Antibiotic susceptibility and genetic relatedness of *Shigella* species isolated from food and human stool samples in Qazvin, Iran

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**Abstract**

**Objective:** The aim of this study was to investigate the genetic relatedness and antimicrobial resistance among *Shigella* species isolated from food and stool samples. Using cross sectional study method, *Shigella* spp. were isolated from food and clinical samples using culture-based, biochemical and serological methods. Antimicrobial susceptibility and genetic relatedness among the isolates were evaluated using disk diffusion and RAPD-PCR methods respectively.

**Results:** The prevalence of *Shigella* spp. were 4.84 and 7.7% in food and stool samples respectively. All food isolates were *Sh. sonnei*. 91.42% of the *Shigella* stool isolates were *Sh. sonnei*. 62.5% of food isolates were resistant to tetracycline. 46.8, 50 and 65.8% of clinical isolates were resistant to imipenem, amikacin and azithromycin respectively. 50 and 85.7% of the food and clinical isolates respectively were MDR. Dendrogram generated by RAPD-PCR showed that the isolates from food and stool samples were categorized in a same group. Close genetic relatedness between MDR *Shigella* isolates from food and clinical samples indicate that foods can be considered as one of the main vehicles for transmission of MDR *Shigella* to human causing acute diseases. Survey of MDR *Shigella* among food and clinical samples is strongly suggested to be implemented.

**Keywords:** *Shigella* species, Genetic relatedness, Antimicrobial susceptibility, Food samples, Stool specimens

**Introduction**

*Shigella* are non-motile, gram-negative and rod-shaped bacteria belonging to the *Enterobacteriaceae* family. During an epidemic outbreak, in 1898, *Shigella* was isolated for the first time from bacillary dysentery cases by Kiyoshi Shiga in Japan [1]. *Shigella* comprises four species including *Sh. dysenteriae*, *Sh. flexneri*, *Sh. boydii* and *Sh. sonnei* [2]. *Shigella* spp. contribute to intestinal disorders including mild to severe bloody diarrhea in children and adults [3]. However, *Sh. dysenteriae* type 1 cause hemorrhagic uremic syndrome (HUS) as an extraintestinal pathogen via releasing shiga-toxins [4]. Prevalence of *Shigella* spp. is geographically different, as *Sh. sonnei* and *Sh. flexneri* have more been isolated from patients in industrialized and developing countries respectively. Shigellosis have commonly been seen among the children under the age of 5 years old especially in low and middle-income countries. *Shigella* is an important foodborne pathogen and sometimes is transmitted via animals. Regarding the remarkable reduction in mortality of this pathogen in the recent decades, there are still more than 160,000 deaths annually caused by *Shigella* worldwide [5].
Drug resistance of human pathogens is one of the main concerns of public health. Multidrug resistant (MDR) is defined when a pathogen is resistant to two or more antibiotics from different groups. Several studies have been highlighting the importance of MDR human and animal pathogens [6–11]. Spread of MDR Shigella is associated with severe outcomes and leads to failure in treatment. Foods have been considered as an important vehicle for transmission of pathogens. A few studies demonstrated a close genetic relatedness between the Shigella isolates from food and clinical samples indicating that Shigella may be transmitted through food to human leading to intestinal and extraintestinal infectious diseases [12, 13].

Random amplified polymorphic DNA (RAPD) method has widely been used to investigate the genetic relatedness between the pathogenic isolates from different sources [14]. Many researchers used RAPD-PCR to study the genetic diversity among pathogenic isolates [15]. Several studies have been performed to characterize the antibiotic resistance in MDR Shigella isolates from food and clinical samples [13]; however, there are limited literature available that provides the genetic relatedness among MDR Shigella isolates from food and clinical samples. The objective of the present study was to investigate the genetic relatedness among the MDR Shigella spp. isolated from food samples and stool specimens from the patients with diarrhea.

**Main text**

**Bacterial isolates**

453 children between the age of 2–5 years old, including 242 males and 211 females, who visited the department of children health and diseases (Qazvin children hospital, Qazvin, Iran) for diarrhea and dysentery from November 2019 to July 2020 were enrolled into the study and forwarded to the clinical central Lab of Qazvin children hospital, Qazvin, Iran. All stool specimens were collected into clean, sterile and disposal containers according to the protocol described by WHO Laboratory investigations, manual of Enteric infections for fecal sample preparation [11]. Totally 165 food samples, consisting of 55 samples of each food item, including minced meat (100 g), vegetable salad (500 g) and raw milk (1 L) were collected from local stores, located in different areas of Qazvin city, from September to December 2019. All clinical and food samples were kept at 4 °C prior to bacterial isolation and were immediately transported to the central Lab. *S. sonnei* ATCC 25,931; *S. boydii* ATCC 12,030; and *S. flexneri* ATCC 12,022 were purchased from Pasteur institute of Iran (Pasteur institute, Tehran, Iran) and used as the reference strains in this study. All reference strains were activated by inoculation into Bovine Heart Infusion (BHI) broth and incubation at 37 °C for 24 h.

