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Antimicrobial investigation on the multi-state outbreak of salmonellosis and shigellosis in Iran

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Abstract

Background: Foodborne diseases are caused by indigestion of contaminated food. In some cases they may result in either hospitalization or death. The Centers for Disease Control (CDC) and Prevention in 2017 stated that 10% reduction in foodborne illness would prevent nearly five million illnesses every year. Approximately one out of six Americans become ill from contaminated foods or beverages every year. Another problem is drug resistance which is responsible for approximately 2 million illnesses and around 23000 dead every year. Nearly 400,000 Americans acquire antibiotic-resistant *Salmonella* or *Campylobacter* each year. The aim of this study was to evaluate the outbreak of salmonellosis and shigellosis along with their antibiotic susceptibility patterns in different provinces of Iran.

Methods: Over a period of 2 years from 2015 to 2016, a total of 1055 cases in 249 outbreaks reported in 20 provinces of Iran, as a part of surveillance by the National Institute of Health (NIH). The stool samples of patients were taken and tested for *Salmonella spp.* and *Shigella spp.* by conventional standard techniques. Disk diffusion was used for the antibiotic sensitivity test.

Results: Of 1055 cases, 118 (11.2%) contained *Shigella* and 74 (7%) contained *Salmonella*. Antibiotic susceptibility tests showed that entirely 100% of *Salmonella* and *Shigella* isolates were susceptible to ciprofloxacin; whereas 12.2% of *Salmonella* and 98.2% of *Shigella* were resistant to cotrimoxazole.

Conclusion: Our results show that there is a need for more food handling practices to minimize the exposure of consumers to *Salmonella* and *Shigella*, at all points along the distribution chain.

Keywords: Foodborne, Outbreaks, Salmonellosis, Shigellosis

Conflicts of Interest: None declared
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Introduction

Foodborne illness outbreaks are defined when two or more people get same illness from same contaminated food. Foodborne illnesses are important public health concerns despite the fact that all effort has been made to prevent it (1, 2). Foodborne diseases are caused by the indigestion of contaminated food. In some cases they may result in either hospitalization or death. Nearly 250 etiologic agents of foodborne illnesses had been recognized so

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↑What is "already known" in this topic:
Salmonella spp. and *Shigella spp.* are the two most important bacteria causing foodborne diseases. Therefore special attention should be paid to these bacteria in any foodborne outbreak.

→What this article adds:
The finding of this research could be used for the epidemiological purpose in Iran.

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far (3). Most people infected with *Salmonella* develop symptoms such as diarrhea, fever, and abdominal cramps between 12 to 72 hours after digestion of contaminated food. The illness is self-limited and recovery takes between 4 to 7 days. But in some cases, diarrhea may be severe and needs hospitalization (4). Out of approximately 5.2 million cases of bacterial diarrhea that occur each year in the United States, 80% is related to foodborne illnesses (5). It is estimated that bacterial enteropathogens are responsible for 76 million illnesses, including 325,000 hospitalizations, and 5,000 deaths in the United States each year (6). The four most commonly reported bacterial enteropathogens in the United States are *Campylobacter*; non-typhoid *Salmonella*, Shiga toxin-producing *E. coli*, and *Shigella* (7). According to data from Centers for Disease Control and Prevention, infections with the following bacterial pathogens were detected in descending order of rates per 100,000 people in the United States in 2012: Salmonella, 16.4 cases; *Campylobacter*, 14.3 cases; Shiga toxin-producing *Escherichia coli* O157:H7 strain, 1.1 cases; *Vibrio*, 0.4 cases; and *Yersinia*, 0.3 cases. The rate of *Shigella* infection in the United States was 2.3 cases per 100,000 in 2011 (8). Salmonellosis outbreaks can occur in a wide range of foods such as fresh vegetables, fruits, meats, puffed cereal snacks, pre-packaged pot pies, and peanut (9).

Shigellosis is usually transmitted from person to person, but in some cases, the main cause of the disease is contaminated food such as water, milk, vegetables, and ice cream (10, 11). Signs and symptoms of food-borne diseases are abdominal cramps, nausea, vomiting, diarrhea, fever, and headache, depending on the severity of the disease. Symptoms usually appear between 24 to 48 hours, and stay for one or two days (11). Food-borne diseases can be dangerous in children, neonatal pregnant women, their fetuses, and persons with weakened immune systems (12, 13). A study carried out by Masoumi Asl et al. in Iran indicated that the outbreak rate has increased from 0.07/100000 to 1.38/100000 population during 2006 and rates of outbreaks in three provinces of Khuzestan, Kermanshah and Qazvin were more than expected (10). The use of antimicrobial agents in any environment might result in the survival of antibiotic-resistant pathogens (14). The use of antimicrobial agents to domestic livestock for the growth, prevention and treatment of diseases is an important factor in the emergence of antibiotic-resistant pathogens that subsequently transfer to humans through the food chain (15, 16). Nerveless most of antimicrobial-resistant *Salmonella* are acquired by eating contaminated foods of animal origin (17, 18). This research was conducted to evaluate the surveillance of the multi-state outbreak of salmonellosis and shigellosis in Iran.

