Molecular Analysis of *Shigella sonnei* Strains Isolated from Hospitals of Tehran and Ilam Cities Using Repetitive Extragenic Palindromic Polymerase Chain Reaction (REP-PCR)

Shiva Hosseini, Abbas Maleki, Sobhan Ghafourian, Hossein Kazemian, Mohammad Hossein Maleki, Reza Mohebi, Noorkhoda Sadeghifard

Clinical Microbiology Research Center, Ilam University of Medical Science, Ilam, Iran

ABSTRACT

Background and Objective: *Shigella* species belong to the family Enterobacteriaceae, which cause dysentery with abdominal pain and tenesmus in human. Our country is one of the endemic and occasional epidemic areas of the disease. In the present study, Repetitive Extragenic Palindromic Polymerase Chain Reaction (REP-PCR) technique that has high differentiation and specificity power compared to phenotypic markers, was used to investigate diversity and geographical distribution of colones as well as typing of *Shigella* strains.

Methods: In this study, a total of 40 *Shigella sonnei* samples were isolated from stool of patients with diarrhea and divided into 5 groups. After identification and confirmation of the isolates by biochemical and serotyping methods, one colony of each isolate was cultured on LB medium and after DNA extraction, PCR was performed. After electrophoresis of the PCR product, gel images were saved in the computer for further analysis and typing of the isolates.

Result: In this research, 40 isolates were examined using REP-PCR and a similarity matrix was constructed based on Dice's coefficient. According to this matrix, some isolates were completely similar (genetic similarity coefficient= 1) and some isolates showed the least similarity (genetic similarity coefficient= 0). The dendrogram was obtained using the UPGMA algorithm. The calculated Cophenetic correlation coefficient for this dendrogram was 0.91645.

Conclusion: From the results of the present study, it was concluded that we can type the *Shigella sonnei* strains using palindromic repetitive sequences. The extent of polymorphism indicates that REP-PCR technique is a useful method for genetic variation analysis in molecular typing of *shigella sonnei* strains. Although, some strains were completely similar, a high genetic variation was found among the studied population of *Shigella sonnei*. This level of variation is probably due to wide geographic distribution of this species in Iran.

Keywords: S*higella sonnei*, REP-PCR, Molecular typing

Corresponding Information: Dr Noorkhoda Sadeghifar, Clinical Microbiology Research Center, Ilam University of Medical Science, Ilam, Iran. Email: sadeghifard@gmail.com

Introduction

Shigella is a non-motile, non-spore-forming, capsuleless, Gram-negative, and facultatively anaerobic coccobacillus, which is 2-3 μm in size. This bacterium belongs to the family Enterobacteriaceae and humans are the only reservoir of the bacterium (1).

Our country is one of the endemic areas of the disease, wherein occasional epidemic outbreaks occur (2). In other countries, some researches have been conducted on recording molecular types of endemic and epidemic strains in order to trace the origin of the infection, but given

the limited studies in Iran, no epidemiological relationship was found among the isolates (3).

Phenotypic and genotypic techniques are used to design effective strategies for the control of infections caused by this bacterium. Phenotypic techniques include evaluation of biochemical characteristics, phage typing, bacteriocin typing, immunoblotting, protein analysis, and identification of antimicrobial resistance patterns (4). These techniques are not powerful enough for identification and differentiation of *S. sonnei* strains and cannot determine the source and ways of distribution, and effective measures for controlling the spread of infection and prevalent isolates (5).

In recent years, genotypic techniques by specific molecular markers are used for epidemiological studies and typing of *shigella* species. These techniques include ribotyping, plasmid analysis, PFGE, chromosomal RFLP, RAPD-PCR, AP-PCR, REP-PCR, and ERIC-PCR (6-8). Among these techniques, REP-PCR has high validity and appropriate power for differentiation of isolates (9). Repetitive sequences of double-stranded DNA, which are called symmetric elements, are often seen in non-coding sections of DNA, (10).

