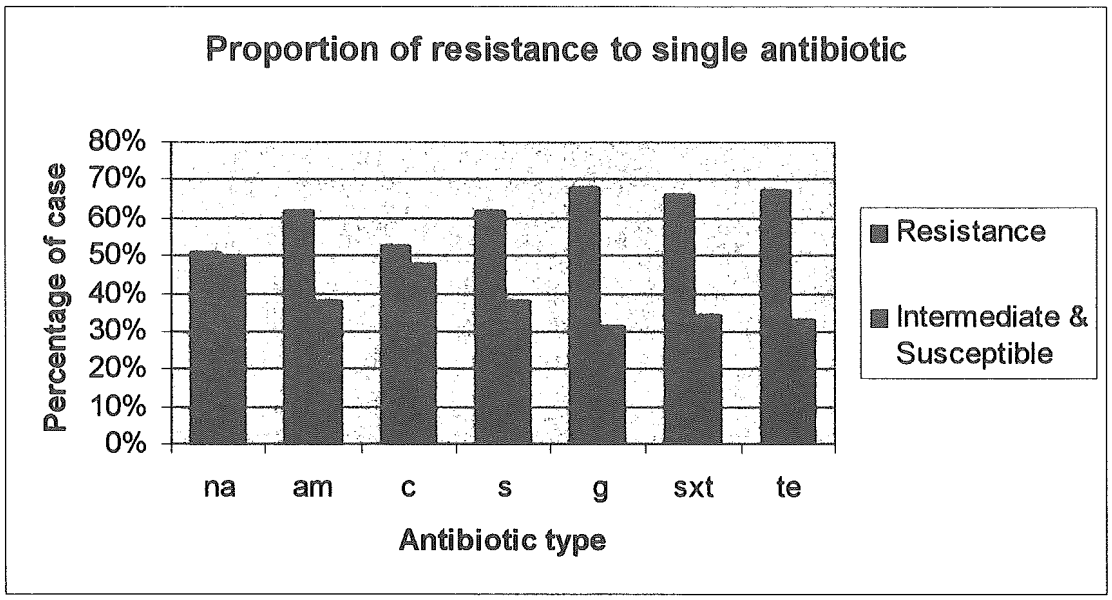
Map1. Proportion of resistance to single antibiotics among 98 *Salmonella enterica* serovar.Typhi strains.



na: nalidixic acid; am: ampicillin; c: Cloramphenicol; s: Streptomycine; g: Gentamycine; sxt : sulphamethoxazole; te: tetracycline.

A total of 98 isolates, 50% of them were resistant to nalidixic acid, 61.2% were resistant to ampicillin, 52% isolates were resistant to chloramphenicol, 61.2% isolates were resistant to streptomycin, 65% were resistant to sulfamethoxazole and 66% isolates were resistant to tetracycline (Map.1)

Although MDR patterns have been characterized in some other study in VN, however, the outcome of our study should be note that the implementation of suitable measure in the field of public health in order to prevent MDR of typhoid fever patterns, such as clonal spreading of MDR S. serovar Typhi of carriers rather than multiple source of infections.

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Arbeit R.D. 1999. Laboratory proceduces for the epidemiologic analysis of microorganisms, p. 116-137. Manual of clinical microbilology, 7th ed. American Society for Microbiology, Washington, D.C.)

Publication list for this work

1) **Analysis of *Salmonella enterica* Serotype Typhi Pulsed-Field Gel Electrophoresis Patterns in Different Regions in Vietnam. Will be submitted.**

Acknowledgement

We would like to thank our colleagues from different Hospitals and Centers for preventive medicine in Vietnam for the supply of *Salmonella* strains.

This study was kindly supported by grant of Ministry of Health, Welfare and Labor of Japan through the grant on "Research for emerging and re-emerging infections".

TITLE: **Subtyping and surveillance of *Salmonella* spp in Malaysia**

Project Leader: Professor Dr Haruo Watanabe
Deputy-Director General, National Institute of Infectious Diseases, Japan

Researchers : 1.Prof Dr Kwai Lin Thong,
Microbiology Division, Institute of Biological Sciences,
Faculty of Science, University of Malaya, Kuala Lumpur,
Malaysia.

2. Dr. Norazah Ahmad.
Head, Bacteriology Unit, Institute for Medical Research,
Kuala Lumpur, Malaysia.

3. Dr Rohani Md Yasin,
Head, Diagnostic Services Division, Institute for Medical Research, Kuala Lumpur, Malaysia.

SUMMARY

Salmonella Typhi remains the top *Salmonella* serovar in Malaysia. A total of 682 *S.* Typhi strains were submitted to the Institute for Medical Research (IMR) for phage typing. In 2005, 13 phage types were observed with the predominant PT B1.
Most of these strains were obtained from a recent outbreak in one particular State in the Peninsular Malaysia. To further differentiate these isolates, PFGE was applied on 85 isolates. Nine different profiles were observed. The predominant pulsotype was an endemic strain seen in the population in the affected area since 1990. The exact cause of the outbreak was not identified but most probably transmitted through carriers who are food handlers. The outbreak occurred during a festival.
The present observation concurred with our previous report that strains associated the outbreaks are genetically more homogenous as compared with those from sporadic cases of typhoid fever (Thong et al, JCM,1994).
We participated in the standardization of PFGE so that the DNA fingerprints obtained can be compared among members of the PulseNet Asia Pacific.
Using this modified Protocol we have subtyped about 260 strains from the most common nontyphoidal *Salmonella* (*S.*Enteritidis, *S.* Typhimurium. *S.* Weltevreden, *S.* Corvalis, *S.* Paratyphi B, *S.* Tshiongwe) in Malaysia. The diversity of the strains were determined and analysed by GelCompar software.
Overall each *Salmonella* serovar has its own characteristic PFGE profiles. Some serovars are more conserved like S. Enteritidis while others are more diverse.

