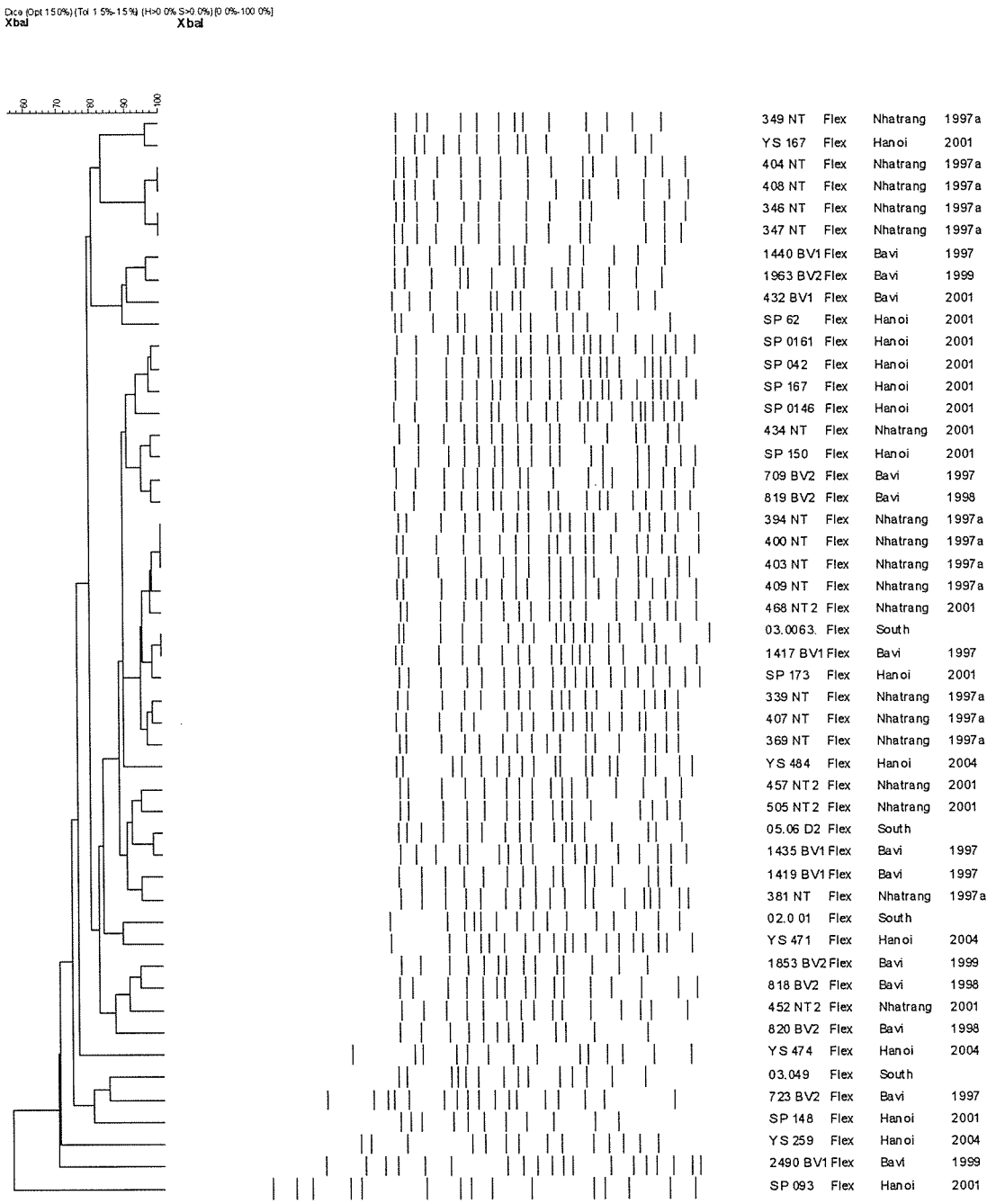


Fig.1. *S. flexneri* PFGE patterns

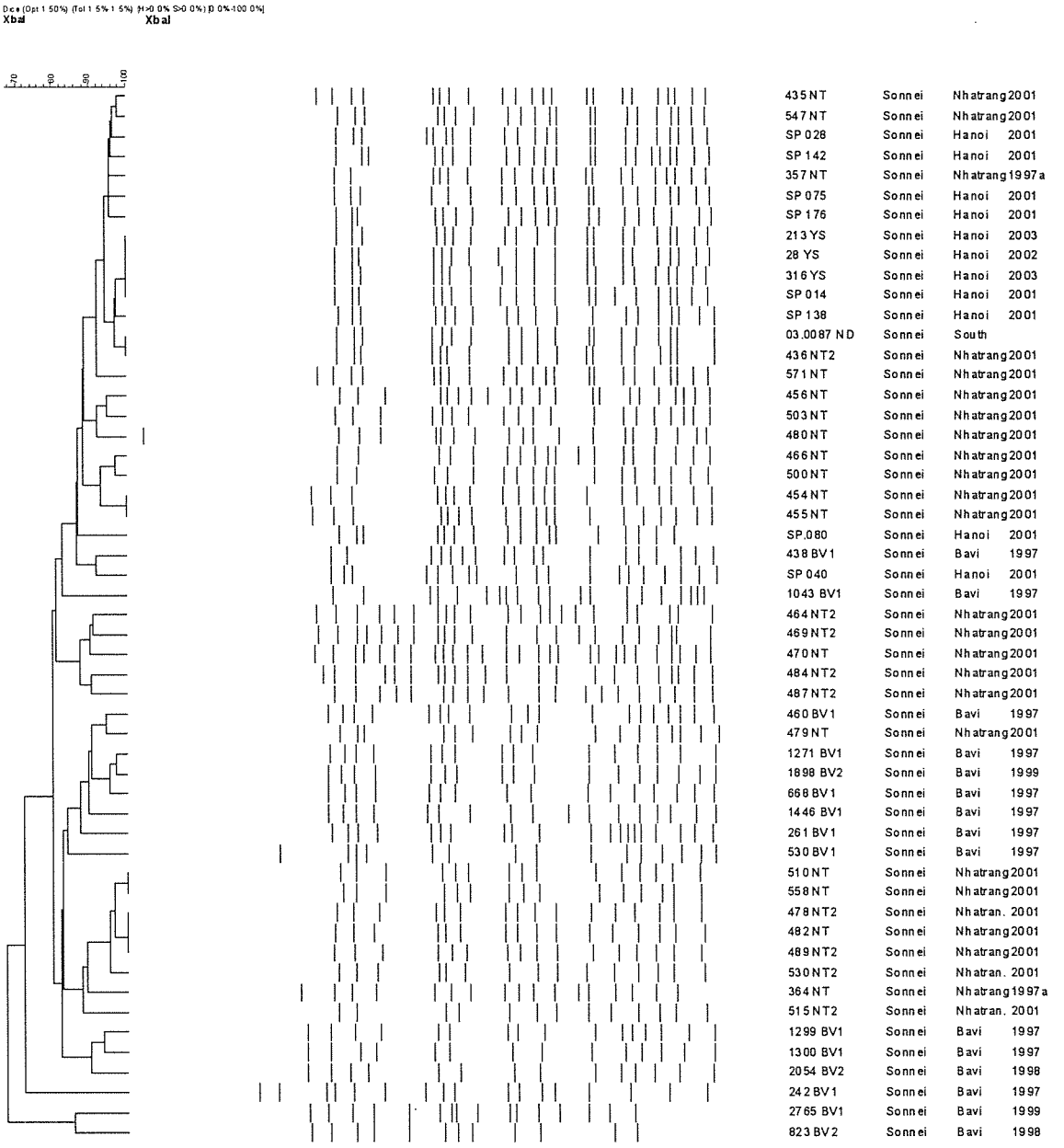


Fig.2. *S. sonnei* PFGE patterns

