Quinolone resistant *Salmonella* species isolated from pediatric patients with diarrhea in central Iran

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**Abstract**

**Background:** This study aimed to investigate the frequency and the antibiotic resistance patterns of *Salmonella* species that were isolated from infectious diarrhea samples taken from pediatric patients in central Iran.

**Methods:** The study analyzed 230 stool specimens that were cultured on XLD, MacConkey agar and GN broth. Polymerase chain reaction (PCR) assay was used to identify the *Salmonella* genus. The antibiotic resistance profiles and the frequency of quinolone and integron genes were obtained.

**Results:** Out of 230 samples of infectious diarrhea, 21 (9.1%) cases of *Salmonella* spp. were identified using culture methods. Another 28 (12.1%) samples had positive PCR results, with *S*. serovar Paratyphi B and C (9/21; 42.8%) and *S*. Typhi (3/21; 14.3%) being the most recognized. The highest antibiotic resistance rates were found for nalidixic acid (15/21; 71.4%), tetracycline (9/21; 42.8%). However, six (28.5%) of isolates were found resistant to cotrimoxazole, ampicillin and chloramphenicol. Among the plasmid-mediated quinolone resistance (PMQR) determinants, *qnrS*, *qnrA*, and *qnrB* were positive in (9/15; 60%), (6/15; 40%) and (3/15; 20%) of the isolates, respectively. Class 1 and 2 integrons were identified in 15 (71.4%) and 3 (14.3%) isolates, respectively.

**Conclusion:** High rates of quinolone resistant and low frequency of MDR *Salmonella* spp. isolates were identified in central Iran, similar to findings in other parts of Asia. To prevent the spread of these resistant strains, the antimicrobial resistance of *Salmonella* spp. isolates should be under constant surveillance, and empiric antibiotic therapy should be adapted appropriately.

**Keywords:** *Salmonella* spp., Diarrhea, Antibiotic, Quinolone resistance, Iran

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(trimethoprim-sulfamethoxazole) and chloramphenicol, and bacteria with simultaneous resistance to all three types of antibiotics were characterized as multi-drug resistant (MDR) [6]. Currently, MDR typhoid is in decline in the Asian regions, where there is a high level of resistance to second-line drugs, such as quinolones and fluoroquinolones [6]. This increased antimicrobial resistance has decreased the number of effective treatment options and, consequently, increased the treatment costs, the risk of complications and the death rate, especially in the pediatric age group [3].

In the summer, diarrhea and dysentery are so common among the pediatric patients at central Iran [7–11]. It is not efficient to identify typhoidal Salmonella in clinical detection labs, and specific information about the scale of typhoidal Salmonella in Iran’s central region is not available. Therefore, this study has been conducted to examine in depth the abundance, the phenotypic antimicrobial resistance levels and the resistance gene content of the region’s Salmonella species by examining diarrhea samples from patients.

Materials and methods
Sample collection
This study protocol was approved by the ethics committee of the Arak University of Medical Sciences (ARAKMU.REC. 93-176-30 and 1395.83). All methods were performed in accordance with the relevant guidelines and regulations. For this cross-sectional, descriptive study, 230 samples of diarrhea were gathered from pediatric patients who were referred to the Children’s Educational-Therapeutic Center affiliated with Arak University of Medical Sciences (in the city of Arak, Iran) due to diarrhea from May 2015 to May 2016. The parent/guardian consent form was provided for participants under 16 years old.

The inclusion criteria for this study were as follows: a completed consent form and a questionnaire was filled out by the patient or the patient’s parents and caregivers; observation of more than five white blood cells per high-power field (HPF) in a stool specimen [12] and the patient had not taken antibiotics for a week before consultation at the hospital.

Phenotypic investigation
The fecal samples were cultured in Gram-negative (GN) broth, xylose lysine deoxycholate (XLD) and MacConkey media (Merck, Hamburg, Germany); then biochemical and serological tests were performed [7]. Application programming interface (API) testing (Biomeriux, France) was used to confirm the presence of Salmonella spp. isolated. S. enterica subsp. enterica PTCC 1709, S. enterica subsp. enterica serovar Paratyphi A PTCC 1230, S. enterica subsp. enterica serovar Paratyphi B PTCC 1231 and S. Typhi PTCC 1609 were used as controls in each assay (obtained from the Iranian Research Organization for Science and Technology). S. enterica subsp. enterica serovar Paratyphi C control strains were acquired from the microbiology department of the Arak University of Medical Sciences.

