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Tables and figures

Table 1. Sources and year of collection for *Salmonella* Typhi isolates.

Country	Year of isolation (no. isolates)			Total
	2007	2008	2009	
Bangladesh	38			38
Indonesia	3	19	34	56
Taiwan	15	15	9	39
Vietnam	26	25		51
Total	82	59	43	184

Table 2. Antimicrobial resistance rate (%) in *Salmonella* Typhi isolates from 4 Asian countries.

Antimicrobials	Bangladesh (n = 38)	Indonesia (n = 56)	Taiwan (n = 39)	Vietnam (n = 51)	Total (n = 184)
Aztreonam	0.0	0.0	0.0	0.0	0.0
Cefotaxime	0.0	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0	0.0	0.0
Ceftriaxone	0.0	0.0	0.0	0.0	0.0
Imipenem	0.0	0.0	0.0	0.0	0.0
Nalidixic Acid	81.6	1.8	0.0	19.6	22.8
Ciprofloxacin	39.5	0.0	0.0	0.0	8.2
Gentamicin	0.0	1.8	0.0	0.0	0.5
Kanamycin	0.0	0.0	0.0	0.0	0.0
Ampicillin	68.4	1.8	5.1	80.4	38.0
Chloramphenicol	57.9	3.6	2.6	80.4	35.9
Streptomycin	76.3	21.4	25.6	84.3	51.1
Sulfamethoxazole	68.4	3.6	7.7	80.4	39.1
Tetracycline	21.1	3.6	5.1	84.3	29.9
Trimethoprim	57.9	1.8	5.1	80.4	35.9
Trimethoprim / sulfamethoxazole	57.9	1.8	2.6	78.4	34.8

Table 3. Clonal distribution of *Salmonella* Typhi isolates from 4 Asian countries

Country	Clone A	Clone B	Clone C	Clone D	Miscelleous	Total
Bangladesh	1	28	5	0	4	38
Indonesia	3	0	4	48	1	56
Taiwan	14	1	16	5	3	39
Vietnam	0	50	0	0	1	51
Total	18	50	25	53	9	184

Table 4. Antimicrobial resistance rate in *Salmonella* Typhi clones

Antimicrobial	Resistance rate (%)					Total (n = 184)
	Clone A (n =18)	Clone B (n = 50)	Clone C (n = 25)	Clone D (n = 53)	Miscellaneous (n = 9)	
Aztreonam	0.0	0.0	0.0	0.0	0.0	0.0
Cefotaxime	0.0	0.0	0.0	0.0	0.0	0.0
Ceftazidime	0.0	0.0	0.0	0.0	0.0	0.0
Ceftriaxone	0.0	0.0	0.0	0.0	0.0	0.0
Imipenam	0.0	0.0	0.0	0.0	0.0	0.0
Nalidixic Acid	5.6	44.3	8.0	1.9	33.3	22.8
Ciprofloxacin	0.0	19.0	0.0	0.0	0.0	8.2
Gentamicin	0.0	0.0	0.0	1.9	0.0	0.5
Kanamycin	0.0	0.0	0.0	0.0	0.0	0.0
Ampicillin	0.0	83.5	8.0	3.8	0.0	38.0
Chloramphenicol	0.0	78.5	8.0	3.8	0.0	35.9
Streptomycin	22.2	84.8	44.0	18.9	22.2	51.1
Sulfamethoxazole	5.6	83.5	8.0	5.7	0.0	39.1
Tetracycline	0.0	63.3	8.0	5.7	0.0	29.9
Trimethoprim	5.6	78.5	4.0	3.8	0.0	35.9
Trimethoprim / sulfamethoxazole	0.0	77.2	4.0	3.8	0.0	34.8

