V. cholerae (O1 and O139): 1274 strains (636 patterns of *Not*I), in them 632 strains with 426 patterns of *Sfi*I;

E. coli O157:H7: 300 strains (161 patterns of *Xba*I);

Shigella: 904 strains (including 238 *S.sonnei* strains with 45 *Xba*I patterns, and 666 *S.flexneri* strains with 210 *Not*I patterns);

Y. enterocolitica: 506 strains (in them 258 strains of O:3, O:8 and O:9, 107 *Not*I patterns);

L. monocytogenes: 296 strains (133 patterns of *Asc*I);

S. suis: 108 strains (61 patterns of *Swa*I);

N. meningitidis: 212 strains (43 patterns of *Nhe*I);

Leptospira: 268 strains (107 patterns of *Not*I).

PulseNet China is trying to optimize the PFGE protocol of *Leptospira interrogans*. The *Not* I patterns of partial isolates of serogroup *interohaemorrhagiae* in the surveillance in Sichuan province were analyzed.

Now more than 10 provincial CDC laboratories have been equipped with PFGE instruments. And more than 60 professional technicians from 29 province- and city-level CDC have been trained in the central laboratory, including specialized training, to do PFGE analysis of the strains collected historically in surveillance, and the strains from outbreak response.

2, Identification of *Shigella flexneri* 4c emerged as dominant serotype of *Shigella*

species in China

Shigellosis is one of the major diarrheal diseases afflicting humans in the developing and underdeveloped parts of the world, more ubiquitous than previously thought. In China, about 0.8 to 1.7 million episodes of bacillary dysentery occurred in recent years, even rapid economic development and the subsequent improvement of water supply and sanitation. In China, *S. flexneri* was the was the predominant serogroup(86%) , followed by *S. sonnei* (12%). The predominant serotype among *S. flexneri* was *S. flexneri* 2a (80%). Here, we found that in Henan province of China, the *S.flexneri* 4c has become predominant serotypes since 2002, and transmitted to several other provinces. 99% of *S.flexneri* 4c isolates were found resistant to ampicillin. When the phylogenetic tree was constructed by using the concatenated sequences of 14 house keep genes used for MLST analysis, *S.flexneri* 4c was found to be evolved from *S.flexneri* 2a. The serotype shift of *Shigella* species lead to protection failure of vaccine strain specific for *S.flexneri* 2a. The epidemiological, clinical, MLST, PFGE and genomic analysis of the emerged *S.flexneri* 4c are underway.

Publications:

Zhang, J., B. Diao, N. Zhang, Z. Cui, L. Zhang, J. Xu, and B. Kan. 2007. Comparison of different electrophoretic parameters of Pulse-Field Gel Electrophoresis for Vibrio cholerae subtyping. J. Microbiol. Methods 71:15-22.

STUDY TITLE: **Organization of PFGE Workshop for Training and Technology transfer for Asia Pacific countries/ areas in February 2008**

STUDY FACILITY: (Name and address of institute)

Public Health Laboratory Centre, 7/F,

382 Nam Cheong Street, Shek Kip Mei, Kowloon, Hong Kong.

STUDY DIRECTOR: (name of the research director) Signature, date

Dr. Kai Man KAM, M.D.

Project leader:

Haruo Watanabe, M.D. Ph.D.
Deputy Director-General
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Toyama 1-23-1, Shinjuku-ku
Tokyo 162-8640, Japan

:

Issue Date: February 22, 2008.

1) **OBJECTIVE:**

(1) To organize a Workshop to enhance the laboratory capacity of countries/ areas in Asia and Pan Pacific region in performing PFGE in February 2008;

(2) To train up key laboratory personnel so they acquire the ability to build up the laboratory capacities in their own country/ area;

(3) To build up a network of trainer and trainees that have shared experiences in PFGE laboratory work, and which can work together in partnership during outbreak investigations.

2) **STUDY DESIGN:**

(1) Organization of Workshop in the Public Health Laboratory Centre in Hong Kong for training of laboratory personnel in the Asia Pacific Region in February 19- 22, 2008.

(2) Co-organizers were: NIID, Japan; CDC, USA.