Considering the presence of these repetitive extragenic palindromic sequences, by designing an appropriate primer according to desired sequence using PCR, electrophoresis of the resultant PCR products, and gel analysis by computer (6-9) and given the number of sequences in each strain and their difference in length, the number of bonds obtained by PCR for each strain is different, and therefore the strains are classified based on diversity of bonds. Although, use of PFGE is considered as a gold standard method, the REP-PCR is a simpler and lower-cost method and provides more rapid results (11). Considering that in our country, molecular studies on molecular epidemiology of *shigella* species are limited to specific regions, this research was carried out with the purpose

of investigating genetic variation of *S. sonnei* strains using REP-PCR method (10).

Materials and methods

Isolation and cultivation

In the present study, a total of 40 *S. sonnei* isolates isolated from patients with diarrhea, were analyzed. Then, the isolates were cultured on selective media, such as MacConkey agar and deoxycholate agar and confirmed by biochemical tests. A specific antiserum was used to differentiate the *S. sonnei* from other species.

DNA extraction

In order to extract DNA, first, one colony of the bacterium in LB media was incubated at 37°C for 18-24 hours. After incubation, the bacterial suspension was centrifuged at 3000 rpm for 5 min and the sediment was used for DNA extraction. DNA of the samples was extracted using Diatom kit, which has been designed based on GuSCN-Silica Gel method.

PCR

After DNA extraction, each sample was stored at -20°C.

The primer sequences used in PCR reaction were as follows:

Forward: REP1RI 5'– III ICG ICG ICA TCI GGC-3'
Reverse: REP2 5'– ICG ICT TATCIG GCC TAC-3'

In order to amplify the desired sequence, in a volume of 50 μl, 2.5 units of Taq polymerase, 50 pM of each primer, 200 μM of each four deoxynucleotides, 1 μl of template DNA, 1.5 μM of $MgCl_2$, 10 mM of Tris-Hcl (pH 8.3), and 50 mM of KCl, were used.

PCR was performed in a thermocycler with

initial denaturation of 95°C for 5 min, then 40 cycles in 90°C for 30 s, 40°C for 1 min, 65°C for 8 min, and finally 75°C for 15 min for amplification of incomplete segments.

Observation of PCR products

About 10 µl of each PCR product was transferred to each well of the gel and electrophoresis was carried out for 1 h at 90 V. The electrophoresis gel was placed on a UV transilluminator and image was captured from the observed bonds. The gel images were stored in computer for further analysis and typing of the isolates.

Results

In this study, 40 *Shigella* isolates, which were identified as *S. sonnei* using biochemical and serotype confirmation, were evaluated by molecular typing using REP-PCR method. To investigate the genetic variation of the *S. sonnei* strains isolated from hospitals of Tehran, a pair of specific primers from a series of REP-PCR

primers, were employed.

In this research, the similarity matrix was constructed based on dice method. According to this matrix, the strains with numbers (1, 2, 3, 4, 5, 11, 12, 13, and 14), (25, 26, 27, 28, and 29), (16 and 17), (19 and 21), (32, 33, 34, 38, and 39), and (36, 37) were completely similar (genetic similarity coefficient = 1) and strains (14 and 23), (13 and 23), (12 and 23), (11 and 23), (10 and 23), (9 and 23), (8 and 23), (6 and 23), (5 and 23), (4 and 23), (3 and 23), (2 and 23), and (1 and 23) showed the least genetic similarity (genetic
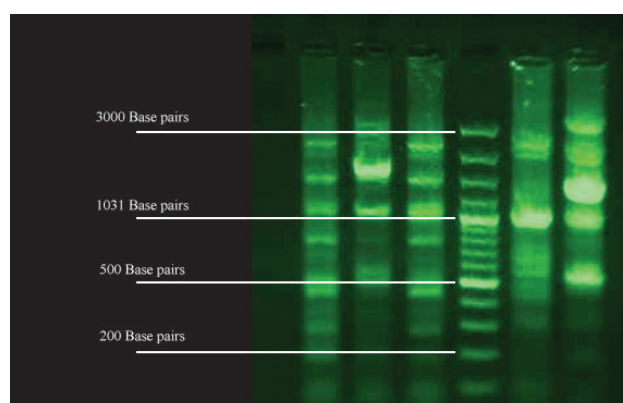

