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Molecular Epidemiological Study of a Shigellosis Outbreak in Kuanhsi Township, Hsinchu County

Abstract

A Shigellosis outbreak occurred on October 15, 1997 at a primary school in Kuanhsi Township of Hsinchu County. Both the plasmid profile analysis and the polymerase chain reaction methods were used to investigate study the 50 strains of Shigella sonnei isolated in the outbreak, to compare them with the Shigella sonnei strains isolated at different times from the central part of Taiwan, for the molecular epidemiological study of this Shigellosis outbreak. The findings by plasmid profile analysis indicated that three different molecular types could be differentiated; and by ploymerase chain reaction, six molecular types could be differentiated. Although most Shigella sonnei strains in this outbreak were of the same serotype (plasmid type I or polymerase chain reaction type E), some strains were of other plasmid profile or polymerase chain reaction molecular type. The Shigella sonnei strains isolated from this outbreak were of different molecular types from strains isolated elsewhere at different times. This fact indicated that the outbreak was a local incident. Strains isolated from family members, by analysis, were of the same molecular type, indicating that the mode of transmission was also by way of familial contact. By analysis of transmissions through school and family contacts, it was noted that molecular typing was useful in identifying major strains of shigellosis outbreaks. Though the present outbreak was caused primarily by a single strain, strains of other molecular types

were also found in school children. A further study of the inapparent Shigellae in the environment and their anti-microbial drugs resistance would be useful in the control of shigellosis outbreaks.

Introduction

A Shigellosis outbreak occurred at a primary school in Kuanhsi Township of Hsinchu County on October 15, 1997. Laboratory testing of specimens showed that 123 school children and 4 teaching staff of the school were positive for Shigella sonnei. The underground water specimens were also found to be positive for Shigella sonnei. Through overall epidemiological survey and laboratory testing, it was further noted that 40 family members of the positive cases were also positive for Shigella sonnei, including four children of the neighboring primary school A, one of the neighboring primary school B, two of kindergarten A, six of kindergarten B, one of kindergarten C, nine of a nursing school, and 17 other family members[1].

The purpose of the present study was, by using the molecular epidemiological approach, to identify the pathogenic agents of the outbreak, to determine if only one determine type of Shigella sonnei was isolated in the present outbreak, to compare them with the Shigella sonnei strains isolated from different parts of the island at different times, and thus to decide if the incident was a local occurrence or an island-wide epidemic.

Methods generally used for the molecular typing of Shigella sonnei are 1) plasmid profile analysis, 2) pulse-field gel electrophoresis, 3) ribotyping, and 4) polymerase chain reaction. These methods have been used extensively[2-9]. Lin et al.[10] of the Division of Bacterial Diseases, Center for Disease Control, Department of Health, used both the plasmid profile analysis and the pulse-field gel electrophoresis at the same time for the molecular typing of Shigella sonnei and found that, the use of two combined methods was more effective than one method alone. Liu et al.[11] of the Taichung Veterans' General Hospital, by comparing the four methods, found that the polymerase chain reaction and the pulse-field gel electrophoresis were equally effective methods of molecular typing. The present study, therefore, used both the plamid profile analysis and the polymerase chain reaction to study these strains of Shigella sonnei.

Materials and Method

Strain isolates: 50 isolates of Shigella sonnei were selected for study. All of them were confirmed again by biochemical reactions to be Shigella spp., and by serological analysis to be Shigella group D, that is, Shigella sonnei.

Plasmid profile analysis: Small-scale preparation of plasmid DNA for detection and restriction enzyme digestion was performed by a rapid alkaline lysis procedure [12]. The DNA was cleaved with endonuclease *Eco* RI under conditions

according to the supplier's recommendations (BRL, USA). Plasmid DNA and the digested DNA were separated by electrophoresis in 0.7% horizontal agarose gels containing 0.5 μg/ml ethidium bromide and photographed under ultraviolet light illumination. Supercoiled ladder and bacteriophge-λ DNA with a known *Hind* III (λ DNA /*Hind* III) cleavage pattern (BRL) were used as size standard.