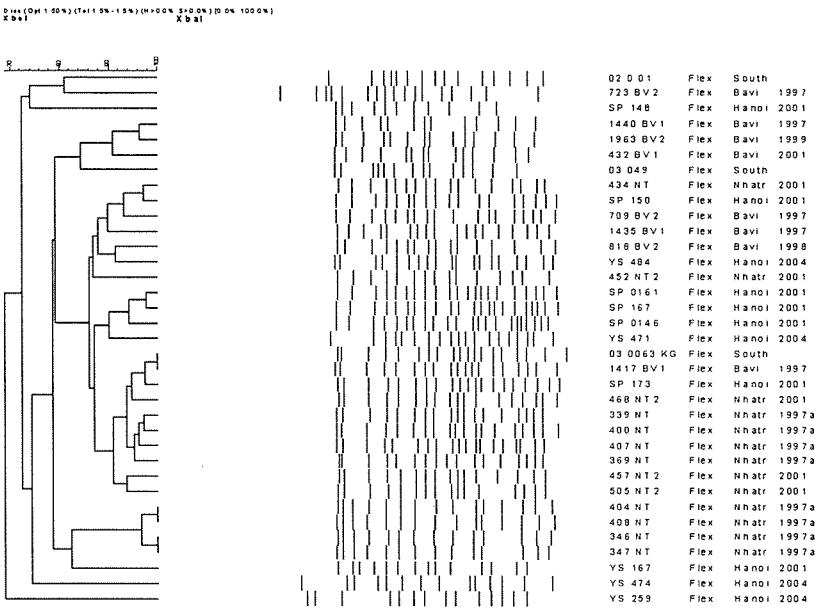


Fig.3. 35 *S. flexneri* MDR to 4 types of drug

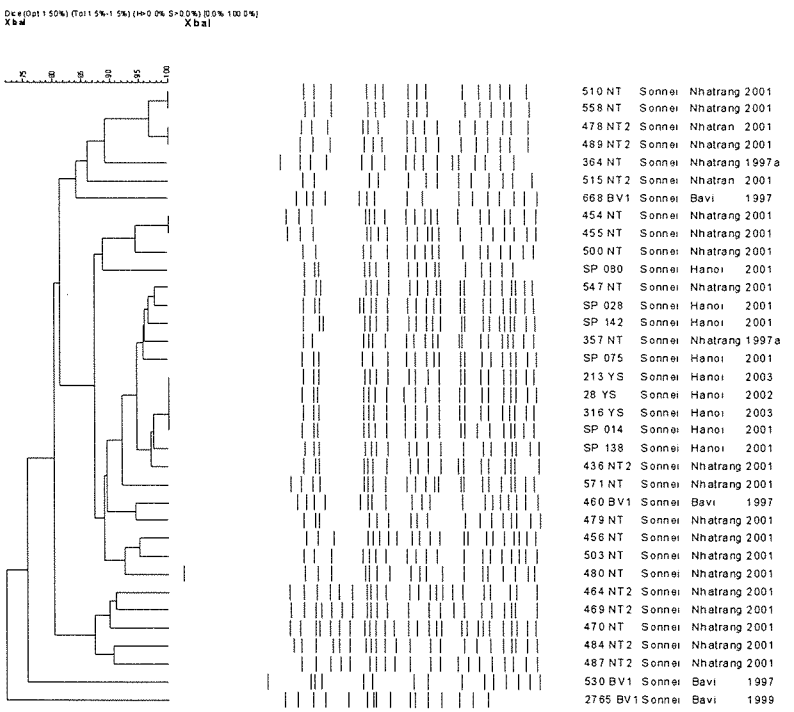
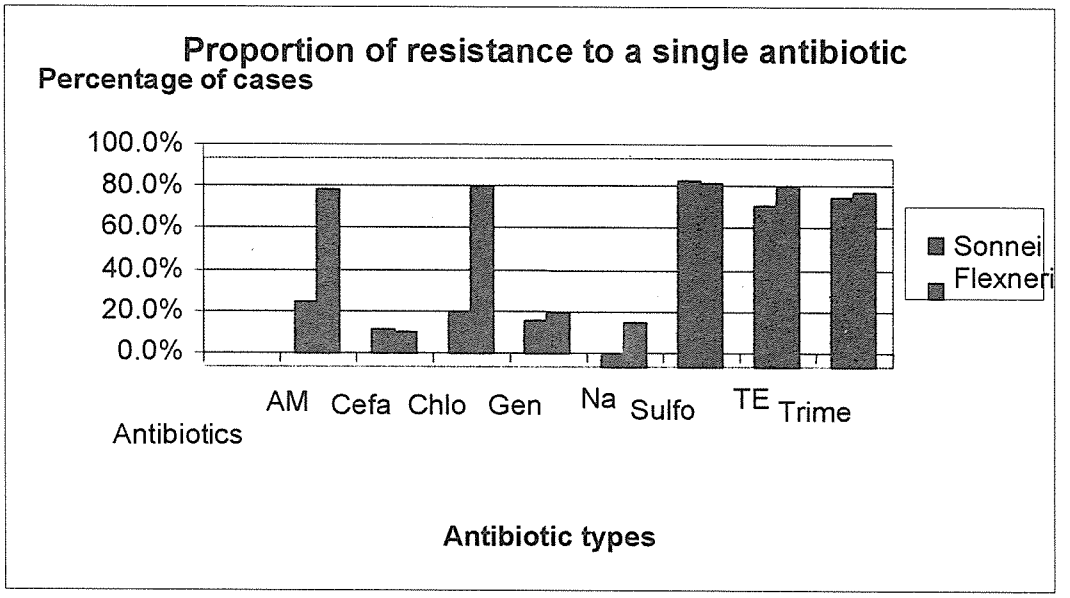


Fig.4. 35 *S.sonnei* MDR to 3 types of drug

Map1. Proportion of resistance to single antibiotic among 102 *S. sonnei* and *flexneri* strains.



