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Original Article

High Prevalence of Integron-Mediated Resistance in Clinical Isolates of *Salmonella enterica*

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SUMMARY: *Salmonella enterica* has become progressively resistant to antimicrobial agents worldwide as a result of genes carried on different classes of integrons. The aim of the current study was to investigate the molecular diversity of these integrons and their association with antimicrobial resistance in clinical *S. enterica* isolates from Tehran, Iran. Antimicrobial susceptibility testing was performed according to the Clinical and Laboratory Standards Institute. The presence of integrons was investigated by PCR using specific primers. Integrons were detected in 65 (47.1%) strains, with classes 1 and 2 being observed in 54 (39%) and 11 (8%) strains, respectively. Integron-positive isolates belonged to seven different *S. enterica* serovars, and all showed a multidrug-resistant (MDR) phenotype. Our findings show that integrons are widely disseminated among *S. enterica* strains from Tehran. Furthermore, the results that class 1 integrons were more prevalent than class 2 in *Salmonella* isolates, and that a statistical association with MDR patterns was observed, suggest that they are more likely to be important in conferring a resistant phenotype to *Salmonella* strains.

INTRODUCTION

Salmonella enterica is one of the most important causes of food-borne disease worldwide (1,2). Antimicrobial drug resistance is an increasing problem in *Salmonella* strains (3). The prevalence of such resistance is mainly a result of the horizontal transfer of antibiotic-resistance genes, partly via mobile genetic elements (4,5). Integrons are known to contribute to the dissemination of antibiotic resistance among bacteria (6). The role of integrons and gene cassettes in the dissemination of multidrug resistance in Gram-negative bacteria is well established (7). Integron classes 1 and 2 are widely distributed among Gram-negative bacteria, including the different serovars of *S. enterica* (8–10).

Class 1 integrons consist of two conserved segments (5′-CS and 3′-CS) separated by a variable region that usually contains one or more gene cassettes. The 5′-CS region contains the integrase gene (*intI1*), the integration site (*attI1*), and a promoter region (P_c) that allows a number of gene cassettes inserted at the *attI1* in a suitable orientation to be expressed. The 3′-CS region includes one gene, *qacEΔ1*, which confers resistance to quaternary ammonium compounds and another, *sul1*, which confers resistance to sulfonamides (11). Class 2 integron is similar to class 1, but it is associated with transposons Tn*7* and is known to carry six different resistance cassettes (10,12,13). Class 2 integrons are less common than class 1 and have been reported in Gram-negative bacteria, including salmonellae (14,15). A gene cassette contains a single antibiotic-resistance gene and a 59-base element (or *attC* site) downstream of the gene, which is responsible for recombination events (16). Numerous resistance genes have been reported in the gene cassettes of *Salmonella*, either alone or in combination with other resistance genes (9,17).

The need for systematic epidemiological studies regarding the role of integrons in antimicrobial drug resistance in bacteria has been emphasized recently (18). Reports from some Asian countries have noted a high prevalence of class 1 and 2 integrons in Gram-negative clinical isolates (19,20). These data suggest that such integrons are relatively common in this continent, especially among *Enterobacteriaceae*, and that they contribute to the spread of antimicrobial drug resistance in healthcare settings. However, few studies have assessed the association between integron carriage and antimicrobial resistance patterns among bacterial species in Iran. In this study we have investigated the molecular diversity of integrons in clinical *S. enterica* isolates in Tehran, Iran and their association with resistance to antimicrobial agents.

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MATERIALS AND METHODS

Bacterial isolates and antimicrobial susceptibility: The study included all *Salmonella* isolates recovered from patients with *Salmonella* infections hospitalized in several hospitals in Tehran, Iran in the period 2006–2008. These isolates were identified in our previous study by conventional biochemical methods and serotyped by slide agglutination with specific antisera (Staten Serum Institute, Copenhagen, Denmark).

