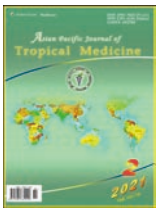

Original Article

Asian Pacific Journal of Tropical Medicine

apjtm.org

doi: 10.4103/1995-7645.281529

Impact Factor: 1.94

Antimicrobial resistance patterns and prevalence of integrons in *Shigella* species isolated from children with diarrhea in southwest Iran

Nabi Jomehzadeh[1], Maryam Afzali[2], Khadijeh Ahmadi[1,2]✉, Shokrollah Salmanzadeh[2], Fateme Jahangiri Mehr[3]

[1]*Abadan Faculty of Medical Sciences, Abadan, Iran*

[2]*Infectious and Tropical Diseases Research Center, Health Research Institue, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

[3]*Biostatistics and Epidemiology Department, Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran*

ABSTRACT

Objective: To investigate the antimicrobial resistance patterns and prevalence of integrons in *Shigella* species isolated from children with diarrhea in southwest Iran.

Methods: In this study, 1 530 stool samples were collected from children under 15 years with diarrhea referred to teaching hospitals in Ahvaz and Abadan, southwest Iran. *Shigella* spp. were identified by standard biochemical tests and PCR. The antibiotic resistance pattern of all *Shigella* isolates was determined by the disk diffusion method and minimum inhibitory concentration (MIC) by E-test.

Results: Of 1 530 stool samples, 91 (5.9%, 91/1 530) were positive for *Shigella* spp. the most common *Shigella* isolates were *Shigella flexneri* 47 (51.6%, 47/1 530). Antibiotic susceptibility tests showed that the highest antibiotic resistance was related to trimethoprim-sulfamethoxazole (87.9%, 80/91) and ampicillin (86.8%, 79/91). Multiplex PCR results revealed that 56% and 86.9% of *Shigella* isolates carried integron class Ⅰ and integron class Ⅱ genes, respectively. None of the isolates included the integron class Ⅲ gene.

Conclusions: The high prevalence of multi-drug resistance in *Shigella* isolates in our area increases the concerns about dissemination of the antibiotic-resistant isolates in this bacterium.

KEYWORDS: Integrons; *Shigella* spp.; Multi-drug resistance; PCR

1. Introduction

Shigellosis is a major health-care concern in the world, especially in developing countries with poor hygiene particularly among children under 5 years old. The incidence of this infection in developing countries, to be 163 million annually[1,2]. The most common symptoms of shigellosis are vomiting, fever, watery diarrhea, tenesmus, and abdominal pain[1,2]. *Shigella* spp. are classified by four serogroups, including *Shigella* (*S.*) *flexneri*, *S. boydii*, *S. dysenteriae*, and *S. sonnei*. *S. sonnei* and *S. flexneri* are the most commonly found in developing countries, such as Iran[3,4]. Treatment with antibiotics can reduce the duration of shigellosis but, resistance to antibiotics has been increasing. In the last decades, multidrug-resistance (MDR) has increased among *Shigella* spp. MDR phenotype achieves by many different mechanisms in clinical isolates. One of the important mechanisms for the increase of resistance to antibiotics is the horizontal transmission of genetic factors. Integrons are mobile genetic elements that could lead to the spread of the MDR phenotype[5]. Integron class Ⅰ (*int Ⅰ*)and integron class Ⅱ (*int Ⅱ*) are the most prevalent genes among the *Shigella* spp. and the relationship between the presence of integrons and resistance to some antibiotics has been demonstrated. Integrons are frequently associated with the resistance of *Shigella* spp. to sulfamethoxazole, trimethoprim, streptomycin, chloramphenicol, tetracycline, and ampicillin[5,6]. Although integrons play an important role in the presence of MDR in *Shigella* spp. there are not any data available to describe the prevalence of integrons of *Shigella*

✉To whom correspondence may be addressed. E-mail:ahmadi.kh.2109@gmil.com

How to cite this article: Jomehzadeh N, Afzali M, Ahmadi K, Salmanzadeh S, Mehr FJ. Antimicrobial resistance patterns and prevalence of integrons in *Shigella* species isolated from children with diarrhea in southwest Iran. Asian Pac J Trop Med 2021; 14(2): 78-82.