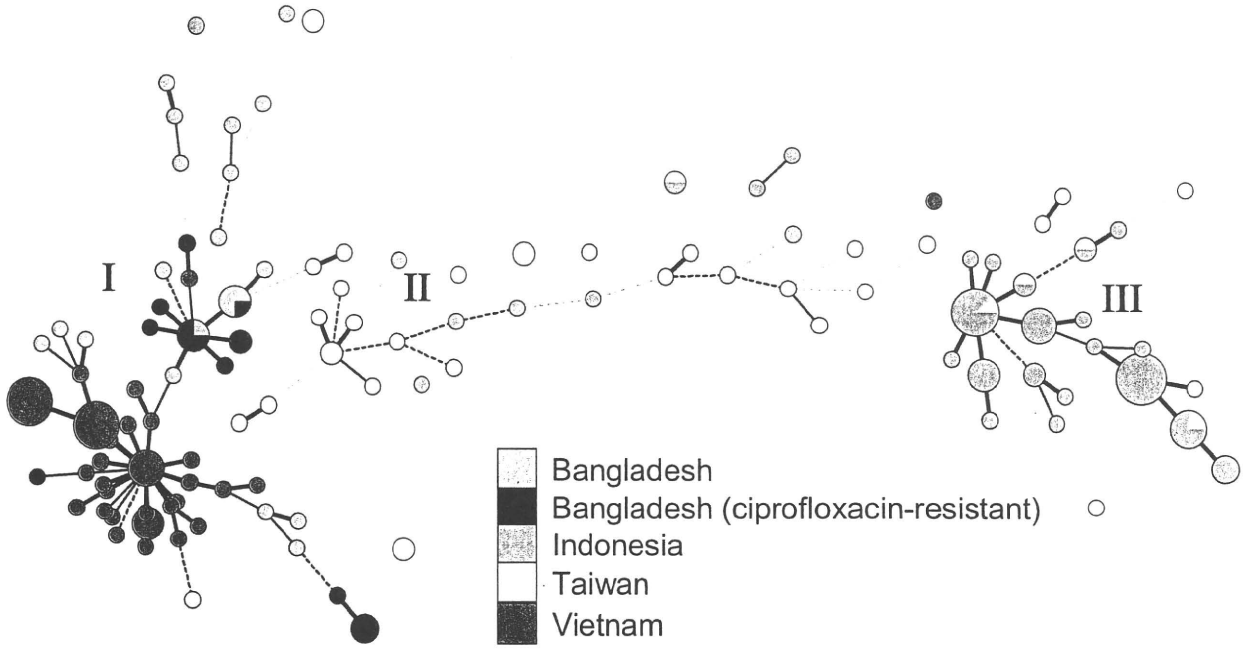

Fig. 1. Clonal relationships among 184 *Salmonella* Typhi isolates from four Asian countries. The tree was constructed using MLVA11 profiles by minimum spanning tree algorithm. Circle size is proportional to the number of isolates belonging to an MLVA type. Two or more MLVA types differing at three or fewer loci are regarded as a group and are marked in gray shadow. There are three MLVA clusters, I, II and III indicated. The country of origin for isolate is marked in celeste for Bangladesh, deep blue for Bangladesh with isolate resistant to cirpofloxacin, green for Indonesia, white for Taiwan and pink for Vietnam.

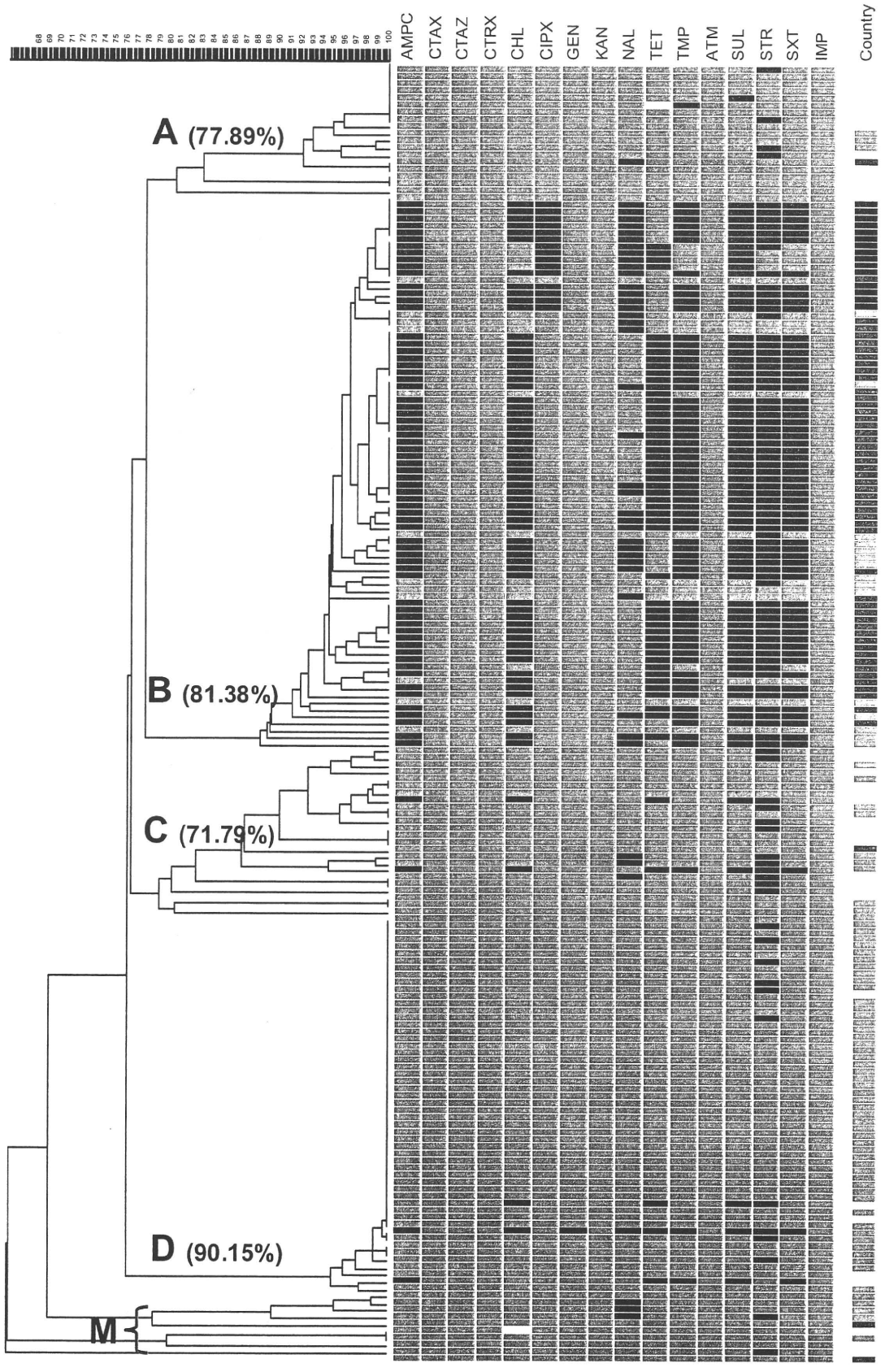
AMPC
CTAX
CTAZ
CTRX
CHL
CIPX
GEN
KAN
NAL
TET
TMP
ATM
SUL
STR
SXT
IMP
Country
A (77.89%)
B (81.38%)
C (71.79%)
D (90.15%)
M

