Determination of ESBLs, pAmpC-beta-lactamase genes, and plasmid replicon types among *Shigella* species from different cities in Iran

Abstract

Shigella species (spp) are the common gram-negative bacilli isolated from patients with diarrhea. Infection treatment of these genus of bacteria due to increasing resistance to antibiotic agents remains a global challenge. Herein, we determined the frequency of ESBLs, plasmid-mediated AmpC-beta-lactamase (pAmpC) genes, and plasmid replicon types in 210 clinical isolates of *Shigella* spp from different cities in Iran. The antibacterial susceptibility of isolates to antibiotic agents and ESBLs production were determined according to the Clinical & Laboratory Standards Institute (CLSI) recommendations. ESBLs, pAmpC genes, and plasmid replicon types of isolates were detected using PCR and multiplex PCR methods. The highest rate of antibiotic resistance was observed to trimethoprim-sulfamethoxazole and the lowest rate of resistance was observed to cefoxitin. Fifty-four percent of the isolates were considered as ESBL-producers. Beta-lactamase genes including $bla_{\text{CTX-M}}$, bla_{TEM}, and bla_{DHA} were detected in 93 (44%), 84 (40%), and 3 (1.4%) of isolates, respectively. Ten various plasmid replicon types including I1-Iγ, K, W, FIB, Y, P, FIC, FIA, HI1, and B/O were identified among the isolates. The study sheds light on the persistent challenges posed by multidrug-resistant (MDR) shigellosis to public health in different regions in Iran. Despite advancements in hygiene practices, the prevalence and population composition of *Shigella* species have remained largely unchanged. Also, the spread of beta-lactamase genes and various plasmid replicon types are increasing among the *Shigella* spp in our country which can be challenging to treat their infection and more efficient strategies, and monitoring should be considered to prevent the spread of them.

Keywords: *Shigella* species, ESBLs, AmpC beta-lactamase, Plasmid replicon types

1. Introduction

Foodborne bacterial pathogens such as *Shigella* species (spp) are the most critical public health concerns around the world (1). The *Shigella* spp including *Shigella dysenteriae*, *Shigella flexneri*, *Shigella sonnei*, and *Shigella boydii* are a member of the Enterobacteriaceae family and caused acute gastroenteritis with high morbidity and mortality especially in children in developing countries (2). Shigellosis caused by *Shigella* spp is an acute enteric infection which is usually characterized by watery and mucoid bloody diarrhea is highly contagious due to low dose. Antibiotic therapy that usually use for infants, elderly, and immunocompromised patients can reduce the duration, severity of shigellosis and the risk of infection transmission. Ciprofloxacin, pivmecillinam, ceftriaxone, and azithromycin are some antibiotic agents for treatment of shigellosis especially in patients with bloody diarrhea (3). However, antibiotic resistance is increasing among *Shigella* spp due to misuse or overuse of antibiotic agents in treatment of shigellosis and multidrug resistant (MDR) of *Shigella* isolates was reported in some countries (4). The ability of this organism to acquire different antibiotic resistance genes through mobile genetic elements such as integrons, transposons, and plasmids are the major factors in spread and emergence of MDR isolates. Most MDR *Shigella* isolates are resistant to cephalosporins, ciprofloxacin, and azithromycin through acquire plasmid-mediated resistance mechanisms (4). Detection of ESBL and AmpC positive among *Shigella* spp are importance because they are usually MDR and therapeutic options for them are limited. Hence, update of our data on the rate and mechanism of resistance to antibiotic agents in *Shigella* spp. are

essential for using effective and justified therapy to decrease the morbidity and mortality rates associated with shigellosis. Conjugative plasmids play an important role in the evolution and dissemination of antibiotic resistance and virulence factors among the bacteria through facilitating horizontal gene transfer (3). Due to the importance of *Shigella* spp in causing gastrointestinal infections and the leading challenges in the treatment of infections resulting from MDR isolates this study was conducted to investigate the presence of ESBLs, pAmpC beta-lactamase genes, and plasmid replicon types among *Shigella* isolates in different cities in Iran.

2. Material and Methods

2.1. Sampling, Culture, and Shigella spp. isolates

This study was performed on 210 clinical isolates of *Shigella* spp. that were collected from patients with diarrhea (A single specimen) and kept at -80 °C in the Tryptic Soy Broth (TSB) containing 30% glycerol from 2019 to 2021. The bacterial isolates were obtained from six cities of Ahvaz, Kerman, Shahr-e Kord, Tabriz, Urmia, and Ardabil in Iran, and were confirmed using standard microbial tests and PCR by specific primers. The sequence and annealing temperature of the primers are shown in Table 1.