Article history: Received 14 August 2019 Revision 27 February 2020
Accepted 27 February 2020 Available online 27 March 2020

strains in southwest Iran, therefore, this study aimed to investigate the antimicrobial resistance patterns and prevalence of integrons in *Shigella* species isolated from children with diarrheal infection in the southwest of Iran.

2. Materials and methods

2.1. Bacterial isolates

In this cross-sectional study, during 18 months from April 2017 to September 2018, 1 530 stool samples were collected from children under 15 years with diarrhea referred to teaching hospitals in Ahvaz and Abadan, southwest Iran. Patients with a history of fever, vomiting, abdominal cramps, watery diarrhea and dysentery were included in our study. Dysentery was characterized by frequent excretion (usually 10 to 13 times/day) of small volume stools consisting of blood, mucus, and pus; often accompanied by abdominal cramps and tenesmus. Diarrhea was defined as the excretion of 3 or more watery stools without blood and mucus in a 24 h period. Patients who were treated with antibiotics at the time of sampling were excluded. These specimens were inoculated into Gram-negative broth tubes as an enrichment medium and immediately transferred to the Laboratory of Microbiology Department of Medicine School of Ahvaz, Iran.

All specimens were cultured in differential media, including xylose lysine desoxycholate (XLD) agar and Hektoen enteric agar (HEA) (Merck, Germany), and then incubated at 37 ℃ overnight. All grown suspected colonies were selected and identified by the biochemical and bacteriological tests such as Triple-sugar Iron Agar (TSI), Sulfide-indole-motility (SIM), Urea Agar, and Simmons Citrate Agar (Merck, Germany) for detection of Shigella strains[7]. All isolates confirmed as *Shigella* spp. were stored in Tryptic Soy Broth (TSB) (Merck, Germany), containing glycerol (30%) at -70 ℃ for antimicrobial susceptibility testing and molecular investigation.

2.2. Antimicrobial susceptibility

Antimicrobial susceptibility was performed on all *Shigella* spp. by Kirby-Bauer disc diffusion method on Muller-Hinton agar medium (Merck, Germany), according to the guidelines of the Clinical and Laboratory Standards Institute[8]. The antibiotic included ceftriaxone (30 μg), trimethoprim/sulfamethoxazole (1.25/23.75 μg), amikacin (30 μg), gentamycin (10 μg), ceftazidime (30 μg), cefotaxime (30 μg), ciprofloxacin (5 μg), azithromycin (15 μg), and ampicillin (10 μg) (Mast Ltd., UK.). Also, *E. coli* ATCC 25922 was used as the control strain. The phenotype of *Shigella* spp. was defined as MDR according to the International Expert proposal for Interim Standards Guidelines[9]. The minimum inhibitory concentrations (MICs) for ceftriaxone, ceftazidime, cefotaxime, ciprofloxacin, amikacin, and gentamicin were determined by E-test (AB Biodisk, Sweden).

2.2. Molecular confirmation of Shigella strains

The whole-genome DNA was extracted using the boiling method as described in previous study[10]. All *Shigella* isolates were confirmed by the PCR method. PCR amplification was performed to detect the *ipaH* gene in *Shigella* isolates. The sequences of primers and annealing temperatures of the *ipaH* gene are shown in Table 1. PCR conditions were examined according to the protocol as described previously[11]. *S. flexnery* ATCC 12122 was used as a positive PCR control for the *ipaH* gene.

2.3. PCR assay for molecular identification of Shigella species

PCR was carried out on all *Shigella* strains to evaluate the prevalence of the *Shigella* species. The primers used to detect *rfc*, *wbgZ*, *rfpB*, and hypothetical protein genes were as previously described[12,13]. The specific primers and annealing temperatures of

Table 1. Primers used in this study to detect *Shigella* spp. and integrons genes.

