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ORIGINAL ARTICLE

Multi-locus sequence type analysis of *Shigellas* pp. isolates from Tehran, Iran

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ABSTRACT

Background and Objectives: Strains of *Shigella* spp. can cause shigellosis, or bacillary dysentery.that is a public health problem worldwide. The aim of this study was to describe the population structure and genetic relatedness of multidrug resistant *S. sonnei* and *S. flexneri* isolated during a one year period from children with diarrhea in Tehran, Iran.
Materials and Methods: A total of 70 *Shigella* spp. were detected during the study period . Twenty MDR isolates of *Shigella* spp. were randomly selected and used in this study. Bacterial identification was performed by conventional biochemical and serological and confirmed by molecular method. After antimicrobial susceptibility testing, we used Multilocus sequence typing (MLST) for subtyping isolates.
Results: We found 14 *Shigella sonnei* and 6 *Shigella flexneri* isolates. Results of MLST showed five sequence types (ST) (145, 152, 241, 245, 1502) and BURST analysis revealed the largest number of single locus variant (SLV) and highest frequency (FREQ) for ST152.
ST 152 with nine members was predicted as the founder by BURST. Frequency for ST 1502 and ST 245 was four isolates and the least frequency was seen for ST 241 and 145 with one and two members, respectively. ST 145 and ST 245 were described as singletons in BURST. All isolates with ST145 and ST245 were identified as *Shigella flexneri*.
Conclusion: Annual Multi locus sequence typing of MDR *Shigella* would help us in better understanding of dominant species and comparing our results with the same studies in other countries especially our neighbor countries in source tracking purposes.

Keywords: *Shigella*, Multilocus sequence typing, Multidrug resistant

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INTRODUCTION

Shigella is a genus in the family Entrobacteriaceae that can cause shigellosis with diarrhea, fever and abdominal cramps. Shigellosis may vary from self-limited disease in healthy adults to severe in young children, elderly and immune compromised patients.

Severe cases can be associated with seizures in children less than 2 years old, Reiter's syndrome and Haemolytic Uremic Syndrome (1).

Epidemiological studies reveal 1,000,000 deaths per year caused by shigellosis in world (2). A total of 500000 illnesses have been generated by *Shigella* in the United States per year whereas shigellosis incidence in six country of Asia was about 100-folds higher than in industrialized nations (2-3).

Different rate of morbidity, mortality and prevalence of *Shigella* spp. have been observed in various geographical regions.

The most cases of shigellosis usually recover without antibiotic treatment but it can be lethal for people with immunosuppressed systems. Therefore, application of a treatment plan is imperative (2). Treatment challenges may especially occur in severe cases with multidrug resistant (MDR) isolates which are characterized as resistance to more than three antibiotic classes (3-4). Antimicrobial resistance patterns and prevalence of serotypes vary in different geographicalregions (5). Bacteria can acquire and disseminate genes for resistance to antibiotics through mobile genetic elements in their evolutionary pathways. The genetic structure of natural population of bacteria changes according to pattern and frequency of recombination and horizontal exchange of these element (4, 6, 7).

Shigella are human adapted *E. coli* that have obtained the new abilities (8). Virulence and invasion plasmid antigen H (*ipaH*) genes are located on the chromosome and the large plasmid PINV (9).

There are many typing methods based on phenotypic or genotypic properties of bacteria that are selected based on the epidemiological approaches. MLST method is based on sequencing of at least seven housekeeping genes, assigning the alleles at the seven loci and allocate the sequence type of each isolate. The provided databases are comparable with other data in the world and allow molecular typing of bacteria via internet (10). MLST is a gold standard method for epidemiological studies and was proved to be discriminatory, accurate, portable and reproducible over the years (11).

The aim of this study was to describe the population structure and genetic relatedness of multidrug resistant *S. sonnei* and *S. flexneri* isolated during a one year period from children with diarrhea in Tehran, Iran.