Among 102 *S. flexneri* and *S. sonnei* isolates, 51 isolates (50%) were resistant to ampicillin, 49 isolates (48%) were resistant to chloramphenicol, 17 isolates (16.6%) were resistant to gentamycin, 13 isolates (12.7%) were resistant to nalidixic acid, 83 isolates (81.4%) were resistant to tetracycline, 11 isolates (10.8%) were resistant to cefalothin, 90 (88.2%) isolates were resistant to sulphamethoxazole (Map.1).

Our findings have shown a high prevalence of drug resistance in both *S. flexneri* and *S. sonnei* which is correspondent with several studies reviewed by Yiu-wai Chu et al. (1998). This indicates that drug resistance among *Shigella* has widely spread. Also, multidrug resistance was common in *Shigella* according to AA Lima et al.(1998) and Kaisar A. Talukder wt al. (2006).

Discussion

In the same geographic region, almost all patients were infected by locally epidemic strains of *Shigella*. Antimicrobial agents such as Ampicillin,

Chloramphenicol, Tetracyclin and Trimethoprim-sulphametoxazole should no longer be used for treatment of *Shigella* infection in Vietnam. On the other hand, multidrug resistant (MDR) strains of *Shigella* are found in different provinces, suggest that the MDR strains may spread to other parts of Vietnam very rapidly. The travel and commercial communications are developing year by year which will actively contribute to the spreading out the MDR. Furthermore, the abuse and misuse of antibiotic is still a big issue in health care in Vietnam.

Acknowledgement

We would like to thank the National Institute of Infectious Disease, Tokyo Japan for financial support.

We also would like to thank our colleagues from NIHE and different hospitals and Centers for preventive medicine in Vietnam for the supply of *Shigella* strains

Reference

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Kaisar A. Talukder, Zhahirul Islam, Dilip K. Dutta, M. Aminul Islam, Bijay K. Khajanchi, Ishrat J. Azmi, Mohd S. Iqbal, M.A.Hossain, A.S.G. Faruque, G.Balakrish Nair and David A. Sack "Antibiotic resistance and genetic diversity of

Shigella sonnei isolates from patient with diarrhoea between 1999 and 2003 in Bangladesh".

REPORT OF RESEARCH SPONSORED BY GRANT FROM NATIONAL INSTITUTE OF INFECTIOUS DISEASES, JAPAN

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FEBRUARY 2007

FINAL REPORT OF RESEARCH SPONSORED BY THE NATIONAL INSTITUTE OF INFECTIOUS DISEASES, JAPAN

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STUDY TITLE: Genotyping of *Salmonella* and *Burkholderia pseudomallei* in Malaysia

STUDY FACILITY: Institute of Biological Sciences, Faculty of Science, University of Malaya, 50603, Kuala Lumpur, Malaysia.

OBJECTIVES OF STUDY

1. To determine the phenotypic and genotypic characterization of major *Salmonella* serovars in Malaysia: antibiotic resistance, integron and PFGE subtypes.

2. To determine the molecular differences between strains *Burkholderia pseudomallei,* an emergent potential agent of bioterrorism of public health concern, isolated from hyperendemic states and normal states in Malaysia.

3. To continue dissemination of PFGE technique as an epidemiological tool for foodborne bacterial pathogens in Malaysia.

BACKGROUND

Salmonella strains were isolated from the environment, animals and various food types. Clinical strains were obtained from the Bacteriology Unit of Institute of Medical Research. These clinical strains were isolated from patients at the clinical laboratories in Government hospitals. As compared to the first year of study, more sources of *Salmonella* were investigated for the second year of study so as to provide a better picture of the diversity and distribution of molecular subtypes of *Salmonella* serovars studied. To ensure the quality of PFGE data are comparable to co-researchers in the PulseNet Asia Pacific region and to facilitate exchange of gel images, we participated in the external quality assurance assessment headed by the National Public Health Laboratory, Hong Kong. Prof Thong recently attended the PulseNet training course on BioNumerics in

Hong Kong (15-17 February, 2007) to learn the use of the software in analyzing PFGE gel images.

