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Table1. Prevalence serotypes of 542 *Vibrio parahaemolyticus* strains isolated from patients in Thailand during year 2005 - 2006.

O Group	O:K serotypes (No. of strains)				
O1 (71)	O1:K25 (3)	O1:K38 (2)	O1:K56 (31)	O1:K69 (1)	O1:KUT (34)
O2 (17)	O2:K3 (14)	O2:K28 (2)	O2:KUT (1)		
O3 (200)	O3:K5 (4)	**O3:K6 (154)**	O3:K7 (1)	O3:K29 (20)	O3:K45 (1)
	O3:K54 (3)	O3:K58 (12)	O3:KUT (5)		
O4 (198)	O4:K4 (1)	O4:K8 (33)	**O4:K9 (52)**	O4:K11 (7)	O4:K12 (3)
	O4:K13 (3)	O4:K34 (1)	**O4:K55 (39)**	O4:K63 (6)	**O4:K68 (46)**
	O4:KUT (7)				
O5 (14)	O5:K15 (3)	O5:KUT (11)			
O6 (1)	O6:K18 (1)				
O8 (25)	O8:K21 (13)	O8:K39 (1)	O8:K41 (2)	O8:KUT (9)	
O10 (6)	O10:KUT (6)				
O11 (5)	O11:KUT (5)				
OUT (5)	OUT:KUT (5)				

Note: Serotype of the 4 outbreak strains were shown in bold character.

Table2. Serotypes and virulence factors of 542 *V. parahaemolyticus* isolated from patients during year 2005-2006.

Serotypes	Virulence factors			No. of strains
	Urease production	Haemolysin genes		
		tdh	*trh*	
O3:K6	-	+	-	**154**
O4:K9	-	+	-	**52**
O4:K68	-	+	-	**46**
O4:K55	-	+	-	**39**
O4:K8	-	+	-	32
O1:KUT	-	+	-	29
O1:K56	-	+	-	27
O3:K29	-	+	-	20
O2:K3	-	+	-	14
O8:K21	-	+	-	13
O3:K58	-	+	-	12
Other 15 serotypes[1]	-	+	-	50
O1:K56	+	+	+	3
O1:K69	+	+	+	1
O1:KUT	+	+	+	4
O6:K18	+	+	+	1
O10:KUT	+	+	+	1
O5:K15	+	+	+	1
O11:KUT	-	-	-	5
O3:KUT	-	-	-	4
O4:KUT	-	-	-	4
O10:KUT	-	-	-	4
OUT:KUT	-	-	-	4
Other 15 serotypes[2]	-	-	-	21
O1:K56	+	-	-	1

Note: Serotype of the 4 outbreak strains were shown in bold character.

[1]Including serotypes: O3:K5, O1:K25, O1:K38, O3:KUT, O4:K11, O4:K12, O4:K13, O4:K63, O4:KUT, O5:K15, O5:KUT, O8:K41, O8:KUT, O10:KUT, and OUT:KUT

[2]Including serotypes: O1:KUT, O1:K25, O2:K28, O2:KUT, O3:K5, O3:K7, O3:K45, O3:K54, O4:K4, O4:K8, O4:K12, O4:K34, O4:K63, O5:KUT, and O8:K39

Table3. Serogroups and enterotoxin genes of Enterotoxigenic *E. coli* isolated from patient with diarrhea symptoms in year 2005-2006.

O-serogroup	No. of positive strains by PCR (%)			Total
	eltIA	*stIA*	*eltIA* + *stIA*	
O169	1	1	-	2
O167	-	2	-	2
O159	-	4	-	4
O148	-	1	-	1
O126	-	1	-	1
O78	-	1	-	1
O18	2	-	-	2
O15	1	5	-	6
O8	6	-	-	6
O6	-	3	3	6
O1	1	-	-	1
O-rough	-	-	1	1
Untypable	15	8	1	24
Total	**26 (45.6)**	**26 (45.6)**	**5 (8.8)**	**57**

Note: *eltIA* = heat-labile enterotoxin genes, *stIA* = heat-stable enterotoxin genes

Table4. Serogroups and virulence genes of Shiga toxin-producing *E. coli* isolated from patient with diarrhea symptoms in year 2005-2006.

Year	Patient	Symptom	Serotype	Virulence genes
2005	Child	Watery diarrhea	O157:HNM	*stx*1, *stx*2, *eae*A, *hyl*
2006	New born infant	Watery diarrhea	O111:HNM	*stx*1, *eae*A

Note: *stx* = shiga toxin genes, *eae*A = intimin encoding gene, *hyl* = enterohaemolysin gene

Table 5. Prevalence of Phage types of 92 *S. aureus* strains in year 2005-2006

Group	No. of strains (%)		
	2005	2006	Total
I	0	0	0
II	2	7	9 (9.8)
III	1	9	10 (10.9)
V	1	0	1 (1.1)
Miscellaneous	1	1	2 (2.2)
Mixed	5	53	58 (63.0)
Typable	**10 (55.6)**	**70 (94.6)**	**80 (87.0)**
Untypable	8 (44.4)	4 (5.4)	12 (13.0)
Total	**18 (100)**	**74 (100)**	**92 (100)**

Table6. Prevalence of Enterotoxin genes of 92 strains of *S. aureus* in year 2005-2006

Enterotoxin genes	2005 (*n=18*)	2006 (*n=74*)	Total (%) (*n=92*)
sea	1	0	0
seb	0	5	5 (5.4)
sec	0	2	2 (2.2)
sed	0	1	1(1.1)
see	0	22	22(23.9)
Toxin producing	**1**	**29**	**30 (32.6)**
None	**17**	**45**	**62 (67.4)**