Antimicrobial drug resistance was determined using the disk diffusion method on Mueller-Hinton agar according to the Clinical and Laboratory Standards Institute's recommendations (21) using antibiotic disks (Oxoid, Hampshire, UK) including amikacin (30 μg), amoxicillin-clavulanic acid (20 + 10 μg), ampicillin (10 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftizoxime (30 μg), ceftriaxone (30 μg), cephalothin (30 μg), chloramphenicol (30 μg), ciprofloxacin (5 μg), doxycycline (30 μg), gentamicin (10 μg), imipenem (10 μg), kanamycin (30 μg), nalidixic acid (30 μg), neomycin (30 μg), piperacillin (100 μg), streptomycin (10 μg), tetracycline (30 μg), ticarcillin (75 μg), tobramycin (10 μg), and trimethoprim-sulfamethoxazole (1.25 + 23.75 μg). *Escherichia coli* (ATCC 25922) was used for quality control.

Integron analysis: *Salmonella* isolates were analyzed by polymerase chain reaction (PCR) amplification techniques to determine whether a class 1 or 2 integron was present. The oligonucleotide primers hep58, 5′-TCA TGG CTT GTT ATG ACT GT-3′ (5′ upstream conserved sequences [5′-CS] of *intI1*) and hep59, 5′-GTA GGG CTT ATT ATG CAC GC-3 (3′ upstream CS [3′-CS] of *qacEΔ1*) (22) were used during PCR to amplify the genes contained in the class 1 integron. Likewise, the primer pair hep74, 5′-CGG GAT CCC GGA CGG CAT GCA CGA TTT GTA-3′ and hep51, 5′-GAT GCC ATC GCA AGT ACG AG-3′ (10) was used to amplify class 2 integrons. Amplifications were performed as described previously (10,22). All PCR amplicons were visualized by agarose gel electrophoresis after staining the gels with ethidium bromide. Statistical significance (P value) was calculated using Pearson χ^2 test or Fisher's exact test, when necessary, to assess the association between the resistance/intermediate resistance (nonsusceptible) pattern and the integron-positive genotype. A P value of less than 0.05 was taken to indicate statistical significance.

RESULTS

The *Salmonella* isolates used in this study belonged to different serovars, including Enteritidis (57 isolates); Infantis (40 isolates); Typhimurium (21 isolates); Albany and Muenchen (4 isolates each); Hadar, Havana, and Newport (2 isolates each); and Haifa, Kentucky, Paratyphi B, Orion, Reading, and Richmond (one isolate each). Overall, 47.1% (65/138) of strains harbored integrons. Class 1 integrons were found in 54 (39.1%) isolates, including seven different serovars of *S. enterica*, namely Albany (3), Enteritidis (5), Haifa (1), Infantis (26), Muenchen (2), Reading (1), and Typhimurium (16). PCR amplification of class 1 integrons showed seven diverse bands of 2.1, 1.9, 1.75, 1.6, 1.25, 1.1, and 0.85 kb (Fig. 1).

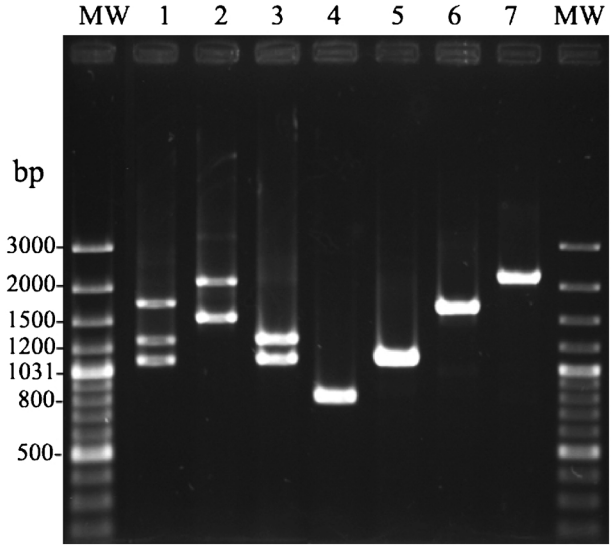

Fig. 1. Different types of PCR amplicon of class 1 integron in *Salmonella* spp. Lanes 1–7 are amplified DNA bands belonging to seven representative *Salmonella* strains. Lanes MW are molecular size marker (100 bp).

Eleven (8%) isolates contained a 2.16-kb class 2 integron, and class 2 integron-positive isolates were distributed in two serovars of *S. enterica*, namely Infantis (6) and Typhimurium (5).

