



Evaluation of Class 1 and 2 Integrons and Antibiotic Resistance Pattern in *Salmonella enterica* Isolated from Diarrheal Food-Borne Outbreaks in Iran

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HIGHLIGHTS

- Overall, the 27 *Salmonella enterica* strains were characterized as 14 *S. Paratyphi C*, 7 *S. Enteritidis*, 5 *S. Paratyphi D*, and 1 *S. Paratyphi A* serovars.
- Class 1 integron presented in all and class 2 integron in three strains.
- Most *Salmonella* strains from diarrheal outbreak of Iran were multiple resistant to the highlighted antimicrobials.

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Acronyms and abbreviations

PCR=Polymerase Chain Reaction

ABSTRACT

Background: *Salmonella* spp. are major causes of food-borne disease and have been identified among many diarrheal outbreaks. The major aim of the current investigation was to evaluate the class 1 and 2 integrons and antibiotic resistance pattern in *Salmonella enterica* isolated from diarrheal food-borne outbreaks in Iran.

Methods: This study was carried out on 115 diarrheal feces samples obtained from food-borne outbreak in 2016 in Iran. Antimicrobial resistance patterns of 27 isolated *S. enterica* serovars and presence of class 1 and class 2 integrons in the serovars were investigated using conventional and molecular methods. Results were statistically analyzed using SPSS software v. 21 and Chi-Square test.

Results: Overall, 27 *S. enterica* were characterized as 14 *S. Paratyphi C*, 7 *S. Enteritidis*, 5 *S. Paratyphi D*, and 1 *S. Paratyphi A* serovars. Results of molecular assay showed that class 1 integron presented in all and class 2 integron in three strains. All isolates with class 2 integron genes were resistant to almost all the antimicrobials.

Conclusion: Most studied *Salmonella* strains from diarrheal food-borne outbreak of Iran in 2016 were multiple resistant to the highlighted antimicrobials. Knowledge about risk factor involving the salmonellosis and their control measures could help the national authorities to prevent the outbreaks. Further comprehensive studies with larger sample sizes are necessary to acquire more data about risk factors of multiple resistant *Salmonella* outbreaks in the country.

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Introduction

Nowadays, acute infectious diarrhea is one of the most important challenges for public health in developing

countries (Alizadeh-Hesar et al., 2014). A recent World Health Organization (WHO) report estimates that 1.7

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billion cases of diarrhea occur annually in children aged up to five years (WHO, 2017).

Salmonella spp. are major causes of food-borne diseases and have been identified among many diarrheagenic outbreaks (Liang et al., 2015; Sousa et al., 2013). The *S. enterica* consists of a large division of hydrogen-sulfide producing Gram-negative bacteria (Porwollik et al., 2004). Some serovars of *S. enterica* such as Typhi can cause systemic infections and typhoid fever, whereas other serovars such as Typhimurium cause gastroenteritis (McClelland et al., 2001).

Nowadays, extensive use of antimicrobial has resulted in rapid development of microbial resistance to almost all available antimicrobials by various mechanisms (Ratajczak et al., 2010). These mechanisms mostly rely on genetic elements such as integrons. Three classes of integrons have been identified, which are related to the antibiotic resistance (Ploy et al., 2003). Integrons have been detected in various nontyphoidal serovars and Typhi serovar of *S. enterica* (Gassama-Sow et al., 2004). Class 1 integron structure includes 5' and 3' conserved segments as well as a variable region. Class 2 integron includes a similar structure but is associated with transposon Tn7. It has been shown that class 2 integron carries three different gene cassettes of *dfrA1*, *sat1*, and *aadA1*, confer drug resistance proteins to streptomycin, streptomycin/spectinomycin as well as trimethoprim, respectively (Ahmed et al., 2005).

Therefore, the major aim of the current investigation was to evaluate the class 1 and 2 integrons and antibiotic resistance pattern in *S. enterica* isolated from diarrheal food-borne outbreaks in Iran.

