RESEARCH OUTPUTS AND CONTRIBUTION OF PROJECT

1.**Training of students**:
Trained 5 postgraduates (Masters level) (1 graduated) and 4 B.Sc (Hons) (2 graduated).

2. **Conference Paper Presentation**:
(i) Thong KL. Identification of Class I Integrons and genomic island SGI1 in multidrug resistant *Salmonella enteric* serovara in Malaysia. Paper presented at the 9th A- IBMN Conference & 16th MSMBB Scientific Meeting, Kuala Lumpur, 3-5th September 2006,

(ii) Thong KL., Maria, J., Dahlia, SR Fatimah, SA Intan, MJ Norshuhaidah, SH Sharifah. Antimicrobial resistanceof zoonotic Salmonella enterica in Malaysia. Paper presented at 42nd Annual Scientific Seminar Malaysian Society of Parasitology and Tropical Medicine ~Exploring New Frontiers: Emerging and Reemerging Zoonosis" 1-2 March 2006.

iii. Thong KL. Surveillance and Subtyping of *Salmonella* spp in Malaysia. Paper presented at the PulseNet USA Update Meeting, Miami, Florida, USA, 3-6 April, 2006.

iv. Thong KL. First paper: Genotypic Characterisation of *Salmonella* and *Burkholderia*. Second paper: Further development of PFGE in Malaysia. Both papers presented at the PulseNet Asia Pacific Update Meeting, Nanjing, China, 19-21 December, 2006.

v. Thong KL. Molecular Epidemiology of *Salmonella enterica* in Malaysia. Paper presented the PulseNet Asia Pacific training course, Hong Kong. 15-17 February, 2007.

vi. Thong KL et al. Molecular Characterisation of drug resistant *Salmonella* Typhimurium in Malaysia. Paper to be presented at the International Symposium of Antimicrobial Agents Resistance (ISAAR) 2007, Singapore, 6-9 March, 2007.

3. PFGE training Workshop in Malaysia
As part of the activities towards developing PulseNet in Malaysia, a hands-on training workshop was successfully conducted on 17-20 April, 2006 at the Institute for Medical Research, Kuala Lumpur. The objective of the workshop was to train more researchers and public health officers from Public Health Institutions to use PFGE as a subtyping tool. A total of 14 personnel from the various state public health institutions attended.
The workshop involved a series of lectures on principles and application of PFGE and a 3 days' hands-on practical of preparation of DNA using the standardized PulseNet Protocol.

As part of the initiative to enable more users to use PFGE, training was also given to medical officers in the teaching hospitals. In addition, 5 postgraduates were taught PFGE to subtype *S.* Typhimurium, *S.* Enterititidis, *S.* Typhi , *S.* Corvalis, and *Burkholderia pseudomallei*.

4. Publications: At least 5 manuscripts are being drafted for possible publication.

28/2/07

Title: Prediction of Penner serotype and species of *Campylobacter* based on PFGE profiles.

Name of researcher: Brent Gilpin

Affiliation: Institute of Environmental Science & Research Limited, Christchurch, New Zealand.

Summary: The aim of this study was to evaluate the potential of PFGE genotyping of *Campylobacter* to predict Penner serotypes and species. From an initial examination of isolates, the most informative isolates missing either a *Kpn*I PFGE profile or a Penner serotype, were analysed in the lab using the appropriate technique. *Sma*I PFGE types correlated with assigned species of over 96% of the isolates examined. PFGE profiles also were predictive of Penner serotype for over 93% of isolates. Only four SmaI:KpnI combinations predicted more than one serotype. At a minimum this narrows the options down to 2 or 3 serotypes. It is also possible that Penner serotype has been incorrectly assigned. To determine how widely predictive these are, a much larger set of isolates needs to be examined. This collection of data and analysis of isolates is ongoing.

Purpose:

The PulseNet Aotearoa New Zealand *Campylobacter* database contains more than 1500 isolates, all of which have a PFGE profile generated using *Sma*I restriction enzyme. Up until 2004, most isolates were also Penner serotyped, and the "serogenotype" was used to evaluate relatedness of isolates. As highlighted in our recent publication (Gilpin et al. 2006 J. Clinical Microbiology 44:406-412) we now believe that PFGE using *Sma*I and *Kpn*I is a superior approach, and progressively have reduced the amount of Penner serotyping performed. However there is a large body of information on Penner serotypes of *Campylobacter*, and it would be useful to be able to be able to compare current PFGE genotyping with previous Penner serotyping and serogentypes. The purpose of this study was to evaluate the potential of PFGE genotyping of *Campylobacter jejuni* to predict Penner serotypes. We also sought to evaluate the potential of PFGE to predict species of *Campylobacter*. If reliable this could speed up outbreak investigations, and save resources.

Methods:

Database fields of isolates in current *Campylobacter* PulseNet Aotearoa New Zealand

database were exported to excel spreadsheet and data analysed. Where subtyping data was incomplete, selected were analysed by PFGE analysis with a second enzyme (*Kpn*I) or Penner serotyping was performed.

Results:

The server Campylobacter database contained 1556 isolates on the 2nd February 2007 (when exported data for this analysis). Of these 1244 were reported as *C. jejuni*, 108 as *C. coli*, 1 as *C. lari* and 203 had no species reported.

All 1556 isolates in the database have been analysed by PFGE using *Sma*I. Sixteen (16) isolates, of which none had species reported, were unable to be digested using this enzyme. The remaining 1550 were assigned to one of 263 *Sma*I PFGE types. Fifteen (15) *Sma*I PFGE types, representing a total of 21 isolates, did not have species information reported. The single *C. lari* isolate in the database had a unique *Sma*I PFGE type (Sm0230). Twenty-seven (27) *Sma*I PFGE types were only associated with *C. coli* isolates and these types accounted for 57 (53%) of the *C. coli* isolates. These *C. coli* specific *Sma*I PFGE types are summarised in Table 1 and most represent only one or two isolates. Forty-nine (49) *Sma*I PFGE types were only associated with *C. jejuni* isolates although for 33 of these types there are isolates for which species information is missing. The number of isolates represented by *C. jejuni*-associated *Sma*I PFGE types are summarised in Table 2.