Integron carriage was then compared with the resistance profile. All strains tested were found to be susceptible to ciprofloxacin, gentamicin, and imipenem. However, susceptibility to amoxicillin-clavulanic acid, ampicillin, chloramphenicol, doxycycline, kanamycin, neomycin, piperacillin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole differed between class 1 integron-positive and -negative isolates. Thus, the frequency of resistance to ampicillin, amoxicillin-clavulanic acid, piperacillin, streptomycin, kanamycin, neomycin, trimethoprim-sulfamethoxazole, tetracycline, doxycycline, and chloramphenicol was significantly ($P < 0.05$) higher in the class 1 integron-positive strains than in the integron-negative strains (Table 1).

All integron-positive *Salmonella* spp. were multidrug resistant. Some of these isolates contained two or three integrons, and resistance to more than 10 antimicrobial agents was observed in eight integron-positive strains.

DISCUSSION

Multidrug resistance in bacterial pathogens is now a common phenomenon in developing countries, including Iran (23–25). This finding is most likely related to the frequent use of over-the-counter drugs with little or no medical supervision (26).

Our findings indicate that antibiotic resistance in *Salmonella* strains is increasing alarmingly since more than 68% of isolates have a multidrug-resistant (MDR) phenotype. MDR *Salmonella* have previously been reported from different parts of the world (27,28). A study carried out in India found an increase in MDR from 53.6 to 63.9% from 1997 to 2001 (27). This is in accordance with Chung et al. who tested 1,334

Table 1. Antibiotic susceptibility of class 1 integron-positive and -negative strains of *Salmonella*

Antibiotic (μg)[1]	Total (n = 138)	Integron-negative (n = 84)			Integron-positive (n = 54)			P value[4]
	%R (no.)[2]	%R[3]	%I	%S	%R	%I	%S	
β-Lactams								
Ampicillin (10)	15.9 (22)	2.38	2.38	95.24	37.04	9.26	53.7	<0.05
Amoxicillin-clavulanic acid (30)	21 (29)	4.76	11.91	83.33	46.3	11.11	42.59	<0.05
Ticarcillin (75)	3.6 (5)	2.38	0	97.62	5.55	0	94.45	NS[5]
Piperacillin (100)	23.2 (32)	8.33	26.19	65.47	46.3	11.11	42.59	<0.05
Cephalothin (30)	4.3 (6)	2.38	2.38	95.24	7.4	3.7	88.9	NS
Ceftriaxone (30)	4.3 (6)	2.38	2.38	95.24	7.4	0	92.6	NS
Cefotaxime (30)	4.3 (6)	2.38	3.57	94.05	7.4	0	92.6	NS
Ceftazidime (30)	4.3 (6)	2.38	1.2	96.42	7.4	0	92.6	NS
Ceftizoxime (30)	2.9 (4)	1.2	2.38	96.42	5.55	1.85	92.6	NS
Aminoglycosides								
Streptomycin (10)	42.7 (59)	16.67	26.19	57.14	83.34	11.11	5.55	<0.05
Kanamycin (30)	22.5 (31)	8.33	8.33	83.33	44.45	1.85	53.7	<0.05
Neomycin (30)	19.6 (27)	8.33	28.57	63.1	37.04	18.51	44.45	<0.05
Tobramycin (10)	0.7 (1)	0	0	100	1.85	0	98.15	NS
Amikacin (30)	1.4 (2)	0	1.2	98.8	3.7	0	96.3	NS
Quinolone								
Nalidixic acid (30)	64.5 (89)	59.52	1.2	39.28	72.23	0	27.77	NS
Antifolate								
Trimethoprim-sulfamethoxazole (25)	20.3 (28)	7.14	16.67	76.19	40.74	7.4	51.86	<0.05
Others								
Tetracycline (30)	50.7 (70)	21.44	39.28	39.28	96.3	1.85	1.85	<0.05
Doxycycline (30)	67.4 (93)	54.76	22.62	22.62	87.04	12.96	0	<0.05
Chloramphenicol (30)	13 (18)	1.2	1.2	97.6	31.48	0	68.51	<0.05

[1]: Concentration of disks.
[2]: Number of resistant isolates.
[3]: R, resistance; I, intermediate resistance; S, susceptible.
[4]: Statistical significance (P value) was calculated using Pearson χ^2 test in terms of number of resistance/intermediate resistance (nonsusceptible) strains and susceptible strains in the class 1 integron-positive and -negative groups.
NS, not statistically significant.

