International Scholars Journals

Advanced Journal of Microbiology Research ISSN 2241-9837 Vol. 13 (3), pp. 001-005, March, 2019. Available online at www.internationalscholarsjournals.org © International Scholars Journals

Full Length Research Paper

Expanding drug resistance through integron acquisition in *Salmonella* spp. isolates obtained in Iran

Bahareh Rajaei[1], Seyed Davar Siadat[1,2*], Mohamad Reza Razavi[3], Mohammad Reza Aghasadeghi [2], Nahid Sepehri Rad[1], Farzad Badmasti[4], Somieh Khanjani Jafroodi[3], Taraneh Rajaei[5], Arfa Moshiri[6] and Saifuddin Javadian[7]

[1]Department of Biology, Science and Research Branch, Islamic Azad University, Tehran, Iran.
[2]Department of Hepatitis and AIDS, Pasteur Institute of Iran, Tehran, Iran.
[3]Department of Parasitology, Pasteur Institute of Iran, Tehran, Iran.
[4]Department of Microbiology, Pasteur Institute of Iran, Tehran, Iran.
[5]Department of Immunology and Allergy, Mashhad University of Medical Sciences, Mashhad, Iran.
[6]Department of Biotechnology, Faculty of Allied Sciences, Tehran University of Medical Sciences, Tehran, Iran.
[7]Department of Biochemistry, Pasteur Institute of Iran, Tehran, Iran.

Accepted 15 January, 2019

A total eighty four epidemiologically unrelated clinical isolates of *Salmonella enterica* serovars were subjected to antimicrobial susceptibility testing and molecular detection of class 1 and 2 integrons. Eleven isolates (13.1%) which were resistant to at least 4 groups of antimicrobial agents considered as MDR (multidrug resistant) *Salmonella* serovars. PCR assays detected *intI1* and *intI2* genes in 50 (59.5%) and 14 (16.7%) of *Salmonella* clinical isolates respectively. Emergence of MDR *Salmonella* serovars demonstrates that antimicrobial selection pressure is widespread and increased distribution of integron carrying gene cassettes which confer resistance to different antibiotics confirms that integron-mediated antibiotic resistance is considerable in our clinical settings.

Key words: *Salmonella* spp., integron, multidrug resistance (MDR).

INTRODUCTION

Salmonella is involved in a wide variety of infections ranging from life-threatening typhoid to gastroenteritis and bacteremia (Boyd and Hartl, 1998). Antibiotic resistance in *Salmonella* is an emerging problem during the last decades. The intensive use of antibiotics in both human and veterinary medicine, as well as in agriculture, has caused bacteria to develop resistance mechanisms against therapeutic drugs (Stokes and Hall, 1989; Rodriguez et al., 2008). Dissemination of antibiotic resistance genes by horizontal gene transfer has led to the rapid emergence of antibiotic resistance among bacteria, thus complicating the treatment of infections (Gu et al., 2008). The worldwide emergence of multidrug-resistant (MDR) phenotypes among *Salmonella* serotypes is of increasing concern for both the scientific community and the general public (Havlickova et al., 2009; Yang et al., 2009).

Acquired resistance evolves via horizontal transfer of antimicrobial resistance genes located on various types of mobile DNA elements (Yang et al., 2009). A key genetic system involved in spreading antibiotic multiresistance is the integron, a genetic element that, although normally immobile itself, can be transferred in companion with mobile genetic elements (Tamang et al., 2007)

Integrons were first identified by virtue of their important role in the acquisition and expression of genes conferring antibiotic resistance. Integrons are composed of three key elements necessary for the capture of exogenous genes: a gene (*intI*) encoding an integrase belonging to the tyrosine-recombinase family; a primary recombination site (*attI*) located immediately adjacent to *intI*; and a strong promoter (Pc) that directs transcription of the captured genes (Carattoli, 2001; Mazel, 2006).

*Corresponding author. E-mail: d.siadat@gmail.com. Tel: 00982144865100. Fax: 00982144865105.

Table 1. Information about number of MDR and resistant isolates on the basis of the MIC determination according to guidelines of CLSI (2009).

