Prevalence and Pattern of Antimicrobial Resistance of *Shigella* species among Patients with Acute Diarrhoea in Karaj, Tehran, Iran

Koorosh MoezArdalan¹, Mohammad Reza Zali¹, Mohammad Mehdi Soltan Dallal², Mohsen Rezaei Hemami¹, and Siavosh Salmanzadeh-Ahrabi¹

¹National Research Department of Foodborne Diseases, Research Center of Gastroenterology and Liver Diseases, Shaheed Beheshti University of Medical Sciences and ²Department of Microbiology, School of Public Health, Tehran University of Medical Sciences, Tehran, Iran

**ABSTRACT**

The study was carried out to determine the prevalence and pattern of antimicrobial resistance of *Shigella* species among patients with acute diarrhoea in Karaj, Tehran, Iran. The study included all acute diarrhoea patients who visited the hospitals and treatment centres of Karaj during November 2001-October 2002. Of 734 stool samples collected from patients with acute diarrhoea and analyzed for *Shigella* spp., 123 (16.8%) yielded *Shigella* spp. (7.5% *S. flexneri*, 5.2% *S. sonnei*, 2.6% *S. dysenteriae*, and 1.5% *S. boydii*). Of the *Shigella* isolates, 90.8% were resistant to one or more antimicrobial agent(s), and 87.8% were multidrug resistant. The most common resistance was to tetracycline (73.5%), trimethoprim-sulphamethoxazole (70.4%), and amoxicillin-clavulanic acid (50.0%). Resistance to cefixime, ciprofloxacin, ceftriaxone, and nalidixic acid was observed in 6.1%, 3.1%, 2.0%, and 1.0% of the isolates respectively. These findings suggest that *Shigella* spp. may be an important aetiological agent of diarrhoea with a high rate of drug resistance in this region, which requires further study.

**Key words:** *Shigella*; Dysentery, Bacillary; Drug resistance, Microbial; Epidemiology; Diarrhoea, Acute; Iran

**INTRODUCTION**

In many developing countries, diarrheal diseases are one of the main causes of death, especially among younger children (1). In the developing world, diarrheal diseases kill at least five million children each year. Endemic bacillary dysentery accounts for about 10% of the disease in these areas with children aged less than five years at the greatest risk (2,3). Among the different pathogens responsible for diarrhoea, *Shigella* spp. play an important role in causing inflammatory diarrhoea and dysentery and can be considered a global health problem (3-7), causing significant morbidity and mortality in developing countries (8,9). Shigellosis, an acute diarrheal disease, is caused by Gram-negative rods, *Shigella*, belonging to the family Enterobacteriaceae. Supply of untreated water, poor sanitation, and overcrowding all contribute to the spread of shigellosis both by human contacts and supplies of contaminated water (10).

Over the past decades, *Shigella* spp. have become progressively resistant to the most widely-used and inexpensive antimicrobials (7,11-13). Moreover, changes in the incidence of *Shigella* spp. from time to time make it difficult to formulate a drug of choice for the treatment of shigellosis (14).

The present study is the first longitudinal survey based on the analysis of stool specimens from patients visiting medical facilities (hospitals, health...
administering network, and private clinics) in Karaj. Our objective was to determine the prevalence and pattern of antimicrobial resistance of *Shigella* spp. among patients with acute diarrhoea in Karaj, a city near Tehran, the capital city of Iran.

**MATERIALS AND METHODS**

**Study area and population**
Karaj, a city in northern Iran with an area of 830 sq km, on the Karaj River, at the southern foot of the Alburz Mountains, is located 48 km west of Tehran (or Teheran). Karaj, with a population of 1,379,360, has an increasing rate of development and population growth. Karaj city consists of six urban districts, and three rural areas with 93 villages, all considered to be summer resorts for the people of Tehran and Karaj. The Karaj dam meets a major part of the water requirements of Tehran.

