1. Large number of bands produced with each enzyme
2. Lack of diversity among isolates

To address the large number of bands decision has been made to modify electrophoresis conditions and focus on the upper end of the gel. Therefore will make the 78.2 kb band the bottom band on the gel (Figure 2).

A BioNumerics *Yersinia enteroliticus* database with NZ and international isolates has been established and currently has 89 isolates in it from NZ, China, USA and Argentina. While there are some indistinguishable isolates between countries with one enzyme, use of a second enzyme indicates that there is some separation of isolates between countries.

Discussion:

Improved protocols for PFGE analysis of *Yersinia enteroliticus* have been developed, and a PulseNet protocol will be published. Conditions suitable for making plugs have been identified, and from enzymes tested, 1st choice enzyme is NotI, followed by 2nd enzyme of ApaI.

The large number of bands produced by NotI has resulted in decision to modify electrophoresis conditions to focus on upper area of gel. A protocol will be published on www.pulsenetinternational.org once validation is completed.

The issue of diversity of PFGE genotypes is less easily solved. To date it would seem that biotypes 3 and 4 have very low diversity, meaning that indistinguishable PFGE types may have no epidemiological significance. Therefore a more discriminatory technique such as multiple-locus variable-number tandem-repeat analysis may be needed (Gierczyski et al. 2007).

References:

Gierczyski, R., A. Golubov, H. Neubaurer, J. N. Pham, and A. Rakin. 2007. Development of multiple-locus variable-number tandem-repeat analysis for Yersinia enterocolitica subspecies palearctica and its application to bioserogroup 4/O3 subtyping. J. Clin. Microbiol. 45:2508-2515

Publication list for this work:

1. PulseNet validated protocol (In development)
2. Paper describing protocol (in preparation)

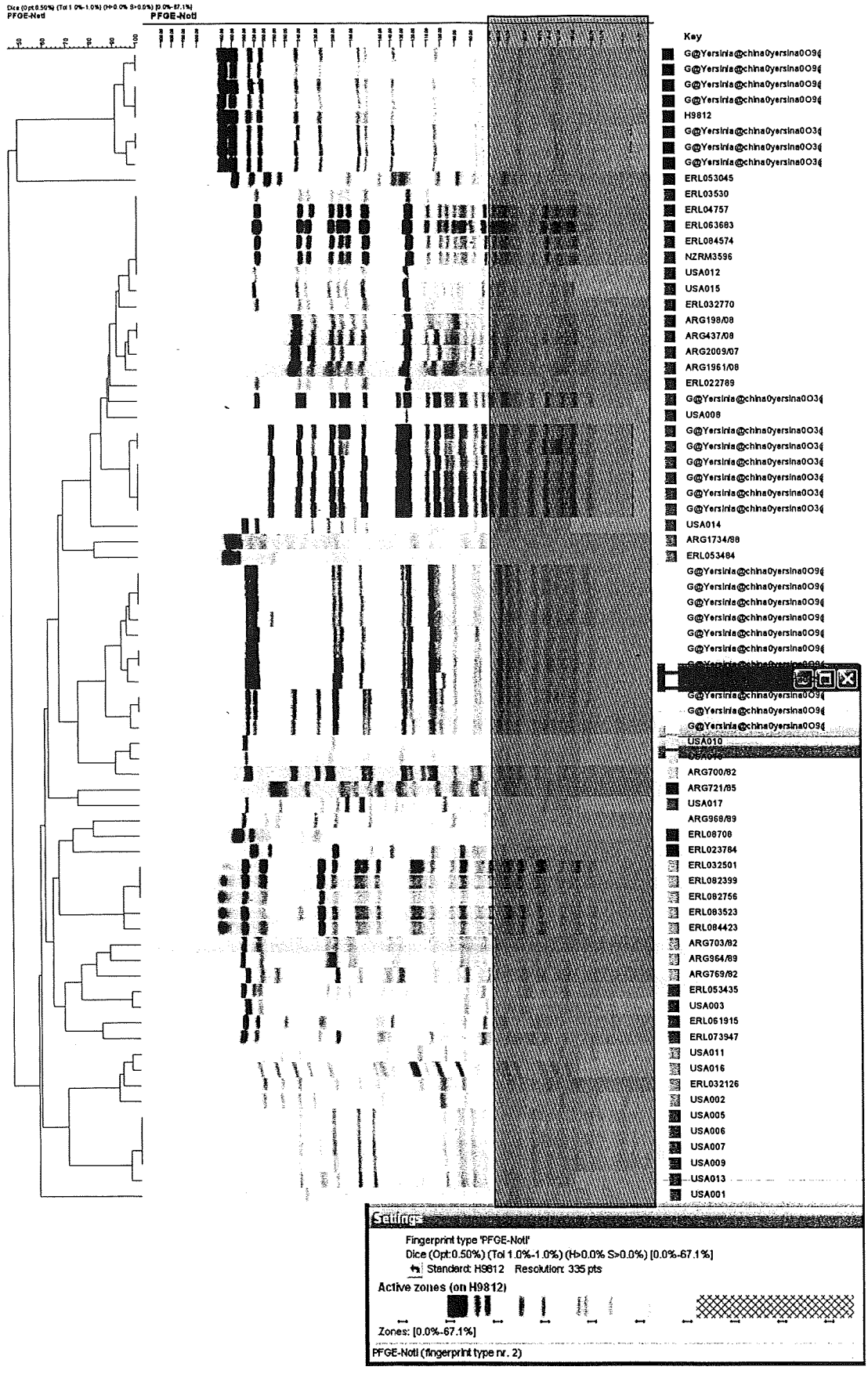

Figure 2. Proposed area for PFGE analysis of *Yersinia* with *Not*I (Shaded area indicates excluded region).