Fig. 2. Dendrogram of 184 *Salmonella* Typhi isolates constructed using PFGE patterns and MLVA5 profiles in 1:1 weights ratios by UPGMA algorithm. MLVA5 is based on 5 slowly-evolved VNTRs (Sty2, Sty3, Sty20, Sty39 and Sty42). The results of antimicrobial susceptibility testing are marked in red for resistance, yellow for intermediate and green for susceptibility. The country of origin for isolate is marked in celeste for Bangladesh, deep blue for Bangladesh with isolate resistant to cirpofloxacin, green for Indonesia, white for Taiwan and pink for Vietnam.

REPORT for RESEARCH SPONSORED BY NATIONAL INSTITUTE OF INFECTIOUS DISEASES, TOKYO JAPAN

Title: Inter-laboratory *Shigella sonnei* MLVA Validation Study

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Summary:

1) Background and Objective: *Shigella sonnei* is common in most countries of PulseNet Asia Pacific. Infection is often associated with foodborne transmission and international travel. PFGE is a standardized subtyping tool for *S. sonnei* used in PulseNet laboratories. MLVA is powerful in discriminating isolates but occasionally is insufficient to distinguish some epidemiologically unrelated isolates. In 2007, an MLVA method, consisting of 26 VNTRs, was developed for *S. sonnei*. The method displays higher discriminatory power than PFGE in discriminating among isolates, especially PFGE-indistinguishable but epidemiologically unrelated isolates. The discriminatory power for closely related isolates is primarily attributed to the highly variable VNTRs. MLVA assay based on a small number of high variability VNTRs displays higher resolving power than PFGE in discriminating isolates for cluster identification and outbreak investigation. In the 6th PNAP meeting held in December 2009 at Bangkok, Thailand, participating members agreed to set up a Shigella Working Group that shall initiate collaborative projects.

Hence the objectives of the study were:

1. To evaluate the set of VNTRs provided by Dr Chiou from CDC , Taiwan.
2. To apply the VNTRs to study Malaysian indigenous strains.
3. To determine the PFGE profiles and compare with MLVA data

2) Study Design:

The inter-laboratory validation consisted of two phases. In phase I, MLVA was tested on 30 well-characterized strains supplied by Taiwan CDC to ensure that our results are comparable and in concordance with the results obtained at Taiwan CDC. The raw data (fragment lists) generated was sent to Taiwan CDC for analysis/comparison. The detailed protocol which is based on the ABI Prism 3130 platform and the PCR primers was provided by Taiwan CDC.

After completion of phase I, we proceeded to phase II in which a prospective evaluation of MLVA was done against PFGE (using *XbaI* and *NotI* restriction enzymes). Data generated are sent to Taiwan CDC for compilation..

3)Methods

3.1. Collection of bacterial cultures. Bacterial cultures were obtained from collaborators. Glycerol stocks of cultures will be kept for further study. Concurrently, we obtained *Shigella* spp from the Institute for Medical Research, Kuala Lumpur. Stocked cultures were also retrieved from culture collection of the Laboratory. All the revived strains were re-confirmed by the commercial identification kit, API from BioMerieux.