2.2. Antimicrobial susceptibility of isolates and detection of ESBL-producing isolates

Disk diffusion method was used to evaluate the antibiotic susceptibility pattern of isolates to different antibiotic agents (Padtan Teb Laboratory Instruments, Co, Iran) including ceftazidime (CAZ, 30 µg), ampicillin (AMP, 10 µg), cefotaxime (CTX, 30 µg), cefoxitin (FOX, 30 µg),

ceftriaxone (CRO, 30 µg), gentamicin (GEN, 10 µg), levofloxacin (LEV, 5 µg), amikacin (AMK, 30 µg), streptomycin (STR, 10 µg), ciprofloxacin (CIP, 5 µg), chloramphenicol (CHL, 30 µg), nalidixic acid (NAL, 30µg), azithromycin (AZM, 15 µg), tetracycline (TET, 30µg), and trimethoprim-sulfamethoxazole (SXT, 1.25/23.75 µg), according to the clinical laboratory standards institute (CLSI) (5). *Shigella* isolates that were resistant to one of the third generations of cephalosporins including ceftazidime, cefotaxime, and ceftriaxone were selected for confirmation ESBL production according to CLSI using the combination disc test with clavulanic acid (5).

2.3. DNA extraction

The DNA of the isolates was extracted by boiling method. Briefly, a single colony from each isolate was suspended in 400 µL DNase and RNase free water and heated at 100°C for 10 minutes. Then, lysates were centrifuged at 12000g for 10 min and the supernatants were used as DNA template for the PCR and multiplex PCR experiment.

2.4. Detection of ESBLs and pAmpC among *Shigella* spp

ESBL resistance genes including bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$, were screened using PCR among ESBL-positive isolates, and cefoxitin-resistant isolates were also selected for identifying pAmpC mediated beta-lactamase genes including bla_{MOX}, bla_{ACC}, bla_{FOX}, bla_{CMY}, bla_{EBC}, and bla_{DHA} using multiplex-PCR amplification technique previously described by Pérez-Pérez and Hanson (6). The PCR products were electrophoresed on 1.5 % agarose gel for 45 min at 100 V and the electrophoresed gel was analyzed by a gel documentation imaging

system. The sequence and annealing temperature of the primers used for the detection of beta-lactamase genes are presented in Table 1.

2.5. Plasmid replicon type profiling

PCR-based plasmid replicon typing (PBRT) was performed based on the Carattoli *et al.,* method, using 18 pairs of specific primers to identify replicons FIA, FIB, FIC, HI1, HI2, I1-Iγ, N, L/M, P, W, A/C, T, K, B/O, X, Y, FIIAs, and Frep in five multiplex-PCR and three simplex-PCR (7). The sequence of primers used in PBRT techniques is presented in Table 2.

2.6. Statistical analysis

The results were statistically evaluated by SPSS version 24 and P-values ≤0.05 were regarded as statistically significant using the chi-square and Fisher's exact tests.

3. Results

Shigella spp. isolates

In the present study, a total of 210 *Shigella* spp. isolates were collected from 6 different cities, including cities Kerman [n= 64 (30.4%)], Ahvaz [n=67 (33.5%)], Ardabil [n=18 (8.5%)], Urmia [n=19 (9%)], Tabriz [n=20 (9.5%)], and Shahr-e Kord [n=22 (10.4%)], that *S. dysenteriae* [n=5 (2%)], *S. flexneri* [n=102 (49%)], *S. boydii* [n=28 (13%)], and *S. sonnei* [n=75 (36%)] were reported. Prevalence of *Shigella* spp. in different cities in Iran were showed in Table 3.

3.1. Result of antimicrobial susceptibility

In this study, the resistance rate to antibiotics agents was trimethoprim-sulfamethoxazole (97%), streptomycin (94%), ampicillin (80%), tetracycline (77%), chloramphenicol (62%) and cefotaxime (57%), ceftriaxone (49%), nalidixic acid (31%), ceftazidime (30%), gentamicin (13%), azithromycin (12%), levofloxacin (6%), amikacin (6%), ciprofloxacin (6%), and cefoxitin (5%). Interestingly, all isolates were resistant to at least three classes of antibiotics that were considered MDR isolates. The rate of antibiotic resistance in different cities is presented in Table 4.