Methodologies

i. Strains collection: Representative environmental and food samples were processed to isolate *Salmonella* by conventional methods. Strains were confirmed by biochemical tests, API commercial kits and serotyped by Kauffman-White scheme. Zoonotic and clinical Salmonellae were obtained from the Veterinary Institutes and Institute for Medical Research respectively. Confirmation of purity of strains was carried out by streaking onto selective BSA plates. Salmonellae cultures were then stored in glycerol at minus 20°Celsius.

ii. Antimicrobial susceptibility tests

a. The antimicrobial susceptibility of the strains was carried out according to the NCCLS protocol by the Kirby-Bauer Disc Diffiusion method. Determination of MICs was carried out on selected resistant strains.

iii. Determination of resistant genes

a. PCR based detection of antimicrobial genes was determined and correlated to the phenotypes.

iv. **Determinationof integrons and Salmonella Genomic Islands (SGI1)**

a. Prevalence of integron and SGI were deterimined by polymerase chain reaction (PCR) using published sequences.
b. DNA content of the integrons was determined by DNA sequencing.

v. **PulseNet Asia Pacific Activities**

a. Adoption of standardized PulseNet Protocol for PFGE analysis
b. Training of manpower in application of PFGE

1. Isolation and identification of Salmonella serovars from Malaysia from various sources

Number of strains isolated and cultured between May 2006 to December 2006. Some of the strains were isolated prospectively while some were collected restropectively from the Veterinary Research Institute and Institute of Medical Research. We have isolated about 100 Salmonella strains from various Serotypes including S. Typhi, S. Typhimurium, S.Enteritdis, S.Corvallis. At least 50 strains were isolated from 150 food samples (raw meat, beef,chicken,and RTE).

2. **Antimicrobial Susceptibility Patterns**

Antimicrobial Resistance Profiles of *Salmonella* Corvallis

Resistant Phenotype	No. of strains
A, C, SxT, S, T	1
A, S, T	2
SxT, S T	2
SxT, T	1
S, T	69
T	1
sensitive	7

A ampicillin, C chloramphenicol, SxTsulphomazoxale S streptomycin,

Nx Nalidixic acid, T tetracycline,Su sulphonamide, cip ciproxacin

Antimicrobial Resistance Profiles *Salmonella* Typhimurium

Antimicrobial resistant profiles	No of Strains
T	1
S	4
SxT T	2
AST	4
ST	9
ACST	6
ACSTSxT	2
CSxTST	1
CSxTST	4
AKST	1
SxTKST	2
ASxTST	1
Sensitive	18

Antibiograms of zoonotic *Salmonella* Enteritidis

Pattern of resistance	No of strains
S	2
T	10
Nx T	1
S T Su	1
SXT Su T	1
A S TE Su	1
Cip SXT Su T	1
C Cip Nx SXT Su	2
C Cip S Nx SXT Su	4
sensitivie	42

Table of antibiograms of clinical *Salmonella* Enteriditis

Antibiograms	**No of strains**
Nx	2
T	5
Su	1
Nx, Su	1
A, Nx, Su	2
Su, T	1
A, Nx	1
A, Gen, Km, Nx	1
A, Na, Su, Tm, SXT, T	1
Su, Tm, SxT T	11
Kan, Nx, T	1
A, Km, Nx, Su, Tm, T	1
sensitive	3

Isolation, detection, confirmation and antibiogram of *Salmonella* spp from a variety of foods (May –Dec 2006)

Food items	No of Salmonella spp	antibiograms
50 seafoods	2	AKT (n=2)
43 leafy salad RTE	1	Su T
52 poultry products	14	S Su T (n=3) S Su T C Su T (n=2) Cip T Sm T C Tm T SXT Su C Tm T Su A S Tm T Nx SxT Cip Su A C S Tm T SxT Nx Su C Cip T
51 raw meat (beef and beef products, meat	7	sensitive (n=3) Cip Su K, Cip T (n=2) Su

3 Determination of integron, integrase gene and *Salmonella* Genomic Islands in selected *Salmonella* species

The emergence of multidrug resistance (MDR) among bacteria is an increasing problem worldwide. An efficient route of acquisition and dissemination of antimicrobial resistance is through R-plasmids, transposons and integrons. Class 1 integrons contain antimicrobial resistance genes in the internal variable region and is found near the 3' end of Salmonella Genomic Island 1(SGI1). In this study, SGI1, class 1 integrons, and integrase gene (*intI*) were determined in 36 MDR strains of *Salmonella* Typhimurium.

Eleven isolates (31 %) were positive for left junction of *S*GI1, 500 bp amplicon and right junction of *S*GI1 with retron sequence, 515 bp amplicon. All the strains were negative for right junction without retron sequence. One strain (3 %), STM 252/01 with ChlSSulSXTTetTmp antibiogram, produced 640 bp amplicon by U7-L12 and LJ-R1 primers specifically designed for detecting left junction of *S*GI1. Blast results showed that 640 bp amplicon was part of *S.* Typhimurium Bacteriophage ST104 sequence .