Title: **Application of Multilocus variable-number tandem repeat analysis for typing of *Shigella* spp.**

Name of researcher; **Mi-Sun Park**
Affiliation; Division of Enteric Bacterial Infections, National Institute of Health, Korea Center for Disease Control and Prevention, Nokbun-dong 5, Eunpyung-gu, 122-701, Seoul, Korea.

Summary:

In this study, we constructed the database with MLVA patterns for the 556 *S. sonnei* isolates and using this database, we also analyzed genetic relatedness between 556 isolates. From the phylogenic analysis result, we can conclude that the *S. sonnei* isolated during the big prevalence stage, after 1998 are genetically apart from the 1991~1997 isolates and also apart from the imported isolates either. We also confirm the ESBL producing ability of *S. sonnei* was came from horizontal transfer. In this study, we also set up the MLVA method for the *S. flexneri* isolates in Korea. We tested the availability of 26 VNTR loci for *S. flexneri,* those used for *S. sonnei*, and we set up the fragment analysis condition for 20 VNTR loci. From the comparison of allele diversity of each VNTR loci, SS01, SS05, SS08, SS09, SS11 and SS12 showed high variability and these 6 loci MLVA typing showed high discriminating ability.

Purpose:

a. Development of a next generation molecular typing method to identify *Shigella* spp. and its application
b. Validation of the usefulness of multilocus variable-number tandem repeat

analysis (MLVA) for typing of *S. flexneri* isolates in Korea

c. Database build-up with MLVA profiles of *S. flexneri*

d. Increment of MLVA database of *S. sonnei* and evaluation of transmission pattern of shigellosis in Korea by analysis of MLVA result.

Methods:

Bacterial Isolates

A retrospective analysis was performed for *S. sonnei* and *S. flexneri* isolates, those collected by public health network of Korea Centers for diseases Control from 1998 to 2009, using the PFGE database of the Korean National Institute of Health (KNIH). A total 556 *S. sonnei* and 153 *S. flexneri* isolates were selected based on their *XbaI*-digested PFGE type and epidemiological history to construct the database of MLVA patterns for *S. sonnei* and compare the discriminatory powers of MLVA and PFGE for *S. flexneri*.

The MacConkey agar, as selective media, used for isolation of *Shigella* spp., For primary identification, the isolates were characterized by conducting standard physiological and biochemical tests with an VITEK GNI plus card and VITEK system (Bio Meriux VITEK, MO, USA). Grouping and Typing antisera (DenkaSeiken, Tokyo, Japan) used for the serological identification of *Shigella sonnei*.

Preparation of crude bacterial DNA.

Plugs prepared for PFGE were used for the preparation of bacterial DNA. 2mm size of plug fragment which cut lengthwise was soaked in 100ul of 10mM Tris (pH 7.4) and boiled for 10min. After centrifugation at 13,000rpm for 10 min, the supernatant was used for template.

MLVA

The information about selected VNTR loci and the sequence of primer sets for PCR amplification of the 26 VNTR loci for MLVA of *S. sonnei* was kindly provided from Dr. Chiou in Taiwan CDC (Liang et. al.), and synthesized from Applied BioSystems (Foster City, CA).

The PCR conditions were slightly modified from Liang's method. Briefly, M1 to M6 were performed with a denaturing step at 94°C for 5 min, followed by 30 cycles of amplification at 94°C for 45 s, 55°C for 50 s, and 72°C for 60 s. M7 was performed under the same conditions except that the annealing temperature was set at 64°C.

Before the fragment analysis, the amplicons were diluted in de-ionized distilled water in a 1: 10 or 1:100 ratio. For the capillary electrophoresis, 1ul of diluted solution, 1ul of GeneScan 500 LIZ standard (Applied Biosystems), and 8.9 $\mu\ell$ Hi-Di-formamide were mixed and denaturated at 95℃. Capillary electrophoresis was performed by ABI Prism 3730xl genetic analyzer.

Data were collected, and the lengths of the amplicons were determined with GeneScan data analysis software, v. 2.0 (Applied Biosystems). All amplicons of different lengths from each locus were subjected to nucleotide sequence determination to verify the repeat sequence and the number of repeat units in the amplicons.

Data analysis of MLVA

The number of repeat units for each locus was saved as "character type" data in BioNumerics software (version 5.1; Applied Maths, Kortrijk, Belgium) and then subjected to cluster analysis using the minimum spanning tree method.

Results:

1. Construction and Analysis of Database with MLVA patterns for *S. sonnei*

a. Allelic diversity of VNTR loci for *S. sonnei*

In this study, 556 *S. sonnei* isolates were tested with MLVA, and we also evaluated the allelic diversity of 25 VNTR loci (Table 1).

VNTR loci SS06, SS03 and SS01 showed high allelic diversity. This result is similar to the result of previous study. In the previous year study, VNTR loci SS10 showed high variability when tested for 62 *S. sonnei* isolates, but when we increased test isolates to 556 isolates, SS10 showed low allelic diversity. The 4th higher variable VNTR loci was SS09 and SS12, SS11 were next.