3.2. Prevalence of ESBLs and pAmpC beta-lactamase genes (*bla* genes)

Based on the results, 114 isolates (54%) of the isolates were ESBLs positive, and bla_{TEM} and $bla_{\text{CTX-M}}$ were detected in 84 (40%), and 93 (44%) of ESBL isolates, respectively. bla_{DHA} as one of the pAmpC-associated genes was only detected in three isolates (1.4%), two isolates belonged to *S. flexneri* and one of them belonged to *S. sonnei*. The prevalence of β-lactamase genes among *Shigella* spp in different cities in Iran is shown in Table 5.

3.3. Prevalence of plasmid replicon types

In this study, the replicon types of I1-Iγ (72.8%), K (54.2%), W (47.1%), FIB (30.9%), Y (21.9%), P (13.3%), FIC (6.6%), FIA (4.2%), HI1 (1.9%), and B/O (1.4%) were reported Distribution of *bla* genes and plasmid replicon types among *Shigella* spp. in Kerman, Ardabil, Shahr-e Kord, Tabriz, Ahvaz, and Urmia in Iran are presented in Tables 6 and 7.

4. Discussion

Shigellosis presents a considerable challenge to global human health and is particularly prevalent in developing countries. Administering antibiotics has been shown to reduce both the severity and duration of the infection, as well as the excretion of the organism in feces, thereby aiding in the prevention of its continued transmission. This study offers insights into the molecular epidemiology of antibiotic resistance profiles, ESBLs, AmpC, and plasmid replicon types among *Shigella* spp. isolated from diarrhea in Iran.

In the current study, *S. flexneri* (49%) and *S. sonne*i (36%) were identified as the most common species and our results were largely consistent with the results of recent studies in Iran and various countries. In several studies between 2001-2019, *S. flexneri* and *S. sonnei* were the predominant species of *Shigella* in Ahvaz and Tehran in Iran (8, 9). As regards *S. flexneri* and *S. sonnei* are predominant species in developing and developed countries respectively, the so-mentioned studies are consistent with the results of our study. The results of the current study showed that despite the high standard of hygiene in our region in recent years, we observed no noticeable change in the prevalence and population composition of isolated *Shigella* spp.

In recent years, there has been an epidemiological transition observed in the prevalence of *Shigella* serogroups. Specifically, there has been a notable emergence of *S. sonnei* in regions where *S. flexneri* historically prevailed. This shift has been documented across various areas in Asia, Latin America, and the Middle East (10). Notably, in the United States, *S. flexneri* was the predominant serotype in the early 1960s, but it was supplanted by *S. sonnei* between 1964

and 1968 the cause of which is unknown (10). The increasing prevalence of *S. sonnei* in developing countries could potentially be attributed to improvements in water quality and sanitation practices. These improvements might limit the passive immunization typically conferred by *P. shigelloides*, which is commonly found in contaminated water sources. Furthermore, the amoeba *Acanthamoeba castellani* serves as a reservoir for *S. sonnei*, enabling its persistence even in highly chlorinated environments where *S. flexneri* struggles to thrive. Additionally, *S. sonnei* demonstrates a greater propensity for acquiring resistance compared to *S. flexneri*, giving it a competitive edge, particularly in regions with limited antimicrobial usage (11).

Shigella spp. can easily acquire and spread antimicrobial resistance genes as in recent years, MDR-positive *Shigella* spp. has been reported abundantly throughout the world. In this study, all isolates were resistant to at least three classes of antibiotics and considered MDR. Also, 54% of isolates were ESBL-produced which could be a serious threat to public health. The frequency of ESBL-producing *Shigella* isolates was reported 7.5% by Ranjbar et al., in 2013 in Tehran, Iran, which our results in comparison to their findings represent a significant increase in ESBL-producing *Shigella* isolates in other regions in Iran (12). In a cross-sectional study in Ardabil, Iran, from 2019 to 2020, 10.2% of *Shigella* species were ESBL positive, and various beta-lactamase genes including $bla_{\text{CTX-M}}$ and bla_{TEM} found among them (13). In another study in Tehran, Iran from 2015-2017 $bla_{\text{CTX-M-15}}$ (10.7 %), bla_{SHV} (28 %), and bla_{TEM} (21.3 %) were reported among *Shigella* isolates (14).

In the present study, *bla*$_{TEM}$ and *bla*$_{CTX-M}$ were the common ESBL genes among the isolates with frequencies 84 (40%) and 93 (44%), respectively, which was similar to findings in Argentina, Turkey, Lebanon, China, Korea, and Japan which they reported *bla*$_{TEM}$ and *bla*$_{CTX}$ as predominant ESBL genes in *Shigella* isolates (15-17).