Gene/protein	Primer sequence (5'-3')	Amplicon size (bp)	Annealing temperature (℃)	References
ipaH	F-GTTCCTTGACCGCCTTTCCGATACCGTC	619	58	
	R-GCCGGTCAGCCACCCTCTGAGAGTAC			[11]
Hypothetical protein	F- GAGCACGGAAACAGAGAGCGCC	240	63	
	R- GGTGCGTTCTTCCGGTGTTCTG			[12]
wbgZ	F- TCTGAATATGCCCTCTAC	430	60	
	R- GACAGAGCCCGAAGAACCG			[13]
rfpB	F- TCTCAATAATAGGGAACACAGC	537	60	
	R- CATAAATCACCAGCAAGGTT			[13]
rfc	F- TTTATGGCTTCTTTGTCG	211	59	
	R- CTGCGTGATCCGACCATG			
int I	F- CCTCCCGCACGATGATC	280	54	
	R- TCCACGCATCGTCAGGC			[14]
int II	F- CACGGATATGCGACAAAAAGGT	789	54	
	R- GTAGCAAACGAGTGACGAAATG			
int III	F- GCCTCCGGCAGCGACTTTCAG	979	54	
	R- ACGGATCTGCCAAACCTGACT			[15]

Shigella spp. genes are listed in Table 1. The total volume of PCR reaction was 25 μL prepared as follows: 12.5 μL of 2X Master Mix, 1 μL of each primer (Cinna gene Company, Iran), 1 μL of template DNA, and distilled water to reach a total volume of 25 μL. Amplification reaction was programmed by a thermal cycler (Eppendorf, Germany) as follows: initial denaturation at 94 ℃ for 5 min, 35 cycles of 94 ℃ for 60 s, annealing (Table 1) for 90 s, extension 72 ℃ for 1 min and final extension 72 ℃ for 7 min. *S. flexneri* ATCC29903, *S. sonnei* ATCC25931, *S. boydii* ATCC8700, and *S. dysenteriae* ATCC13313 were used as a positive control.

2.4. Amplification of integrons genes

PCR was performed for the detection of *int Ⅰ*, *int Ⅱ* and *int Ⅲ* genes. The PCR conditions were similar to the previous study[14]. The sequences of primers and annealing temperatures are shown in Table 1. *S. flexneri* ATCC 12022, *S. sonnei* ATCC 9290 were used as a positive control and *E. coli* ATCC 25922 was used as the negative control.

2.5. Ethics

The study was approved by the Research Ethics Committee of the Abadan School of medical sciences (Ethical code: IR.ABADANUMS.REC1398.023), Abadan, Iran. Written informed consent was obtained from all the children's parents.

3. Results

3.1. Bacterial isolation

In this study, 5.9% (*n*=91) of 1 530 stool samples were positive for *Shigella* spp. Of the 1 530 patients, 47.1% (*n*=720) and 52.9% (*n*=810) were males and females, respectively. The patients have had various clinical symptoms, including vomiting (31.5%, *n*=482), fever (60.9%, *n*=932), abdominal pain (83.1%, *n*=1 271), watery diarrhea (77.9%, *n*=1 193), and dysentery (21.2%, *n*=324).

From a total of 91 *Shigella* spp., 56.0% (*n*=51) and 44.0% (*n*=40) were isolated from male and female patients, respectively. No significant differences in *Shigella* infection were found between male and female patient (P>0.05). Distribution of *Shigella* spp. isolated from the 91 diarrheic children according to age were: 1-5 years, 59.3% (*n*=54); 6-10 years, 24.1% (*n*=22); 11-15 years, 16.5% (*n*=15). Bloody diarrhea, mucoid diarrhea and watery diarrhea were found in 13(14.3%), 7(7.7%), 57(62.6%) patients, respectively. Of these 91 positive samples, 51.6% (*n*=47), 39.6% (*n*=36) and 8.8% (*n*=8) samples were identified as *S. flexneri*, *S. sonnei*, and *S. boydii* respectively. Distribution of Shigella strains according to age group and species are shown in Table 2.

