The Prevalence of Shiga toxin-1 in Shigella spp. Isolates Collected from Diarrhea Patients, Ahvaz, Iran

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Research

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Abstract

Objective

Shigellosis as one of the substantial causative agents of microbial dysentery still has a remarkable prevalence in areas with poor hygienic infrastructures. The probable existence of the deadly Shiga toxin-1(Stx1) in some Shigella strains would manifest life-threatening clinical symptoms of the infection. The aim of this study was to determine the presence of Stx1 in isolated from patients with diarrhea.

Results

Totally, 227 Shigella species including 60 *S. flexneri*, 157 *S. sonnei,* and 10 *S. boydii* were collected from diarrheal patients in tropical infectious diseases research center of Ahvaz, Iran, from 2013 to 2015. The isolates were collected mostly from the intensive care unit, infectious disease, and surgery department. The isolates were identified and the polymerase chain reaction (PCR) was performed to detect the *stx* gene. The results indicated that none of them encode the *stx* gene. In conclusion, isolates of this study were not capable of *stx1* encoding.

1. Introduction

Shigella is a gram-negative, non-motile bacillus which serves as a major enteropathogen. *Shigella* infections mostly have been associated with watery diarrhea, dysentery, as well as some severe complications such as encephalopathy (1). This type of bacteria has four species including *S. flexneri, S. sonnei,* and *S. boydii*. These species are distributed worldwide. For instance, *S. flexneri* has been reported to high prevalence in India and Rwanda, while *S. sonnei* was commonly distributed in Thailand, Israel, and the USA (2-6). Shigella species is mainly diagnosed based on some symptoms such as fever, blood, mucous diarrhea and abdominal pain. Routine microscopy of fresh stool is a simple, cheap, rapid, and easy test to detect Shigella infections (7). For the treatment of diagnosed Shigella infections, ciprofloxacin is the first-line and pivmecillinam, ceftriaxone or azithromycin are the second-lines of treatment (8).

Shiga toxin (Stx) as a potent bacterial toxin was initially founded in *S. dysenteriae and after that, it also was founded in Escherichia coli.* Stx at least has two types including Stx1 and Stx2. Despite diverse genetically and immunologically origins, they have the same mechanism of action. Structural analysis indicated that Stxs consist of two major subunits including catalytic domains (A) and binding domain (B) to make an AB5 complex inhibiting eukaryotic protein synthesis (9). In addition, this toxin has also been shown to trigger cytokine production and induce host cell apoptosis (10). Stx toxicity is responsible for severe human disorders such as hemorrhagic colitis and hemolytic uremic syndrome (11).

The gene encoding Stx in *S. dysenteriae* is located on its chromosome but this gene in *E. coli* is associated to be a prophage (9). However, recently it has been shown that Stx-converting phages are capable of transferring *stx* genes from cell to cell through a transduction process called horizontal gene

transferring. The exact mechanism underlying such gene transferring is partially unknown however, it has been suggested that after infection with a Stx-converting phage, the integrated *stx* genes mostly remain silent in the lysogens. In the presence of DNA damaging agents or other factors that induce the bacterial S.O.S. response, the lytic cycle was activated. Infected cell lysis eventually resulted in the releasing of phage particles that enhance *stx* genes transferring to other bacteria (12, 13). A growing body of evidence indicated that the *stx* gene has the potential to be transferred to other Shigella species like *S. flexneri*, *S. sonnei*, and *S. boydii* but with a rare prevalence (14-16). Therefore, the presence of stx genes is one of the most important challenges in clinical microbiology. Here, we identified 227 Shigella isolates including 60 *S. flexneri*, 157 *S. sonnei* and 10 *S. boydii* which all have the potentials to encode *stx1.* The PCR analyses determined that none of the samples encodes *stx1*.

2. Materials And Methods

2.1.1 Sampling, isolation, and identification

The specimens (N=227) were collected between January 2013 to October 2015 from patients with diarrhea in the infectious and tropical diseases research center of Ahvaz Jundishapur University of Medical Sciences, Ahvaz, Iran. The patients were characterized by 3 episodes of diarrhea with or without blood, mucus, and gastric cramps. For bacterial isolation, the specimens were cultured in Brain Heart Infusion agar followed by biochemical tests including oxidase, urease, Triple Sugar Iron agar (TSI), motility, and carbohydrate fermentation. The isolates were consists of 60 *S. flexneri*, 157 *S. sonnei*, and 10 *S. boydii*.

