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

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**Background:** *Salmonella* spp 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

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**1. Background**

*Salmonella* spp 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.

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**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), cefazidime (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 ESBL 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 an ESBL 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 ESBL isolates by the E-test (AB Biobase, 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 °C 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

<table>
<thead>
<tr>
<th>Primers</th>
<th>Nucleotide Sequence (5' to 3')</th>
<th>Amplicon, bp</th>
<th>PCR Condition</th>
<th>References</th>
</tr>
</thead>
<tbody>
<tr>
<td>Int1</td>
<td></td>
<td></td>
<td>Denaturin</td>
<td>Annealin</td>
</tr>
<tr>
<td>F</td>
<td>CAGTGGACATAAGCCTGTTC</td>
<td>160</td>
<td>94,30 s</td>
<td>55,30 s</td>
</tr>
<tr>
<td>R</td>
<td>CCCGAGCCATAGCTGTA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int2</td>
<td></td>
<td>788</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>CACGGATATGCGACAAAAAGGT</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>GTAGCAAACGAGTGACGAATG</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Int3</td>
<td></td>
<td>979</td>
<td></td>
<td></td>
</tr>
<tr>
<td>F</td>
<td>GCCCTCCGGCAGCGACTTTTCA</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>R</td>
<td>ACGGATCTCGCCTAAACCTGAC</td>
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<td></td>
</tr>
</tbody>
</table>
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 alone among integron-positive isolates. Primers IntI1F and IntI1R were used to amplify a 160 bp fragment of the intI1 gene for the class 1 integrase, the primers IntI2F and IntI2R amplified a fragment of 288 bp, specific for the intI2 gene and primers IntI3F and IntI3R were used to amplify a specific intI3 gene. Table 3 shows an apparent association between antibiotic resistance and presence of integrons among Salmonella isolates.

Table 2. Percentage of isolates Susceptible, Moderately Susceptible or Resistant to Each Antibiotic a,b

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

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

b Data are presented as No. (%).

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 with 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 class 1 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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