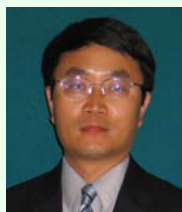


## Novel methods for typing *Salmonella* Typhimurium – a common cause of food poisoning in Australia



Dr Qinning Wang  
Postdoctoral Fellow,  
CIDM Public Health

Tel: (02) 9845 6255

Email:  
qinningw@icpmr.wsahs.nsw.gov.au

Other members of the study group included Robert Chiew, Peter Howard, Vitali Sintchenko and Lyn Gilbert.

*Salmonella enterica* serovar Typhimurium (STM) is one of more than 2000 serovars in the genus *Salmonella* and a major bacterial pathogen that causes food-borne infection (salmonellosis) all over the world. For many years, STM has been the commonest serovar isolated from humans and animals in Australia. As it is common and genetically varied, further subtyping is needed for surveillance and outbreak investigations.

Phage typing is the most widely used method, but is subjective and not particularly discriminatory. It requires interstate transportation of isolates, leading to delay in obtaining results (~3 weeks), which significantly reduces the chance of recognizing and identifying the sources of outbreaks. Molecular typing methods vary but many - such as pulse field gel electrophoresis - are slow, expensive and often no more discriminatory than phage typing. Recently a promising new method, known as multilocus variable number tandem repeats analysis (MLVA) has been applied to several *Salmonella* serovars.

At CIDM Public Health, we developed a multiplex PCR (mPCR)-based reverse line blot (RLB) hybridization assay that can identify most phage types and distinguish strains within them. It is relatively rapid and objective and potentially suitable for routine surveillance and outbreak investigations. We compared it with phage typing and MLVA, using STM isolates belonging to many phage types, including outbreak isolates.

### Isolates tested

We tested 168 sporadic STM isolates, representing 48 phage types, collected between 2000 and 2005 in NSW and 79 isolates associated with 2 outbreak investigations. The latter comprised a) 14 isolates from humans and one food isolate from three small, apparently related restaurant outbreaks and 22 sporadic isolates belonging to the same phage type (PT 170) and b) 25 isolates from humans involved in a hospital outbreak and 15 sporadic isolates belonging to same phage type (PT 135).

### Methods

The mPCR/RLB method involves identification of 32 genetic targets - 26 sequences from various salmonella phages and 6 putative phage type-specific fragments. Primers for each target are combined in the multiplex PCR, which amplifies DNA from the salmonella isolates of interest. The PCR products are hybridized to a membrane, to which probes for each of the 32 target sequences have been fixed. The presence of the target sequences is indicated by a signal, which develops on a light sensitive film exposed to the membrane, due to the reaction between amplified DNA and the chemiluminescent labelled probe (see Figure 1). Up to 43 salmonella isolates can be tested on a single membrane.

MLVA involves PCR amplification of 5 different target sequences in the salmonella genome, which are characterised by variable numbers of tandem repeats. The numbers of repeats at each site vary in different strains and are inferred from the lengths of the PCR amplicons. This gives a 5-digit "formula" for each strain, which is objective and easily compared between laboratories and over time.

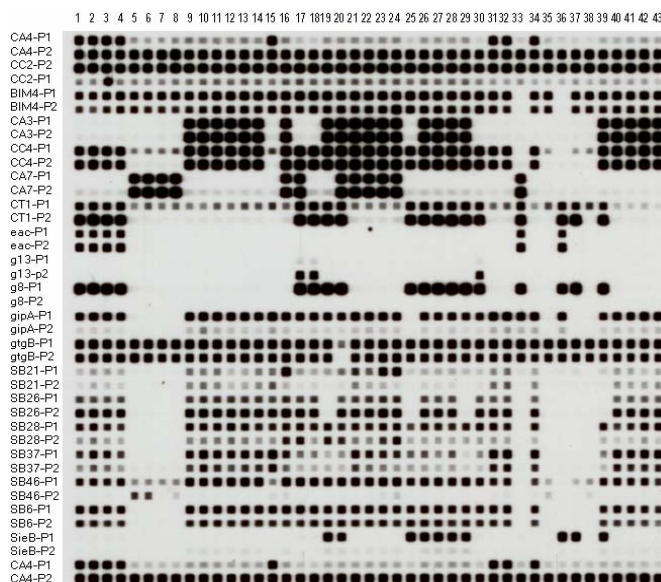


Figure 1. RLB patterns of different phage types (PT) of STM. Columns: 1-4, PT 64; 5-8, PT 6var 1; 9-13, PT 99; 14-16, RDNC; 17-20, PT U302; 21-24, PT U307; 25-28, Untypable; 29-30, PT 186; 31-32, PT 9; 33, PT 20var1; 34, PT 30; 35, PT 140 var 1; 36, PT 177; 37, PT 179; 38, PT 197; 39, PT 12; 40-43, PT 135. Left column: Probes

## Results

### a) Sporadic isolates

The 168 STM isolates gave 104 different RLB patterns, most of which correspond to different phage types. 93 isolates belonged to RLB types with multiple representatives and 75 isolates were unique. The RLB patterns clustered into five larger groups (A to E), two of which contain the more common phage types, such as PT 170, 135, 135a, 126, 12, 9.