This study revealed that more than 50% of the *Shigella* isolates exhibited resistance to ampicillin, tetracycline, trimethoprim-sulfamethoxazole, streptomycin, chloramphenicol, cefotaxime, and ceftriaxone. Many reports have highlighted a significant prevalence of resistance to ampicillin and trimethoprim-sulfamethoxazole among *Shigella* isolates that based on these reports trimethoprim-sulfamethoxazole and ampicillin are not appropriate choices for the treatment of shigellosis (18, 19). A high prevalence of resistance to nalidixic acid, trimethoprim/sulfamethoxazole, and ampicillin was reported in *Shigella* isolates among pediatric patients in different regions of Iran (14). So, findings in different regions in Iran similar to our results showed that the rate of resistance to ampicillin, nalidixic acid, and trimethoprim/sulfamethoxazole is high.

In contrast to the findings by Xing et al. in China, in our study resistance to nalidixic acid (31%) was low (18). This difference can be due to the less use or no use of this drug in the treatment of shigellosis and other gastrointestinal tract infections in our country. GU et al., demonstrated a significant increase in ciprofloxacin resistance in Asia and African countries over 12 years, whereas resistance to this antibiotic and third-generation cephalosporins below 1% in the United States and some European countries were reported. Ceftriaxone is presently the primary

treatment for shigellosis in hospitalized patients. However, the irregular use of antibiotics has contributed to the development of resistant strains. Resistance to third-generation cephalosporins, particularly ceftriaxone, has been notably high in countries like Vietnam, China, and Iran. Despite reports of increasing antibiotic resistance in *Shigella* spp, the high resistance rate (49%) to ceftriaxone in our study is noteworthy.

In a study conducted by Jafari et al., in 2009 in Tehran, Iran, more than 90% of *Shigella* isolates were found to be susceptible to ceftriaxone, ceftazidime, cefepime, and ciprofloxacin (20). Conversely, Mostafavi et al., (21) reported a very high level of resistance among *Shigella* serotypes to trimethoprim-sulfamethoxazole, ampicillin, and third-generation cephalosporins. Studies conducted in Iran over the past 20 years consistently revealed a high level of resistance to trimethoprim-sulfamethoxazole and ampicillin in Iran (21-24).

In this study, a significant relationship was observed between all the *S. sonnei* strains and resistance to trimethoprim-sulfamethoxazole ($P \leq 0.5$). Also in the current study, *S. boydii*, *S. flexneri*, and *S. dysenteriae* strains had a significant relationship with resistance to each of the antibiotics trimethoprim-sulfamethoxazole, ampicillin, and streptomycin, ($P \leq 0.05$) that this could mostly indicate a clonal spread of this strains due to the horizontal transfer of plasmids carrying multiple resistance genes, in our country hence it can indicates a key role of horizontal transfer of multiple drugs resistance genes associated with endemic plasmids. Overall, the low resistance to ciprofloxacin (6%) in our study suggest that fluoroquinolones remain effective for treating shigellosis in different regions in our country. However, due to the limitations of

fluoroquinolone prescription in children because of their side effects some cephalosporins are often used as an alternative treatment for shigellosis.

The PBRT method is an applied tool for the evaluation relatedness of each isolate in epidemiological studies. In this study, plasmid profiling showed that 72.8% of isolates were harboring I1-Iγ replicon type as the most abundant replicon type. I1-Iγ replicons are limited to Enterobacteria hosts that are carrying the $bla_{\text{CTX-M}}$, bla_{SHV}, bla_{CMY}, bla_{TEM} genes, integrons, and resistance genes to arsenic, tetracycline, and streptomycin.

In the study by Ruekit *et al.*, in India that was conducted on 29 *S. sonnei* isolates, only B/O (34.4%) and I1-Iγ (13.7%) replicon types were reported while in our study I1-Iγ and B/O in 72.8% and 1.4% of isolates were reported, respectively (25). Due to the geographical distribution and species diversity of our isolates, clonal dissemination of *Shigella* isolates carrying the I1-Iγ replicon is not very likely. Also, in the present study, most ESBL isolates carried the I1-Iγ replicon, so maybe there is a co-relationship between these two factors. Results of this study indicate that probably I1-Iγ replicon is more compatible with ESBL-positive *Shigella* isolates than other plasmid replicon types.

In the present study, multi-replicon types harboring isolates were observed abundantly, so that among plasmid-carrying isolates, 59.3% of the isolates had at least three replicons simultaneously. Also, two isolates that both belonged to *S. soneii* from Kerman carried eight replicons types. The multi-replicons phenomenon among the isolates is one of the main factors in successful exchanges genetic among isolates, therefore while increasing the chance of

horizontal gene transfer among isolates (a broad host range), it increases the possibility of genetic recombination between different plasmids in each isolate which can cause the evolution and diversity among these bacteria with new pathogenicity and resistance profile.

