Profiling of Virulence-associated Factors in Shigella Species Isolated from Acute Pediatric Diarrheal Samples in Tehran, Iran

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**Objectives:** The genus *Shigella* comprises the most infectious and diarrheagenic bacteria causing severe diseases, mostly in children under five years of age. This study aimed to detect nine virulence genes (*ipaBCD*, *VirA*, *sen*, *set1A*, *set1B*, *ial*, *ipaH*, *stx*, and *sat*) in *Shigella* species (spp.) using multiplex polymerase chain reaction (MPCR) and to determine the relation of *Shigella* spp. from pediatric diarrheal samples with hospitalization and bloody diarrhea in Tehran, Iran.

**Methods:** *Shigella* spp. were isolated and identified using standard microbiological and serological methods. The virulence genes were detected using MPCR.

**Results:** Seventy-five *Shigella* spp. (40 *S. sonnei*, 33 *S. flexneri*, 1 *S. dysenteriae*, and 1 *S. boydii*) were isolated in this study. The prevalence of *ial*, *sen*, *sat*, *set1A*, and *set1B* was 74.7%, 45.4%, 28%, 24%, and 24%, respectively. All *S. flexneri* isolates, while no *S. sonnei*, *S. dysenteriae*, or *S. boydii* isolates, contained *sat*, *set1A*, and *set1B*. All isolates were positive for *ipaH*, *ipaBCD*, and *virA*, while one (1.4%) of the isolates contained *stx*. The highest prevalence of virulence determinants was found in *S. flexneri* serotype 2a. Nineteen (57.6%) of 33 *S. flexneri* isolates were positive for *ipaBCD*, *ipaH*, *virA*, *ial*, and *sat*. The *sen* determinants were found to be statistically significantly associated with hospitalization and bloody diarrhea (*p* = 0.001).

**Conclusion:** This study revealed a high prevalence of enterotoxin genes in *S. flexneri*, especially in serotype 2a, and has presented relations between a few clinical features of shigellosis and numerous virulence determinants of clinical isolates of *Shigella* spp.

**Key Words:** virulence gene, *Shigella*, pediatrics, diarrhea, gene profile, Iran

**INTRODUCTION**

Shigellosis, or bacillary dysentery, continues to be a public health concern worldwide, mainly in the underdeveloped and developing regions with poor hygiene and limited access to clean drinking water [1,2]. The genus *Shigella* is divided into four serogroups—*S. dysenteriae* (serogroup A), *S. flexneri* (serogroup B), *S. boydii* (serogroup C), and *S. sonnei* (serogroup D) [3]. Shigellosis is an invasive illness of the human colon that leads to varied clinical symptoms ranging from mild watery diarrhea to severe colitis [4]. The pathogenesis of shigellosis is related to...
various virulence factors located in the chromosome or large virulent inv plasmids [5]. Colonization—in which epithelial cell penetration and modification of the host response towards infection for dissemination from cell to cell occurs—is mediated by an invasion-associated locus (ial) and the invasion plasmid antigen H (ipaH) genes, respectively [6,7]. Chromosomal genes, set1A and set1B, encode the Shigella enterotoxin 1 (ShET-1), and are among the factors associated with the watery phase of diarrhea [8]. Shigella enterotoxin 2 (ShET-2) is involved in invasion and is located in large virulent plasmids [8].

ShET-1 and ShET-2, in addition to their enterotoxigenic activity, play an important role in the transport of electrolytes and water in the intestine [9]. Plasmid-encoded toxin (pet), secreted autotransporter toxin (sat), and Shigella IgA-like protease homologue (SigA) are members of the class 1 serine protease autotransporters of Enterobacteriaceae (SPATES) [8,9]. VirA are located on large virulent plasmids and act as virulence determinants in intercellular spreading and invasion [8–10]. Two distinct shiga toxins, stx-1 and stx-2, are encoded by chromosomal genes and expressed only by S. dysenteriae serotype 1 and are similar to the shiga-like toxins of enterohemorrhagic Escherichia coli (EHEC) [11]. These toxins lead to the expansion of vascular lesions in the kidney, central nervous system, and colon in a large number of cell types [12]. Because of the high toxicity of the shiga toxin, infections with S. dysenteriae serotype 1 commonly have life-threatening complications [13].

