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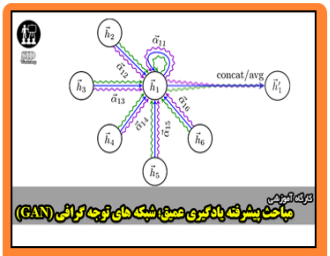

مباحث پیشرفته یادگیری عمیق؛
شبکه های توجه گرافی
(Graph Attention Networks)

Analysis of Clonal Relationships among Shigella spp. Isolated from Children with Shigellosis in Ahvaz, Iran

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ABSTRACT

Shigellosis is one of the important gastrointestinal bacterial infections, particularly among children of developing countries such as Iran. Antibiotic susceptibility pattern and genetic typing for epidemiological purposes are of significant issues in Shigella infectious control. The aim of this study was to investigate the antibiotic susceptibility and genetic relationship among Shigella strains isolated from children with shigellosis at paediatric hospital in Ahvaz, south west of Iran. This study included all Shigella strains isolated from paediatric patients with diarrhea admitted to Abuzar pediatric hospitals in Ahvaz, during January-June 2015. Shigella isolates were identified using standard microbiological and serological methods. Shigella spp strains also were studied by antimicrobial susceptibility testing and Enterobacterial Repetitive Intergenic Consensus (ERIC) - PCR analysis. Total of 50 Shigella strains were isolated from children with dysentery diarrhea. In total, 31 (62%) were identified as Shigella flexneri, 16(32%) and 3 (6%) were Shigella sonnei and Shigella boydii respectively. High level resistance were detected against ampicillin, trimethoprim-sulfamethoxazole and cephalotine. All isolates were sensitive to ceftriaxone, imipenem gentamicin and amikacin. The results of ERIC-PCR data analysis showed 11 different types of Shigella with four closely-related patterns. S. flexneri was the predominant serogroup of Shigella spp. in children in the referral pediatric hospital in Ahvaz. Ampicillin and trimethoprim-sulfamethoxazole is no longer recommended for shigellosis empirical treatment and should be replaced by other antibiotics such as ceftriaxone or ciprofloxcacin. Diverse but genetically close strains of shigella were responsible for shigellosis in paediatric patients in Ahvaz, south west of Iran.

Keywords: Shigella, Clonal relationships, ERIC-PCR

INTRODUCTION

Shigellosis is a gastrointestinal infection caused by Shigella. Shigella is a Gram-negative, anaerobic, non-spore forming and non-motile bacilli belong to the Enterobacteriaceae family [1]. Clinically important species of Shigella genus are S. flexneri, S. dysenteriae, S. sonnei, S. boydii, which can cause shigellosis or bacillary dysentery (bloody diarrhea) [1].

Though shigellosis involve people at any age in both developing and developed countries, they are more prevalent among children with two to three years of age due to lack of personal hygiene, specifically the case of hand washing. Shigella is one of the main causes of death among children worldwide, including Iran. [2,3] Antibiotic resistance in Shigella spp is increasing and emerge of multi resistant strains has become a public health challenge in recent

years [4]. Khuzestan province in the south west of Iran is an endemic area for shigella infections in children.

Epidemiological studies have suggested molecular typing as useful tools for tracing the infection resources and genetic relationship of the bacteria [5-6].

PCR-based typing methods, such as REP-PCR is an alternative technique to produce fingerprint directly without treatment by endonucleases. This technique is rapid, reproducible and has a high discriminatory power. Nowadays, REP-PCR, a genetic fingerprinting method, has been widely used for genetic typing [8]. Oligonucleotide primers are designed based on short repeated sequences in prokaryotes [7]. In the REP-PCR, three types of primer sequences that are complementary with three types of repetitive elements are used: first, the ERIC sequences, with 126 base pairs (bp), complementary to repetitive intragenic sequences of Enterobacteriaceae family; second, the REP sequences, a 38 bp sequence, complementary to the gene palindrome and the third area called 154 bp repetitive elements box. Position and the number of ERIC sequences in bacteria are different, so they can be used as genetic markers for considering a variety of bacteria [7, 8].

Since Shigella infection is one of the common causes of diarrheal disease among children in Ahvaz city in Khuzestan province and ERIC-PCR is one of the best techniques for epidemiologic studies of bacterial infections [8], thereby, the aim of this study is to investigate the antibiotic susceptibility and genetic relationship by ERIC-PCR technique among Shigella strains isolated from children with shigellosis from the main pediatric hospital in Ahvaz.