In conclusion, the study sheds light on the persistent challenges posed by shigellosis to global public health. Despite advancements in hygiene practices, the prevalence and population composition of *Shigella* species remain largely unchanged. The epidemiological transition from *S. flexneri* to *S. sonnei* observed in various regions underscores the dynamic nature of this infectious disease. Antimicrobial resistance among *Shigella* spp. poses a significant threat, with multi-drug resistant and ESBL-producing isolates increasing worldwide which can reduce treatment options. Notably, the high resistance rate to ceftriaxone in Iran highlights the urgent need for prudent antibiotic stewardship. Also, our study highlights the possible role of plasmid replicon types in the development and dissemination of resistance genes among *Shigella* isolates and the spread of the I1-Iγ replicon which is usually associated with ESBL genes underscores the importance of understanding plasmid dynamics in combating antimicrobial resistance. However, despite the challenges posed by antimicrobial resistance some antibiotics like ciprofloxacin remain effective for treating shigellosis, although we have limitations with it, especially in pediatric patients, and ongoing surveillance and appropriate use of antibiotic agents are essential for managing shigellosis caused by drug-resistant isolates.

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Conflicts of interest

All authors of this manuscript have no conflicts of interest to disclose.

Ethics approval

The present study with Reg. No. 97000972 was approved by the ethical committee of Kerman University of Medical Sciences, Kerman, Iran. The ethics approval code is IR.KMU.REC.1398.199.

Availability of data and material

The datasets used or analyzed during the study are available on reasonable requests from the corresponding author (**Email:** d.kalantar@kmu.ac.ir).

Authors' contributions

S.A.AM, M.M, H.HN, P.M, and D.K-N integrated the data and wrote the draft of the manuscript. M.M, P.M, and D.K-N contributed to data acquisition, interpretation, and analysis of the results. All authors reviewed and approved the final manuscript for publication.

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Table 1. The primer sequences are used for conformation of different species of *Shigella*.

Gene	Primer sequence (5′-3′)	Product size (bp)	Annealing (°C)	Use
Sboy	F-TCTGATGTCACTCTTTGCGA R-GAATCCGGTACCCGTAAGGT	248	59	For confirmation *S. boydii*
Sflex	F-TTTATGGCTTCTTTGTCGGC R-CTGCGTGATCCGACCATG	537	56	For confirmation *S. flexneri*
Sson	F-AATGCCGTAAGGAATGCAAG R-CTTGAAGGAGATTCGCTGCT	503	58	For confirmation *S. sonnei*
Sdys	F-TCTCAATAATAGGGAACACAG R-CATAAATCACCAGCAAGGTT	211	56	For confirmation *S. dysenteriae*
***bla*$_{SHV}$**	F-TCAGCGAAAAACACCTTG R-TCCCGCAGATAAATCACC	471	50	For detection of ESBL genes
***bla*$_{TEM}$**	F- GAGTATTCAACATTTCGTGTC R- TAATCAGTGAGGCACTATCTC	861	60	
***bla*$_{CTX-M}$**	F-CGCTTTGCGATGTGCAG R-ACCGCGATATCGTTGGT	550	60	
***bla*$_{MOX}$**	F-GCTGCTCAAGGAGCACAGGAT R-CACATTGACATAGGTGTGGTGC	520	57	For detection of pAmpC genes using multiplex-PCR
***bla*$_{CIT}$**	F-TGGCCAGAACTGACAGGCAAA R-TTTCTCCTGAACGTGGCTGGC	462		
***bla*$_{DHA}$**	F-AACTTTCACAGGTGTGCTGGGT R-CCGTACGCATACTGGCTTTGC	405		
***bla*$_{ACC}$**	F-AACAGCCTCAGCAGCCGGTTA R-TTCGCCGCAATCATCCCTAGC	346		
***bla*$_{EBC}$**	F-TCGGTAAAGCCGATGTTGCGG R-CTTCCACTGCGGCTGCCAGTT	302		
***bla*$_{FOX}$**	F-AACATGGGGTATCAGGGAGAT R-GCAAAGCGCGTAACCGGATTGG	190		