3.2. Antimicrobial susceptibility test

Among 91 *Shigella* isolates, the highest rates of resistance were to trimethoprim-sulfamethoxazole (87.9%, 80/91), ampicillin (86.8%, 79/91), and tetracycline (80.2%, 73/91). The antimicrobial susceptibility profile of the *Shigella* spp. to 10 antibiotics are shown in Table 3. MIC results were as follows: ciprofloxacin (1-256 μg/L), amikacin and gentamicin (0.5-256 μg/L), ceftriaxone (30-256 μg/L), and cefotaxime, ceftazidime (5-256 μg/L). The majority of isolates 76.9% (*n*=70) were MDR with 20 different patterns.

3.3. Frequency of intI and intII genes

The *int Ⅰ* and *int Ⅱ* genes were detected in 56.0% (*n*=51) and 86.9% (*n*=79) strains of *Shigella*, respectively. None of the isolates had integrin class Ⅲ (*int Ⅲ*) gene. All MDR strains *int Ⅱ* alone or in combination with *int Ⅰ*. The distribution of integrons in different serotype isolates of *Shigella* is shown in Table 4.

Table 2. Distribution of *Shigella* spp. by age [*n* (%)].

Age group (year)	*S. flexneri*	*S. sonnei*	*S. boydii*
1-5	26 (28.6)	22 (24.2)	6 (6.5)
6-10	13 (27.6)	7 (19.4)	2 (2.1)
11-15	8 (17.02)	7 (19.4)	0 (0.0)
Total	47 (51.6)	36 (39.6)	8 (8.8)

N=91.

Table 3. Frequency of antibiotic resistance among *Shigella* isolates[(*n*, %)].

Antibiotics	*S. flexneri* (*n*=47)	*S. sonnei* (*n*=36)	*S. boydii* (*n*=8)	Total (*n*=91)
Trimethoprim/ sulfamethoxazole	42 (89.3)	30 (83.3)	8 (100.0)	80 (87.9)
Ampicillin	43 (91.4)	31(86.1)	5 (62.5)	79 (86.8)
Tetracycline	37 (78.7)	31 (86.1)	5 (62.5)	73 (80.2)
Cefteriaxon	27 (57.4)	17 (47.2)	2 (25.0)	46 (50.5)
Ceftazidime	21 (44.6)	15 (41.6)	2 (25.0)	38 (41.7)
Cefotaxime	21 (44.6)	15 (41.6)	3 (37.5)	39 (42.8)
Ciprofloxacin	5 (10.6)	1 (2.7)	0 (0.0)	6 (6.5)
Amikacin	1 (2.1)	2 (5.5)	0 (0.0)	3 (3.2)
Gentamicin	1 (2.1)	2 (5.5)	0 (0.0)	3 (3.2)
Azithromycin	3 (6.3)	5 (13.8)	0 (0.0)	8 (8.7)

Table 4. Distribution of integrons in different serotype isolates of *Shigella* [*n* (%)].

Species	*int Ⅰ* (*n*=5)	*int Ⅱ* (*n*=33)	*int Ⅲ* (*n*=0)	*int Ⅰ*$^+$+*int Ⅱ*$^+$ (*n*=46)
S. flexneri (*n*=47)	2 (4.2)	5 (10.6)	0 (0.0)	39 (82.9)
S. sonnei (*n*=36)	3 (8.3)	26 (72.2)	0 (0.0)	3 (8.3)
S. boydii (*n*=8)	0 (0%)	2 (25)	0 (0.0)	4 (50.0)