Table 1: *C. coli*-Associated *Sma*I PFGE Types

*Sma*I PFGE Type	No. Isolates	*Sma*I PFGE Type	No. Isolates
Sm0109	2	Sm0249	1
Sm0158	1	Sm0250	17
Sm0177	1	Sm0251	2
Sm0190	1	Sm0252	1
Sm0193	1	Sm0253	1
Sm0200	1	Sm0254	1
Sm0203	1	Sm0255	1
Sm0237	4	Sm0256	1
Sm0238	2	Sm0257	1
Sm0239	2*	Sm0258	1
Sm0240	1	Sm0263	1
Sm0242	1	Sm0265	5
Sm0243	2	Sm0283	1
Sm0244	2		

* additional isolates of this PFGE type did not have species information entered

Most (212, 80.6%) of the *Sma*I PFGE types were associated with only *C. jejuni* isolates. Of these types, 169 (79.7%) represented five or fewer isolates as shown in Table 2. Thirty-three (33) of these *C. jejuni*-associated *Sma*I PFGE types contained isolates without species information.

Table 2: *C. jejuni*-Associated *Sma*I PFGE Types

No. Isolates Represented	No. *Sma*I PFGE Types	Descriptions
1	88	Sm0003, 0017, 0022, 0025, 0027, 0029, 0044, 0054, 0057, 0058, 0059, 0064, 0065, 0066, 0067, 0069, 0070, 0074, 0076, 0079, 0085, 0086, 0088, 0089, 0091, 0097, 0099, 0100, 0103, 0104, 0112, 0114, 0115, 0116, 0117, 0119, 0122, 0124, 0125, 0128, 0129, 0130, 0132, 0133, 0136, 0141, 0154, 0155, 0156, 0162, 0163, 0165, 0166, 0170, 0171, 0174, 0181, 0185, 0191, 0192, 0194, 0195, 0196, 0201, 0204, 0207, 0208, 0209, 0211, 0212, 0213, 0217, 0222, 0227, 0228, 0234, 0241, 0247, 0248, 0260, 0269, 0271, 0272, 0273, 0278, 0281, 0285, 0287
2	33	Sm0008, 0012, 0019, 0028, 0032, 0043, 0056, 0063, 0068, 0072, 0075, 0082*, 0096, 0105*, 0107, 0108, 0110, 0111, 0118, 0121, 0123, 0126, 0140, 0157*, 0167, 0173, 0175, 0178, 0187*, 0205, 0206, 0218, 0221, 0225, 0276, 0277
3	30	Sm0023, 0031, 0047, 0077, 0092, 0093, 0101*, 0106*, 0131, 0137, 0146, 0148, 0149, 0150, 0153, 0179, 0180, 0188, 0223*, 0274
4	7	Sm0011, 0090, 0120, 0138, 0168, 0186, 0189
5	11	Sm0005, 0007, 0020*, 0073, 0087, 0142*, 0145*, 0152, 0164, 0183*, 0184*
6	2	Sm0049, 0144
7	8	Sm0004, 0016*, 0042*, 0043, 0055, 0060, 0127, 0147
9	4	Sm0006, 0041, 0045, 0083
10	5	Sm0034*, 0062, 0080*, 0078, 0094
11	3	Sm0010*, 0052, 0082*
12	3	Sm0048, 0143*, 0161
13	1	Sm0015
15	3	Sm0051, 0151, 0199
16	3	Sm0030*, 0033*, 0061
17	3	Sm0014*, 0039*, 0182
18	1	Sm0026*
19	1	Sm0053
21	4	Sm0013*, 0018*, 0040*, 0098
23	1	Sm0172*
29	1	Sm0036
32	1	Sm0002*
36	1	Sm0081*
38	1	Sm0046*
42	1	Sm0009*
54	1	Sm0038
55	1	Sm0050

* these *Sma*I PFGE types contained isolates without species information

The remaining 8 *Sma*I PFGE types, representing 284 isolates contained both *C. jejuni* and *C. coli* isolates. Of these 3 *Sma*I PFGE types, representing 227 isolates, also contained isolates without species information.

SmaI or SmaI:KpnI profiles correlated with a single serotype for 458 of the isolates with Penner serotyping information (Table 3). An additional 31 of the isolates with Penner serotypes had SmaI:KpnI patterns that correlated with 2 or 3 Penner serotypes (Table 4).

Table 3. Correlation of PFGE patterns with Penner serotypes

SmaI	KpnI	Serotype	Isolates		SmaI	KpnI	Serotype	Isolates
1		1	42	12	110	??	23,36	2
1		2	27	8	111	??	27	2
1		3	55	7	118	??	2	2
1		5	6	5	120	??	37	4
4	any		27	7	123	??	22	2
6	any	4c		9	127	??	33	6
7	any	4c		5	140	??	2	2
9	any	4c		39	142	??	37	4
10	any	4c		5	143	??	8,17	10
13	any	1,44		21	144	??	23,36	2
14	any	1,44		13	144	??	8,17	2
15		38	2	1	145	??	5	3
15		138	19	1	146	??	19	2
16	any	1,44		5	149	??	6	3
21	any		11	8	150	??	2	2
36	any	8c		29	151	??	52	3
37	38,39,104		2	9	152	??	1,44	3
42	any		2	6	153	??	6	3
43	any	4c		7	161	??	1,44	2
45	any	4c		9	167	??	8,17	2
62	any		35	7	172	??	1,44	8
81	any		2	16	178	??	10	2
84	any	1,44		10	179	??	2	3
98		43	1,44	18	180	??	37	2
160	any		11	10	182	??	5	2
164	any		11	5	182	??	24	2
1	??		5	6	182	??	42	2
1	??		21	2	182	??	45	2
1	??		53	3	182	??	1,44	3
20	??	8,17		3	183	??	23,36	4
39	??		2	3	184	??	5	2
53	??		5	17	188	??	19	3
61	??		35	12	189	??	23,36	4
80	??		2	3	199	??	10	12
80	??	1,44		7	206	??	6	2
96	??	1,44		2	218	??	23,36	2
98	??		10	2	221	??	5	2
106	??	8,17		2	223	??	1,44	2
108	??		11	2	275	??	11	2

Table 4. SmaI:KpnI patterns which correlated with more than one Penner serotype.