MARERIAL AND PATIENTS

Sampling

Diarrheal stool samples were collected from patients with diarrhea who had referred to Abuzar paediatric hospital during January to July 2015. Samples were placed in clean stool containers and were transferred to the microbiology laboratory of Department of Microbiology in Ahvaz Jundishapour University. Samples were immediately cultured on xylose lysine deoxycolate (XLD), Hectoen enteric agar and Salmonella- Shigella agar (SS) media and were incubated for 24 h at 37 ° C afterwards. All media were purchased from MERCK (MERCK, Germany). The isolates were identified as Shigella spp. based on conventional morphologic and conventional microbiologic tests. Serological diagnostic tests using specific antiserum (Bahar-Afshan, Iran) were used to determine the Shigella spp. serogroups.

Antimicrobial susceptibility testing

The antimicrobial susceptibility of Shigella strains were determined by Kirby–Bauer disk diffusion method. The antibiotics (Rosco, Denmark) panel including: ampicillin (AMP: 30 µg), ampicillin sulbactam (SAM:30 µg), ciprofloxacin (CIP: 30µg), gentamicin (GEN: 10 µg), nalidixic acid (NI:30µg), cephalothin (CEP:30µg), ceftazidime (CAZ:30µg), imipenem (10µg), ceftriaxone (CTR:30µg), amikacin (AMI;30µg),sulphametoxazole thriimethoprim (SXT:1.25 + 23.75 µg)Zone diameters were measured after 24 h incubation at 35ºC. Results were interpreted according to clinical and laboratory standards institute (CLSI 2014) [9].

DNA Extraction and ERIC–PCR

DNA was extracted from all isolates by DNA extraction kit (Sinacolon, Iran). PCR components and amplification profiles comprise a 300 nM of each primer ERIC (F): 5′- ATG TAA GCT CCT GGG GAT TCA C-3 و′ ERIC (R): 5′- AAG TAA GTG ACT GGG GTG AGC G-3′ (Metabion, Germany); 5.5 mM MgCl2; 200 mM each deoxynucleoside triphosphates (dNTP); and 0.125 U of Taq DNA polymerase.

After preparing the reaction mixture in a volume of 25 µl., PCR was performed using DNA thermocyler (Peq Star, Germany) with the following program: denaturation at 94 °C(3 minutes , 1 cycle), denaturation at 94 ° C(1 minute, 35 cycle), 48 °C (1 minute, 35 cycles), 72°C (2 minutes, 35 cycles), 72 °C (5 minutes ,1 cycle). The PCR products were visualized by electrophoresis in 1.2% agarose gel, stained with ethidium bromide, and examined under ultraviolet gel imaging system. The sizes of the PCR products were determined through comparison with the 1Kb molecular size standards.

ERIC results analysis

For the purpose of detecting the genetic relationship among Shigella isolates based on ERIC area, 20 isolates were selected according to differences in banding pattern and antibiotic resistance phenotypes. The Gel Compare software version VI was used to analyze ERIC results. Band profiles were compared with Dice method and clustering was done by UPGMA program, Gel Compare software, version 4.0.

Statistical analysis

Statistical analysis was conducted using the SPSS version 16 (SPSS, Inc., Chicago, IL, USA). Chi-square test and Fisher's exact test was used for the evaluation relation between qualitative variables. P-value of less than 0.05 was considered as significant.

RESULTS

A sum of 50 Shigella strains were isolated from children suspected to shigellosis who referred to Abouzar hospital in Ahvaz city. From each patient, one strain was isolated. Among isolates, 31(62%) were identified as *S. flexneri*, 16(32%) as *S.sonneii*, and 3 (6 %) as *S.boydii*. Considering the gender of patients, 21 (42%) were female and 29 (58%) were male patients. There was no statistically significant association between gender and species of shigella (p> 0.05). Sixty patients (32%) were under 2 years old and 34 patients (68%) were 2 to 8 years old. There was not any association between age and type of Shigella spp.

Antibiotic resistance patterns

The antimicrobial susceptibility analysis showed that all Shigella strains were susceptible to gentamycin, imipenem, amikacin, and ceftriaxone. High level resistance was observed against ampicillin 50 (100%), cephalexin 33 (66%) and trimethoprim-sulfamethoxazole 41 (82%). The antibiotic resistance rate of Shigella isolates is shown in figure 1.