Table 2. The list of primers for plasmid replicon typing of isolates.			
Replicon type	**Primer sequence (5′-3′)**	**Target site**	**Amplicon size (bp)**
HI1	F-GGAGCGATGGATTACTTCAGTAC R-TGCCGTTTCACCTCGTGAGTA	parA-parB	471
HI2	F-TTTCTCCTGAGTCACCTGTTAACAC R-GGCTCACTACCGTTGTCATCCT	iterons	644
I1	F-CGAAAGCCGGACGGCAGAA R-TCGTCGTTCCGCCAAGTTCGT	RNAI	139
X	F-AACCTTAGAGGCTATTTAAGTTGCTGAT R-TGAGAGTCAATTTTTATCTCATGTTTTAGC	ori g	376
L/M	F-GGATGAAAACTATCAGCATCTGAAG R-CTGCAGGGGCGATTCTTTAGG	repA,B,C	785
N	F-GTCTAACGAGCTTACCGAAG R-GTTTCAACTCTGCCAAGTTC	repA	559
FIA	F-CCATGCTGGTTCTAGAGAAGGTG R-GTATATCCTTACTGGCTTCCGCAG	iterons	462
FIB	F-GGAGTTCTGACACACGATTTTCTG R-CTCCCGTCGCTTCAGGGCATT	repA	702
W	F-CCTAAGAACAACAAAGCCCCCG R-GGTGCGCGGCATAGAACCGT	repA	242
Y	F-AATTCAAACAACACTGTGCAGCCTG R-GCGAGAATGGACGATTACAAAACTTT	repA	765
P	F-CTATGGCCCTGCAAACGCGCCAGAAA R-TCACGCGCCAGGGCGCAGCC	iterons	534
FIC	F-GTGAACTGGCAGATGAGGAAGG R-TTCTCCTCGTCGCCAAACTAGAT	repA2	262
A/C	F-GAGAACCAAAGACAAAGACCTGGA R-ACGACAAACCTGAATTGCCTCCTT	repA	465
T	F-TTGGCCTGTTTGTGCCTAAACCAT R-CGTTGATTACACTTAGCTTTGGAC	repA	750
FIIAS	F-CTGTCGTAAGCTGATGGC R-CTCTGCCACAAACTTCAGC	repA	270
FrepB	F-TGATCGTTTAAGGAATTTTG R-GAAGATCAGTCACACCATCC	RNAI/repA	270
K/B	F-GCGGTCCGGAAAGCCAGAAAAC R-TCTTTCACGAGCCCGCCAAA	RNAI	160
B/O	F-TCTGCGTTCCGCCAAGTTCGA	RNAI	159

Table 3. Prevalence of *Shigella* spp. in different cities in Iran.						
***Shigella* spp.**	**City; n (%)**					
	Ardabil; 18 (%)	**Ahvaz; 67 (%)**	**Kerman; 64 (%)**	**Urmia; 19 (%)**	**Shahre-kord; 22 (%)**	**Tabriz; 20 (%)**
S. dysenteriae	0	0	2 (3.1)	1 (5.3)	0	2 (10)
S. flexneri	10 (55.6)	27 (40.3)	34 (53.2)	8 (42.1)	10 (45.5)	13 (65)
S. boydii	4 (22.2)	10 (14.9)	9 (14.06)	1 (5.3)	3 (13.6)	1 (5)
S. sonnei	4 (22.2)	30 (44.8)	19 (29.6)	9 (47.3)	9 (40.9)	4 (20)
Total	18 (100)	67 (100)	64 (100)	19 (100)	22 (100)	20 (100)

Table 4. The frequency of resistance to different antibiotic agents in different cities in Iran among *Shigella* spp.						
Antibiotic agents	**City; n (%)**					
	Ardabil	**Ahvaz**	**Kerman**	**Urmia**	**Shahre-kord**	**Tabriz**
Cefoxitin	6 (33.3)	-	4 (6.2)	-	-	2 (10)
Ciprofloxacin	3 (16.66)	1 (1.4)	5 (7.8)	1 (5.2)	1 (4.5)	2 (10)
Ampicillin	17 (94.44)	42 (62.6)	57(89)	14 (73.6)	20 (90.9)	19 (95)
Gentamicin	8 (44.44)	2 (2.9)	8 (12.5)	4 (21)	4 (18.1)	2 (10)
Nalidixic acid	9 (50)	24 (35.8)	16 (25)	8 (42.1)	5 (22.7)	4 (20)
Levofloxacin	3 (16.6)	3 (4.4)	4 (6.2)	-	3 (13.6)	1 (5)
Amikacin	-	4 (5.9)	7 (10.9)	-	2 (9)	-
Streptomycin	13 (72.2)	64 (95.5)	64 (100)	18 (94.7)	22 (100)	18 (90)
Azithromycin	4 (22.2)	3 (4.4)	5 (7.8)	5 (26.3)	2 (9)	7 (35)
Tetracycline	9 (50)	63(94)	44 (68.7)	16 (84.2)	16 (72.7)	15 (75)
Trimethoprim-sulfamethoxazole	17 (94.44)	67 (100)	64 (100)	17 (89.4)	21 (95.4)	19 (95)
Chloramphenicol	2 (11.1)	14 (20.8)	26 (40.6)	6 (31.5)	4 (18.1)	4 (20)
Ceftriaxone	11 (61.1)	27 (40.2)	29 (45.3)	10 (52.6)	10 (45.4)	17 (85)
Cefotaxime	14 (77.7)	31 (46.2)	34 (53.1)	10 (52.6)	14 (63.6)	18 (90)
Ceftazidime	8 (44.4)	26 (38.8)	4 (6.2)	3 (15.7)	12 (54.5)	11 (55)