SmaI	KpnI	Serotypes
Sm0002	Kp0004	37 (1), 58 (4), 4c (1)
Sm0005	Kp0128	10 (1), 4c (4)
Sm0038	Kp0037	2 (1), 1,44 (16), 23,36 (2)
Sm0055	Kp0052	2 (1), 23,36 (1)

Numbers in bracket refer to number of isolates.

Discussion: *Sma*I PFGE types correlated with assigned species of over 96% of the isolates examined. PFGE profiles also were predictive of Penner serotype for over 93% of isolates. Only four SmaI:KpnI combinations predicted more than one serotype. At a minimum this narrows the options down to 2 or 3 serotypes. It is also possible that Penner serotype has been incorrectly assigned. To determine how widely predictive these are, a much larger set of isolates needs to be examined. This collection of data and analysis of isolates is ongoing.

Reference list:

Gilpin B, Cornelius A, Robson B, Boxall N, Ferguson A, Nicol C, Henderson T. 2006. Application of pulsed-field gel electrophoresis to identify potential outbreaks of campylobacteriosis in New Zealand. J Clin Microbiol. **44**:406-12.

Publication list for this work:

1) Cornelius, Robson, Nicol and Gilpin. Predicting Penner serotypes of Campylobacter jejuni on the basis of pulsed field gel electrophoresis patterns. In preparation for Journal of Molecular Methods.

Title: **PFGE Standardization and molecular epidemiological study of *Vibrio vulnificus.***

Name of researcher; **Bok K won Lee**
Affiliation; Lab. 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:

Vibrio vunificus (*V. vulnificus*) is a gram negative and estuarine bacterium commonly found in coastal waters and in association with shellfish and fish. This bacterium is known to cause septicemia and severe wound infections in patients with chronic liver diseases or immuno-compromised condition. In order to present current epidemiological status of *V. vulnificus* cases in Korea, monthly occurrence of clinical and environmental *V. vulnificus* and morbidity and mortality during 2001 to 2005 year were analyzed. We performed comparison of pulsed-field gel electrophoresis (PFGE) and randomly amplified polymorphic DNA (RAPD) for the analysis of 78 clinical *V. vulnificus* isolates during 2000 to 2004. We used *Not* I restriction enzyme for the digestion of genomic DNA, and the best condition of PFGE for *V. vulnificus* was initial pulsing time: 4.16 sec, final pulsing time: 40 sec, voltage: 6 v/cm, running temperature: 14℃, and running time: 18 hrs with CHEF Mapper system (Bio-Rad Laboratories, CA, USA). In addition to PFGE analysis, we performed RAPD analysis for the fine clustering of the isolates. In this study, we describe high-level variation in PFGE profiles among 78 clinical isolates, as determined by RAPD-PCR.

Purpose:

1. PFGE analysis of clinical *Vibrio vulnificus* isolates obtained in 2000-2004 in Korea.
2. Randomly amplified polymorphic DNA (RAPD) analysis of clinical *Vibrio vulnificus* isolates obtained in 2000-2004 in Korea.

Methods:

Data Collection

A retrospective analysis was performed for *V. vulnificus* sepsis cases nationwide from 2000 to 2005, using the *V. vulnificus* database of the Korean National Institute of Health (KNIH). Because *V. vulnificus* sepsis is categorized as a class 3 notifiable disease in Korea, the laboratory of enteric pathogens in KNIH has collected the demographic data and isolates of the reported cases. Collected isolates were cultured again and analyzed for the microbial characteristics including molecular subtyping. Using these data collected from 2000 to 2005, place of residence and data of disease occurrence were studied.

Bacterial Isolates

The thiosulfate-citrate-bile salts-sucrose (TCBS) agar, as selective media, used for isolation of *V. vulnificus*. For primary identification, the isolates were characterized by conducting standard physiological and biochemical tests with an API 20E kit (Bio Meriux SA, France). PCR amplification of the *vvhA* gene, specific to *V. vulnificus*, was also used for confirmation of the isolates.

Pulsed-Field Gel Electrophoresis for genetic relatedness (PFGE).

The preparation of genomic DNA blocks and digestion with a restriction enzyme were carried out, as described by Gautom and PFGE protocols suggested by CDC. *V. vulnificus* isolates were tested firstly and analysed by using restriction enzymes *Not* I

(New England Biolabs, MA, USA). Typing by PFGE of genomic DNA digested with *Not* I was carried out in a CHEF Mapper system (Bio-Rad Laboratories, CA, USA). The PFGE pulsing and running conditions were changed independently for various running times and 6 Volts/cm at 14℃ for the optimization and standardization. *Salmonella* Braenderup BAA664 was used as a molecular size marker strain. After electrophoresis, the gels were stained with ethidium bromide for 20 minutes and were photographed using Gel Doc 2000 (Bio-Rad Laboratories, CA, USA).

RAPD analysis.

Ten 10-bp oligonucleotide primers (Bioneer, Daejeon, Korea) with G+C contents of 50% were screened for the ability to provide a suitable band pattern with various *V. vulnificus* strains. The primer selected had the following sequence: 5'GGATCTGAAC3'. RAPD-PCR amplification of the DNA was performed using a AccuPower® PCR PreMix (Bioneer). The cycling profile was as follows: one cycle consisting of 94°C for 5 min, 35 cycles consisting of 94°C for 40 sec, 43°C for 40 sec, and 72°C for 1 min, and a final cycle consisting of 72°C for 5 min. The RAPD products were electrophoresed at 100 V for approximately 1 h on a 2.0% agarose gel. A 100-bp plus ladder (Bioneer) was used as a molecular size marker.