**Data collection and stool sampling**
Sampling was performed during November 2001-October 2002, covering all the medical centres of Karaj, which are responsible for the treatment of diarrhoea patients and which are uniformly distributed in the city. The health service facilities comprised all seven medical centres of the health administration network, three main hospitals (Emam Khomeini, Bahonar, and Rajayee) of Karaj, and three private clinics. We were unable to cover offices of private physicians due to problems in transportation and coordination. All patients with acute diarrhoea who visited the above-mentioned centres were sampled during the one-year study period. A stool sample was obtained from each patient who had acute diarrhoea diagnosed by the physician of the medical centre.

**Bacteriological procedures**
Faecal samples from patients with acute diarrhoea were transported to the laboratory in the Cary-Blair transport medium and were processed within two hours. For optimal isolation, three different media and an enrichment medium were used. The samples were inoculated directly on MacConkey agar, xylose-lysine deoxycholate agar, and *Salmonella-Shigella* agar. Enrichment was done in Selenite F broth and incubated at 37 °C overnight. Sub-cultures were done on the media mentioned above and were further incubated at 37 °C overnight. Colonies morphologically resembling *Shigella* species were further identified by biochemical reactions according to the standard methods (15) and confirmed by slide agglutination test using commercially-available antisera from MAST Group Ltd. (MAST House, Derby Road, Bootle, Merseyside, L201EA, UK). These antisera were *Shigella dysenteriae* poly A, *S. dysenteriae* poly A1, *S. flexneri* poly B, *S. boydii* poly C, *S. boydii* poly C1, *S. boydii* poly C2, *S. boydii* poly C3, and *S. sonnei* poly D.

**Drug susceptibility test**
*Shigella* isolates were examined for their susceptibilities to ampicillin, amoxicillin-clavulanic acid, amikacin, chloramphenicol, cephalothin, gentamicin, kanamycin, nalidixic acid, co-trimoxazole, tetracycline, cefixime, ciprofloxacin, and ceftriaxone by the standard disc-diffusion method (16).

**Statistics**
The prevalence of *Shigella* spp. was calculated as the frequency of visits by the patients with acute diarrhoea who attended the urban and rural medical centres of Karaj. The frequency of diarrhoea is reported as mean±SD. Median and range are reported for the age of the patients. The differences in clinical symptoms, treatments used, and isolation frequencies of *Shigella* spp., among different age groups were determined by a two-tailed chi-square distribution analysis with Yates’ correction at the 95% level of significance. The SPSS/PC 11.0 software (SPSS Inc., Chicago, IL) was used for analysis of data.

**RESULTS**
In total, 734 stool samples were collected from patients with acute diarrhoea (median age: 3.0 years, range: two months-97 years). The stool samples were analyzed for *Shigella* spp. The age distribution of the patients was as follows: 204 (27.8%) were aged less than one year, 218 (29.7%) were aged one or more than one to less than five year(s), 75 (10.2%) were aged five or more than five to less than 12 years, and 237 (32.3%) were aged 12 or more than 12 years. The ratio of males to females was 1.21. Dysentery was observed in 63 (8.6%) patients. The age distribution of 123 patients (median age: 13.0 years, range two months-88 years), whose specimens yielded *Shigella* spp., was as follows: 19 (15.4%) were aged less than one year, 27 (22.0%) were aged one or more than one to less than five year(s), 75 (60.2%) were aged five or more than five to less than 12 years, and 237 (32.3%) were aged 12 or more than 12 years. The clinical symptoms and other characteristics of the 123 patients with *Shigella* spp. isolated from their stool specimens are shown in
The prevalence of *Shigella* spp. isolated from the patients and their age distribution are shown in Table 2. The species of the 123 (16.8%) *Shigella* isolates comprised

### Table 1. Clinical symptoms, other characteristics, and age distribution of 123 patients with *Shigella* infection presented at hospitals or clinics in Karaj, Tehran, Iran