MATERIALS AND METHODS

Bacterial strains. A total of 70 *Shigella* spp. isolates were detected during study period from 5291 investigated stool samples (1.32 %) and these included *S. sonnei* (n=61, 87.14%) and *S. flexneri*. (n=8), (11.43%) based on the serological and molecular methods used for identification. As low as 1.43% of isolates were characterized as *S. boydii* and no *S. dysentriae* was recovered from the patients. The majority of isolates were isolated during November and December 2012 and August, September and October 2013 among which 20 *Shigella* spp. were randomly selected and used in this study. Selection was performed according to frequency of isolation of *Shigella* spp. in each month and accordingly, 14 (70%) *S. sonnei* and 6 (30%) *S. flexneri* were selected (Table 1). Bacterial isolates were identified using both conventional biochemical tests and serology using agglutination with specific A-D antisera (Baharafshan Institute of Research & Development, Tehran, Iran) (12). The identity of isolates was also confirmed by PCR amplification of *ipaH, wbgZ* and *rfc*genes, specific for *Shigella* spp., *S. sonnei* and *S. flexneri*, respectively (13-14).

The primer sequences used in this study are shown in Table 2 and all of primers were synthesizedby TAGC Company (Tag Copenhagen A/S Kong GeorgsVej 12 DK-2000 Frederiksberg Denmark). PCR was performed in a reaction with total volume of 25 µL, containing 2.5 µL 10x Taq polymerase buffer, 0.3 µL dNTPs (10 $mmol.L^{-1}$), 1 U Taq DNA polymerase (Fermentas, Lithuania), 0.6 µL $MgCl_2$ (50 $mmol.L^{-1}$) and 0.3 $mol.L^{-1}$ of each primer. PCR was done as follows: initial denaturation step at 94°C for 5 min, followed by 30 cycles consisting of denaturation (at 94°C for 1 min), annealing (1 min, separately adjusted for each set of primer pairs according Table 2), extension (at 72°C for1 min), followed by a final extension step at 72°C for 5 min. Finally PCR products were assessed for specific band on agarose gel (90 volt, 45min).

Antimicrobial susceptibility testing. Antimicrobial susceptibility testing was performed for minocycline (30µg), tetracycline (30µg), doxycycline (30µg), ampicillin (10µg), trimethoprim-sulfamethoxazole (25µg), nalidixic acid (30µg), norfloxacin (10µg), ciprofloxacin (5µg), levofloxacin (5µg) and streptomycin (10µg) by disk diffusion method ac-

cording to CLSI guidelines. *E. coli* 25922 was used as the control strain.

Multi-locus sequence typing (MLST). The genomic DNA from *Shigella* isolates was extracted using Exgene Cell SV kit (Genen all Biotechonolgy Co. Ltd Korea) that were grown in LB broth medium at 37°C overnight.

Seven housekeeping genes: *adk* (adenylate kinase), *fumC* (fumaratehydratase), *gyrB* (DNA gyrase), *icd*

Table 1. Bacterial isolates under study

Date of Isolation	Number of diarrheal stool samples	No. of *Shigella* spp. isolated	Number of *Shigella* spp. Selected for MLST analysis
November/2012	959	30	7
December/2012	693	18	4
January/2013	292	0	0
February/2013	312	2	1
March/2013	333	0	0
April/2013	356	0	0
May/2013	415	0	0
June/2013	403	1	1
July/2013	353	2	1
August/2013	385	4	2
September/2013	414	5	2
October/2013	357	8	2
Total	5291	70	20

Table 2. Oligonucleotide primers used in this study.

