Isolates collected from different provinces in 2000s and 1990s are being analyzed with PFGE. The epidemiological information and patterns of strains are being adding into the database of *V. cholerae*.

b) Outbreak investigation

Two outbreaks in village dinner parties, caused by serogroup O139 *V. cholerae*, appeared in Jiangxi in May. Strains isolated from patients, carriers and environmental water and marine lives were sent to the center laboratories for the molecular typing. The results were returned to the laboratory in Jiangxi CDC. The cholera toxin genes were detected in these isolates by PCR. Four PFGE patterns were obtained. In these outbreaks there was a dominant PFGE pattern. The pattern of isolates from turtles and bullfrogs could be matched with it, although the samples of turtles and bullfrogs which were used in the dinners could not be obtained, the consumed turtles and bullfrogs were bought from the same market. It suggested that the turtles and bullfrogs which carried toxigenic strains were the source of these outbreaks.

(2) *S. typhi* and *S. paratyphi A*

The routine surveillance of typhoid fever was continued in the provinces of high incidence. This year the *S. typhi* and *S.paratyphi* strain databank was in updating, and the PFGE analysis of these collected strains were in progress. Two hundred and twenty *S. typhi* and *S. paratyphi A* isolates from Guizhou in the previous twenty years were analyzed with PFGE in the center laboratory in China CDC, and the data was added into the database. The strains from an outbreak caused by *S. typhi* in Xinjiang in the autumn of 2006 were also sent to the center lab, the analysis is undergoing.

(3) *Shigella* spp.

a) Database

In 2006, 510 strains were collected from Anhui, Sichuan, Gansu, Shanxi, Jiangxi and Hubei, which are the enhanced *Shigella* surveillance provinces, including 127 strains from 2005 in Anhui Province. Serotype F4c is the dominant (28.8% of the collection), others include F1a, F2a and F2b, et al. Virulence genes *ipaH*, *ial*, *set* and *sen* are detected with PCR, the PFGE analysis is in progress.

b) Outbreaks investigation

Two outbreaks caused by *Shigella sonnei* in Sichuan Province are reported and the strains were collected for analysis of PFGE. One outbreak occured in September 3, 2006 in one primary school of Chongzhou City in Sichuan. Three hundred and thirty-nine laboratory confirmed cases were found. Thirty-four strains including one strain from food were analyzed by PFGE. Four patterns were gotten, one was predominant (26 of 34 strains belonged to this pattern). The food isolate was mathed with the predominant pattern, suggesting the source of this outbreak. However the raw materials (celery and the Chinese onion) of the food could not be obtained further.

Another outbreak appeared in one village primary school in Dayi County of Sichuan, starting from September 8, 2006. Seventy-two laboratory confirmed cases were reported. Eighteen isolates were used in PFGE and 8 patterns were obtained, with one predominant pattern (10 of 18 strains).

The patterns of these strains which were isolated from two outbreaks were quite different, which suggests that there's no etiological relationship.

(4) *Yersinia enterolitica*

In 2006 the surveillance was continued in selected provinces, including Jiangsu, Henan, Anhui, Shandong and Jilin. Samples of dirrheal patients, poultry, livestock and some medical insects were collected for isolation of *Y. enterolitica*. Two hundred and seventy-six strains were obtained. Twenty-one strains were isolated from 2498 diarrheal patients, with the positive rate of 0.84%, 183 strains were isolated from 2789 animals (positive rate 6.56%), 22 were from 631 insects (positive rate 3.49%), and 50 were from 604 food samples (positive rate 8.28%). Virulence genes of *foxA*, *ail*, *ystA* and *ystB* were detected by PCR. In clinical isolates, *foxA* was all positive, positive rate of *ystB* was 50%, *ail* and *ystA* were all negative. In the food isolates, *foxA* was 100% positive and *ystB* gene was positive in 63.6% of strains. The positive rates of *foxA*, *ail*, *ystA* and *ystB* in the animal and insects isolates were 100%, 2.48%, 2.48% and 74.4% respectively. The PFGE analysis of these isolates are in progress.