Clustering of isolates

PFGE patterns and RAPD profiles of *V. vulnificus* isolates were visually compared and numbered in sequence according to the molecular sizes of the bands. Coefficients of dice similarity were calculated, and cluster analysis was performed with the unweighted pair group method with arithmetic averages (UPGMA) algorithm in the BioNumerics software (Applied Maths BVBA, Belgium) by using a 1.0% tolerance for the band migration distance.

Results:

1) Epidemiological analysis.

The number of *Vibrio vulnificus* isolates from patients was obtained had a clear seasonal peak during the summer months (Figure 1). The greatest frequency occurred in August or September every year. The number of notified *V. vulnificus* sepsis cases was about 50 or more yearly. Mortality of the patients was over 50% (Figure 2).

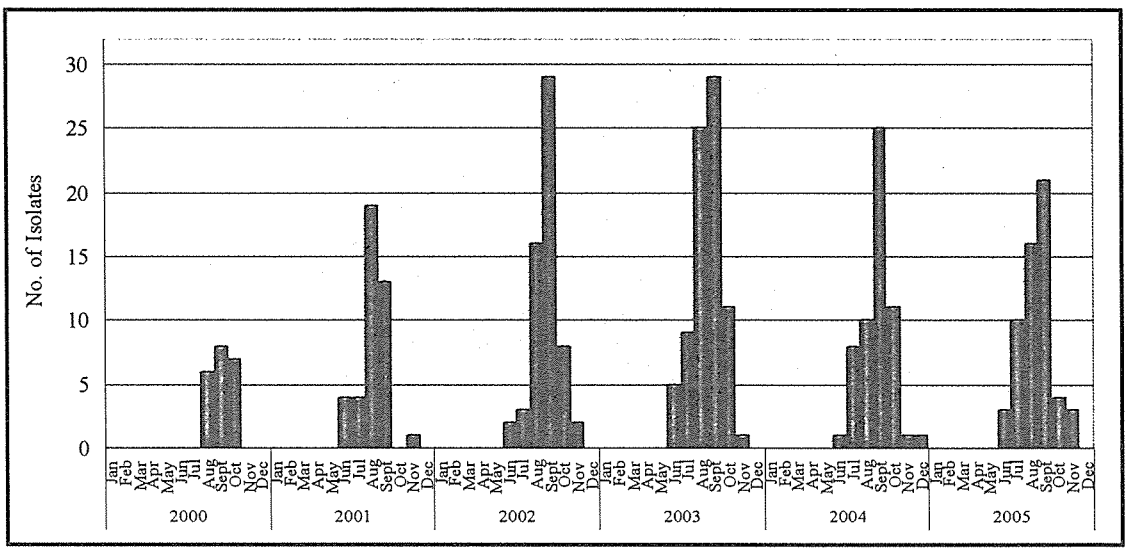

Figure 1. Monthly occurrence of *Vibrio vulnificus* sepsis for recent 6 yr

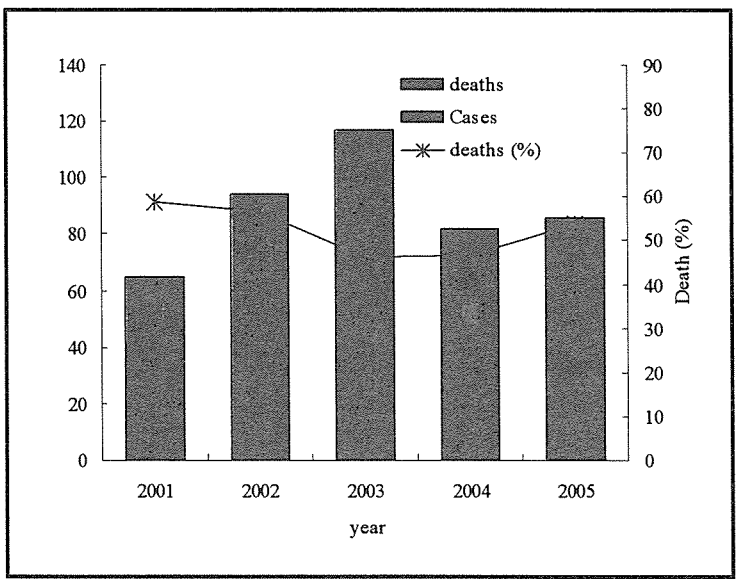

Figure 2. Occurrence and lethality of *Vibrio vulnificus* sepsis for recent 5 yr.

The *Vibrio vulnificus* sepsis was 295 reports from 2001 to 2005. We received 81 (27.5%) reports of *Vibrio vulnificus* sepsis from Jeonnam, 48 (16.3%) from Gyeongnam, 33 (11.2%) from Gyeonggi (Figure 3).