Primers	Sequence (5'→3') Annealing	Annealing °C	Amplicon size (bp)
ipaH-F	GTTCCTTGACCGCCTTTCCGATACCGTC	60	619
ipaH-R	GCCGGTCAGCCACCCTCTGAGAGTAC		
wbgZ-F	TCT GAATATGCCCTCTACGCT	60	430
wbgZ-R	GACAGAGCCCGAAGAACCG		
rfc-F	TTTATGGCTTCTTTGTCGG	60	537
rfc-F	CTGCGTGATCCGACCATG		
adkF	ATTCTGCTTGGCGCTCCGGG	57	583
adkR	CCGTCAACTTTCGCGTATTT		
fumCF	TCACAGGTCGCCAGCGCTTC	57	805
fumCR	GTACGCAGCGAAAAAGATTC		
gyrBF	TCGGCGACACGGATGACGGC	59	879
gyrBR	ATCAGGCCTTCACGCGCATC		
icdF	ATGGAAAGTAAAGTAGTTGTTCCGGCA	58	877
icdR	GGACGCAGCAGGATCTGTT		
mdhF	AGCGCGTTCTGTTCAAATGC	56	798
mdhR	CAGGTTCAGAACTCTCTCTGT		
purAF	TCGGTAACGGTGTTGTGCTG	57	816
purAR	CATACGGTAAGCCACGCAGA		
recAF	ACCTTTGTAGCTGTACCACG	56	634
recAR	AGCGTGAAGGTAAAACCTGTG		

(isocitrate/isopropylmalate dehydrogenase), *mdh* (malate dehydrogenase), *pur*A (adenylosuccinate dehydrogenase), *recA* (ATP/GTP binding motif) were amplified using the primers and PCR conditions described in protocols introduced for MLST of *E. coli* (15).

Primers sequences are shown in Table 2. PCR products were purified and both DNA strands were sequenced using ABI 3730X capillary sequencer (Macrogen, Seoul, Korea).

Data analysis. Sequenced data were read by Lasergene 66 software and trimmed according to database of MLST Home and National Center for Biotechnology Information (NCBI) for each housekeeping genes. Sequences were compared with MLST databases of *E. coli* and were identified allelic profiles and Sequence Types. Data of MLST were analyzed by BURSTalgorithm and clonal complex were determined based on SLV(16).

We used the Minimum spanning tree (MST), a graphical tool according to Prim's algorithm, to draw a tree using MLST allelic profile data. Phylogenetic analysis was done by MLST Philip program based on neighbor-joining software using sequences of the seven housekeeping genes for each STs.

RESULTS

Bacterial strains. Twenty isolates of *Shigella* spp. were selected and used in this study consisting of 14 (70%) *S. sonnei* and 6 (30%) *S. flexneri.*

All *Shigella* spp. harboured *ipaH* gene specific for *Shigella* genus with an amplification band of 619bp, while simultaneously all *S. sonnei* and *S. flexneri* isolates produced 430bp and 537bp bands related to *wbgZ* and *rfc*, respectively, followed by sequencing which confirmed the identity of both species (Table 2). The results of serogrouping were in agreement with molecular identification method.

Antimicrobial susceptibility testing. The results of antimicrobial susceptibility revealed high prevalence of resistance to streptomycin, trimethoprim-sulfamethoxazole and tetracycline. All isolates were susceptible to quinolones including norfloxacin, levofloxacin and ciprofloxacin. The majority of isolates were multi drug resistant (MDR) with seven patterns of resistance. The result of antimicrobial susceptibility of isolates is shown in Table 3.

MLST, BURST, minimum spanning tree and phylogenetic tree. Electrophoresis of PCR products revealed the bands that specified by 879bp (*adk*), 805bp (*fum*), 879bp (*gyr*), 877bp (*icd*), 798bp (*mdh*), 816bp (*pur*) and 634bp (*rec*A) (Table 2).

A comparison between the *E. coli* sequences of seven housekeeping genes available at MLST and data obtained in this study, the allelic profiles, STs and ST complexes were determined. A total of five STs (145, 152, 1502, 241, 245) were found for twenty isolates while no new ST was detected in this study (Table 4).