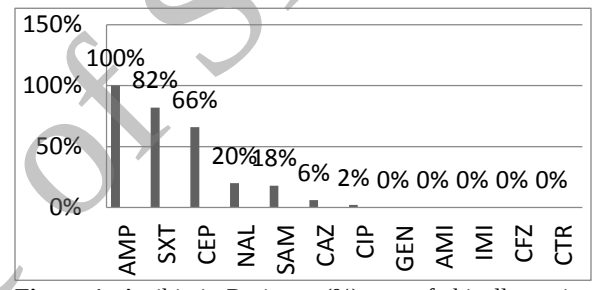

Figure 1. Antibiotic Resistant (%) rate of shigella strains isolated from children with shigellosis in Ahvaz, south west of Iran.

The majority of shigella isolates [68%] were resistant to three or more antibiotic classes [10] from different antibiotic families and were considered as multidrug-resistant (MDR) isolates. Our results also showed a co-resistance to cephalotin and trimethoprim-sulfamethoxazol (CEP /SXT) was the most common co-resistance pattern (phenotypes) among entire shigella spp. 34 (68%) as well as each species (table 1). The second most common co-resistance pattern was found against cephalotin / sulfamethoxazole - trimethoprim / nalidixic acid (CEP / SXT / NAL) with rate of 14%. (Table 1). There was no association between antibiotic resistance pattern and types of Shigella spp.

Table 1. Antibiotic Resistant Patten of Shigella spp. Isolated from children with shigellosis in Ahvaz

Antibiotic Resistance Patterns	Resistance Frequency (%)
AMP	100%
AMP/SXT/CEP	68%
AMP/SXT/ SAM	2%
AMP/SXT/CEP/NAL	14%
AMP/SXT/CEP/SAM	13%
AMP/SXT/CEP/CAZ	2%
AMP/SXT/CEP/SAM/NAL	6%
AMP/SXT/CEP/SAM/NAL/CAZ	2%

REP (ERIC)-PCR

Considering the REP (ERIC)-PCR results, the amplified fragment (bands) sizes varied from 200 bp to more than 1 kb. Through analysis of electrophoresis results, the genetic diversity among Shigella isolates was revealed. Each of Shigella species showed unique REP-PCR patterns. Although, the banding patterns of *S. flexneri*, *S. sonnei* and *S. boydii* were different from each other, high genetic relationship was detected among strains of each species. An average of three bands, four bands and five bands were observed in *Shigella flexneri*, *Shigella boydii* and *Shigella sonnei* *respectively*. Based on REP-PCR analysis of studied shigella isolates, a total of 4 clusters and eleven different types were identified. Each of *Shigella flexneri* and *S. sonnei* was set in two separated clusters. *S.boydii* isolates were not set in any cluster (Figure 2).

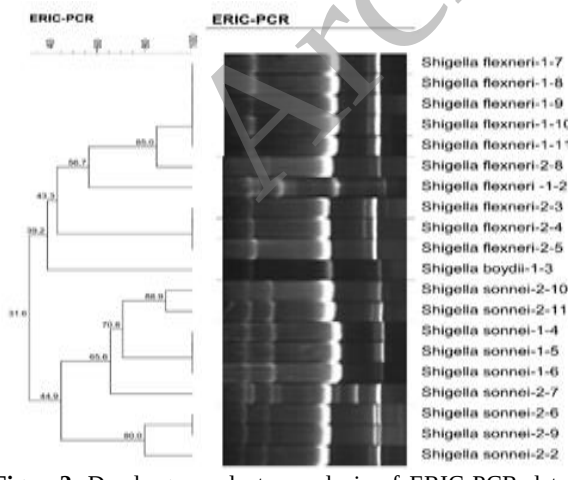

Figure2. Dendrogram cluster analysis of ERIC-PCR data for 20 selected Shigella strains isolated from children with shigellosis in Ahvaz,Iran

DISCUSSION

S.flexneri is one of the sources of shigellosis in developing countries, whereas *S.sonnei* has been known as the predominant species isolated from clinical cases in developed countries. Our findings revealed that *S. flexneri* is the major cause of shigellosis in studied children (63%) in Ahvaz area which is in accordance with the other study in region, in which Khaghani et al have showed *S. flexneri* as common cause of shigellosis among children in Ahvaz during 2008 to 2010 [11]. However, some reports from Iran indicate an increasing replacement of *Shigella flexneri* with *Shigella sonnei* in recent years; for example, in Ranjbar et al study, *S. sonnei* was the most common Shigella species isolated from patients admitted in Tehran hospitals [7]. In general, we can conclude that based on certain circumstances, the predominant shigella species could be different even in different parts of one country

Though children are more at risk for shigellosis and development of severe clinical symptoms, the risk can vary depending on the age. According to our findings, 32 % of shigellosis cases belonged to children under two years of age, hence, the more vulnerability of this age group to getting shigellosis rather than other age groups.