4. Discussion

Shigellosis is a significant public health problem in the world, especially in developing countries and causes 5 to 10% diarrhea in different regions and recently, in Asia, the incidence of this infection cause 414 000 deaths per year[15]. In endemic regions of the developing countries, shigellosis is predominantly a pediatric disease. In our study, the prevalence of shigellosis was 5.9%, which is similar to some studies[16-18], but higher than previous reports[19,20]. It seems that the difference in the distribution of *Shigella* strains in various studies is due to the difference in geographic and socioeconomic variables, laboratory mistake in identifying isolates, time, and study conditions. The most frequent age group in our study was age 1-5 years, similar to other studies[21,22]. The reason might be children in this age group being susceptible to microorganisms, poor hygiene, and lower immune responses in this age group[23]. The geographical distribution of the four *Shigella* spp. varies in different regions, *S. flexneri* was the major bacteria that caused diarrhea in most Asian countries[24]. Our study showed that *S. flexneri* 47 (51.6%) was the predominant species among *Shigella* strains in Ahvaz and Abadan, which is comparable with previous studies in Iran and other countries[4,17,24], although others studies have shown the most common serotype isolated was *S. sonnei*[2,4]. Antibiotics are often used for children with bloody and chronic diarrhea to reduce the duration of the disease. Because shigellosis is very contagious, information about the antimicrobial susceptibility is very important for suitable treatment and management of the disease[25]. The antibiotic resistance pattern of *Shigella* spp. varies in different geographic regions. The emergence of MDR strains in *Shigella* spp. is a growing concern around the world[26]. In this study majority of *Shigella* isolates were resistant to trimethoprim/sulfamethoxazole (87.9%), ampicillin (86.8%), and tetracycline (80.2%), which is similar to the previous study from Iran and other countries[26-28]. According to these results, these antibiotics are not appropriate to treat shigellosis in these regions. The results showed that gentamicin, amikacin, and ciprofloxacin were the best antibiotics against *Shigella* isolates. The increasing prevalence of MDR to *Shigella* spp. is a serious problem in developing countries. In our study, the prevalence of MDR in *Shigella* spp. isolates were (76.9%). Other studies reported a high percentage of MDR to *Shigella* spp. [16,29], but our results showed that MDR rates were higher than the previous study in the southwest, Iran[17]. It seems that abuse and overuse of antibiotics for the treatment of diarrhea is one of the main causes of high levels of MDR. Antibiotic resistance in *Shigella* spp. generally occurs due to mobile genetic elements such as transposons, plasmids, and integrons. Mobile genetic elements can cause a distribution of drug resistance genes among different bacteria. MDR in *Shigella* spp. sometimes can be caused by *int Ⅰ* and *int Ⅱ* genes[14]. In the current study (56%) and (86.9%) of *Shigella* isolates carried intI and *int Ⅱ* genes, respectively. None of the isolates had the *int Ⅲ* gene. These results are similar to previous studies[24,30,31]. Our results showed that the prevalence of class 2 is significantly higher than in class 1. The results showed that *Shigella* isolates with both classes of integrons 1 and 2 had a high prevalence of MDR. Also, the prevalence of *int Ⅱ* genes was noticeably associated with MDR in the *Shigella* isolates. These results suggest that there is a relationship between the *int Ⅱ* gene and other antibiotic-resistant genes that require further studies on molecular level studies. More continuous surveillance studies should be conducted in other parts of the world to investigate the true distribution of *Shigella* isolates carrying the *int Ⅱ* gene.

In conclusion, antibiotic resistance has increased in *Shigella* spp. due to misuse and overuse of antibiotics. The high prevalence of multi-drug resistance in *Shigella* isolates in our area increases the concerns about dissemination of the antibiotic-resistant isolates in this bacterium.

Avoiding the distribution of antibiotic resistance and the spread of the integrons in *Shigella* spp. is an immediate issue. Therefore, regular monitoring programs to prevent further spread of MDR *Shigella* isolates is essential.

Conflict of interest statement

The authors report no conflicts of interest in this work.

Acknowledgments

This work was supported by the Vice-Chancellor for Research grant (Grant No. U98-564) of Abadan University of Medical Science. The authors of this manuscript would like to acknowledge the laboratory and nursing personnel of children and infants ward in teaching hospitals in Ahvaz, Abadan, and Khorramshahr, who assisted to collect the clinical specimens.

Authors' contributions

MA developed the original idea and the protocol, performed the experiments, KA was involved in data collection and wrote the preliminary draft, FJ analyzed the data, NJ revised the manuscript, SS was the advisor.

References

[1] Alipour M, Talebjannat M, Nabiuni M. Polymerase chain reaction method for the rapid detection of virulent *Shigella* spp. *Int J Mol Clin Microbiol* 2012; **2**(1): 134-137.