The largest number of single locus variant (SLV), n=2, and the highest frequency (FREQ) for ST152, n=9, were revealed by BRUST analysis (Table 5). ST 152 and ST 241 together fell in ST complex 152, all of which were identified as *S. sonnei* and constituted about 50% of total isolates under study (Table 4).

ST 152 with the greatest number of SLV was as predicted founder. Frequency for ST 145 and ST 245 was low. Allelic profile of ST145 and ST245 were more different compared to other isolates so described as singletons in BURST (Table 5). All isolates in ST145 and ST245 were identified as *S. flexneri* (Table 4). ST 152 was predicted as the founder (ST that has the greatest number of single-locus variants) by BURST with 9 isolates falling in this ST. Frequency for both ST 1502 and ST 245 was four, while one and two isolates fell in ST 241 and 145 respectively. Allelic profile of ST145 and ST245 were more substantially different compared to other isolates so described as singletons in BURST (Table 5). All isolates with

Table 3. Antimicrobial susceptibility testing

Antibiotics	Sensitive %	Intermediate %	Resistant %
Ampicillin	76	0	24
Ciprofloxacin	98	2	0
Doxycycline	4	38	58
Levofloxacin	98	2	0
Minocycline	38	62	0
Nalidixic Acid	80	2	38
Norfloxacin	100	0	0
Streptomycin	0	0	100
Tetracycline	4	5	96
Trimethoprim	2	0	98

these 2 allelic profiles (n=6) were identified as *S. flexneri* (Table 4).

Output of MST indicated five circles (STs) which are linked together. In topological arrangement of MST, each circle represents one ST, also length of lines show distance between sequence types. Analysis of STs according to MST also revealed the highest priority for ST152 with the largest SLV in this study (Fig. 1).

Phylogenetic analysis also revealed the ST152 as the root of tree that was predicted as ancestor for *Shigella* isolates of this study.

Similar results of BURST, Minimum spanning tree and phylogenetic tree also emphasize on ST152 as the predicted founder of our isolates (Fig. 1 and Table 5).

DISCUSSION

According to previous studies in Iran, *S. flexneri* was the most common serogroup during 2001-2006, while the predominant serogroup was *S. sonnei* during 2008-2012 (17). The present study revealed a higher frequency of *S. sonnei*, compared to other *Shigella* species in Iran during 2012-2013, which imitates the infection profile of more developed countries. One reason for the rise in the incidence of *S. sonnei* can be the improving hygiene level in Iran and the industrialization of the capital city. Although, the increased environmental adaptation of *S. sonnei* should not be ignored (18-19).

The majority of *Shigella* isolates (95%) in this study fulfilled the MDR criteria, however the antimicrobial resistance pattern was different among the isolates.

The results of antimicrobial susceptibility assay revealed a high resistance, among the *Shigella* isolates, against streptomycin, trimethoprim-sulfamethoxazole and tetracycline. Despite the CLSI recommendation for the use of trimethoprim-sulfamethoxazole (TMP) for fecal isolates of *Shigella*, its use is nowadays limited due to the high resistance of this organism (20). Previous reports in Iran have reported a 92.2% to 94%, resistance to TMP during 2000-2011(1, 18, 21). A high level resistance to TMP has also been reported from Nepal (81.54%), and the USA (66%) (22-23). The wide distribution of resistance to TMP can be attributed to over prescription or misuse of the antibiotics in clinics (24). All TMP resistant isolates in the current study were also resistant to streptomycin (Table 3). Simultaneous resistance to streptomycin and TMP can probably be related to a 6.3kb plasmid that was investigated by Barman and colleagues (2010) or other mobile resistance genetic elements (25).

As with tetracycline, 10% of the isolates were sus-

Table 4. Sequence types, ST complexes and allelic profiles in relation to Shigellasero groups and antimicrobial resistance patterns.