Table 1. Allelic diversity of *S. sonnei* VNTRs

VNTR locus	Length of repeat unit (bp)	No. of repeat unit (range)	No. alleles (n=556)	Allelic diversity (n=556)
SS 01	7	1-21	16	0.84
SS 02	9	1-2	2	0.30
SS 03	7	2-30	22	0.85
SS 04	7	2-3	2	0.04
SS 05	7	1-4	3	0.05
SS 06	7	2-28	25	0.86
SS 07	7	2-6	3	0.08
SS 08	60	1-2	2	0.01
SS 09	6	5-17	13	0.63
SS 10	6	2-7	6	0.18
SS 11	6	2-9	7	0.34
SS 12	9	1-4	4	0.36
SS 13	6	2-5	4	0.16
SS 14	9	1-10	4	0.04
SS 15	6	2-3	2	0.04
SS 16	17	1-7	4	0.08
SS 17	6	2-3	2	0.04
SS 18	5	2-7	4	0.04
SS 19	5	2-3	2	0.27
SS 20	40	1-2	2	0.05
SS 21	18	1-2	2	0.01
SS 22	11	1-2	2	0.05
SS 23	16	2-6	5	0.24
SS 25	135	1-2	2	0.04

b. Analysis of MLVA patterns for *S. sonnei* Korean isolates using 4 most variable VNTR loci

The discriminating power between MLVA and PFGE was compared using 556 *S. sonnei* isolates (Table 2), and the MLVA, using the 4 most variable loci showed more strong discriminating power than that of PFGE

Table 2. Comparison of typing methods

Methods	No. of genotypes	DI	VNTR locus
PFGE	175	0.9122	
4 loci	347		SS01, 03, 06, 09
7 loci	356		SS01, 03, 06, 09, 02, 11, 12
24 loci	445	0.9775	

Using the 4 most variable VNTR loci, the MLVA pattern of 556 *S. sonnei* isolates showed three major clonal complexes, but between the complexes, no clear relation according to the isolated region and time was shown (Fig 1).

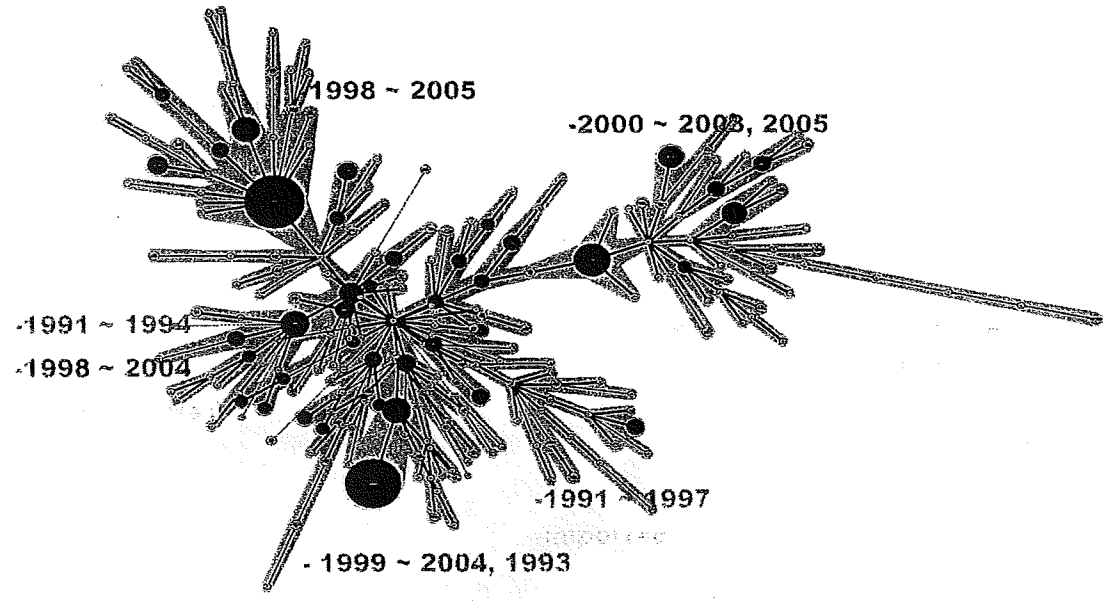

Figure 1. Phylogenic tree constructed by the MLVA patterns by using 4 VNTR loci.

c. Analysis of MLVA patterns for *S. sonnei* Korean isolates using 7 most variable VNTR loci and 12 most variable VNTR loci

From the phylogenic analysis using the 7 most variable VNTR loci, the MLVA pattern of 556 *S. sonnei* isolates showed more diversity than 4 loci MLVA. The MLVA patterns of *S. sonnei* isolates, those are isolated between 1991 to 1997, are branch off as a cluster from the MLVA patterns of after 1998 isolates (Fig. 2). But except this result, there was no clear relation according to the isolated region and time

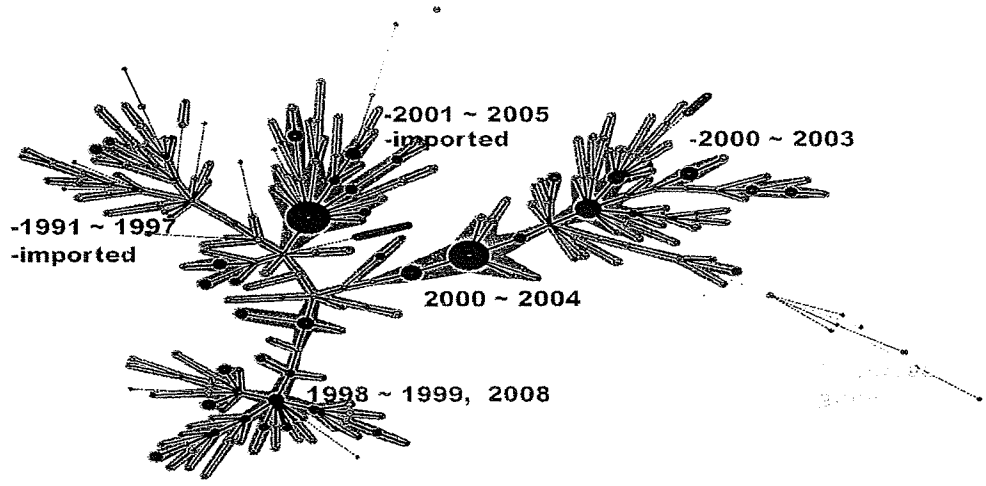

Figure 2. Phylogenic tree constructed by the MLVA patterns by using 7 VNTR loci.