(3) The Public Health Laboratory Centre in Hong Kong was responsible for the use of fund monies of 2,000,000 yen in the overall organization of the Workshop.

(4) The Workshop took place in the Public Health Laboratory Centre in Hong Kong.

(5) The Workshop lasted 4 days and covered the setting up of PFGE laboratory data analysis software, requisite computer technique, commonly encountered problems, quality control/ quality assurance issues, and network requirements.

3) **RESULTS:**

(1) Participants of the Workshop had hands-on experience in performing PFGE' data analysis and management. (listed Appendix I)

(2) PFGE Data input and analysis in relation to outbreak investigations were also covered in the Workshop. (Schedule in Appendix II)

(3) Key trainers from advanced institutions (including NIID, Japan and CDC, USA) were invited to participate in the Workshop.

(4) Evaluation of the Laboratory Workshop by participants were done to gather experiences for development of future work in the Asia Pacific Region. (Appendix III)

(5) A report was generated after the Workshop.

Appendix I

Participants List for PulseNet Asia Pacific PFGE Workshop 2008

		Name	Email	From	Departure	
					Date	Date
1	**Trainer**	Dr. Peter Gerner-Smidt	plg5@CDC.GOV	USA	18 Feb	23 Feb
2	**Trainer**	Michele Parsons	zcp9@CDC.GOV	USA	17 Feb	23 Feb
3	**Trainer**	Grant Williams	fid4@CDC.GOV	USA	17 Feb	23 Feb
4	**Trainer**	Mr. Jun Terajima	terajima@nih.go.jp	Japan	18 Feb	23 Feb
5	Trainee	Ms Celia C. Carlos	celia.carlos@yahoo.com	Philippines	18 Feb	23 Feb
6	Trainee	Mr. Manuel Jamoralin Jr.		Philippines	18 Feb	23 Feb
7	Trainee	Ms Marietta Lagrada		Philippines	18 Feb	23 Feb
8	Trainee	Miss Piyada Wangroongsarb	piyada@dmsc.moph.go.th	Thailand	18 Feb	23 Feb
9	Trainee	Miss Kritsana Poorikittichai	poorikittchai@hotmail.com	Thailand	18 Feb	23 Feb
10	Trainee	Mr. Bo PANG	cdcpangbo@gmail.com	PR China	18 Feb	23 Feb
11	Trainee	Mrs. Xuemei BAI		PR China	18 Feb	23 Feb
12	Trainee	Mr. Abdus Salam Mondol	biosalam1023@yahoo.co.uk	Bangladesh	18 Feb	23 Feb
13	Trainee	Mr. Muhammad Atiqul Islam	atikmicro@yahoo.com	Bangladesh	18 Feb	23 Feb

Appendix II

Agenda for PulseNet Asia Pacific PFGE Workshop Hong Kong 2008

Date: February 19 - 22, 2008
Venue: Conference Room at Public Health Laboratory Centre (PHLC), Hong Kong

February 19, 2008 (Tuesday)

Chairperson of the day: Dr. Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrival PHLC	
9:05 - 9:10 am	Welcome	KM Kam
9:10 - 9:15 am	Overview of workshop	Danny Cheung
9:15 - 9:30 am	Installation of BioNumerics	CDC, USA
9:30 - 9:50 am	Overview of BioNumerics and MasterScripts v4.0	CDC, USA
9:50 - 10:30 am	Analyzation of PFGE Gel Images, Linking Gel Lanes, and Entering Data	CDC, USA
10:30 - 11:00 am	Coffee Break	
11:00 - 12:00 pm	Exercise 1: Analyze a PFGE Gel Image and Link Entries to Database	CDC, USA~~ALL~~
~~12:00 - 1:00 pm~~	~~Extended Forum (Open to all colleagues)~~	~~Peter Gerner-Smidt~~
1:00 - 2:00 pm	Lunch	
2:00 - 2:30 pm	Exercise 1: Analyze a PFGE Gel Image and Link Entries to Database (Continued)	CDC, USA~~ALL~~
2:30 – 2:50 pm	PulseNet USA Communication	CDC, USA
2:50 – 3:05 pm	Creation and File Location of PulseNet Bundle Files	PHLC, HK
3:05 – 4:05 pm	Exercise 2: Analyze a PFGE Gel Image; Prepare and Create a PulseNet Bundle File for Distribution	CDC, USA~~ALL~~
4:05 – 4:20 pm	Coffee Break	
4:20 - 4:35 pm	Laboratory Experience Sharing	Participant country presentation - Bangladesh
4:35 - 4:50 pm	Laboratory Experience Sharing	Participant country presentation - China