(5) *Leptospira*

The protocol of PFGE for *Leptospira* using *Not*I as the restriction enzyme was

optimized and the parameters were fixed. The protocol was verified by 180 strains from different year, provinces and hosts. Now the database has been constructed and the background information of the isolates was obtained.

(6) Training

Technicians from 4 provincial CDCs (Jiangxi, Guizhou, Chongqin and Sichuan) were trained for the PFGE analysis this year. From December 19 to 21 of 2006, PulseNet Asia Pacific annual meeting was held in Nanjing, China. Staff from 15 provincial and city-level CDCs attended the meeting as the sitter-in. On December 22 after this meeting, PulseNet China symposium was also held, to discuss the future work of PulseNet China.

**Comments:**

The database of strain analysis of PulseNet China has been constructed, the data is continuing to update. Up to now the technicians from more than 8 provinces are trained in the center lab in China CDC. Some provinces which have the equipment can perform PFGE in their own labs and the patterns obtained from their labs can be recognized and compared by BioNumerics. Now the data transfer depend on email exchange. And some provinces which are trained in the center lab but do not have PFGE equipment can send their isolates to the center lab. It is an effective manner of work in the response of outbreak.

The PFGE technology has been successfully applied in several outbreak investigations. Combination of timely laboratory and epidemiological analysis may strengthen the outbreak investigation and control. We also noticed that in the foodborne disease outbreak investigation, the strains isolated from food and environmental samples are lacked. So the in time sampling, standard collection of food and environmental samples and sensitive isolation will be emphasized in the network laboratories in the future.

Real-time is necessary in the emergence response. It depends on the equipment of the network laboratories and their PFGE operation abilities. The training will be

continued in the next year to strengthen the abilities of the network laboratories, to provide the useful laboratory information in the routine surveillance and response of outbreaks.

**Publication list for this work:**

Jin Dong, Cui Zhi-gang, Xiao Yu-chun, Wang Xin, Gu Feng, Xia Sheng-li, Hu Wan-fu, Yang Jin-chuan, Wang Hua, Gu Ling, Xu Jian-guo, Kan Biao, Jing Huai-qi. Molecular typing of the pathogenic Yersinia enterocolitica strains with pulsed field electrophoresis isolated in China. Chin J Epidemiol. 2006, 27(8): 677-680.

| | |
|---|---|
| STUDY TITLE: | Organization of PFGE Workshop for Training and Technology transfer for Asia Pacific countries/ areas in February 2007 |
| STUDY FACILITY: (Name and address of institute) | Public Health Laboratory Centre, 7/F,<br>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.<br>**Deputy Director-General**<br>**National Institute of Infectious Diseases**<br>**Toyama 1-23-1, Shinjuku-ku**<br>**Tokyo 162-8640, Japan** |

Issue Date: February 22, 2007.

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 2007;

(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 12- 15, 2007.

(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 Workshop , February 12- 15, 2007

| | | Name | From | Arrival Date | Departure Date |
|---|---|---|---|---|---|
| 1 | **Trainer** | Ms Kelley B. Hise | USA | 10 Feb | 16 Feb |
| 2 | **Trainer** | Dr. Kara Cooper | USA | 10 Feb | 16 Feb |
| 3 | **Trainer** | Ms Jana Lockett | USA | 10 Feb | 16 Feb |
| 4 | **Trainer** | Mr. Jun Terajima | Japan | 11 Feb | 16 Feb |
| 5 | Trainee | Dr. Kaisar Talukder | Bangladesh | 11 Feb | 17 Feb |
| 6 | Trainee | Dr. Munirul Alam | Bangladesh | 11 Feb | 17 Feb |
| 7 | Trainee | Dr. Kwai-Lin Thong | Malaysia | 11 Feb | 16 Feb |
| 8 | Trainee | Mr. Zhigang CUI | PR China | 11 Feb | 16 Feb |
| 9 | Trainee | Ms Jing LOU | PR China | 11 Feb | 16 Feb |
| 10 | Trainee | Dr. Orn-Anong Ratchtrachenchai | Thailand | 11 Feb | 15 Feb |
| 11 | Trainee | Miss Sriwanna Huttayananon | Thailand | 11 Feb | 15 Feb |