<table>
<thead>
<tr>
<th>Clinical and other characteristics</th>
<th>&lt;1 year (n=19)</th>
<th>≥1-&lt;5 year(s) (n=27)</th>
<th>≥5-&lt;12 years (n=13)</th>
<th>≥12 years (n=64)</th>
<th>Total (n=123)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Diarrhoea Frequency*</td>
<td>6.9±0.9</td>
<td>4.9±0.7</td>
<td>5.0±2.4</td>
<td>6.4±3.2</td>
<td>6.0±2.6</td>
</tr>
<tr>
<td>Watery</td>
<td>11 (57.9)</td>
<td>16 (59.3)</td>
<td>7 (53.8)</td>
<td>42 (65.6)</td>
<td>76 (61.8)</td>
</tr>
<tr>
<td>Mucus</td>
<td>3 (15.8)</td>
<td>7 (25.9)</td>
<td>3 (23.1)</td>
<td>24 (37.5)</td>
<td>37 (30.1)</td>
</tr>
<tr>
<td>Blood</td>
<td>0</td>
<td>1 (3.7)</td>
<td>1 (7.7)</td>
<td>21 (32.8)</td>
<td>23 (18.7)</td>
</tr>
<tr>
<td>Vomiting</td>
<td>16 (84.2)</td>
<td>20 (74.1)</td>
<td>10 (76.9)</td>
<td>29 (45.3)</td>
<td>75 (61.0)</td>
</tr>
<tr>
<td>Anorexia</td>
<td>1 (5.3)</td>
<td>5 (18.5)</td>
<td>2 (15.4)</td>
<td>35 (54.7)</td>
<td>43 (35.0)</td>
</tr>
<tr>
<td>Lethargy</td>
<td>8 (42.1)</td>
<td>14 (51.9)</td>
<td>6 (46.2)</td>
<td>41 (64.1)</td>
<td>69 (56.1)</td>
</tr>
<tr>
<td>Abdominal pain</td>
<td>0</td>
<td>5 (18.5)</td>
<td>1 (7.7)</td>
<td>54 (84.4)</td>
<td>60 (48.8)</td>
</tr>
<tr>
<td>Temperature ≥38.5 °C</td>
<td>2 (10.5)</td>
<td>4 (14.8)</td>
<td>0</td>
<td>30 (46.9)</td>
<td>36 (29.3)</td>
</tr>
<tr>
<td>Therapy received</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Intravenous fluid</td>
<td>10 (52.6)</td>
<td>16 (59.3)</td>
<td>8 (61.5)</td>
<td>28 (43.8)</td>
<td>62 (50.4)</td>
</tr>
<tr>
<td>Oral rehydration solution</td>
<td>10 (52.6)</td>
<td>13 (48.1)</td>
<td>8 (61.5)</td>
<td>8 (12.5)</td>
<td>39 (31.7)</td>
</tr>
<tr>
<td>Antibiotics</td>
<td>0</td>
<td>0</td>
<td>0</td>
<td>10 (15.6)</td>
<td>10 (8.1)</td>
</tr>
</tbody>
</table>

* Mean±SD number of loose or watery stools per 24 hours

Figures in parentheses indicate percentages

lower (p<0.04) in patients whose specimens yielded *Shigella* spp. compared to the rest of the patients. Other characteristics and clinical symptoms did not show a significant difference between the above-mentioned groups. Among the patients with *Shigella*, fever, blood in stool, anorexia, and abdominal pain were significantly higher among those who were aged 12 or more than 12 years (p<0.001), while vomiting was more prevalent among those aged less than 12 years (p<0.001). The physicians prescribed oral rehydration therapy (ORT) to a significantly lower and antibiotics to a significantly higher number of patients aged 12 or more than 12 years (p<0.0001 and p<0.01 respectively).

