

Evaluation of a PCR based approach to study the relatedness among *Shigella sonnei* strains

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ABSTRACT

Background: Infections caused by *Shigella* are a major cause of diarrheal disease in the developing and developed countries. The present study was conducted to apply and evaluate arbitrarily primed PCR (AP-PCR) for investigation of genetic relatedness among the strains of *Shigella sonnei* isolated from cases of acute diarrhea in Tehran.

Patients and methods: Totally, 60 *S. sonnei* strains isolated from children hospitalized due to enteritis at five hospitals in Tehran during 2003 and two sporadic isolates recovered in 1984 were investigated. Molecular typing was performed by AP-PCR. Depending on the number and size of amplified DNA bands, the strains were clustered into AP-PCR profiles.

Results: All strains of *S. sonnei* were typeable with this approach. AP-PCR generated nine indistinguishable bands ranged from 0.35 to 2.5 kbp in all studied strains. Only a single AP-PCR pattern was observed among the *S. sonnei* strains recovered in 2003. Two sporadic isolates recovered in 1984 showed different AP-PCR patterns compared to recent clinical isolates.

Conclusion: Results suggest that a very homogeneous AP-PCR cluster types might be responsible for shigellosis caused by *S. sonnei* in Tehran in 2003. Further molecular analysis conducted on a larger selection of isolates could confirm our findings.

Keywords: Acute diarrhea, *Shigella sonnei*, Molecular epidemiology, AP-PCR.
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INTRODUCTION

Shigella is among the most common causes of bacterial diarrheal diseases. Endemic *Shigella* is responsible for approximately 10% of all diarrheal episodes among children younger than five years living in developing countries and up to 75% of

diarrheal death (1). Children and the elderly are at greatest fatal risk from shigellosis. Shigellosis is one of the major causes of morbidity in children with diarrhea in Iran (2-5) but there are scanty studies addressing its molecular epidemiology.

Development of molecular typing methods has given the clinical microbiology laboratory powerful tools tracking pathogens in outbreaks. Many traditional and molecular typing methods

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such as biotyping, phage typing, serotyping, ribotyping, restriction fragment length polymorphisms, pulsed field gel electrophoresis (PFGE) typing have been used for the epidemiological investigation of *Shigella sonnei* (2, 6,7).

AP-PCR uses oligonucleotide primers with arbitrary sequences that can serve molecular typing of several bacterial species. This technique is less laborious and time-consuming than other DNA-based typing techniques. This method has been widely used for the molecular typing of different bacteria in epidemiological studies, and its advantages and disadvantages are well known (8-10).

Recently we have investigated the distribution of *Shigella* species in Tehran and found *S. sonnei* as the most prevalent *Shigella* species (3). In current study, we applied AP-PCR technique to investigate the genetic relatedness among the strains of *S. sonnei* isolated from cases of acute diarrhea occurred in Tehran, Iran during 2003.

PATIENTS and METHODS

Bacterial strains: A total of 178 strains of *S. sonnei* isolated from children hospitalized due to enteritis at Children Medical Center and Mofid hospital, two reference pediatric hospitals, and three other large hospitals (Baqiyatallah, Milad and Firozabadi) in Tehran, were selected among which 60 isolates were randomly selected for AP-PCR analysis. All strains had been identified at a genus level by previously described procedures (11) while agglutination with specific antiserum from MAST Group LTD (Mast House, Derby Road, Bootle, Merseyside, L20 1EA, UK) was used to identify the species.