ORIGINAL OBJECTIVES OF PROJECT

1. To maintain surveillance and molecular tracking of *Salmonella* spp from clinical and environmental sources in Malaysia.

2. To perform phenotypic, serological, antimicrobial resistance and molecular analysis of resistant genes of the stains under surveillance during the study period

3. To subtype the strains by pulsed field gel electrophoresis according to the PulseNet Asia Pacific Group Protocol

4.To train more researchers in the public health laboratories in the use of standardize method to ensure continuity and success of PulseNet Asia Pacific.

5.To participate in the establishment of the PulseNet Asia Pacific through comparison and exchanges of profiles of strains from Malaysia and member countries to identify outbreak strains and new emerging clones.

Status of project

1. On-going
2. manuscripts in preparation

Prepared by Prof Dr Kwai Lin Thong
University of Malaya, Kuala Lumpur, Malaysia

RESEARCH OUTPUTS

1. SURVEILLANCE OF *SALMONELLA* INFECTION IN MALAYSIA

Background

In Malaysia, the current foodborne disease surveillance data collected is mainly through physician based surveillance and outbreak investigations. Through this system, notification is received from government health facilities consisting of health canters, outpatient departments and hospitals and also from the private hospitals and general medical practitioners. The food borne disease included are cholera, typhoid, hepatitis A and dysentery have been less than 5/100,000 population, sporadic in nature with outbreaks confined to certain areas only. In the case of food poisoning, cases have mainly been institutions ranging from 20 to 30/100,000 population (Bulletin MKAK,2005).

In addition, the Ministry of Health, Malaysia also implements a syndromic approach to infectious disease notification and laboratory investigation to strengthen surveillance and rapid response to emerging infectious diseases. In the laboratory-based surveillance, 3 pathogens, that is *Salmonella* spp, *Salmonella* Typhi *and Salmonella* Paratyphi were monitored as foodborne pathogens. This system requires participating hospitals to send their isolates to the Institute for Medical Research (IMR) for re-culture and typing. The Surveillance section will publish periodic bulletins to communicate to the relevant authorities. However, the database may not be nationally representative since it covers only government hospital laboratories (*per com. Dr.Wan MH, Deputy Director, Disease Control Div., Public Health Dept, Ministry of Health*).

Table 1 gives the number of *Salmonella* serotypes data from 2003 up to October 2005. The number of *Salmonella* isolates sent to Bacteriology Unit, IMR and Enteric Pathogens in Public Health Laboratory for serotyping were 915 in 2004, 824 in 2005 and 552 in January-October 2005. Fifteen of the most common serotypes in order of frequency are shown in Table 1.

Table 2 gives the number and types of Phage Types of S. Typhi recently isolated in 2005. Thirteen different phage types (PT) were found. The predominant PTs were B1 and E1. Majority of the strains that were phage typed were from an outbreak in Kelantan, a northern State in the Peninsular Malaysia.. Almost al the strains were susceptible to all the antibiotics tested. Only one S. Typhi was a multidrug strain

Table 1 Top 15 Salmonellas identified and serotypes identified and reported to the National Laboratory based Surveillance ***(Source: Bulletin Survelan Makmal Isu 5/2005).***

	serotypes	No	%	serotypes	No	%	serotypes	No	%
1	Enteritidis	244	26.7	Enteritidis	206	25.0	Enteritidis	155	28.1
2	Weltevreden	200	21.9	Weltevreden	165	20.0	Weltevreden	142	25.6
3	Corvallis	115	12.6	Corvallis	117	14.2	Corvallis	57	10.3
4	Typhimurium	49	5.4	Typhimurium	43	5.2	Typhimurium	37	6.7
5	Stanley	32	3.5	Albany	37	4.5	Tshionghwe	18	3.3
6	Tshionghwe	29	3.2	Limete	18	2.2	Limete	9	1.6
7	Blegdam	19	2.1	Braenderup	15	1.8	Stanley	8	3.3
8	Albany	17	1.9	Tshionghwe	15	1.8	Agona	7	1.6
9	Braenderup	12	1.3	Stanley	11	1.3	Albany	5	1.4
10	Newport	10	1.3	Bovismorbificans	10	1.2	Rissen	5	0.9
11	Agona	8	1.3	Lagos	10	1.2	Virchow	5	0.9
12	Bareilly	8	0.9	Rissen	10	1.1	Bovismorbificans	4	0.9
13	Bovismorbificans	7	o.8	Eppendorf	9	1.1	Braenderup	4	0.7
14	Berta	6	0.7	Agona	8	1.0	Lagos	4	0.7
15	Eppendorf	5	0.5	Kottbus	8	1.0	Newport	4	0.7
	Subtotal	**761**	**83.2**		**682**	**82.8**		**460**	**83.3**
	Total	915			824			552	