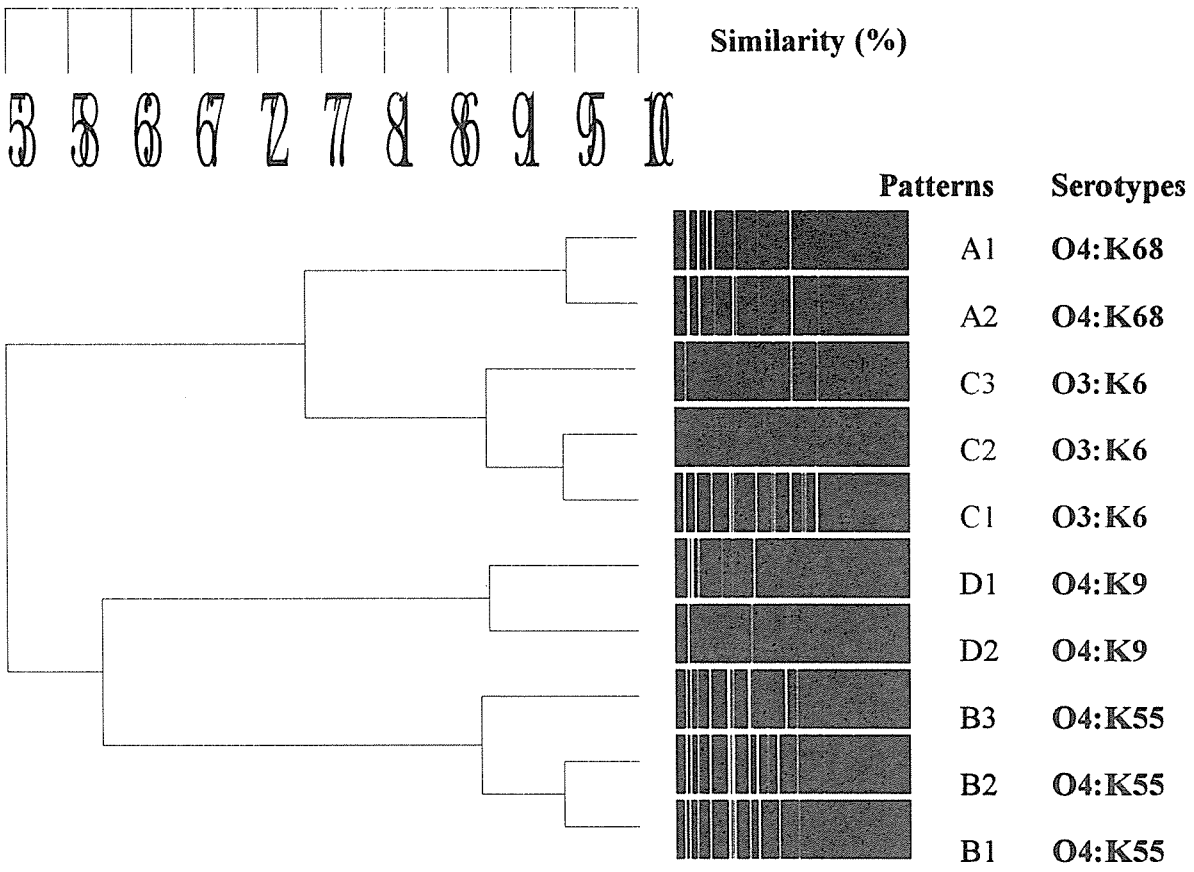

Figure1. Dendrogram and PFGE banding patterns for O4:K68, O4:K55, O3:K6 and O4:K9 outbreak strains isolated during 2005-2006