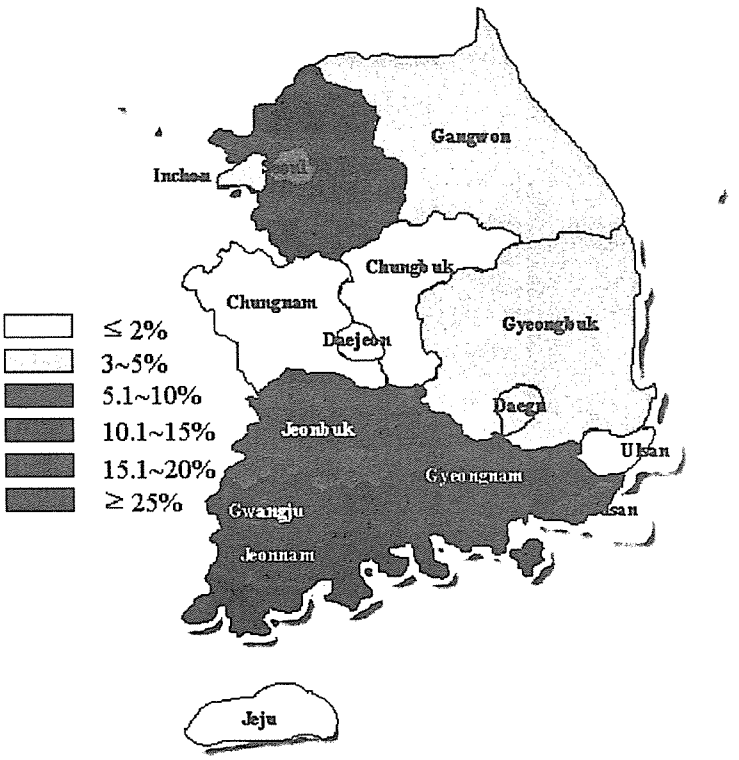

Figure 3. Map of *Vibrio vulnificus* sepsis for recent 5 years.

2) PFGE

We performed comparison of PFGE patterns with 78 clinical isolates obtained during 2000 to 2004 year (Table 1). We used Not I restriction enzyme for the digestion of genomic DNA, and the best condition of PFGE for *V. vulnificus* was initial pulsing time: 4.16 sec, final pulsing time: 40 sec, voltage: 6 v/cm, running temperature: 14□, and running time: 18 hrs with CHEF Mapper system (Bio-Rad Laboratories, CA, USA) (Figure 4). Based on the Not I PFGE patterns, dendrograms were produced (Figure 5). We observed high-level diversity among PFGE profiles of clinical *V. vulnificus* isolates with similarities as low as 44%. When we compared the PFGE patterns of *V. vulnificus* isolates from high-risk area and low-risk area respectively, certain specific cluster has not defined (Figure 6, 7). In the high-risk area, the highest similarity among the PFGE patterns was 79%. These results indicate that PFGE was too discriminatory to define clusters that distinguished clinical strains from environmental strains.

Table 1. The sources of *Vibrio vulnificus* clinical isolates used in this study

Isolate No.	Strain	Date of Isolation	Isolation site
	Clinical		
VV01	00-01944	August/00	Gyeongnam
VV02	00-02132	August/00	Jeonbuk
VV03	00-02133	August/00	Jeonbuk
VV04	00-02581	August/00	Jeonbuk
VV05	00-02611	August/00	Jeonnam
VV06	00-02725	August/00	Jeonnam
VV07	00-02735	August/00	Jeonnam
VV08	00-02737	September/00	Jeonnam
VV09	00-02813	September/00	Jeonnam
VV10	00-02858	September/00	Gyeongnam
VV11	00-02921	September/00	Jeonbuk
VV12	00-03176	September/00	Gyeongnam
VV13	00-03177	September/00	Gyeongnam
VV14	00-03178	September/00	Gyeongnam
VV15	00-03191	September/00	Gyeonggi
VV16	00-03195	October/00	Jeonbuk
VV17	00-03670	October/00	Gyeongnam
VV18	00-04197	October/00	Gyeongbuk
VV19	01-01269	July/01	Jeonbuk
VV20	01-01300	July/01	Chungnam
VV21	01-01305	July/01	Jeonbuk
VV22	01-01566	July/01	Seoul
VV23	01-01672	July/01	Gyeonggi
VV24	01-01673	July/01	Gyeonggi
VV25	01-01691	August/01	Jeonnam
VV26	01-01831	August/01	Gyeongnam
VV27	01-01832	August/01	Gyeongnam
VV28	01-02079	August/01	Gyeonggi
VV29	01-02990	August/01	Gyeonggi
VV30	02-01768	June/02	Jeonnam
VV31	02-01829	June/02	Gyeonggi
VV32	02-01872	June/02	Jeonnam
VV33	02-02053	July/02	Jeonnam
VV34	02-02177	August/02	Gyeongnam
VV35	02-02439	August/02	Gyeongnam
VV36	02-02440	August/02	Jeonnam
VV37	02-02490	August/02	Jeonnam
VV38	02-02491	August/02	Jeonnam
VV39	02-03002	September/02	Ulsan
VV40	02-03003	September/02	Ulsan
VV41	02-03135	September/02	Gyeongnam

Isolate No.	Strain	Date of Isolation	Isolation site
	Clinical		
VV42	02-03136	September/02	Gyeongnam
VV43	02-03138	September/02	Gyeongnam
VV44	02-03139	September/02	Gyeongnam
VV45	02-03140	September/02	Gyeongnam
VV46	02-03158	September/02	Jeonbuk
VV47	02-03159	September/02	Jeonbuk
VV48	02-03187	September/02	Jeonnam
VV49	02-03227	September/02	Busan
VV50	02-03244	September/02	Gyeonggi
VV51	02-03316	September/02	Gyeongnam
VV52	03-01533	July/03	Gyeongnam
VV53	03-01534	August/03	Jeonnam
VV54	03-01833	July/03	Incheon
VV55	03-02401	August/03	Busan
VV56	03-02403	August/03	Gyeongnam
VV57	03-02578	August/03	Gyeongnam
VV58	03-02594	August/03	Busan
VV59	03-02595	August/03	Busan
VV60	03-02613	August/03	Incheon
VV61	03-02754	August/03	Busan
VV62	03-02755	September/03	Jeonbuk
VV63	03-02859	August/03	Jeonbuk
VV64	03-02924	September/03	Gyeonggi
VV65	03-02925	September/03	Gyeonganm
VV66	03-02926	September/03	Busan
VV67	03-02959	September/03	Gyeonganm
VV68	03-03152	September/03	Gyeonganm
VV69	03-03495	October/03	Busan
VV70	04-16824	July/04	Gyeonggi
VV71	04-16825	July/04	Gyeonggi
VV72	04-17027	July/04	Daegu
VV73	04-17408	July/04	Jeonnam
VV74	04-17911	August/04	Busan
VV75	04-17912	August/04	Busan
VV76	04-17913	August/04	Busan
VV77	04-18630	August/04	Busan
VV78	04-18634	August/04	Busan