Integron positive isolates	No. of resistant isolates to the following antibiotics[a] on the basis of MIC									MDR[b]
	SMX	SXT	TMP	CIP	NAL	AMP	CAZ	CHL	STR	
intI1 positive isolates(50)	20	45	22	1	32	5	2	21	20	11
intI2 positive isolates(14)	10	12	11	0	11	0	1	4	6	1

[a]Abbreviation of mentioned antibiotics and breakpoint for resistance (µg/ml) : AMP, ampicillin (32); CAZ, ceftazidime (16); CHL, chloramphenicol (32); CIP, ciprofloxacin (4); NAL, nalidexic acid (32); STR, streptomycin (64); TMP, trimethoprim (4); SMX, sulfamethoxazole (512); SXT, sulfamethoxazole-trimethoprim (4/76). Breakpoints were adopted from CLSI (Clinical and Laboratory Standards Institute), except for streptomycin, which has no CLSI breakpoint.

[b] In this study isolates which were resistant to at least 4 groups of antimicrobial agents considered as MDR *Salmonella* isolates.

On the basis of nucleotide sequence of the integrase gene (*intI*), nine classes of integrons have been identified (Moura et al., 2007; Macedo-Vinas et al., 2009) but, to date, only those of class 1 and 2 have been reported in *S. enterica* (Rodriguez et al., 2008).

The objective of this study was to evaluate the antimicrobial resistance profile and to investigate the contribution of class 1 and 2 integrons in multidrug resistant *Salmonella* isolates.

MATERIALS AND METHODS Isolation

and identification of bacteria

A total of eighty four *S. enterica* isolates attributed to Typhi (n=40), Paratyphi A (n=2), Typhymurium (n=12), and non Typhi (n=30) serovars were studied. The clinical isolates were recovered from stool (n= 69), blood (n= 6), bone marrow (n= 3), synovial fluid (n= 3), ascites (n= 1), abces (n= 1), urine (n= 1). All isolates were identified by standard microbiological techniques as previously described (Martin et al., 2008; Ahmed et al., 2009). The serogroup was checked with O antisera by the slide agglutination method (Difco Laboratories, Detroit, MI).

Antimicrobial susceptibility test

The antimicrobial susceptibility test was performed using the standard disk diffusion method on Muller- Hinton agar and the Minimum Inhibitory Concentrations (MICs) were determined by broth microdilution method according to the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2009). Disks prepared by MAST company (Mast Co, Merseyside, UK) were used to determine the susceptibility of isolates to ampicillin (10 µg), tetracycline (30 µg), chloramphenicol (30 µg), trimethoprim (5 µg), sulfamethoxazole-trimethoprim (30 µg), streptomycine (10 µg), nalidexic acid (30 µg), ciprofloxacin (5 µg), ofloxacin (5 µg), levofloxacin (5 µg), norfloxacin (5 µg), gatifloxacin (5 µg), moxifloxacin (5 µg), cefotaxime (30 µg), cefixime (5 µg), ceftriaxone (30 µg), cefepime (30 µg), ceftazidime (30 µg), amikacin (30 µg), azithromycin (15 µg), spectinomycin (100 µg), gentamicin (10 µg), colistin-sulfate (10 µg), imipenem (10 µg). The MICs of ampicillin, chloramphenicol, streptomycin, nalidixic acid, ciprofloxacin, ceftazidime, trimethoprim, sulfamethoxazole and sulfamethoxazole-trimethoprim were carried out against all clinical isolates. The breakpoints used were those defined by the CLSI for Enterobacteriaceae, with the exception of streptomycin (32 _g/mL) (CLSI, 2009) (Table 1). *E. coli* ATCC 25922 was used as a quality control organism in antimicrobial susceptibility test.

Detection of class 1 and 2 integrons

Chromosome and plasmid DNA templates were prepared by phenol-chloroform methods as previously described (Sambrook et al., 1989) . All isolates were screened for detection of class 1 and 2 integrons by the primers described by Goldstein et al. (2001) designed for the *intI1* and *intI2* genes respectively. The amplification program was performed by termocycler (Eppendorf Mastercycler®, MA) and started with initial denaturation of 4 min at 94°C and programmed with 35 cycles of each: 1min at 94°C, 30 s at 60°C, 1 min at 72°C. The program finished with the final extension of 10min at 72°C.

