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BRIEF REPORT

Occurrence of class 2 integrons among multi-drug resistant *Shigella sonnei* isolated from Tehran, Iran in 2005

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ABSTRACT

Background: Shigellosis is one of the major causes of morbidity in children with diarrhea in Iran. Integrons play an important role in the evolution and dissemination of multidrug resistance in gram-negative bacteria. The occurrence of integrons among *Shigella* spp. is frequently reported throughout the world. The aim of this study was to assess the occurrence of class 2 integrons among the multi drug resistant *S. sonnei* isolated from Iranian children in 2005.

Materials and methods: The study was conducted in two major pediatric hospitals in Tehran, Iran. Fecal specimens and rectal swab collected from patients were cultured and identified as *Shigella* by the conventional methods. Antimicrobial susceptibility test was performed according to the standard CLSI guideline. Multi-drug resistant isolates of *S. sonnei* were further examined for the presence of class 2 integron by PCR using specific primers. Amplicons were confirmed by restriction endonuclease analysis.

Results: A total of 83 multi-drug resistant *S. sonnei* strains were isolated. Of these, 45 (54%) exhibited a class 2 integron of 2.1 kbp, and 34 (41%) a class 2 integron of 1.3 kbp. Class 2 integrons were not detected in four isolates.

Conclusion: The results showed an increased occurrence of class 2 integron carrying *S. sonnei* isolated from children in Tehran in 2005.

Keywords: *Shigella sonnei, Class 2 integron, Multi-drug resistant.*
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INTRODUCTION

Infections caused by *Shigella* species are an important cause of diarrheal disease, in both developing and developed countries. Shigellosis is usually associated with a significant increase in the morbidity and mortality rates (1,2). It is one of the major causes of morbidity in children with diarrhea in Iran (3-8).

Integrons as mobile genetic elements are able to disseminate antibiotic resistance genes by horizontal or vertical transfer and have been shown to play an important role in the evolution and dissemination of multidrug resistance in gram-

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negative bacteria (9,10). These genetic elements have been frequently reported among members of *Enterobacteriaceae* (11).

Several classes of integrons have been identified, each with a distinct integrase gene, associated with gene cassettes coding for antimicrobial resistance genes, however integron classes 1 and 2 are the most frequent in gram-negative bacteria (12).

Class 1 integrons are frequently reported in clinical isolates and their gene cassette arrays are similar to those observed in the transposon *Tn7* (13). The class 2 integron has an organization similar to that of class 1 but it is associated with transposon *Tn7*, and it is known to carry three classic gene cassettes, *dfrA1*, *sat1* and *aadA1*, which confer resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively (14).

Class 3 integrons are rare. Class 4 is a distinctive class of integrons located in the *Vibrio cholerae* genome and is not known to be associated with antibiotic resistance (15).

Recently, several reports have shown that *S. sonnei* carrying a class 2 integron is responsible for some epidemic shigellosis in different countries (16-21).

In our previous study (3), genetic relatedness among isolates of *S. sonnei* carrying class 2 integrons has been investigated in Tehran, Iran during 2002-2003 and has been shown that class 2 integron carrying *S. sonnei* emerged in our geographic area. To assess the new situation in 2005, we conducted the present study to investigate the occurrence of class 2 integron among the multi drug resistant strains of *S. sonnei* isolated from pediatric patients admitted to two major pediatric hospitals in Tehran, Iran.

PATIENTS and METHODS

Bacterial strains and antimicrobial susceptibility testing: We conducted the study in two major pediatric hospitals (Children Medial Center and Mofid hospital) in Tehran, Iran, in 2005. The study included all patients less than 12 years of age with diarrhea (three times or more watery or soft defecations per 24 h) that had lasted ≤7 days, fever, abdominal pain, tenesmus with or without nausea and vomiting.

Fecal specimens and rectal swab collected from patients were cultured according to standard microbiological methods. All *Shigella* strains were identified at a genus level by conventional methods by previously described procedures (3,22) while agglutination with specific antiserum from MAST Group LTD (Mast House, Derby Road, Bootle, Merseyside, L20 1EA, UK) was used to identify *S. sonnei*. Antimicrobial susceptibility test was performed according to the standard CLSI guideline (23) using ampicillin, ceftriaxone, nalidixic acid, kanamycin, ceftizoxime, ceftazidime, ciprofloxacin, cephalothin, cefotaxime, co-trimoxazole, streptomycin and tetracycline.