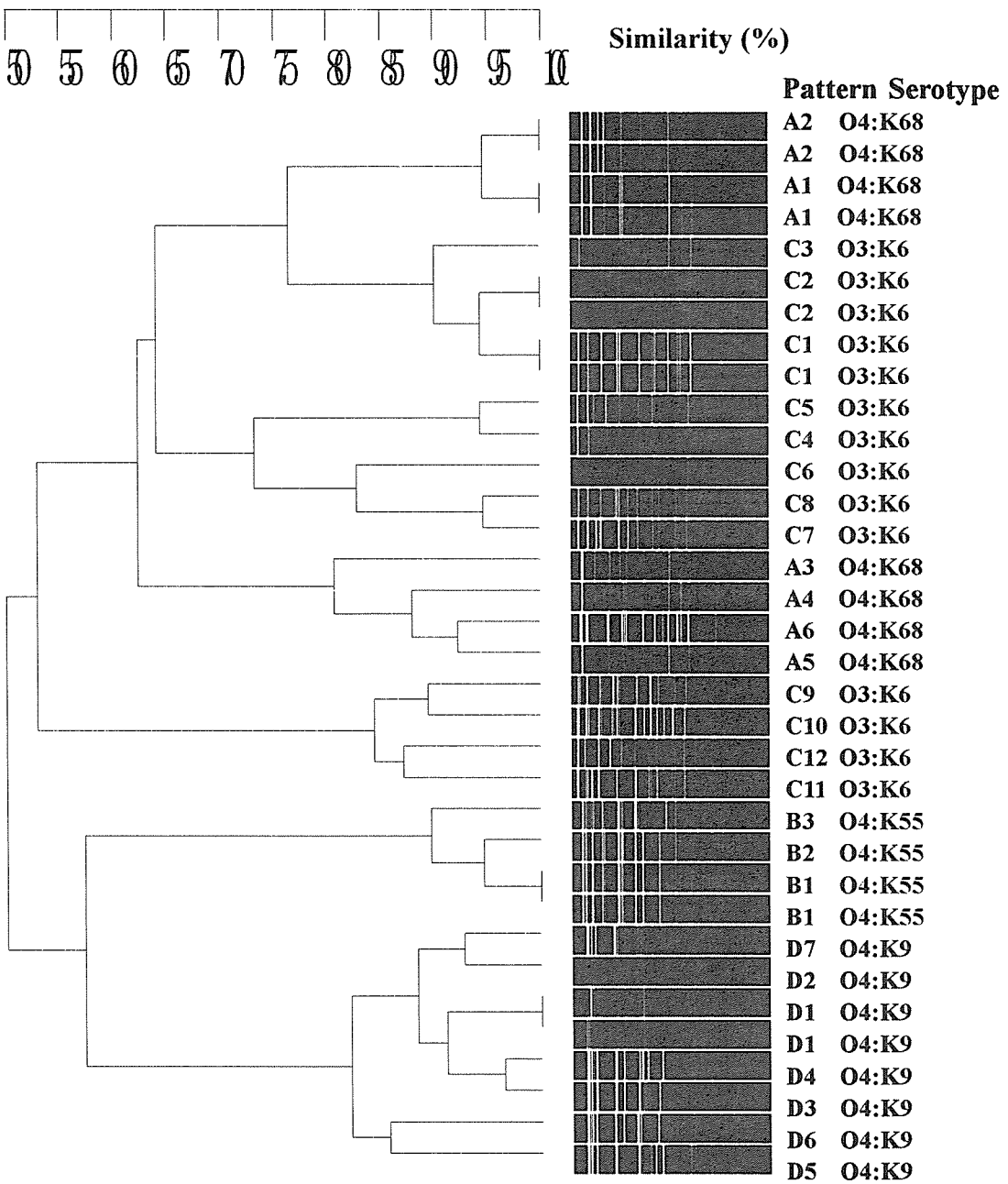

Figure2. Dendrogram and banding patterns for O4:K68, O4:K55, O3:K6 and O4:K9 outbreak and non-outbreak strains isolated during 2003-2006

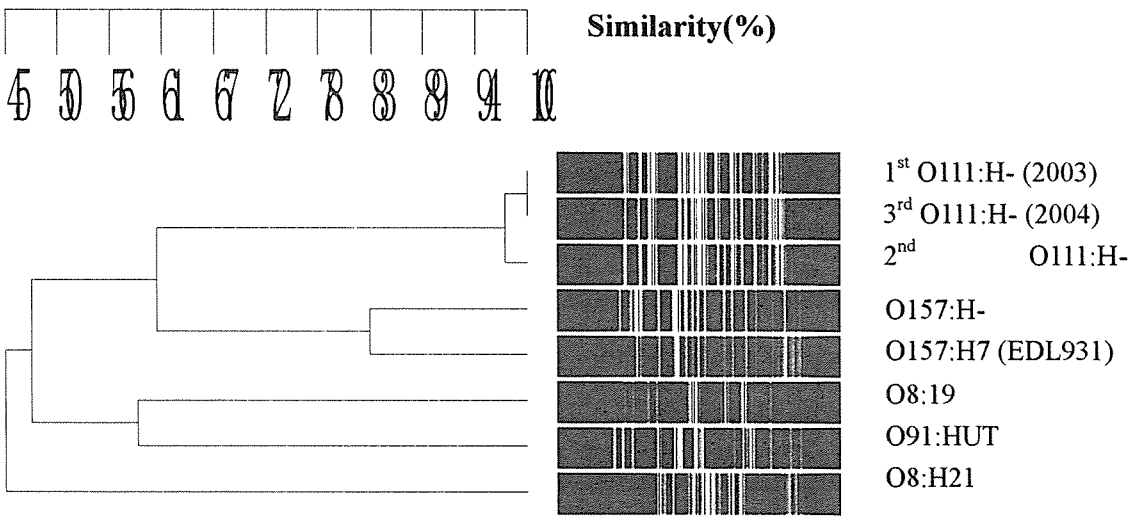

Figure3. Dendogram and banding of PFGE of *Xba*I digested of Shiga toxin-producing *E. coli*

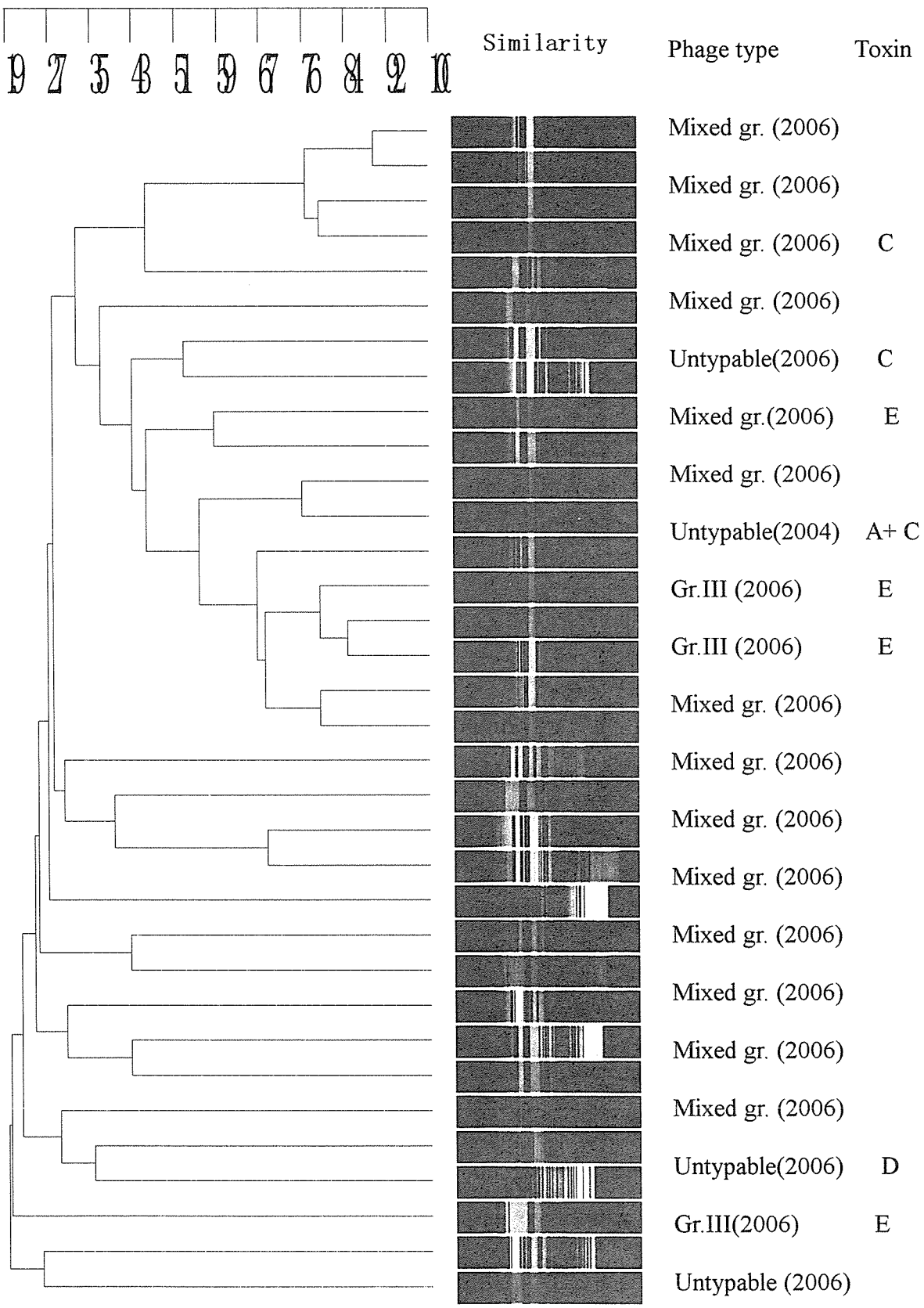
Similarity
Phage type
Toxin
Mixed gr. (2006)
Mixed gr. (2006)
Mixed gr. (2006) C
Mixed gr. (2006)
Untypable(2006) C
Mixed gr.(2006) E
Mixed gr. (2006)
Untypable(2004) A+ C
Gr.III (2006) E
Gr.III (2006) E
Mixed gr. (2006)
Mixed gr. (2006)
Mixed gr. (2006)
Mixed gr. (2006)
Mixed gr. (2006)
Mixed gr. (2006)
Mixed gr. (2006)
Mixed gr. (2006)
Mixed gr. (2006)
Untypable(2006) D
Gr.III(2006) E
Untypable (2006)

