Appendix I

Participants List for PulseNet Asia Pacific PFGE Workshop 2010 (23 -26 FEB 2010)

		Name	Sex	From	Arrival Date	Departure Date
1	Trainer	Dr. Kara Cooper (Microbiologist)	F	PulseNet Methods Development and Validation Laboratory , PulseNet Program, Enteric Diseases Laboratory Branch, Centers for Disease Control and Prevention, USA	20 Feb	27 Feb
2	Trainer	Dr. Mariana Pichel	F	PulseNet Latin America	22 Feb	27 Feb
3	Trainer	Dr. Jun Terajima	M	Department of Bacteriology National Institute of Infectious Diseases, Japan		
4	Trainee	Mst. Fatema-Tuz-Johura (Research Officer)	F	Enteric and Food Microbiology Laboratory Laboratory Sciences Division ICDDR,B, Bangladesh	22 Feb	27 Feb
5	Trainee	Zhahirul Islam (Senior Research Officer)	M	Enteric Microbiology Laboratory Laboratory Sciences Division ICDDR,B, Bangladesh	22 Feb	27 Feb
6	Trainee	Srirat Pornruangwong (Medical Scientist)	F	WHO National Salmonella and Shigella Center National Institute of Health Department of Medical Sciences Ministry of Public Health, Thailand		
7	Trainee	Phattharaphon Chaichana (Medical Scientist)	F	WHO National Salmonella and Shigella Center National Institute of Health Department of Medical Sciences Ministry of Public Health, Thailand		
8	Trainee	Shengli Xia (Associate professor)	M	Institute for Infectious Disease Control and Prevention Henan Provincial Centers for Disease Control and Prevention, PR China		
9	Trainee	Wanfu Hu (Associate professor)	M	Anhui Provincial Center for Disease Control and Prevention, PR China		
10	Trainee	Haijian Zhou (Assistant professor)	M	National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, PR China	22 Feb	27 Feb
11	Trainee	Xiaoxia Tao (Technician-in-charge)	F	National Institute for Communicable Disease Control and Prevention, Chinese Center for Disease Control and Prevention, PR China	22 Feb	27 Feb
12	Trainee	Dr. Kwai-Lin Thong (Professor)	F	Institute of Biological Science Faculty of Science and Biomedical Science and Molecular Microbiology Laboratory UMBIO Cluster, Institute of Graduate Studies University of Malaya, Kuala Lumpur, Malaysia		
13	Trainee	Miss Agnes Ye Zhengyu	F	Veterinary Public Health Centre Agri-Food and Veterinary Authority Singapore		

Appendix II
Agenda for PulseNet Asia Pacific PFGE Workshop Hong Kong 2010

Report of the PulseNet Asia Pacific PFGE Workshop, Hong Kong, February 23-26, 2010

Date: February 23- 26, 2010
Venue: Conference Room at Public Health Laboratory Centre (PHLC), Hong Kong

February 23, 2010 (Tuesday)

Chairperson of the day: Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrive at PHLC	
9:05 – 9:15 am	Registration	
9:15 – 9:30 am	Welcome remarks, expectations of the Workshop	KM Kam, PHLC, HK Kara Cooper, CDC, USA Jun Terajima, NIID, Japan Mariana Pichel, PulseNet Latin America
9:30 – 9:45 am	Overview of Workshop	Danny Cheung, PHLC, HK
9:45 – 10:20 am	Installation and Overview of BioNumerics/ MasterScripts	Kara Cooper, CDC, USA
10:20 – 10:30 am	Group Photo	
10:30 – 11:00 am	**Coffee Break**	
11:00 – 11:40 am	Analysis of PFGE Gel Images, Linking Gel Lanes, and Entering Data	Alf Chu, PHLC, HK
11:40 – 1:00 pm	Exercise 1: Analyze a PFGE Gel Image and Link Entries to a Database	Alf Chu, PHLC, HK
1:00 – 2:00 pm	**Lunch**	
2:00 – 2:20 pm	PulseNet USA: Overview of Molecular Subtyping Network for Foodborne Diseases Surveillance	Kara Cooper, CDC, USA
2:20 – 2:35 pm	Creation and File Location of PulseNet Bundle Files	Cindy Luey, PHLC, HK
2:35 – 3:35 pm	Exercise 2: Prepare and Create a PulseNet Bundle File for Distribution	Cindy Luey, PHLC, HK
3:35 – 3:50 pm	**Coffee Break**	
3:50 – 4:20 pm	PFGE Experience in Japan	Jun Terajima, NIID, Japan
4:20 – 4:35 pm	Laboratory Experience Sharing	Participant presentation – Bangladesh
4:35 – 4:50 pm	Laboratory Experience Sharing	Participant presentation – China
4:50 pm	Q and A	
5:00 pm	End of Day 1 – Shuttle back to Hotel	

February 24, 2010 (Wednesday)