Integron analysis: The PCR reaction mixture was prepared as previously described (3). For integron analysis, PCR was performed with the specific primer pair hep74 (5'-CGGGATCCCGGAGGCATGCACGATTTGTA-3') and hep51 (5'-ATGCCATCGCAAGTACGAG-3'). Primer hep74 binds to *attI*2, and hep51 binds to *orf*X, situated at the right end of the cassette region within transposon *Tn7* (24).

Amplification was performed by using the following temperature profile: pre-denaturation at 94°C for 10 min, followed by 35 cycles of 94°C for 1 min, 55°C for 1 min, and 72°C for 5 min, with a final extension step at 72°C for 5 min. The amplified DNA products were analyzed by conventional 1% agarose gel electrophoresis in 1× TBE buffer and stained with ethidium bromide.

The sequence similarity among the 2.2-kb amplicons was confirmed by restriction analysis with the restriction endonucleases HincII (20). Approximately 5 to 10 units of endonuclease per

10 micrograms of DNA were used for DNA digestion under the conditions recommended by the manufacturer (Roche Diagnostics, Mannheim, Germany). The digestion products were analyzed on a. 1% agarose gel stained with ethidium bromide.

RESULTS

All strains were resistant to streptomycin, co-trimoxazole and tetracycline, while 10% of the strains were resistant to ampicillin, 8.3% to nalidixic acid and 5% to kanamycin. None of the isolates were resistant to ceftriaxone, ceftizoxime, ceftazidime, ciprofloxacin, cephalothin and cefotaxime. All 83 multi-drug resistant (streptomycin, sulfamethoxazole-trimethoprim and tetracycline) *S. sonnei* were selected for integron analysis. As shown in figure 1, two different class 2 integron structures were identified among *S. sonnei* isolates, 45 strains (54%) contained a 2.2 Kbp class 2 integron and 34 strains (41%) had a different class 2 integron of 1.3 Kbp. Class 2 integrons were not detected in 4 strains. Restriction analysis of 2.2-kb amplicons using restriction endonuclease *Hinc*II produced the expected restriction pattern.

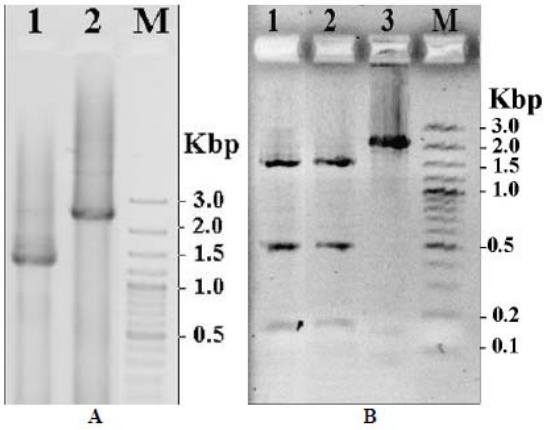

Figure 1. (A) Class 2 integron in *S. sonnei* strains in Iran in 2004. Lanes: 1 and 2 are representative strains carrying class 2 integron of 2.2 kbp and 1.3 kbp respectively; M, 100-bp DNA ladder.
(B) Results of restriction analysis of the 2.2-kb amplicons of a representative class 2 integron-positive *S. sonnei* strain with *Hinc*II (lane 1). Lanes 2 contains the undigested amplicon. Lane M, 100 bp DNA ladder.

DISCUSSION

Indiscriminate use of antibiotics and horizontal gene transfer has led to the development of resistance of *Shigella* spp against to commonly used antibiotics. Multi-resistance to the antimicrobial agents used in treatment of shigellosis has been reported in Europe (25), Africa (26), Asia (27) and South America (28,29).

Dissemination of multi-resistant strains of *S. sonnei* carrying a class 2 integron has been recently reported in many countries (16-22) suggesting a worldwide occurrence of cases due to this organism. The previous study confirmed this situation in Iran since *S. sonnei* isolated in the years 2002 and 2003 attributed to a few predominant clusters including strains with PFGE types B and C carrying a 2161 bp class 2 integron, and PFGE types A carrying a 1371 bp class 2 integron (3).