RESULTS

Disk diffusion and minimum inhibitory concentration (MIC)

A total of eighty four *Salmonella* isolates including *S.* Typhi (n = 40), *S*.Typhymurium (n=12), *S*. Paratyphi A (n = 2), non-Typhi (n=30) serovars were studied. According to the Disk diffusion and minimum inhibitory concentration (MIC), antimicrobial resistance patterns were as follow: 25 isolates (29.8%) were resistant to streptomycin, 25 isolates (29.8%) to sulfamethoxazole-trimethoprim, 30 isolates (35.7%) to trimethoprim, 23 isolates (27.4%) to chloramphenicol, 57 isolates (67.9%) to tetracycline, 6 isolates (7.1%) to ampicillin, 54 isolates (64.3%) to nalidexic acid, 1 isolate (1.2%) to ciprofloxacin, 6 isolates (7.2%) to cefotaxime, 8 isolates (9.5%) to cefexime, 6 isolates (7.2%) to ceftriaxon, 2 isolates (2.4%) to ceftazidime, 2 isolates (2.4%) to colistin-sulfate, 3 isolates (3.6%) to gentamicin, 24 isolates (28.6%) to spectinomycin, 5 isolates (5.9%) to azithromy-cin, 2 isolates (2.4%) to amikacin. All the isolates were sensitive to imipenem, ofloxacin, levofloxacin, norfloxacin, gatifloxacin, moxifloxacin. Of the 84 isolates only 4 isolates (4.7%) were sensitive to the all of the tested antimicrobial agents.

Identification of multidrug resistant (MDR) isolates

Multi-drug resistance was defined as resistance to at least 4 groups of antimicrobial agents. Of the eighty four

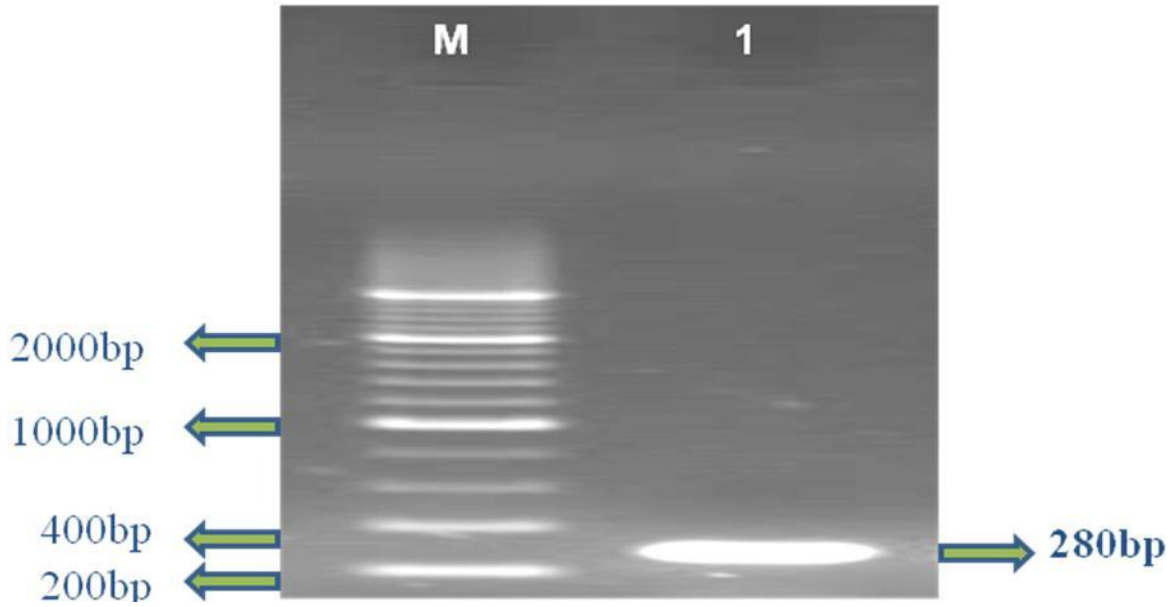

Figure 1. PCR Amplification of *intI1* Gene. Lane M: 200bp DNA Ladder. Lane 1: *int1* PCR product.