Table 2. Phage types of *S.* Typhi isolated in 2005

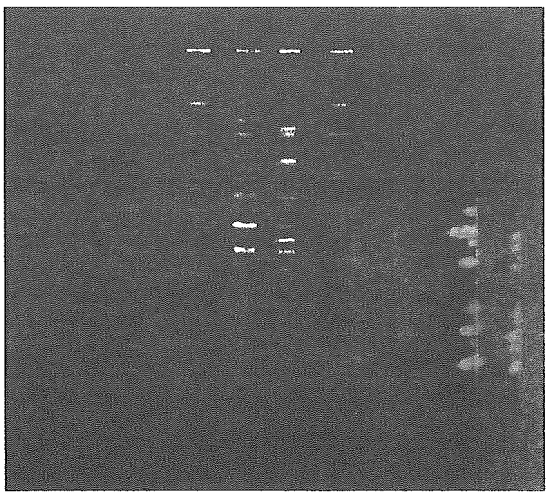
Phage types	No (%)
A1	11 (6.5)
B1	56 (32.9)
C10	1 (0.6)
C4	7 (4.1)
D1	2 (1.2)
D2	5 (2.9)
DVS	16 (9.4)
E1	44 (25.9)
UVS	6 (3.5)
UVS1	8 (4.8)
UVS2	5 (2.9)
UVS4	4 (2.5)
VNS	5 (2.9)

2. Molecular Subtyping of *Salmonella* spp in Malaysia

1. Standardization of PFGE protocol according to PulseNet consensus

We have successfully implemented the One-Day protocol to subtype the recent *Salmonella* strains in Malaysia. We have changed our protocol according to PulseNet Salmonella protocol. Figure 1 shows the PFGE profiles of test strains from Hong Kong Public health Lab as performed by our laboratory.

Figure 1.



Lanes 1-4:
SM1, SM2,
SM3, SM4

Output of interlaboratory evaluation of PFGE protocol : Results sent to Hong Kong Public Health Laboratory (c/o Dr Kam) for evaluation. November 2005

2. *Salmonella* Enteritidis

S. Enteritidis is the most common nontyphoidal *Salmonella* serovar in Malaysia since 2000. Since the start of this project we have subtyped 40 strains and 16 pulsotypes (SEXba1-16) were obtained. One predominant profile, SEXba1 was prevalent comprising of strains isolated from different parts of the country.

Fig 1. Representative profiles of *Salm* Enteritidis

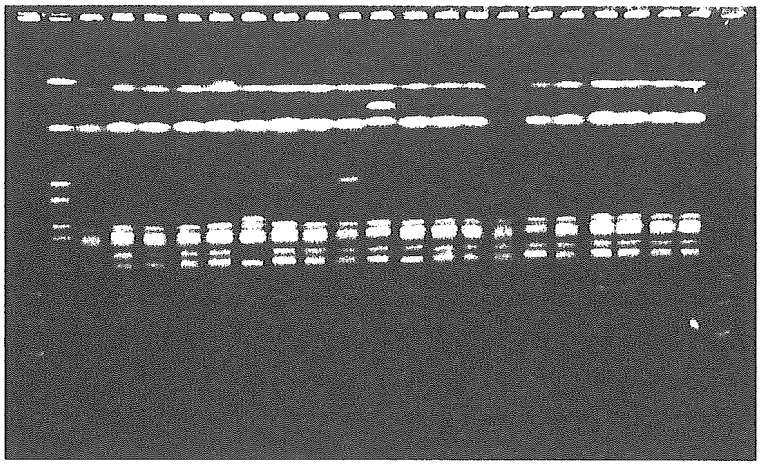
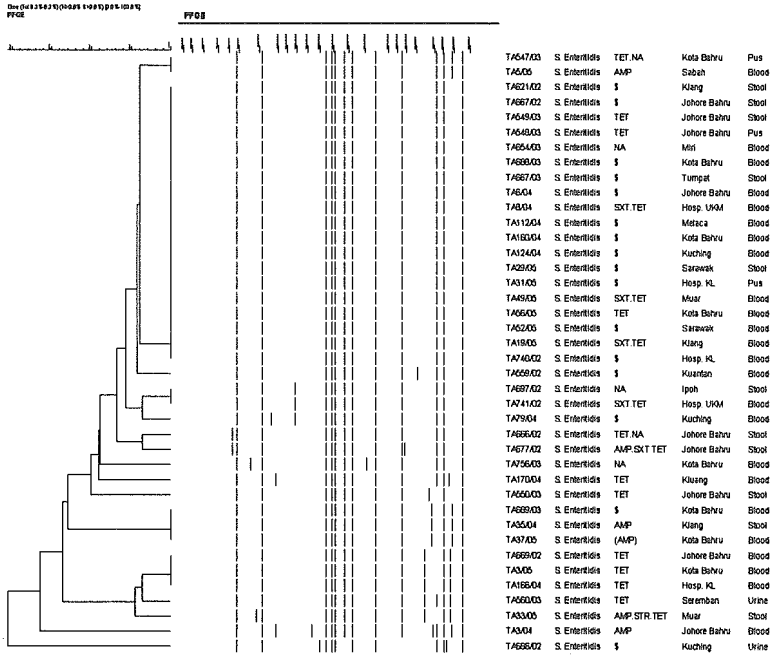


Fig. 2: Dendrogram of Salm Enteritidis showing the clustering of the strains



3. *Salmonella* Typhimurium

Thirty seven strains isolated between 2002-2005 were subtyped by PFGE and 20 pulsotypes were obtained. Two main pulsotypes, STMXba1 and STMXba2, which differed only in one band (30kb) was found in 17 strains (46%). The number of DNA restriction fragments varied from 11 to 15.