Chairperson of the day: Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrive at PHLC	
9:05 – 9:20 am	Data Importing into and Exporting from BioNumerics	Cindy Luey, PHLC, HK
9:20 – 10:30 am	Exercise 3: Analyze a PFGE Gel Image; Data Import into and from BioNumerics	Cindy Luey, PHLC, HK
10:30 – 11:00 am	**Coffee Break**	
11:00 – 11:15 am	Queries of Local Databases	Cindy Luey, PHLC, HK
11:15 – 11:45 am	Basics Behind Comparisons and Clustering	Kara Cooper, CDC, USA
11:45 – 12:05 pm	Performing Comparisons in BioNumerics	Cindy Luey, PHLC, HK
12:05 – 1:00 pm	Exercise 4: Performing Queries and Creating Comparisons	Cindy Luey, PHLC, HK
1:00 – 2:00 pm	**Lunch**	
2:00 – 2:20 pm	Advanced Queries of Local Databases	Cindy Luey, PHLC, HK
2:20 – 3:00 pm	Exercise 5: Query the Database Using the Advanced Query Tools	Cindy Luey, PHLC, HK
3:00 – 3:30 pm	Life of A PulseNet Cluster	Kara Cooper, CDC, USA
3:30 – 3:45 pm	**Coffee Break**	
3:45 – 4:15 pm	QA/QC and Factors that Influence Data Analysis	Kara Cooper, CDC, USA
4:15 – 4:35 pm	PulseNet Latin America Experience	Mariana Pichel, PulseNet Latin America
4:35 – 4:50 pm	Laboratory Experience Sharing	Participant presentation – Malaysia
4:50 – 5:00 pm	Q and A	
5:00 pm	End of Day 2 – Shuttle back to Hotel	

February 25, 2010 (Thursday)

Chairperson of the day: Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrive at PHLC	
9:05 – 9:35 am	PIC WG Update	Dr. Edman Lam, PHLC, HK
9:35 – 9:55 am	Database Management: Settings, Pick List Use and Modification, Layout Modification, Changing Fields, Printing Reports	Cindy Luey, PHLC, HK
9:55 – 10:15 am	Exercise 6: Change Layout/Settings, Print Preview Reports	Cindy Luey, PHLC, HK
10:15 – 10:30 am	PulseNet USA Communication	Kara Cooper, CDC, USA
10:30 – 11:00 am	**Coffee Break**	
11:00 – 11:20 am	Naming Patterns and Creating Local Unique Pattern Lists	Cindy Luey, PHLC, HK
11:20 – 12:15 pm	Exercise 7: Identifying and Naming Unique Patterns in the database	Cindy Luey, PHLC, HK
12:15 – 12:30 pm	Working with Subsets	Cindy Luey, PHLC, HK
12:30 – 1:00 pm	Exercise 8: Create Subsets for Serotype in *Salmonella* Database	Cindy Luey, PHLC, HK
1:00 – 2:00 pm	**Lunch**	
2:00 – 2:20 pm	Use of Groups and the Chart and Statistics Tool	Cindy Luey, PHLC, HK
2:20 – 3:00 pm	Exercise 9: Create Charts and Graphs to Create Reports	Cindy Luey, PHLC, HK
3:00 – 3:30 pm	Composite Data Sets	Alf Chu, PHLC, HK
3:30 – 4:00 pm	Exercise 10: Cluster analysis using a composite data set for *Salmonella*	Alf Chu, PHLC, HK
4:00 – 4:15 pm	**Coffee Break**	
4:15 – 4:30 pm	Laboratory Experience Sharing	Participant presentation – Singapore
4:30 – 4:45 pm	Laboratory Experience Sharing	Participant presentation – Thailand
4:45 – 5:00 pm	Q and A	
5:00 pm	End of Day 3 – Shuttle back to Hotel	

February 26, 2010 (Friday)

Chairperson of the day: Jun Terajima

Time	Activities	Speakers/Modulators
8:30 am	Shuttle from Hotel to PHLC	
9:00 am	Arrive at PHLC	
9:00 – 9:30 am	Extended Forum (Open to all PHLC colleagues) *(Title: to be determined by speaker)*	Kara Cooper, CDC, USA
9:30 – 10:00 am	Extended Forum (Open to all PHLC colleagues) *(Title: to be determined by speaker)*	Jun Terajima, NIID, Japan
10:00– 10:30 am	Extended Forum (Open to all PHLC colleagues) Development of PulseNet Standardized Protocol for Subtyping *Shigella flexneri* by PFGE	Mariana Pichel, PulseNet Latin America
10:30– 11:00 am	Coffee Break	
11:00 – 1:00 pm	*<Split group session>* Demo on *Shigella flexneri* PFGE protocols (Group A) Practical Session on BioNumerics with vibrio*s* (Group B)	Demo by PulseNet Latin America and PHLC, HK BioNumerics practices by CDC, USA
1:00 – 2:00 pm	Lunch	
2:00 – 4:00 pm	*<Split group session>* Demo on *Shigella flexneri* PFGE protocols (Group B) Practical Session on BioNumerics with vibrio*s* (Group A)	Demo by PulseNet Latin America and PHLC, HK BioNumerics practices by CDC, USA
4:00 – 4:15 pm	Coffee Break	
4:15 – 5:00 pm	Discussion Summary Certificate presentation	All participants KM Kam, PHLC, HK Kara Cooper, CDC, USA Jun Terajima, NIID, Japan Mariana Pichel, PulseNet Latin America
5:00 pm	End of Workshop – Shuttle back to Hotel	

