MASF IV-2(Carlin & Lindberg, 1987). However, when we sequenced the genome of one clinical isolate 2002017 from an ill child, the gene encoding for the serotype IV antigenicity could not be observed, which determines specific glucosyltransferase *gtrIV*, by addition of a glucosyl group to the first sugar, N-acetylglucosamine, of the O antigen tetrasaccharide backbone(Adams *et al.*, 2001). In contrast, the gene *gtrX* encoding the type X specific glucosyltransferase, which involves in the addition of a glucosyl group to the first rhamnose of the O antigen tetrasaccharide backbone, was found in the 2002017 genome(Verma *et al.*, 1993). Therefore, we named it Fxb as a new serotype in *S. flexneri* because it has the type X specific gene *gtrX* rather than *gtrIV*. We then found that the serotype conversion bacteriophage SfX of strain 2002017 was inducible, and could experimentally convert a serotype Fy strain which carries the unmodified O antigen to seroytpe Fx(Simmons & Romanowska, 1987).

Isolation frequencies of *S. flexneri*

In a total of 1890 isolates from Henan province alone were serotyped, F2a was found as the predominant serotype (26%) in 2000 and 2001, started to decline from 2002, up to less than 10% in 2004, started to rise in 2005, and increased to 42% and became most dominant serotype again in 2007. The Fxb, first appeared in 2001, outcompeted F2a from 2003 to 2006 accounting for 14%, 35%, 47% and 48% isolations. But then, it declined to only 15% in 2007, back to the 2002 level. National surveillance, established in 2005 covering 2 surveillance posts in each of 10 selected provinces, revealed that Fxb become the prevalent serotype in Shanxi in 2006 (67%)

and 2007 (33%), and in Gansu, Anhui and Shanghai in 2007 with 67%, 54%, and 35% respectively.

***S. flexneri* Fxb is multi-drug resistance**

Resistances to the commonly used antibiotics were tested in local CDC stations and antibiotics resistances of 116 selected isolates were confirmed by the National Laboratory in Beijing. It was found that all of the 116 isolates were resistant to ampicillin and nalidixic acid. The resistance to ampicillin was significantly increased, from 53% reported in China and 84% in Asia as compared with other serotypes in total (von *et al.*, 2006; Wang *et al.*, 2006). In the ampicillin and nalidixic acid resistance isolates, 96 % were cross resistant to chloramphenicol and tetrcycline and 89.7% resistance to trimethorpim-sulfamethoxzole.

Genes gained, lost and mutated in *Sh.flexneri* FXb

In order to reveal the genomic changes of Fxb emerged, one isolate, 2002017, from a 2 year old infant patient isolated in 2002 in Henan Province was sequenced (GenBank access number CP001383-CP001388). Its genome is composed of one chromosome and 5 plasmids including the large virulence plasmid . One of the plasmids encodes genes for sulfonamide and streptomycin resistance.

As compared with published genomes of *Sh.flexneri*, the FXb Strain 2002017 had acquired 3 genomic islands. The first one is a 37,006 bp *Shigella* serotype conversion island, which is a prophage, encoding genes for O antigen modification. The 2002017 serotype conversion island is similar to and located at the same site as other O antigen island of *Shigella* for serotype conversion reported previously (Yang *et al.*, 2006; Ingersoll *et al.*, 2002). The 2002017 erotype conversion island contains the *gtr* genes

for serotype Fx conversion as the *gtr* sequences are identical to the SfX *gtr* genes previously published(Guan *et al.*, 1999; Verma, Verma, Huan & Lindberg, 1993). The island also contains phage morphology genes with overall similarity to lambdoid phages(Oberto *et al.*, 1989). The O antigen island bacteriophage (SfX) was induced from 2002017, which converted a Fy strain to Fx serotype experimentally. It was named as FXb SHI-O.

