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Burden of Food-Related Illness Caused by Resistant *Salmonella* spp. and *Shigella* spp.: Harbingers of Multistate Outbreaks in 2012 and 2013

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Abstract

Background: In many countries, *Salmonella* and *Shigella* species are frequently found to cause gastroenteritis outbreaks.
Objectives: We describe nationwide data on infections with *Salmonella* spp. and *Shigella* spp. in Iran.
Materials and methods: During a two-year period (2012 to 2013), rectal-swab samples were analyzed for the presence of bacteria. Sensitivity of the bacterial isolates to antimicrobial agents was tested according to clinical and laboratory standards institute (CLSI) guidelines.
Results: Twenty-nine states reported 249 outbreaks of foodborne illnesses. In total, 1055 patients (604 males and 451 females, age range: < 1 and > 60 years) were enrolled in this study, of whom 18 died. Seventy-four culture-confirmed cases of infection with *Salmonella* spp. were identified, of which 10.8%, 6.8%, 68.9%, and 13.5% corresponded to *Salmonella* serotype A, B, C, or D respectively. Similarly, *Shigella* spp. were responsible for 118 cases of the foodborne illnesses; among them, *Shigella sonnei* (with 105 cases, 89%) was the leading serovar. Ciprofloxacin (100%) was the most effective antibacterial agent against *Salmonella* spp. followed by amikacin. Nalidixic acid and gentamycin were the least effective antibacterial agents against *Salmonella* spp. Similarly, *Shigella* spp. were also highly sensitive to ciprofloxacin (100%), whereas tetracycline and ampicillin were the least effective antibacterial agents against *Shigella* spp.
Conclusions: These are the first recognized and confirmed outbreaks of foodborne illnesses in Iran. *Salmonella* and *Shigella* infections represent a considerable disease burden in our country. Therefore, efforts to reduce transmission of these pathogens via food and other routes must be implemented on a national scale. It is noteworthy that the outbreaks of *Shigella* and *Salmonella* infections in our country also pose a threat of antibiotic resistance.

Keywords: Outbreak, Antibiotic Resistance, *Shigella*, *Salmonella*

1. Background

In recent years, widely distributed outbreaks of foodborne illnesses have been recognized as an emerging public-health problem worldwide (1-3). Iran is no exception and an increasing number of outbreaks of foodborne infections have been reported (4-6): these outbreaks provide a unique opportunity to learn more about foodborne illnesses. In Iran, the data on the incidence of foodborne-illness outbreaks have been collected by the Center for Disease Control since 2006.

Foodborne illnesses, which result from food contaminated with infectious or toxigenic microorganisms, are a major cause of morbidity and a significant cause of mortality (7-9). Although viruses account for the half of all foodborne illnesses, most hospitalizations and deaths related to foodborne infections are due to bacteria (10, 11). Among bacterial infections, diseases caused by *Shigella* and *Salmonella* species continue to be important causes of diarrhea (12, 13).

2. Objectives

There are insufficient national data on foodborne-illness outbreaks in Iran; accordingly, we describe large nationwide sampling and analysis of outbreaks of infections with *Salmonella* spp. or *Shigella* spp.

3. Materials and Methods

3.1. Outbreak Investigations in Iran

During a two-year period (2012 to 2013), after state and local health departments reported outbreaks to the National Institute of Health (NIH) of Iran, rectal-swab sam-

ples were either transported to the referral laboratory of the NIH immediately or placed into a transport medium and analyzed for the presence of bacteria. Briefly, for the isolation of *Salmonella* and *Shigella*, the swabs were inoculated into Selenite F, incubated overnight, and then streaked on Hektoen and xylose lysine deoxycholate (XLD) agar and incubated overnight at 37°C. Then, the bacteria were identified according to standard biochemical tests (14), and finally, these microbes were confirmed to be *Salmonella* and *Shigella* by the API-20E assay.