4:50 pm	Q and A	
5:00 pm	End of Day 1 – Shuttle back to Hotel	

February 20, 2008 (Wednesday)

Chairperson of the day: Dr. Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrival PHLC	
9:05 - 9:35 am	Japan experience on PFGE	Jun Terajima, NIID, Japan
9:35 - 9:50 am	Queries of Local Databases	PHLC. HK
9:50 - 10:15 am	Basics Behind Comparisons and Clustering	CDC, USA
10:15 - 10:35 am	Performing Comparisons in BioNumerics	CDC, USA
10:35 - 11:05 am	Coffee Break	
11:05 - 12:00 pm	Exercise 3: Query the Database and Perform Comparisons	CDC, USA~~ALL~~
12:00 - 12:20 pm	Advanced Queries of Local Databases	PHLC, HK
12:20 - 1:00 pm	Exercise 4: Query the Database Using the Advanced Query Tools	CDC, USA~~ALL~~
1:00 - 2:00 pm	Lunch	
2:00 - 2:30 pm	Composite Data Sets	CDC, USA
2:30 - 3:30 pm	Exercise 5: Work with Composite Data Sets	CDC, USA~~ALL~~
3:30 - 3:45 pm	Coffee Break	
3:45 – 4:15 pm	Data Interpretation	CDC, USA
4:15 – 4:30 pm	Laboratory Experience Sharing	Participant country presentation – Malaysia
4:30 – 4:45 pm	Laboratory Experience Sharing	Participant country presentation - Philippines
4:45 pm	Q and A	
5:00 pm	End of Day 2 – Shuttle back to Hotel	

February 21, 2007 (Thursday)

Chairperson of the day: Dr. Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrival PHLC	
9:05 - 9:25 am	Importing into BioNumerics; Exporting Data from BioNumerics	PHLC, HK
9:25 - 10:30 am	Exercise 6: Analyze a PFGE Gel Image; Import Data from Excel	CDC, USA~~ALL~~
10:30 - 11:00 am	Coffee Break	
11:00 - 11:20 am	Settings, Pick List Use and Modification, Layout Modification, Changing Fields, Printing Reports	CDC, USA
11:20 - 11:40 am	Exercise 7: Change Layout/Settings, Print Preview Reports	CDC, USA~~ALL~~
11:40 - 12:00 pm	Naming Patterns and Creating Local Unique Pattern Lists	CDC, USA
12:00 - 1:00 pm	Exercise 8: Name Patterns and Create a Local Unique Pattern List	CDC, USA~~ALL~~
1:00 - 2:00 pm	Lunch	
2:00 - 2:15 pm	Working with Subsets	PHLC, HK
2:15 - 2:40 pm	Exercise 9: Create Subsets for Serotypes in *Vibrio parahaemolyticus* Database	CDC, USA~~ALL~~
2:40 - 3:00 pm	Using Groups/Colors and the Chart and Statistics Tool	CDC, USA
3:00 - 3:30 pm	Exercise 10: Create Charts and Graphs	CDC, USA~~ALL~~
3:30 - 3:40 pm	Coffee Break	
3:40 - 4:10 pm	HK experience	Janice Lo, PHLSB, HK
4:10 - 4:40 pm	Active and Long-term Surveillance	CDC, USA
4:40 - 4:55 pm	Laboratory Experience Sharing	Participant country presentation - Thailand
4:55 pm	Q and A	
5:00 pm	End of Day 3 – Shuttle back to Hotel	

February 22, 2007 (Friday)