3.2. Receipt of 30 DNA Samples from Taiwan: The main collaborating

center in Taiwan provided DNA samples to evaluate the primers for 8 VNTRS.

3.3. MLVA analysis. Since this is an interlaboratory evaluation exercise, the preparation, execution and analysis for MLVA will be as indicated by the Coordinator. This involved DNA extraction to prepare template for PCR. PCR conditions adopted will be as provided by the main coordinator (Dr Chiou). PCR amplicons will be checked on agarose gel electrophoresis. Fragment analysis preparation and loading of samples will follow standard protocols. Data to be sent to main collaborator.

3.4. MLVA and PFGE of indigenous strains.

A total of 50 *Shigella* strains will be analyzed using the same protocol and data will be compared with those from other collaborating centres in India, Taiwan and Japan. New set of fluorescent primers were synthesized. DNA extraction was carried out. PFGE was also carried accordingly to the standardized protocols of PULSENET INTERNATIONAL.

3.5 DATA Analysis

Both the MLVA data and PFGE data are analysed using the BioNumerics Version 3.5

4) Results

4.1 Evaluation of VNTRS.

Phase 1 of *Shigella sonnei* mlva SOP validation was completed. Thirty strains obtained from Dr. Chiou (CDC, Taiwan) were used in this phase. Eight VNTR loci were tested: SS1, SS3, SS6, SS9, SS10, SS11, SS12 and SS13.

The SOP was followed completely without the need of primer concentration modification for the PCR amplification. However when we used a 20X dilution for PCR product, off-scales were encountered in the analysis. Therefore, further optimization for loading quantity was carried out by using a serial dilution of 20X, 40X, 60X, 80X, and 100X PCR amplicons. Only the 40X dilution gave the best peak in the electrophorerogram. Hence, subsequent analysis was carried out using this condition. The values obtained for SS1 of some strains were incorrect. They were all of copy number 1. The correct amplicon sizes were obtained after repeated experiments. Some loci (especially for loci SS12) have double bands detected; the correct ones were those with higher height or area. Our results showed that some of the ghost bands have similar height with the actual amplicon, although the height is slightly lower. The results of this evaluation was submitted to the Coordinator in Taiwan in November/December, 2010. We were advised to exclude SS12 from the VNTR set (7 loci are sufficiently discriminatory) for phase II of this work. Feedback from Dr. Chiou

commented that the amplicon sizes obtained were quite close to the average and could be assigned the copy numbers.

PCR runs were made in two multiplex as indicated in Table 1 and the expected sizes of the amplicons are indicated in Table 2.

Table1 mPCR for 8 VNTRs

Primer mix (100 µl)	Locus	Primer/H20	Stock soln µM	Volume µl	Final conc. in primer mix µM	Final conc. in PCR reaction µM
M1	SS1	SS1-F (ViC)	100	1	1	0,05
		SS1-R	100	1	1	0,05
	SS3	SS3-F (6FAM)	100	2	2	0,1
		SS3-R	100	2	2	0,1
	SS6	SS6-F (NED)	100	4	4	0,2
		SS6-R	100	4	4	0,2
	SS9	SS9-F (PET)	100	2	2	0,1
		SS9-R	100	2	2	0,1
		H20		82		
M2	SS10	SS10-F (VIC)	100	1	1	0,05
		SS10-R	100	1	1	0,05
	SS11	SS11-F (6FAM)	100	1	1	0,05
		SS11-R	100	1	1	0,05
	SS12	SS12-F (NED)	100	5	5	0,25
		SS12-R	100	5	5	0,25
	SS13	SS13-F(PET)	100	1	1	0,05
		SS13-R	100	1	1	0,05
		H20		84		

Table 2: Sizes of the expected amplicons compared to the Standards

Multiplex	Locus	Repeat _bp	Copy no.	Size_amplicon _bp	Size estimated by		
					500LIZ*	600LIZ	ROX625
M1	SS1	7	15	230	216.01	213.95	216.48
	SS3	7	17	233	230.02	227.65	231.29
	SS6	7	24	320	314.83	311.44	317.5
	SS9	6	9	215	215.6	213.31	216.11
M2	SS10	6	3	198	194.61	191.6	194.39
	SS11	6	4	199	197.21	194.33	197.04
	SS12	9	2	188	184.93	181.54	184.74
	SS13	6	3	236	236.73	234.79	238.73

The summary of the evaluation data is in Table 3.