Numerous studies have been conducted on the prevalence and antimicrobial resistance of Shigella species, both in Iran and other countries [13,14]. The aim of the present study was to detect nine virulence genes (ipaBCD, VirA, sen, set1A, set1B, ial, ipaH, stx, and sat) in Shigella species (sp.) using the multiplex polymerase chain reaction (MPCR) and to determine the relation of Shigella spp. from pediatric diarrheal samples with hospitalization and bloody diarrhea in Tehran, Iran.

MATERIALS AND METHODS

1. Clinical samples and laboratory identification

Seventy-five Shigella strains, including S. sonnei (n = 40), S. flexneri (n = 33), S. dysenteriae (n = 1), and S. boydii (n = 1), were used in this cross-sectional study. These strains were isolated from 946 non-duplicate stool samples from pediatric patients with diarrhea in Tehran, Iran, during an 18-month period from May 2015 to October 2016. The presence or absence of bloody diarrhea and any history of hospitalization were reported by the individual responsible for the clinical evaluation.

Cary-Blair transport medium (Oxoid, Basingstoke, Hampshire, UK) was used for sample transportation to the laboratory, where each sample was subjected to immediate testing. In the laboratory, all specimens were cultured in different differential media, including xylose lysine deoxycholate (XLD) agar and Hektoen enteric agar (HEA) (Merck, Darmstadt, Germany), and then incubated at 37°C for 24 hours. All grown colonies were identified using a conventional biochemical culture base and a microbiological API 20E kit (bioMérieux, Marcy l’Etoile, France). Serological tests were performed on the Shigella strains using the slide agglutination method [14]. The serotypes of all Shigella isolates were determined with commercially available polyclonal- and monoclonal-specific antisera (Denka Seiken, Tokyo, Japan) against all Shigella serotypes, including S. sonnei 1 and 2, polysen, S. flexneri, S. dysenteriae 1, and polyvalent S. boydii. S. boydii ATCC 9207, S. dysenteriae ATCC 13313, S. sonnei ATCC 1202, and S. flexneri ATCC 9290 were used as quality controls in each test. All strains were stored in Luria-Bertani broth containing 50% glycerol at −80°C until use.

2. MPCR method

Each sample was subjected to MPCR amplification using 14 pairs (nine virulence genes and five species-specific genes) of different primers (Table 1 [15–18]). MPCR with various Tm details are shown in Table 1. MPCR was performed using a polymerase chain reaction (PCR) instrument with mastercycler gradient (PEQLAB, Erlangen, Germany) for the detection of various virulence- and species-specific genes (set1A/set1B, ial/virA, sen/ipaBCD, sat, stx, and ipaH). The overnight-grown colonies on the XLD agar plates were picked for template genomic DNA extraction by the boiling method. The total volume of the MPCR mixture was 20 μL, containing 0.5 μL extracted template DNA, 2.0 μL 10× PCR buffer, 0.5 μL MgCl2, (50 mM), 0.5 μL deoxynucleotides (10 mM), 0.5 μL each virulence gene primer, 0.5 μL Taq DNA polymerase (5 U/μL) (Amplicon Co., Copenhagen, Denmark), and 13 μL dH2O (In set1A/set1B, 2 μL H2O was added). The MPCR conditions for the amplification of virulence genes included an initial denaturation at 94°C for 60 seconds, 35 cycles of denaturation at 94°C for 60 seconds, annealing at 58°C (variable) for 90 seconds, and extension at 72°C for 60 seconds, as well as a final extension at 72°C for 7 minutes. The reaction mixture was completed in a thermal gradient cycler (PEQLAB) for the detection of species-specific genes using the following MPCR procedure: pre-denaturation at 95°C for 1 minutes, 35 cycles with denaturation at 94°C for 35 seconds, annealing at 55°C for 90 seconds, extension at 72°C for 30 seconds, and final extension at 72°C for 7 minutes. The PCR products were subjected to electrophoresis using 1.0% agarose gel, stained with ethidium bromide, and observed under ultraviolet light.
Statistical analysis was then conducted for each of the virulence determinants. The analysis included cross-tabulation and the performance of the Pearson chi-square test of independence. Levels of significance were determined between the two clinical features (hospitalization and bloody diarrhea) and enterotoxin genes.