Chairperson of the day: Dr. Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrival PHLC	
9:05 - 9:30 am	Standardized Protocols for Subtyping Foodborne Bacterial Pathogens by PFGE	CDC, USA
9:30 - 10:00 am	PFGE Troubleshooting - With emphasis on *Vibrio* PFGE protocols	CDC, USA
10:00 - 10:30 am	Coffee Break	
10:30 - 12:30 pm	***<Split group session>*** **Demo on Vibrio PFGE protocol (Group A)** **Practical Session on BioNumerics with *Vibrio cholerae*** (Group B)	Demo by PHLC, HK BioNumerics practices by CDC, USA
12:30 - 1:30 pm	Lunch	
1:30 - 2:00 pm	QA/QC and Factors that Influence Data Analysis: Gel Quality, Band Marking, etc.	CDC, USA
2:00 - 2:10 pm	Discussion	Concerns/ Issues raised during morning session (to avoid repeat Q and A on One specific topics)
2:10 - 4:10 pm	***<Split group session>*** **Demo on Vibrio PFGE protocol (Group B)** **Practical Session on BioNumerics with *Vibrio cholerae*** (Group A)	Demo by PHLC, HK BioNumerics practices by CDC, USA
4:10 – 5:00 pm	Summary Certificate presentation Group photo	Peter Gerner-Smidt Jun Terajima, NIID, Japan KM Kam All participants / PHLC
5:00 pm	End of Workshop – Shuttle back to Hotel	

Appendix III

WORKSHOP EVALUATION

Course name: The Fifth PulseNet Asia Pacific PFGE Workshop

Location: Public Health Laboratory Centre (PHLC), 382 Nam Cheong Street, Shek Kip Mei, Kowloon, Hong Kong

Dates: February 19- 22, 2008

Offered by: Public Health Laboratories Centre (PHLC), Department of Health, Hong Kong
Association of Public Health Laboratories (APHL)
National Institute of Infectious Diseases (NIID), Department of Bacteriology, Japan
PulseNet Program, Foodborne and Diarrheal Diseases Branch (FDDB),
Centers for Disease Control and Prevention (CDC)

Please complete this evaluation so that we can improve this workshop when it is given again.

1. What is your overall evaluation of this course?

Excellent_____ Good_____ Satisfactory_____ Unsatisfactory_____

2. Were the objectives of the course clearly defined? Yes____ No____

3. Were the objectives of the course met? Yes____ No____

4. Please rate the quality and usefulness of handouts.

Excellent_____ Good_____ Satisfactory_____ Unsatisfactory_____

4b. Please rate the quality and usefulness of the practices.

Excellent_____ Good_____ Satisfactory_____ Unsatisfactory_____

5. Please rate how this course will influence your ability to perform and interpret molecular subtyping of *Salmonella* serotypes, *E. coli, Shigella, Listeria, Campylobacter, Vibrio* and other organisms in the future.

Very positively____ Positively____ Not much____ Not at all____

6. Would you recommend this course to others in public health laboratories? Yes____ No____
Please explain:

7. Please rate each of the following lectures:

"Subject Matter": 1 = material was not at all pertinent; 5 = it was very pertinent
"Presentation": 1 = material was not at all clear; 5 = it was very clear
"Time Allotted": TS = lecture was too short; S = short; R = right amount of time; L = long; TL = lecture was too long

Date: Feb 19, 2008

	Subject Matter					Presentation					Time Allotted				
A. Installation of BioNumerics	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
B. Overview of BioNumerics and MasterScripts v3.0	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
C. Analyzing of PFGE gel images, Linking, gel lanes, and entering data	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
D. Exercise 1: Analyzing a PFGE gel image and link entries to database	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
E. QA/QC and Factors that influence data analysis: Gel quality, band marking etc.	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
F. Creation and File Location of PulseNet Bundle Files	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
G. Exercise 2: Analyzed a PFGE gel image, prepare and create a PulseNet Bundle file for Distribution	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
H. PulseNet USA Communication	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL

Date: Feb 20, 2008

	Subject Matter					Presentation					Time Allotted				
A. PFGE protocol	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
B. Queries of Local Databases	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
C. Basics behind comparisons and clustering	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
D. Performing Comparisons in BioNumerics	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL