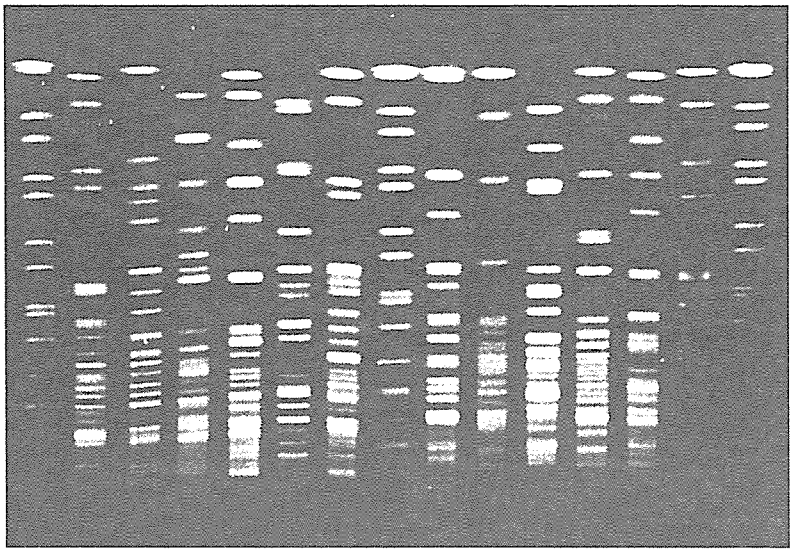

Figure 4. Representative *Not* I digested PFGE patterns of clinical *V. vulnificus* isolates.

Lane 1, 8, 15, *S.* Breanderup ATCC BAA-664; Lane 2, 00-01944; Lane 3, 00-12132; Lane 4, 00-02133; Lane 5, 00-02581; Lane 6, 00-02611; Lane 7, 00-02725; Lane 9, 00-02735; Lane 10, 00-02737 ; Lane 11, 00-02813; Lane 12, 00-02858; Lane 13, 00-02921; Lane 14, 00-03176.

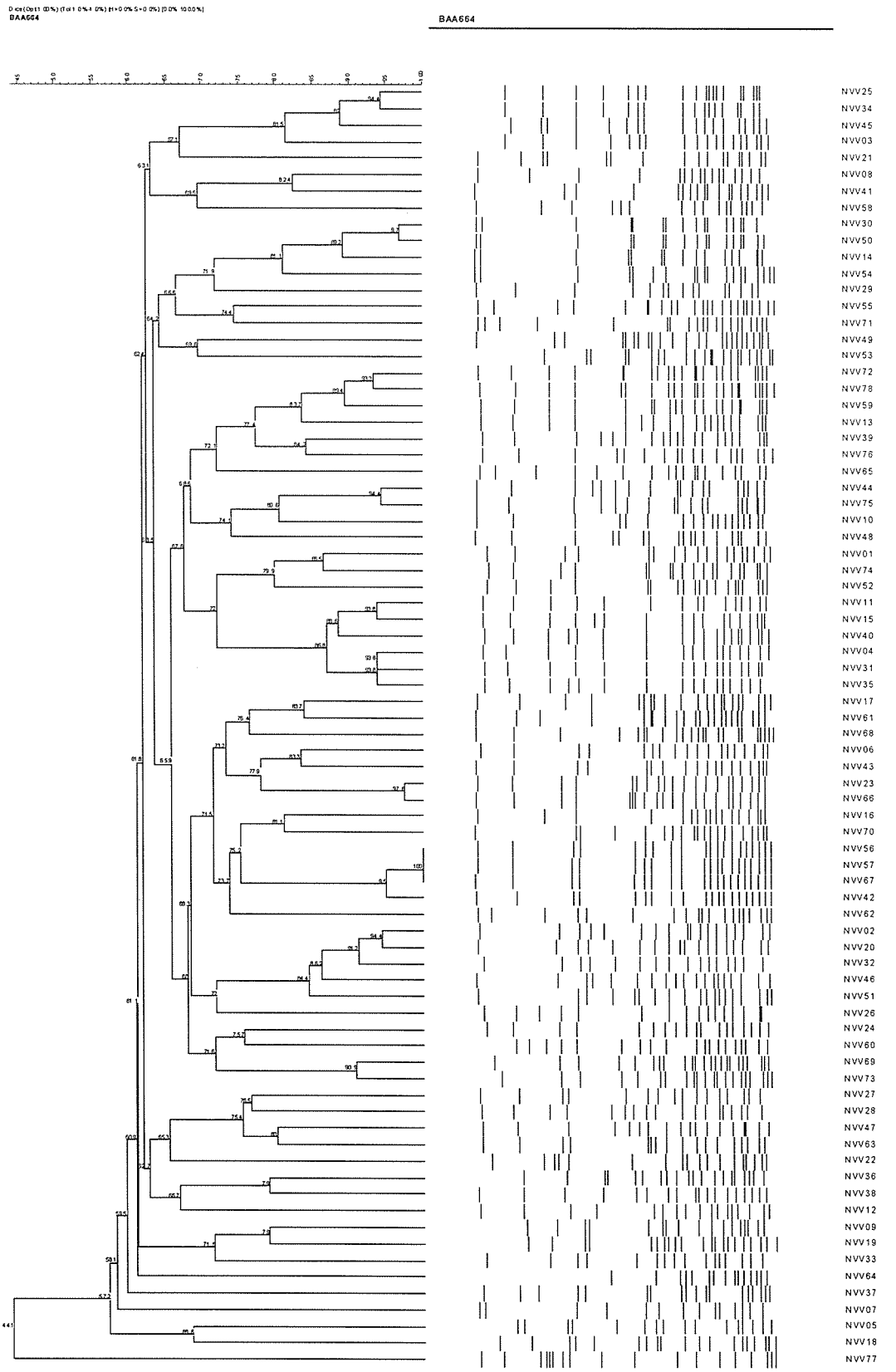

Figure 5. Dendrogram of *Not* I digested PFGE patterns of total *V. vulnificus* isolates in Korea from 2000-2004.