The second acquired genomic island is a multi antibiotics resistance island encoding for tetracycline, chloramphenicol, amplicillin and streptomycin resistance. It is similar to the *Shigella* resistance locus (SRL) island initially discovered in a *S. flexneri* 2a strain, YSH6000(Luck *et al.*, 2001; Turner *et al.*, 2004; Turner *et al.*, 2001; Turner *et al.*, 2003). The first 8 kb and the last 7 kb of multi antibiotics resistance island are almost identical between 2002017 and YSH600 . However, the island of 2002017 had gained an additional set of tetracycline resistance genes, extra 22 genes of various functions, lost the iron acquisition system. It was named as FXb SRL.

The third is also a multi antibiotics resistance island, mediated by transposon(Lichtenstein & Brenner, 1982; Sundstrom & Skold, 1990). It has 15,360 bp in size, carrying gene cassettes for dihydrofolate reductase (*dfrA1*), streptothricin acetyltransferase (*sat1*) and aminoglycoside adenyltransferase (*aadA1*), conferring resistance to trimethoprim, streptothricin and streptomycin/spectinomycin, respectively. It was named as FXb SRL2. In addition to these islands, FXb 2002017 gained 13 genes of which 11 are single gene gains when compared with the three published genomes. The majority of these genes are of unknown function.

Shigella as a host adapted pathogen has undergone considerable genome decay(Jin *et al.*, 2002; Wei *et al.*, 2003). The loss of gene functions seems to be continuing with 37 new pseudogenes in 2002017, in addition to the 194 pseudogenes shared with 2457T Thirty seven percent of the losses are genes of phage or IS origin or unknown functions. Of the 7 new pseudogenes that can be found in the ShiBASE database of

Shigella genomes, 3 (*avtA* , *fdnG* and *xdhA*) are also pseuodgenes in one of the non-*S. flexneri Shigella* genomes sequenced up to now while 4 (*fdoG, treB*, *hemE* and *folE*) are intact in all other *Shigella* genomes(Yang *et al.*, 2005; Yang, Chen, Yu, Sun & Jin, 2006).

Public Health Significance

Our mice experiments showed no cross protection between Fxb and F2a (data not shown) and thus the only vaccine currently in use against *S. flexneri* in China, a live F2a vaccine, is expected to offer no protection against the Fxb serotype, which was designed to be specific for serotype F2a. Since a novel serotype can appear and increase to a high prevalence in a very short time frame and serotypes can interconvert frequently, a rapid vaccine development cycle would be most beneficial to provide timely protection against newly emerged serotypes such as Fxb. Further, the choice of vaccine candidates should be from among the currently circulating epidemic clones.

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STUDY TITLE:	Organization of PFGE Workshop for Training and Technology transfer for Asia Pacific countries/ areas in February 2009
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** **National Institute of Infectious Diseases** **Toyama 1-23-1, Shinjuku-ku** **Tokyo 162-8640, Japan**

:

Issue Date: February 23, 2009.

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 2009;

(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 10- 13, 2009.

(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 10- 13, 2009

Name	From	Arrival Date	Departure Date
Molly Leeper	PulseNet National Database Team CDC/NCZVED/DFBMD/EDLB/NFLORT USA	8 Feb	13 Feb
Ms Kristy Kubota Husband	American Public Health Association USA	7 Feb	14 Feb
Dr. Jun Terajima	Department of Bacteriology National Institute of Infectious Diseases Japan	9 Feb	14 Feb
Ms Wai Yee LEE	Animal and Plant Health Centre Agri-Food and Veterinary Authority Singapore	9 Feb	14 Feb
Ms Wei Ling TAN	Veterinary Public Health Centre Agri-Food and Veterinary Authority Singapore	9 Feb	15 Feb
Dr. T. Ramamurthy	Division of Bacteriology National Institute of Cholera and Enteric Diseases India	9 Feb	14 Feb
Mr. Goutam Chowdhury	Division of Bacteriology National Institute of Cholera and Enteric Diseases India	9 Feb	14 Feb
Mr. Manash Roy	Division of Bacteriology National Institute of Cholera and Enteric Diseases India	9 Feb	14 Feb
Mr. Kabir Uddin Ahmed	Enteric Microbiology Laboratory Laboratory Sciences Division ICDDR,B Bangladesh	9 Feb	15 Feb
Mohammad Aslam	Enteric and Food Microbiology Laboratory Laboratory Sciences Division ICDDR,B Bangladesh	9 Feb	15 Feb
Mr. Chaiwat Pulsrikarn	National Institue of Health Department of Medical Sciences Ministry of Public Health Thailand	9 Feb	14 Feb
Dr. Seong-Han Kim	Division of Enteric Bacterial Infections Centers for Infectious Disease Research National Institute of Health Republic of Korea	9 Feb	14 Feb
Dr. Junyoung Kim	Division of Enteric Bacterial Infections Centers for Infectious Disease Research National Institute of Health Republic of Korea	9 Feb	14 Feb
Dr. Deng xiaoling	Institute of Microbiology Center for Disease Control and Prevention of Guangdong Province	9 Feb	13 Feb
Dr. Lan quanxue	Center for Disease Control and Prevention of Shenzhen City	9 Feb	13 Feb

