase, the primers *Int*2F and *Int*2R amplified a fragment of 288 bp, specific for the *intI*2 gene and primers *Int*3F and *Int*3R were used to amplify a specific *int*I3 gene. Table 3 shows an apparent association between antibiotic resistance and presence of integrons among *Salmonella* isolates.

Table 3. Antibiotic Susceptibility Pattern Among Integron Positive Isolates [a]

Antibiotic Resistance Phenotype	Integron			Total
	Class 1	Class 2	Class 1 and 2	
SXT	5	-	-	5
NA, SXT	5	-	1	6
SXT, T	1	-	-	1
NA, CAZ	1	-	-	1
NA, SXT, AMP	1	-	-	1
NA, SXT, OFX	3	-	-	3
NA, SXT, T	1	-	3	4
AMP, CHL, T	1	-	-	1
SXT, AMP, CHL, T	3	-	-	3
NA, SXT, CHL, T, FOX	1	-	-	1
SXT, AMP, CRO, CAZ, ATM	1	-	1	2
NA, SXT, CHL, T, CRO, CTX, CAZ, ATM	1	-	1	2
NA, SXT, AMP, T, CRO, CTX, CAZ, ATM	1	-	-	1
Sensitive to all antibiotics	4	-	1	5
Total	29	-	7	36

[a] Abbreviations: NA, nalidixic acid; SXT, trimethoprim-sulfamethoxazole; OFX, ofloxacin; AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; IPM, imipenem; T, tetracycline; CRO, ceftriaxon; CTX, cefotaxime; CAZ, ceftazidime; ATM, aztreonam; FOX, cefoxitin; AK, amikacin; GM, gentamicin..

5. Discussion

Extended-spectrum cephalosporins and fluoroquinolones are commonly used to treat infections of *Salmonella*. Bacteria resistance to these important drugs has dramatically increased (18). The ability of integrons to integrate resistance gene cassettes makes them prime pools for the further dissemination of antibiotic resistance among clinical isolates of gram-negative bacteria, including *Salmonella* isolates (6). Our findings indicated that the 32.7 % of isolated *Salmonella* were integron positive, this rate of integron positive in our isolates are similar with published reports that *Salmonella* harbors high prevalence of integron class 1, lower class 2 and no class 3 (6, 7, 19-21). The lack of integron class 3 may indicate its null role in antibiotic resistance. However, in other Enterobacteriaceae, was showed the role integron class 3 in antibiotic resistance (22). As mentioned above, the prevalence of class 1 integron, as compared to class 2 may imply that class 1 integron is more important in capturing resistant determinants. Resistance to nalidixic acid and trimethoprim-sulfamethoxazole were high (75% and 50% respectively). This rate of resistance in our isolates is similar to the rate found by others in recent years of our country but was different withwith the data of other countries (6, 12, 23-26). In this study, the observed high resistance to co-trimoxazole and nalidixic acid among the isolates with integron, suggested probable dominance of the associated resistance gene cassettes within variable region of class1 integrons. Fortunately, among integron posi-

tive isolates of this study resistance to aminoglycosides antibiotics was not detected. Of course, in other studies in Iran and world resistance to aminoglycosides was detected among integron-positive *Salmonella* isolates (6, 12, 24). Also, in this study all isolates were susceptible to ciprofloxacin. However, in a report from Tehran, *Salmonella Enteritidis* with resistance to ciprofloxacin from a boy was isolated (18). In this study, all isolates were sensitive to imipenem. This resulted from restricted prescription of carbapenems in Iran (27, 28). The present study indicated the highest resistance in the collected *Salmonella* isolates was to trimethoprim-sulfamethoxazole (63.6%), followed by nalidixic acid (47.3%), tetracycline (33.6%), chloramphenicol (27.3%), and ampicillin (24.5%). Resistance in *Salmonella* strains to amoxicillin, trimethoprim-sulfamethoxazole (co-trimoxazole) and chloramphenicol has posed an issue to therapy of systemic salmonellosis (5). So this data alerts the proper use of antibiotics in the medicine and agriculture. Among isolates with ESBL phenotype-positive, three isolates have integron. Two isolates exhibited class 1 and 2 integrons and one exhibited class 1 integron and only one isolate was no integron. Perhaps, this result shows the importance of integrons in carrying and transfer of resistance genes in ESBL-producing bacteria. Due to the presence of class I of integrons in drug resistance to antibiotics such as beta-lactams, aminoglycosides and tetracycline, it is essential more attention to them. The variable presence of integrons among extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae species (0 to 66%) is described (29). Five (13.9%) integron positive isolates were susceptible to all antimicrobials tested and 26 (72.2%) were multidrug-resistant (MDR) and showed resistance to more than two antimicrobial families and this result can demonstrates strong association between MDR *Salmonella* phenotype and presence of integron class 1 (5, 6, 8, 9, 12, 22, 24-26, 29-32). Nevertheless, to genetically confirm this association, sequencing and amplification of class 1 and 2 integrons cassette regions should to be performed. As a conclusion; the high prevalence of integron-positive isolates in our MDR *Salmonella* isolates indicates that these mobile genetic elements are common among different *Salmonella spp*. and associate with reduced susceptibility to the first-line antimicrobial drugs.