3.2. Antibiotic Sensitivity Test

Sensitivity to antimicrobial agents was analyzed according to Clinical and Laboratory Standards Institute (CLSI) guidelines (15). The antibiotic disks (Mast Antibiotic Disc; UK) that we used contained gentamicin (gen) 10 μg/mL, tetracycline (tet) 30 μg/mL, ciprofloxacin (cip) 2.5 μg/mL, ampicillin (amp) 25 μg/mL, nalidixic acid (nal) 30 μg/mL, cefotaxime (cefo) 5 μg/mL, cephalexin (ceph) 5 μg/mL, ceftriaxone (cfx) 5 μg/mL, or amikacin (amk) 30 μg/mL.

4. Results

During the study period, 29 states reported 249 outbreaks of foodborne illnesses. In total, 1055 patients (604 males and 451 females, age range: < 1 and > 60 years) were enrolled in this study, of whom 18 died (Table 1). The highest incidence of foodborne illnesses was seen in the age group 7 - 12 years (Table 2).

Seventy-four (18 in 2012 and 56 in 2013) culture-confirmed cases of *Salmonella* spp. infections were identified, of which 10.8%, 6.8%, 68.9%, and 13.5% represented *Salmonella* serotype A, B, C, and D, respectively (Table 3). Similarly, *Shigella* spp. were responsible for 118 cases of foodborne illnesses (22 in 2012 and 96 in 2013); among them, *Shigella sonnei* (with 105 cases, 89%) was the leading serovar.

Ciprofloxacin (100%) was the most effective antibacterial agent against *Salmonella* spp. followed by amikacin. Nalidixic acid and gentamycin were the least effective antibacterial agents against *Salmonella* spp. Similarly, *Shigella* spp. were highly sensitive to ciprofloxacin (100%), whereas tetracycline and ampicillin were the least effective antibacterial agents against *Shigella* spp. (Table 4).

Table 1. The Percentage of People With Foodborne Illnesses by Gender and Age

	Year 2012	Year 2013	Total
Gender [a]			
Male	186 (61.8)	418 (55.2)	604 (57.25)
Female	115 (38.2)	336 (44.5)	451 (42.74)
Results of statistics test			
Chi-square	111.684	214.329	22.189
df	5	1	1
P Value	< 0.001	< 0.001	< 0.00

[a]Values are presented as No. (%).

Table 2. The Percentage of People of All Ages, and the Incidence of Foodborne Illnesses During the Study Period[a]

Age Groups, y	No. of Patients, %		
	2012	2013	Total
< 1	7 (02.32)	7 (0.92)	14 (01.32)
1 - 7	17 (05.64)	46 (06.10)	63 (05.97)
7 - 12	68 (22.59)	168 (21.22)	228 (21.61)
12 - 18	62 (20.59)	155 (20.55)	217 (20.56)
18 - 25	60 (19.93)	145 (19.23)	205 (19.43)
25 - 35	30 (09.96)	92 (12.20)	122 (11.56)
35 - 45	23 (07.64)	65 (08.62)	88 (08.34)
45 - 55	17(05.64)	53 (07.02)	70 (06.63)
55 - 65	7 (02.32)	23 (03.05)	30 (02.84)
> 65	8 (02.65)	10 (01.32)	18 (01.70)
Total	301 (100)	754 (100)	1055 (100)

[a]The results of the chi-square test showed that there was a significant correlation between incidence of foodborne illnesses and different age groups in both years (P < 0.0001).

Table 3. The Number and Proportion of Cases of *Salmonella* and-*Shigella* spp. Infections During the Foodborne-Illness Outbreaks in Iran in 2012 and 2013[a]

Bacteria	2012	2013	Total
***Salmonella* serogroup A**	2.0 (0.7)	6.0 (0.8)	8.0 (0.8)
***Salmonella* serogroup B**	0.0 (0.0)	5.0 (0.7)	5.0 (0.5)
***Salmonella* serogroup C**	9.0 (3.0)	42 (5.6)	51 (4.8)
***Salmonella* serogroup D**	7.0 (2.3)	3.0 (0.4)	10 (0.9)
Shigella sonnei	16.0 (5.3)	89 (11.8)	105 (9.9)
Shigella flexneri	6.0 (2.0)	7.0 (0.9)	13 (1.2)
Shigella dysentery	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)
Shigella boydii	0.0 (0.0)	0.0 (0.0)	0.0 (0.0)

[a]Values are presented as No. (%).