## Appendix II

# Agenda for PulseNet Asia Pacific PFGE Workshop Hong Kong 2007

***Date: February 12- 15, 2007***
***Venue: Public Health Laboratory Centre (PHLC), Hong Kong***

**February 12, 2007 (Monday)**

**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 | Dr. KM Kam |
| 9:10-9:15 am | Overview of workshop | Danny Cheung |
| 9:15-9:30 am | Installation of BioNumerics | Jana Lockett, PulseNet USA |
| 9:30-9:50 am | Overview of BioNumerics and MasterScripts v3.0 | Jana Lockett, PulseNet USA |
| 9:50-10:30 am | Analyzation of PFGE Gel Images, Linking Gel Lanes, and Entering Data | Kelley Hise, PulseNet USA |
| 10:30-11:00 am | Coffee Break | |
| 11:00-12:30 pm | Exercise 1: Analyze a PFGE Gel Image and Link Entries to Database | Kelley Hise, PulseNet USA |
| 12:30-1:00 pm | QA/QC and Factors that Influence Data Analysis: Gel Quality, Band Marking, etc. | Kara Cooper, PulseNet USA |
| 1:00 – 2:00 pm | Lunch | |
| 2:00-2:15 pm | Creation and File Location of PulseNet Bundle Files | Jana Lockett, PulseNet USA |
| 3:30 – 3:45 pm | Coffee Break | |
| 3:45-4:05 pm | PulseNet USA Communications | Kelley Hise, PulseNet USA |
| 4:05-4:30 pm | Laboratory Experience Sharing | Participants (Thailand) |
| 4:30 pm | Laboratory Experience Sharing | Participants (Malaysia) |
| 4:50 pm | Q and A | |

| | | |
|---|---|---|
| 5:00 pm | End of Day 1 – Shuttle back to Hotel | |

## February 13, 2007 (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 | |
| 9:05-9:50 am | PFGE Protocols | Kara Cooper, PulseNet USA |
| 9:50-10:05 am | Queries of Local Databases | Jana Lockett, PulseNet USA |
| 10:05-10:30 am | Basics Behind Comparisons and Clustering | Kelley Hise, PulseNet USA |
| 10:30-11:00 am | Coffee Break | |
| 11:00-11:20 am | Performing Comparisons in BioNumerics | Kelley Hise, PulseNet USA |
| 11:20-12:00 pm | Exercise 3: Query the Database and Perform Comparisons | Kelley Hise, PulseNet USA |
| 12:00-12:20 pm | Advanced Queries of Local Databases | Jana Lockett, PulseNet USA |
| 12:20-1:00 pm | Exercise 4: Query the Database Using the Advanced Query Tools | Jana Lockett, PulseNet USA |
| 1:00 – 2:00 pm | Lunch | |
| 2:30-3:30 pm | Exercise 5: Work with Composite Datasets | Kelley Hise, PulseNet USA |
| 3:30 – 3:45 pm | Coffee Break | |
| 3:45-4:30 pm | PFGE Troubleshooting | Kara Cooper, PulseNet USA |
| 4:30 pm | Laboratory Experience Sharing | Participants (Beijing, China) |
| 4:50 pm | Q and A | |
| 5:00 pm | End of Day 2 – Shuttle back to Hotel | |