**RESULTS**

1. **Shigella species**

Of the 946 diarrheal samples, 75 isolates of *Shigella* spp. were obtained using conventional biochemical and microbiological tests. All isolates were confirmed by the *Shigella* genus-specific PCR. The prevalence of the *Shigella* species is shown in Table 2. The species-specific amplification test showed that 40, 33, 1, and 1 strains of *S. sonnei*, *S. flexneri*, *S. dysenteriae*, and *S. boydii*, respectively, were isolated from all the tested samples. The study was performed on children aged 1–15 years; as anticipated, children over one year of age were more affected by *Shigella* than the younger children were. The prevalence rate of *Shigella* spp. varied in different age groups; *S. sonnei* was identified in 12 (30.0%) isolates in the ≤ 5 years age group and 28 (70.0%) isolates in the < 5 years of age group, while *S. flexneri* was found in 17 (51.5%) isolates in the ≤ 5 year age group, but this difference was not statistically significant (*p* = 0.16). Of the total isolates, 45.3% and 54.7% of isolates were associated with males and females, respectively, but this distribution was not significant (*p* = 0.19).

2. **Virulence factors**

All isolates were positive for the *ipaH*, *ipaBCD*, and *virA*, while only one (1.4%) of all the isolates was positive for the *stx* (Table 2). The prevalence of the *ial*, *sen*, *sat*, *set1A*, and *set1B* was 74.7%, 45.4%, 28.0%, 24.0%, and 24.0%, respectively; the results are shown in Table 2. *set1A*, *set1B*, and *sat* were only detected in *S. flexneri* isolates. *stx* is carried by *S. dysenteriae*. The highest prevalence of virulence determinants was found in *S. flexneri*. One interesting finding was the simultaneous presence of the *ipaBCD*, *ipaH*, *virA*, and *ial* in 31 isolates (77.5%) of *S. sonnei*, while these genes were not found in the nine remaining isolates. In addition, 19 (57.6%) of the 33 *S. flexneri* isolates were simultaneously positive for the *ipaBCD*, *ipaH*, *virA*, *ial*, and *sat*. All *S. flexneri* while no *S. sonnei*, *S. dysenteriae*, and *S. boydii* isolates harbored *sat*, *set1A*, and *set1B*. Between the two *Shigella* enterotoxin genes, *sen*
was found to be statistically significant and associated with hospitalization and bloody diarrhea \( p = 0.001 \), as shown in Table 3. The remainder of the calculations yielded values in which \( p < 0.05 \), and thus were considered to be statistically insignificant in this study.

**DISCUSSION**

In the current study, 75 *Shigella* isolates were obtained from all the tested stool samples. Conventionally identified isolates of *Shigella* were confirmed using *ipaH*-specific PCR assay. In our study, similar to Binet et al’s study [19], *ipaH* was detected in all *Shigella* culture-positive specimens [20]. In accordance with these results, Vu et al. [21] showed that *ipaH* is carried by all four *Shigella* species as well as by enteroinvasive *E. coli* (EIEC). In agreement with Casabonne et al’s [22] and Cruz et al’s [23] results, the results of our study revealed that *virA* and *ipaBCD* were found to be positive in all the strains. *Shigella* attaches to the target region through the two receptors, hyaluronan receptor CD44 and integrin \( \alpha_5\beta_1 \) [24]. *Shigella* attaches to CD44 through the *IpaB* determinant, while the *IpaBCD* complex interacts via \( \alpha_5\beta_1 \) integrin receptor. Finally, invasion and cytoskeleton reformation occur by the binding of *Shigella* to the receptors [24,25].