4.2 MLVA of Malaysian *Shigella sonnei*

Forty indigenous Malaysian strains of *Shigella sonnei* were available for analysis using the MLVA protocol. Strains were from human stool samples from years 1997 to 2000a and 2007 to 2010. All the strains were from different localities in Malaysia, with patient age distribution of 2-87 years old. The results obtained showed that loci SS1, SS3, SS6, and SS9 were highly variable, with number of alleles of 14, 17, 17, and 12, respectively; loci SS10, SS11, and SS13 were relatively less heterogeneous (number of alleles = 6, 8, and 3 respectively). More alleles were detected for loci SS1, SS3, and SS9 compared to the strains tested in phase I. No strains with identical profile of copy number were observed. More strains are being added into the collection to make the sample size larger. The work is near completion.

The summary of data is in Table 4.

Table 3: Result for phase I evaluation

Strain	SS1 (Standard average)	SS1	SS3 (Standard average)	SS3	SS6 (Standard average)	SS6	SS9 (Standard average)	SS9	SS10 (Standard average)	SS10	SS11 (Standard average)	SS11	SS13 (Standard average)	SS13
N05.0011	130.13	130.14	229.86	229.84	165.68	165.7	204.38	204.68	207.05	207.27	197.20	196.73	230.79	230.89
C05.1923	130.13	130.54	216.36	216.22	321.37	321.43	210.17	210.29	212.85	213.12	202.83	202.49	230.79	230.94
sh22703	130.13	130.13	209.66	209.57	307.10	307.23	215.96	216.04	212.85	213.07	202.83	202.47	230.79	230.9
sh09727	130.13	130.2	182.45	182.33	212.21	212.66	233.27	233.53	207.05	207.38	208.77	208.37	230.79	230.97
sh30284	130.13	130	257.31	257.21	265.99	266.26	215.96	216.07	212.85	212.25	214.12	214.33	230.79	231.43
sh15371	130.13	130.21	223.03	223.14	192.64	192.86	215.96	216.14	207.05	207.44	219.96	220.12	230.79	231.08
sh07156	130.13	130.2	202.87	202.61	265.99	266.01	227.48	227.79	207.05	207.31	214.12	214.13	230.79	230.93
sh07150	130.13	130.1	202.87	202.54	272.76	273.11	227.48	227.95	207.05	207.33	214.12	214.08	230.79	230.96
sh10288	130.13	130.21	189.32	189.17	165.68	165.91	227.48	227.7	207.05	207.33	197.20	196.69	230.79	230.96
sh22738	130.13	130.22	223.03	223.05	165.68	165.77	268.49	268.79	207.05	207.26	197.20	196.69	230.79	230.93
N04.0829	162.69	163.2	236.62	236.64	259.22	259.44	204.38	204.53	194.72	194.9	197.20	196.66	236.89	237
C04.1101	199.29	200.16	313.60	313.46	265.99	266.17	204.38	204.52	194.72	194.83	197.20	196.67	236.89	236.99
sh12324	175.26	175.69	243.61	243.57	192.64	192.92	210.17	210.32	194.72	194.89	197.20	196.73	236.89	237.02
C07.0755	216.87	218.32	229.86	229.84	314.33	314.35	215.96	216.19	194.72	194.96	197.20	196.7	236.89	237.1
C07.0894	223.63	224.04	229.86	229.87	314.33	314.45	215.96	216.08	194.72	194.93	197.20	196.68	236.89	236.97
C07.4098	216.87	218.3	229.86	229.94	360.99	361.32	215.96	216.03	194.72	194.92	197.20	196.64	236.89	236.99
sh24464	180.86	181.96	216.36	216.22	265.99	266.17	204.38	204.51	194.72	194.93	202.83	202.51	236.89	236.97
sh25550	135.95	136.31	285.18	284.9	239.14	239.1	204.38	204.68	223.40	223.71	208.77	208.32	236.89	237.04
sh23752	135.95	136.3	271.23	271.01	279.56	279.63	204.38	204.52	223.40	223.77	208.77	208.34	236.89	237.05
sh26840	135.95	136.22	291.92	291.84	279.56	279.64	204.38	204.53	223.40	223.58	208.77	208.07	236.89	236.91
89e1015	135.95	136.33	250.40	250.36	286.12	286.51	204.38	204.71	223.40	222.83	208.77	208.38	236.89	237.08
89e1237	135.95	136.21	257.31	257.19	286.12	286.66	204.38	204.77	223.40	223.71	208.77	208.29	236.89	236.75
sh23679	135.95	136.31	271.23	271.11	286.12	286.54	204.38	204.53	223.40	223.68	208.77	208.33	236.89	237.05
sh26364	135.95	136.21	291.92	291.8	293.18	293.29	204.38	204.64	223.40	223.63	208.77	208.17	236.89	236.98
sh27192	135.95	136.23	264.20	264.1	279.56	279.67	210.17	210.33	223.40	223.76	208.77	208.31	236.89	237.08
sh25405	135.95	136.23	257.31	257.31	286.12	286.45	204.38	204.58	223.40	223.66	214.12	214.08	236.89	237.08
C05.2119	168.92	169.33	257.31	257.31	219.19	219.27	221.70	221.9	188.94	189.09	197.20	196.66	242.96	243.14
S05.2456	193.33	194.04	264.20	264.2	232.26	232.42	215.96	216.22	188.94	189.07	197.20	196.68	249.08	249.25
04-901-748065	162.69	163.18	209.66	209.7	192.64	192.55	204.38	204.59	200.49	200.66	197.20	196.67	249.08	249.25
N05.0012	187.15	187.97	189.32	189.22	252.57	252.66	210.17	210.28	200.49	200.66	197.20	196.69	249.08	249.32
allele no.	11		16		17		7		6		5		4	