Salmonella isolates in Korea and found that 65.9% of them were MDR (28). In addition, Antunes et al. reported that 21% of 1,183 *Salmonella* strains isolated in Portugal were MDR (12).

We found that more than 96% of class 1 integron-positive isolates were resistant to tetracycline, 87% to doxycycline, 83% to streptomycin, 46% to piperacillin, 44% to kanamycin, 40% to trimethoprim-sulfamethoxazole, 37% to neomycin, 37% to ampicillin, and 31% to chloramphenicol, whereas the corresponding figures for integron-negative isolates were 21, 54, 16, 8, 8, 7, 8, 2, and 1%, respectively.

Jin et al. studied 834 *Salmonella* isolates in Hong Kong and found that 90% of integron-positive isolates were resistant to sulfamethoxazole and 89% to tetracycline (19). Chang et al. showed that 100% of integron-positive isolates were resistant to sulfamethoxazole, 50% to tetracycline, 50% to trimethoprim, 29% to streptomycin, and 25% to chloramphenicol (29). Interestingly, ampicillin, chloramphenicol, kanamycin, streptomycin, tetracycline, and trimethoprim-sulfamethoxazole are no longer, or only infrequently, used in a clinical setting for the treatment of *Salmonella* infection. It is thus possible that the higher resistance rates of *Salmonella* to these agents may be largely attributed to use of these antibiotics in domestic animals, either therapeutically or for the purpose of growth promotion (30).

Integrons are a major vehicle for the spread of multiple-antibiotic resistance (31,32). Class 1 integrons were identified in 54 (39.1%) strains herein, a higher prevalence than that reported by Cabrera et al., who found that 25% of *S. enterica* strains in Spain contained class 1 integrons (33). Other studies found that the prevalence of class 1 integrons in *Salmonella* spp. was 20.4% in the United Kingdom (34), 13% in Hong Kong (19), and 13 % in Vietnam (35). In contrast, only 11 (8%) of the 138 isolates contained class 2 integrons. Previous studies have found that class 2 integrons have a more limited distribution than that reported here (12,32).

Our study showed an inverse, but not statistically significant, association between integron presence and resistance to cephalosporins and nalidixic acid in *Salmonella*, thereby suggestig that the resistance determinants for these antimicrobial agents are not frequently associated with integrons. Cephalosporin resistance may be encoded by different extended-spectrum β-lactamases (ESBLs) as most are derivatives of the TEM and SHV β-lactamase families, whereas other groups, such as CTX-M, PER, and KPC, have been described in the past few years (36–38). Machado et al. found no association between integron carriage and β-lactam resistance in ESBL-producing *E. coli* strains unless the strains contained metallo-β-lactamases (39). Quinolones stabilize the breaks in the DNA induced by DNA *gyrA*se or topoisomerase IV, and the resulting drug-enzyme-

DNA complex inhibits DNA synthesis (40). More than 64% of the *Salmonella* spp. studied herein were resistant to nalidixic acid. Resistance to this antimicrobial agent has mainly been observed in isolates belonging to serovar Enteritidis. The wide use of quinolones, such as nalidixic acid, for the treatment of infections in this region has been correlated with an increased resistance to these agents (24,41).

In summary, we have shown that integrons are widely disseminated among *S. enterica* isolated from clinical samples in Tehran. Class 1 integrons were found to be more prevalent than class 2 in *Salmonella* spp. isolates, and to be associated with MDR phenotypes, thereby suggesting their importance in conferring this resistance profile.

Surveillance and monitoring of antimicrobial drug resistance, including screening for integrons as likely indicators of drug resistance and acquisition of new resistance traits, are required to plan effective strategies to contain this phenomenon in food-borne organisms. Further studies on the prevalence of integrons should be carried out in other regions in Iran to estimate the occurrence of these genetic elements in *Salmonella* spp. in this country more reliably.

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Conflict of interest None to declare.

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