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References

1. McGhie EJ, Brawn LC, Hume PJ, Humphreys D, Koronakis V. Salmonella takes control: effector-driven manipulation of the host. *Curr Opin Microbiol.* 2009;**12**(1):117–24.
2. Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, et al. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. *Antimicrob Agents Chemother.* 2001;**45**(3):723–6.
3. Guerra B, Soto S, Cal S, Mendoza MC. Antimicrobial resistance and spread of class 1 integrons among Salmonella serotypes. *Antimicrob Agents Chemother.* 2000;**44**(8):2166–9.
4. Sandvang D, Aarestrup FM, Jensen LB. Characterisation of integrons and antibiotic resistance genes in Danish multiresistant Salmonella enterica Typhimurium DT104. *FEMS Microbiol Lett.* 1998;**160**(1):37–41.
5. Firoozeh F, Shahcheraghi F, Zahraei Salehi T, Karimi V, Aslani MM. Antimicrobial resistance profile and presence of class I integrongs among Salmonella enterica serovars isolated from human clinical specimens in Tehran, Iran. *Iran J Microbiol.* 2011;**3**(3):112–7.
6. Ranjbar R, Giammanco GM, Farshad S, Owlia P, Aleo A, Mammina C. Serotypes, antibiotic resistance, and class 1 integrons in Salmonella isolates from pediatric cases of enteritis in Tehran, Iran. *Foodborne Pathog Dis.* 2011;**8**(4):547–53.
7. Ebner P, Garner K, Mathew A. Class 1 integrons in various Salmonella enterica serovars isolated from animals and identification of genomic island SGI1 in Salmonella enterica var. Meleagridis. *J Antimicrob Chemother.* 2004;**53**(6):1004–9.
8. Ranjbar R, Giammanco GM, Aleo A, Plano MR, Naghoni A, Owlia P, et al. Characterization of the first extended-spectrum beta-lactamase-producing nontyphoidal Salmonella strains isolated in Tehran, Iran. *Foodborne Pathog Dis.* 2010;**7**(1):91–5.
9. Ploy MC, Chainier D, Tran Thi NH, Poilane I, Cruaud P, Denis F, et al. Integron-associated antibiotic resistance in Salmonella enterica serovar typhi from Asia. *Antimicrob Agents Chemother.* 2003;**47**(4):1427–9.
10. Carattoli A. Plasmid-mediated antimicrobial resistance in Salmonella enterica. *Curr Issues Mol Biol.* 2003;**5**(4):113–22.
11. Carattoli A. Importance of integrons in the diffusion of resistance. *Vet Res.* 2001;**32**(3-4):243–59.
12. Carattoli A, Villa L, Pezzella C, Bordi E, Visca P. Expanding drug resistance through integron acquisition by IncFI plasmids of Salmonella enterica Typhimurium. *Emerg Infect Dis.* 2001;**7**(3):444–7.
13. Fluit AC, Schmitz FJ. Resistance integrons and super-integrons. *Clin Microbiol Infect.* 2004;**10**(4):272–88.
14. Clinical and Laboratory Standards Institute.. Performance standards for antimicrobial susceptibility testing, 20th information supplement (M100-S20. *CLSI.* 2011;**30**(1).
15. Doi Y, Adams-Haduch JM, Shivannavar CT, Paterson DL, Gaddad SM, Muzaheed.. Faecal carriage of CTX-M-15-producing Klebsiella pneumoniae in patients with acute gastroenteritis. *Indian J Med Res.* 2009;**129**(5):599–602.
16. Fiett J, Baraniak A, Mrowka A, Fleischer M, Drulis-Kawa Z, Naumiuk L, et al. Molecular epidemiology of acquired-metallo-beta-lactamase-producing bacteria in Poland. *Antimicrob Agents Chemother.* 2006;**50**(3):880–6.
17. Xin-mian W, Yao-guang W, Feng-zhi B, Yu-guo S, Xiu-feng Z, Yan-fang Z, et al. High prevalence of atypical class 1 integrons and class 2 integrons in multi-drug resistance Shigella flexneri isolated from China. *Afr J Microbiol Res.* 2012;**6**(42):6987–93.
18. Firoozeh F, Shahcheraghi F, Zahraei-salehi T, Aslani MM, Banisaeed R. First CTX-M type β-lactamase producing and ciprofloxacin resistant Salmonella infection acquired by a child in Iran. *Int J Enteric Pathog.* 2013;**1**(2):76–8.
19. Eshraghi S, Dalall S, Mehdi M, Fardsanei F, Zahraii Salehi T, Nikmanesh B, et al. Salmonella enteritidis and antibiotic resistance patterns: a study on 1950 children with diarrhea. *Tehran Univ Med J.* 2010;**67**(12):876–82.
20. Zahraii Salehi T, Mahzounieh M, Saeedzadeh A. The Isolation of Antibiotic-Resistant Salmonella from Intestine and Liver of Poultry in Shiraz Province of Iran. *Int J Poult Sci.* 2005;**4**(5):320–2.
21. Lindstedt BA, Heir E, Nygard I, Kapperud G. Characterization of class I integrons in clinical strains of Salmonella enterica subsp. enterica serovars Typhimurium and Enteritidis from Norwegian hospitals. *J Med Microbiol.* 2003;**52**(Pt 2):141–9.
22. Barraud O, Casellas M, Dagot C, Ploy MC. An antibiotic-resistant