Appendix II

Agenda for PulseNet Asia Pacific PFGE Workshop Hong Kong 2009

Date: February 10-13, 2009
Venue: Conference Room at Public Health Laboratory Centre (PHLC), Hong Kong

February 10, 2009 (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 Molly Leeper CDC, USA Kristy Kubota, APHL Jun Terajima, NIID, Japan
9:30 – 9:45 am	Overview of Workshop	Danny Cheung, PHLC, HK
9:45 – 10:20 am	Installation and Overview of BioNumerics/ MasterScripts	Molly Leeper, CDC, USA
10:20 – 10:35 am	Group Photo	
10:35 – 11:00 am	Coffee Break	
11:00 – 11:40 am	Analyzation 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 Communication	Molly Leeper, CDC, USA
2:20 – 2:35 pm	Creation and File Location of PulseNet Bundle Files	Molly Leeper, CDC, USA
2:35 – 3:35 pm	Exercise 2: Prepare and Create a PulseNet Bundle File for Distribution	Molly Leeper, CDC, USA
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 – Thailand
4:35 – 4:50 pm	Laboratory Experience Sharing	Participant presentation – Singapore
4:50 pm	Q and A	
5:00 pm	End of Day 1 – Shuttle back to Hotel	

February 11, 2009 (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; Import Data from Excel	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	Molly Leeper, CDC, USA
11:45 – 12:05 pm	Performing Comparisons in BioNumerics	Molly Leeper, CDC, USA
12:05 – 1:00 pm	Exercise 4: Performing Queries and Creating Comparisons	Molly Leeper, CDC, USA
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	Molly Leeper, CDC, USA
3:30 – 3:45 pm	Coffee Break	
3:45 – 4:15 pm	QA/QC and Factors that Influence Data Analysis	Molly Leeper, CDC, USA
4:15 – 4:30 pm	Laboratory Experience Sharing	Participant presentation – Korea
4:30 – 4:45 pm	Laboratory Experience Sharing	Participant presentation – India
4:45 – 5:00 pm	Q and A	
5:00 pm	End of Day 2 – Shuttle back to Hotel	

February 12, 2009 (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	Hong Kong PFGE Experience	Alf Chu, 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 – 10:45 am	Coffee Break	
10:45 – 11:05 am	Naming Patterns and Creating Local Unique Pattern Lists	Kristy Kubota, APHL
11:05 – 12:00 pm	Exercise 7: Identifying and Naming Unique Patterns in the database	Kristy Kubota, APHL
12:00 – 12:15 pm	Working with Subsets	Cindy Luey, PHLC, HK
12: 15 – 12:35 pm	Exercise 8: Create Subsets	Cindy Luey, PHLC, HK
12:35 – 1:35 pm	Lunch	
1:35 – 1:55 pm	Use of Groups and the Chart and Statistics Tool	Molly Leeper, CDC, USA
1:55 – 2:30 pm	Exercise 9: Create Charts and Graphs to Create Reports	Molly Leeper, CDC, USA
2:30 – 3:00 pm	Composite Data Sets	Alf Chu, PHLC, HK
3:00 – 3:30 pm	Exercise 10: Cluster analysis using a composite data set for *Salmonella*	Alf Chu, PHLC, HK
3:30 – 4:00 pm	Coffee Break	
4:00 – 4:15 pm	BioNumerics V5.0	Molly Leeper, CDC, USA
4:15 – 4:30 pm	Laboratory Experience Sharing	Participant presentation – China (Guangdong)
4:30 – 4:45 pm	Laboratory Experience Sharing	Participant presentation – Bangladesh
4:45 – 5:00 pm	Q and A	
5:00 pm	End of Day 3 – Shuttle back to Hotel	