Methods

Laboratory Investigation

During the 2-year period from 2015 to 2016, a total of 1055 cases in 249 outbreaks reported in twenty providences of Iran, as a part of surveillance conducted by the Ministry of Health and Medical Education in Iran (10).

The outbreak samples were collected from health centers during the outbreaks and were sent to us, as we were the collaborator laboratory of the Ministry of Health and Medical Education.

Stool samples of patients with the following symptoms, diarrhea, fever, abdominal pain, vomiting, and nausea were collected from different providences of Iran and tested in the laboratory of the department of public health for isolation and identification of *Salmonella* and *Shigella* by means of standard techniques and API 20E kit. For isolation of *Salmonella*, samples were inoculated in Selenite-F broth at 37 °C for 12 to18 hours and for *Shigella* directly were streaked onto Hektoen enteric agar (Merck, Hamburg, Germany)-and incubated at 37 °C for 24 hours. The suspicious gram-negative and oxidase-negative colonies were selected for identification using conventional biochemical tests, such as TSI, SIM, ODC, LIA, Simmons Citrate and Urea. The phenotypic biochemical profiles of isolates were compared with WHO criteria for *Salmonella* and *Shigella* (12).

Susceptibility Testing

Disc diffusion kit manufactured by MAST Company (Bootle, Merseyside, UK) was used for the susceptibility test according to guidelines by Clinical and Laboratory Standards Institute (19). A colony of the bacteria was inoculated in a test tube containing broth media. Broth culture was incubated at 35 °C until it reached 0.5 McFarland turbidity. Broth was cultured on Mueller-Hilton agar plates (Merck) and antimicrobial disks were placed on the plates and incubated at 37 °C for 16-18 hours. Antimicrobial disks were used including: ampicillin (10 mcg), amikacin (30 cg), chloramphenicol (30 mcg), cefotaxim (30 mcg), gentamicin (10 mcg), nalidixic acid (30 mcg), cotrimoxazole (10 mcg), tetracycline (30 mcg), cephalexin (30 mcg), ciprofloxacin (5 mcg), imipenem (10 mcg), and ceftriaxon (30 mcg), cefixim (30 mcg), and clindamycin (30 mcg).

Ethics

This research was approved by the Ethics Committee of Tehran University of Medical Sciences (TUMS) (project number: 91-04-172-20345).

Statistical Analysis

Statistical analysis of results was performed using SPSS/PC 11.5 software (SPSS, Chicago, IL). Chi-square and Fisher's exact two-tailed tests were used. A P-value less than 0.05 was considered as statistically significant.

Results

Among the 1055 persons infected in 20 provinces, 249 (23.6%) were hospitalized and 18 (1.71%) were died. The percentage of *Shigella* and *Salmonella* isolates was 118(11.2%) and 74(7%) respectively. The results of isolation of *Salmonella spp.* and *Shigella spp.* are shown in Table 1. Sources of contamination were food (36.1%), water (9.7%), and uncertain sources (54.2%). The demographic results (sex, age, job, and seasonal) are shown in Table 2.

Salmonellosis occurred mostly in spring (67.6%) and

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Table 1. Percent frequency of *Salmonella* and *Shigella* species isolated from outbreaks

Bacteria	Frequency	%
Salmonella serogroup A	8	10.8
Salmonella serogroup B	5	6.8
Salmonella serogroup C	51	68.9
Salmonella serogroup D	10	13.5
Shigella sonnei	105	89.0
Shigella flexneri	13	11.0
Shigella dysenteriae	0	0.0
Shigella boydii	0	0.0

Table 2. Demographic distribution in outbreaks of *Salmonella* and *Shigella*

Demographic information	Level	%
Sex	Male	61.5
	Female	38.5
Age	< 1	1.6
	1-7	6.2
	7-25	56.8
	25-65	29.2
	> 65	6.2
Season	Spring	24.0
	Summer	42.2
	Autumn	21.8
	Winter	12.0
Location	Urban	78.1
	Rural	21.9

Table 3. Results of analyzed outbreak of *Salmonella* (n=74) and *Shigella* (n=118) antimicrobial susceptibility testing, Iran

Antibiotic	*Salmonella* No.	%	*Shigella* No.	%
Ciprofloxacin	74	100	118	100
Imipenem	70	94.6	-	-
Amikacin	66	89.2	74	62.7
Cloramphenicol	64	86.5	-	-
Cotrimoxazol	59	79.7	-	-
Ampicilin	56	75.7	65	55.1
Ceftriaxon	56	75.7	107	90.7
Cefotaxim	46	62.2	100	84.7
Tetracyclin	31	41.9	18	15.3
Cephalexin	19	25.7	96	81.4
Gentamaycin	13	17.6	90	76.3
Nalidixic Acid	9	12.2	82	69.5
Cefixim	-	-	112	94.9
Clindamycin	-	-	19	16.1

Shigellosis in summer (50.8%). Male patients were dominated (57.3%). Statistical analysis of K2 (chi-square) test indicates that the relation between risk of outbreak by water, food, season in which outbreak occurred, and the gender are significant ($p<0.001$). Most salmonellosis (70.3%) occurred in Hamadan and shigellosis (54.2%) occurred in Yazd province respectively. The outbreaks were happened in schools, restaurants, workplaces, domestic houses, dormitories, barracks, nursing houses, nurseries. All strains of *Salmonella* and *Shigella* were susceptible to ciprofloxacin, whereas 88% of *Samonella* and 30.5% of *Shigella* were resistant to nalidixic acid. The antibiotic resistance results are shown in Table 3.