E. Exercise 3: Query the Database and Perform Comparisons 1 2 3 4 5 1 2 3 4 5 TS S R L TL

F. Advanced Queries of Local Databases 1 2 3 4 5 1 2 3 4 5 TS S R L TL

G. Exercise 4: Query the Database Using the Advanced Query Tools 1 2 3 4 5 1 2 3 4 5 TS S R L TL

H. Composite Databases 1 2 3 4 5 1 2 3 4 5 TS S R L TL

I. Exercise 5: Work with composite Datasets 1 2 3 4 5 1 2 3 4 5 TS S R L TL

J. PFGE Troubleshooting 1 2 3 4 5 1 2 3 4 5 TS S R L TL

Date: Feb 21, 2008

	Subject Matter	Presentation	Time Allotted
A. Importing into BioNumerics; Exporting data from BioNumerics	1 2 3 4 5	1 2 3 4 5	TS S R L TL
B. Exercise 6: Analyze a PFGE gel image; import data from Excel	1 2 3 4 5	1 2 3 4 5	TS S R L TL
C. Setting, Pick list use and Modification, Layout modification Changing fields, printing reports	1 2 3 4 5	1 2 3 4 5	TS S R L TL
D. Exercise 7: Change layout/settings, print preview reports	1 2 3 4 5	1 2 3 4 5	TS S R L TL
E. Naming patterns and creating local unique patterns List	1 2 3 4 5	1 2 3 4 5	TS S R L TL
F. Exercise 8: Name patterns and create a local unique pattern list	1 2 3 4 5	1 2 3 4 5	TS S R L TL
G. Working with subsets	1 2 3 4 5	1 2 3 4 5	TS S R L TL
H. Exercise 9: Create subsets for serotypes in Salmonella database	1 2 3 4 5	1 2 3 4 5	TS S R L TL
I. Using Groups/colors and the Chart and Statistics Tool	1 2 3 4 5	1 2 3 4 5	TS S R L TL
J. Exercise 10: Create charts and Graphs	1 2 3 4 5	1 2 3 4 5	TS S R L TL
K. PFGE troubleshooting	1 2 3 4 5	1 2 3 4 5	TS S R L TL

Date: Feb 22, 2008

	Subject Matter	Presentation	Time Allotted
A. Data interpretation	1 2 3 4 5	1 2 3 4 5	TS S R L TL
B. Outbreak investigation	1 2 3 4 5	1 2 3 4 5	TS S R L TL
C. Active and Long-term surveillance	1 2 3 4 5	1 2 3 4 5	TS S R L TL
D. Japan Experience	1 2 3 4 5	1 2 3 4 5	TS S R L TL

8. Do you have suggestions for any topics that were not included in this course that should be included in future courses?

9a. What activities did you find most helpful in the computer laboratory?

9b. What activities did you find least helpful in the computer laboratory?

10. Was the time allotted for each topic or practice session appropriate? Yes____ No____

a. For which activities should more time be allowed?

b. For which activities should less time be allowed?

11. In your opinion, should we have this course again for other PulseNet participating Laboratories? Yes____ No____

12. Other comments about course:

Name (Optional):_____ Date:_____

Vibrio cholerae outbreak in North Vietnam in 2007- Antibiotic resistances and PFGE pattern

Phung Dac Cam[1], Chien-Shun Chiou[2], Nguyen Thi Phong Lan[1], Luong Minh Hoa[1] and Haruo Watanabe[3].

1. Division of Enteric Infections, National Institute of Hygiene and Epidemology, 1 Yersin street, Hanoi 10 000, Vietnam
2. Central Branch Office, Center for disease control, Taiwan, 5F 20 Wen-sin South 3rd Road, Taichung City 408, Taiwan.
3. National Infectious Diseases, 1-23-1, Toyama, Shinjuku-ku, Tokyo 162-8640, Japan.