WORKSHOP EVALUATION

Course name: The Seventh PulseNet Asia Pacific PFGE Workshop

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

Dates: February 23-26, 2010

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, Enteric Diseases Laboratory Branch (EDLB),
Centers for Disease Control and Prevention (CDC), USA

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____

4a. 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 23, 2010

	Subject Matter					Presentation					Time Allotted			
A. Installation and Overview of BioNumerics/ MasterScripts	1	2	3	4	5	1	2	3	4	5	TS	S	R	L TL
B. 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
C. 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
D. PulseNet USA: Overview of Molecular Subtyping Network for Foodborne Diseases Surveillance	1	2	3	4	5	1	2	3	4	5	TS	S	R L	TL
E. Creation and File Location of PulseNet Bundle Files	1	2	3	4	5	1	2	3	4	5	TS	S	R L	TL
F. Exercise 2: Prepare and Create a PulseNet Bundle file for Distribution	1	2	3	4	5	1	2	3	4	5	TS	S	R L	TL
G. PFGE Experience in Japn	1	2	3	4	5	1	2	3	4	5	TS	S	R L	TL

Date: Feb 24, 2010

	Subject Matter					Presentation					Time Allotted			
A. Data Importing into and Exporting from BioNumerics	1	2	3	4	5	1	2	3	4	5	TS	S	R L	TL
B. Exercise 3: 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. Queries of Local Databases	1	2	3	4	5	1	2	3	4	5	TS	S	R	L TL
D. Basics Behind Comparisons and Clustering	1	2	3	4	5	1	2	3	4	5	TS	S	R	L TL
E. Performing Comparisons in BioNumerics	1	2	3	4	5	1	2	3	4	5	TS	S	R	L TL
F. Exercise 4: Performing Queries and Creating Comparisons	1	2	3	4	5	1	2	3	4	5	TS	S	R L	TL
G. Advanced Queries of Local Databases	1	2	3	4	5	1	2	3	4	5	TS	S	R	L TL
H. Exercise 5: Query the Database Using the Advanced Query Tools	1	2	3	4	5	1	2	3	4	5	TS	S	R L	TL

R L TL

J. Life of A PulseNet Cluster 1 2 3 4 5 1 2 3 4 5 TS S R L TL

K. QA/QC and Factors that Influence Data Analysis 1 2 3 4 5 1 2 3 4 5 TS S R L TL

L. PulseNet Latin America Experience 1 2 3 4 5 1 2 3 4 5 TS S R L TL

Date: Feb 25, 2010

Subject Matter Presentation
Time Allotted

A. PIC WG Update 1 2 3 4 5 1 2 3 4 5 TS S R L TL

B. Database Management Tools 1 2 3 4 5 1 2 3 4 5 TS S R L TL

C. Exercise 6: Database Settings and Layout, Pick List 1 2 3 4 5 1 2 3 4 5 TS S R L TL
Printing Reports

D. PulseNet USA Communication 1 2 3 4 5 1 2 3 4 5 TS S R L TL

E. Naming Patterns and Creating Local Unique Patterns Lists 1 2 3 4 5 1 2 3 4 5 TS S R L TL

F. Exercise 7: Identifying and Naming Unique Patterns in the database 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 8: Create Subsets for Serotype in Salmonella Database 1 2 3 4 5 1 2 3 4 5 TS S R L TL

I. Use of Groups and the Chart and Statistics Tool 1 2 3 4 5 1 2 3 4 5 TS S R L TL

J. Exercise 9: Create Charts and Graphs to Create Reports 1 2 3 4 5 1 2 3 4 5 TS S R L TL

K. Composite Data Sets 1 2 3 4 5 1 2 3 4 5 TS S R L TL

L. Exercise 10: Cluster analysis using a Composite Data Set 1 2 3 4 5 1 2 3 4 5 TS S R L TL
For *Salmonella*

Date: Feb 26, 2010

Subject Matter Presentation
Time Allotted

A. PulseNet Laboratory Updates 1 2 3 4 5 1 2 3 4 5 TS S R L TL

B. MLVA typing of *E. coli* non- O157 strains in Japan 1 2 3 4 5 1 2 3 4 5 TS S R L TL

C. Development of PulseNet Standardized Protocol 1 2 3 4 5 1 2 3 4 5 TS S R L TL
For Subtyping *Shigella flexneri* by PFGE

D. Demo on *Shigella flexneri* PFGE protocol	1	2	3	4	5	1	2	3	4	5	TS	S	R	L	TL
E. Practical Session on BioNumerics with *Vibrios*	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: _____

Identification the new combinations of beta-lactam antibiotics and β-lactamase inhibitors of *Shigella* isolates from 2007-2009 in Vietnam