Investigating Salmonella antibiotic resistance by disk diffusion
Using the Clinical and Laboratory Standards Institute (CLSI) 2017 guidelines [13], an antibiogram assay was performed on the isolated Salmonella spp. colonies. The antibiotic discs contained nalidixic acid (30 μg), tetracycline (30 μg), cotrimoxazole (25 μg), ampicillin (10 μg), chloramphenicol (30 μg), ceftriaxone (30 μg), cepotaxime (30 μg), ceftizoxime (30 μg), ceftazidime (30 μg), cefoxitin (30 μg), cefepime (30 μg), gentamicin (10 μg), azithromycin (15 μg), ciprofloxacin (5 μg) and imipenem (10 μg) (Mast Diagnostics, United Kingdom).

Genotypic investigations
DNA extraction
DNA was directly extracted from the fecal samples and the reference Salmonella spp. isolates using the QIAamp DNA stool mini kit (Qiagen GmbH, Hilden, Germany), according to the manufacturer’s protocol. The amount and purity of the extracted DNA were measured with a NanoDrop apparatus (Thermo Fisher Scientific, Waltham, Massachusetts, United States) and confirmed using the universal primers for the bacterial 16S rRNA gene [8].

Genotypic identification
PCR of the inlA gene was performed to confirm the Salmonella genus [14]. PCR of the qnr determinant genes qnrS, qnrA, and qnrB was performed to amplify the plasmid-mediated quinolone resistance (PMQQR) targets. Mutations in the gyrA and parC genes of the quinolone-resistant Salmonella spp. isolates were also identified using DNA sequencing techniques [7]. Sul1,2 for sulphonamide resistance and quartenary ammonium compounds (qac) resistance genes were investigated using PCR method (Table 1) [15].

Integron detection
To investigate class 1, 2 and 3 integrons, PCR assay was performed as previously described in the literature (Table 1) [7].
**Results**

Of the 230 analyzed samples, 21 (9.1%) and 28 (12.1%) were found to be positive for *Salmonella* spp. using the exclusive culture and PCR methods, respectively. All the culture-positive samples were identified as positive using PCR; and seven of the samples that were culture-negative were also identified as positive using PCR. Of the 21 patients (9.1%) afflicted with *Salmonella* spp., 9 (42.8%) were female and 12 (57.1%) were male, resulting in a female-to-male infection ratio of 1:1.3. The average age of the people afflicted with salmonellosis was 4 years and 5 months. The youngest diseased person was an 8-month-old girl; the oldest was a 12-year-old boy. The clinical symptoms among the people suffering from salmonellosis are given in Table 2.

**Phenotypic and genotypic investigation**

Of the 21 cultured *Salmonella* spp. isolates, 9 (42.8%) were identified as *S. Paratyphi B*, 9 (42.8%) were identified as *S. Paratyphi C*, and 3 (14.3%) was identified as *S. Typhi*; no case of *S. Paratyphi A* was found.

**Phenotypic and genotypic antibiotic resistance determination**

Using the CLSI 2017 guidelines, the highest resistance rates in *Salmonella* spp. were observed against nalidixic acid (15/21; 71.4%), tetracycline (9/21; 42.8%),

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**Table 1** The primers used in this study