Summary

The last cholera outbreak occurred in Vietnam in 2002. The 2007 outbreak has come unexpectedly in Hanoi since 23 October 2007. It spread out quickly and within 2 weeks more than 1000 cases were detected in all districts of Hanoi city. Right after, the outbreak spread to 13 neighbouring provinces and the number of cases rose up to 2000. The outbreak lasts until the end of January 2008.
This outbreak has the following prominent characteristics:

- Quick spreading
- Severe cases with typical dehydration symptoms
- Caused by contaminated water sources, which then contaminating green vegetables and other foods
- *V. cholerae* O1, biotype El Tor, serotype Ogawa has been isolated.
- PFGE image shown one big cluster with 100% similarity from bacteria isolated in the patients in 4 different provinces and other small cluster is less than 94.5% of similarity including strains isolated from waste water.

Introduction

Cholera is a clinical epidemiologic syndrome caused by *Vibrio cholerae*, usually of serotype O1. Cholera remains an important public health in developing countries . In Vietnam, cholera was still endemic in 35 of Vietnam's 45 provinces in mid-1980s. The last 60 sporadic cases of cholera found in the fall of the year 2004 (1). Cholera outbreak unexpectedly happened in January 2007 in Hanoi city. After two week the outbreak spreading out quickly to

the whole city (Fig.1). Then the outbreak quickly transmitted to neighbouring provinces (Fig.2). The cases was increased with severe symptoms included severe dehydration . Our purposes of study is to identify the cause of the cholera outbreak in 2007 and to detect the antibiotic resistances of the *V.cholerae* and PFGE profile.

Figure 1. Accumulative number of *Vibrio.cholerae* cases through the time

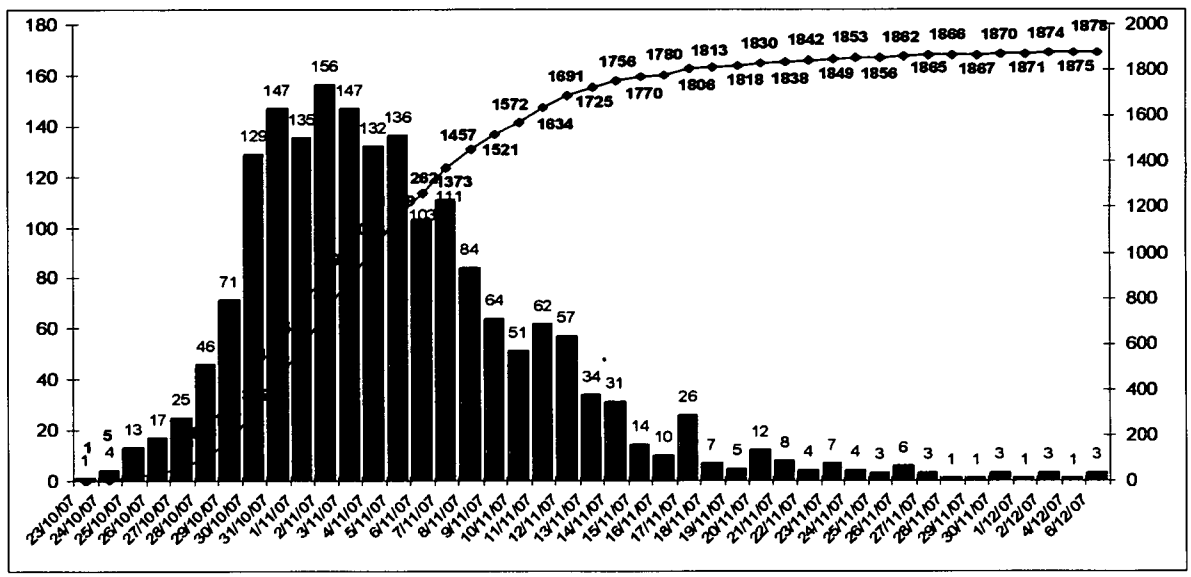
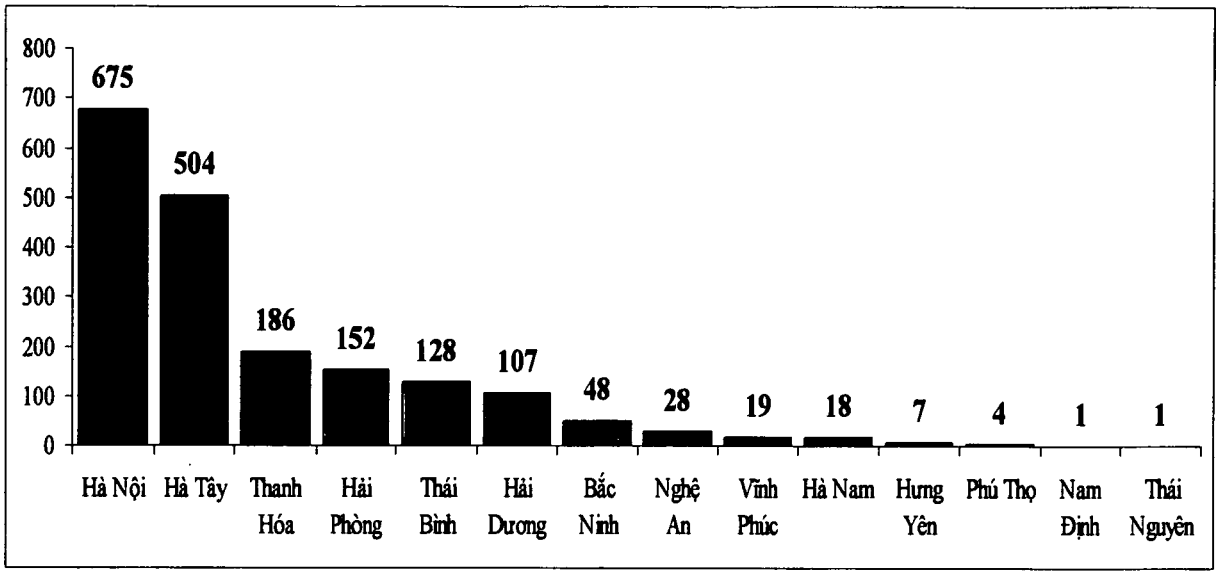