ST	Code number	ST complex	Species	Resistance profile
152	5	152	*S. sonnei*	Te,sxT,S
152	6	152	*S. sonnei*	Te,sxT,S
152	2	152	*S. sonnei*	Te,sxT,S
152	17	152	*S. sonnei*	Te,D,sxT,S
152	12	152	*S. sonnei*	Te,D,sxT,S
152	16	152	*S. sonnei*	Te,D,sxT,S
152	18	152	*S. sonnei*	Te,D,sxT,S
152	15	152	*S. sonnei*	Te,D,sxT,S,,NA
152	9	152	*S. sonnei*	Te,D,sxT,S,,NA
241	8	152	*S. sonnei*	Te,D,sxT,S,,NA
1502	1	None	*S. sonnei*	Te,sxT,S
1502	11	None	*S. sonnei*	Te,D,sxT,S
1502	20	None	*S. sonnei*	Te,D,sxT,S
1502	13	None	*S. sonnei*	Te,D,sxT,S,,Am
245	3	245	*S. flexneri*	Te,sxT,S,Am
245	10	245	*S. flexneri*	Te,sxT,S,Am
245	4	245	*S. flexneri*	Te,D,S,Am
245	7	245	*S. flexneri*	Te,sxT,S,,Am
145	14	243	*S. flexneri*	sxT,S,Am,NA
145	19	243	*S. flexneri*	sxT,S

Table 5. Results of BURST analysis.

ST	No. of isolates	FREQ	SLV	DLV	TLV	Average Distance
Group	**14**					
152*		9	2	0	0	1.0
1502		4	1	1	0	1.5
241		1	1	1	0	1.5
Singleton	**6**					
145		2				
245		4				

* Predicted Founder; ST, sequence type;FREQ, Frequency; SLV, Single -Locus Variant; DLV, Double -Locus Variant; TLV, Triple- Locus Variant.

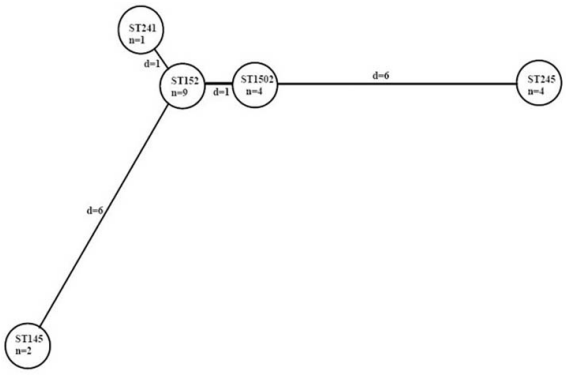

Fig. 1. Minimum spanning treeprim: Minimum spanning tree with cost, d: distance, n: number, ST: Sequence type. Each ST is represented by a circle.

ceptible to doxycycline. According to the previous studies in Iran and other countries, resistance to tetracycline might be an intrinsic characteristic among *Shigella* spp, being observed in the majority of clinically isolated strains (18, 26).

Two isolates which were recognized as ST145 (*S. flexneri*) were susceptible to tetracycline which rules out the intrinsic characteristic of resistance to this antibiotic among *S. flexneri* strains.

The results showed 25% and 5% resistance to ampicillin among *S. flexneri* and *S. sonnei*, respectively. This phenomenon which was also reported by Seidlein and colleagues (2006) reveals a higher ampicillin resistance among *S. flexneri* compared with *S. sonnei* isolates (3).

Resistance to nalidixic acid was shown to be 12.5% among *S. flexneri* isolates and 16.5% among the *S. sonnei* isolates. Approximately similar results were obtained for both species. Due to the rise in antimicrobial resistance among *Shigella* isolates it can be concluded that antimicrobial susceptibility testing must be done to warrant the effectiveness of prescribed antimicrobial agents. Fortunately, all isolates in this study were susceptible to fluoroquinolones including; ciprofloxacin, levofloxacin and norfloxacin. Other studies in Iran have revealed similar results for fluoroquinolones (18, 19, 21), except for Gharibi and colleagues (2012) which reported a 4.25% resistance to ciprofloxacin among *Shigella* isolates in Bushehr province of Iran (27). There also exist some discrete reports on resistance to these drugs in other countries (28). Excessive use of ciprofloxacin as the first line treatment for shigellosis may be the cause of increasing resistance to this drug. Totally, the low rate of resistance to fluoroquinolones makes these drugs a better for the treatment.