Table 4: Amplicon size and copy number of MLVA type of Malaysian Strains for Phase II

Strain	SS1	Copy no.	SS3	Copy no.	SS6	Copy no.	SS9	Copy no.	SS10	Copy no.	SS11	Copy no.	SS13	Copy no.
TC 16/98	223.1	15	223.1	16	252.59	15	216.1	9	194.78	3	191.79	3	237.08	3
TC 8/00	142.83	3	257.18	21	239.12	13	227.44	11	212.18	6	209.28	6	237.03	3
TC 9/99	175.58	8	175.5	9	165.82	2	233.32	12	207.24	5	191.85	3	230.85	2
TC 33/98	130.92	1	306.45	28	279.72	19	221.74	10	207.34	5	208.07	6	230.9	2
TC 6/99	130.22	1	271.1	23	225.82	11	204.4	7	207.34	5	209.21	6	230.9	2
TC 8/98	243.4	18	243.4	19	272.78	18	210.4	8	207.36	5	209.27	6	230.9	2
TH 3/07	130.19	1	202.73	13	293.09	21	245.32	14	218.7	7	226.77	9	230.88	2
TC 5/97	181.71	9	229.88	17	286.27	20	268.7	18	217.75	7	203.44	5	236.98	3
TC 6/00	175.49	8	175.31	9	245.95	14	222.07	10	218.8	7	220.89	8	230.87	2
TC 2/00	154.7	5	154.43	6	219.14	10	221.74	10	201.55	4	215.01	7	230.83	2
TC 4/99	129.99	1	154.46	6	165.92	2	239.66	13	207.2	5	197.72	4	231.07	2
TC 23/98	130.29	1	243.56	19	266.09	17	210.2	8	207.19	5	208.03	6	230.9	2
TC 2/99	189.28	10	189.19	11	232.62	12	239.43	13	212.83	6	209.36	6	230.95	2
TC 15/98	192.99	11	271.07	23	286.46	20	227.52	11	217.85	7	184.8	2	236.92	3
TC 11/99	196.2	12	195.95	12	165.83	2	256.65	16	207.22	5	190.6	3	230.88	2
TC 7/00	195.93	12	195.93	12	266.06	17	215.9	9	207.13	5	208.01	6	230.84	2
TC 11/00	195.78	12	195.78	12	272.83	18	216.01	9	207.29	5	207.99	6	230.83	2
TC 1/97	168.86	7	168.51	8	259.31	16	256.52	16	213.04	6	213.71	7	230.74	2
TC 17/98	180.97	9	223	16	245.89	14	210.1	8	194.72	3	191.86	3	236.98	3
TC 24/98	130.11	1	168.45	8	165.82	2	227.5	11	201.5	4	191.74	3	230.82	2
TC 4/00	161.68	6	161.59	7	165.82	2	227.4	11	207.29	5	191.78	3	230.79	2
TC 25/98	175.84	8	175.58	9	165.91	2	227.42	11	207.29	5	197.7	4	230.86	2
TC 6/98	168.9	7	168.55	8	219.2	10	210.1	8	194.87	3	191.85	3	236.92	3
TC 8/99	130.29	1	175.42	9	307.37	23	233.31	12	223.55	8	191.82	3	230.96	2
TC 1/00	168.56	7	168.56	8	239.14	13	221.66	10	218.65	7	220.91	8	230.91	2
TC 7/98	168.55	7	168.55	8	239.23	13	210.11	8	194.62	3	191.79	3	236.99	3
TC 3/99	154.42	5	154.42	6	239.22	13	210.13	8	207.31	5	215.05	7	230.85	2
TC 5/00	209.41	13	209.24	14	239.02	13	204.54	7	194.79	3	191.83	3	236.99	3
TH 6/07	236.68	17	236.68	18	259.3	16	216	9	212.99	6	213.82	7	230.87	2
TC 11/98	202.93	12	202.68	13	165.88	2	198.66	6	213.11	6	184.67	2	230.79	2
TC 7/99	202.71	12	202.62	13	219.05	10	233.29	12	207.24	5	203.47	5	230.83	2
TH 3/08	130.22	1	250.36	20	245.9	14	216.15	9	212.97	6	214.95	7	230.87	2
TC 4/98	202.94	12	202.68	13	335.55	27	227.46	11	224.52	8	226.8	9	230.88	2
TH 2/09	161.76	6	161.5	7	165.81	2	233.31	12	207.33	5	191.81	3	230.86	2
TH 4/08	175.7	8	182.27	10	165.8	2	215.89	9	207.31	5	191.79	3	230.91	2
TC 32/98	168.7	7	243.47	19	314.66	24	262.42	17	223.49	8	203.46	5	236.98	3
TC 10/00	209.72	13	209.46	14	348.53	29	227.49	11	218.76	7	226.73	9	230.9	2
TH 2/08	222.99	15	222.98	16	272.84	18	215.99	9	194.82	3	197.64	4	243.01	4
TC 7/97	223.09	15	222.92	16	286.53	20	244.96	14	217.8	7	203.44	5	236.94	3
TH 7/08	161.51	6	161.51	7	279.68	19	210.14	8	212.87	6	215.09	7	230.93	2
TC 3/98	196.07	12	195.98	12	286.38	20	204.35	7	212.88	6	220.82	8	230.86	2
allele no.		14		17		17		12		6		8		3