### Table 2. Prevalence of *Shigella* spp. isolated and age distribution of patients presented at hospitals or clinics in Karaj, Tehran, Iran

<table>
<thead>
<tr>
<th>Pathogen</th>
<th>&lt;1 year (n=204)</th>
<th>≥1-&lt;5 year(s) (n=218)</th>
<th>≥5-&lt;12 years (n=75)</th>
<th>≥12 years (n=237)</th>
<th>Total (n=734)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Shigella</em> spp.</td>
<td>19 (9.3)</td>
<td>27 (12.4)</td>
<td>13 (17.3)</td>
<td>64 (27.0)</td>
<td>123 (16.8)</td>
</tr>
<tr>
<td><em>S. flexneri</em></td>
<td>5 (2.5)</td>
<td>15 (6.9)</td>
<td>6 (8.0)</td>
<td>29 (12.2)</td>
<td>55 (7.5)</td>
</tr>
<tr>
<td><em>S. sonnei</em></td>
<td>7 (3.4)</td>
<td>8 (3.7)</td>
<td>6 (8.0)</td>
<td>17 (7.2)</td>
<td>38 (5.2)</td>
</tr>
<tr>
<td><em>S. dysenteriae</em></td>
<td>2 (1.0)</td>
<td>3 (1.4)</td>
<td>0</td>
<td>14 (5.9)</td>
<td>19 (2.6)</td>
</tr>
<tr>
<td><em>S. boydii</em></td>
<td>5 (2.5)</td>
<td>1 (0.5)</td>
<td>1 (1.3)</td>
<td>4 (1.7)</td>
<td>11 (1.5)</td>
</tr>
</tbody>
</table>

Figures in parentheses indicate percentages

55 (7.5%) *S. flexneri*, the most common isolate, 38 (5.2%) *S. sonnei*, 19 (2.6%) *S. dysenteriae*, and 11 (1.5%) *S. boydii*. The frequency of isolation of *Shigella* spp. from patients aged 12 or more than 12 years was significantly higher than from the lower age groups (p<0.0001). Of the *Shigella* spp., *S. flexneri* and *S. dysenteriae* occurred at a higher frequency in those who were aged 12 or more than 12 years (29 and 14 strains respectively) (p<0.01 and p<0.001 respectively).

The number of specimens received and the number of *Shigella* spp. strains isolated in each month are shown in the figure. The figure shows that the highest number of *Shigella* spp. was isolated in June, September, and October, and the least was observed in March, April,
Epidemiology of Shigella in Iran

November, and December. The isolation rate of Shigella spp. in spring, summer, fall, and winter was 23.4%, 24.2%, 17.6%, and 18.4% respectively. However, more data in the subsequent years are needed to elucidate the seasonality of shigellosis in this region.

We studied the prevalence of Shigella spp. in a distinctive area of the central region of Iran, with water supply and sanitary conditions similar to Tehran. The specimens were obtained from patients whose disease was severe enough to bring them to a medical facility. Although our sample may not reflect the genuine spectrum of Shigella spp. in the community, this survey is still significant because, to our knowledge, this is among the first longitudinal studies to define the prevalence and antimicrobial resistance of Shigella in Iran.

The drug susceptibility patterns of 98 isolates of Shigella spp. were determined (Table 3). Of the Shigella isolates, 90.8% were resistant to one or more agent(s), and 87.8% were multidrug-resistant. The most common resistance among Shigella spp. was to tetracycline (73.5%), trimethoprim-sulphamethoxazole (70.4%), and amoxicillin-clavulanic acid (50.0%). S. flexneri isolates were most frequently resistant to tetracycline (82.2%), amoxicillin-clavulanic acid (82.2%), and trimethoprim-sulphamethoxazole (77.7%). S. sonnei isolates were most frequently resistant to tetracycline (86.2%), trimethoprim-sulphamethoxazole (79.3%), and cephalothin (58.6%). Resistance to cefixime, ciprofloxacin, ceftriaxone, and nalidixic acid was observed in 6.1%, 3.1%, 2.0%, and 1.0% of the isolates respectively.