2.1.2 DNA extraction and molecular identification

DNA of Shigella species isolates was extracted using the boiling method as described previously. In brief, three bacterial colonies were dissolved in 1000 µl of LB (Luria Broth) medium. The samples were placed in boiling water for 10 min followed by incubation in -20 ºC for 10 min. This freeze-thaw cycle was repeated again followed by centrifugation at 10,000 rpm for 5 min. The supernatant was then discarded and the pellets were dissolved in ethanol. The samples were finally air-dried followed by dissolving in distilled water and storing in -20 ºC until PCR examination.

2.1.3 PCR analysis

The PCR analysis was performed similarly to the previous study(17). The extracted DNA was subjected to PCR using the following primers according to manufactures instruction. Forward *stx1*: 5' CTG TGG CAA GAG CGA TGT TA 3', Reverse *stx1*:5' GCC GGA CAC ATA GAA GGA AA 3'. In order to perform PCR analysis, 12 µL of PCR master mix 8 µL of sterile distilled water, 2 µL reverse and forward primers (15 Picomole of each one) and 3 µL of extracted DNA (50 Nanogram/ml) were added to each vial. The PCR procedures were as follow; the initial denaturation at 95°C for 5 minutes, 35 cycles at 95°C for one min, annealing at 55°C for one min, extension at 72°C for 30 seconds, and a final extension at 72°C for 10 min.

The PCR products were finally subjected to gel electrophoresis on 2% agarose followed by ethidium bromide visualization.

3. Result And Discussion

PCR analysis was performed to investigate the presence of *stx1* gene. *S. dysentery* was also used as a positive control (RITCC1875). As indicated in Fig. 1, our data revealed that all 227 isolates were negative for the *stx1* gene. This is the only prospective study incorporating molecular methods for the specific detection of stx genes in stools diarrhea patients in Iran. No prevalence of stx in patients of this study is in parallel with low rates in other countries.The infections caused by Shigella species are globally distributed and prevalent in developing countries such as Bangladesh, and Afghanistan (18, 19). However, it has been shown that infection with Shigella species is prevalent in Iran (20).

To date, infections with Stx-producing organisms in Iran are generally caused by *Escherichia coli (21, 22)*. On the other hand, it has been reported that more than 70 percent of shigellosis are caused by *S. sonnei* however, *S. dysenteriae* infections as the main source of Stx-1among Shigella species, are rare (23). In addition, other spices such as *Enterobacter cloacae, Citrobacter freundii, Aeromonas hydrophila* and *Aeromonas caviae* as well as some spices of *E.coli* have been suggested to encode *stx* gene (24, 25). The presence of the *stx* gene in such bacteria supporting the hypothesis of horizontal gene transferring. In addition, the study of Gray et al further confirmed that new *stx*-encoding prophages were in circulating within geographical area, and they spread to other continents, by immigrants (26).

4. Limitation

The aim of this study was to determine the prevalence of *stx1* gene in Shigella isolates and, if available, to the cytotoxicity of positive isolates on the cells were supposed to be evaluated. However, we did not find any positive isolates for the *stx1* gene in this study. As the main limitation, indeed this study was not enough to introduce Iran as a country without strains of *stx1* coding species. This achievement requires further studies, especially in areas where bacteria *Shigella* are endemic. In addition, due to phage transferring probability to other bacteria such as *Enterobacter* and *Aeromonas* other bacteria can be tested to evaluate the presence *stx1* gene.

5. Conclusion

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Abbreviations

Stx1: Shiga toxin-1

PCR: polymerase chain reaction

TSI: Triple Sugar Iron

LB: Luria Broth

Declarations

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Competing interests

The authors declare that there is no conflict of interests in the publication of this study.

Ethics approval and consent to participate

The Ethics issues of this study were approved by the ethics committee of Ilam University of Medical Sciences. The written informed consent was obtained from all the participants, and informed consent obtained was written.

Availability of data and materials

The source of data is available on request to the corresponding author

Authors' Contributions

N.M designed and performed the experiments, analyzed data, and wrote the manuscript. **H.V** designed and performed experiments. **A.Gh** and **A.M** designed experiments, analyzed data, and wrote the manuscript. **N.S** designed the study, analyzed data, and wrote the manuscript. All authors read and approved the final manuscript.

Consent for publication

Not applicable.

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Figures

Figure 1

The representative image of PCR product for stx1 gene. Lane 1 was positive control of S. dysentery, Lane 2 was negative control, Lane 3, 4 and 5 were S. sonnei, Lane 6, 7 and 8 were S. flexneri, and Lane 9 was S. boydii