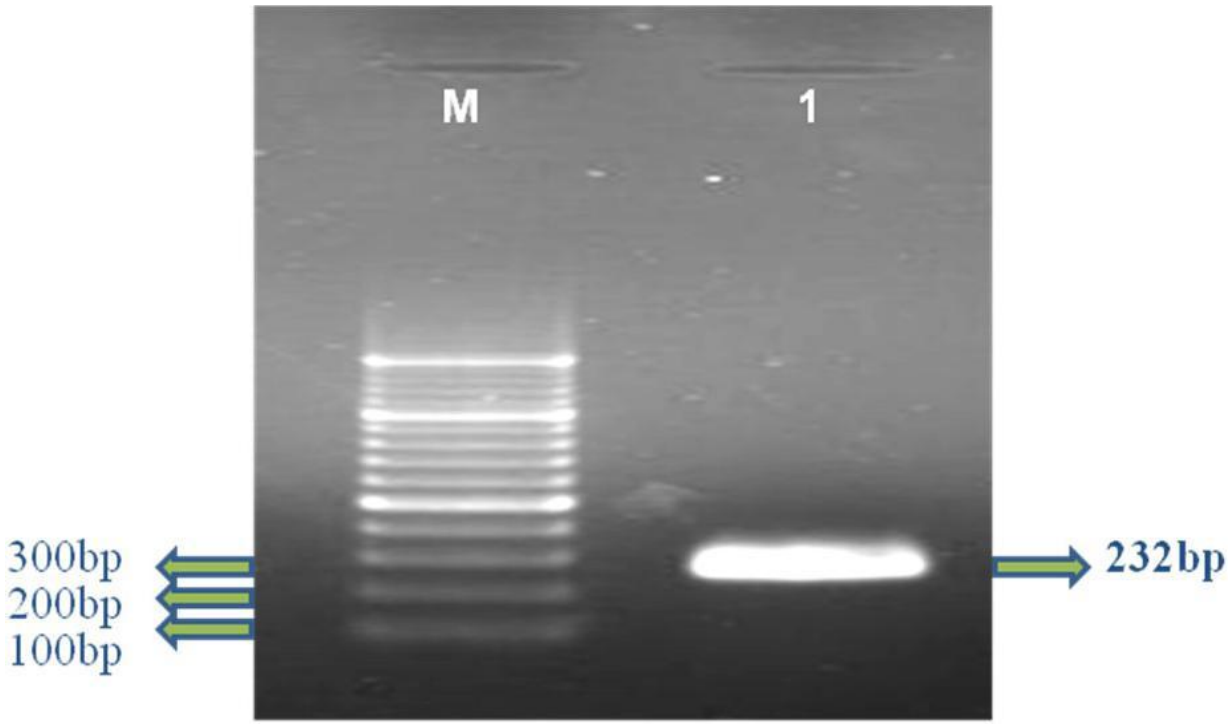

Figure 2. PCR Amplification of *intI2* Gene. Lane M: 100bp DNA Ladder. Lane 1: *int2* PCR product.

isolates, eleven isolates (13.1%) were considered as MDR *Salmonella* serovars (1). These MDR isolates were attributed to *S. Typhi* (n=3), *S. Typhimurium* (n=3), other non-Typhi serovars (n=5). One of the MDR isolates were recovered from bone marrow and the rest ten MDR isolates were originated from stool.

Detection of class 1 and 2 Integrons

Fifty (59.5%) isolates amplified *intI1* gene with the amplicon size of 280bp and fourteen (16.7%) isolates carried *intI2* gene with the amplicon size of 232bp (Figures 1 and 2). Of 50 isolates carrying class 1 integrons, 8 (16%)

Table 2. Information about integron positive isolates among different sources of *Salmonella* isolates.

Isolation source	No. of *intI1* positive isolates (%)	No. of *inti2* positive isolates (%)	No. of *intI1* and *inti2* positive isolates (%)
Stool(69)	41	13	13
Blood(6)	3	0	0
Bone marrow(3)	2	0	0
Synovial fluid(3)	1	0	0
Abces(1)	1	0	0
Ascites(1)	1	0	0
Urine(1)	1	1	1
Total(84)	50(59.5)	14(16.7)	14(16.7)

Table 3. Information about integron positive isolates among serovars of *Salmonella* spp.

