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Clonal dissemination of a single *Shigella sonnei* strain among Iranian children during Fall 2012 in Tehran, I.R. Iran

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ABSTRACT

Shigella species are a common cause of bacterial diarrhea worldwide and the disease is characterized by seasonality. *Shigella* has been encountered by widespread resistance to commonly used antibiotics which is a serious concern. The aim of this study was to analyze the epidemiological relatedness of *Shigella* strains isolated from children during one year period by PFGE method and to investigate antimicrobial resistance determinants and cassettes among *Shigella* species. The occurrence of *Shigella* spp. in the present study was 1.32% during the study period and the majority of cases (56 (80%)) were occurred during autumn while *Shigella sonnei* was the most prevalent species identified. Multi-drug resistance phenotype was seen in 98.5% of total isolates with $SXT^r/TE^r/TMP^r$ resistance pattern. Among the 70 *Shigella* spp. analyzed in this study, 16 isolates were positive for class I integron (*int1*$^+$) with two types of gene cassette arrays (*dfrA17*/*aadA5* and *dfrA7*).The class 2 integron was more frequently detected among the isolates (85.71%) with *dfrA1*/*sat1*/*aadA1* (10%) and *dfrA1*/*sat1* (75.71%) gene cassettes. The *tetA* and *tetB* determinants were observed in 75.7% and 21.42% of *Shigella* isolates and *tet*(*A*) was the foremost in *S. sonnei* and *Shigella flexneri* population. In this study 5 tetracycline resistant isolates had no tetracycline resistance gene (A–D) and no association was recognized between the value of MIC against tetracycline and the *tet* genes content of isolates. Fifty three of total *Shigella* isolates (75.7%) showed an identical PFGE patterns. Seven PFGE clusters observed in our study were composed of members with one to three band variations, which is indicative of closely related isolates. The major cluster (cluster C) constituted 75.7% of total isolates, all of which (except eight isolates) consonantly showed identical class 2 integron of 1500 bp which strongly suggests the dissemination of a single *S. sonnei* clone among the pediatric population in 2012 autumn in Tehran, Iran, in comparison with the equal data from the comparable time period from recent years.

1. Introduction

Shigellosis still remains a serious public health problem and an important cause of morbidity and mortality in developed as well as undeveloped countries (Bennish and Wojtyniak, 1991).The species of *Shigella* that cause the diarrheal disease have been divided into 4 subgroups (A–D), with the common classification as *Shigella dysenteriae*, *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei*, respectively (van der Ploeg et al., 2010). In industrialized countries *S. sonnei* is the epidemic subgroup of *Shigella* and repeatedly is considered as the dominant subgroup in these countries (Kotloff et al., 1999). In epidemiological studies, finding the source of an infection is a prominent step in controlling the transmission and spread of the epidemic strain (Mammina et al., 2005).

Information about the molecular mechanisms of antimicrobial resistance is implemented for intervention strategies. Among the genetic elements contributing in the antimicrobial resistance, integrons as mobile elements with genetic platforms for gene cassettes encoding and transferring determinants of antimicrobial resistance play important role, especially in Gram negative bacteria including *Shigella* spp. (Gootz and Marra, 2008).

Changes within *Shigella* spp. genome and increase of multi drug resistance (MDR) among *Shigella* are one of the major concerns in developing countries (Wong et al., 2010). This high resistance is due to the acquisition and transfer of resistance determinants via horizontal transfer of the resistance plasmids, transposons and integrons that may lead to higher fatality rates (Spicknall et al., 2013).

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Tetracycline which is a broad-spectrum antibiotic that can inhibit protein synthesis in a wide range of Gram positive and Gram negative organisms (Nelson and Levy, 1999; Nelson et al., 1993).Currently, the use of this antibiotic in the treatment of human infections and prevention of bacterial infections in agriculture and livestock increases the rate of resistance to this class of antimicrobial agents (Chopra and Roberts, 2001).One of the major mechanisms involved in the resistance to tetracycline is the efflux pump systems including *tet*(*A*), (B), (C), (D), (E), (G), (I), (M) and (K) genes, the first four genes are more widespread in Gram negative bacteria including *Shigella* spp. (Roberts, 1996).

Recently, accesses at the molecular level have been used to estimate the relatedness of bacterial isolates. Pulsed-field gel electrophoresis (PFGE) is one of the most discriminatory, sensitive and reliable typing method that is used to detect a high degree of DNA polymorphism in epidemiological studies (Woodward et al., 2005).

The objectives of this study were (i) to analyze the epidemiological relatedness among *Shigella* strains isolated from children during one year period on the basis of PFGE patterns and (ii) a more comprehensive study of species-specific distribution of *tet* determinants and integron associated resistance gene cassettes in *Shigella* genus.