## February 14, 2007 (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 | |
| 9:05-9:25 am | Importing into BioNumerics; Exporting Data from BioNumerics | Jana Lockett, PulseNet USA |
| 9:25-10:30 am | Exercise 6: Analyze a PFGE Gel Image; Import Data from Excel | Jana Lockett, PulseNet USA |
| 10:30-11:00 am | Coffee Break | |
| 11:00-11:20 am | Settings, Pick List Use and Modification, Layout Modification, Changing Fields, Printing Reports | Kelley Hise, PulseNet USA |
| 11:20-11:40 am | Exercise 7: Change Layout/Settings, Print Preview Reports | Kelley Hise, PulseNet USA |
| 11:40-12:00 pm | Naming Patterns and Creating Local Unique Pattern Lists | Kelley Hise, PulseNet USA |
| 12:00-1:00 pm | Exercise 8: Name Patterns and Create a Local Unique Pattern List | Kelley Hise, PulseNet USA |
| 1:00 – 2:00 pm | Lunch | |
| 2:00-2:15 pm | Working with Subsets | Jana Lockett, PulseNet USA |
| 2:15-2:40 pm | Exercise 9: Create Subsets for Serotypes in *Salmonella* Database | Jana Lockett, PulseNet USA |
| 2:40-3:00 pm | Using Groups/Colors and the Chart and Statistics Tool | Jana Lockett, PulseNet USA |
| 3:00-3:30 pm | Exercise 10: Create Charts and Graphs | Jana Lockett, PulseNet USA |
| 3:30-3:45 pm | Coffee Break | |
| 3:45-4:30 pm | PFGE Troubleshooting | Kara Cooper, PulseNet USA |
| 4:30-4:50 pm | Laboratory Experience Sharing | Participants (ICDDR, B) |
| 4:50-5:00 pm | Q and A | |
| 5:00 pm | End of Day 3 – Shuttle back to Hotel | |

## February 15, 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 | |
| 9:05-9:35 am | Data Interpretation | Kelley Hise, PulseNet USA |
| 9:35-10:05 am | Outbreak Investigation | Jana Lockett, PulseNet USA |
| 10:05-10:30 am | Active and Long-term Surveillance | Kelley Hise, PulseNet USA |
| 10:30-11:00 am | Coffee Break | |
| 11:00-11:25 am | Japan experience | Dr. Jun Terajima, NIID, Japan |
| 11:25-11:45 am | Discussion | PHLC / HK |
| 11:45-12:00 noon | Final Q and A | |
| 12:00 – 1:00 pm | Summary<br>Certificate presentation<br>Group photo | Dr. Jun Terajima, NIID, Japan<br>Dr. KM Kam<br>All participants/ PHLC |
| 1:00 pm | End of Workshop – Shuttle back to Hotel | |

Chairperson of the day will lead Q and A sessions, and ensure timing/ smooth running of the proceedings.

## Appendix III

### WORKSHOP EVALUATION CONSOLIDATION (SUMMARY)

**Course name:** The Fourth PulseNet Asia Pacific PFGE Workshop

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

**Dates:** February 12-15, 2007

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

**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? (6 evaluation from participants and 1 from trainer)

Excellent__**5 + 1**___ Good_**1**____ Satisfactory_____ Unsatisfactory_____

2. Were the objectives of the course clearly defined? Yes_**6 +1**_ No____

3. Were the objectives of the course met? Yes___**5+1**_ No____ Not answered **1**

4. Please rate the quality and usefulness of handouts.

Excellent___**5+1**__ Good_____ Satisfactory__**1**___ Unsatisfactory_____

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

Excellent_**4**____ Good__**2**___ 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__**5**__ Positively__**1**__ Not much____ Not at all____

6. Would you recommend this course to others in public health laboratories? Yes__**5+1**__ No **1** **(suggest to clarify with participant)**_

Please explain:

1. **This course is very helpful**
2. **Trainers have excellent experience with actual cases, hence they were able to share real/actual situations in epidemiological investigation. Definitely would recommend this to others in PHL to understand the key issues involved.**
4. **This will extend to other laboratories**