Table 5. Prevalence of β-lactamase genes among *Shigella* spp in different cities in Iran.				
City	**β-lactamase genes, n (%)**			
	***bla*$_{CTX-M}$**	***bla*$_{SHV}$**	***bla*$_{TEM}$**	***bla*$_{DHA}$**
Ardabil (18)	11(61.1)	0	7 (38.8)	1 (5.5)
Ahvaz (67)	16 (23.8)	0	25 (37.3)	0
Kerman (64)	29 (45.3)	0	19 (29.6)	2 (3.1)
Urmia (19)	10 (52.6)	0	7 (36.8)	0
Shahr-e Kord (22)	10 (45.45)	0	14 (63.6)	0
Total (190)	76 (40)	0	72 (37.8)	3 (1.57)

Table 6. Prevalence of plasmid replicon types among *Shigella* spp in different cities in Iran.										
City (n)	**Plasmid replicon types, n (%)**									
	HI1	**I1-Iγ**	**FIA**	**FIB**	**W**	**Y**	**P**	**FIC**	**K**	**B/O**
Ardabil (18)	0	15 (83.3)	0	7 (38.8)	12 (66.6)	1 (5.5)	1 (5.5)	0	10 (55.5)	0
Ahvaz (67)	1 (1.4)	43(64.1)	5 (7.4)	18 (26.8)	12 (17.9)	1 (1.49)	0	0	33 (49.2)	2 (2.9)
Kerman (64)	0	48(75)	2 (3.1)	10 (5.6)	41	29 (45.3)	18 (28.1)	11 (17.1)	40 (62.5)	3 (4.6)
Urmia (19)	2 (10.5)	16 (84.2)	0	6 (31.5)	8 (42.1)	5 (26.3)	1 (5.2)	1 (5.2)	7 (36.8)	0
Shahre-kord (22)	1 (1.4)	15 (68.1)	0	14 (63.6)	14 (63.6)	5 (22.7)	4 (18.1)	4 (18.1)	11 (50)	0
Total (190)	4 (2.1)	137 (72.1)	7 (3.68)	55 (28.9)	87 (45.7)	41 (21.57)	24 (12.63)	16 (8.42)	101 (53.1)	5 (2.63)

Table 7. Distribution of *bla* genes and plasmid replicon types among *Shigella* spp. in Kerman, Ardabil, Shahr-e Kord, Tabriz, Ahvaz, and Urmia in Iran.