*VirA*, *IcsA/VirG*, *SopA/IscP*, and *PhoN2* are important determinants for bacterial penetration into host cells and actin nucleation at one end of the bacterium [8]. Some of the virulence factors mentioned above are also situated in large virulent plasmids. Fifty-six (74.7%) *Shigella* strains were found to carry *ial*, in our study; these results are approximately consistent with those of Casabonne et al’s [22] and Hosseini Nave et al’s [26] studies. This contrast may be because *ial* is only located on the virulent plasmid and can cause deletion mutations [26]. *Sat* was described first in uropathogenic *E. coli* (UPEC), but has now also been found in *Shigella* spp. The prevalence of *sat* in *S. flexneri* has been found to be 21 (63.6%) [8]. This data conflicts with that of studies conducted in India [27] (56/65, 86.2%) and India 72/75 (96.0%) [28]. A large invasion plasmid gene (*sen*), which encodes ShET-2, has also been reported in numerous *Shigella* spp. In this study, 45.4% (34/75; 19 *S. sonnei* and 15 *S. flexneri*) isolates carried *sen* [29]. Similarly, *sen* has been detected in 37 (66.1%) *Shigella* isolates in Kerman, Iran [26]. Casabonne et al. [22] showed that of the 100 *Shigella* isolates, 29 *S. flexneri* and 11 *S. sonnei* carried the gene encoding ShET-2.

The conflict is likely because of the loss of the large plasmid that contains the gene in different *Shigella* serogroups and the number of samples. *Shigella* enterotoxin 1 (ShET-1) is encoded by *set* located on the chromosomes of several clinical strains of

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**Table 2. Prevalence and combination of virulence genes among 75 *Shigella* isolates**

| Virulence gene | *Shigella* (n = 75) | *S. sonnei* (n = 40) | *S. flexneri* (n = 33) | *S. boydii* (n = 1) | *S. dysenteriae* (n = 1) | *Total* |
|----------------|---------------------|----------------------|-----------------------|---------------------|-------------------------|---------|
| *ipaBCD* | 75 (100) | 40 (100) | 33 (100) | 1 (100) | 1 (100) | 75 (100) |
| *setI* | 75 (100) | 40 (100) | 33 (100) | 1 (100) | 1 (100) | 75 (100) |
| *setII* | 75 (100) | 40 (100) | 33 (100) | 1 (100) | 1 (100) | 75 (100) |
| *setIII* | 75 (100) | 40 (100) | 33 (100) | 1 (100) | 1 (100) | 75 (100) |
| *setIV* | 75 (100) | 40 (100) | 33 (100) | 1 (100) | 1 (100) | 75 (100) |
| *setV* | 75 (100) | 40 (100) | 33 (100) | 1 (100) | 1 (100) | 75 (100) |

Values are presented as number (%). NA, not available. Combining of genes: I: *ipaBCD* + *virA*; II: *ipaBCD* + *ipaH* + *virA* + *iaI*; III: *ipaBCD* + *virA* + *iaI* + *sen*; IV: *ipaBCD* + *ipaH* + *virA* + *iaI* + *set1I* + *set1II* + *set1III* + *set1IV* + *set1V*; V: *ipaBCD* + *ial* + *ipaH* + *virA* + *set1I* + *set1II* + *set1III* + *set1IV* + *set1V*.

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### Table 3. Assessing major *Shigella* virulence genes associated with main symptoms of shigellosis

| Virulence gene | Hospitalization | OR (CI) | p-value | Bloody diarrhea | OR (CI) | p-value |
|----------------|-----------------|---------|---------|-----------------|---------|---------|
|                | Positive        | Negative|         |                 | Positive| Negative|       |
| SET.1A         | 3 (30.0)        | 15 (23.8)| 0.7 (0.16–3.1) | NS               | 16 (88.9)  | 20 (36.4) | 0.001** |
|                | Negative        | 7 (70.0)  | 48 (76.2)|                 | 2 (11.1)    | 35 (63.6) |       |
| SET.1B         | 3 (30.0)        | 15 (23.8)| 0.7 (0.16–3.1) | NS               | 16 (88.9)  | 20 (36.4) | 0.001** |
|                | Negative        | 7 (70.0)  | 48 (76.2)|                 | 2 (11.1)    | 35 (63.6) |       |
| SEN            | 10 (100)        | 24 (38.1)| 1.1 (0.6–2.1)| 0.001**         | 24 (70.6)  | 12 (30.8) | 3.2 (1.9–7.2) | 0.001** |
|                | Negative        | 0         | 39 (61.9)|                 | 10 (29.4)   | 27 (69.2) |       |
| SAT            | 3 (30.0)        | 18 (28.6)| 0.9 (0.6–1.4)| NS               | 18 (85.7)  | 18 (34.6) | NR      | NR     |
|                | Negative        | 7 (70.0)  | 45 (71.4)|                 | 3 (14.3)    | 34 (65.4) |       |

Values are presented as number (%).
OR, odds ratio; CI, confidence interval; NS, not significant; NR, not reported.