2. Materials and methods

2.1. Bacterial strains and detection of Shigella by biochemical test and API20E strip

From 2012 November to 2013 October, 5291 stool specimens were collected from 3 major hospitals in Tehran, Iran. The specimens were collected from children under 5 years of age with diarrhea characterized by $\geqslant 3$ episode of loose or watery stools with or without blood, mucus and stomach cramps. For preliminarily detection of *Shigella* spp., the specimen were cultured on selective agar plates including *Salmonella-Shigella* agar and XLD agar (Merck, Hamburg, Germany) (Ewing, 1986). Putative *Shigella* organisms were selected and preliminarily characterized by biochemical tests (oxidase, urease, TSI, motility and carbohydrate fermentation) (Perilla et al., 2003) and confirmed by API-20E strip kit according to manufacturer's instructions (API-bioMérieux, Inc., La Balme les Grottes, France). *S. sonnei* ATCC 9290, *S. flexneri* ATCC 12022 were used as controls in each assay. *Shigella boydi* and *S. dysenteriae* control strains were kindly provided by World Health Organization (WHO).

2.2. Molecular identification of Shigella spp. and serogroup determination

The isolates were identified by the conventional biochemical tests and presumptive isolates were confirmed as *Shigella* spp. by PCR for *ipaH* gene. Species identification was performed with additional biochemical tests and serogrouping method. For this purpose, *Shigella* isolates were subcultured and tested for agglutination on glass slides by means of commercially available polyclonal antisera (Baharafshan Institute of Research & Development, Tehran, Iran) using standard slide agglutination method (Guardabassi et al., 2000).

Additional primer pairs specific for *S. sonnei* and *S. flexneri* were used to confirm the accuracy of two most commonly found *Shigella* species. The primer names, their sequences and amplification conditions are presented in Table 1.

2.3. Antimicrobial susceptibility test

Antimicrobial susceptibility testing was performed by the disc diffusion method for augmentin (25 μg), co-trimoxazole (25 μg), tetracycline (30 μg), minocycline (30 μg), gentamicin (10 μg), streptomycin (10 μg), ciprofloxacin (5 μg) and nalidixic acid (30 μg) (Difco Laboratories, Detroit, USA) according to CLSI guideline (CLSI, 2012). *Escherichia coli* ATCC 25922 was used for quality control in antimicrobial susceptibility testing.

2.4. Minimal inhibitory concentration (MIC) of tetracycline

Extended serial dilutions ranging from 2 to 256 μg/ml was prepared and MIC of tetracycline was determined for 12–16 h culture of each isolate through agar dilution method according to CLSI guidelines (CLSI, 2006).

2.5. Determination of resistance associated genes and cassettes

The presence of class 1 integron was investigated by PCR amplification of the conserved integrase gene with specific set of primers (*int*). Furthermore, the content of integron was explored using PCR

Table 1
Primer sequences used in this study.

Target genes	Primer sequence	Amplicon size (bp)	Annealing temp.	References
ipaH	GTTCCTTGACCGCCTTTCCGATACCGTC GCCGGTCAGCCACCCTCTGAGAGTAC	619	55 °C	Sethabutr et al. (1993)
tet(*A*)	GTGAAACCCAACATACCCC GAAGGCAAGCAGGATGTAG	927	55 °C	Ng et al. (1999)
tet(*B*)	CCTTATCATGCCAGTCTTGC ACTGCCGTTTTTTCGCC	659	53 °C	Ng et al. (1999)
tet(*C*)	CTTGAGAGCCTTCAACCCAG ATGGTCGTCATCTACCTGCC	418	55 °C	Ng et al. (1999)
tet(*D*)	TGGGCAGATGGTCAGATAAG CAGCACACCCTGTAGTTTTC	787	53 °C	Ng et al. (1999
Int1	TGCGTGTAAATCATCGTCGT CAAGGTTCTGGACAGTTGC	900	53 °C	Adabi et al. (2009)
In	GGCATCCAAGCAGCAAGC AAGCAGACTTGACCTGAT	Variable	55 °C	Collis and Hall (1992)
hep	CGGGATCCCGGACGGCATGCACGATTTGTA GATGCCATCGCAAGTACGAG	Variable	55 °C	White et al. (2001b)
Sflex	TTTATGGCTTCTTTGTCGGC CTGCGTGATCCGACCATG	537	56 °C	Ojha et al. (2013)
Sson	TCT GAATATGCCCTCTACGCT GACAGAGCCCGAAGAACCG	430	56 °C	Ojha et al. (2013)
qacEΔ1-F sul1-B	ATCGCAATAGTTGGCGAAGT GCAAGGCGGAAACCCGCGCC	800	60	Stokes and Hall (1989) Sundström et al. (1988)