[2] Ranjbar R, Dallal MMS, Talebi M, Pourshafie MR. Increased isolation and characterization of *Shigella sonnei* obtained from hospitalized

children in Tehran, Iran. *J Health Popul Nutr* 2008; **26**(4): 426.

[3] Koppolu V, Osaka I, Skredenske JM, Kettle B, Hefty PS, Li J, et al. Small-molecule inhibitor of the *Shigella flexneri* master virulence regulator *VirF*. *Infect Immun* 2013; **81**(11): 4220-4231.

[4] Cruz CBNd, Souza MCSd, Serra PT, Santos I, Balieiro A, Pieri FA, et al. Virulence factors associated with pediatric shigellosis in Brazilian Amazon. *Biomed Res Int* 2014; **2014**: 539697. doi: 10.1155/2014/539697.

[5] Pan JC, Ye R, Meng DM, Zhang W, Wang HQ, Liu KZ. Molecular characteristics of class 1 and class 2 integrons and their relationships to antibiotic resistance in clinical isolates of *Shigella sonnei* and *Shigella flexneri*. *J Antimicrob Chemother* 2006; **58**(2): 288-296.

[6] Barrantes K, Achí R. The importance of integrons for development and propagation of resistance in *Shigella*: The case of Latin America. *Braz J Microbiol* 2016; **47**(4): 800-806.

[7] Taneja N, Mewara A, Kumar A, Verma G, Sharma M. Cephalosporin-resistant *Shigella flexneri* over 9 years (2001–09) in India. *J Antimicrob Chemother* 2012; **67**(6): 1347-1353.

[8] Clinical and Laboratory Standards Institute (CLSI). *Performance standards for antimicrobial disk sceptibility testing*. Approved standard. 9th ed. CLSI document M2-A9.26:1. Wayne: Clinical and Laboratory Standards Institute; 2018.

[9] Magiorakos AP, Srinivasan A, Carey R, Carmeli Y, Falagas M, Giske C, et al. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 2012; **18**(3): 268-281.

[10]Li P, Li H, Lei H, Liu W, Zhao X, Guo L, et al. Rapid detection of *Acinetobacter baumannii* and molecular epidemiology of carbapenem-resistant *A. baumannii* in two comprehensive hospitals of Beijing, China. *Front Microbiol* 2015; **6**: 997.

[11]Abbasi P, Kargar M, Doosti A, Mardaneh J, Dalini SG, Dehyadegari MA. Real time pcr for characterization of enteroinvasive *E. coli* (eiec) in children with diarrhea in shiraz. *Ann Colorectal Res* 2014; **2**(3): e22721.

[12]Ojha SC, Yean Yean C, Ismail A, Banga Singh KK. A pentaplex PCR assay for the detection and differentiation of *Shigella* species. *Biomed Res Int* 2013; **2013**: 412370. doi: 10.1155/2013/412370.

[13]Kim HJ, Ryu JO, Song JY, Kim HY. Multiplex polymerase chain reaction for identification of shigellae and four *Shigella* species using novel genetic markers screened by comparative genomics. *Foodborne Pathog Dis* 2017; **14**(7): 400-406.

[14]Dallal MMS, Omidi S, Douraghi M, Ashtiani MTH, Yazdi MKS, Okazi A. Molecular analysis of integrons and antimicrobial resistance profile in *Shigella* spp. isolated from acute pediatric diarrhea patients. *GMS Hyg Infect Control* 2018; **13**: 1-6.

[15]Ke X, Gu B, Pan S, Tong M. Epidemiology and molecular mechanism of integron-mediated antibiotic resistance in *Shigella*. *Arch Microbiol* 2011; **193**(11): 767.

[16]Opintan J, Newman MJ. Distribution of serogroups and serotypes of multiple drug resistant *Shigella* isolates. *Ghana Med J* 2007; **41**(1): 8.

[17]Jomezadeh N, Babamoradi S, Kalantar E, Javaherizadeh H. Isolation and antibiotic susceptibility of *Shigella* species from stool samples among hospitalized children in Abadan, Iran. *Gastroenterol Hepatol Bed Bench* 2014; **7**(4): 218-223.