Result of MLST Philip was more suitable for this study and ST152 was considered as a root in this tree and the branch lengths were similar to MST.

Our results revealed five ST (145, 152, 241, 245, and 1502) among 20 *Shigella* isolates with ST 152 comprising the most frequent type which was indicated as predicted founder in this study. ST 152 for *Shigella* isolates was also reported from other countries of all continents (Table 6). The ST152 as the dominant ST with the MDR phenotype is of great significance which can render the treatment of shigellosis more challenging.

ST152 was closely related to ST1502 and ST241 which were two single locus variant for ST152. All the strains in ST152, ST1502 and ST 241 were recognized as *S. sonnei.* This results is in agreement with the studies by Cao & Wei and Inouye et al. (2012) for ST 241 and ST 1502 (Table 6). In these studies, *Shigella* spp. were typed to ST152 and ST241 with ST152 comprising the predicted founder which is in consistent with our study, however both *S. flexneri* and *S. sonnei* species were included in ST152 in the above mentioned studies (29), which was in contrast with the results of our study.

Two isolates fell in ST145 (ST complex 243) and four isolates in ST245 (ST complex 245), both of which were identified as singletons. In the present study, all strains in ST145 and ST245 were identified as *S. flexneri*, aphenomenon which was also reported by Wirth et al. (2006). However, ST 245 encompassed *S. boydii* and *S. flexneriin* the study of Cao & Wei (2012). This suggests that probably *S. flexneri* is derived from a distinct parental clone (8).

It is notable that only isolates with ST145 properties were susceptible to tetracycline which suggests the hypothesis that ST specification of the isolates may affect the acquisition and/or expression of antimicrobial resistance properties.

All the STs in the current study have been previously reported in other studies for *Shigella* and so we did not find any new sequence type which may be related to i) population size or ii) geographic distribution of specific *Shigella* sequence types or to iii) the *Shigella* genome stability (2, 15, 29). Although other sequence types of *Shigella* spp. have been identified throughout the world (Table 6) but there are still insufficient data on the incidence of *Shigella* STs in different geograph-

ical locations which emphasizes the need to annual monitoring and MLST sequence typing of outbreak and sporadic *Shigella* strains for better understanding of distribution and dominant sequence types in the world.