The present study was undertaken to assess new situation in 2005 in Iran. Antimicrobial susceptibility testing showed a high degree of resistance to traditionally used antibiotics (streptomycin, tetracycline, and trimethoprim-sulfamethoxazole) in the area. This result agreed with several earlier studies (11,30).

Eighty three multi-drug (streptomycin, sulfamethoxazole-trimethoprim and tetracycline) resistant *S. sonnei* strains isolated in the study were analyzed for the occurrence of class 2 integrons. Fifty four percent of the strains contained a 2.2 kbp class 2 integron while 41% carried 1.3 Kbp class 2 integron. Class 2 integrons were not detected in 4 strains.

As suggested by other investigators, resistance to streptomycin and trimethoprim seems to be attributable to existence of genes contained in class 2 integrons, although other mechanisms are possible. Their gene array, generally including the

ORF *dhfrI*, *sat* and *aad*, is rather stable, due to the presence of a defective integrase. It is established that these genes are highly prevalent among Shigella spp. from other countries (11,17,19,21)

As suggested in a previously published report (3), our finding showed the simultaneous circulation of two groups of strains carrying a classes 2 integron of 2.2 kbp bp and a shorter integron of 1.3 kbp,

The proportion of class 2 integron-positive isolates is comparable with those strains isolated from Tehran during 2002-03. In a recent study, among 54 streptomycin, sulfamethoxazole-trimethoprim and tetracycline resistant *S. sonnei* isolates, 28 (52%) exhibited a class 2 integron of 2161 bp, and 24 (44%) a class 2 integron of 1371 bp, respectively. Class 2 integrons were not detected in two isolates (3).

When comparing the results with previous studies, our findings reinforce the establishment and/or dissemination of a few well defined multi-resistant clones of *S. sonnei* in the country.

We hope the results obtained from current study will be useful in epidemiological investigation of *Shigella* spp., particularly *S. sonnei* in Iran. More continuous surveillance studies should be conducted in other parts of world in order to investigate the true distribution of *S. sonnei* carrying the class 2 integron in the country and its impact on the epidemiological behavior of this organism on a global scale.

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REFERENCES

1. Bennish ML, Wojtyniak BJ. Mortality due to shigellosis: community and hospital data. Rev Infect Dis. 1991;13: S245–S251.

2. Kotloff KL, Winickoff JP, Ivanoff B, Clemens JD, Swerdlow DL, Sansonetti PJ, et al. Global burden of Shiglla infections: implications for vaccine development and implementation of control strategies. Bull World Health Organ. 1999;77:651-66.

3. 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.

4. 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.

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

6. Ranjbar R, Soltan-Dallal MM, Pourshafie, Mammina C. Antibiotic resistance among *Shigella* serogroups isolated in Tehran, Iran (2002-2004). J Infect Dev Ctries. 2009;3:647-48.

7. Ranjbar R, Sadeghifard N, Soltan Dallal MM, Farshad S. Evaluation of a PCR based approach to study the relatedness among *S. sonnei* strains isolated in Tehran. Iranian Journal of Clinical Infectious Diseases 2009; 4:163-66.

8. Hosseini MJ, Ranjbar R, Ghasemi H, Jalilian HR. The prevalence and antibiotic resistance of *Shigella spp*. recovered from patients admitted to Bouali hospital, Tehran, Iran during 1999-2000. Pak J Biol Sci. 2007; 10: 2778-80.

9. Hall RM, Stokes HW. Integrons: novel DNA elements which capture genes by site-specific recombination. Genetica. 1993;90:115–32.

10. Liebert CA, Hall RM, Summers AO. Transposon Tn21, flagship of the floating genome. Micobiol Mol Biol Rev. 1999;63:507–22.

11. Peirano G, AgersoY , Aarestrup FM, dos Reis EM ,dos Prazeres Rodrigues D. Occurrence of integrons and antimicrobial resistance genes among *Salmonella enterica* from Brazil. J Antimicrob Chemother. 2006; 58:305-9.

12. Goldstein C, Lee MD, Sanchez S, Hudson C, Phillips B, Register B, et al. Incidence of class 1 and 2 integrases in clinical and commensal bacteria from livestock, companion animals, and exotics. Antimicrob Agents Chemother. 2001;45:723-26.