Phung Dac Cam[1], Nguyen Thuy Tram[1], Hoang Thu Ha[1] and Haruo Watanabe[2]

1. **National Institute of Hygiene and Epidemiology, 1 Yersin street, Hanoi 10 000, Vietnam**
2. **National Institute of Infectious Diseases (NIID), 1-23-1, Toyama, Shinjuku, Tokyo 162-8640, Japan.**

SUMMARY

Extended-spectrum β-lactamase (ESBL) producing *Shigella* are growing concern in human medicine today. The basis of ampicillin resistance in *Shigella* spp. was analyzed for a number of isolates. Both *S.flexneri* and *S. sonnei* resistant to ampicillin and amoxicillin were inhibited by clavulanic acid (AmC-30) and piperacillin with Tazobactam (TZP-110). In addition, ESBL-producing bacteria are frequently resistant to many classes of non- beta-lactam antibiotics, resulting in difficult-to-treat infections. This report provides presence of ESBL producing *Shigella sonnei* among collected *Shigella* strains in Vietnam.

INTRODUCTION

Shigella species currently remain a world wide public health problem. Shigellosis is one of the major causes of bloody diarrhoea associated with high morbidity and mortality, especially in paediatric patients. The adequate antibiotic treatment of *Shigella* infections may limit the clinical course of illness and the duration of faecal excretion of bacteria. Antimicrobial resistance, a problem of increasing proportion, occurs in many classes of bacteria in all areas of the world Therefore, the emergence of resistance in *Shigella* spp. is a matter of great public health concern, mainly in developing countries (Sack et al., 2004). There are few recently reported studies about antimicrobial resistance among diarrheagenic *E. coli* and *Shigella* strains in children in Vietnam (Cao *et al.*, 2002; Isenbarger *et al.*, 2002). At first, the treatment of choice for shigellosis is TMP/SMX for susceptible strains (Griffin *et al.*, 1989). *Shigella* strains became progressively resistant to multiple antimicrobial agents, shortly after they became commercially available, initially to sulfonamides, then to tetracycline, chloramphenicol, and streptomycin less than 10 years after each was introduced, and subsequently to ampicillin, kanamycin, and TMP/SMX because of rapid dissemination of resistance plasmids. In most countries in Asia and Africa, in certain Latin American countries, and in Israel, more than 50% of all *Shigella* isolates are resistant to ampicillin and TMP/SMX (Bennish *et al.*, 1992). Due to this high incidence of resistance, alternative options such as the use of oral expanded spectrum β-lactamase (ESBL) have been recommended, since the late 1990s, as the empirical antibiotic treatment of shigellosis in paediatric patients (Lopez 1999 and Lopez *et al.*, 2000). In fact, the occurrence of an ESBL enzyme in *S. flexneri* isolates from a study in Argentina was reported (Walther-Rasmussen *et al.*, 2004). There is a need to address resistance to expanded-spectrum β-lactamase (ESBL) in this pathogen. We therefore conducted a study to aim at the extension

of genetic diversity to more recent isolations of *Shigella* strains from Vietnam with respect to antimicrobial patterns and PFGE profiles.

MATERIALS AND METHODS

Bacterial strains

In Vietnam, *Shigella* strains isolated from human patients by peripheral clinical laboratories are transferred to the National Institute of Hygiene and Epidemiology for serotyping. From 2007 to 2009, 113 *Shigella* strains were collected and serotyped by slide agglutination with commercial antisera from the Denmark. These organisms were screened for the presence of ESBLs and then investigated for the presence of CTM-M β–lactamases that have been commonly observed in Vietnam.

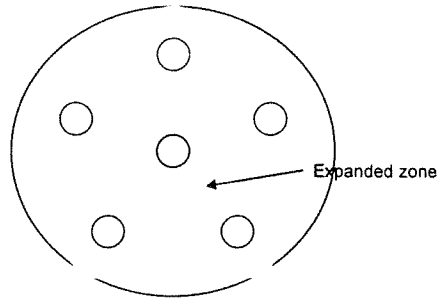
Antimicrobial susceptibility testing of *Shigella sonnei* and *Shigella flexneri* isolates

In the first period (2009), the antibiotic susceptibility of *S. sonnei* and *S.flexneri* strains was tested. The susceptibility of 14 antibiotics was determined by the disc diffusion (Kirby- Bauer) and Minimal Inhibitory Concentration (MIC) methods following the recommendations of the Clinical and Laboratory Standards Institute (CLSI; formerly NCCLS). The antibiotics tested were ampicillin-10 (AM), amoxicillin-10 (AML), Azitjromycin-15 (AZM), ceftazidime-30 (CAZ), cefdinir-5 (CDN), cefditoren-5 (CDR), cefixime-5 (CFM), ciprofloxacin-5 (CIP), cefpodoxime-10 (CPD), ceftriaxone-30 (CRO), ceftibuten-30 (CTB), cefotaxime-30 (CTX), faropenem-5 (FAR), mecillinam-10 (MEL) (Oxoid discs). Interpretation of inhibition zones was performed according to the CLSI criteria, and quality control were performed using the *Escherichia coli* ATCC 25922, *E.coli* ATCC 35218, *Pseudomonas aeruginosa* ATCC 27853, *Staphylococcus aureus* ATCC 29213, and *K.pneumoniae* ATCC 700603 reference strains.