Analyzed by Fisher’s exact test; **significant difference.

*S. flexneri* serotype 2 and rarely on those of other serotypes [28]. *ShET-1* has been found to stimulate fluid secretion into the intestine, thus, contributing to the watery phase of diarrhea [28,30]. In our study, 18 (24.0%) isolates were found to carry both set1A and set1B. Casabonne et al. [22], Vargas et al. [15], and Cruz et al. [23] showed that the prevalence of set1A and set1B was 7.0% (7/100), 3.92 (2/51), and 36.6 (11/30), respectively. In agreement with previous studies, the present study showed that set1A and set1B were detected only in *S. flexneri* strains [26].

*stx* is another virulence determinant related to *S. dysenteriae*: it is not excreted by the bacteria, but is released only during cell lysis [31]. Only one (1.4%) *S. dysenteriae* isolate carries *stx*. Bekal et al.’s [32] study detected *S. flexneri* isolates harboring the Shiga toxin 1-producing gene. In Gray et al.’s study [33], 21% of the isolates, including *S. flexneri* 2a, *S. flexneri* Y, and *S. dysenteriae* 4, were found to harbor and produce *stx*. Among *Shigella* enterotoxin genes, both *sen* and set enterotoxins are significantly associated with bloody diarrhea. In Cruz et al.’s study [23], *ShET-2* was found to contribute to intestinal injury and bloody diarrhea.

Farrán et al. [34] reported that the *ShET-2* coding *sen* is responsible for epithelial inflammation; in this research found a combination of the *ipabCD*, *ipah*, *virA*, and *ial* in 31 (77.5%) *S. sonnei* isolates. In addition, Zhang et al. [16] found that 19 (57.6%) of 33 *S. flexneri* isolates were positive for *ipabCD*, *ipah*, *virA*, *ial*, and *sat* simultaneously; however, only *sat*, set1A, and set1B were detected in *S. flexneri* strains. They also showed that 2, 123, 8, 12, and 53 of 198 *Shigella* isolates carried *ial1/ipah/virA*, *ial1/ipah/virA/set1A/sen*, *ial1/ipah/virA/set1A/sen*, and *ial1/ipah/virA/set1A/sat1B/sen*, respectively [16]. Of the 100 *Shigella* isolates, 24 *S. flexneri* were found to carry *set* and *sen* in Casabonne et al.’s study [22]. To the best of our knowledge, this is the first study on the distribution of virulence gene combinations, and these genes are related with hospitalization and bloody diarrhea among *Shigella* species in Tehran, Iran. In conclusion, this work has demonstrated the high prevalence of two enterotoxins, *ShET-1* and *ShET-2*, in *S. flexneri*, especially, among the hospitalized pediatric patients who were included in the study population. Among *Shigella* serotypes, *S. flexneri* serotype 2a was found to have a high number of virulence determinants. Bloody diarrhea and hospitalization were also found to be associated with the number of virulence determinants. Future studies should investigate the relations between shigellosis symptoms and virulence determinants in Iran.

**CONFLICTS OF INTEREST**

No potential conflict of interest relevant to this article was reported.

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1. Ranjbar R, Soltan Dallal MM, Talebi M, et al. Increased isolation and characterization of Shigella sonnei obtained from hospitalized children in Tehran, Iran. J Health Popul Nutr 2008;26:426-30.

2. Soltan Dallal MM, Ranjbar R, Pourshafie MR. The study of antimicrobial resistance among Shigella flexneri strains isolated in Tehran, Iran. J Pediatr Infect Dis 2011;6:125-9. https://doi.org/10.3233/JPI-2011-0307

3. Ranjbar R, Behnood V, Memariani H, et al. Molecular characterisation of quinolone-resistant Shigella strains isolated in Tehran, Iran. J Glob Antimicrob Resist 2016;5:26-30. https://doi.org/10.1016/j.jgar.2016.01.010

4. Soltan Dallal MM, Eghbal M, Shafarianpour A, et al. Prevalence and multiple drug resistance of Shigella sonnei isolated from diarrheal stool of children. J Med Bacteriol 2015;4:24-9.