13. Tosini F, Visca P, Luzzi I, Dionisi AM, Pezzella C, Petrucca A, et al. Class 1 integron-borne multiple-

antibiotic resistance carried by IncFI and IncL/M plasmids in *Salmonella enterica* serotype Typhimurium. Antimicrob Agents Chemother. 1998; 42:3053-58.

14. Hansson K, Sundström L, Pelletier A, Roy PH. IntI2 integron integrase in Tn7. J Bacteriol. 2002;184:1712-21.

15. Mazel D, Dychinco B, Webb VA, Davies J. A distinctive class of integron in the Vibrio cholerae genome. Science. 1998;280:605-8.

16. Centers for Disease Control and Prevention (CDC). (2004) Day care related outbreaks of rhamnose-negative *Shigella sonnei*– six states, June 2001-March 2003. MMWR Morb Mortal Wkly Rep 2003;53:60-63.

17. DeLappe N, O'Halloran F, Fanning S, Corbett-Feeney G, Cheasty T Cormican M. Antimicrobial resistance and genetic diversity of *Shigella sonnei* isolates from western Ireland, an area of low incidence of infection. J Clin Microbiol. 2003;41:1919-24.

18. Hoe CH, Yasin RM, Koh YT, Thong KL. Antimicrobial susceptibility and pulsed-field gel electrophoresis of *Shigella sonnei* strains in Malaysia (1997–2000). J Appl Microbiol. 2005;99:133-40.

19. McIver CJ, White PA, Jones LA, Karagiannis T, Harkness J, Marriott D, Rawlinson WD. Epidemic strains of *Shigella sonnei* biotype g carrying integrons. J Clin Microbiol. 2002;40:1538-40.

20. Mammina C, Pontello M, Dal Vecchio A, Nastasi A, *Shigella sonnei* Working Group. Identification of *Shigella sonnei* biotype g isolates carrying class 2 integrons in Italy (2001 to 2003). J Clin Microbiol. 2005;43:2467-70.

21. Oh JY, Yu HS, Kim SK, Seol SY, Cho DT, Lee JC. Changes in patterns of antimicrobial susceptibility and integron carriage among *Shigella sonnei* isolates from southwestern Korea during epidemic periods. J Clin Microbiol. 2003;41:421-23.

22. Terajima J, Tamura K, Hirose K, Izumiya H, Miyahara M, Konuma H, et al. A multi-prefectural outbreak of *Shigella sonnei* infections associated with eating oysters in Japan. Microbiol Immunol. 2004; 48:49-52.

23. National Committee for Clinical Laboratory Standards. (2002) Performance standards for antimicrobial susceptibility testing. In Twelfth information supplement. M100-S12 Philadelphia: NCCLS.

24. White PA, McIver CJ, Rawlinson WD. Integrons and gene cassettes in the Enterobacteriaceae. Antimicrob Agents Chemother. 2001;45:2658-61.

25. Maraki S, Georgiladakis A, Christidou A, Scoulica E, Tselentis Y. Antimicrobial susceptibilities and b-lactamase production of *Shigella* isolates in Crete, Greece, during the period 1991–1995. APMIS 1998; 106: 879–83.

26. Egah DZ, Banwat EB, Audu ES, Allanana JA, Danung ML, Damen JG, et al. Multiple drug resistant strains of *Shigella* isolated in Jos, central Nigeria. Niger Postgrad Med J. 2003;10:154–56.

27. Lee JC, Oh JY, Kim KS, Jeong YW, Cho JW, Park JC, et al. Antimicrobial resistance of *Shigella sonnei* in Korea during the last two decades. APMIS 2001;109: 228–34.

28. Lima AAM, Lima NL, Pinho MCN, Barros EA, Teixeira MJ, Martins MCV, et al. High frequency of strains multiply resistant to ampicillin, trimethoprim-sulfamethoxazole, streptomycin, chloramphenicol, and tetracycline isolated from patients with shigellosis in northeastern Brazil during the period 1988 to 1993. Antimicrob Agents Chemother. 1995; 39:256–259.

29. Flores A, Araque M, Vizcaya L. Multi-resistant *Shigella* species isolated from pediatric patients with acute diarrheal disease. Am J Med Sci. 1998;316:379–84.

30. Fulla N, Prado V, Duran C, Lagos R, Levine MM. Surveillance for antimicrobial resistance profiles among *Shigella* species isolated from a semirural community in the northern administrative area of Santiago, Chile. Am J Trop Med Hyg. 2005;72:851-54.