Screening for and confirmation of ESBLs

The presence of ESBLs was evaluated in both the control strains and recent clinical isolates. Screening and disk confirmation tests using CPD-10 (10μg), CAZ-30 (30μg), CTX-30 (30μg), CRO-30 (30μg), amoxicillin with clavulanic acid (AmC-30) and piperacillin/Tazobactam (TZP-110) were performed and interpreted by NCCLS criteria for ESBL screening and disk confirmation tests. Disks for ESBL confirmation tests were obtained from Oxoid Inc.

Selection of *Shigella* spp. isolates whose one inhibitors results of CPD 10μg ≤ 17 mm, or CAZ 30μg ≤ 22 mm, or CTX 30μg ≤ 27mm, or CRO 30μg ≤ 25 mm were reviewed for testing ESBL-screening. ESBL production is inferred when the beta-lactam zone is expanded by the β-lactamase inhibitors, as determined by gross visual inspection, and interpreted as ESBL-positive, at least one expanded zone of five beta-lactams by two β-lactamase inhibitors (Figure 1)



The standard *Escherichia coli* ATCC 25922 and *K.pneumoniae* ATCC 700603 were used as negative and positive controls, respectively. Detection of ESBL-producing *Shigella* spp. by Double Disk Synergy Testing is done together with test organisms as a check on the activity of the antimicrobial disks and on the reproducibility of the test. The control results are shown in the table below.

Table 1

Antimicrobial Disk	**Beta-lactam one is expended by the β–lactamase inhibitors (Pos/Neg)**			
	***E.coli* ATCC 25922**		***K.pneumoniae* ATCC 700603**	
	Amoxicillin/ Clavulanic acid **AmC-30**	Piperacillin/ Tazobactam **TZP-110**	Amoxicillin/ Clavulanic acid **AmC-30**	Piperacillin/ Tazobactam **TZP-110**
Betalactamase disk 1	Neg	Neg	Pos	Pos
Betalactamase disk 2	Neg	Neg	Pos	Pos
Betalactamase disk 3	Neg	Neg	Pos	Pos
Betalactamase disk 4	Neg	Neg	Pos	Pos
Betalactamase disk 5	Neg	Neg	Pos	Pos

Molecular analysis of antibiotic-resistant determinants.

Total DNA was extracted using a Qiagen DNA mini kit following the manufacturer's recommendations. Antibiotic resistance determinants and integrons were detected by PCR using the specific primers listed below.

CTX-M-IV-F: GCT GGA GAA AAG CAG GGG AG
CTX-M-IV-R: GAT AGC TGA CGC AAC GTC TG

The PCR contained a 0.5μL of 20mM concentration of each primer, 2.5μLof 10x PCR buffer II (Bio-Rad), 1.9μL of 25mM concentrations of $MgCl_2$, 2.0μL of each of the four deoxynucleoside triphosphates at a concentration of 10 mM, and 0.2μL of *Taq* polymerase (Qiagen).

Amplifications were performed with a final volume of 25µL on a MyCycler (Bio-Rad) using the following temperature programs: initial denaturation at 96°C for 4 mins, 30 cycles of 96°C for 30s, annealing for 30 s at 62°C, and extension for 1 min at 72°C, and a final extension step at 72°C for 10 mins. PCR products (10µL) were separated by electrophoresis using a 1% agarose gel and visualized under UV light after staining in a 1µg/ ml ethidium bromide solution.