Table 6. Geographical distribution of *Shigella* spp. sequence types

	species	Allelic profile	Country/ Continent	Year of isolated	References
152	*S. sonnei*	11 63 7 1 14 7 7	Iran	2012	This study
152	*S. sonnei*	11 63 7 1 14 7 7	China	2009	Cao, Y. & Wei, D. (2012)
152	*S. flexneri*	11 63 7 1 14 7 7	China	2010	Cao, Y. & Wei, D. (2012)
152	*S. sonnei*	11 63 7 1 14 7 7	Germany	1997	Cao, Y. & Wei, D. (2012)
152	*S. sonnei*	11 63 7 1 14 7 7	Asia, Africa America, Europe	1943-2008	Inouye et al (2012)
152	*S. sonnei*	11 63 7 1 14 7 7	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
241	*S. sonnei*	11 63 6 1 14 7 7	China	1997	Cao, Y. & Wei, D. (2012)
1502	*S. sonnei*	6 63 7 1 14 7 7	Asia, Africa America, Europe	1943-2008	Inouye et al (2012)
245	*S. flexneri*	6 61 6 11 13 3 50	Iran	2012	This study
245	*S. flexneri*	6 61 6 11 13 3 50	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
245	*S. flexneri*	6 61 6 11 13 3 50	China	1983	Cao, Y. & Wei, D. (2012)
245	*S. flexneri*	6 61 6 11 13 3 50	Germany	1997	Cao, Y. & Wei, D. (2012)
245	*S. flexneri*	6 61 6 11 13 3 50	Canada	1982	Cao, Y. & Wei, D. (2012)
245	*S. flexneri*	6 61 6 11 13 3 50	Asia, Africa America, Europe	1943-2008	Inouye et al (2012)
245	*S.boydii*	6 61 6 11 13 3 50	China	2009	Cao, Y. & Wei, D. (2012)
145	*S. flexneri*	1 10 1 1 1 1 1	Asia+Pacific, Africa America, Europe		Wirth et al. (2006)
149	*S. boydii*	6 60 60 3 6 6 3	Asia+Pacific, Africa America, Europe		Wirth et al. (2006)
151	*S. sonnei*	11 62 7 1 14 7 7	Germany	1997	Cao, Y. & Wei, D. (2012)
240	*S. flexneri*	6 61 4 11 13 3 50	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
243	*S. boydii*	1 4 1 1 1 1 1	Asia, Africa America, Europe	1943-2008	Inouye et al (2012)
243	*S.boydii, S.flexneri S.dysenteriae*	1 4 1 1 1 1 1	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
248	*S. flexneri*	6 74 6 66 13 3 50	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
250	*S.boydii, S.dysenteriae*	6 59 60 3 47 3 3	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
255	*S. flexneri*	6 61 4 11 48 3 50	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
259	*S. flexneri*	6 78 6 11 13 3 50	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
262	*S. flexneri*	1 6 1 1 1 1 1	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
264	*S. flexneri*	6 61 68 11 13 3 55	Asia+Pacific, Africa America, Europe		Wirth et al., 2006
412	*S.boydii*	95 111 91 99 68 70 76	Bangladesh		Cao, Y. & Wei, D. (2012)
626	*S. flexneri*	116 61 6 11 1 3 50	Taiwan	1991	Choi et al (2007)
627	*S. flexneri*	6 61 6 11 13 97 50	Korea	2002	Choi et al (2007)
628	*S. flexneri*	6 61 6 11 13 3 55	Philippine	1981-2000	Choi et al (2007)
629	*S. flexneri*	6 145 6 11 13 3 55	Korea		Choi et al (2007)
630	*S. boydii*	6 61 6 11 6 95 7	Asia+Pacific, ,Africa America,Europe		Wirth et al., 2006
631	*S. flexneri*	6 74 6 123 13 3 50	France		Choi et al (2007)
632	*S. flexneri*	1 10 1 1 1 96 1	Taiwan		Choi et al (2007)
633	*S. flexneri*	6 61 6 11 1 3 98 50	Japan	2006	Choi et al (2007)
634	*S. flexneri*	6 61 6 123 13 3 50	China	2002	Nie et al., 2006
651	*S. flexneri*	6 14 9 6 11 13 3 50	China	1943-2008	Choi et al (2007)
1025	*S. boydii*	6 61 6 174 13 3 50	Asia, Africa America, Europe	1943-2008	Inouye et al (2012)
1504	*S. sonnei*	11 63 7 1 14 17 2 7	Asia, Africa, America ,Europe	1943-2008	Inouye et al (2012)
1505	*S. sonnei*	11 63 7 1 14 17 3 7	Asia, Africa America, Europe	2010	Inouye et al (2012)
2208	*S. sonnei*	45 240 192 99 91 159 142	China		Cao, Y. & Wei, D. (2012)

CONCLUSION

This study was the first report of MLST genotyping of MDR resistant *Shigella* spp. in Iran which determined five sequence types with ST 152 as the dominant sequence type being composed of *S. sonnei* isolates. Resistance to ampicillin was most frequently observed in ST245 and susceptibility to tetracycline was only found in ST 145 which could indicate a common mechanism of resistance acquisition. These results emphasized the need to monitor and evaluate the resistance profile change among sequence types for better prevention, control and treatment of shigellosis.

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