Materials and methods

Samples

This study was carried out on 115 diarrheal feces samples obtained from food-borne outbreak in 2016 in Iran which were sent to Food-borne Outbreaks Laboratory, Department of Pathobiology, School of Health, Tehran University of Medical Sciences, Tehran, Iran. The samples were previously gathered by health centers of local medical sciences universities in four Iranian metropolitans, including Karaj (Alborz Province), Tehran (Tehran Province), Ghazvin (Ghazvin Province), and Yazd (Yazd Province).

Bacterial isolation

Samples were cultured on Selenite-F agar and incubated at 37 °C for 8-12 h. Then, they were cultured on Xylose Lysine Deoxycholate (XLD; Merck, Germany) and Hektoen Enteric (HE; Merck, Germany) agars and incu-

bated at 37 °C for 24 h. After incubation, colonies were chosen for selective tests, including urease, Indole Methyl-red Voges-Proskauer Citrate (IMViC), motility and H₂S production in Sulfur Indole Motility agar (SIM; Merck, Germany), lysine decarboxylation in Lysine Iron Agar (LIA; Merck, Germany), and fermentation of sugars in Triple-Sugar Iron agar (TSI; Merck, Germany). The isolates were characterized to serovar level using serological tests (Sifin, Germany).

Antimicrobial resistance

The disk diffusion method was used for antimicrobial susceptibility assessment of the bacterial isolates according to CLSI (2015). At first, Mueller-Hinton Agar (MHA; Merck, Germany) was inoculated with bacterial suspension equivalent to 0.5 McFarland turbidity. The antimicrobial disks (Difco, USA), including amoxicillin, ceftazidime, cefotaxime, tetracycline, nalidixic acid, ciprofloxacin, streptomycin, gentamicin, chloramphenicol, nitrofurantoin, and trimethoprim-sulfamethoxazole were set on Mueller-Hinton plates with sufficient distances; and incubated at 35 °C for 18 h. After that, the plates were studied for resistance patterns and the inhibition zones were recorded. Standard strains of *S. enterica* serotype Enteritidis (ATCC 13076) and *S. enterica* serotype Typhimurium (ATCC 14028) were used as controls.

DNA extraction

DNA was extracted using precipitation method and DNA extraction filter-column kit (CinnaGen, Iran). Quality of the extracted DNA was assessed by gel electrophoresis and then the DNA was stored at -70 °C until use.

Polymerase Chain Reaction (PCR)

For the molecular identification of class 1 and class 2 integrons, primer sequences were obtained from previous reports (Firoozeh et al., 2011; Rahmani et al., 2013) as shown in Table 1. Total volume of each PCR reaction mixture included 20 µl and the PCR condition included an initial denaturation step at 95 °C for 5 min; then, 30 cycles of 30 s at 95 °C, 30 s at 60 °C, and 60 s at 72 °C were done followed by a final extension step at 72 °C for 5 min. The PCR products were electrophoresed on 1% gels and visualized under UV. Hence, the *S. enterica* serotype Enteritidis (ATCC 13076) was considered as control. The fragment with the size of 558 bp in length showed the presence of class I integrin.

Statistical analysis

Data were statistically analyzed using SPSS Software v. 21 (IBM Analytics, USA) with Chi-Square test. Differences were reported as significant when $p \leq 0.05$.

Results

In this study, 27 *S. enterica* strains were used to assess antimicrobial resistance profiles of the bacteria and the existence of class 1 and class 2 integrons. These strains were characterized as 14 *S. Paratyphi C*, 7 *S. Enteritidis*, 5 *S. Paratyphi D*, and 1 *S. Paratyphi A* serovars.

The PCR study detected class 1 integron in all *Salmonella* strains (Figure 1), but only three of the strains had class 2 integron, including *S. Paratyphi D* (n=2) and *S. Enteritidis* (n=1) serovars. No association ($p>0.05$) was found between the class 1 and class 2 integrons and

Salmonella seravors.