In recent years, resistance against commonly available antibiotics such as ampicillin, tetracycline and sulfonamides is at rise and even multiple drug resistance Shigella species has been reported [12]. Ampicillin and trimethoprim-sulfamethoxazole are the first recommended choices for shigellosis empirical treatment. Considering the result of the current study in which all isolates were resistant against

ampicillin and also remarkable number (88%) were resistant to trimethoprim-sulfamethoxazole, it is concluded that they are not suggested for empirical treatment of shigellosis in the studied area any more. As the same result can be seen in other regions, the antibiogram test seems necessary for using ampicillin and trimethoprim-sulfamethoxazole for treatment of shigellosis cases.

Ciprofloxacin, third-generation cephalosporins and fluoroquinolones are known as the second-line antibiotics for shigellosis treatment. Farshad et al and Ranjbar et al have reported a very low level or no- resistance to ciprofloxacin in Iranian isolates [2, 13]. We found 2% resistance to ciprofloxacin and 6% to ceftazidim and high levels of susceptibility to gentamicin and amikacin, proving our study to be in accordance with previous studies, and their effective action against Shigella spp [13, 7].

Epidemiological investigation of gastrointestinal infection due to Shigella spp. are quite significant issues because they are public health-threatening diseases and children are extremely vulnerable to this infections. It has been proven that molecular typing method will be able to solve many epidemiological problems. Among molecular typing methods for epidemiological studies of shigella spp., the PFGE technique is the gold standard; however, this technique is very expensive and sometimes time-consuming. In this study ERIC-PCR was used which is much easier and cheaper to perform, particularly in Enterobacteriaceae family, including Shigella spp [14-16]. Moreover, the reliability, adequate rapidity and discriminatory power of this method have been documented for typing of Shigella species [7, 17-19].

The ERIC-PCR was used to study the genetic relatedness among endemic *shigella spp* strains isolated from paediatric patients in Ahvaz, Iran. This technique categorized isolates into four different clusters (E1-E4) and 11 different types with different antibiotic resistance patterns. ERIC-PCR results manifested close genetic linkage among strains from each species. This result may indicate common sources of shigellosis in Ahvaz. Our finding also showed diversity in ERIC regions among *S.flexneri, S. sonnei and S. boydii* strains. Moreover, S. *flexneri* and *S.sonnei and S. boyidii* exhibited unique ERIC patterns. According to these findings we may use ERIC-PCR as a potent technique for molecular typing and discriminating the shigella spp. In accordance with our study, Ranjbar and colleagues have documented that ERIC-PCR is a useful molecular technique for subtyping and epidemiological studies of shigella spp in Iran [7, 17]. About 70% of isolates showed the same antibiotic resistance pattern; therefore, it is concluded that Shigella spp with close antibiotic resistance and genetic linkage are cause of shigellosis in children in Ahvaz.

One of the goals of molecular typing is detecting the common source of infection, such as contaminated food or water. In this study, it was not possible to get information from presumptive sources of shigellosis infection due to the limitation in sampling from wide area in a big city like Ahvaz and also due to having patients referring from others cities of province and neighbour Provinces; nevertheless,, typing results indicated that there should be probably some common source of infection caused by each species of Shigella.

CONCLUSIONS

To sum up, *S. flexneri is* identified as the most common cause of shigellosis in children in the Ahvaz, south west of Iran. Fluoroquinolones, aminoglycosides and third generation cephalosporins antibiotics showed greater action against Shigella spp. While majority of isolates were resistant against ampicillin and sulphametoxazle trimethoprim, we found that different clones of Shigella spp. were

responsible for shigellosis in children and are circulating in Ahvaz. Moreover, the current study showed that REP-PCR molecular typing would be beneficial to epidemiological and surveillance studies of shigella spp. to peruse a better differentiation of strains, specifically among children living in high-risk endemic areas like Ahvaz.