Figure 2. *Vibrio cholerae* isolated from provinces



Materials and methods

1. Fecal samples

Stool samples were taken directly from patients with acute watery diarrhea. Put 1-2 grams of stool in clean and well-capped vials, then put them in the cold box and immediately transported to the Lab.

2. Bacterial isolation and identification

The stool samples will put into alkaline peptone broth, then incubated into incubator at 37° C for 18 h. The screening process will be repeated 3 times in alkaline peptone broth. Then plate directly into TCBS agar, incubated at 37° C for 18 h. Then the yellow colonies will be pick up and plate in nutrient agar for biochemical test and slide agglutination (2).

3. Antibiotic susceptibility test using the performance standards for Susceptibility testing; seventeenth Informational Supplement. Clinical and Laboratory Standards Institute;M100-S17; Vol.27 No 1, January 2007. Antibiotic used for testing is amoxicillin-clavulauic acid (AMC), cefuroxime (CXM), ceftriaxone (CRO), Imipenem (IPM), Erythromycine (E), Clindamycine

(CM), Gentamycin (GM), Ciprofloxacine (CIF), Nalidixic acid (NA), Chloramphenicol (C), Tetracyclin (TE), Trimethoprim-Sulphametoxazone (SXT).

4. PFGE profile following the method of Cooper, 2006 (3)

Results

1. Serotype of the *V.cholerae* isolates

122 isolates of *Vibrio cholerae* is belonged to serogroup O1 , biotype El Tor and serotype Ogawa.