Polymerase chain reaction (PCR) analysis: ERIC (enterobacterial repetitive intergenic consensus sequence)-PCR was performed as described by Liu et al., [11] with modification. Five colonies obtained from a fresh 18-h culture on nutrient agar were harvested into 100 microliter of sterile distilled water, boiled for 5 min, and then centrifuged for 5 min at 14,000×g. The supernatant fluid was collected for use as DNA template. The PCR reaction mixture containing 2 μl of DNA template, 1 U of *Taq* polymerase (DyNAzyme ^{TM} II DNA Polymerase, Finnzymes Oy, Finland), 10 mM Tris-HCl (pH 8.8), 1.5 mM $MgCl_2$, 50 mM KCl, 0.1% Triton X-100, 250 μM (each) deoxynucleoside triphosphates, and 1 μM concentration of a single primer. The primer used in this study was ERIC-1 (5'-GTGAATCCCCAGGAGCTTACAT-3'). The amplification of *Shigella* DNA was performed in a Perkin-Elmer thermal cycler (model: U.S.A), with a temperature range as follows: 95^0C for 5 min to denature the template; four low-stringency cycles of 94^0C for 1 min, 26^0C for 1 min, and 72^0C for 2 min, 40 cycles of 94^0C for 30 sec, 40^0C for 30 sec, and 72^0C for 1 min, and finally, 72^0C for 15 min. Negative controls with no DNA template were included in each run. The PCR products (10 μl) were analyzed by agarose gel electrophoresis in 1.6% agarose gels in TBE buffer containing ethidium bromide (1 μl/ml) at 50 V for 4 hr and were visualized under a UV transilluminator. The PCR patterns were considered to be identical on the basis of similar numbers and matching positions of all major bands. Small differences in the intensities of faint bands were ignored.

Results

Three different plasmid patterns were found. Representative plasmid profiles from each *S. sonnei* isolate were shown in Fig. 1. Only plasmids of less than 20 kb were used for comparison of plasmid profile. The patterns were distinct from each other. The pasmid patterns contained one to six plasmids each, which ranged in molecular size from 1.7 to 20 kb. Six bands with size from 1.7 kb to 20.0 kb were present in pattern I (lane 1), three bands with size between 2.3 kb to 20.0 kb were present in pattern II (lane 2), and only one band with size around 20.0 kb was present in pattern III (lane 3). The *S. sonnei* strain isolated from underground water demonstrated the pattern I (lane 4). Other *S. sonnei* isolates from a different area (Taichung County, in central Taiwan) and time point showed a distinct pattern (lane 5), which differed from those isolates in this outbreak. The different *Shigella* serotype isolates, *Shigella flexneri* (lane 6), were also analyzed for comparison. The *Shigella flexneri* strain also showed a distinct plasmid pattern from those of *S. sonnei* strains.

Figure 2 showed the representative data of these different isolates, one to six bands with different molecular size were found in the ERIC-PCR fingerprint. Three bands were presented in PCR fingerprint pattern A (lane 1), two bands were found in pattern B (lane 2), five bands were found in pattern C (lane 3), other five bands were found in pattern D (lane 4). The pattern D is different from pattern C with bands of different size. Four bands of PCR products were found in pattern E (lane 5), and three bands of PCR products were found in pattern F (lane 7). The PCR fingerprint pattern of *S. sonnei* isolate from underground water (lane 6) showed the same pattern as pattern E.

Table 1 summarized the plasmid patterns and the ERIC-PCR fingerprint identified from 50 *S. sonnei* isolates. Most of the plasmids (47/50) including the plasmid isolated from underground water sample were identical and belonged to patterns I. However different patterns of plasmid were also identified from *S. sonnei* isolates of this outbreak. Among these plasmids analyzed, 1 of 50 belonged to patterns II, and 2 of 50 to patterns III. Among the 50 *S. sonnei* isolates analyzed, 5 isolates were ERIC-PCR fingerprint pattern A, 2 isolates were pattern B, 1 isolates were pattern C, 1 isolate was pattern D, 40 isolate was pattern E and 1 isolate was pattern F. The ERIC-PCR fingerprint pattern E was found in cases from most of the classes in the primary school as well as the kindergartens and nursery, and was the mainly causative agent in the whole event of shigellosis.

A further study of family members showed that the children of the same family or their cousins demonstrated similar molecular types. Table 2 showed that three brothers in family A had the same ERIC-PCR fingerprint (pattern E) and plasmid profiles (pattern I), the dates of illness being October 10, October 17 and November 02, 1997 respectively. In family B, two cousins, whose dates of illness were October 25 and November 02, 1997 respectively had the same molecular type. In family C, two sisters and one cousin also had an identical ERIC-PCR fingerprint (pattern E) and plasmid profiles (pattern I), the dates of illness onset being October 16, November 02 and November 05, 1997 respectively.