Four integrons were identified in *S.* Typhimurium. Seven isolates (19 %) showed 650 and 1950 bp class integrons. Four isolates (11 %) showed 1000 bp integron. Eight isolates (22%) with AmpChlSSulTet antibiogram showed 1000 and 1200 bp integrons. One strain (3%) with AmpSSulSXTTmp showed a 170 bp amplicon. Fifteen isolates showed 1310 bp amplicon. DNA sequencing showed that 170 bp amplicon was part of integron and but the 1310 bp amplicon was not integron.

From blast results of DNA sequences, the 650 bp class 1 integron harbored streptothricin acetyltransferase (*sat*) gene that encoded the enzyme streptothricin acetyltransferase and conferred resistance to streptothricin; 1000 bp class 1 integron harbored *aadA2* gene that encoded the enzyme aminoglycoside adenyltransferase AAD(3") and conferred resistance to streptomycin and spectinomycin, and it was part

of *S*GI1; 1200 bp class 1 integron harbored *pse-1*gene that encoded the enzyme β-lactamase and conferred resistance to ampicillin, and it was part of *S*GI1; 1950 bp class 1 integron harbored *dhfrXII* gene at the 5' end that encoded the enzyme dihydrofolate reductase and conferred resistance to trimethoprim and *aadA2* gene at the 3' end that encoded the enzyme aminoglycoside adenyltransferase AAD(3") and conferred resistance to streptomycin and spectinomycin, and it was part of *S*GI1. The 170 bp amplicon is part of class 1 integron which harbored partial of *aadA*2 gene. However, 1310 bp amplicon was not a class 1 integron although it was part of the *S.* Typhimurium genome.

Nineteen isolates (53%) were positive results for integrase (*intI1*) gene.
(results summarised in Table 1)

A manuscript in preparation for publication.

TabLe 1. Prevalence of *S*GI1, integron, and integrase genes in *Salmonella* Typhimurium

Antibiogram		*S*GI1 left junction (500 bp)		*S*GI1 right junction without retron (460 bp)		*S*GI1 right junction with retron (515 bp)		Integron			Integrase gene (558 bp)	
								+ve		-ve		
	No.	+ ve	- ve	+ve	-ve	+ ve	- ve	(bp)	No.		+ve	-ve
AmpChlSSulTet	8 (22%)	8 (22%)	0 (0%)	0 (0%)	8 (22%)	8 (22%)	0 (0%)	1000, 1200	8 (22%)	0 (0%)	8 (22%)	0 (0%)
AmpChlSSXTTetTmp	2 (6%)	0 (0%)	2 (6%)	0 (0%)	2 (6%)	0 (0%)	2 (6%)	1950, 650	2 (6%)	0 (0%)	2 (6%)	0 (0%)
AmpSSulSXTTmp	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (1%)	0 (0%)	1 (3%)	170[a]	1 (3%)	0 (0%)	0 (0%)	1 (3%)
AmpSSulTet	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	1310[b]	0 (0%)	1 (3%)	0 (0%)	1 (3%)
AmpSTet	2 (6%)	0 (0%)	2 (6%)	0 (0%)	2 (6%)	0 (0%)	2 (6%)	1310[b]	2 (6%)	0 (0%)	0 (0%)	2 (6%)
Amp(S)	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	1310[b]	1 (3%)	0 (0%)	0 (0%)	1 (3%)
ChlKanSSulSXTTetTmp	3	0	3	0	3	0	3	1950, 650	3	0	3	0

	(8%)	(0%)	(8%)	(0%)	(8%)	(0%)	(8%)		(8%)	(0%)	(8%)	(0%)
ChlKanSSulTet	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	1000	1 (3%)	0 (0%)	1 (3%)	0 (0%)
ChlSSulSXTTetTmp	1 (3%)	1[c] (3%)	0 (0%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	1310[b]	1 (3%)	0 (0%)	0 (0%)	1 (3%)
ChlSSXTTetTmp	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	1310[b]	1 (3%)	0 (0%)	0 (0%)	1 (3%)
KanSSulSXTTetTmp	2 (6%)	0 (0%)	2 (6%)	0 (0%)	2 (6%)	0 (0%)	2 (6%)	1950, 650	2 (6%)	0 (0%)	2 (6%)	0 (0%)

Antibiogram		*SGI1* left junction (500 bp)		*SGI1* right junction without retron (460 bp)		*SGI1* right junction with retron (515 bp)		Integron			Integrase gene (558 bp)	
								+ve		-ve		
	No.	+ ve	- ve	+ve	-ve	+ ve	- ve	(bp)	No.		+ve	-ve
SSulSXTTmp	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	0 (0%)	1 (3%)	-	0 (0%)	1 (3%)	0 (0%)	1 (3%)
STet	12 (33%)	3 (8%)	0 (0%)	0 (0%)	3 (8%)	3 (8%)	0 (0%)	1000	3 (8%)	0 (0%)	3 (8%)	0 (0%)
		0 (0%)	9 (25%)	0 (0%)	9 (25%)	0 (0%)	9 (25%)	1310[b]	9 (25%)	0 (0%)	0 (0%)	9 (25%)