Figure4. Dendrogram and banding of *Sma*I PFGE patterns of *Staphylococcus aureus* strains isolates were related with 80-90 %similarity

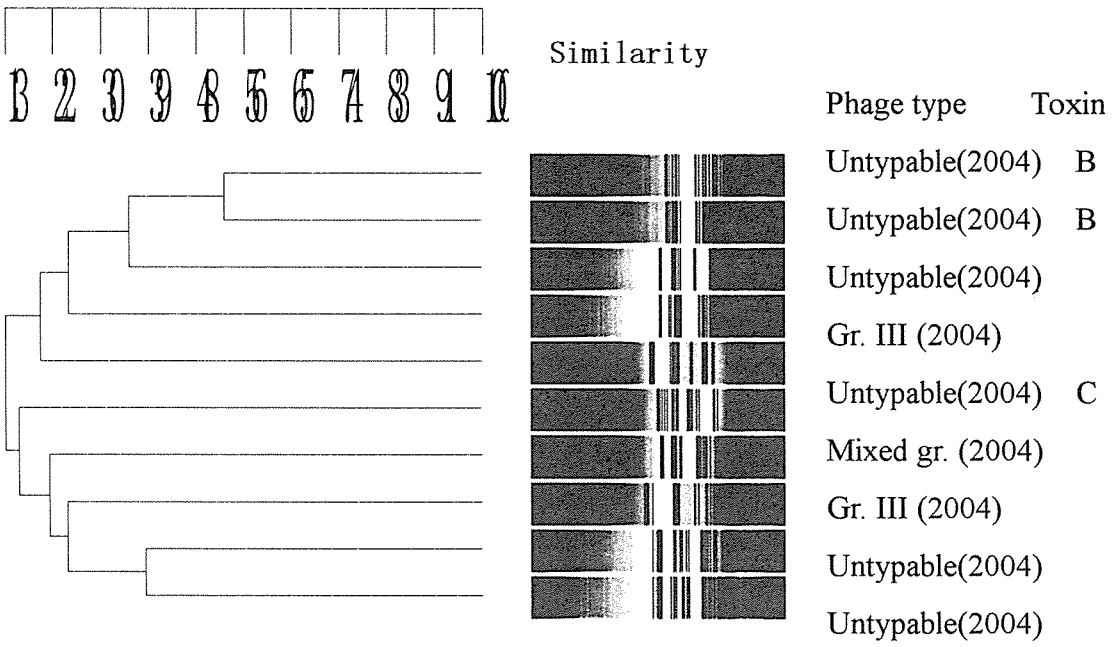

Figure5. Dendrogram and banding of *Sma*I PFGE patterns of *Staphylococcus aureus* strains isolated from sporadic cases in year 2004.