4.3 PFGE of Malaysian *Shigella sonnei*

1. The standardized PulseNet protocol for *Shigella sonnei* was used. Conditions: 2.16 s - 54.17 s, 25 hrs, 6 V/cm, 1% gel
2. Only 1 enzyme (*Xbal*) was used, as this enzme was very discriminative.
3. PFGE was highly discriminative. Each strain was subtyped into a unique pulsotype (Fig 1 and 2).
4. The discriminative ability of both PFGE and MLVA was comparable as all the strains were clearly distinguished.

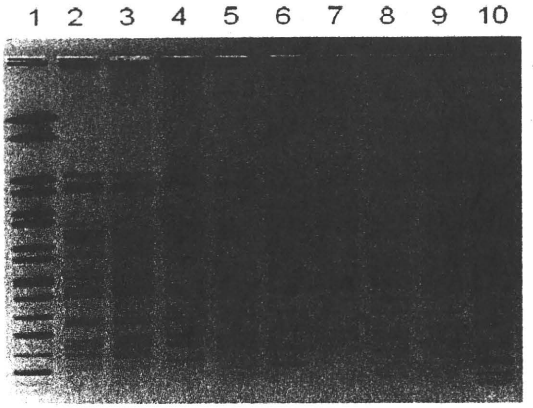

Fig 1 Representative *S. sonnei Xbal* pulsotypes

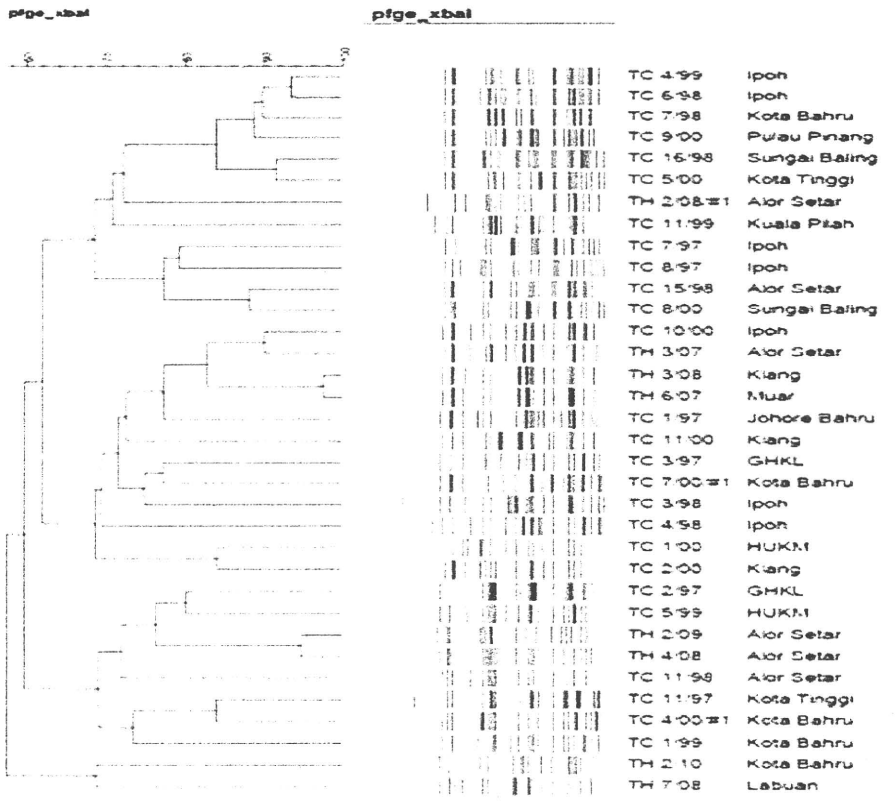

Fig 2. Cluster analysis of Malaysian *Shigella sonnei*

Part 2 of research work

5.0 Multilocus Variable Number Tandem Repeats Analysis (MLVA) of *Salmonella* Typhimurium

This is the continuation of year 2009/10 work.