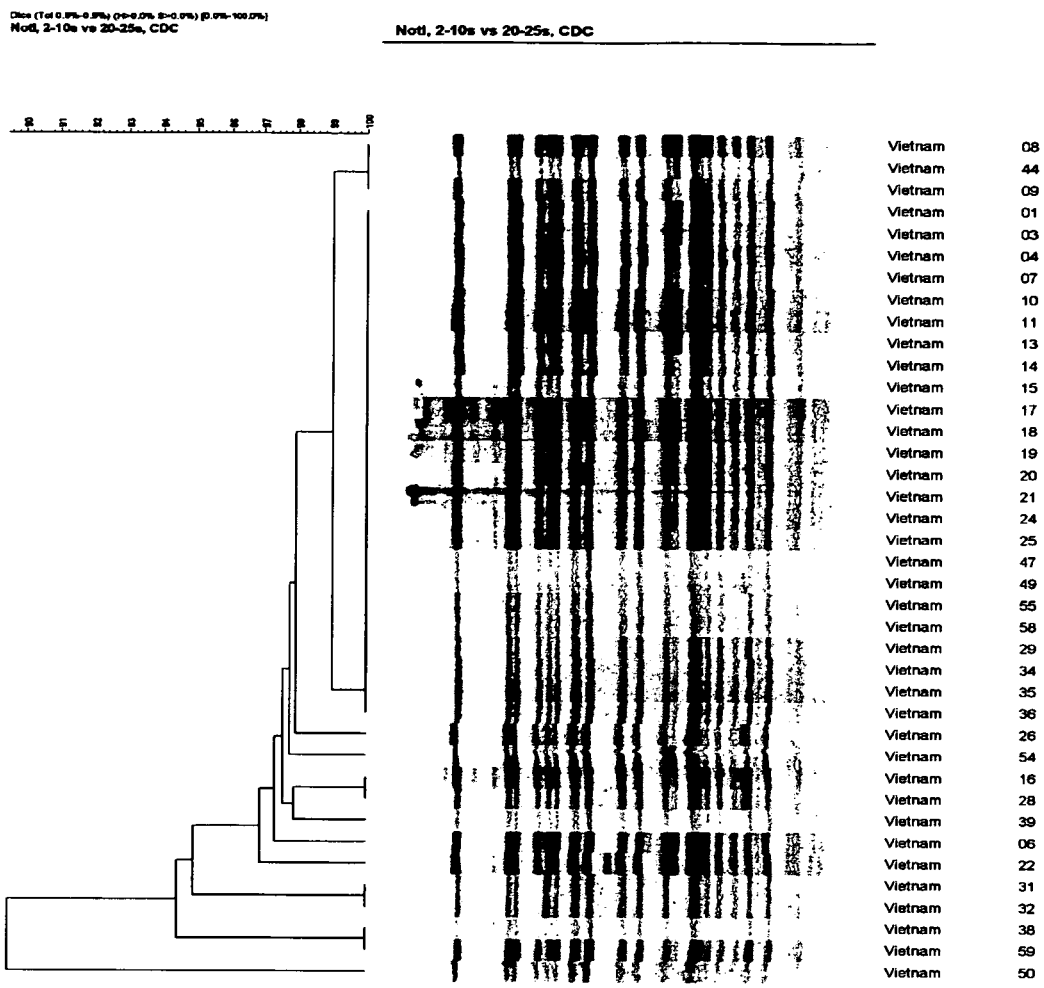
2. Antibiotic susceptibility

Table 1. Antibiotic resistance of *V. cholerae*

Antibiotics	**N**	**Sensitivity**	**Antibiotic resistance**
AMC	122	100%	
CMX	122	100%	
CRO	122	100%	
IPM	122	100%	
E	87		100%
CM	60	100%	
GM	122	100%	
CIP	122	100%	
NA	122	2.5%	97.5%
C	122	100%	
TE	122		100%
SXT	120	0.8%	99.2%

Vibrio choleare isolates are still very sensible to the new groups of antibiotics (Table 1). The bacteria resisted 100% with erythromycin, tetracycline and 97.5% of *V.cholerae* resisted to nalidixic acid. In this outbreak the physicians preferred to use Ciprofloxacine for treatment.

3. PFGE pattern



In this outbreak, total of 39 isolates (37 isolates from human, 2 isolates from waste water) were analyzed by pulsed-field gel electrophoresis (PFGE) with *Xba*I following PulseNet protocols and analysis guidelines. Eleven different PFGE patterns were identified at similarity level of 89%, showing low diversity among the trains from 4 different provinces. Several clusters were detected, which included 36 of 37 patient isolates had similar PFGE pattern at similarity level of 95%; 24 of 39 (61.5%) patient isolates had exactly the same PFGE patterns (100% genetic similarity), and they obtained isolates from every of 4 provinces (Ha noi, Hung yen, Hai phong, Ha tay), suggesting