[18]Jesudason MV. *Shigella* isolation in Vellore, south India (1997-2001). *Indian J Med Re* 2002; **115**: 11-13.

[19]Jafari F, Hamidian M, Salmanzadeh-Ahrabi S, Bolfion M, Kharaziha P, Yaghobi M, et al. Molecular diagnosis and antimicrobial resistance pattern of *Shigella* spp. isolated from patients with acute diarrhea in Tehran, Iran. *Gastroenterol Hepatol Bed Bench* 2009; **1**(1): 11-17.

[20]MoezArdalan K, Zali MR, Dallal MMS, Hemami MR, Salmanzadeh-Ahrabi S. Prevalence and pattern of antimicrobial resistance of *Shigella* species among patients with acute diarrhoea in Karaj, Tehran, Iran. *J Health Popul Nutr* 2003; **21**(2): 96-102.

[21]Rolfo F, Marin GH, Silberman M, Pattin J, Giugnio S, Gatti B, et al. Epidemiological study of shigellosis in an urban area of Argentina. *J Infect Dev Ctries* 2012; **6**(4): 324-328.

[22]Sousa MÂB, Mendes EN, Collares GB, Péret-Filho LA, Penna FJ, Magalhães PP. *Shigella* in Brazilian children with acute diarrhoea: Prevalence, antimicrobial resistance and virulence genes. *Mem Inst Oswaldo Cruz* 2013; **108**(1): 30-35.

[23]Orrett FA. Prevalence of *Shigella* serogroups and their antimicrobial resistance patterns in southern Trinidad. *J Health, Popul & Nutr* 2008; **26**(4): 456.

[24]Shen Y, Qian H, Gong J, Deng F, Dong C, Zhou L, et al. High prevalence of antibiotic resistance and molecular characterization of integrons among *Shigella* isolates in Eastern China. *Antimicrob Agents Chemother* 2013; **57**(3): 1549-1551.

[25]Singh KKB, Ojha SC, Deris ZZ, Rahman RA. A 9-year study of shigellosis in Northeast Malaysia: Antimicrobial susceptibility and shifting species dominance. *J Public Health* 2011; **19**(3): 231-236.

[26]Zamanlou S, Ahangarzadeh Rezaee M, Aghazadeh M, Ghotaslou R, Babaie F, Khalili Y. Characterization of integrons, extended-spectrum β-lactamases, AmpC cephalosporinase, quinolone resistance, and molecular typing of *Shigella* spp. from Iran. *Infect Dis* 2018; **50**(8): 616-624.

[27]Pourakbari B, Mamishi S, Mashoori N, Mahboobi N, Ashtiani MH, Afsharpaiman S, et al. Frequency and antimicrobial susceptibility of *Shigella* species isolated in Children Medical Center Hospital, Tehran, Iran, 2001-2006. *Braz J Infect Dis* 2010; **14**(2): 153-157.

[28]Nikfar R, Shamsizadeh A, Darbor M, Khaghani S, Moghaddam M. A study of prevalence of *Shigella* species and antimicrobial resistance patterns in paediatric medical center, Ahvaz, Iran. *Iran J Microbiol* 2017; **9**(5): 277.

[29]Jing YZ, Guang CD, Hai YY, Qing TF, Yuan LX. Multi-drug resistance and characteristic of integrons in *Shigella* spp. isolated from China. *Biomed Environ Sci* 2011; **24**(1): 56-61.

[30]Ranjbar R, Aleo A, Giammanco GM, Dionisi AM, Sadeghifard N, Mammina C. Genetic relatedness among isolates of *Shigella sonnei* carrying class 2 integrons in Tehran, Iran, 2002–2003. *BMC Infect Dis* 2007; **7**(1): 62.

[31]Nógrády N, Király M, Borbás K, Tóth Á, Pászti J, Tóth I. Antimicrobial resistance and genetic characteristics of integron-carrier shigellae isolated in Hungary (1998–2008). *JMM* 2013; **62**(10): 1545-1551.