| Target gene description | Primer | Sequence 5′ → 3′ | Amplicon size (bp) | Annealing temperature | References |
|-------------------------|--------|------------------|--------------------|-----------------------|-----------|
| Universal DNA bacterial  | 16s-rRNA-F 16s-rRNA-R | S-AGGAGGTGATCCAAACCGCA-35-ACCTGGAGGAAATTTTGGAT-3 | 367 | 55 | [8] |
| Salmonella spp.          | invA-F invA-R | S-TTGTTACCGCTATTGATACG-35-CTGACTGTACCTGT-3 | 521 | 60 | [14] |
| Fluoroquinolone          | gyrA-F gyrA-R | S-AAACTCGCCGCTGTGTTGTC-35-GCCATAACCTACGGGAT-3 | 344 | 55 | [7] |
|                         | parC-F parC-R | S-CTGAACTGCAACCCGTAAT-35-AGCGGATGTC-3 | 168 | 55 | [7] |
|                         | qnrS-F qnrS-R | S-TGGAACCTGACAGCTGATCG-35-TTGAGTCAGGACA-3 | 656 | 60 | [7] |
|                         | qnrA-F qnrA-R | S-GTAAAGTTTTTCGCAAGG-35-ATCCAGATCGCAGACA-3 | 593 | 60 | [7] |
|                         | qnrB-F qnrB-R | S-GTGGGCGAAAAATTGCAACAGA-35-ATCCGAGTTCAGATGC-3 | 264 | 53 | [7] |
| Integrase1               | Int1-F Int1-R | S-CAATGCGATACAGCCTGTT-35-CCTGACAGCTATGAG-3 | 160 | 55 | [7] |
| Integrase2               | Int2-F Int2-R | S-TGGCAGGATCTACATTAGG-35-CCGCCTGACTGAG-3 | 288 | 55 | [7] |
| Integrase3               | Int3-F Int3-R | S-GGCGCAAGCGGACTTTCAG-35-ACCGGATCTGCAACCTGACT-3 | 979 | 59 | [7] |
| Sulphonamide resistance  | Sul1-F Sul1-R | S-TCGGAGGAGTTCGCAGT-35-CCGCCTGACTGAG-3 | 331 | 65 | [7] |
|                         | Sul2-F Sul2-R | S-GTCATTCAGGACAGACAGA-35-GAAGGCGACGGCAGC-3 | 435 | 58 | [7] |
| Quaternary ammonium compounds | qac-F qac-R | S-GCTTCAGACAAATGGGAGA-35-CTCCGCTACCA-3 | 370 | 55 | [15] |

**Table 2** Frequency of clinical symptoms in pediatric patients with *Salmonella* spp.

| Salmonella spp | Mucus in the stool | Abdominal pain | Vomiting | Fever | Blood in the stool |
|----------------|--------------------|----------------|----------|-------|-------------------|
| *S. Paratyphi B* | 9/9 (100%) | 9/9 (100%) | 6/9 (66.6%) | 5/9 (55.5%) | 6/9 (66.6%) |
| *S. Paratyphi C* | 9/9 (100%) | 7/9 (77.7%) | 3/9 (33.3%) | 8/9 (88.8%) | 8/9 (88.8%) |
| *S. Typhi* | 3/3 (100%) | 3/3 (100%) | 1/3 (33.3%) | 3/3 (100%) | 3/3 (100%) |
| *S. Paratyphi A* | – | – | – | – | – |
cotrimoxazole (6/21; 28.5%), ampicillin (6/21; 28.5%), and chloramphenicol (6/21; 28.5%) (Table 3). All of the *Salmonella* isolates were susceptible to cefixime, ceftriaxone, cefotaxime, ceftizoxime, cefoxitin, cefepime, gentamicin, azithromycin, ciprofloxacin, and imipenem. No cases of MDR were observed. All isolates carrying PMQR contain similar mutations in *parC* at amino acid 80 (replacement of serine with isoleucine; GenBank accession no. HM068910) and *gyrA* at amino acid 83 (replacement of serine with leucine). The frequency of antibiotic resistance genes among *Salmonella* spp. was given in Table 4.

**Discussion**

The frequency of salmonellosis, as determined by bacterial culture and PCR, was 9.1% and 12.1%, respectively. Of these two methods, the sensitivity of the PCR method was higher [16, 17]. Other studies conducted in Sudan, Iran (Tehran), Iraq reported frequencies of 4%, 7%, and 14.8%, respectively [14, 18, 19]. These differences in the frequency of salmonellosis may be related to a variety of factors, including exposure to the natural reservoirs of *Salmonella* species in these geographical areas, differences in climate and many other environmental conditions, as well as age differences, and differences in the level of economic development, the level of individual hygiene, and contamination via food preparers who are chronic carriers of *Salmonella* [5].