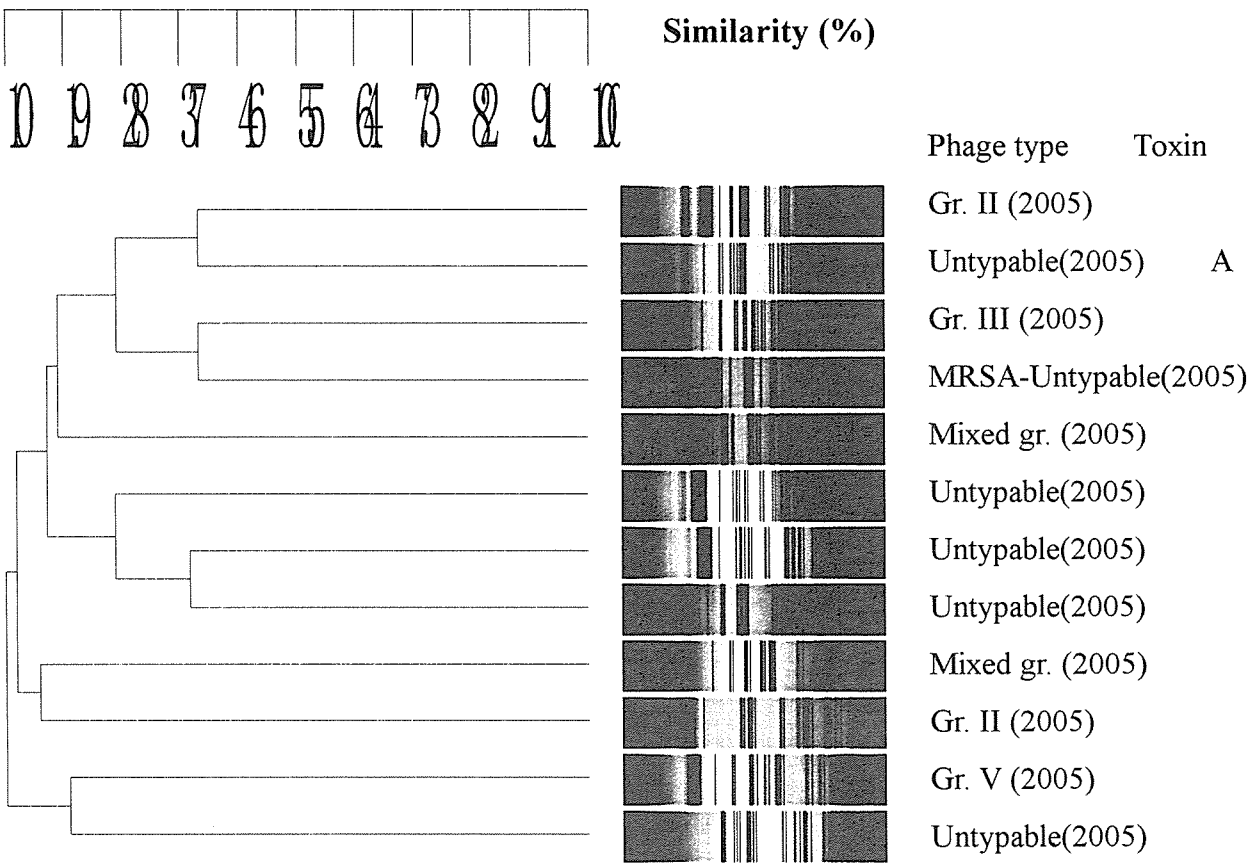

Figure6. Dendrogram and banding of *Sma*I PFGE patterns of *Staphylococcus aureus* strains isolated from different provinces in year 2005.

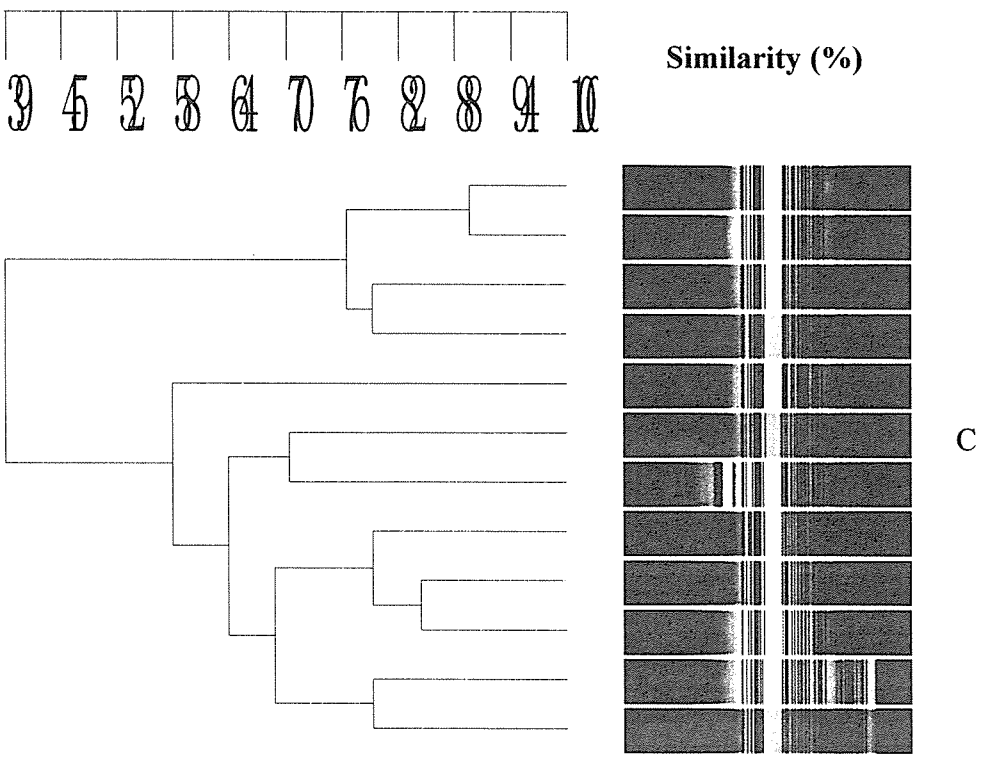

Figure7. Dendrogram and banding of *Sma*I PFGE patterns of *Staphylococcus aureus* strains isolated from outbreak in the same province in year 2006. All isolates were phage type Mixed gr. Only one isolate was produced enterotoxin C.