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 12, 2007**

| | Subject Matter | Presentation | Time Allotted |
|---|---|---|---|
| A. Installation of BioNumerics | 1 2 3 4 5<br>**1 1 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| B. Overview of BioNumerics and MasterScripts v3.0 | 1 2 3 4 5<br>**1 1 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| C. Analyzing of PFGE Gel Images, Linking Gel Lanes, and Entering Data | 1 2 3 4 5<br>**1 1 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| D. Exercise 1: Analyzing a PFGE Gel Image and Link Entries to Database | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| E. QA/QC and Factors that Influence Data Analysis: Gel Quality, Band Marking etc. | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| F. Creation and File Location of PulseNet Bundle Files | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| G. Exercise 2: Analyzed a PFGE Gel Image, Prepare and Create a PulseNet Bundle file for Distributio | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**1 5** |
| H. PulseNet USA Communication | 1 2 3 4 5<br>**1 5** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**1 5** |

**Date: Feb 13, 2007**

| | Subject Matter | Presentation | Time Allotted |
|---|---|---|---|
| A. Standardized Protocols for Subtyping Foodborne Bacterial Pathogens by PFGE | 1 2 3 4 5<br>**1 5** | 1 2 3 4 5<br>**1 5** | TS S R L TL<br>**6** |
| B. Queries of Local Databases | 1 2 3 4 5<br>**1 5** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| C. Basics Behind Comparisons and Clustering | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| D. Performing Comparisons in BioNumerics | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| E. Exercise 3: Query the Database and Perform Comparisons | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| F. Advanced Queries of Local Databases | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| G. Exercise 4: Query the Database Using the Advanced Query Tools | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| H. Composite Data Sets | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| I. Exercise 5: Work with Composite Data Sets | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |
| J. PFGE Troubleshooting | 1 2 3 4 5<br>**1 5** | 1 2 3 4 5<br>**1 5** | TS S R L TL<br>**1 5** |

**Date: Feb 14, 2007**

| | Subject Matter | Presentation | Time Allotted |
|---|---|---|---|
| A. Importing into BioNumerics; Exporting Data from BioNumerics | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| B. Exercise 6: Analyze a PFGE Gel Image; Import Data from Excel | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| C. Settings, Pick List Use and Modification, Layout Modification Changing Fields, Printing Reports | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| D. Exercise 7: Change Layout/Settings, Print Preview Reports | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| E. Naming Patterns and Creating Local Unique Patterns Lists | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| F. Exercise 8: Name Patterns and Create a Local Unique Pattern List | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| G. Working with Subsets | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| H. Exercise 9: Create Subsets for Serotypes in *Salmonella* Database | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**6** |
| I. Using Groups/Colors and the Chart and Statistics Tool | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**1 5** |
| J. Exercise 10: Create Charts and Graphs | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 1 4** | TS S R L TL<br>**1 5** |
| K. PFGE troubleshooting | 1 2 3 4 5<br>**1 5** | 1 2 3 4 5<br>**2 4** | TS S R L TL<br>**6** |

**Date: Feb 15, 2007**

| | | Subject Matter | Presentation | Time Allotted |
|---|---|---|---|---|
| A. PFGE Data Interpretation Issues | | 1 2 3 4 5<br>**1 5** | 1 2 3 4 5<br>**1 5** | TS S R L TL<br>**1 5** |
| B. Outbreak investigation | | 1 2 3 4 5<br>**2 4** | 1 2 3 4 5<br>**1 5** | TS S R L TL<br>**1 5** |
| C. Active and Long-term surveillance | | 1 2 3 4 5<br>**1 5** | 1 2 3 4 5<br>**1 5** | TS S R L TL<br>**1 5** |
| D. Japan Experience | Not answered<br>**1** | 1 2 3 4 5<br>**1 4+1** | 1 2 3 4 5<br>**1+1 4** | TS S R L TL<br>**5+1** |

8. Do you have suggestions for any topics that were not included in this course that should be included in future courses?
   1. **Not answered**
   2. **None**
   3. **Not answered**
   4. **we can include unidentified pathogenic organism which showed virulence properties**
   5. **Not answered**
   6. **Not answered**

9a. What activities did you find most helpful in the computer laboratory?
   1. **All**
   2. **Everything**
   3. **All**
   4. **OK**
   5. **THE entire program**
   6. **Not answered**