In the present study, the most prevalent *Salmonella enterica* serovar isolates were *S*. Paratyphi B and *S*. Paratyphi C (42.8%). In other studies, *S*. Typhi and *S*. Paratyphi B were reported to be the predominant serogroup in Iran (Tehran) and Ethiopia, respectively [19, 20]. General hygiene, socioeconomic conditions, and ecological conditions affect the frequency of *Salmonella* spp. serogroups [4].

Because salmonellosis is spread by food, any information regarding the frequency and the antimicrobial resistance of the isolates is a public health concern [21]. The antibiotic resistance properties of *Salmonella* spp have been reported to vary and be regionally distinct [22]. The present study is the first to report on the frequency of salmonellosis and its associated resistance patterns for a panel of 16 antibiotics in central Iran.

The traditional first-line drugs used to treat *Salmonella* spp. are chloramphenicol, ampicillin, and cotrimoxazole [6]. In Iran (Tehran) and Mexico, 11% and 33% of the strains have been reported to be resistant to chloramphenicol, respectively [3, 23]. In Iran (Tehran), Mexico, and Pakistan 13.5%, 20%, and 66.1% of the strains were found to be resistant to ampicillin [23–25]. In Iran (Tehran), Mexico, and Pakistan 23%, 28.8%, and 66.5% of the strains were resistant to cotrimoxazole, respectively [23–25]. These differences indicate that resistance to *Salmonella* first-choice agents may be related to presentation from different sources [26]. In Iran (Tehran), *Sul1* was found to be resistant in 32% of the strains [3]. In India, *Sul1* and *Sul2* were found to be resistant in 100% and 77.7% of the strains,

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**Table 3** Phenotypic antibiotic resistance rates in *Salmonella* spp.

| Antibiotic       | *Salmonella* spp. | *S*. Paratyphi B | *S*. Paratyphi C | *S*. Typhi |
|------------------|-------------------|-----------------|-----------------|-----------|
|                  | n:21              | n:9             | n:9             | n:3       |
| Nalidixic acid   | 15 (71.4%)        | 6 (66.6%)       | 6 (66.6%)       | 3 (100%)  |
| Tetracycline     | 9 (42.8%)         | 5 (55.5%)       | 2 (22.2%)       | 2 (66.6%) |
| Cotrimoxazole    | 6 (28.3%)         | 2 (22.2%)       | 3 (33.3%)       | 1 (33.3%) |
| Ampicillin       | 6 (28.5%)         | 4 (44.4%)       | 1 (11.1%)       | 1 (33.3%) |
| Chloramphenicol  | 6 (28.5%)         | 3 (33.3%)       | 2 (22.2%)       | 1 (33.3%) |

**Table 4** The frequency of antibiotic resistance genes among *Salmonella* spp.

| Resistance       | Target gene | *Salmonella* spp. | *S*. Paratyphi B | *S*. Paratyphi C | *S*. Typhi |
|------------------|-------------|-------------------|-----------------|-----------------|-----------|
| Sulfonamide      | *Sul1*      | 6/6 (100%)        | 2/2 (100%)      | 3/3 (100%)      | 1/1 (100%) |
|                  | *Sul2*      | 3/6 (50%)         | 2/2 (100%)      | 1/3 (33.3%)     | 0%        |
| Fluoroquinolone  | *gyrA*      | 15/15 (100%)     | 6/6 (100%)      | 6/6 (100%)      | 3/3 (100%) |
|                  | *parC*      | 15/15 (100%)     | 6/6 (100%)      | 6/6 (100%)      | 3/3 (100%) |
|                  | *qnrS*      | 9/15 (60%)       | 4/6 (66.6%)     | 2/6 (33.3%)     | 3/3 (100%) |
|                  | *qnrA*      | 6/15 (40%)       | 2/6 (33.3%)     | 1/6 (16.6%)     | 3/3 (100%) |
|                  | *qnrB*      | 3/15 (20%)       | 2/6 (33.3%)     | 0%              | 1/3 (33.3%) |
| Integrase        | *int1*      | 15/21 (71.4%)    | 7/9 (77.7%)     | 6/9 (66.6%)     | 2/3 (66.6%) |
|                  | *int2*      | 3/21 (14.3%)     | 3/9 (33.3%)     | 0%              | 0%        |
|                  | *int3*      | 0%               | 0%              | 0%              | 0%        |
| Quaternary amm.  | *qac*       | 9/21 (42.8%)     | 5/9 (55.5%)     | 3/9 (33.3%)     | 1/3 (33.3%) |
respectively [27]. The difference in frequency of sulfonamide-resistant in different regions mainly relates to different antibiotic usage patterns [5]. However, MDR Salmonella spp. are a worldwide concern but is not very common [6]. In Iran (Tehran), Malaysia, and Nigeria, 51.8%, 42%, and 65.9% of the strains were resistant to tetracycline, respectively [28–30].