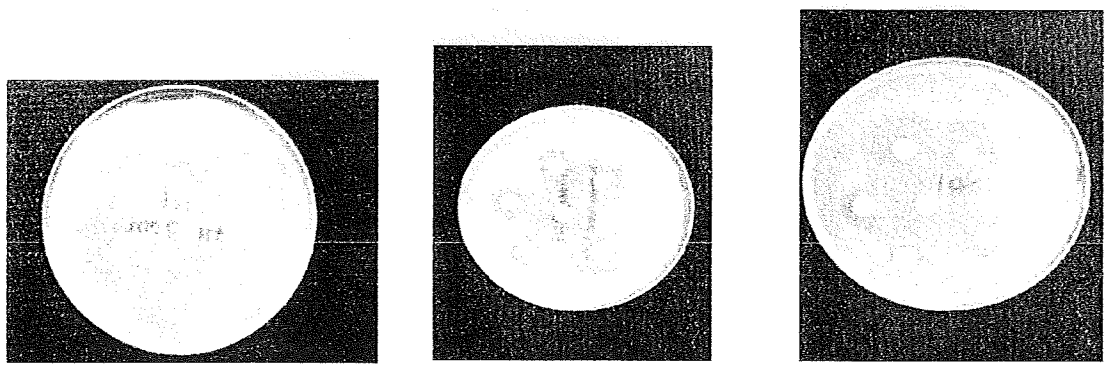
PFGE analysis

S. sonnei and *S.flexneri* strains were analyzed by pulsed-field gel electrophoresis (PFGE) according to the standardized PFGE protocol that was developed for PulseNet by U.S Center for Disease Control and Prevention (www.cdc.gov/pulsenet) and digested with the restriction endonuclease *XbaI* (New England Biolabs, Inc). *Salmonella enterica* serovar Braenderup H9812 was used as a size marker. BioNumerics software was used to compare PFGE profiles. The generated bands were analyzed by using the Dice coefficient and the unweighted-pair group method using average linkages, with a tolerance of 2%.

RESULTS AND DISCUSSION

A total of 54 *Shigella* strains were analyzed in 2009, 45 isolates of *Shigella flexeri* and 9 isolates of *Shigella sonnei*. The fraction of *Shigella* isolates tested in this time resistant was 100% to ampicillin and amoxicillin. Fraction of isolates resistant to cefditoren, cefdinir, cefpodoxime and cetriaxone was 16.7%, resistant to cefixime, cefotaxime and mecillinam were 14.8%, 9.3% and 1.9%, respectively. All *Shigella* isolates were susceptible to azithromycin, ceftazidime, ceftibuten, ciprofloxacin, and faropenem. Our findings are incorporated with the study done by Trung *et al.*, 2007. a potential pathogen was identified in 67.3% of children with diarrhea where the prevalence of *Shigella* spp was 4.7% and predominant in children older than two years of age. Among 162 diarrheagenic *Escherichia coli* strains and 28 *Shigella* strains were determined on the basis of NCCLS guidelines, more than 75% of the strains were resistant to ampicillin, chloramphenicol (53.6% of Shigella strains), and trimethoprim-sulfamethoxazole. Multiresistance was detected in 89.5% of *E. coli* strains and 78.6% of *Shigella* strains.

Nine isolates of *Shigella sonnei* were ESBL-producing positive by Double Disk Synergy tests and the Combination Disk test (Table 2)



ESBL-negative ESBL-positive ESBL-positive (*S.sonnei* isolate)

The ESBLs-producing *Shigella* strains were confirmed by the combination disk and Minimal Inhibitory Concentration Assays. The results are shown in the table 3.

The nine ESBL-positive *Shigella sonnei* isolates were molecular typed for genes encoding CTX-M-IV by PCR. All nine *S.sonnei* isolates were found to harbor β–lactamases CTX-M group IV. The amplified CTX-M- group IV of these nine isolates will be cloned and the sequenced in the next period.

By September 2009, a total of 14 sporadic diarrhea cases have been reported though NIHE. Isolates from all 14 patients were analyzed by PFGE to compare the patterns of generated band. Among those isolates, one was ESBL producing *Shigella sonnei* and its PFGE pattern was 92% similarity to two non-beta lactam isolates (see lanes 12, 13, 14).

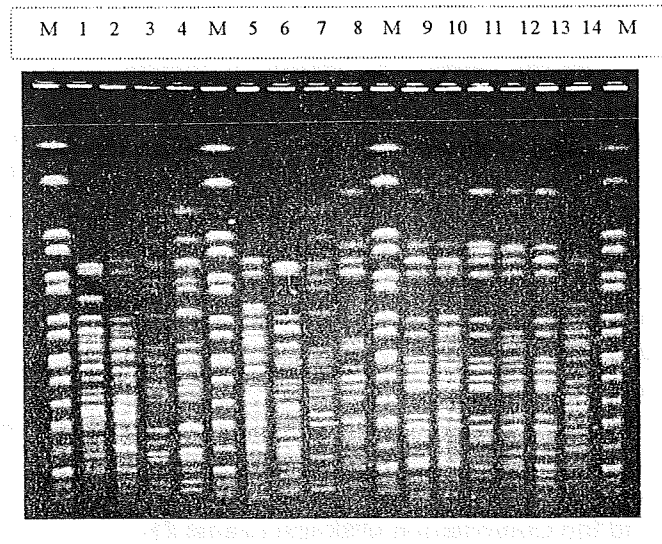
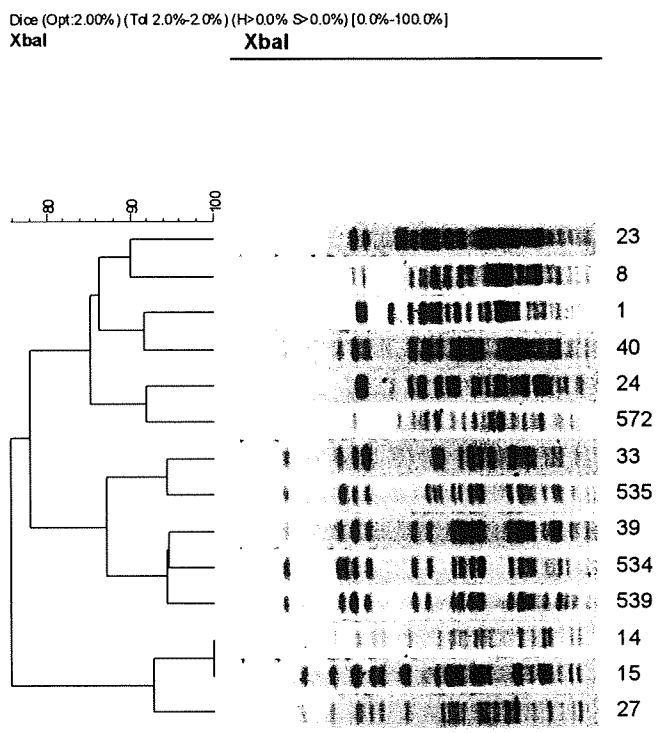