Fig. 1
A sample of the resultant electrophoresis gel of REP-PCR. M: molecular marker, column 1 is related to the negative control and columns 2 to 8 is for group 1 to 5.
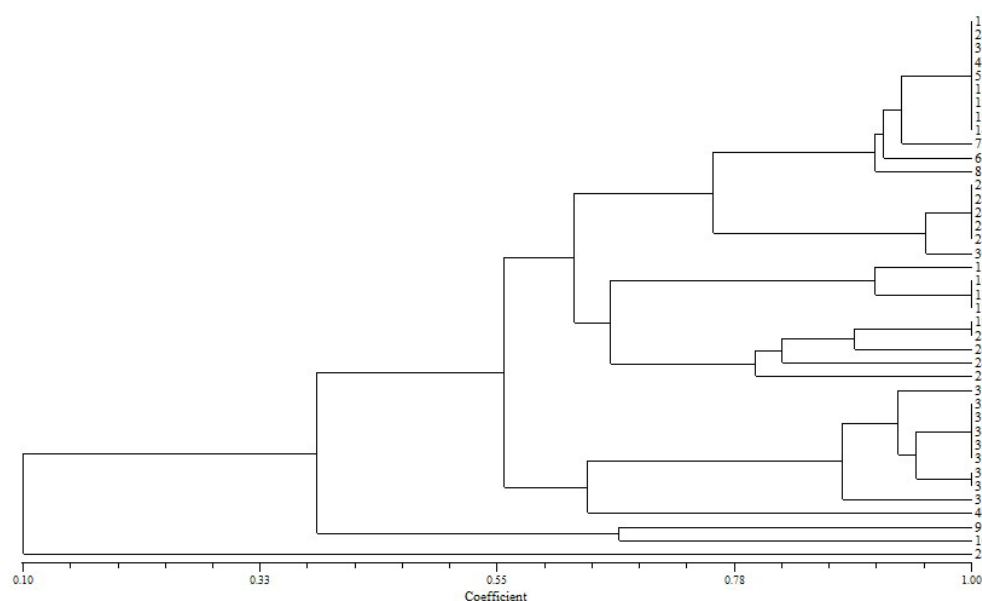

Fig. 2
Antibiotic selection of MCF7 cell clones. A: Primary stages of clone formation. B and C: growth of cells. D: formation of monolayer

similarity coefficient = 0). As can be seen in Fig. 1, dendrogram was calculated using the UPGMA algorithm. The obtained cophenetic correlation coefficient for this dendrogram was 0.91645.

Discussion

Seventeen scoreable fragments were produced using a pair of specific primer. Fig. 2 illustrates an electrophoresis pattern produced by PCR using REP-PCR primers. This level of polymorphism shows that REP-PCR technique is a useful method for analysis of genetic variation in molecular typing of *S. sonnei* strains. Estimates of genetic similarity based on this method revealed that some strains has 100% genetic similarity and there was no genetic similarity between the strain 23 and other strains. Estimation of genetic similarity using DNA fingerprinting data is a useful method for assessment of diversity and distribution of the isolates and strains involved in outbreaks and determination of the epidemiological relationship among different *S. sonnei* isolates.

In a study by Jersek et al. (1999), 64 isolates of Listeria monocytogenes isolated from human, animals, and food, were typed and classified into 4 genetic groups of A, B, B, and D (12)

Snelling et al. (1996) investigated acinetobacter baumannii strains by REP-PCR typing and cloning boiling method and their results indicated that typing by this method is rapid and convenient (13).