February 13, 2009 (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) **Emerging Diarrheal Infections**	T. Ramamurthy, NICED, India
9:30 – 10:00 am	Extended Forum (Open to all PHLC colleagues) **Subtyping of bacterial isolates**	Jun Terajima, NIID, Japan
10:00 – 10:30 am	Coffee Break	
10:30 – 11:00 am	Subtyping Foodborne Bacterial Pathogens by PFGE (Standardized Protocols and Troubleshooting)	Kristy Kubota, APHL
11:00 – 1:00 pm	*<Split group session>* Demo on *Vibrio* PFGE protocols (Group A) Practical Session on BioNumerics with *Vibrio parahaemolyticus* (Group B)	Demo by PHLC, HK BioNumerics practices by APHL
1:00 – 2:00 pm	Lunch	
2:00 – 4:00 pm	*<Split group session>* Demo on *Vibrio* PFGE protocols (Group B) Practical Session on BioNumerics with *Vibrio parahaemolyticus*(Group A)	Demo by PHLC, HK BioNumerics practices by APHL
4:00 – 4:15 pm	Coffee Break	
4:15 – 5:00 pm	Final Q&A/ Discussion Summary of PNAP Workshop Certificate presentation	All participants Kristy Kubota, APHL Jun Terajima, NIID, Japan KM Kam, PHLC, HK
5:00 pm	End of Workshop – Shuttle back to Hotel	

Appendix III

WORKSHOP EVALUATION – Summary

Course name: The Sixth PulseNet Asia Pacific PFGE Workshop

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

Dates: February 10-13, 2009

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 **12** Good____ Satisfactory____ Unsatisfactory____

2. Were the objectives of the course clearly defined? Yes **12** No____

3. Were the objectives of the course met? Yes **12** No____

4a. Please rate the quality and usefulness of handouts.

Excellent **10** Good **2** Satisfactory____ Unsatisfactory____

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

Excellent **10** 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 **11** Positively **1** Not much____ Not at all____

6. Would you recommend this course to others in public health laboratories? Yes 9 No 3– **No further comment**_

Please explain:

1. It is a great tool share the information quickly from one to another.
2. No answered.
3. Not answered.
4. A very comprehensive, well-organized workshop with theory (lectures) and practical sessions. The wet-lab demo was useful too. Cindy is very experienced.
5. Not answered.
[redacted]
7. The Teaching way.
[redacted]
9. Current group size is just nice and participants can get sufficient attention and help from trainers. Detailed explanations with PFGE and the use of BioNumerics. Trainers are very experienced and willing to share with the participants.
10. Whenever the Applied Maths BioNumerics software is available or in use.
[redacted]
12. This course let me know how to use BioNumerics.

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. Not answered.
3. No.
4. The basic of BioNumerics was well-covered for PFGE analysis.
5. MLVA wet lab. practice, analysis by BN.
6. Not answered.
7. No.
8. Not answered.
9. Presentation of troubleshooting for PFGE perhaps not necessary to go thro as many points are already mentioned during practical demo. Just provide the notes and participants can read thro' at their own time. More exercises could be included for participants can read thro/ at their our time. More excises could be included for participants to do in their spare time.
10. Selection criteria of strains for cluster analysis. Epidemiological significance of cluster analysis and use of BioNumerics software.
11. All the topics of that are fulfilled in this course
12. No.

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

1.Not answered.
2. Researches from PHLC helped to understand the topic and practice.
3. Naming pattern and creating local unique pattern lists.
4. The practical sessions after each lecture topics were useful as hand-on practice enhance learnings.
5. Personal practice for each participant.
6. Not answered.
7. Practice.
8. Not answered.
9. Practise session were extremely helpful.
10. All most all the topics included in this workshop.
11. Analyze a PFGE gel image and basic behind comparison and clustering analysis is most helpful in our laboratory.
12. Exercise.