Figure 1: Representative PFGE patterns of *Shigella sonnei* and *Shigella flexneri* isolates from Vietnam. Lanes M are *Salmonella enterica* serovar Braenderup H9812 was used as a size marker. Lanes 1-14 are isolates from patients



Acknowledgement

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Table 2 (A and B). ESBL screening tests

A

Code	ESBL screening tests								
	Initial Screening by disk diffusion test								
	CPD (≤ 17)		CAZ (≤ 22)		CTX (≤ 27)		CRO (≤ 25)		Interpret
VN 4	6	Pos	24	Neg	13	Pos	13	Pos	**Pos**
VN 6	6	Pos	24	Neg	13	Pos	13	Pos	**Pos**
VN 7	6	Pos	24	Neg	12	Pos	13	Pos	**Pos**
VN 12	6	Pos	23	Neg	13	Pos	13	Pos	**Pos**
VN 19	6	Pos	25	Neg	12	Pos	12	Pos	**Pos**
VN 25	6	Pos	24	Neg	12	Pos	11	Pos	**Pos**
VN 26	6	Pos	29	Neg	14	Pos	12	Pos	**Pos**
VN 27	6	Pos	27	Neg	15	Pos	11	Pos	**Pos**
VN 32	6	Pos	30	Neg	16	Pos	13	Pos	**Pos**

B

Code	ESBL screening tests										
	Double Disk Synergy Test (Pos = expanded zone)										
	AmC					TZP					
	ATM	CPD	CAZ	CTX	CRO	ATM	CPD	CAZ	CTX	CRO	Interpret
VN 4	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**
VN 6	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**
VN 7	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**
VN 12	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**
VN 19	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**
VN 25	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**
VN 26	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**
VN 27	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**
VN 32	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	Pos	**Pos**

Table 3. ESBL confirmatory tests

Code	ESBL confirmatory tests															
	Combination disk (Pos ≥5 mm increase)							MIC (Pos ≥3 twofold concentration decrease)								
	CTX	CTX-CLA	Result	CAZ	CAZ-CLA	Result	Interpret	CTX	CTX-CLA	CTX-TAZ	Result	CTX	CTX-CLA	CTX-TAZ	Result	Interpret
VN 4	12	27	pos	24	29	pos	**pos**	1024	0.06	0.06	pos	8	0.06	0.25	pos	**pos**
VN 6	12	29	pos	23	29	pos	**pos**	512	0.06	0.06	pos	8	0.125	0.25	pos	**pos**
VN 7	12	29	pos	24	30	pos	**pos**	1024	0.06	0.06	pos	8	0.125	0.25	pos	**pos**
VN 12	12	28	pos	24	31	pos	**pos**	1024	0.06	0.06	pos	8	0.125	0.25	pos	**pos**
VN 19	15	30	pos	25	30	pos	**pos**	1024	0.06	0.06	pos	8	0.125	0.25	pos	**pos**
VN 25	14	29	pos	24	30	pos	**pos**	2048	0.06	0.06	pos	1	0.125	0.25	pos	**pos**
VN 26	14	27	pos	28	31	pos	**pos**	1024	0.06	0.06	pos	4	0.125	0.25	pos	**pos**
VN 27	13	25	pos	26	29	pos	**pos**	1024	0.06	0.06	pos	4	0.125	0.25	pos	**pos**
VN 32	16	28	pos	28	29	pos	**pos**	256	0.06	0.06	pos	2	0.125	0.5	pos	**pos**

Title: Development of PFGE protocol for *Yersinia enterolitica*

Name of researcher; Brent Gilpin

Affiliation; Institute of Environmental Science & Research Limited, New Zealand

Summary:

The objective of this research project is to develop a PFGE protocol for *Yersinia enteroliticus*. Following evaluations of PFGE protocols, conditions suitable for making plugs have been identified, and from enzymes tested, 1st choice enzyme is *Not*I, followed by 2nd enzyme of *Apa*I. 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.

A lack of diversity among isolates was identified. 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.

Purpose:

The genus *Yersinia* includes three human pathogens; *Y. pestis*, *Y. pseudotuberculosis* and *Y. enterocolitica*. The incidence of yersiniosis in NZ in 2008 was 12.4 cases per 100,000 people making it the 3rd most frequently notified enteric disease in NZ.