Discussion

Customarily, for the confirmation of the pathogenic agents in Shigellosis outbreaks, standard culture method is used for the isolation of bacteria, and then serological methods are used for typing to confirm which strain of Shigella is responsible. Plasmid profile analysis is often used to help identify the clinically isolated strains. It is also used as a molecular epidemiological tool in the study of Shigellosis outbreaks. In particular, when there is only one serotype of Shigella sonnei in the outbreak, plasmid profile analysis is used for the molecular typing of

Shigella sonnei, to help identify epidemiologically associated and non-associated strains[13,14].

Liu et al.[11] compared the four molecular typing methods and found that ERIC-PCR fingerprint analysis technique was a quick and simple molecular typing method for Shigella sonnei. Its effect was comparable to that of the pulse-field gel electrophoresis, and better than that of the plasmid profile analysis. The present study, therefore, used both the plasmid profile analysis and the polymerase chain reaction.

Though studies have demonstrated that ERIC-PCR fingerprint analysis gives a better differentiation effect than the plasmid profile analysis, the two methods used in the present study were able to differentiate the pathogenic agents of this Shigella sonnei outbreak. The serological method, however, can differentiate only one type, that is Shigella sonnei.

Study results showed that although some different molecular types were found to exist in one class, the isolates of Shigella sonnei from children of the same class were generally of the same type, and were of the same type as the isolates from the underground water. This fact suggested that it was likely that there were a few asymptomatic carriers that were detected in the school-wide screenings. The special features of these strains and their anti-microbial drugs resistance deserve further study.

That isolates of Shigella sonnei from patients of the same family were of the same serotype, and that their dates of onset were close to each other indicated that familial contact among family members was the primary mode of transmission. The present outbreak was the outcome of the common source of infection and transmission by personal contact.

The molecular epidemiological method can be successfully used in the study of the pathogenic agents of Shigellosis outbreaks. It is also useful in the identification of strains. Most importantly, however, the basic information of the strains of each outbreak should be established for comparison with strains isolated from different places and at different times[15] for similarities and differences, and for their anti-microbial drug resistance. Indigenous information on disease control can thus be established for future reference in disease control.

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Figure 1. A Typical Shigella sonnei Plasmid Profile

M: λ/Hind III as a marker for the size of molecular weight; first bar, type I; second bar, type II; third bar, type III; fourth bar, strains isolated from underground water, the same as type I; fifth bar, strains of Shigella sonnei isolated from the central part of Taiwan in 1997; sixth bar, Shigella flexneri, for comparison.

Figure 2. A Typical molecular type of Shigella sonnei by Polymerase chain reaction Fingerprint.

M: 1/Hind III as a marker for the size of molecular weight. First bar, type A; second bar, type B; third bar, type C; fourth bar, type D; fifth bar, type E; sixth bar, type E, (strains isolated from underground water); seventh bar, type F; eighth bar, using sterilized distilled water as DNA negative control.

Table 1. Molecular Types of 50 Shigella sonnei Strains

Class	PCR (No of strains)						Plasmid Profile (No of strains)		
	A	B	C	D	E	F	I	II	III
1 A					1		1		
1 B						1	1		
1 C	1						1		
2 A				4			5		
2 B	3[#]	2				2	5		2
3 A	1				4		5		
3 B					4		3	1	
4 A				1*	1		2		
4 B					4		4		
5 A					2		2		
5 B					3		3		
6 A					2		2		
6 B					2		2		
Underground water					1		1		
Other schools					1		1		
Kindergarten A				1		1			
Kindergarten B				1		1			
Nursing school				2		2			
Cared at home					5		5		
Total	5	2	1	1	40	1	47	1	2

#Two of them were the same cases as those of plasmid profile type III.
*asymtomatic

Table 2. Familial Infections of Shigella sonnei*

Family	Cases (School, Class)	Sex	Age (years)	PCR Typing	Plasmid Profile	Date of Onset
A	2 A	male	7	E	I	Oct 10, 97
	1 A	male	6	E	I	Oct 17,97
	Nursing school	male	3	E	I	Nov 2,97
B	1 A	female	6	E	I	Oct 25,97
	Kindergarten A	male	4	E	I	Nov 13,97
C	4 A	female	9	E	I	Oct 16,97
	Kindergarten B	female	5	E	I	Nov 2,97
	Cared at home	female	2	E	I	Nov 5,97

*Family A: three brothers, two in classes 2 A and 1 A of the same school; one in nursing school.

Family B: two cousins, one in class 1 A, one in kindergarten A.

Family C: two sisters and one cousin; elder sister in class 4 A, younger sister cared at home; cousin in kindergarten B. All isolates of Shigella sonnei were of plasmid profile type I and PCR type E (same as the strains isolated from underground water).