Fig 3. Representative profiles of S. Typhimurium

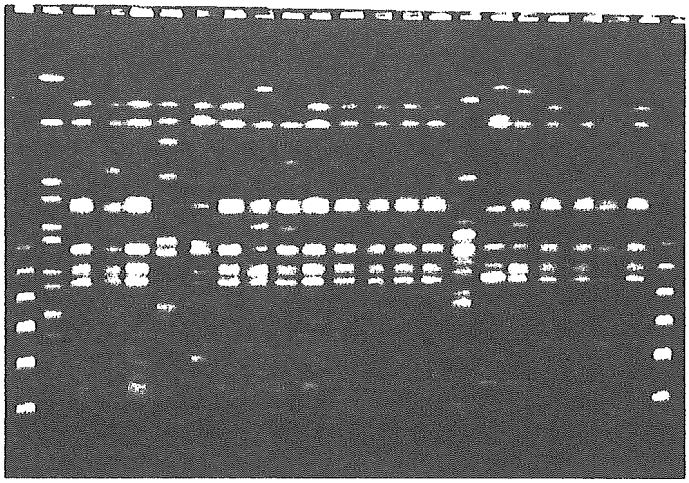
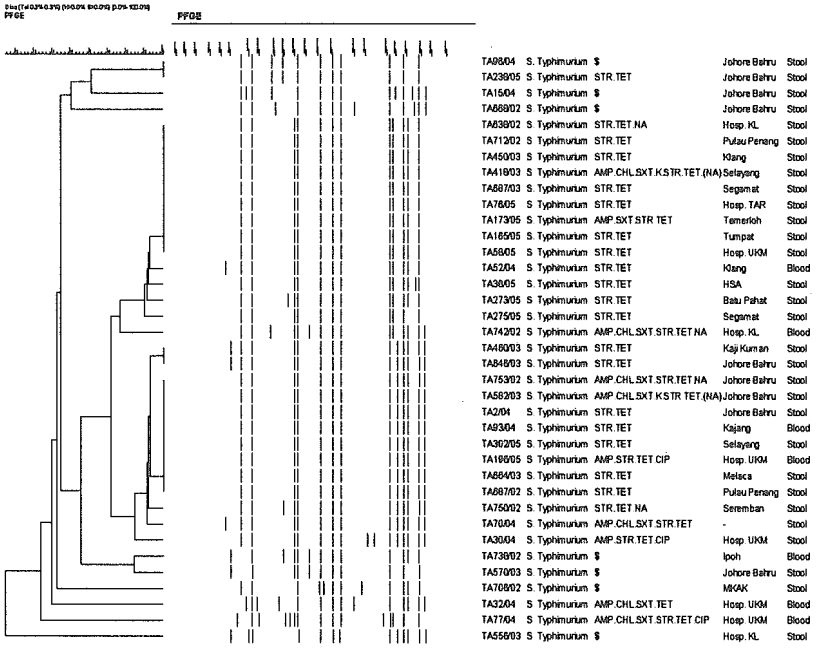


Fig. 4 Dendrogram showing the clustering of strains.



4. *Salmonella* Weltevreden

Thirty seven strains isolated between 2002 to 2005 were subtyped into 32 pulsotypes. Members of this serovar are genetically more diversed as compared to other serovars. No predominant profile was observed.

Fig. 5 Representative profiles of Salm Weltevreden

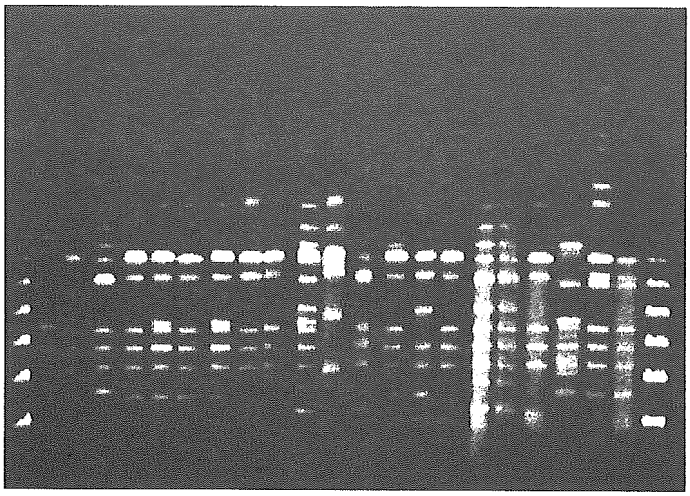
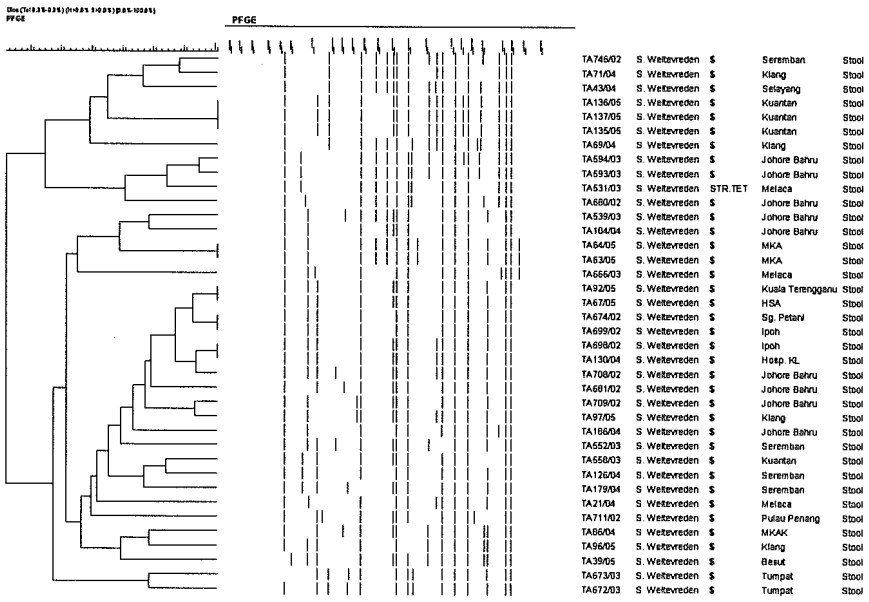