At the moment typing of *Y. enterocolitica* in NZ is restricted to biotyping according to biochemical activity (biogroups). In NZ biogroup 4 isolates account for >90% of cases, with biogroups 1B, 2, 3 and 5 also associated with human infection. Biogroup 1A isolates are regarded as avirulent or 'environmental', though they may be opportunistic pathogens. The lack of discrimination biotyping offers restricts the ability to identify outbreaks and to understand disease epidemiology. The objective of this research project is to develop a PFGE protocol for *Yersinia enteroliticus*.

Methods:

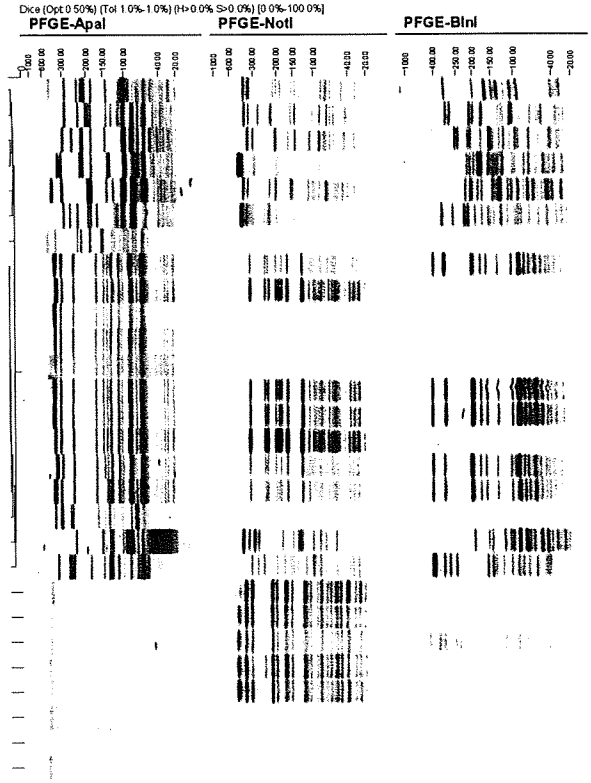
This has been a collaborative project with CDC Atlanta and PulseNet Latin America. Methodological approach

- Select a range of Yersinia isolates to evaluate
- Test PulseNet E. coli plug preparation protocol
- Test range of enzymes
- Validate protocol
- Establish BioNumerics Database

We selected initially 28 isolates covering a range of years, sources and biotypes. Conditions for evaluation of plugs were tested. The plugs were digested with *Apa*I, *Not*I, *Bln*I and *Sbf*I. A range of electrophoresis programmes were tested, and comparisons made with isolates from USA and Argentina.

Results:

The PulseNet *E. coli* protocol was found to produce genomic DNA plugs which could then be digested with restriction enzymes (Figure 1).



Key	Biotype	Additional Information	Year Isolated
ERL053435	1A	Human travel to Fiji	2005
ERL061915	1B	Human	2006
ERL023784	1B	Human	2002
ERL053484	1A	Avian	2005
ERL073947	1A	Ovine	2007
ERL08708	1A	Travel to Asia	2008
NZRM2603		type strain ATCC9610	
ERL032770	4	Human blood isolate	2003
ERL04757	4	Human blood isolate	2004
Fenwick Z39		O:3 from pigs, pre 2000	pre 2000
ERL08109	4	Human blood isolate	2008
ERL073627	4	Human blood isolate	2007
ERL084574	4	Human blood isolate	2008
NZRM3596	4	Human isolate Biotype 4, serotype 0:3 ER95.	1995
ERL063683	4	Human blood isolate	2006
ERL022789	4	Canine	2002
ERL03530	5	Human	2003
NZRM767	3	NCTC 10460 Biotype 3, serotype 1 (2a, 3)	
ERL053045	1B	Human	2005
ERL032126	2	Meat	2003
ERL032501	2	Human blood isolate	2003
ERL082399	2	Human blood isolate	2008
ERL082756	3	Human blood isolate	2008
ERL083523	3	Human blood isolate	2008
ERL084423	3	Travel to Australia	2008
ERL08599	3	Travel to Australia	2008
NZRM1000		strain myo syn. Pasteurella x, serotype 3	
NZRM1001		strain 79b university of lund, serotype 9	

Figure 1. PFGE images from NZ isolates of *Yersinia* digested with *Apa*I, *Not*I and *Bln*I.

Costs of enzymes selected for comparison are listed below ($NZ).

Enzyme			per unit	cost per digest	
ApaI	Roche	$152/5000units	$ 0.03	$ 1.50	50 units/digest
NotI	Roche	$398/1000units	$ 0.40	$ 11.94	30 units/digest
BlnI	Roche	$624/1000 units	$ 0.62	$ 18.72	30 units/digest
SbfI	NEB	$210/500 units	$ 0.42	$ 12.60	30 units/digest

Two key issues emerged.