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

1.Not answered.
2. Little hurry.
3. BioNumerics V5.0
4. Nil.
5. Internet service for email and Large Monitor Screen.
6. Not answered.
7. Too much talking less practice.
8. Not answered.
9. All the activities were useful.
10. Nothing specials as all are useful topics.
11. Prepare and create a PulseNet bundle files that may be very helpful in our laboratory.
12. Lecture.

10. Was the time allotted for each topic or practice session appropriate? Yes **10** No___ Not answered **2**

a. For which activities should **more time** be allowed?

1. Not answered.
2. Not answered.
3. Use of Groups and the Chart and Statistic tool.
4. Nil.
5. Not answered.
6. Not answered.
7. Life of a PulesNet Cluster and BioNumerics V5.0. Please focus more some new or changed place in the V5.0.
8. Not answered.
9. Analysis of gel images and lanes.
10. Analyzing a PFGE gel image and link entries to database. Prepare and create bundle file for distribution.
11. Analysis of PFGE Gel image.
12.Not answered.

b. For which activities should **less time** be allowed?

1. Not answered.
2. Not answered.
3. BioNumerics V5.0
4. Nil.
5. All appropriate.
6. Not answered.
7. No.
8. Not answered.
9. Nil.
10. All the activities had enough time except for the share time.
11. Performing Queries and Creating comparisons.
12. Not answered.

11. In your opinion, should we have **this course again** for other PulseNet participating Laboratories?
Yes 11 No____ Not answered 1

1. Not answered.
2. Not answered.
3. Not answered.
4. Not answered.
5. For the time being, it is required.
6. Not answered.
7. Yes.
8. Not answered.
9. Try to arrange the heavy presentations before lunch so that participants will not be so sleepy.
10. Not answered.
11. Not answered.
12. Not answered.

12. **Other comments** about course:
1. Not answered.
2. Not answered.
3. Not answered.
4. Nil.
5. Thank you for the perfect organization of all training schedule and passion of trainers.
6. Not answered.
7. I hope the training course can be held each year.
8. Not answered.
9. A big thank you to all the organizers and people who have helped to make this course so successful and enjoyable.
10. The course was well-organized and useful for us. The staff of APHL helped in clarifying the basic doubts and the practical sessions are wonderfully managed.
11. No. Thanks.
12. I have learned a lot from the course. Thank for PHLC, Hong Kong.

Application of pulse-field gel electrophoresis to the characterization of pathogenic *Vibrio paraheamolyticus* isolates obtained from clinical sources in Nha Trang, Vietnam.

Phung Dac Cam[1], Nguyen Thi Phong Lan[1], Tran Van Tung[2], Nguyen Thuy Tram[1] and Haruo Watanabe[3]

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Summary:

Vibrio paraheamolyticus is one of the most important food-borne pathogens in a coastal area in Vietnam. The aim of the present study was to characterize pathogenic *V.paraheamolyticus* isolates from clinical sources in Nha Trang, Vietnam in order to have a better understanding of the seroprevalence and genotype differentiation among them. The method incorporated cultivation and isolation of food and faecal samples by serologic typing of O and K antigen and molecular typing (PFGE). Among 31 *V. paraheamolyticus* isolates, isolates were belonged either to O3 : K6 or O4: K68 or O1 : K25 by serotyping. By molecular typing (PFGE), 16/31 (52%) shared a coefficient of similarity at $\geq$ 85% . 5/31 (16%) had the same PFGE pattern similarity at 100%. Among these isolates, 3 isolates obtained in 1997, 2 isolates obtained from 1996 and 1 isolate in 1999. 3/31 (similarity at 100%) obtained isolates of 1999, 2001 and 1997. Almost 50% of strains isolated were belong to pandemic strains that emerged around 1996 and spread to many countries. Obviously a transition of major serovars occurs among the pandemic strains represented by emergence of O3:K6 in 1997, O4:K8 in 1998 and O1:K25 in 1998 and 1999.