**DISCUSSION**

Like many other developing countries, diarrhoeal diseases are among the main health problems in Iran (1,6,17), and endemic bacillary dysentery accounts for about 10% of the disease incidence in the study areas (2,3). Our study focused on Shigella because Shigella spp. plays a significant role in the disease burden of many developing countries (5,7,18-20). We studied the prevalence of Shigella spp. in a distinctive area of the central region of Iran, with water supply and sanitary conditions similar to Tehran. The specimens were obtained from patients whose disease was severe enough...
diarrhoea patients and represents 50-70% of all *Shigella* isolates (29). Our findings show remarkable agreement with those of studies in Iran and other developing countries (18-22) and are in contrast with studies in developed countries where *S. sonnei* is dominant and *S. flexneri* is the second most prevalent isolate (27,28).

However, one report from central and western regions of Iran indicated a higher frequency for *S. sonnei* (42.4%) among *Shigella* spp. isolated (7).

The average age of the patients with *Shigella* infection in our study was lower compared to some reports from the United States, indicating a rise in the average age of *Shigella* infection to 24 years (30). However, the group of patients with the highest frequency of isolation of *Shigella* spp. in our study were older (aged 12 or more than 12 years) compared to some reports from other developing countries, where the group of one or more than one to less than five years of age had the highest frequency of isolation (18,31,32).

There are some differences in the approximate prevalence of the signs and symptoms in this study compared to other studies on *Shigella* spp. (33-36). In our study, the patients had a higher prevalence of vomiting and watery diarrhoea and a lower prevalence of mucus and blood in stools. This might be due to the relatively greater number of younger children in our study and their different clinical presentation, which comprises a shorter duration of illness with watery diarrhoea and more vomiting (33). This different clinical presentation in children is consistent with the finding of one report from central and western regions (33).

Table 3: Drug susceptibility patterns of *Shigella* spp.* isolated from patients presenting at hospitals or clinics in Karaj, Iran

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th><em>S. flexneri</em> R</th>
<th><em>S. sonnei</em> R</th>
<th><em>S. boydii</em> R</th>
<th><em>S. dysenteriae</em> R</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>R  I  S</td>
<td>R  I  S</td>
<td>R  I  S</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>24</td>
<td>18</td>
<td>3</td>
<td>6</td>
</tr>
<tr>
<td>Amoxicillin-clavulanic acid</td>
<td>37</td>
<td>5</td>
<td>3</td>
<td>5</td>
</tr>
<tr>
<td>Amikacin</td>
<td>8</td>
<td>8</td>
<td>29</td>
<td>4</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>26</td>
<td>7</td>
<td>12</td>
<td>1</td>
</tr>
<tr>
<td>Cephalothin</td>
<td>24</td>
<td>7</td>
<td>14</td>
<td>17</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0</td>
<td>13</td>
<td>32</td>
<td>1</td>
</tr>
<tr>
<td>Kanamycin</td>
<td>7</td>
<td>17</td>
<td>21</td>
<td>5</td>
</tr>
<tr>
<td>Nalidixic acid</td>
<td>1</td>
<td>7</td>
<td>37</td>
<td>0</td>
</tr>
<tr>
<td>Co-trimoxazole</td>
<td>35</td>
<td>1</td>
<td>9</td>
<td>23</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>37</td>
<td>5</td>
<td>3</td>
<td>25</td>
</tr>
<tr>
<td>Cefixime</td>
<td>4</td>
<td>4</td>
<td>37</td>
<td>2</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>2</td>
<td>3</td>
<td>40</td>
<td>1</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>2</td>
<td>4</td>
<td>39</td>
<td>0</td>
</tr>
</tbody>
</table>