MLVA patterns using 12 VNTR loci, showed similar results when using 7 VNTR loci. 1991 ~ 1997 isolates made their own clonal complex and imported isolates are separated as small complex (Fig. 3)..

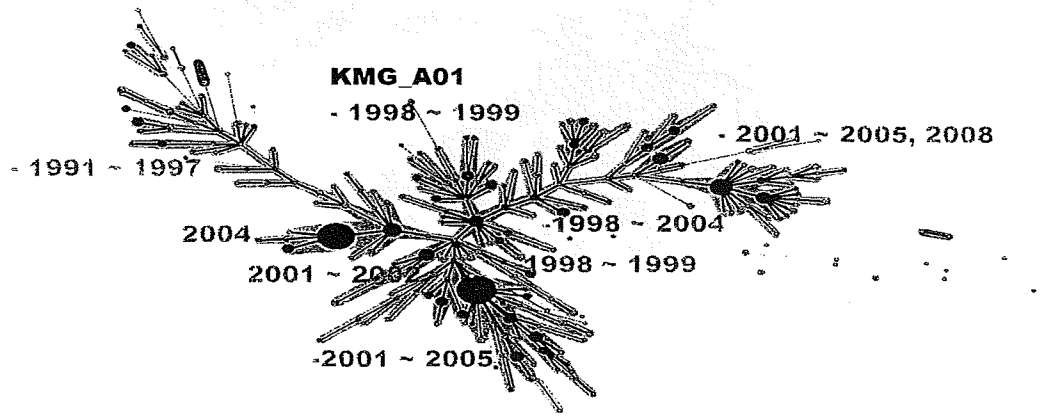

Figure 3. Phylogenic tree constructed by the MLVA patterns by using 12 VNTR loci.

d. Analysis of MLVA patterns for *S. sonnei* Korean isolates using 24 VNTR loci

From the phylogenic tree using the 24 VNTR loci, we can conclude that, the 1991 ~ 1997 *S. sonnei* isolates are genetically not related the big prevalence of shigellosis after 1998 (Fig. 4).

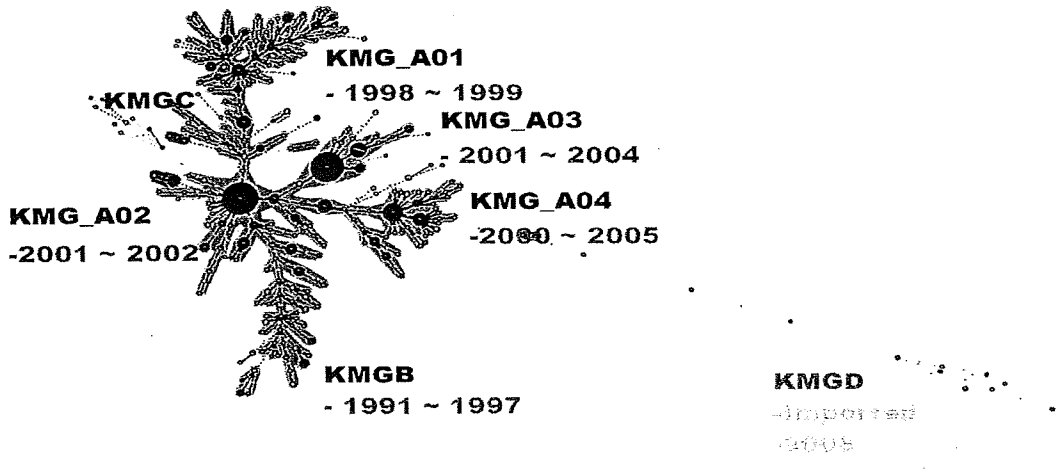

Figure 4. Phylogenic tree constructed by the MLVA patterns by using 24 VNTR loci.

e. Analysis using MLVA for the indistinguishable PFGE pattern of *S. sonnei*

The PFGE pattern SNZX 006 is a major pattern of *S. sonnei* isolates after year 2000 in Korea. This pattern was reported from whole of the country, and this pattern is also related the outbreak caused by ESBL producing *S. sonnei* in 2004.

Using 24 full VNTR loci, the PFGE pattern SNZX 006 divided into many different MLVA patterns, but as can see at the phylogenic tree (fig. 5), almost of the MLVA patterns are on the same genealogy. This phylogenic tree also showed clear sequence by the isolation year also.

The ESBL producing isolates constructed big clonal complex and this complex is on the same linage as other PFGE pattern SNZX 006 isolates.