Discussion

There are many reasons for the spread of food-borne diseases including increased number of travels and tourism, eating outside home, and change of eating habits (4, 20). *Salmonella* is a common cause of foodborne disease and is responsible for nearly 18% of outbreaks (21). Previous investigators have demonstrated that *Salmonella* was the cause of about 50% of food-borne diseases, followed by pathogenic *Escherichia coli* (33%), *Shigella* (17%) (22), and some other isolates of *Salmonella* (2.9%) (23). Variety of foods such as peanut butter, leafy vegetables, sprouts, grains/beans, fruits/nuts, fungi, dairy, eggs, fish, pork, beef and chicken could be contaminated with *Salmonellas* and cause salmonellosis (9, 11, 24, 25). In this study, the percentage of *Salmonella* isolate was 11%. Among the four isolated serotypes of *Salmonella*, serotype C (68.9%) was the most common and serotype B (6.8%) the least common serotypes. The highest incidence of outbreaks was observed during spring (67.6%) and the lowest number in winter; this might be due to the higher consumption of fruits and vegetables in winter. Between the sources of contamination, 36.1% of *Salmonella* was isolated from foods, which is alarming. Shigellosis can spread easily in childcare facilities, and homeless shelters; and the outbreaks of shigellosis are difficult to control due to the ease of person-to-person transmission among young children (8). The results of this study suggest that effective control including participation in the intervention, supervised hand washing for children in schools, childcare centers, and homeless shelters can prevent the transmission of shigellosis into the community (26, 27). The most affected age ranges between 7 to 25 years (61%) and the lowest was over 65 years (6%). Male gender was dominated (male 61.8% and female 38.2%). The percentage of outbreaks in the urban areas (78%) compared to rural areas (22%) indicates that the risk of outbreaks can be reduced by using proper hygiene. All of the isolated *Salmonella* and *Shigella* were sensitive to ciprofloxacin. In contrast to our results, studies conducted in Turkey showed a reduction in sensitivity to ciprofloxacin (28, 29). The result of this study showed that some of the isolates *Shigella* spp were resistance to ampicilin which is commonly used for the treatment of *Shigella* infections. Various studies have reported an increase in antimicrobial resistance amongst different species of *Shigella spp*. (30). Based on our findings, the most commonly observed resistance of *Shigella* strains were to tetracycline (84.7%), ampicillin (44.9%) and clindamycin (83.9%) and lowest resistant was found to ciprofloxacin (100%). However, a previous study reported the highest resistance rates (61.8%) to tetracycline (25). The role of antimicrobials in the control of epidemic shigellosis are not a substitute for hygienic measures in reducing the secondary spread of shigellosis. Antimicrobials should be reserved for treatment of patients only when clinically indicated, to prevent the resistant strains of *Shigella* (30, 31). Prophylactic cannot be recommended to prevent illness in persons who are exposed but not ill. Furthermore using antimicrobials to treat patients with mild shigellosis to reduce the spread of secondary infections is not more effective in preventing *Shigella* infections than hand washing with soap and water (27, 32). Since resistance patterns may change, antimicrobial selection should be based on antimicrobial resistance results of strains. Shigellosis outbreaks are more common in the summertime (33, 34). Most *Salmonella* infections in

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humans result from the ingestion of contaminated food such as poultry, beef, pork, eggs and milk (11, 24, 25). Intestinal Salmonellosis is self-limited and recovery will be obtained in several days without antibiotics. When the infection spreads beyond the intestinal tract, antibiotics such as ciprofloxacin in adults and ceftriaxone in children could be used for the treatment. Since antimicrobial agents are given to domestic livestock for the prevention and treatment of disease, antimicrobial-resistant *Salmonella* could transfer from contaminated foods of animal origin (11, 27, 33). All stains of *Salmonella* were susceptible to ciprofloxacin, imipenem (94.6%), amikiacin (89.2%), and only (12.2%) to nalidixic acid. In another study, all *Salmonella* isolates were susceptible to nalidixic acid (35), compared to our results (12.2%). These findings demonstrate the multidrug-resistance strains of *Salmonella* including nalidixic acid resistant isolates (23). The results of this study indicate that proper strategy is necessary to reduce the prevalence of antimicrobial resistant *Salmonella* in food by means of proper guidelines for the use of antibacterial agents in animal foods.

Conclusion

Our two-year period analysis of food poisoning cases, illustrated that *Salmonella* and *Shigella* species were the most common causative agent of food poisoning. Therefore proper food safety instruction should apply for either food manufacturer or suppliers in order to prevent foodborne disease outbreaks.

Conflict of Interests

The authors declare that they have no competing interests.

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