class 3 integron in an Enterobacter cloacae isolate from hospital effluent. *Clin Microbiol Infect.* 2013;**19**(7):E306–8.
23. Hamidian M, Tajbakhsh M, Walther-Rasmussen J, Zali MR. Emergence of extended-spectrum beta-lactamases in clinical isolates of Salmonella enterica in Tehran, Iran. *Jpn J Infect Dis.* 2009;**62**(5):368–71.
24. Dessie HK, Bae DH, Lee YJ. Characterization of integrons and their cassettes in Escherichia coli and Salmonella isolates from poultry in Korea. *Poult Sci.* 2013;**92**(11):3036–43.
25. Riano I, Moreno MA, Teshager T, Saenz Y, Dominguez L, Torres C. Detection and characterization of extended-spectrum beta-lactamases in Salmonella enterica strains of healthy food animals in Spain. *J Antimicrob Chemother.* 2006;**58**(4):844–7.
26. Dolejska M, Bierosova B, Kohoutova L, Literak I, Cizek A. Antibiotic-resistant Salmonella and Escherichia coli isolates with integrons and extended-spectrum beta-lactamases in surface water and sympatric black-headed gulls. *J Appl Microbiol.* 2009;**106**(6):1941–50.
27. Naghoni A, Ranjbar R, Tabaraie B, Farshad S, Owlia P, Safiri Z, et al. High prevalence of integron-mediated resistance in clinical isolates of Salmonella enterica. *Jpn J Infect Dis.* 2010;**63**(6):417–21.
28. Poulad far G, Foroozand F, Pourabbas B. *Antimicrobial drug resistance patterns of Salmonella strains in patients with systemic salmonellosis in Southern Iran*. USA: International Society for Infectious Diseases; 2010. Available from: http://www.xcdsystem.com/icid_ise2012/ISE.401.html.
29. Machado E, Ferreira J, Novais A, Peixe L, Canton R, Baquero F, et al. Preservation of integron types among Enterobacteriaceae producing extended-spectrum beta-lactamases in a Spanish hospital over a 15-year period (1988 to 2003). *Antimicrob Agents Chemother.* 2007;**51**(6):2201–4.
30. 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.
31. Rotimi VO, Jamal W, Pal T, Sovenned A, Albert MJ. Emergence of CTX-M-15 type extended-spectrum beta-lactamase-producing Salmonella spp. in Kuwait and the United Arab Emirates. *J Med Microbiol.* 2008;**57**(Pt 7):881–6.
32. Ranjbar R, Naghoni A. Frequency of class 2 Integrons among clinical strains of Salmonella enteric Isolated in Tehran. *Iran J Infect Dis Tropic Med.* 2011.