* In total, 98 *Shigella* strains were tested
† Zone diameters in millimeters: ampicillin (R: ≤13, I: 14-16, S: ≥17), amoxicillin-clavulanic acid (R: ≤13, I: 14-17, S: ≥18), amikacin (R: ≤14, I: 15-16, S: ≥17), chloramphenicol (R: ≤12, I: 13-17, S: ≥18), cephalothin (R: ≤14, I: 15-17, S: ≥18), gentamicin (R: ≤12, I: 13-14, S: ≥15), kanamycin (R: ≤13, I: 14-17, S: ≥18), nalidixic acid (R: ≤13, I: 14-18, S: ≥19), co-trimoxazole (R: ≤10, I: 11-15, S: ≥16), tetracycline (R: ≤14, I: 15-18, S: ≥19), cefixime (R: ≤15, I: 16-18, S: ≥19), ciprofloxacin (R: ≤15, I: 16-20, S: ≥21), and ceftriaxone (R: ≤13, I: 14-20, S: ≥21)
R=Resistant; I=Intermediate; S=Susceptible

of Iran indicated a higher frequency for *S. sonnei* among *Shigella* spp. isolated (7).

The average age of the patients with *Shigella* infection in our study was lower compared to some reports from the United States, indicating a rise in the average age of *Shigella* infection to 24 years (30). However, the group of patients with the highest frequency of isolation of *Shigella* spp. in our study were older (aged 12 or more than 12 years) compared to some reports from other developing countries, where the group of one or more than one to less than five years of age had the highest frequency of isolation (18,31,32).

There are some differences in the approximate prevalence of the signs and symptoms in this study compared to other studies on *Shigella* spp. (33-36). In our study, the patients had a higher prevalence of vomiting and watery diarrhoea and a lower prevalence of mucus and blood in stools. This might be due to the relatively greater number of younger children in our study and their different clinical presentation, which comprises a shorter duration of illness with watery diarrhoea and more vomiting (33). This different clinical presentation in children is consistent with the finding of one report from central and western regions (33).
The results of the antimicrobial susceptibility tests showing a relatively higher number of multidrug-resistant isolates compared well with previous reports from Iran (7,21), and especially the emergence of resistance to fluoroquinolones and third-generation cephalosporins indicates that designing a surveillance system for antimicrobial resistance in Iran and the introduction of integrated guidelines for the appropriate use of antibiotics are urgently needed. According to the susceptibility of the majority of Shigella spp. to cefixime, ciprofloxacin, ceftriaxone, and nalidixic acid in this study, we recommend the more-readily available drug, nalidixic acid, as the drug of choice for shigellosis in both adults and children.

Based on the findings of our study, we conclude that Shigella spp. can be considered an important aetiological agent of diarrhoea, having a high rate of drug resistance in this region. The information about the prevalence of a diarrhoeal pathogen should facilitate the control of the disease in the country. Continued vigilance of the safety of food, health education of food handlers, and close attention to hygiene and sanitary conditions can provide an effective barrier against the spread of shigellosis.

ACKNOWLEDGEMENTS

The budget of this granted research project (No. EPS/00/114), including equipment and personnel, was provided by the Research Center of Gastroenterology and Liver Diseases, Shaheed Beheshti University of Medical Sciences.

We would like to thank the research team of NRDFD (National Research Department of Foodborne Diseases): Haleh Edalatkhah, Sanaz MoezAradal, Fereshteh Jaafari, Koorosh Zolfagharian, Ali Moghaddam Gol Mohammadi, Masoume azimi rad, Effat Habibi, Dr. Bita Nikkhohligh, Soudabeh Taheri, Dr. Hayede Golesorkhi, and Dr. Kambiz Parchamazad, for their sincere help in performing technical parts of the project.

We also thank Dr. Ali Gouya, Director of the Center for Disease Control of Iran, Dr. Ali Ghandi, Deputy of Health, Iran University of Medical Sciences, Dr GholamAli Mohajerin, Dr Arash Nokar, and Dr. Masoud Khoddami Nazar, and all the staff of the health administration network of Karaj, without whose help this work was not possible.

REFERENCES