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"The authors declare no conflict of interest"

REFERENCES

1.Dekker JP, Frank KM. Salmonella, Shigella, and yersinia. Clinical Lab Medicine 2015; 35 (2):225–46.

2. 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 Journal of Microbiology 2014; 8(1):14004.

3.Prevention.National Shigella surveillance annual report, 2012. Atlanta, Georgia: US Department of Health and Human Services, CDC. 2014.

4. Ud-Din AI, Wahid SU, Latif HA, Shahnaij M, Akter M, Azmi IJ, et al. Changing trends in the prevalence of Shigella species: emergence of multi-drug resistant Shigella sonnei biotype g in Bangladesh. PloS one 2013;8 (12):e82601.

5.Bentley CA, Frost JA, Rowe B. Phage typing and drug resistance of *Shigella sonnei* isolated in England and Wales. Epidemiology Infection journal 1996; 116: 295-302.

6.Mendoza MC, Martin MC, Gonzalez-Hevia MA. Usefulness of ribotyping in a molecular epidemiology study of shigellosis. Epidemiology infection journal 1996; 116:127-135.

7.Ranjbar R, Ghazi FM. Antibiotic Sensitivity Patterns and Molecular Typing of *Shigella sonnei* Strains Using ERIC-PCR. Iranian Journal of Public Health 2013; 42(10): 1151-1157

8.Wilson LA Sharp PM. Enterobacterial Repetitive Intergenic Consensus (ERIC) Sequences in Escherichia coli: Evolution and Implications for ERIC-PCR. Molecular Biology and Evolution 2006; 23:1156–1168.

9.CLSI Clinical and Laboratory Standards Institute. Performance Standards for Antimicrobial Susceptibility Testing, Tweny four Information Supplement. 2014.

10. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 2012 1; 18(3):268-81.

11.Khaghani S, Shamsizadeh A, Nikfar R, Hesami A. Shigella flexneri: a three-year antimicrobial resistance monitoring of isolates in a Children Hospital, Ahvaz, Iran. Iranian Journal of Microbiology. 2014; 6 (4):225-9.

12. Song Q, Lin W, Gao H, Yang Y, Xu J, Xu G. Changing antimicrobial resistance patterns and trends of Shigella isolates in Ningbo, mid-east China, 2005–2013. International journal of antimicrobial agents. 2015; 45(5):559.

13.Ranjbar R, Mammina C, Pourshae 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.

14.Sobrino B, Brion M, Carracedo A. SNPs in forensic genetics: a review on SNP typing methodologies. Forensic Science International journal 2005; 154: 181–194.

15.Cooper JE, Feil EJ. Multi locus sequence typing: what is resolved? Trends Microbiology 2004; 12: 373–377.

16.Willems RJ, Van Schaik W. Transition of *Enterococcus faecium* from commensal organism to nosocomial pathogen. Future Microbiology. 2009; 4 (9):1125-35.

17.Ranjbar R, Hosseini MJ, Kaffashian AR, Farshad S. An outbreak of shigellosis due to Shigella flexneri serotype 3a in a prison in Iran. Archive of Iranian medicine 2010; 13: 413-16.

18.Liu PY, Lau YJ, Hu BS, Shyr JM, Shi ZY, Tsai WS et al . Analysis of clonal relationships among isolates of *Shigella sonnei* by different molecular typing methods. Journal of Clinical Microbiology 1995*;* 33: 1779-83.

19.Penatti MP, Hollanda LM, Nakazato G, Campos TA, Lancellotti M, Angellini M, et al Epi-demiology characterization of resistance and PCR typing of *Shigella flexneri* and *Shigella sonnei* strains isolated from bacillary dysentery cases in Southeast Brazil. Brazilian Journal of Medical and Biological Research 2007; 40:294-258.

20.Lee TM, Chang CY, Chang LL, Chen WM, Wang TK, Chang SF One predominant type of genetically closely related *Shigella sonnei* prevalent in four sequential outbreaks in school children. Diagnosis Microbiology Infectious Disease 2007*;* 45:173-181.

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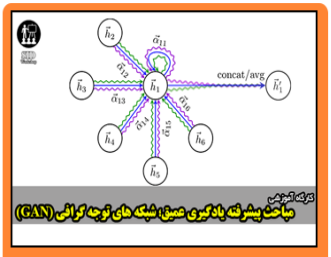

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