Extraction of genomic DNA: Bacterial strains were grown aerobically in trypticase soy broth at 37°C. Two ml of overnight culture was centrifuged at 4,000rpm for 20min. The pellet was resuspended in 620microliter of lysis buffer (10mM Tris-HCl,

50mM EDTA, 100mM NaCl, pH 8) containing 1% SDS and 0.4mg/ml of proteinase K. The mixture was incubated for 1h at 56°C. An equal volume of phenol/chloroform/isoamylalcohol was then added to mixture and centrifuged at 10,000rpm for 10min. The supernatant was added to an equal volume of chloroform and after centrifuging at 10000rpm, the top layer was collected and DNA was precipitated with two volumes of cold isopropanol at -20°C for 10min. The pellet was obtained by centrifugation for 20min and washed with 1.5ml of 70% cold ethanol. Finally, the pellet was resuspended in 100microliter of TE 1X buffer (10mM Tris-Cl, 1mM Na₂ EDTA, pH 8) and 1µl was used for AP-PCR reaction (12,13).

AP-PCR: The AP-PCR fingerprinting was carried out using ARB11 primer (AP-ARB11; 5'-CTAGGACCGC-3') and AP-PG05 (5'-AGCCCA GCTATGAAC-3') (14,15). DNA templates were amplified in a total reaction volume of 50µl containing 2.5U of Taq polymerase, 50pmol of each primer, 200µM of each deoxynucleotide, 1.5mM MgCl₂, 10mM Tris-HCl (pH 8.3), and 50mM KCl.

Amplification was carried out with denaturation at 94°C for 5min, followed by 40 cycles according to the following program: 94°C for 30sec, annealing at 40°C for 1min, and extension at 72°C for 1min, plus a final extension of 10min at 72°C to complete partial polymerizations. The PCR product was run and visualized in 2% agarose gel stained with ethidium bromide (14,15).

RESULTS

All tested strains of *S. sonnei* were typeable by this method. Nine indistinguishable bands were produced by AP-PCR in all recent strains. Generated fragments ranged from 0.35 to 2.5 kbp. As shown in figure 1, only a single AP-PCR pattern was observed among the *S. sonnei* strains recovered in 2003.

Two sporadic isolates recovered in 1984 showed different AP-PCR patterns compared to recent clinical isolates.

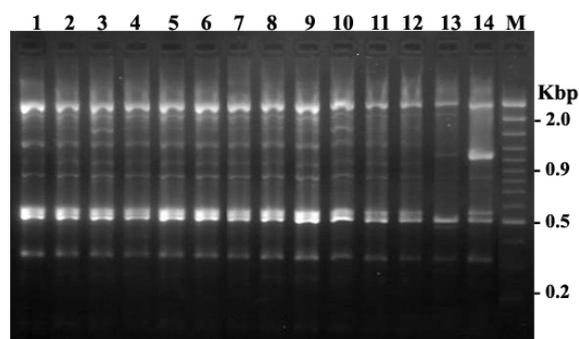


Figure 1. Genomic typing by AP-PCR fingerprinting. Lanes 1-12 are amplified DNA bands belonging to 12 representative *S. sonnei* strains isolated in Tehran in 2003. Lanes 13 and 14 are amplified DNA bands belonging to two sporadic strains isolated from clinical cases in Tehran in 1984. Lane M is molecular size marker (100bp).

DISCUSSION

Current research was carried out to apply AP-PCR for investigation of genetic relatedness among 60 randomly selected strains of *S. sonnei* isolated from cases of acute diarrhea in Tehran during 2003.

Molecular epidemiology provides a crucial contribution to accurately interpret epidemiological evolution of infectious diseases in communities, when screening by phenotypic methods, such as biotyping or drug resistance pattern analysis, is seriously hindered by the homogeneity of circulating strains (2).

We found only a single AP-PCR pattern among the *S. sonnei* strains isolated in 2003. The results suggest that a very homogeneous AP-PCR cluster types might be responsible for shigellosis caused by *S. sonnei* in Tehran in 2003.

In a separate report published in 2007, we have investigated PFGE patterns of some strains of *S. sonnei* used in current study and found *S. sonnei*

isolated in 2003 could be attributed to a few predominant clusters (2).

When considering and comparing the results obtained from these studies on same isolates, our findings reinforce the previous considerations and emphasize the involvement of a limited number or an oligo clone of *S. sonnei* during the study period. Further molecular analysis conducted on a larger selection of isolates could confirm our findings.