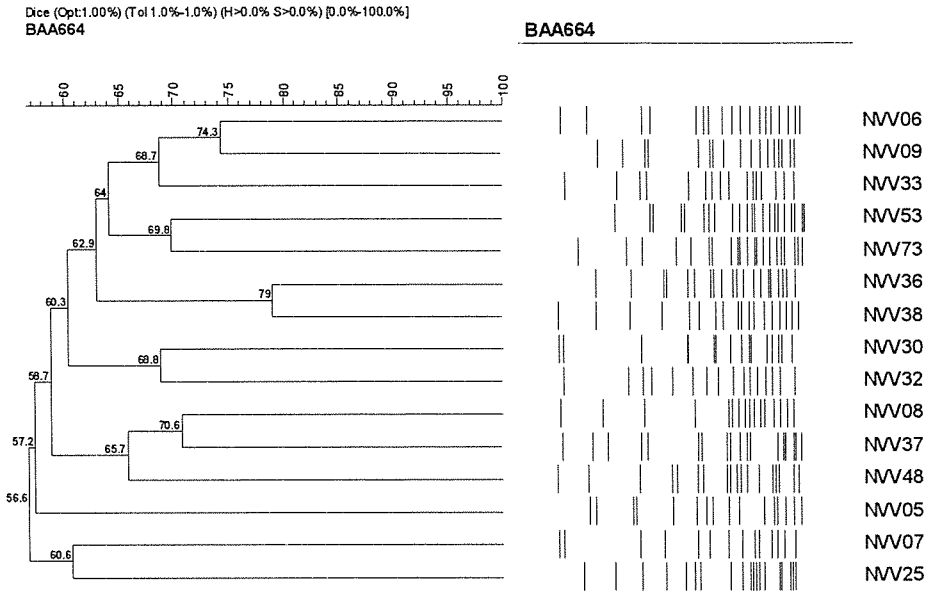

Figure 6. Dendrogram of *Not* I digested PFGE patterns of *V. vulnificus* isolates from high-risk area (Jeonnam province).

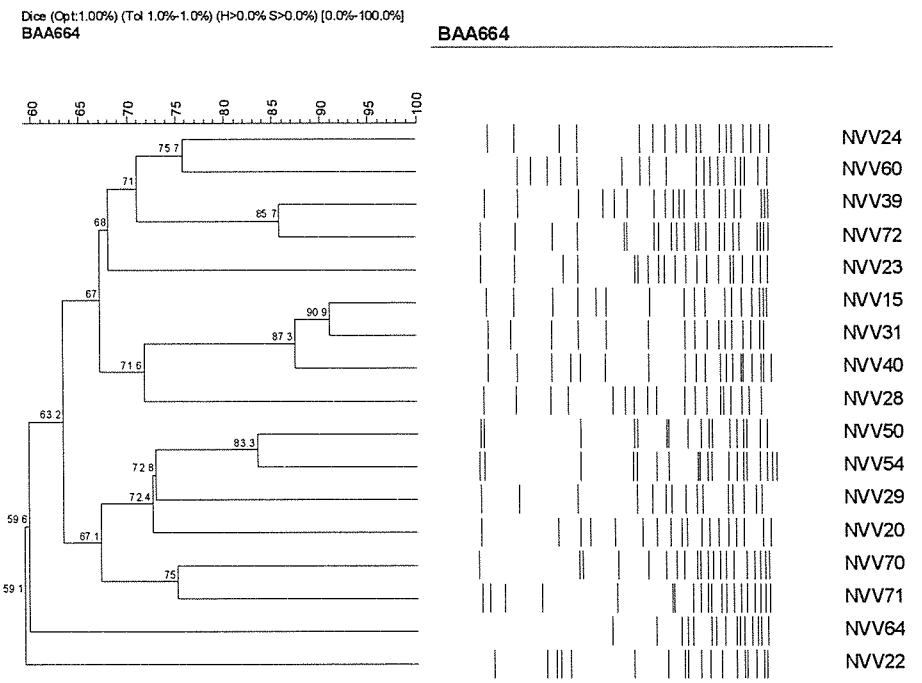

Figure 7. Dendrogram of *Not* I digested PFGE patterns of *V. vulnificus* isolates from low-risk area.

3) RAPD results.

A total of 78 *V. vulnificus* clinical isolates were subjected to RAPD PCR analysis and yielded reproducible profiles. Examples of RAPD PCR are shown in Fig. 8. The results of the cluster analysis of the RAPD PCR profiles are shown in Fig. 9. The 93.6% (73 of 78) of the clinical isolates were located in a single cluster, (under similarity 50%) (Table 2). However, the RAPD profiles analyzed in this study were very heterogeneous, with similarities as low as 24%.