9b. What activities did you find least helpful in the computer laboratory?
   1. **Not answered**
   2. **None**
   3. **None**
   4. **did not find anything**
   5. **Nothing**
   6. **Not answered**

10. Was the time allotted for each topic or practice session appropriate? Yes__**5**__ No____ Not answered **1**

   a. For which activities should more time be allowed?
      1. **Exercise 2**
      2. **Actual lab exercise**
      3. **Not answered**
      4. **Discussion**
      5. **NA**
      6. **Not answered**

   b. For which activities should less time be allowed?
      1. **Not answered**
      2. **None**
      3. **Not answered**
      4. **Not answered**
      5. **NA**
      6. **Not answered**

11. In your opinion, should we have this course again for other PulseNet participating Laboratories? Yes_5+**1**__ No_
    Not answered **1**
    (when need arises)

12. Other comments about course:
   2. **This is an excellent course. Lecturers were excellent. Learn a lot. It would have taken months to learn the same thing on my own. Many thanks to the organizing committee for excellent arrangement, food, facilities etc.**
   4. **Course should be continued**
   5. **It was excellent! Wonderful!!**
   6. **Thank you to everyone. I learn a lot of things in detail.**

Name (Optional):_____ Date:_____

# Antimicrobial susceptibility and Pulsed Field Gel Electrophoresis analysis of *Shigella* strains in Vietnam (1997-2004)

**Name of researcher:**
Nguyen Thi Phong Lan, Phung Dac Cam, Tran Minh Thu[1], Haruo Watanabe[2]

[1]National Institute of Hygiene and Epidemiology, Hanoi, Vietnam

[2] National Institute of Infectious Diseases, Tokyo, Japan

**Summary:** A total of 102 isolates of *S. flexneri* and *S. sonnei* (*n*=49 and n=53 respectively) have obtained from patients with diarrhea in four different provinces of Vietnam from 1997 to 2004. All strains were identified by standard biochemical tests and serotyping. The antimicrobial susceptibility of those strains was determined by the agar diffusion method on Mueller Hinton agar to the 8 antimicrobial agents of Oxoid. PFGE was performed as PulseNet USA protocol using XbaI. Among 49 of *S. flexneri* isolates 26/49 (53%) shared proportion of similarity at ≥ 88% with 12 different PFGE patterns found, 35/49 (71%) of strains of *S.flexneri* were resistant to 4 types of antimicrobial drugs: ampicillin, sulphonamides, trimethoprim and tetracycline, 21/35 resistant strains had the same PFGE pattern (at 86%). Of 53 isolates of *S. sonnei*, 21/53 (40.5%) shared a proportion of similarity at ≥ 88% with 17 PFGE patterns found and 35/53 (66%) of strains of *S. sonnei* were resistant to 3 types of antimicrobial drugs tetracycline, trimethoprim, and sulphonamides, 21/35 resistant strains had the same PFGE pattern ( 88%). Among 102 *Shigella* strains, 100 strains (98 %) were resistant to at least 1 type of drug.

## Introduction

Shigellosis continues to be a major public health problem and remains endemic in Vietnam. In a recent cohort study conducted by the National Institute of Hygiene and Epidemiology, Hanoi (NIHE) to follow the diarrhoeal incidences in a population of Vietnamese children under 5 years old in a northern area, *Shigella*

was among the most commonly found pathogens . Of the *Shigella* isolates, *Shigella flexneri* was the most common serogroup (>70%). Because of misusing and abusing of antibiotics in Vietnam, the situation of antibiotic resistance of *Shigella spp.* increased year by year including multi-drugs resistance is also concerned .

The *Shigella* isolates from different geographic areas of Vietnam will be collected by years (1998-2004). Bacterial identification will be done at the National Reference Laboratory of Enteric pathogens at NIHE. Then the susceptibility test of different kind of antimicrobial agents will be done by Kirby Bauer method. The PFGE will be performed to see whether *Shigella* isolates showing the cluster pattern/or different one in different areas.