All three isolates with class 2 integron demonstrated antimicrobial resistance, except *S. enteritidis* serovar with an intermediate susceptibility to trimethoprim-sulfamethoxazole. There was no significant association ($p>0.05$) between the symptoms of the patients and presence of class 2 integron in the bacterial isolates; in contrast, a significant association ($p<0.05$) was observed between the city and presence of class 2 integron in the isolates (Table 2).

Table 1: Primer sequences used in this study (Firoozeh et al., 2011; Rahmani et al., 2013)

Gene	Sequence (5'-3')	Melting temperature (°C)	base pair (bp)
<i>Int1</i> F	GCCTGGCTGTTCTTCTACGG	60.5	558
<i>Int1</i> R	GATGCCTGCTTGTCTACGG	60.5	
<i>Int2</i> F	CACGGATATGCGACAAAAAGGT	60.3	740
<i>Int2</i> R	GTAGCAAACGAGTGACGAAATG	60.3	

Table 2: Association of symptoms and geographical origin of diarrheal outbreaks with the presence of class 2 integron in the *Salmonella* isolates

	No. of isolates with class 2 integron	p-value
<i>Symptom</i>		
Abdominal cramp	0	-
Fever	3	0.390
Nausea	3	0.692
Vomit	3	0.526
<i>Geographical origin (city)</i>		
Ghazvin	0	-
Karaj	3	0.000
Tehran	0	-
Yazd	0	-

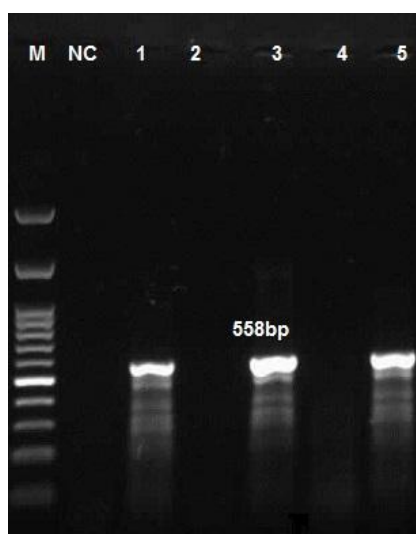


Figure 1: Gel electrophoresis for the PCR product of class 1 integron. M: ladder; NC: negative control; lanes 1 and 3: isolates with class 1 integron with the fragments of 558 bp in length; lane 5: positive control with *S. enterica* serotype Enteritidis (ATCC 13076). Lanes 2 and 4 are empty.

Discussion

In recent years, studies have been carried out in the world in order to investigate health problems and food-borne outbreaks due to *Salmonella* spp. Also, multidrug resistance in *S. enterica* subsp. *Enterica* is rapidly growing (Cummings et al., 2013; Ferrari et al., 2019; Wright et al., 2016). The extensive use of antimicrobial agents in disease prophylaxis and treatment plays a significant role in spread of bacterial resistance to antimicrobials (Edrington et al., 2004; Gassama-Sow et al., 2004). Findings from the present study have shown a high rate of antimicrobial resistance in *Salmonella* strains isolated from food-borne outbreaks occurred in Iran. Furthermore, the results demonstrated the bacterial resistance to at least one antimicrobial, mostly to multiple antimicrobials. An explanation may be based on the inappropriate use of large quantities of antibiotics during the last decades in Iran.