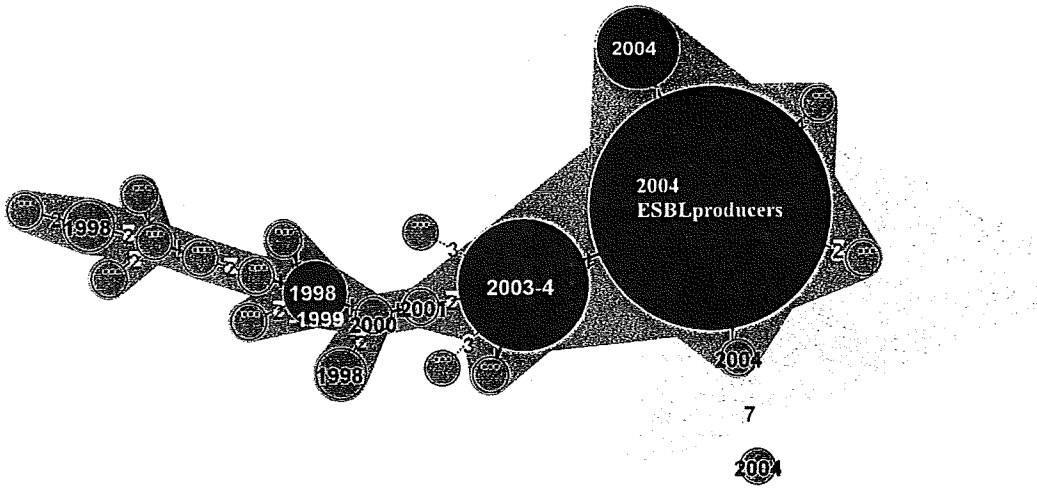

Figure 5. Analysis result of the PFGE pattern SNZ006 by MLVA

2. Development of MLVA methods for *S.flexneri*

a. Set up of the reaction condition for MLVA

Because we used same prime and probe set of the MLVA for the *S. sonnei* and *S. flexneri,* the optimal conditions for PCR is same as that of *S. sonnei*. Briefly, the optimal reaction condition of PCR set M1, M2, M3, M4, M5, and M6 was same as previously published method (Liang et. Al.), but for M7 set, the annealing temperature was modified to 64°C. The optimal results were obtained by using Platinum *Taq* (Invitrogen, Carlsbad, CA).

For the fragment analysis, the optimal amplification cycle was 30 cycle and the optimal dilution scale of amplicon was 1:10 (M1, M2, M3, M4, and M5 set) and 1:100 (M6 set)

b. Variability of each VNTR locus

A total 153 of *S. flexneri* isolates were tested. From the MLVA result of these 153 isolates, VNTR loci SS09 and SS12 were most variable, the allelic diversities of each isolates were 0.83 and 0.76 respectively. The next variable VNTR loci were SS05, SS11, SS01 and SS08 (table 3). But, the VNTR loci SS03, SS07, SS23, SS25 were not amplified in this study.

As expected, these VNTR loci showed low variability than used for the *S. sonnei*, but it has enough discriminating ablility for *S. flexneri*.

Table 3. Allelic diversity of *S. flexneri* VNTRs

VNTR locus	Length of repeat unit (bp)	No. of repeat unit (range)	No. alleles (n=153)	Allele diversity (n=153)
SS 01	7	5-43	7	0.35
SS 02	9	1-5	4	0.08
SS 04	7	3	1	0.00
SS 05	7	1-4	4	0.68
SS 06	7	2-20	3	0.04
SS 08	60	1-6	4	0.34
SS 09	6	4-18	11	0.83
SS 10	6	1-3	3	0.10
SS 11	6	3-40	4	0.36
SS 12	9	2-25	7	0.76
SS 13	6	3-10	4	0.19
SS 14	9	2	1	0.00
SS 15	6	2	1	0.00
SS 16	17	1-2	2	0.01
SS 17	6	2	1	0.00
SS 18	5	3-6	2	0.21
SS 19	5	2-7	4	0.12
SS 20	40	1-2	2	0.03
SS 21	18	1	1	0.00
SS 22	11	1-2	2	0.05

c. Comparison of discriminatory power

153 tested isolates were classified into 69 *NotI*-PFGE type. Major pattern was SZXN11.067, and 26 isolates were included in this pattern (fig. 6). Next prevailed

pattern was SZXN11.060 and SZXN11.052, 13 and 11 isolates were included into these patterns respectively.

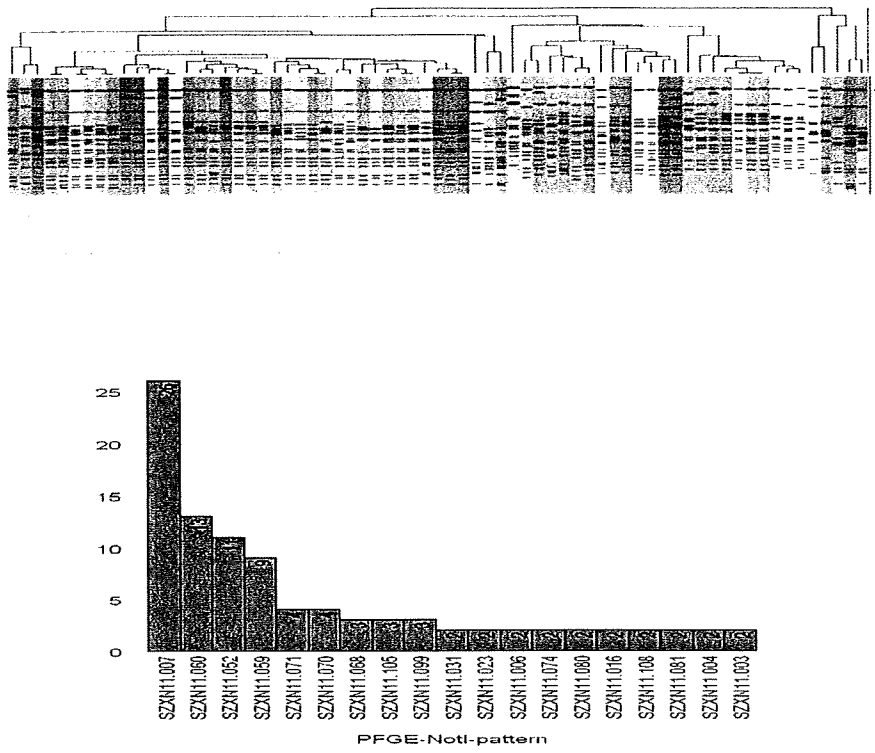

Figure 6. Dendrogram and distribution of *NotI*-PFGE pattern of 153 *S. flexneri* isolates in this study

The discriminating power of MLVA was higher than that of PFGE. From the fragment analysis result of 20 VNTR loci (table 3), MLVA typing with the three VNTR loci (SS05, SS09, SS12), 153 isolates are classified into 70 patterns. It showed almost same discriminating power that of *Not I*- digested PFGE.