Development of Multilocus Variable-Number Tandem Repeat analysis (MLVA) method for molecular subtyping of *Shigella sonnei*

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We developed and evaluated a multilocus variable-number tandem repeat (VNTR) analysis (MLVA) method for subtyping of *S. sonnei* isolates. A total of 26 VNTR loci were identified by comparison of the repeat sequence loci in the genomic sequences of *S. sonnei* strains Ss046 and 53G and by testing on 536 isolates, which had previously been characterized by pulsed-field gel electrophoresis (PFGE). MLVA was more discriminatory than PFGE for the isolates, with discriminatory index of 0.95 and 0.89, respectively. The usefulness of MLVA for epidemiological investigation was evaluated with 151 isolates from 10 shigellosis outbreaks and 22 PFGE-indistinguishable isolates collected from 9 epidemologically-unrelated events. The study showed that MLVA was a powerful subtyping tool to discriminate isolates for outbreak investigation and exhibited a high level of discriminatory power to the PFGE-indistinguishable isolates from different epidemiological events.

Purpose:

Shigellosis, which is caused by *Shigella dysenterae*, *S. flexneri*, *S. boydii* and *S. sonnei*, is a major diarrheal disease in the developing countries. The annual number of *Shigella* episodes throughout the world is estimated to be 164.7 million, of which 163.2 million occur in developing countries (with 1.1 million deaths) and 1.5 million in industrialized countries [1]. *S. flexneri* is the most prevalent species in developing countries, while *S. sonnei* is predominant in industrialized countries [1]. Shigellosis is a notifiable disease in Taiwan. Physicians and hospital authorities have an obligation to report any confirmed case and to send the *Shigella* isolate to the Centers for Disease Control of Taiwan. From 1993 to 2004, 61 to 1,357 shigellosis cases (0.26-5.89 cases per 100,000 population) were confirmed annually [2]. *S. sonnei* and *S. flexneri* were the major species causing shigellosis in Taiwan. *S. dysenterae* and *S. boydii* were rare and only found in imported cases. *S. sonnei* was responsible for most of the large shigellosis outbreaks in the industrialized western area [3] and was frequently associated with imported cases [4]. In contrast, *S. flexneri* was mainly circulating among the aboriginal tribes in the mountainous areas [5]. In central Taiwan, *S. flexneri* caused most of the infections from 1996 to 2000; however, *S. sonnei* replaced *S. flexneri* to become the major cause of the disease from 2001 to 2003. The dramatic increase in case number in 2001-2002 was associated with *S. sonnei*.

Analyses of *Shigella* isolates by various genotyping methods provide useful information for the purposes of outbreak investigation [6, 7], tracing disease

transmission [8, 9], disease surveillance [10], and evolutionary study [11]. Several genotyping methods have been developed for *S. sonnei* [11-14]. Among these genotyping methods, pulsed-field gel electrophoresis (PFGE) has been standardized and used to build an international molecular subtyping network for foodborne disease surveillance [10]. PFGE has been proven to be a powerful tool for discriminating *Shigella* strains in many laboratories. However, PFGE is, at times, too discriminatory for investigating the clonal relationship among *Shigella* strains which have been circulating for years.

To compensate this drawback of PFGE, in a previous study, we previously developed an inter-IS1 spacer typing (IST) method for the subtyping of *S. sonnei* strains [11]. IST exhibits a lower level of discriminatory power than PFGE but is more useful than PFGE for investigating the genetic relationships among *S. sonnei* strains circulating over a longer time span, and also is useful for discriminating certain strains which are indistinguishable by PFGE. Even though IST gets more genetic information for the PFGE-indistinguishable isolates, it does not discriminating all the isolates collected from different epidemiological events. Furthermore, IST generates image-type data as PFGE does; it is difficult to standardize the protocol and compare data between laboratories. Therefore, an alternative method that can be used to subtype *S. sonnei* isolates for disease surveillance and outbreaks investigation, and to do phylogenetic analysis for studying global transmission of *S. sonnei* strains.

Among the next generation subtyping methods, multilocus VNTR analysis (MLVA) has been developed for several bacterial pathogens [15-19]. Studies indicate that MLVA has a similar or higher discriminatory power than PFGE [15, 16]; it can be used to subtype bacterial isolates for outbreak investigation. Also, MLVA data can be used for phylogenetic analysis of bacterial strains for study of global transmission of bacterial clones [15, 17]. The present study, we will aim to develop MLVA methods for subtyping of *S. sonnei*.

Methods:

1) Bacterial strains. *S. sonnei* isolates were collected in central and eastern Taiwan between 1996 and 2005. A collection of 536 isolates was used to compare the discriminatory power of the MLVA and PFGE. The discriminatory power of the two subtyping methods was evaluated by a discriminatory index as calculated and reported by Hunter [20]. Among the collection, 151 isolates derived from 10 shigellosis outbreaks was used to evaluate the usefulness of MLVA and PFGE on the discrimination of isolates collected from different outbreaks (Table 1). The outbreaks occurred at schools, tour groups, and family neighborhoods over a period of weeks or months and the epidemiological relationship among infected cases was collected by epidemiological investigation. Another set of 22 isolates with an indistinguishable PFGE (J16N09.0015) pattern collected from 9 epidemiologically-unrelated events was subjected to MLVA. Twenty of the 22 isolates had been characterized previously with inter-IS1 spacer typing [11].

2) Identification of VNTR loci. The genomes of *S. sonnei* strains Ss046 (GenBank accession no. CP000038) and 53G (obtained from The Welcome Trust Sanger Institute) [21], were explored for potential VNTR loci using VNTRDB computer software developed by Chang et al. [22]. The program, which incorporates the algorithm of the Tandem Repeat Sequence Finder software [23], searches tandem repeat sequence loci from one of the two genomic sequences and then locates the position and counts the number of repeat unit for each of the loci at the other compared genome. The two genomic sequences are used in turn as the "parent" sequence, so that a locus with only one repeat unit at a genome, but with two or more repeat units at the other genome, will not be missed. The computer searches identified 33 potential VNTR candidates. In addition, a locus with 5 repeat sequence units in both genomes was included to evaluate VNTR potentiality. In total, 34 VNTR candidates were tested on 10 genetic distinct strains. Twenty-six loci, which have various number of repeats in the 10 tested isolates were considered to be VNTR loci and were chosen for genotyping *S. sonnei* isolates.