Total	36 (100%)	11 (31%) $1^c = 1$ (3%)	24 (67%)	0 (0%)	36 (3%)	11 (31%)	25 (69%)	650, 1950 bp	7 (19%)	1 (3%)	19 (53%)	17 (47%)
								1000 bp	4 (11%)			
								1000, 1200 bp	8 (22%)			
								Total	19 (53%)			
								170^a	1 (3%)			
								1310^b	15 (42%)			

Note: Amp = Ampicillin; Chl = Chloramphenicol; Kan= Kanamycin; NA = Nalidixic acid; S = Streptomycin; Sul = Sulfonamide compound; SXT = Trimethoprim-sulfamethoxazole; Tet = Tetracycline; Tmp = Trimethoprim; 170^a: part of integron gene; 1310^b: non-specific binding; not integron gene; 1^c: 640 bp (non-specific binding, not *SGI1* left junction).

4. Molecular Epidemiology of *Burkholderia pseudomallei*

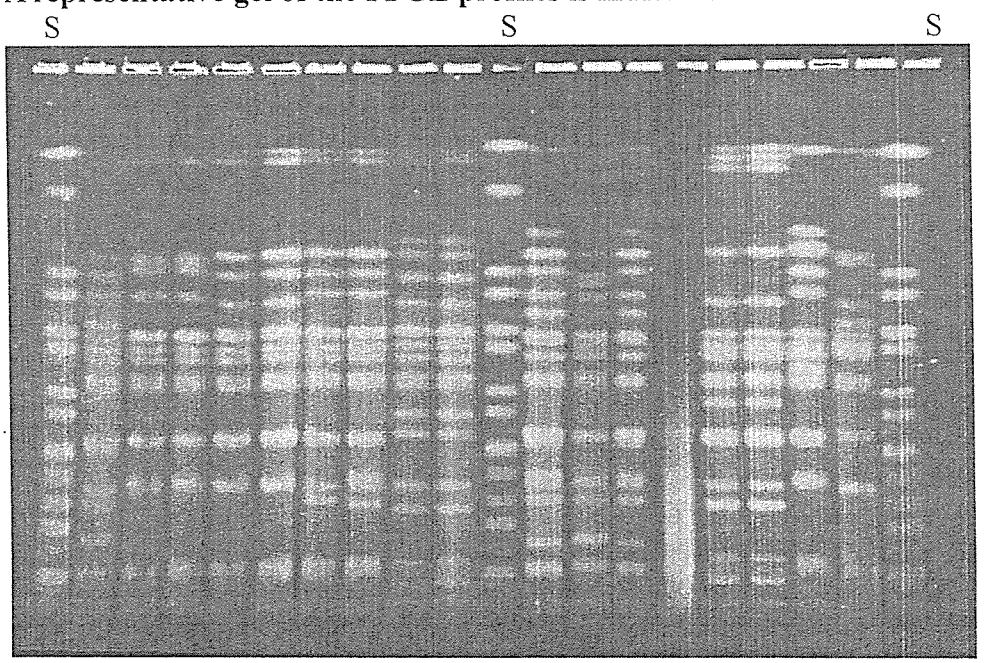
Objective: To determine the molecular differences between strains *Burkholderia pseudomallei,* an emergent potential agent of bioterrorism of public health concern, isolated from hyperendemic states and normal states in Malaysia.

Meliodiosis is endemic in Southeast Asia and Asia. Interest in this disease has increased with the recognition of this bacterium as a potential bio-weapon. Cases of meliodosis are not evenly distributed throughout Malaysia. More cases are reported from some states and these are considered as hyperendemic or hot spots for meliodosis. No studies have been reported on whether unusually virulent or highly infective strains of B. pseudomallei have been responsible of infections in these hot spots. Hence, to map out the epidemiology of *B. pseudomallei*, strains from hyperendemic states (Sabah, Kelantan, Terengganu) will be compared to strains from normal endemic states like Selangor and Johor by PFGE.

Results:
The first part of the work was to develop suitable protocol for the separation of *B. pseudomallei* genome. This was successfully done.

We have analysed at 90 strains of *B.pseudomallei* from various endemic and non-endemic States in Malaysia. Isolates from different states gives different pfge profiles. Work on this project is still on-going by a Master Medical student

A representative gel of the PFGE profiles is indicated.