A total of 97 strains (50 clinical, 26 zoonotic, and 21 food strains) of *S.* Typhimurium were examined in this study. Five VNTR loci were targeted in MLVA assay of these strains, namely STTR3, STTR5, STTR6, STTR9, and STTR10pl. The primers used in this study were adapted from study done by Lindstedt et al. (2004). Results had shown that the polymorphisms of the VNTR loci (STTR5, STTR6, STTR9, and STTR10pl) were lower compared to study done by Lindstedt et al. (2004). However, STTR3 showed higher polymorphism in this study compared to previous study (Lindstedt et al., 2004). All strains were subtyped into 38 MLVA types with a discriminatory power of 0.82. The high genetic homogeneity among strains from different sources suggests that Malaysian S. Typhimurium strains show high clonality and lack genetic diversity.

Inference : VNTR loci selected in this study were insufficient for detailed typing of S. Typhimurium strains in Malaysia which are highly clonal.

Future study : Repeat MLVA assay of *S.* Typhimurium strains using primers recommended by laboratory standard operating procedure for Pulsenet MLVA of *S.* Typhimurium.

Progress : Laboratory standard operating procedure for Pulsenet MLVA of *S.* Typhimurium targeted 7 VNTR loci. Currently, four VNTR loci, namely ST3, ST5, ST7, and STTR10, of 72 strains were evaluated. Evaluation process is still ongoing.

Status : Ongoing

6.0 Multilocus Variable Number Tandem Repeats Analysis (MLVA) of *Salmonella* Enteritidis

A total of 112 strains of *S.* Enteritidis were examined in this study, including 39 clinical and 73 zoonotic strains of domestic origin, isolated from year 2003 to year 2008. The primers used in this study were adapted from the published work of Malorny *et al.* (2008) and Cho *et al.* (2007). Five VNTR loci (SENTR4, SENTR5, SENTR6, SENTR7, and SE7) were tested in this study. The PCR conditions were successfully optimized. All 112 strains were subjected to MLVA assay, and data analyses using GeneMapper and BioNumerics are in progress.

Future study : MLVA assay of *S.* Enteritidis strains is to be completed by adding primers recommended by laboratory standard operating

procedure for Pulsenet MLVA of *S.* Enteritidis.

Status : Ongoing

7.0 Pulsed Field Gel Electrophoresis (PFGE) of *Salmonella* Typhimurium

The *Xba*I pulsotypes of the 97 strains of *S.* Typhimurium examined in the MLVA study are to be confirmed. PFGE typing of 92 strains were completed. Data analyses using BioNumerics are in progress.

Future study : The PFGE typing of the remaining 7 strains is to be completed. The result of PFGE typing of the 92 strains has shown that the strains were subtyped into 74 pulsotypes, with a discriminatory index of 0.99.

Problems :

- The steps involved in PFGE are tedious and require extra care and longer time to be completed.
- Strong backgrounds were observed. Problem was solved by increasing the washing of plugs by TE buffer to 8 times.

Status : Ongoing

Research Output:

1. Soo Tein Ngoi, and Kwai Lin Thong. 2009. Development of Multiple-locus Variable-number Tandem Repeat Analysis (MLVA) for Subtyping of *Salmonella enterica* subsp. *enterica* Serovar Typhimurium. Book of abstracts of International Congress of Malaysian Society for Microbiology (ICMSM 2009), p. 133. Parkroyal Hotel, Penang, Malaysia.
2. Soo Tein Ngoi, and Kwai Lin Thong. 2010. Evaluation of Multilocus Variable Number Tandem Repeat Analysis (MLVA) in Determining Genetic Diversity of *Salmonella enterica* Serovar Typhimurium. Book of abstracts of My1Bio Conference, p. 110. Berjaya Times Square Hotel, Kuala Lumpur, Malaysia.
3. Presentation of Application of MLVA of Shigella sonnei and Salmonella in Malaysia at the 2010 7th PulseNet Asia Pacific Strategic Planning Meeting in Hong Kong, in 20-22 December 2010.
4. **Manuscripts in preparation for work done**. Detailed data analysis is still on going. Once analysis is completed, manuscripts will be prepared for publication.

Verified by Dean,
Faculty of Science

Prepared by
Prof Thong Kwai Lii

プロジェクト２：ウイルス

デング熱