But, using the 6 most variable VNTR loci (SS01, SS05, SS08, SS09, SS11, and SS12), MLVA typing produced 93 genotypes for 153 isolates. MLVA typing with 11 VNTR loci (SS01, SS02, SS05, SS08, SS09, SS10, SS011, SS12, SS13, SS19, SS22) classified 153 tested isolate into 98 patterns. And MLVA typing with 20 full VNTR loci classified 153 tested isolate into 99 patterns. So, in appearance, 6 VNTR loci MLVA typing has similar discriminating power compare to that of 11 VNTR loci and 20 VNTR loci MLVA typing

Table 3. Comparison of typing methods

	No. of genotype	DI	VNTR locus
PFGE	**69**	**0.953**	
20 loci	**99**	**0.992**	
11 loci	**98**		**SS01, SS02, SS05, SS08, SS09, SS10, SS011, SS12, SS13, SS19, SS22**
6 loci	**93**		**SS01, SS05, SS08, SS09, SS011, SS12**
3 loci	**70**		**SS05, SS09, SS12**

d. Analysis of MLVA patterns for *S. flexneri* Korean isolates

As previously mentioned, using the 3 VNTR loci, the MLVA typing showed same discriminating power than that of PFGE for 153 *S. flexneri* isolates. The phylogenic tree constructed by this 3 loci MLVA showed simple relationship between each other, but, there was no clear relationship according to the isolated region and time of each complex .

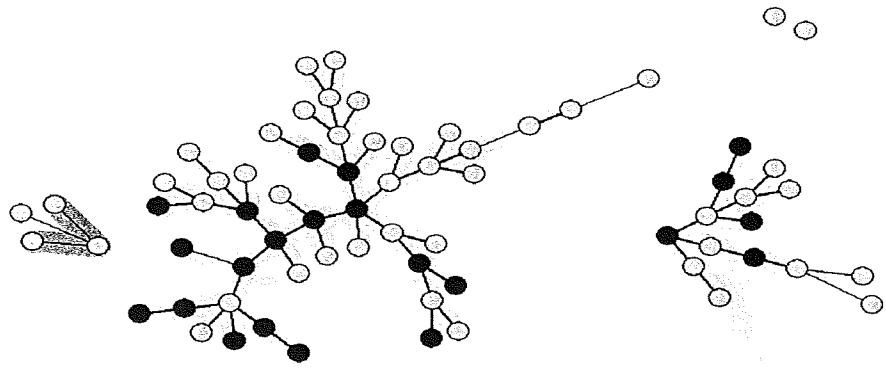

Figure 7. Phylogenic tree constructed by the MLVA patterns for *S. flexneri* by using 3 VNTR loci

As shown in figure 8, 9, and 10, the structure of phylogenic trees are almost similar. And there was no clear relationship according to the isolated region and time

of each complex in all the phylogenic trees.

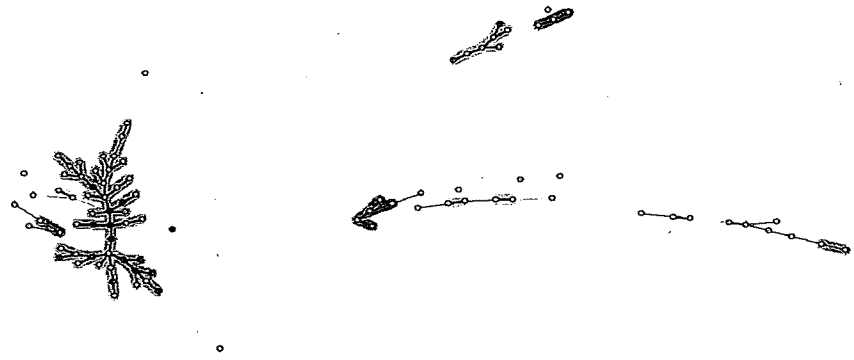

Figure 8. Phylogenic tree constructed by the MLVA patterns for *S. flexneri* by using 6 VNTR loci.

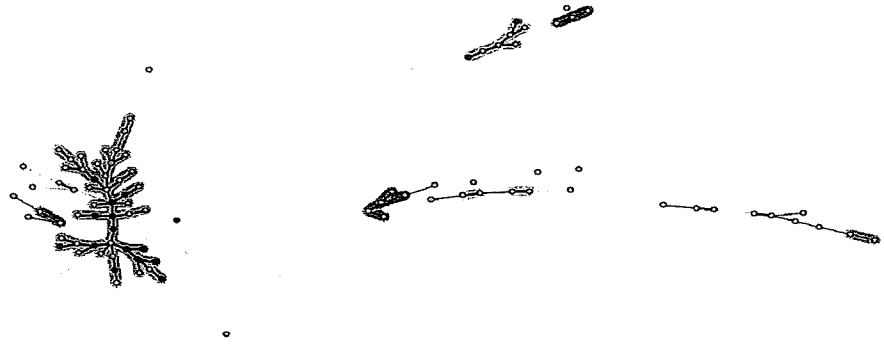

Figure 9. Phylogenic tree constructed by the MLVA patterns for *S. flexneri* by using 11 VNTR loci