5. Shen Y, Qian H, Gong J, et al. High prevalence of antibiotic resistance and molecular characterization of integrons among Shigella isolates in Eastern China. Antimicrob Agents Chemother 2013;57:1549-51. https://doi.org/10.1128/AAC.02102-12

6. Phantouamath B, Sithivong N, Insisengmay S, et al. Pathogenicity of Shigella in healthy carriers: a study in Vientiane, Lao People’s Democratic Republic. Jpn J Infect Dis 2005;58:232-4.

7. Büttner D, Bonas U. Who comes first? How plant pathogenic bacteria orchestrate type III secretion. Curr Opin Microbiol 2003;6:159-66. https://doi.org/10.1016/S0378-1097(03)00761-4

8. Schroeder GN, Hilbi H. Molecular pathogenesis of Shigella spp.: controlling host cell signaling, invasion, and death by type III secretion. Clin Microbiol Rev 2008;21:134-56. https://doi.org/10.1128/CMR.00032-07

9. Zaidi MB, Estrada-García T. Shigella: a highly virulent and elusive pathogen. Curr Trop Med Rep 2014;1:81-7. https://doi.org/10.1007/s40475-014-0019-6

10. Day WA Jr, Maurelli AT. Shigella flexneri LuxS quorum-sensing system modulates virB expression but is not essential for virulence. Infect Immun 2001;69:15-23. https://doi.org/10.1128/IAI.69.1.15-23.2001

11. Unkmeir A, Schmidt H. Structural analysis of phage-borne stx genes and their flanking sequences in shiga toxin-producing Escherichia coli and Shigella dysenteriae type 1 strains. Infect Immun 2000;68:4856-64. https://doi.org/10.1128/IAI.68.4856-4864.2000

12. Cherla RP, Lee SY, Tesh VL. Shiga toxins and apoptosis. FEMS Microbiol Lett 2003;228:159-66. https://doi.org/10.1016/S0014-0106(03)00761-4

13. Ranjbar R, Soltan Dallal MM, Pourshafie MR, et al. Serogroup distribution of Shigella in Tehran. Iranian J Publ Health 2004;33:32-5.

14. Farshad S, Sheikh R, Japoni A, et al. Characterization of Shigella strains in Iran by plasmid profile analysis and PCR amplification of ipa genes. J Clin Microbiol 2006;44:2879-83. https://doi.org/10.1128/JCM.00310-06

15. Vargas M, Gascon J, Jimenez De Anta MT, et al. Prevalence of Shigella enterotoxins 1 and 2 among Shigella strains isolated from patients with traveler’s diarrhea. J Clin Microbiol 1999;37:3608-11.

16. Zhang CL, Liu QZ, Wang J, et al. Epidemic and virulence characteristic of Shigella spp. with extended-spectrum cephalosporin resistance in Xiaoshan district, Hangzhou, China. BMC Infect Dis 2014;14:260. https://doi.org/10.1186/1471-2334-14-260

17. Ruiz J, Navia MM, Vila J, et al. Prevalence of the Sat gene among clinical isolates of Shigella spp. causing travelers’ diarrhea: geographical and specific differences. J Clin Microbiol 2002;40:1565-6. https://doi.org/10.1128/JCM.40.4.1565-1566.2002

18. Faruque SM, Khan R, Kamruzzaman M, et al. Isolation of Shigella dysenteriae type 1 and S. flexneri strains from surface waters in Bangladesh: comparative molecular analysis of environmental Shigella isolates versus clinical strains. Appl Environ Microbiol 2002;68:3908-13. https://doi.org/10.1128/AEM.68.8.3908-3913.2002

19. Binet R, Deer DM, Uhlfelder SJ. Rapid detection of Shigella and enteroinvasive Escherichia coli in produce enrichments by a conventional multiplex PCR assay. Food Microbiol 2014;40:48-54. https://doi.org/10.1016/j.fm.2014.09.002