The numbers of different profiles within each phage type varied and was greater for relatively uncommon phage types. A few phage types could not be distinguished from each other by this method but most were easily identified and showed at least two different RLB profiles (strains).

The 168 selected STM isolates were separated into 100 MLVA types which were further clustered into four groups (A-D). Group D contained the more common phage types (PT170, PT 135, PT 135a, and PT 126), suggesting these phage types are closely related. This typing method distinguished most of 48 phage types tested, although a few shared the same MLVA type.

### b) Outbreak investigations

Results are summarised in the Table 1.

In the first outbreak, all isolates gave the RLB pattern of PT 170 (or PT 108, which is identical). All 14 human isolates and 1 food isolate were indistinguishable from each other and from the commonest of five strains identified among sporadic isolates.

MLVA distinguish two closely related strains among the outbreak isolates, which differed only at one of the 5 loci – one, represented by 2 human isolates and the food isolate, was indistinguishable from the commonest of 7 different MLVA types identified among the sporadic PT 170 isolates. The remaining 12 human isolates belonged to the other strain.

In the second outbreak, 23 isolates produced identical RLB patterns consistent with that of PT 135 and two were identified as PT 126 (and shown not to be involved in the outbreak). There were two distinct patterns among the sporadic PT 135 isolates, one of which was identical with that of the outbreak strain. There were 5 MLVA types among 23 outbreak isolates and 8 among 15 sporadic isolates.

Phage type	No. isolates tested	RLB pattern (N)	MLVA type (N)	Source
PT 170	14	b (14)	01-02-14-05-08 (12) 01-02-13-05-08 (2)	Restaurant outbreaks in NSW (2006)
PT 108	1	b (1)	01-02-13-05-08 (1)	Food isolate from the restaurant (2006)
PT 170	22	a (1) b (17) c (1) d (2) e (1)	01-24-16-11-06 (1) 01-02-13-05-08 (3) 01-03-14-05-08 (1) 01-02-14-05-08 (14) 01-02-15-05-08 (1) 02-08-15-00-07 (1) 02-09-14-00-07 (1)	Sporadic isolates (2000-2005)
Total	37			
PT 135	23	a (23)	01-03-16-03-08 (1) 01-04-16-05-08 (18) 01-04-16-05-00 (2) 01-05-15-01-08 (1) 01-08-13-05-08 (1)	Hospital outbreaks in NSW (2004)
PT 126	2	b (2)	01-09-25-05-08 (1) 01-10-20-05-00 (1)	Hospital outbreaks in NSW (2004)
PT 135	15	a (11), c (4)	01-03-16-05-08 (1) 01-04-16-03-08 (3) 01-04-16-05-08 (1) 01-05-15-03-08 (1) 01-05-16-01-08 (2) 01-05-16-05-08 (5) 01-05-17-03-08 (1) 01-06-15-03-08 (1)	Sporadic isolates (2001-2003)
Total	40			

Table 1. Results of RLB and MLVA typing in two outbreak investigations. Note: PT 170 and 108 are identical.

### Conclusions

- The newly developed mPCR/RLB typing assay for STM is relatively fast, inexpensive and discriminatory and has the potential to be used as an alternative to phage typing.
- MLVA is faster and less labor intensive than mPCR/RLB and has a similar or greater discriminatory power, especially in outbreak investigations.
- Further experience is needed to determine which reflects the epidemiology of STM infections more accurately but either, alone or a combination, can be used for surveillance and outbreak investigation and to identify more outbreaks, more rapidly than phage typing.

## STAFF PROFILE PING ZHU



Ping Zhu  
Culture Curator, CIDM Public Health

Tel: (02) 9845 6255

Email:  
zhup@icpmr.wsahs.nsw.gov.au

Prior to joining CIDM Public Health in 2004, Ping completed a BSc at Guangzhou Teacher's College in China and later a Graduate Diploma in Biotechnology at the University of New South Wales. For two years she worked for Gist-brocades -a Dutch company which specialised in penicillin and yeast production. This company merged into DSM Food Specialties (UNSW) from 1999 where she continued to work for a further 5 years as a research associate. Her work involved the development of starter cultures for the dairy industry in Australia and overseas.

Ping has been involved in the inception and management of the CIDM Public Health culture collection; a central repository for isolates in existing research and reference collections along with important referred and diagnostic isolates. To ensure that they are appropriately preserved for long-term storage and catalogued for easy retrieval, clinical isolates of interest are carefully documented in a database and then freeze-dried and stored at  $-80^{\circ}\text{C}$  for future reference. This is an invaluable resource for CIDM-PH and other researchers.

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