Lane 2 to 6 = from Trenganu; Lane 7 & 8 = from Sk State
Lane 9 (=10) = from J State (same patient) Lane 12 = from Sh State Lane 13, 14(=15), 16(=17), 18, 19= from K State

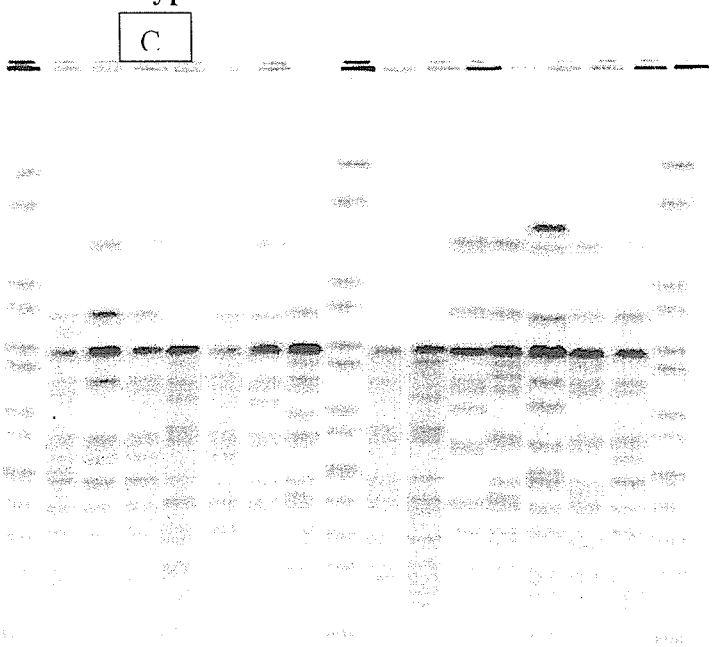
late.

. Bhd.

4. PFGE of *Salmonella* serovars

PFGE typing and analysis is a continuous effort. We have changed our old protocol to adopt the Standardised PulseNet Protocol. A representative of PFGE analysis is as indicated. Once the data is completed analysed, a manuscript will be prepared in two months time to document the diversity of Salmonella spp studied.

Salmonella Typhi

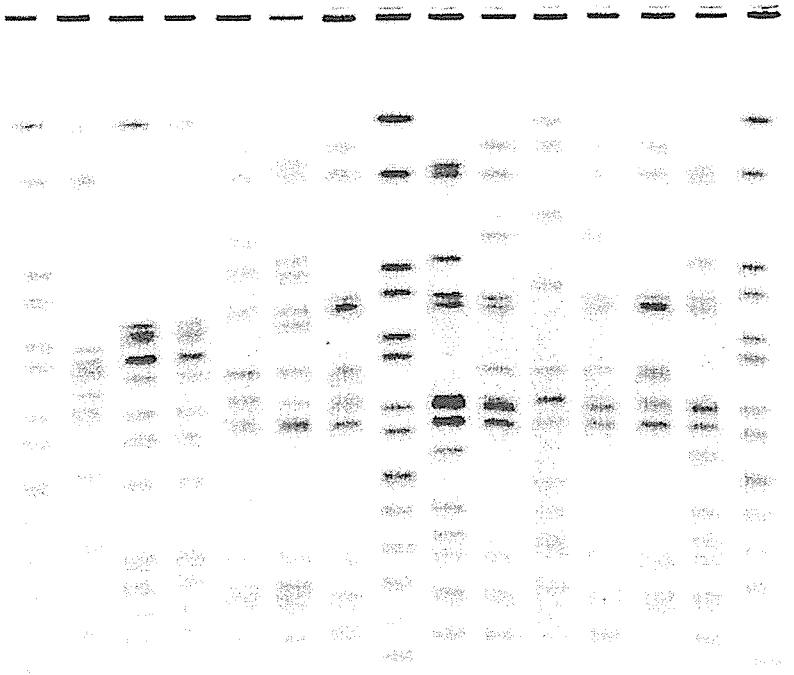


We have analysed 48 strains from an outbreak in a highly endemic State in Malaysia
2 (2005); 48 (2006)
Number of different pattern - 13
The most common pattern of the *Xba* 03 (*C)
Recurrent Pattern

late.

. Bhd.

Salmonella Typhimurium



- We have analysed at least 100 isolates from various sources. Representative profiles from different animal hosts, farms, year
- Animal isolates- 60 (42 different profiles)

Salmonella Enteritidis

Representative profiles of *S.* Enteritidis subtyped Limited patterns obtained among the Malaysian *S.* Enteritidis.