20. Farshad S, Ranjbar R, Hosseini M. Molecular genotyping of Shigella sonnei strains isolated from children with bloody diarrhea using pulsed field gel electrophoresis on the total genome and PCR-RFLP of IpaH and IpaBCD genes. Jundishapur J Microbiol 2014;8:e14004. https://doi.org/10.5812/jim.14004

21. Vu DT, Sethabutr O, Von Seidlein L, et al. Detection of Shigella by a PCR assay targeting the ipaH gene suggests increased prevalence of shigellosis in Nha Trang, Vietnam. J Clin Microbiol 2004;42:2031-5. https://doi.org/10.1128/JCM.42.5.2031-2035.2004

22. Casabonne C, González A, Aquíñ V, et al. Prevalence and virulence genes of Shigella spp. isolated from patients with diarrhea in Rosario, Argentina. Jpn J Infect Dis 2016;69:477-81. https://doi.org/10.7883/yoken.JJID.2015.459

23. Cruz CBN, Souza MCS, Serra PT, et al. Virulence factors associated with pediatric shigellosis in Brazilian Amazon. Biomed Res Int 2014;2014. https://doi.org/10.1155/2014/539697

24. Pizarro-Cerdá J, Cossart P. Bacterial adhesion and entry into host cells. Cell 2006;124:71-27. https://doi.org/10.1016/j.cell.2006.02.012

25. Demali KA, Jue AL, Burridge K. IpaA targets beta integrins and rho to promote actin cytoskeleton rearrangements necessary for Shigella entry. J Biol Chem 2006;281:39534-41. https://doi.org/10.1074/jbc.M605939200

26. Hosseini Nave H, Mansouri S, Emaneini M, et al. Distribution of genes encoding virulence factors and molecular analysis of Shigella spp. isolated from patients with diarrhea in Kerman, Iran. Microb Pathog 2016;92:68-71. https://doi.org/10.1016/j.micpath.2015.11.015

27. Niyogi SK, Vargas M, Vila J. Prevalence of the sat, set and sen genes among diverse serotypes of Shigella flexneri strains isolated from surface waters in Bangladesh: comparative molecular analysis of environmental Shigella isolates versus clinical strains. Clin Microbiol Infect 2004;10:574-6. https://doi.org/10.1111/j.1469-0691.2004.00897.x

28. Roy S, Thanasekaran K, Dutta Roy AR, et al. Distribution of Shigella enterotoxin genes and secreted autotransporter toxin gene among...
diverse species and serotypes of shigella isolated from Andaman Islands, India. Trop Med Int Health 2006;11:1694-8. https://doi.org/10.1111/j.1365-3156.2006.01723.x

29. Sousa MA, Mendes EN, Collares GB, et al. Shigella in Brazilian children with acute diarrhoea: prevalence, antimicrobial resistance and virulence genes. Mem Inst Oswaldo Cruz 2013;108:30-5. https://doi.org/10.1590/S0074-02762013000100005

30. Baraki N, Wodajo A, Abera M, et al; Haramaya University. Foodborne diseases. Degree program for health officers, nurses, environmental health officers and medical laboratory technologists. Ethiopia: EPHTI; 2005.

31. Boerlin P, McEwen SA, Boerlin-Petzold F, et al. Associations between virulence factors of Shiga toxin-producing Escherichia coli and disease in humans. J Clin Microbiol 1999;37:497-503.

32. Bekal S, Pilon PA, Cloutier N, et al. Identification of Shigella flexneri isolates carrying the Shiga toxin 1-producing gene in Quebec, Canada, linked to travel to Haiti. Can J Microbiol 2015;61:995-6. https://doi.org/10.1139/cjm-2015-0538

33. Gray MD, Lacher DW, Leonard SR, et al. Prevalence of Shiga toxin-producing Shigella species isolated from French travellers returning from the Caribbean: an emerging pathogen with international implications. Clin Microbiol Infect 2015;21:765.e9-14. https://doi.org/10.1016/j.cmi.2015.05.006

34. Farfán MJ, Toro CS, Barry EM, et al. Shigella enterotoxin-2 is a type III effector that participates in Shigella-induced interleukin 8 secretion by epithelial cells. FEMS Immunol Med Microbiol 2011;61:332-9. https://doi.org/10.1111/j.1574-695X.2011.00778.x