Table 4. The Antibiotic Resistance Pattern of the Isolates of *Salmonella* spp. and *Shigella* spp. From Outbreaks of Foodborne Illnesses in Iran in 2012 and 2013[a]

Antibiotic	*Salmonella* spp.	*Shigella* spp.
Amikacin	08 (10.8)	44 (37.3)
Ampicillin	17 (23.0)	53 (44.9)
Ceftriaxone	18 (24.3)	11 (9.3)
Cefotaxime	28 (37.8)	16 (13.6)
Tetracycline	43 (58.1)	100 (84.7)
Cephalexin	55 (74.3)	22 (18.6)
Gentamycin	61 (82.4)	28 (23.7)
Nalidixic acid	65 (87.8)	36 (30.5)
Ciprofloxacin	74 (0.0)	118 (0.0)

[a]Values are presented as No. (%).

5. Discussion

Despite advances in food safety, foodborne illnesses remain common worldwide (8, 16, 17). Major pathogens causing foodborne illnesses are *Salmonella* spp. and *Shigella* spp.; these bacteria can rapidly disseminate via food. Therefore, improving food safety and reducing the burden of the infections caused by these pathogens means promoting and implementing effective food safety interventions on a national scale.

We estimated the national burden of *Salmonella* and *Shigella* gastroenteritis outbreaks in Iran by analyzing the data from all 29 states. For most regions, there have been no population-based or multicenter studies. In this study, we estimated the disease burden of laboratory-confirmed *Salmonella* and *Shigella* infections as well as their antibiotic resistance patterns. Thus, we isolated and identified 74 and 118 culture-confirmed cases of infection with *Salmonella* spp. or *Shigella* spp., respectively. These estimates can be used to determine the economic burden imposed by these pathogens.

Outbreaks due to *Salmonella serovars* are reported worldwide. For example, *Salmonella typhimurium* and *Salmonella Virchow* have been the leading pathogens and have played a major role in large outbreaks (18, 19). Although in our study, in all states, *Salmonella* serogroup C ranked as the most common serogroup, other studies suggest that *Salmonella enterica* serovar Virchow rarely causes gastrointestinal infections, accounting for only 0.1% - 0.5% in Italy and 0.8% in the European Union among all *Salmonella* serovars isolated from human cases of *Salmonella* infection (18, 20).

Similarly, among *Shigella* serovars, *Shigella sonnei* was the leading serovar (with 105 cases, 89%). The same dominant *Shigella* species was observed not only in our study but also in Australia and in the United States (2, 21); therefore, this serotype poses a serious threat to public health in Iran.

In recent years, multidrug-resistant foodborne pathogens like *Salmonella* spp. and *Shigella* spp. have emerged in various countries, and the prevalence of the respective infections has increased (22, 23).

By analyzing the drug resistance data, we found that *Salmonella* spp. were completely resistant to nalidixic acid and gentamycin during the 2012 and 2013 outbreaks in Iran; these findings are consistent with the data from other studies in Iran and neighboring countries (24-26). A study by Ashtiani et al. (27) out of Iran showed that the resistance rate to nalidixic acid was 42.3%. Similarly, among *Shigella* isolates, a high frequency of resistance to tetracycline (84.7%) and ampicillin (44.9%) was reported; these results are in consistent to another study out of Iran (28).

Ours seems to be the first report of recognized and confirmed outbreaks of foodborne illnesses in Iran. These outbreaks during 2012 and 2013 represent infective and toxigenic types of foodborne illnesses caused by *Salmonella* spp. and *Shigella* spp.

It is noteworthy that although outbreaks of infections with *Shigella* and *Salmonella* spp. have attracted considerable attention, the principal purpose of this report is to increase awareness of the threat posed by antibiotic resistance and to encourage urgent actions to address this threat.

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Footnotes

Authors' Contribution: Enayatollah Kalantar: writing of the manuscript draft; Mohamad Mehdi Soltan Dallal: isolation, identification, and serotyping of bacteria and help with the first draft of the manuscript; Samaneh Motalebi: help with writing of the manuscript; Hosein Masoomi Asl: management of transportation of the isolates; Abbas Rahimi Forushani: data analysis.

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