City	*bla* genes	Species	Replicon plasmid type profile
Ardabil	bla_{TEM}	*S. flexneri*	I1-Iγ, K
	bla_{TEM}	*S. flexneri*	I1-Iγ, FIB, K
	bla_{CTX-M}	*S. flexneri*	I1-Iγ, FIB, W, K
	bla_{CTX-M}	*S. flexneri*	I1-Iγ, FIB, W, Y, P
	bla_{CTX-M}	*S. flexneri*	I1-Iγ, FIB, W, K
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, FIB, W, K
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, K
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, FIB, W
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, FIB, W
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W, K
	bla_{CTX-M},bla_{TEM}	*S. sonnei*	I1-Iγ, W
	bla_{CTX-M}, bla_{TEM}	*S. sonnei*	I1-Iγ, W
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, FIB, W
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W, K
	bla_{CTX-M}, bla_{TEM}	*S. boydii*	I1-Iγ, K
	bla_{CTX-M}, bla_{TEM}	*S. boydii*	I1-Iγ, W
Urmia	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, FIB, W, FIC, K
	bla_{CTX-M}, bla_{SHV}	*S. flexneri*	I1-Iγ, FIB, W
	bla_{CTX-M}, bla_{TEM}	*S. sonnei*	I1-Iγ, FIB, W
	bla_{CTX-M}, bla_{TEM}	*S. boydii*	I1-Iγ, W, Y
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, FIB, W
	bla_{CTX-M}, bla_{TEM}	*S. dysenteriae*	I1-Iγ, K
	bla_{CTX-M}	*S. flexneri*	I1-Iγ, FIB, W
	bla_{CTX-M}	*S. flexneri*	I1-Iγ Y, K
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, FIB, W
Kerman	bla_{TEM}	*S. flexneri*	I1-Iγ, W
	bla_{TEM}	*S. flexneri*	I1-Iγ, Y, FIC, K
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, W, Y, P
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, K, B/O
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, W, K
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, W, Y, K
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, W, Y, K
	bla_{CTX-M}, bla_{TEM}	*S. flexneri*	I1-Iγ, K, B/O
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W, K
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, FIB, W, Y, P, K
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W, K
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, FIB, K
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, FIB, Y, K
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, FIA, FIB, W, K
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, K, FIC, P, Y, W, FIB, FIA
	bla_{CTX-M}	*S. sonnei*	W, Y, P
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W, K
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, B/O
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W, Y, P, K
	bla_{CTX-M}	*S. sonnei*	K, FIC, Y
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W
	bla_{CTX-M}	*S. sonnei*	I1-Iγ, W, Y, P, K
	bla_{CTX-M}	*S. boydii*	I1-Iγ, FIC, K
	bla_{CTX-M}	*S. boydii*	I1-Iγ, W, P, K
	bla_{CTX-M}, bla_{TEM}	*S. boydii*	I1-Iγ, W, Y, P, K
	bla_{CTX-M}, bla_{TEM}	*S. boydii*	I1-Iγ, FIB, W
	bla_{TEM}	*S. sonnei*	I1-Iγ, K, FIC, P, Y, W, FIB, FIA
	bla_{CTX-M}	*S. sonnei*	K, FIC, Y
	bla_{TEM}	*S. sonnei*	I1-Iγ, W
	bla_{TEM}	*S. sonnei*	I1-Iγ, W, Y, P, K

	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, W, Y, P, FIC, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	W, Y, P
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	W, K
Ahvaz	*bla*$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, FIA
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, FIB
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	W, FIA
	bla$_{TEM}$	*S. flexneri*	FIA
	bla$_{TEM}$	*S. sonnei*	I1-Iγ, FIB, K
	bla$_{TEM}$	*S. sonnei*	I1-Iγ, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, FIB
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, FIB
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, FIB
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, W
	bla$_{TEM}$	*S. boydii*	I1-Iγ, W, K
	bla$_{TEM}$	*S. boydii*	I1IY, W, K
	bla$_{TEM}$	*S. boydii*	I1-Iγ
	bla$_{TEM}$	*S. flexneri*	I1-Iγ, FIA, FIB, W
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ, FIB, K
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, FIB
	bla$_{CTX-M}$	*S. flexneri*	I1-Iγ, K
	bla$_{TEM}$	*S. flexneri*	I1-Iγ, FIB, W
Tabriz	*bla*$_{CTX-M}$	*S. sonnei*	I1-Iγ, FIB, W, K
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ, FIB, W, Y
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ, FIB, W, P, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. boydii*	I1-Iγ, FIA
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. dysenteriae*	I1-Iγ, K
	bla$_{CTX-M}$	*S. flexneri*	I1-Iγ, Y, K
	bla$_{CTX-M}$	*S. flexneri*	I1-Iγ, K
	bla$_{CTX-M}$	*S. flexneri*	I1-Iγ, FIB, W, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, FIB, W
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, W, Y, P, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, FIB, W
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, K, B/O
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, Y
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1IY, FIB, W, P, K
	bla$_{CTX-M}$	*S. flexneri*	W, Y, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, FIB, W, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, FIB, W, Y
Shahre-kord	*bla*$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	I1-Iγ, FIB, W, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. flexneri*	FIB, W, Y, P
	bla$_{TEM}$	*S. flexneri*	FIB, W
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, FIB, W, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. sonnei*	I1-Iγ, K
	bla$_{TEM}$	*S. sonnei*	I1-Iγ, K
	bla$_{TEM}$	*S. flexneri*	I1-Iγ, FIB, W
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ, FIB, W, K
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ, K
	bla$_{CTX-M}$	*S. sonnei*	I1-Iγ, K
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. boydii*	I1-Iγ, FIB, W
	bla$_{CTX-M}$, *bla*$_{TEM}$	*S. boydii*	I1-Iγ, FIB, W, Y, P