We hope the obtained results could pave the way for further epidemiological investigation of *Shigella* spp. particularly *S. sonnei* in Tehran, Iran. Continuous studies should be conducted in this area and other parts of this large country in order to investigate the subtyping of *Shigella* organisms using more discriminating molecular methods.

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REFERENCES

1. Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ, et al. Global burden of *Shigella* infections: implications for vaccine development and implementation of control strategies. *Bull WHO* 1999;77:651-66.
2. Ranjbar R, Aleo A, Giammanco GM, Dionisi AM, Sadeghifard N, Mammina C. Genetic relatedness among isolates of *Shigella sonnei* carrying class 2 integrons in Tehran, Iran, 2002-2003. *BMC Infect Dis* 2007;22:62.
3. Ranjbar R, Mammina C, Pourshafie MR, Soltan-Dallal MM. Characterization of endemic *Shigella boydii* strains isolated in Iran by serotyping, antimicrobial resistance, plasmid profile, ribotyping and pulsed-field gel electrophoresis. *BMC Res Notes* 2008;1:74.
4. Ranjbar R, Soltan Dallal MM, Pourshafie MR, Aslani MM, Majdzadeh R, Khorramizadeh MR. Serogroup distribution of *Shigella* spp. in Tehran. *Iranian J Publ Health* 2004; 33:32-35.

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5. Ranjbar R, Soltan Dallal MM, Pourshafie MR. Epidemiology of shigellosis with special reference to hospital distribution of *Shigella* strains in Tehran. *Iranian J Clin Infect Dis* 2008;3(1):35-8.
6. Hinojosa-Ahumada M, Swaminathan B, Hunter SB, Cameron DN, Kiehlbauch JA, Wachsmuth IK, et al. Restriction fragment length polymorphisms in rRNA operons for subtyping *Shigella sonnei*. *J Clin Microbiol* 1991;29:2380-84.
7. Jamieson AF, Bremner DA, Bergquist PL, Lane HED. Characterization of plasmids from antibiotic-resistant *Shigella* isolates by agarose gel electrophoresis. *J Gen Microbiol* 1979;113:73-81.
8. Welsh J, Rampino N, McClelland M, Perucho M. Fingerprinting genomes using PCR with arbitrary primers. *Nucleic Acids Res* 1990;18:7213-8.
9. Ramadass P, Latha D, Senthilkumar A, Srinivasan P, Saranya N. Arbitrarily primed PCR; A rapid and simple method for typing of leptospiral serovars. *Indian J Med Microbiol* 2002;20:25-8.
10. Wullt M, Burman LG, Laurell MH, Akerlund T. Comparison of AP-PCR typing and PCR-ribotyping for estimation of nosocomial transmission of *Clostridium difficile*. *J Hosp Infect* 2003;55:124-30.
11. Ewing WH, editor. *Edwards and Ewing's identification of Enterobacteriaceae*. Elsevier Science Publishing Co. New York, 1986;p:169-81.
12. Ranjbar R, Pourshafie MR, Sadeghifard N, Karami A, Hamidian M, Izadi M, et al. Molecular characterization of epidemic isolates of *Vibrio cholerae* O1 by Arbitrarily primed PCR (AP-PCR). *Iranian J Publ Health* 2008;37:83-7.
13. Ranjbar R, Sadeghifard N, Ahmadi A, Izadi M, Zaeimi-Yazdi J, Ghasemi A, et al. Antimicrobial susceptibility and AP-PCR typing of Iranian isolates of *Acinetobacter* spp. strains. *Iranian J Publ Health* 2007; 36:50-6.
14. Killgore GE, Kato H. Use of arbitrary primer PCR to type *Clostridium difficile* and comparison of results with those by immunoblot typing. *J Clin Microbiol* 2003; 32:1591-93.
15. Silva JJ, Tang YJ, Gumerlock PH. Genotyping of *Clostridium difficile* isolates. *J Infect Dis* 1994;169:661-64.