**Shigella spp. isolation and identification**

After homogenization, stool samples were directly streaked onto MacConkey and Salmonella-Shigella (SS) agar (Promedia, Spain) and incubated anaerobically at 37 °C for 24 h. H2S negative and non-lactose fermentative colonies were selected for further biochemical tests. Isolation of Shigella spp. from food samples were conducted using the method described by Mokhtari et al. [13]. First, 25 g or mL of each food sample were homogenized using Stomacher BagMixer Lab blender (InterScience, France) for 1 min. Homogenized samples were mixed and diluted with 225 mL of buffered peptone water broth (BPW, Promedia, Spain) and incubated for 24 h at 37 °C. Diluted samples were inoculated into Shigella-broth (Promedia, Spain) and incubated at 37 °C overnight. Enriched samples were streaked onto the MacConkey and SS agar (Promedia, Spain) plates and incubated anaerobically at 37 °C for 24 h. Urease, motility, triple sugar iron, lysin iron decarboxylase and IMViC tests were performed for biochemical confirmation of the presumptive colonies. Serological assay was used for identification of *Shigella* genus using Difco Antiseras Kit (BD-Difco Co. USA). Different species of *Shigella* isolates were also identified by serological tests using *Shigella* species Difco Antiseras Kit (BD-Difco Co. USA). All serological tests were conducted according to the kit manufacturers’ instruction [12].

**Antimicrobial Susceptibility testing**

The antimicrobial susceptibility of the isolates was evaluated using the disk diffusion technique which was previously described by Marami et al. [16] in accordance with the guideline of Clinical and Laboratory Standards Institute (CLSI) [17]. Seven antimicrobial disks (Oxoid, Ltd, UK) including chloramphenicol (30 mg), tetracyclin (30 mg), ampicillin (10 mg), amikacin (30 mg), imipenem (10 mg), azithromycin (15 mg) and nalidixic acid (30 mg) were applied. When the isolates were resistant to two or more different antimicrobial agents, multidrug resistance (MDR) was defined.

**DNA extraction**

All strains were inoculated into trypticase soy broth (TSB, Promedia, Spain), incubated at 37 °C overnight and centrifuged at 3500 rpm for 10 min. After discarding the supernatant, precipitated cell pellets were subjected to DNA extraction using the gram-negative bacterial DNA extraction kit (Sinaclon, Iran) according to the manufacturers’ instruction. Quantity and quality of the extracted genome were measured using NanoDrop Spectrophotometer (Thermo Scientific, USA). Concentration of the DNA templates were adjusted to 50 ng. µL⁻¹.
RAPD-PCR based genotyping and genomic fingerprinting

In this study, primer UBC245: 5’- CGC GTG CCA G-3’ [18], previously used for RAPD-genotyping of *Shigella*, was used. Each reaction tube contained 25 µL of total reaction volume including 12.5 µL of PCR 2X master mix kit (Ampliqon, Denmark), 0.5 µL of primer (50 µM), 1 µL of DNA template (50 ng. µL⁻¹) and deionized sterilized water to the final reaction volume. Thermal cycling was run using Bio-rad T100 machine (Bio-rad, USA) in the following program: initial denaturation 5 min at 95 °C; 35 cycles of 95 °C for 1 min, 36 °C for 1 min, 72 °C for 5 min; and 5 min at 72 °C as the final extension. PCR products were characterized using gel electrophoresis for 2 h at 70 v on 1.5% w/v agarose (Merck, Germany) containing DNA safe stain (CinnaGen, Iran) along with 100-bp DNA ladder. Fluorescent banding patterns were recorded using gel documentation system (Mahazma, Iran). For genomic fingerprinting, DNA banding patterns were interpreted using PyElph software version 1.4 [19]. Similarities were calculated and the unweighted pair group method with arithmetic mean (UPGMA) dendrogram was drawn regarding Dice coefficient by NTSYSpc software version 2.20 (Applied Biostatics Inc., USA).