Serovars (No.)	No. of *intI1* and intI2-positive isolates (%)[c]	No. of *intI2*-positive isolates (%)[b]	No. of *intI1*-positive isolates (%)[a]
S. Typhi (40)	5	5	21
Non-typhi serovars (30)	8	8	20
S. Typhimurium (12)	1	1	7
S. Paratyphi A (2)	0	0	2
Total (84)	14(16.7)	14(16.7)	50 (59.5)

[a] indicates the number and percentage of *intI1*-posetive in *Salmonellae* serovars
[b] indicates the number and percentage of *intI2*-posetive in *Salmonellae* serovars
[c] indicates the number and percentage of serovars carrying both *intI1* and *intI2* genes.

isolates amplified class 1 integrons exclusively on chromosome, 12 (24%) isolates exclusively on plasmid, 30 (60%) isolates both on plasmid and chromosome. Among fourteen isolates harbored class 2 integrons, 3 (21.4%) isolates carried class 2 integrons only on plasmids and 11 (78.6%) isolates both on plasmid and chromosome (Tables 2 and 3).

DISCUSSION

The worldwide emergence of multidrug-resistant (MDR) phenotypes among *Salmonella* serotypes is causing an increasing concern. Use of antimicrobials in clinical and veterinary medicine is a recognized driving force for the selection of resistant bacteria. Selective pressure has resulted in the development of strains that are resistant to more than one antimicrobial agent (Miriagou et al., 2006; Yang et al., 2009). Integrons are genetic elements able to recognize and capture mobile gene cassettes carrying antibiotic resistance genes leading to MDR distribution and subsequently limitation of treatment options for infections (Stokes and Hall, 1989).

In this study eighty four clinical isolates of *Salmonella* spp. were subjected to molecular investigations to detect integron-associated multidrug resistance.

Of 84 isolates, 11 (13.1%) isolates were resistant to more than 4 groups of antimicrobial agents and considered as MDR *Salmonella* serovars (Table 1). Most of the MDR isolates were recovered from stool and related to the non-Typhi serovars of *Salmonella enterica*. Fifty (59.5%) isolates harbored class 1 integrons and amplified *intI1* gene more detected in Typhi serovar and 14 (16.7%) isolates contained class 2 integrons carrying *intI2* gene as well as *intI1* gene which were more considered in non–Typhi serovars of *Salmonella enterica* (Table 3).

Our study indicates that all the MDR isolates harbored class 1 integrons and one of the MDR isolates carried class 2 in addition to class 1 integrons. This result highlights the integron role in MDR distribution (Table 1).

To confirm the previous studies, according to the results of MIC determination, most of the *intI*-positive isolates showed high resistance to nalidixic acid, trimethoprim and trimethoprim–sulphamethoxazole (Chang et al., 2007) and indicated increased MIC to be higher than 512 µg/ml. Limitless use of these antibiotics in hospitals and other health care environments may be involved in the selection and consequently distribution of integron-carrying *Salmonella*. These antibiotics are at risk for future use and would be prescribed with more consideration.

In agreement with other studies, fluoroquinolones, third-generation cephalosporins and imipenem are

suggested to be used as frontline therapeutic drugs in treatment of *Salmonella* infections. Carbapenems are the main class of drugs used for treatment of infections caused by MDR and extended-spectrum -lactamase-producer Gram-negative bacteria such as *Salmonella* (Ahmed et al., 2009).

In conclusion our results support the hypothesis that integron acquisition may lead to dissemination of new antibiotic resistance determinants and high levels of multidrug resistance. Epidemiological studies on the distribution of integrons would be of great use in detecting MDR isolates.

REFERENCES

Ahmed AM, Younis EA, Ishida Y, Shimamoto T (2009). Genetic basis of multidrug resistance in *Salmonella enterica* serovars Enteritidis and Typhimurium isolated from diarrheic calves in Egypt. Acta Trop., 111: 144-149.