It has been shown that there is a strong association between class 1 integron and resistance to specific antimicrobials, normally owing to the presence of resistance gene cassettes within these integrons (Caleja et al., 2011). A study on class 1 integron in traveler's diarrhea showed class 1 integron in 4/16 (25%) of *Salmonella* spp. isolated from feces samples gathered in Spain (Cabrera et al., 2006). Moreover, 12 distinct gene cassettes were detected inside and outside of the class 1 integron. Another research carried out by Vo et al. (2010) on 297 Vietnamese non-typhoid *Salmonella* isolates showed that 13–50% of the isolates with class 1 integron were mostly resistant to multiple antimicrobials of ampicillin, chloramphenicol, gentamicin, kanamycin, nalidixic acid, streptomycin, sulphonamide, tetracycline, and trimethoprim. In addition, nine distinct integron profiles were investigated in nearly 28% of 11 *Salmonella* serovars. In Senegal, Gassama-Sow et al. (2004) investigated contribution of integrons to antimicrobial resistance of eight isolates of *S. enterica*. They detected class 1 integron in all of the bacterial isolates, while no class 2 or class 3 integrons were found. Also, they found that most isolates were resistant to combinations of amikacin, chloramphenicol, gentamicin, netilmicin, spectinomycin, streptomycin, trimethoprim-sulfamethoxazole, tetracycline, as well as tobramycin; that are in agreement with our findings. The results of the present study is similar to the findings of Eshaghi Zadeh et al. (2019) that reported the most spread serotype in 30 *Salmonella* isolates from children diarrheas in Tehran, Iran included Enteritidis (36.7%), followed by Paratyphi C (30%), and Typhimurium (16.7%). These researchers revealed that the most antibacterial resistance was related to nalidixic acid (53.3%), streptomycin (40%), and tetracycline (36.7%).

Foods of animal origin such as red meat, poultry, egg, milk, etc. are reported as major carriers of *Salmonella* spp. causing diarrheal food-borne diseases (Cummings et al., 2013; Jackson et al., 2013). El-Demerdash et al. (2018) reported 19 *Salmonella* spp. within 110 Enterobacteriaceae isolated from broilers. These multidrug resistant *Salmonella* isolates contained class 1 and/or class 2 integrons with different gene cassettes (El-Demerdash et al., 2018). Fardsanei et al. (2017) collected 34 *S. enterica* serovar Enteritidis from various foods in Iran and reported that all isolates were resistant at different levels to cefuroxime (79.4%), nalidixic acid (47%), and ciprofloxacin (44.2%). Same researchers investigated antibiotic susceptibility patterns in 44 *S. enterica* serovar Enteritidis isolates from patients with gastroenteritis in Tehran, Iran (Fardsanei et al., 2018). They found high rates of multiple antimicrobial resistances to ciprofloxacin (90.9%) and nalidixic acid (77.3%). The previous published reports in database about occurrence of diarrhea-causing *Salmonella* serovars in foodstuffs indicated the role of contaminated foods in spreading of the salmonellosis. For instance, Jackson et al. (2013) stated that 83% of egg-associated *Salmonella* outbreaks in United States occurred with serotype Enteritidis during 1998-2008.

We found no significant statistical relationship between symptom of the patients and the presence of class 2 integron in the *Salmonella* isolates. Although class 2 integron only found in the *Salmonella* isolates from Karaj city, there are some doubts about probable association between geographical origin of the isolates and the presence of class 2 integron. This is due to the fact that our sample size was relatively low that could be considered as a technical limitation in this study.

Conclusion

The class 1 integron was found in all *Salmonella* strains isolated from the diarrheal food-borne outbreaks during 2016 in Iran. Furthermore, most strains were multiple resistant to the highlighted antimicrobials. Knowledge about risk factor involving the salmonellosis and their control measures could help the national authorities to prevent the outbreaks. Further comprehensive studies with larger sample sizes are necessary to acquire more data about risk factors of multi-drug resistant *Salmonella* outbreaks in the country.

Author contributions

S.F.S. did the experimental work and drafted the manuscript; M.M.S.D. designed and supervised the

study; S.A. advised scientifically during the study; R.M.N.F. advised technically during the study and revised the manuscript.

Conflicts of interest

All the authors declared that this is no conflict of interest in the study.

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