**Results**

*Shigella* were detected in 8 food samples (4.84%). All of these isolates were *Sh. sonnei* and isolated from vegetable salad samples. *Shigella* spp. were detected in 35 stool samples (7.7%) including 32 *Sh. sonnei* (91.42%), 2 *Sh. flexneri* (5.71%) and 1 *Sh. boydii* (2.85%) isolate. We have not isolated any *Sh. dysenteriae*. As shown in Table 1, 62.5% of food isolates were resistant to tetracycline and all isolates were sensitive to amikacin. 46.8, 50 and 65.8% of clinical isolates were resistant to imipenem, amikacin and azithromycin respectively. Also, all clinical isolates were sensitive to tetracycline, chloramphenicol and nalidixic acid. 4 (50%) and 30 (85.7%) of the food and clinical isolates respectively were MDR. 16 distinct amplicons ranged in size from 60 to 1100 bp were detected by RAPD-PCR formed 22 different RAPD profiles and 3 main genotypic clusters consisting of groups R1, R2 and R3 (Fig. 1). Genotypic categorization and serogroups of the isolates were demonstrated in Table 2. All *Sh. sonnei* isolated from food and stool samples were categorized together in a same cluster (Group R2) with a close genetic relatedness.

**Discussion**

Shigellosis, specially caused by MDR *Shigella* spp., is now considered as one of the main public health concerns. The prevalence of this pathogen is continually increasing specially in developing countries [20]. *Shigella* spp. lead to death in children under the age of 5 years old [12]. In addition to person-to-person transmission, *Shigella* spp. are transmitted via food as a foodborne pathogen to infect human [21]. At the present study, we found higher prevalence rates of MDR *Shigella* isolates with close genetic relatedness in food and stool samples indicating poor hygiene practices and concern to public health.

The overall prevalence of the *Shigella* spp. in food and stool samples were 7.7% and 4.84% respectively. The finding of current study is significantly high compared to previous reports. Bakhshi et al. found *Shigella* spp. in 1.32% of all stool samples, collected from patients with diarrhea in Iran, consisting of *Sh. sonnei* in 92% of all isolates [22]. *Sh. sonnei* have predominantly been isolated in developed countries [23]. We also found *Sh. sonnei* predominantly in 100 and 91.42% of all isolates from food and clinical samples respectively. These findings are comparable to studies conducted before. Ranjbar et al., Nikfar et al., Zamanlou et al. and Abbasi et al. also found *Sh. sonnei* as the predominant species among the food and clinical *Shigella* isolates in Iran [24–27] indicating a recent significant change in predominant *Shigella* spp. from *flexneri* to *sonnei*.

| Antimicrobial agent | Food samples | Stool samples |
|---------------------|--------------|---------------|
|                     | S. sonnei (n = 8) | S. sonnei (n = 32) | S. flexneri (n = 2) | S. boydii (n = 1) |
| Imipenem            | 1 (12.5)     | 11 (46.8)     | 1 (50)          | 1 (100)        |
| Ampicillin          | 3 (37.5)     | 32 (100)      | 2 (100)         | 1 (100)        |
| Tetracycline        | 5 (62.5)     | 0 (0)         | 0 (0)           | 0 (0)          |
| Amikacin            | 0 (0)        | 16 (50)       | 1 (50)          | 1 (100)        |
| Chloramphenicol     | 3 (37.5)     | 0 (0)         | 0 (0)           | 0 (0)          |
| Nalidixic Acid      | 1 (12.5)     | 0 (0)         | 0 (0)           | 0 (0)          |
| Azithromycin        | 3 (37.5)     | 21 (65.6)     | 1 (50)          | 1 (100)        |
Analysis of antimicrobial susceptibility in this study showed that the food isolates were highly resistant to tetracycline and the clinical isolates were strongly resistant to imipenem, amikacin and azithromycin. Higher susceptibility to tetracycline, chloramphenicol and nalidixic acid was also observed in clinical isolates. Shahin et al. isolated *Shigella* spp. from water samples strongly resistance to nalidixic acid and susceptible to chloramphenicol [28]. Marami et al. isolated MDR *Shigella* spp. with 75% resistance to tetracycline [16]. Aklilu et al. observed MDR *Shigella* isolates completely susceptible to amikacin. These differences in antimicrobial susceptibility patterns may be explained by different availability of antibiotics worldwide. We found different antibiotic resistance patterns between food and clinical isolates [29]. Mokhtari et al. also found significantly different antimicrobial susceptibility patterns of *Shigella* isolates obtained from food and clinical samples [13].

There are only few studies regarding the genetic relatedness among *Shigella* isolates obtained from food and clinical samples. We found a close genetic relatedness between the food and clinical isolates as they were clustered in a same group. Mokhtari et al. also demonstrated a clonal relationship amongst food and human stool *Shigella* isolates [13]. These findings indicate that foods are important vehicle of transmission of MDR *Shigella* to human causing acute intestinal and extraintestinal diseases.