Although quinolone/fluoroquinolones are intended to be appropriate drugs against resistant isolates, the enhancement in antimicrobial resistance is a burden in controlling infections caused by Salmonella spp. [31]. In the present study, 71.4% of the Salmonella strains were found to be resistant to nalidixic acid, and none of the strains was resistant to ciprofloxacin. In Iran (Tehran), Nigeria, and India, 66.6%, 59%, and 96% of the strains were resistant to nalidixic acid, respectively [32–34].

In this study, qnrS, qnrA, and qnrB were found at 60%, 40%, and 20%, respectively, in nalidixic acid-resistant Salmonella strains. In Iran (Tehran), qnrS, qnrA, and qnrB were found at 56.5%, 30.4%, and 1.1% in the strains, respectively [32]. In Brazil, qnrS, qnrB, and qnrA were found at 53.3%, 40%, and 0% in the strains, respectively [31], while in India, qnrB was at 70% and none of the strains showed resistance to qnrA and qnrS [35]. Quinolones, and especially fluoroquinolones, are widely used in poultry farms and in the treatment of companion animals in Iran, and this contributes to the risk of resistant zoonotic bacterial agents being spread via the food chain [36]. Generally, studies have determined a direct relationship between quinolone usage in poultry and the frequency of nalidixic acid-resistant Salmonella spp. isolates from humans [37]. Fluoroquinolone resistance is prevalent across Asia, in part because of the widespread consumption of this class of antimicrobials [6].

In the current study, qac were found at 42.8% in Salmonella isolates, while in Iran (Tehran) and Iraq, qac were found at 31% and 60% in the strains, respectively [3,38].

In Iran (Tehran), int1 (32%), int2 (13%), and int3 (0%) were found in the strains [3], while in Hong Kong, int1 was found in 13% of the strains but none showed resistance to int2 and int3 [39]. Of the three categories of integrons pertinent to antimicrobial resistance, the class 1 integron is the most frequently obtained in Gram-negative bacteria [40]. The prevalence of integrons in the enterobacteriaceae family has been varied and has played a significant role in the development of drug-resistant bacteria [41]. Thus, the high prevalence of antibiotic resistance probably relates to the high prevalence of class I and II integrons.

**Conclusion**

To reduce and prevent outbreaks of quinolone resistance, and prevention of the emergence of MDR Salmonella spp., a coherent program needs to be developed for the control and surveillance of antimicrobial resistance in the long run. Further, empiric antibiotic therapy should be adapted appropriately, and Salmonella carriers should be identified and given specific treatment in order to prevent this transmission route.

**Abbreviations**

PCR: Polymerase chain reaction; DNA: Deoxyribonucleic acid; XLD: Xylose lysine deoxycholate; PMQR: Plasmid-mediated quinolone resistance; MDR: Multi-drug resistant; HPF: High-power field; BLAST: Basic local alignment search tool.

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**Authors’ contributions**

EGR conceptualized and designed the study. EA were involved in the data collection, generation, and performed data analysis. All authors have read and approved this version of the manuscript.

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**Availability of data and materials**

All data pertaining to this study are within the manuscript in sections sample collection and results. The datasets analyzed and/or used during the current study are available from the corresponding author on reasonable request.

**Declarations**

**Ethics approval and consent to participate**

This study received ethical approval from the Arak University of Medical Sciences (Numbers: 2137 and 2571). Informed consent was obtained from a parent and/or guardian for participants under 16 years old. A signed consent form was obtained from each patient. There was no access to any information that enabled authors to identify individual patients.

**Consent for publication**

Not applicable.

**Competing interests**

The authors stipulate that they have no conflict of interest in regard to this study.

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