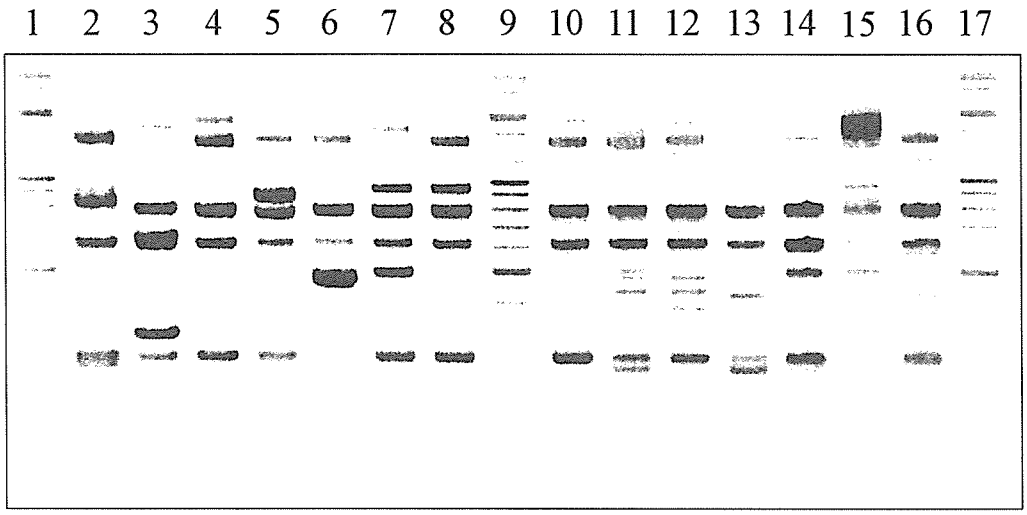

Figure 8. Representative RAPD profiles of clinical *V. vulnificus* isolates.

Lanes 1, 9, 17, 100-bp plus ladder; Lane 2, 00-01944; Lane 3, 00-12132; Lane 4, 00-02133; Lane 5, 00-02581; Lane 6, 00-02611; Lane 7, 00-02725; Lane 8, 00-02735; Lane 10, 00-02737 ; Lane 11, 00-02813; Lane 12, 00-02858; Lane 13, 00-02921; Lane 14, 00-03176; Lane 15, 00-03176; Lane 16, 00-03176.

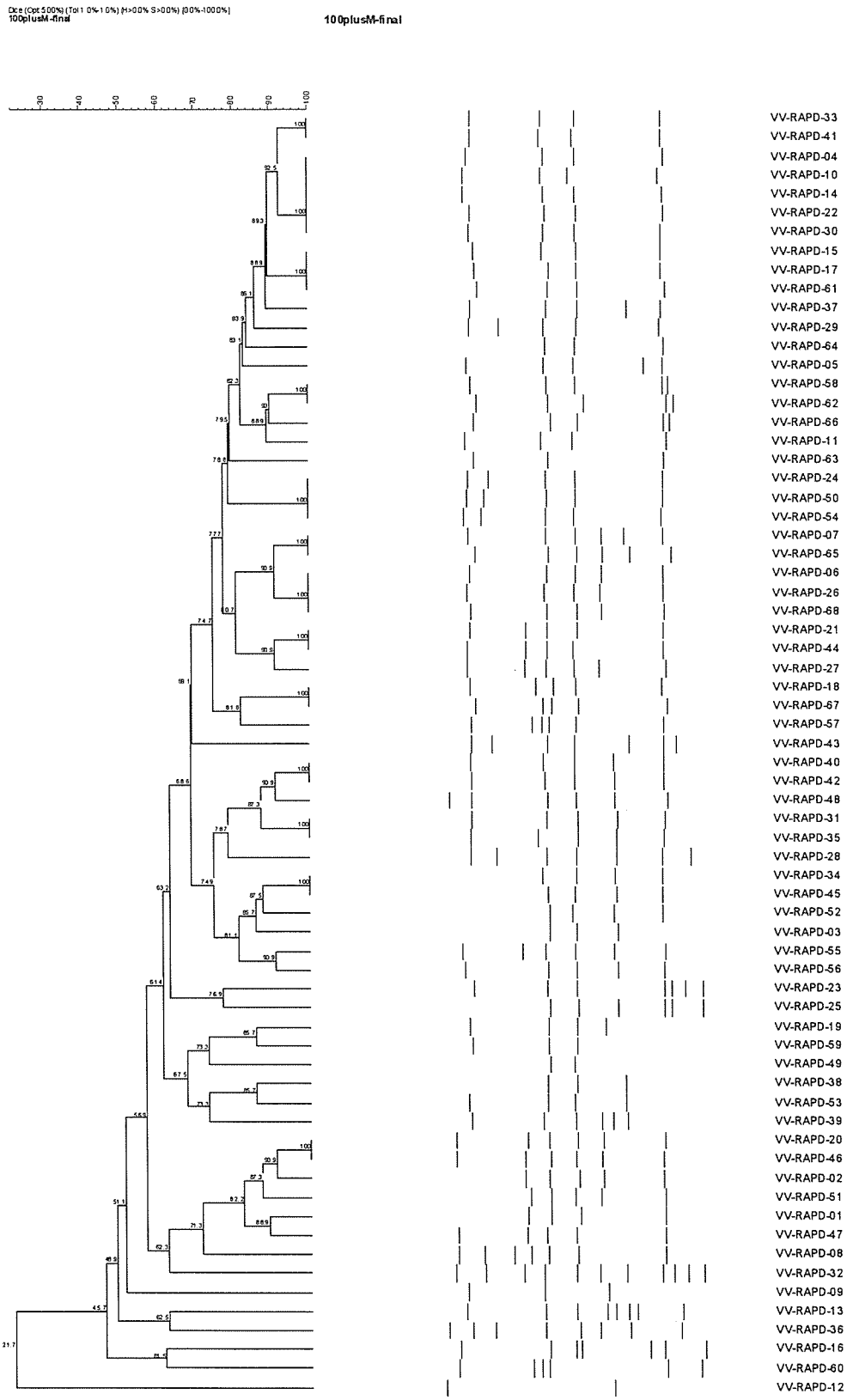

Figure 9. Dendrogram of RAPD patterns of clinical *V. vulnificus* isolates.

Table 2. Percentages of *V. vulnificus* clinical isolates at selected similarity indice as analyzed by RAPD-PCR.

Type of isolates	% of isolates at similarity level of:					
	40%	**50%**	**60%**	**70%**	**80%**	**90%**
Clinical	77(98.7%)	73 (93.6%)	65 (83.3%)	45 (57.7%)	18 (23.1%)	7 (9%)