**Conclusions**

We detected *Shigella* spp. in 4.84 and 7.7% of food and stool samples respectively. We observed *Sh. sonnei* as the predominant species in both food and stool samples. We observed food isolates resistant to tetracycline and the clinical isolates strongly resistant to imipenem, amikacin and azithromycin. All clinical isolates were susceptible to tetracycline, chloramphenicol and nalidixic acid. 50% and 85.7% of the food and clinical isolates respectively were MDR. We found a close genetic relatedness between the isolates from food and clinical samples. The present study revealed a high prevalence of MDR *Shigella* with close genetic relatedness from food and stool samples indicating poor hygiene practices. Our findings also indicate that MDR *Shigella* can be transmitted through foods and cause infectious disease in human. Performing other studies is of great importance to investigate genetic relatedness between MDR *Shigella* isolates from food and clinical samples.

**Limitations**

- 43 *Shigella* isolates from all food and clinical samples are not sufficient to evaluate the genetic relatedness and the antimicrobial susceptibility of the isolates.
- RAPD-PCR method is not adequately precise and reproducible for genotyping of *Shigella* isolates; other sequence based genotyping techniques have been recommended.
Abbreviations

HUS: Hemorrhagic Uremic Syndrome; DNA: DeoxyriboNucleic Acid; PCR: Polymerase Chain Reaction; BHI: Bovine Heart Infusion; RAPD: Random Amplification of Polymorphic DNA; MDR: MultiDrug Resistant; UPGMA: Unweighted Pair Group Method with Arithmetic mean; CLSI: Clinical and Laboratory Standards Institute; spp.: Species.

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Authors’ contributions

BP designed the research; BP, AD and YKM performed all experiments and analysis; BP and RM wrote the first draft of the manuscript; BP and AP and MRM finalized and reviewed the manuscript; All authors reviewed the draft and approved the manuscript.

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Availability of data and materials

All data used and analyzed at the present study are available from the corresponding author on a reasonable request.

Declarations

Ethics approval and consent to participate

The study protocol for clinical samples was evaluated, reviewed, and approved by the ethics committee of Qazvin University of Medical Science (QUMS.14003060). The written consent was taken from the parents of the patients in the local language.

Consent for publication

Not applicable.

Competing interests

The authors declare that they have no competing interest.

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Table 2 Genotypic properties and serogroup of Shigella spp. isolated from food and stool samples

| ID  | Species | Serogroup | Source | RAPD type |
|-----|---------|-----------|--------|-----------|
| SS1 | S. sonnei | D         | Food   | R2        |
| SS2 | S. sonnei | D         | Food   | R2        |
| SS3 | S. sonnei | D         | Food   | R2        |
| SS4 | S. sonnei | D         | Food   | R2        |
| SS5 | S. sonnei | D         | Food   | R2        |
| SS6 | S. sonnei | D         | Food   | R2        |
| SS7 | S. sonnei | D         | Food   | R2        |
| SS8 | S. sonnei | D         | Food   | R2        |
| SS9 | S. sonnei | D         | Stool  | R2        |
| SS10| S. sonnei| D         | Stool  | R2        |
| SS11| S. sonnei| D         | Stool  | R2        |
| SS12| S. sonnei| D         | Stool  | R2        |
| SS13| S. sonnei| D         | Stool  | R2        |
| SS14| S. sonnei| D         | Stool  | R2        |
| SS15| S. sonnei| D         | Stool  | R2        |
| SS16| S. sonnei| D         | Stool  | R2        |
| SS17| S. sonnei| D         | Stool  | R2        |
| SS18| S. sonnei| D         | Stool  | R2        |
| SS19| S. sonnei| D         | Stool  | R2        |
| SS20| S. sonnei| D         | Stool  | R2        |
| SS21| S. sonnei| D         | Stool  | R2        |
| SS22| S. sonnei| D         | Stool  | R2        |
| SS23| S. sonnei| D         | Stool  | R2        |
| SS24| S. sonnei| D         | Stool  | R2        |
| SS25| S. sonnei| D         | Stool  | R2        |
| SS26| S. sonnei| D         | Stool  | R2        |
| SS27| S. sonnei| D         | Stool  | R2        |
| SS28| S. sonnei| D         | Stool  | R2        |
| SS29| S. sonnei| D         | Stool  | R2        |
| SS30| S. sonnei| D         | Stool  | R2        |
| SS31| S. sonnei| D         | Stool  | R2        |
| SS32| S. sonnei| D         | Stool  | R2        |
| SS33| S. sonnei| D         | Stool  | R2        |
| SS34| S. sonnei| D         | Stool  | R2        |
| SS35| S. sonnei| D         | Stool  | R2        |
| SS36| S. sonnei| D         | Stool  | R2        |
| SS37| S. sonnei| D         | Stool  | R2        |
| SS38| S. sonnei| D         | Stool  | R2        |
| SS39| S. sonnei| D         | Stool  | R2        |
| SS40| S. sonnei| D         | Stool  | R2        |
| SF1 | S. flexneri | B         | Stool  | R1        |
| SF2 | S. flexneri | B         | Stool  | R1        |
| SB  | S. boydii  | C         | Stool  | R3        |
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