Fig. 6 Dendrogram showing the clustering of Salm Weltevreden



5.*Salmonella* Corvalis

Thirty-three strains isolated between 2002-2005 were subtyped into 17 pulsotypes. Three main pulsotypes, SCVXba1m SCVXba2 and SCVXba3 were represented by 55% of the strains. These pulsotypes differ in 1 –3 bands only.

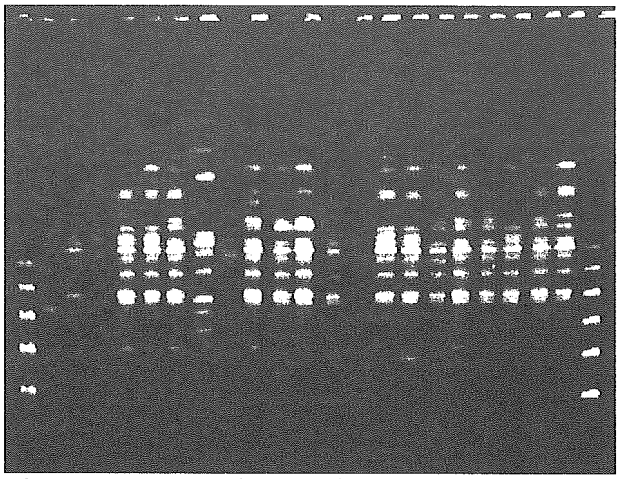
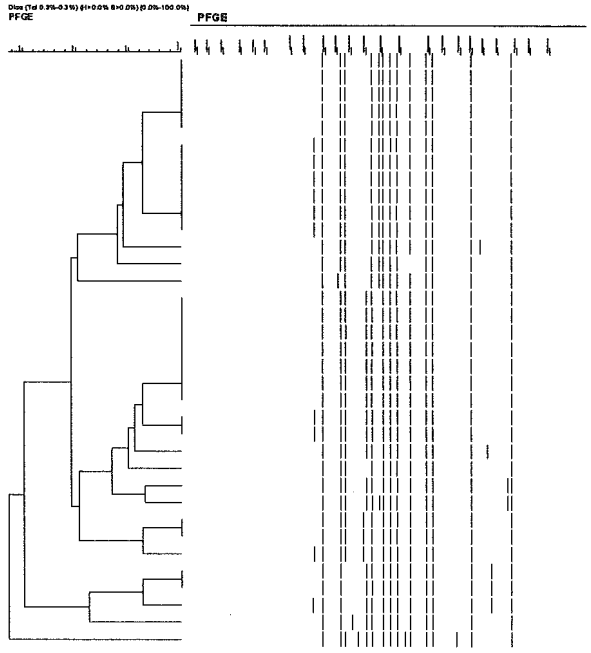


Fig. 7 Representative profiles of Salm Corvalis

Fig8. Dendrogram showing the clustering of strains



TA679/02	S Corvallis	SXT.STR.TET	MKAK	Stool
TA555/03	S Corvallis	STR.TET	Hosp. KL	Stool
TA47/04	S Corvallis	STR.TET	Klang	Stool
TA26/05	S Corvallis	STR TET	Selayang	Stool
TA98/05	S Corvallis	STR TET	Klang	Stool
TA660/02	S Corvallis	SXT.STR.TET	Alor Setar	Stool
TA675/02	S Corvallis	STR.TET	Sg Petani	Sputum
TA587/03	S. Corvallis	STR.TET	Johore Bahru	Stool
TA24/04	S Corvallis	STR.TET	Johore Bahru	Stool
TA130/05	S Corvallis	STR.TET	Seremban	Stool
TA129/05	S Corvallis	STR.TET	Seremban	Stool
TA608/03	S Corvallis	AMP.STR.TET	Hosp. UKM	Stool
TA115/05	S Corvallis	STR.TET	Hosp. UKM	Stool
TA662/03	S Corvallis	STR.TET	Melaca	Stool
TA648/03	S Corvallis	STR.TET	Johore Bahru	Blood
TA683/03	S Corvallis	STR.TET.(NA)	Kaji Kumen	Stool
TA14/04	S Corvallis	STR.TET	Johore Bahru	Stool
TA102/04	S Corvallis	STR.TET	Hosp. KL	Stool
TA25/05	S Corvallis	STR.TET	MKA	Stool
TA93/05	S. Corvallis	STR.TET	Kuala Terengganu	Stool
TA90/04	S. Corvallis	STR.TET	Seremban	Stool
TA683/02	S. Corvallis	STR.TET.NA	Alor Setar	Stool
TA49/04	S Corvallis	STR.TET	Hosp. UKM	Stool
TA635/03	S Corvallis	AMP.STR.TET.(NA)	Hosp. KL	Stool
TA64/04	S Corvallis	STR TET	Hosp. UKM	Stool
TA62/05	S. Corvallis	STR.TET	MKA	Stool
TA94/05	S Corvallis	STR TET	Kuala Terengganu	Stool
TA684/03	S Corvallis	STR TET.(NA)	Kaji Kumen	Stool
TA72/05	S Corvallis	STR.TET	HSA	Blood
TA83/04	S Corvallis	STR.TET	Klang	Stool
TA731/02	S Corvallis	STR.TET	Sg Petani	Stool
TA732/02	S Corvallis	STR.TET	Sg Petani	Stool
TA103/04	S Corvallis	STR TET	Hosp. KL	Stool
TA729/02	S Corvallis	STR.TET	Hosp. KL	Stool
TA651/03	S Corvallis	STR TET.(NA)	Kaji Kumen	Stool