Boyd EF, Hartl DL (1998). *Salmonella* virulence plasmid: Modular acquisition of the *spv* virulence region by an F-plasmid in *Salmonella enterica* subspecies I and insertion into the chromosome of subspecies II, IIIa, IV and VII isolates. Genetics, 149: 1183-1190.

Carattoli A (2001). Importance of integrons in the diffusion of resistance. Vet. Res., 32: 243-259.

Chang YC, Shih DYC, Wang JY, Yang SS (2007). Molecular characterization of class 1 integrons and antimicrobial resistance in *Aeromonas* strains from foodborne outbreak-suspect samples and environmental sources in Taiwan. Diagn. Microbiol. Infect. Dis., 59: 191-197.

Clinical and Laboratory Standards Institute (CLSI) (2009). Performance standards for antimicrobial susceptibility testing; nineteenth informational supplement. M100-S19, CLSI, Wayne, PA.

Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, Grady M, Liebert C, Summers AO, White DG, Maurer JJ (2001). Incidence of Class 1 and 2 Integrases in Clinical and Commensal Bacteria from Livestock, Companion Animals, and Exotics. Antimicrob. Agents Chemother., 45: 723–726.

Gu B, Pan S, Wang T, Zhao W, Mei Y, Huang P, Tong M (2008). Novel cassette arrays of integrons in clinical strains of Enterobacteriaceae in China. Int. J. Antimicrob. Ag., 32: 529–533.

Havlickova H, Hradecka H, Bernardyova I, Rychlik I (2009). Distribution of integrons and SGI1 among antibiotic-resistant *Salmonella enterica* isolates of animal origin. Vet. Microbiol., 133: 193-198.

Macedo-Vinas M, Cordeiro NF, Bado I, Herrera-Leon S, Vola M, Robino L, Gonzalez-Sanz R, Mateos S, Schelotto F, Algorta G, Ayala JA, Echeita A, Vignoli R (2009). Surveillance of antibiotic resistance evolution and detection of class 1 and 2 integrons in human isolates of multi-resistant *Salmonella* Typhimurium obtained in Uruguay between 1976 and 2000. Int. J. Infect. Dis., 13: 342-348.

Martin BS, Lapierre L, Cornejo J, Bucarey S (2008). Characterization of antibiotic resistance genes linked to class 1 and 2 integrons in strains of *Salmonella* spp. isolated from swine. Can. J. Microbiol., 54: 569-576.

Mazel D (2006). Integrons: agents of bacterial evolution. Nat. Rev. Microbiol., 4:608-620.

Miriagou V, Carattoli A, Fanning S (2006). Antimicrobial resistance islands: resistance gene clusters in *Salmonella* chromosome and plasmids. Microbes Infect., 8: 1923-1930.

Moura A, Henriques I, Ribeiro R, Correia A (2007). Prevalence and characterization of integrons from bacteria isolated from a slaughterhouse wastewater treatment plant. J. Antimicrob. Chemother., 60: 1243-1250.

Rodriguez I, Rodicio MR, Herrera-Leon S, Echeita A, Mendoza MC (2008). Class 1 integrons in multidrug-resistant non-typhoidal *Salmonella enterica* isolated in Spain between 2002 and 2004. Int. J. Antimicrob. Ag., 32: 158-164.

Sambrook J, Fritsch EF, Manaiatis T (1989). Molecular Cloning: A Laboratory Manual, 2nd ed. Cold Spring Harbor Laboratory Press, Cold Spring Harbor, NY.

Stokes HW, Hall RM (1989). A novel family of potentially mobile DNA elements encoding site- specific gene-integration functions: integrons. Mol. Microbiol., 3: 1669-1683.

Tamang MD, Oh JY, Seol SY, Kang HY, Lee JC, Lee YC, Cho DT, Kim J (2007). Emergence of multidrug-resistant *Salmonella enterica* serovar Typhi associated with a class 1 integron carrying the *dfrA7* gene cassette in Nepal. Int. J. Antimicrob. Ag., 30: 330-335.

Yang BW, Zheng J, Brown EW, Zhao SH, Meng JH (2009). Characterisation of antimicrobial resistance-associated integrons and mismatch repair gene mutations in *Salmonella* serotypes. Int. J. Antimicrob. Ag., 33: 120-124.