3) Preparation of crude bacterial DNA. *S. sonnei* isolates, stored at –70 °C, were plated onto trytic soy agar and incubated overnight at 37 °C. A loopful (10 μl) of bacterial growth was removed from the plate, suspended in 100 μl of TE buffer (10 mM Tris-Cl, 1 mM EDTA, pH 8.0) in an Eppendorf tube, and boiled for 10 min. After centrifugation at 3700 *g* for 10 min, the supernatant was transferred to a new tube for use.

4) PCR amplification and analysis of VNTR regions. The primer sets specific to the 26 VNTR regions are listed on Supplemental material 1. The primers were designed using the free program available at the Primer3 website [24]. The forward primer of each primer set was labeled on its 5' end with an ABI-compatible dye, 6-FAM, NED, VIC or PET by the manufacture (Applied BioSystems, Foster City, CA, USA). For PCR amplification, each 10-μl PCR mixture contained 1x PCR buffer, 3 mM $MgCl_2$, 0.05-0.4 μM each primer, 200 μM each deoxyribonucleotide, 1.0 unit of the recombinant SuperNew Taq DNA polymerase (Jier Sheng Company, Taipei, Taiwan), and 1 μl of DNA template prepared as above-mentioned. Seven multiplex PCR combinations (M1-M7) were carried out for MLVA analysis of each *S. sonnei* isolate. The combinations and concentration of primers were listed as the followed: M1 [SS12 (0.4 μM) + SS14 (0.1) + SS16 (0.1) +SS21 (0.1)]; M2 [SS1 (0.05) + SS10 (0.1) + SS11 (0.1) +SS22 (0.1)]; M3 [SS3 (0.1) + SS6 (0.2) + SS9 (0.05) +SS23 (0.1)]; M4 [SS5 (0.1) + SS7 (0.05) + SS8 (0.05) +SS20 (0.1)]; M5 [SS4 (0.1) + SS13 (0.05) + SS18 (0.1) +SS25 (0.1)]; M6 [SS2 (0.1) + SS15 (0.1) + SS17 (0.1) +SS19 (0.2)]; M7 [SS24 (0.1) + SS26 (0.1)]. The PCR reaction was carried on a GeneAmp PCR System 9600 (Applied BioSystems). M1-M6 were performed with a denaturing step at 94 °C for 5 min, followed by 30 cycles of amplification step at 94 °C for 45 s, at 55 °C for 50 s, and at 72 °C for 60 s. M7 was performed with the above condition except annealing temperature was set at 62 °C.

Before size analysis of the fluorescent amplicons were diluted in water at a 1:10 (M7) or 1:100 (M1-M6) ratio, then transferred 1 μl of the solution into 10 μl formide. After

denatured by heating, the amplicons were separated by capillary electrophoresis on an ABI Prism 3130 Genetic Analyzer with GeneScan 500 LIZ Size Standard (cat # 4322682; Applied BioSystems). Data were collected and lengths of amplicons were determined with GeneScan Data Analysis Software, ver 3.7 (Applied BioSystems). All amplicons with different lengths from each locus were subjected to nucleotide sequence determination to verify the repeat sequence and the numbers of repeat units in the amplicons. The primers (without dye label) used for nucleotide sequence determination were the same as the primer sets used for PCR amplification. DNA sequencing was performed using the ABI Prism Big Dye Terminator cycle sequencing ready reaction kit and an ABI Prism 3130 Genetic Analyzer. The numbers of repeat units for the 26 VNTR loci and the predicted sizes of amplicons for the *S. sonnei* strains Ss046 and 53G were taken as the standards to infer the number of repeat unit of each locus for the isolates tested.

5) Data analysis. The numbers of repeat units for each locus were saved as "Character Type" data in BioNumerics software (version 3.5; Applied Maths, Kortrijk, Belgium) and then subjected to cluster analysis using the Minimum Spanning Tree method. The polymorphism information index or Nei's diversity index (DI) was calculated for evaluating allele diversity as $1-\Sigma(\text{allele frequency})^2$.

Results

1) VNTR loci. In total, 26 VNTR loci were identified after testing on 10 genetic distant strains (Table 1). Most of the loci have short repeat sequences with range from 5 to 9 bp. Eight loci have only one repeat unit in strain Ss046 or strain 53G. Locus SS1 is quite unusual; it has one repeat unit in strain 53G but 10 units in strain Ss046. By testing on 536 isolates, 2 to 20 alleles were found for the loci. Among them, 6 loci had a number of alleles of 6 or more and exhibited a high level of allele diversity, with Nei's diversity index from 0.72 to 0.92.

2) MLVA genotyping. The MLVA genotyping conducted on 536 *S. sonnei* isolates identified 126 MLVA types. The discriminatory index was 0.95. By PFGE method, the isolates were discriminated into 101 PFGE types with discriminatory index of 0.89.

3) MLVA analysis for isolates from outbreaks. The 26 VNTR loci were tested on 151 *S. sonnei* isolates collected from 10 outbreaks (Table 2). The isolates were previously characterized by PFGE and IST [11]. Of the 10 outbreaks, 6 were identified with multiple MLVA types in the collected isolates. Except outbreak O9, each outbreak with multiple MLVA types existed with a predominant subtype. MLVA identified fewer subtypes than PFGE for the 151 isolates.