6. *Salmonella* Paratyphi B

Thirty-seven strains isolated between 2002-2005 were subtyped into 29 pulsotypes. Based on these restriction markers, members of this serovar are more diverse. No predominant pulsotype was found.

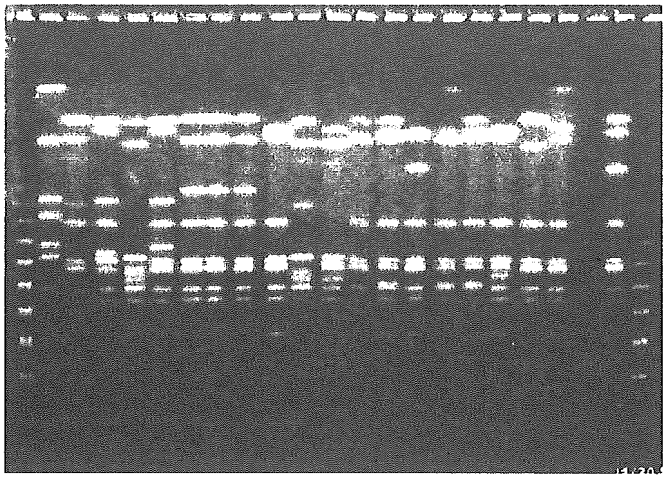
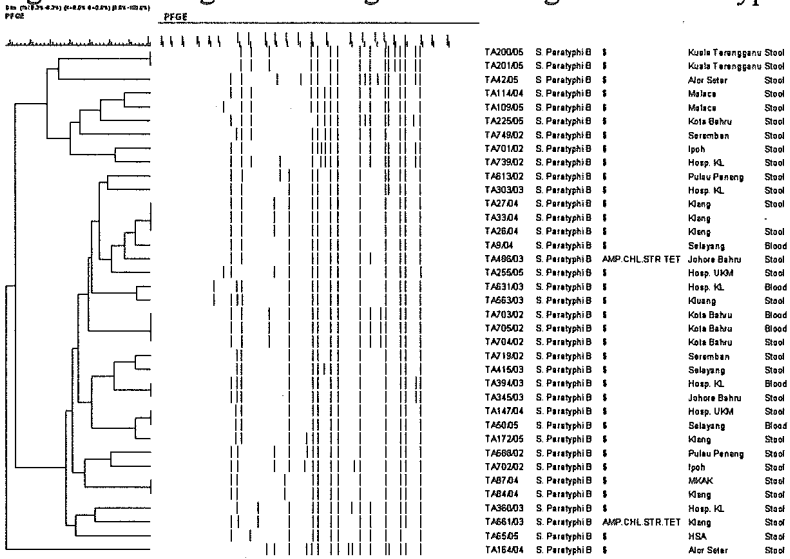


Fig. 9 Representative profiles of *Salm* Paratyphi B

Fig. 10. Dendrogram showing the clustering of Salm Paratyphi B



Salmonella Tshiongwe

A total of 34 strains were subtyped into 24 pulsotypes. At 80% similarity, 4 subclusters were observed. No predominant pulsotype was obtained and a wide genetic diversity exists among these strains. One small cluster of strains isolated from Tumpat could be part of a small outbreak.