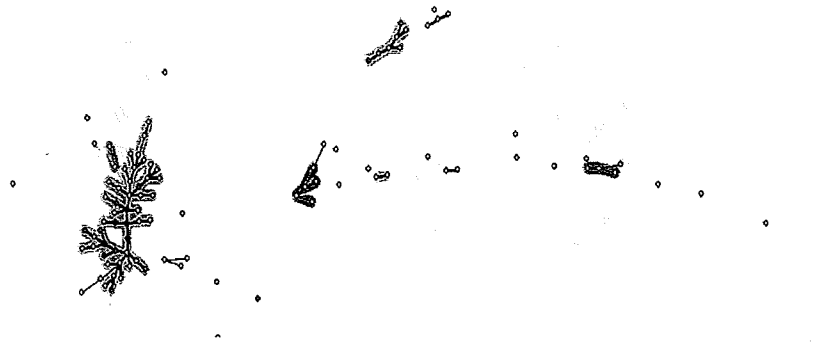

Figure 10. Phylogenic tree constructed by the MLVA patterns for *S. flexneri* by using 20 VNTR loci

Discussion

Before the 1997, shigellosis was not a serious infectious disease in Korea, because the annual reported cases were below 20 for 10 years. But, after 1998, unexpected abrupt increase of shigellosis was reported in Korea. In the short period, the abrupt increase of incidence made it difficult to identify the genetic relatedness even using PFGE. From this reason, in this study we introduced the next generation molecular subtyping methods, MLVA for the evaluation of genetic relatedness of *Shiella* spp. isolated in Korea.

In the previous year study, we set up the MLVA method for the *S. sonnei* and confirm the availability of this method for *S. sonnei*. In this year study, we constructed the database with MLVA patterns for the 556 *S. sonnei* isolates and analyzed the genetic relatedness between these isolates.

Compare to the previous year results, the variability VNTR locus SS10 was not so good when we tested 556 isolates. So for the 4 VNTR loci MLVA typing, we substitute VNTR locus SS10 to SS09 in this year study.

MLVA using 4 most variable VNTR loci, it has strong and enough discriminating power compare to the PFGE and 8 or 12 VNTR loci MLVA typing. So we can use it for the shigellosis surveillance and outbreak investigation as expected in previous year.

But in the phylogenic analysis, 4 loci MLVA didn't showed clear relatedness. In this study, if we using more VNTR loci, we can get the more information about the phylogeny in *S. sonnei* isolated in Korea. Finally we can conclude that, the big shigellosis prevalence isolates after 1998 are genetically apart from the 1991 ~ 1997 isolates, and apart from the imported isolates either.

From this result, we can try to identify the genetic factor related in prevalence of shigellosis, and we can develop the genetic marker relate to the prevalence of some bacterial enteric infections.

From the MLVA analysis for the indistinguishable PFGE pattern SNZ006, almost al the MLVA patterns on the same lineage, include the ESBL producing isolated those

caused big outbreak in 2004. therefore the ESBL producing isolates probably get the ESBL producting ability by the horizontal transfer.

In this study, we also set up the MLVA method for the *S. flexneri* isolated in Korea, and evaluated its usefulness. Among the 26 VNTR loci those tested for variability, VNTR loci SS03, SS07, SS23, SS24, SS25, and SS26 were not amplified. Therefore we set up the condition for the 20 VNTR loci.

VNTR loci SS01, SS05, SS08, SS09, SS11 and SS12 were highly variable for *S. flexneri* isolates tested in this study. This result is slightly different from *S. sonnei* those used same VNTR loci, because we used VNTR loci for developed *S. sonnei*, we didn't expected high discriminating power for the S. *flexneri*, and in the other study (Chiou et al.,) the VNTR loci for the *S. sonnei* can not work for the *S. flexneri*. But using 6 most variable VNTR loci, this method showed enough discriminating power also.

From the result of this study, MLVA typing method is really useful molecular tool for the surveillance and outbreak investigation for the shigellosis and it can compensate the limit of PFGE , and also MLVA can be used as a useful tool for the phylogenic analysis for the *S. sonnei*.

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1) **B. Lindstedt.** 2005. Multiple-locus variable number tandem repeats analysis for genetic fingerprinting of pathogenic bacteria. Electrophoresis. 26: 2567-2582.
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5) **Liang, S., Watanabe, H., Terajima, Jun., Li, C., Liao, J., Tung, S., and C. Chiou.** 2007. Multilocus variable number tandem repeat analysis for molecular typing of *Shigella sonnei*. J. Clin. Microbiol. 45:3574-3580.

6) **Chiou, C., Wei, H., Wang, Y., Liao, J., and C. Oliver**. 2006. Usefulness of inter-IS1 spacer polymorphisms for subtyping of . of *Shigella sonnei*. J. Clin. Microbiol. 44:928-3933

7) **Chiou, C., Watanabe, H., Wang, Y., Wang, W., Terajima, J., Tong, K., Phung, D., and S. Tung**. 2009. Utility of Multilocus variable number tandem repeat analysis as a molecular tool for the phylogenetic analysis of *Shigella sonnei*. J. Clin. Microbiol. 47:1149-31154

Publication list for this work:

1) **The Genetic relatedness and evolution of *Shigella sonnei* during prevalent shigellosis stage in Korea (in preparation).**

PROGRESS REPORT:

1. *Escherichia coli*:

Phenotypic and genotypic characterization of different virotypes of *Escherichia coli* present in diarrheal patients and non-diarrheal controls

Investigators: Talukder KA, Azmi IJ, Aslam M, Salam MA, Howlader AM, Akter M, Amin MB, Haque R, Endtz HP and Cravioto A

Introduction:

Escherichia coli are versatile organism that can cause secretory and bloody diarrhoea as well as persistent diarrhoea. The pathogenesis of the different serotypes of *E. coli* is self explanatory in producing different types of diarrhoea. The diarrhoea caused by *E. coli* is a worldwide phenomenon from Middle East to Latin America and from Europe and Asia to Africa and affects both the developed and developing country (Qadri *et al.*, 2005). Human and most worm blooded animals carry *E. coli* as a part of the normal intestinal flora. A group of these organisms have developed the ability to cause both intestinal and extra intestinal disease (Robins-Bromne *et al;* 2002, Nataro *et al,* 1998). Strains associated with the presence of diarrhoea have been grouped according to shared pathogenic characteristics.(Kaper *et al.*, 2004) To date, the five major groups are 1) enterotoxignic (ETEC) that causes infantile and traveler's diarrhoea; infant diarrhoea; 2) enteropathogenic (EPEC) that causes infant diarrhoea; 3) enteroinvasive (EIEC) that cause dysentry; 4) enteroaggregative (EAEC) that is associated with bloody and persistent diarrhoea and 5) enteropathogenic (EHEC) that cause hemorrhagic colitis and hemolytic urmic syndrome.(Levine, 1987). These different clinical manifestations are related with the presence of virulence genes present in plasmids and in defined groups of genes, called pathogenicity islands, present in the chromosomes of these bacteria. Therefore, the purpose of the present study was to determine the prevalence of different virotypes of *E. coli* isolated from diarrhoeal patients and healthy individuals without having diarrhoea and to characterize these strains especially EPEC and EAEC phenotypically and genotypically.

Methodology:

A total of 166 *E. coli* strains were isolated from both the diarrhoeal patients (n=71) and healthy individuals (n=95) during a community based study conducted by ICDDR, B at Mirpur in Dhaka City between November 2008 and February 2009. Of these, 21 strains were found to be EPEC and EAEC which were identified by Multiplex PCR. Out of 21 strains, 10 were from diarrhoeal patients (CDS samples) and 11 were from non-diarrhoeal controls (CMS samples). Their Antimicrobial Susceptibility were tested by the disk diffusion method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS, 1999). Their Plasmid profile analysis was also performed.

All identified EAEC and EPEC strains were analyzed by pulsed-field gel electrophoresis (PFGE) to reveal the clonal relationship among the strains. PFGE was performed by the standardized protocol developed by CDC for *E. coli* (CDC protocol, 2004). PFGE analysis of *Xba*-I digested chromosomal DNA of the strains yielded 12 to 16 reproducible DNA fragments ranging in size approximately from 30 to 600Kb (Figure 1 and 2). Analysis of the gel images was carried out by both the Tenovar theory (Tenover *et al.*, 1995) and BioNumerics software (Applied Maths, Belgium) using the dice coefficient and unweighted-pair group method (UPGMA) using average linkages to generate dendrograms with 1.0% tolerance values.

Results and Conclusion:

Among 10 CDS isolates, 6 were identified as EAEC and 4 were EPEC strains whereas 11 CMS isolates include 4 EAEC and 7 EPEC strains. Most of the *E. coli* strains isolated from diarrhoeal patients were resistant to Amoxicillin (AMC), Mecillinam (MEL) and Trimethoprim-sulfamethoxazole (SXT). The same result was found with strains from non-diarrhoeal controls. Of the 21 strains, 3 EAEC were resistant to Ciprofloxacin (CIP).

Among the total 10 EAEC strains, 2 ESBL (Extended spectrum ß-lactamase) producing *E. coli* were found from diarrhoeal patients whereas only 1 ESBL producing *E. coli* was from non-diarrheal controls. No ESBL producing *E. coli* was found from EPEC strains. The susceptibility test data showed that all the ESBL producing strains were resistant to third generation cephalosporin [ceftriaxone (CRO), cefotaxime, ceftazidime] and Nalidixic acid (NA) [Table 1 & 2].

In plasmid profile analysis different plasmid patterns were found in the EPEC and EAEC strains. All typical EPEC strains harbored 60 to 90 MDa plasmid known as EPEC adherence factor (EAF) plasmid.

Dendrogram analysis of all the EAEC strains showed that they were completely heterogeneous. Dendrogram analysis of the ESBL producing E. coli showed that 2 of those strains contained 41.38% homology (Figure 1). In case of EPEC strains, dendrogram analysis showed that 3 strains contained identical PFGE pattern, of which 2 strains were isolated from non-diarrhoeal controls and the rest one from diarrhoeal patient (Figure 2).

Table 1: Summary of all characteristic patterns of EAEC strains

Strain ID	Plasmid Pattern	Antibiotic Susceptibility Profile	PFGE Pattern	ESBL
7207 CDS-2	P3	$AMC^{I}SXT^{R}MEL^{S}NA^{R}CIP^{I}CRO^{S}$	A	-
7207 CMS-5	P10	$AMC^{I}SXT^{R}MEL^{S}NA^{R}CIP^{S}CRO^{S}$	B	-
7123 CDS-2	P8	$AMC^{S}SXT^{S}MEL^{S}NA^{S}CIP^{S}CRO^{R}$	C	+
17136 CDS-4	P5	$AMC^{R}SXT^{R}MEL^{S}NA^{R}\ CIP^{S}CRO^{S}$	D	-
7212 CMS-3	P12	$AMC^{R}SXT^{S}MEL^{S}NA^{S}CIP^{S}CRO^{S}$	E	-
8070 CMS-1	P9	$AMC^{S}SXT^{S}ME^{S}NA^{R}CIP^{S}CRO^{S}$	F	-
7160 CMS-7	P11	$AMC^{I}SXT^{R}MEL^{S}NA^{R}CIP^{S}CRO^{S}$	G	+
7191 CDS-1	P4	$AMC^{I}SXT^{R}MEL^{I}NA^{R}\ CIP^{S}CRO^{S}$	H	+

Key: I=Intermediate, R=Resistant, S=Sensitive