Beyer et al. (1998) used REP-PCR method to identify epidemic and non-epidemic isolates of Salmonella enterica that had source variation. Most of the epidemic isolates were assigned to groups 1 and 2, and non-epidemic strains were assigned to group 3 (14). In this study, it was concluded that *S. sonnei* strains could be typed using repetitive palindromic sequences.

Acknowledgment

Conflict of interest

The author declare that there is no conflict of interests.

References

1. Hong KL, Sooter LJ. Single-Stranded DNA Aptamers against Pathogens and Toxins: Identification and Biosensing Applications. Biomed Res Int 2015;2015:419318.

2. Ranjbar R, Memariani M, Memariani H. Diversity of Variable Number Tandem Repeat Loci in Shigella Species Isolated from Pediatric Patients. Int J Mol Cell Med 2015;4(3):174–181.

3. Boschi-Pinto C, Velebit L, Shibuya K. Estimating child mortality due to diarrhoea in developing countries. Bull World Health Organ 2008;86(9):710-7.

4. Ranjbar R, Ghazi FM, Farshad S, Giammanco GM, Aleo A, Owlia P, et al. The occurrence of extendedspectrum beta-lactamase producing Shigella spp. in Tehran, Iran. Iran J Microbiol 2013;5(2):108-12.

5. Thompson CN, Duy PT, Baker S. The Rising Dominance of Shigella sonnei: An Intercontinental Shift in the Etiology of Bacillary Dysentery. PLoS Negl Trop Dis 2015;9(6):e0003708.

6. Dibaj R, Aslani MM, Heidarieh P, Hashemi A, Ataei B, Daei-Naser A, et al. Conventional and molecular investigation of Shigella isolates in relation to an outbreak in the area of Isfahan, Iran. Iran J Microbiol 2013;5(4):339-44.

7. 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 2015;8(1):e14004.

8. Kosek M, Yori PP, Gilman RH, Vela H, Olortegui MP, Chavez CB, et al. Facilitated molecular typing of Shigella isolates using ERIC-PCR. Am J Trop Med Hyg 2012;86(6):1018-25.

9. Ranjbar R, Memariani M. Multilocus variablenumber tandem-repeat analysis for genotyping of Shigella sonnei

strains isolated from pediatric patients. Gastroenterol Hepatol Bed Bench 2015;8(3):225-32.

10. Li S, Sun Q, Wei X, Klena JD, Wang J, Liu Y, et al. Genetic characterization of Shigella flexneri isolates in Guizhou Province, China. PLoS One 2015;10(1):e0116708.

11. Navia MM, Capitano L, Ruiz J, Vargas M, Urassa H, Schellemberg D, et al. Typing and characterization of mechanisms of resistance of Shigella spp. isolated from feces of children under 5 years of age from Ifakara, Tanzania. J Clin Microbiol 1999;37(10):3113-7.

12. Jersek B, Gilot P, Gubina M, Klun N, Mehle J, Tcherneva E, et al. Typing of Listeria monocytogenes strains by repetitive element sequence-based PCR. J Clin Microbiol 1999;37(1):103-9.

13. Snelling AM, Gerner-Smidt P, Hawkey PM, Heritage J, Parnell P, Porter C, et al. Validation of use of whole-cell repetitive extragenic palindromic sequencebased PCR (REP-PCR) for typing strains belonging to the Acinetobacter calcoaceticus-Acinetobacter baumannii complex and application of the method to the investigation of a hospital outbreak. J Clin Microbiol 1996;34(5):1193-202.

14. Beyer W, Mukendi FM, Kimmig P, Bohm R. Suitability of repetitive-DNA-sequence-based PCR fingerprinting for characterizing epidemic isolates of Salmonella enterica serovar Saintpaul. J Clin Microbiol 1998;36(6):1549-54.

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