Fig. 12. Representative profiles of *Salm.* Tshiongwe

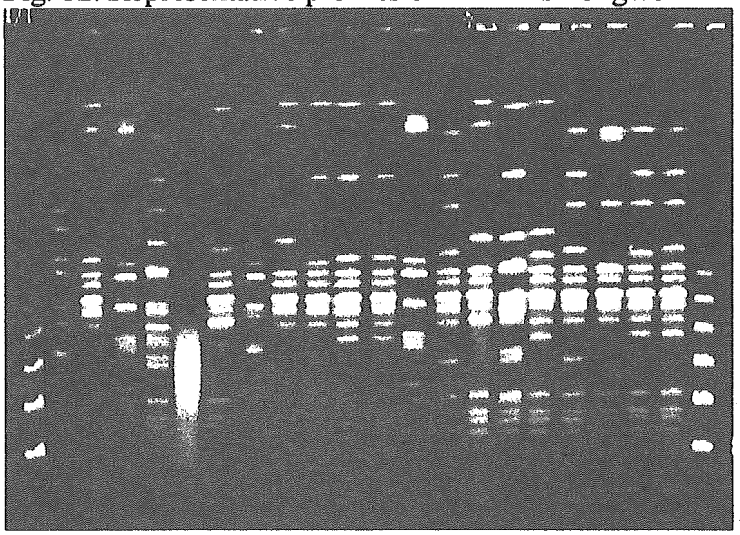
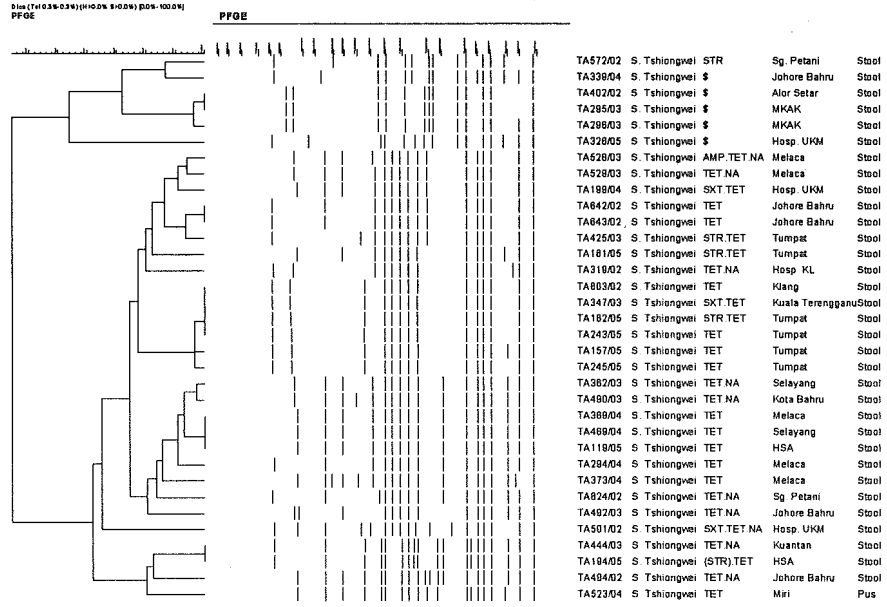


Fig. 13 Dendrogram of Salm. Tshiongwe



Salmonella **Typhi**

S.Typhi remains the top *Salmonella* serovar reported. In 2005, there was an outbreak of typhoid fever in Kelantan. The district hospital submitted the strains to the Institute for Medical Research for further subtyping. All the strains were of phage type B1 and were drug-susceptible. PFGE analysis showed that all the outbreak strains were of one endemic clone. Retrospective analysis of the previously isolated strains from the same locality showed the 2005 outbreak profile was similar to those strains isolated since 1994. The source of the outbreak could not be identified although it was a person to person infection and, most probably originated from carriers who are food handlers.

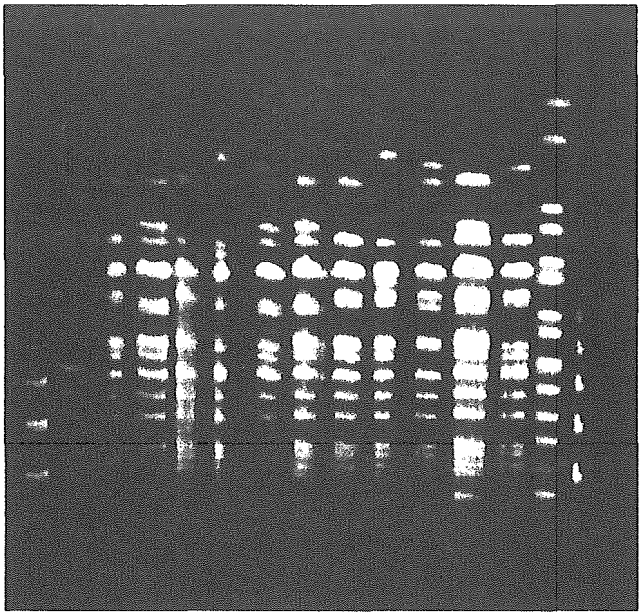


Figure 14
Lanes 1-15: Lambda DNA marker, H9812 12/05;48/05/142/05;131/05;181/05;45/02;75/02;81/02;103/02;108/02;H9812;lambda DNA

3. RESISTANT GENES DETERMINATION

To determine the prevalence of resistance genes

Figure 15 Percentage of resistant S Typhi strains (data available for up to 2001)

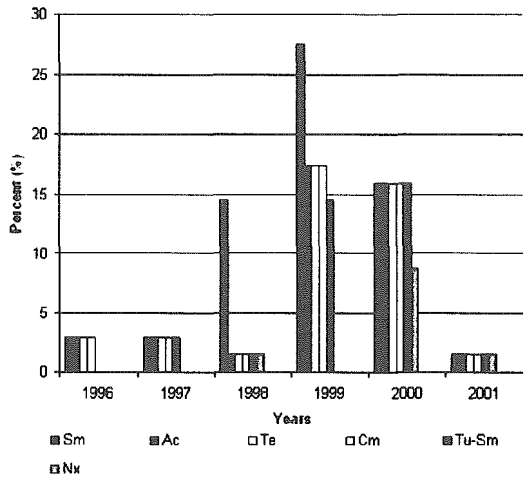
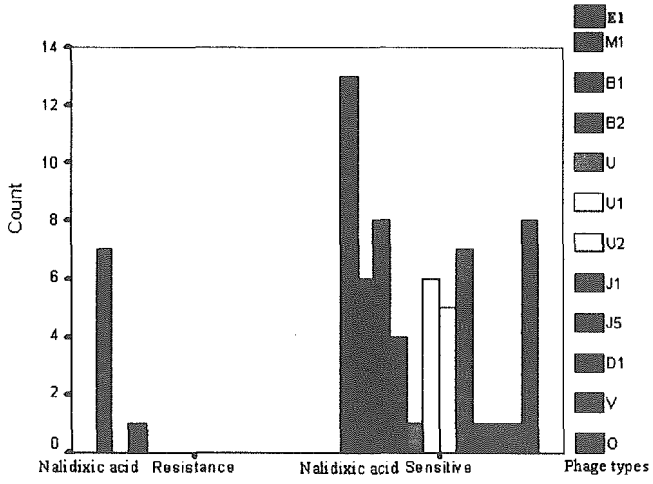