**Objective**

The objectives of this study were to examine the susceptibility of the collection of *Shigella* strains isolated from different geographic areas of Vietnam (1998-2004) and to characterize the PFGE pattern of these *Shigella* strains.

**Materials and Methods**

*Laboratory investigation*

Bacterial strains

The Laboratory of Enteric Infections of the National Institute of Hygiene and Epidemiology have collected stool samples of diarrheal patients from different provinces. At the Laboratory, the isolates were identified by standard biochemical methods and serotyping of *Shigella*. For PFGE, *S. flexneri* and *S. sonnei* isolates were grown overnight at $37^0$C on Trypticase soy agar plates. A total of 102 isolates were selected between 1997 and 2004 from 4 different provinces, included 49 *S. flexneri* and 53 *S. sonnei*.

**Antimicrobial susceptibility testing**

The antimicrobial susceptibility of the strains was test by the standard disk diffusion methods (National Commitee for Clinical Laboratory Standards.1997. Performance standards for antimicrobial disk susceptibility tests – approved

standard) on Muller-Hinton agar (Oxoid) to the following 8 antimicrobial agents of Oxoid: Ampicillin (Am-10), Chloramphenicol (C-30), Gentamycin (Gm-10), Nalidixic acid (NA-30), Tetracyclin (Te-30), Cefalothin (KF-30), Sulphamethoxazole (RL-25) , and Trimethoprim (W-5).

**PFGE**

PFGE was performed for all strains as Pulse Net USA protocol with the restriction enzyme XbaI: One-Day (24-28 h) Standardized Laboratory Protocol for Molecular Subtyping of *Escherichia coli* O157:H7, non-typhoidal *Salmonella* serotypes, and *Shigella sonnei* by Pulsed Field Gel Electrophoresis (PFGE).

Calculation of similarity indices

The BioNumerics software version 3.0 (Applied Maths, Kontrijk, Belgium) was used for calculating the Disc similarity indices (tolerance 1.0%, unweighted pair group method using arithmetic indices) in the cluster analysis. *Salmonella* serotype Braenderup (H9812) was used as a reference standard to normalize gels. DNA size standard using Lambda Ladder – Bio-Rad 170-3635

## Results

### PFGE analysis

### PFGE pattern associated *Shigella* source.

We characterized by antimicrobial susceptibility testing and PFGE a total 102 *Shigella* isolates from patient with diarrhea from 4 different provinces as Hanoi, Bavi, Nha Trang, Ho chi Minh from 1997 to 2004. Twenty five isolates were obtained from patients in Hanoi, 28 isolates from Bavi, 44 isolates from Nha Trang, and 5 isolates from Ho Chi Minh city.

In each of the *S. flexneri* and *S. sonnei* groups, cluster analysis of all profiles showed that many but not all strain from the same geographical region grouped together, 9/11 of *S. sonnei* strains from Hanoi had the same PFGE patterns (similarity at 95%); 20/25 *S. sonnei* strains from Nha Trang shared a proportion of similarity at 83.67%, 15/22 *S. flexneri* of Nha Trang had a similarity at 86.74%. (Figure 1 & 2).

**Multidrug resistance cases**

Among 102 *Shigella* strains, only two strains (2%) of *S. sonnei* were fully susceptible to all 8 antibiotics whereas another 98% were resistant to at least one type of the 8 commonly used available drugs: ampicillin, chloramphenicol, gentamycin, nalidixic acid, tetracyclin, cefalothin, suphonamides, and trimethoprim. Only one strain of *S. flexneri* was resistant to 8 antimicrobial drugs.

Of the 49 *S. flexneri* isolates, 35/49 (71%) were resistant to all 4 commonly used antimicrobial drugs: ampicillin, sulphonamides, and trimethoprim and tetracycline and 21/35 resistant strains had the same PFGE pattern ( at 86%).
Among 53 of *S. sonnei*, 35/53 (66%) were resistant to 3 types of antimicrobial drugs: tetracycline, trimethoprim and sulphonamides, 21/35 had the same PFGE pattern (at 88%). (Figure 3 & 4).