The clustering analysis of the MLVA types by minimum spanning tree algorithm demonstrated the usefulness of MLVA in discriminating *S. sonnei* strains collected from different outbreak (Fig. 1). The analysis indicated that strains with different IST

genotypes were located in distinct clusters. Six outbreaks were identified with multiple MLVA types. For each outbreak with multiple types, the minor type(s) was mostly a single locus variant of the major type. Four outbreaks were caused by IST1 clone, two by IST21 clone. Strains of IST1 clone were detected for the first time in Taiwan in 2000, since then IST1 strain spread out and caused many outbreaks in central and eastern Taiwan [25]. Outbreak O5 was the first one of the four outbreaks caused by strains of IST1 clone. The isolates of outbreak O5 were identified with 3 MLVA types, the predominant type was distinct from the major types for the three outbreaks O5, O7 and O8. Outbreak O7 contained 5 MLVA types. The major type of outbreak O7 could not be distinguished from the major type for outbreak O8 and one of the two major types for outbreak O9. Outbreak O9 was the last one of the four outbreaks caused by IST1 clone. One of the two major types for outbreak O9 was distinct from the major types for the outbreaks O5, O7 and O8. Outbreaks O2 and O4 shared a common MLVA type. Both outbreaks occurred in central and northern Taiwan during a close time, they were epidemiological related.

4) MLVA analysis for isolates with indistinguishable PFGE pattern. A total of 22 *S. sonnei* isolates were collected from 9 epidemiologically-unrelated events. Infections occurred in five countries and the collected isolates were indistinguishable by PFGE with NotI and XbaI. In a previous study [11], 20 isolates from 8 of the events were characterized and identified with five IST genotypes. Isolates from two events (E2 and E3) occurred in Taiwan and three events (E6-E8) in Vietnam and Cambodia were not distinguished by IST typing. In contrast with, MLVA exhibited a high discriminatory power to the isolates. As shown in Fig. 2, the isolates from different events were discriminated into distinct MLVA genotypes. The genetic relationship between the MLVA types constructed by minimum spanning tree algorithm revealed that the strains from closer geographic locations had closer genetic relationship, such as the strains from Vietnam and Cambodia. Two events were identified with two MLVA types. To each event, the two MLVA profiles differed in only one locus.

Discussion

The VNTRDB program is a useful tool for searching VNTR candidates from multiple genomic sequences **[22]**. It searches repeat sequence loci in a genomic sequence and locates the position and counts the number of repeat unit for each of the loci at the other genomic sequences. Each of genomic sequences is used in turn as the "parent" sequence, so that a locus with only one repeat unit at a genome, but with two or more repeat units at the other genomes, will not be missed. Furthermore, many repeat loci can be identified to be VNTR loci by comparison of two or more genomic sequences that saves tremendous work on testing a large number of repeat sequence loci for identification of VNTR loci. To date, two genomic sequences of *S. sonnei* strains Ss046 and 53G have been finished [21, 26]. In this study, VNTRDB program identified 36 VNTR loci in the two genomic sequences. In total, we only evaluated 34 VNTR candidates and confirmed 26 VNTR loci. Of the 26 loci, 8 loci had only one repeat unit in either strain Ss046 or 53G (Table 1). The loci could be missed if only a

sequence is available. By exploring repeat sequence loci with VNTRDB program, more VNTR loci could be found when more genomic sequences of *S. sonnei* strains are available.

The MLVA method developed in this study is useful to subtype *S. sonnei* isolates for outbreak investigation. For the 10 outbreaks, MLVA clearly distinguished the major outbreak strains with different IST genotypes. MLVA can also discriminate outbreak strains derived from a common clone. Outbreaks O5, O7, O8 and O9 were caused by strains derived from IST1 clone. IST1 strain was identified for the time in Taiwan in 2000 and then spread to eastern and central Taiwan. The present study shows that MLVA distinguishes the major outbreak strains for outbreak O5, O7 and O9. The isolates collected from the 10 outbreaks also characterized by PFGE in a previous study [11]. PFGE discriminates the isolates into more subtypes than MLVA, but the PFGE does not clearly distinguish the outbreak strains for outbreaks O5, O7 and O9. The three outbreaks have a common major PFGE subtype [11].

This study showed that the MLVA method is powerful to discriminate PFGE-indistinguishable strains collected from different epidemiological events. In a previous study, 20 of the 22 isolates were discriminated into 5 IST genotypes. The IST genotyping provides more information, but the data are still not able to discriminate some events (Fig. 2). MLVA method provides more helpful data that can clearly discriminate the isolates collected from different epidemiological events.

One of the purposes of development of MLVA method is to replace PFGE for fine typing of isolates for disease surveillance and outbreak investigation. Although a total of 26 VNTR loci are identified, it is not necessary to analyze all the loci for the purpose. Which loci are good to include in MLVA for subtyping of isolates needs to be determined by testing on isolates from various geographic regions. In this study, isolates from 6 of the 10 outbreaks were identified with multiple MLVA types. The variations occurred on loci SS1, SS3, SS6 and SS9. Another analysis conducted on 22 PFGE-indistinguishable isolates detected variations on 8 loci (SS1, SS3, SS6, SS9, SS11, SS13, SS16 and SS23). The 8 loci had a high to moderate level of allele diversity (Table 1). The data suggests that the 8 loci are good candidates to be included in a MLVA typing scheme for disease surveillance and outbreak investigation.

The other purpose to develop a MLVA method is for investigation of genetic relationship of strains evolved over a longer time span. A previous study shows that PFGE is too discriminatory [11]. PFGE data are not appropriate to build genetic relationship of strains evolved over years. Currently, MLST is a gold standard to build phylogenetic relationship of bacterial species [19]. Up to date, there is still no MLST method developed for *S. sonnei*. MLST is costly; it is not practical to be used as a routine genotyping method. MLVA can be an alternative method for phylogenetic study for bacterial species [15]. The present study identifies 26 VNTR loci, which are a basis to build clonal relationship of *S. sonnei* strains for investigation of global transmission of *S. sonnei* clones.

In conclusion, a MLVA method with 26 VNTR loci has been developed. The method is more discriminatory than PFGE. MLVA can be used for fine typing of *S. sonnei*

isolates for epidemiological investigation, and also provides data for building phylogenetic relationship among *S. sonnei* strains. However, study on more strains from various geographical regions is needed to determine which loci are needed for the two purposes.

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