Figure 2 2

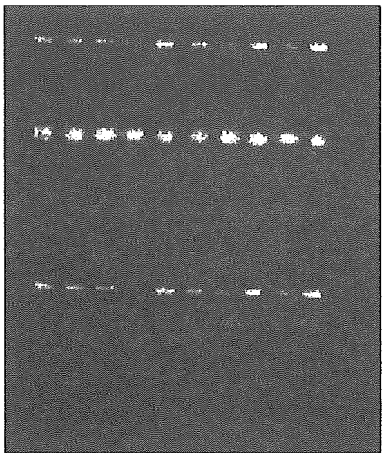
Resistance profiles percent versus the years (1996-2001)

Figure 16: Prevalence of Nalidixic acid resistance in S. Typhi



Detection of integrons in MDR *S.* Typhi

All the MDR S. Typhi showed three amplicons when PCRed using Class I integron. Not Class 2 integron was detected.

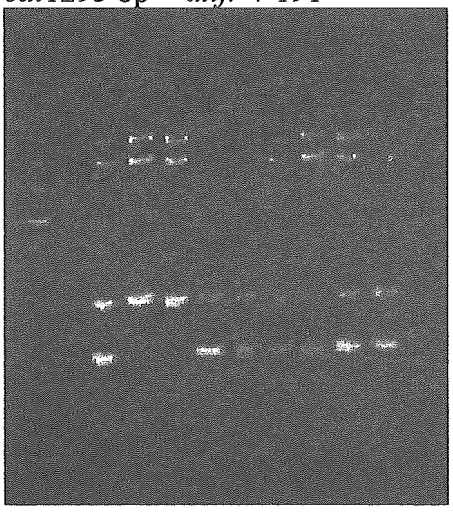


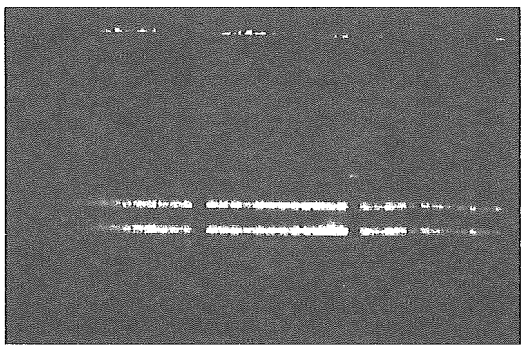
3 amplicons amplified by 5CS and 3CS class 1 integron primers in MDR Typhi

Detection of resistance genes in MDR S. Typhi

Correlation between resistant phenotypes with the presence of the genes as detected by PCR.

*tem*1 798bp *tet*A 678bp
*cat*1293 bp *dhfr*-7 191





dps 308 bp *dhfr*-7 191

CONCLUSIONS

1. Surveillance of *Salmonella* infection is ongoing
2. Continuous effort to subtype the top 5 *Salmonella* serovars to develop the database of PFGE pulsotypes
3. Manuscripts for the work are in preparation.

Prepared
Prof Dr Kwai Lin Thong

Title: Evaluation of the utility of two enzymes for PFGE subtyping of *Campylobacter*

Name of researcher; Brent Gilpin
Affiliation; Institute of Environmental Science & Research Limited, Christchurch, New Zealand.

Summary:

Campylobacteriosis has emerged worldwide as a significant cause of gastric illness, and New Zealand has one of the highest rates of campylobacteriosis in the developed world with 327.4 cases per 100,000 notified in 2004 (1). In this study we evaluated the PulseNet protocol for *Kpn*I digestion, analysed isolates in the New Zealand databases, and performed additional subtyping where appropriate to determine the utility of using both *Sma*I and *Kpn*I for analysis of isolates of *Campylobacter*.

The PulseNet protocol works well, although use of *Kpn*I from NEB produced often poor results, with consistently better profiles achieved using *Kpn*I enzyme from Invitrogen.

Examination of the *Sma*I PFGE subtyping of *Campylobacter* indicated that digestion with *Sma*I alone, is sufficient to show *differences* between most isolates, but in many instances is insufficient to demonstrate *similarity* between isolates. For example the most frequent *Sma*I pattern in the database - Sm0001 consists of only five bands. Some variation is evident within this *Sma*I pattern, but this variation is insufficient to reproducibly differentiate the isolates. Digestion with *Kpn*I however clearly distinguishes the isolates into clonal groups.

*Sma*I enzyme is less discriminatory than *Kpn*I with diversity index in one defined group of isolates of 0.31 for *Sma*I compared to 0.39 for *Kpn*I. When compared the diversity index increased to 0.42.

Key findings of this study are:

- Reproducible PFGE of *Campylobacter* using *Kpn*I is possible.
- Comparison of isolates using profiles from two enzymes simplifies comparisons, and strengthens greatly any conclusions on similarity or difference.
- Campylobacters produce a range of PFGE profiles. For analysis, PFGE profiles should only be grouped where patterns with two enzymes are indistinguishable or where differences in bands can be explained by logical mutations in bands.

A paper is in preparation on the value of two enzyme analysis.