

The Broad Street Pump

SALMONELLA

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The outbreak of enterohaemorrhagic *Escherichia coli* in Germany last year and recent reports about the emergence of *Salmonella* Kentucky as a “new super-bug” have re-focused public attention on food-borne diseases. Salmonellosis is an important cause of food-borne disease with significant economic burden to the population and healthcare systems. In the last century, microbiologists have identified hundreds of different types of salmonella that can infect humans and warm-blooded animals and are wide-spread, especially in areas with substandard farming and supplies of drinking water. They can cause acute gastroenteritis following ingestion of contaminated water or undercooked food of animal origin. The human disease caused by these bacteria ranges from mild abdominal discomfort to vomiting and dehydration and can be life-threatening for children and the elderly. It has become a major public health problem internationally. The global human health impact of non-typhoidal *Salmonella* infection can be as high as 10^8 cases and 155,000 deaths each year. Many cases of salmonellosis would be prevented if common outbreak sources could be identified rapidly by enabling earlier public health interventions. However, changes in food-borne disease epidemiology have complicated recognition and investigation of outbreaks. The increased mobility of people, complex food production, processing and distribution systems and the plethora of retail fast food outlets necessitate more efficient and effective ways to identify sources of food-borne outbreaks. Salmonella-related outbreaks are increasingly associated with a diverse range of food sources, yet the mechanisms of contamination often remain poorly understood and the true rates of infection are likely to be under-reported. In this issue, CIDM-PH collaborators share the results of their translational research on public health aspects of salmonella infection.



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HAI & Infection Prevention & Control Workshop

May 3 and 4, 2012

Medical Entomology in Australia

June 29, 2012

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The role of plasmids in the acquisition and spread of antibiotic resistance in *Salmonella* Typhimurium

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Salmonella infections of humans and warm-blooded animals are mostly due to a relatively small number of the >1500 recognised serovars of *Salmonella enterica* subspecies *enterica* [1], some of which infect a broad range of animal hosts while others are host specific [2]. These infections most commonly follow consumption of contaminated water or food [3].

Salmonella pathogenesis:

Once ingested, *Salmonella* pass through the gastrointestinal tract and adhere to intestinal epithelial cells by fimbriae present on the bacterial surface. Their uptake into the cell is facilitated by a type III secretion system (T3SS) encoded on the *Salmonella* pathogenicity island 1 (SPI-1). Virulence proteins are translocated from the bacterial cytoplasm into the host cell where they induce fluid secretion into the intestinal tract and inflammation and initiate internalization of the bacteria into membrane bound *Salmonella*-containing vesicles (SCV) [2-3]. The SCV are essential for the survival of *Salmonella* within infected epithelial cells and are transported to the basal membrane where they enter phagocytic immune cells leading to systemic infection [3]. Within the SCV *Salmonella* express a T3SS encoded on *Salmonella* pathogenicity island 2 (SPI-2), which transfers virulence proteins into the host cell where they induce apoptosis and, in immune cells, suppress antigen presentation thus limiting the immune response [2-3].

S. typhimurium and antibiotic resistance:

S. enterica serovar Typhimurium (STM) is the most commonly isolated *Salmonella* from humans in Australia, and notifications across Australia are increasing [4]. It can cause life-threatening systemic infection, especially in immunocompromised patients [2], for which antibiotic therapy is essential. Previously, ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole were effective, but resistance to these antibiotics is now as high as 70% in some parts of the world [1]. Multidrug resistance in *Salmonella* is becoming a global problem attributable to resistance regions located on both the chromosome and plasmids [1, 5].

Antibiotic resistance plasmids in Enterobacteriaceae:

Plasmids are self-replicating extrachromosomal DNA elements that carry genes responsible for their autonomous replication, copy number within each cell and stable inheritance [6]. Some plasmids confer specific survival advan-

tages on their host such as resistance to various antibiotics or toxic metals, or traits that enhance fitness or virulence [7-8]. Some large conjugative plasmids carry multi-resistance regions consisting of multiple mobile genetic elements and many resistance determinants [9-10]. The most prevalent of these in the *Enterobacteriaceae* are known as IncF-type, IncA/C, IncL/M and Inc1, denoting their replication systems [6]. Such plasmids spread β -lactam, quinolone and aminoglycoside resistance genes [6] and their prevalence is generally linked to antibiotic use, which selects for commonly associated resistance genes. However, IncF-type plasmids are found in >50% of the intestinal flora of humans irrespective of resistance genes and appear to be the dominant type in species like *E. coli* [6, 11].

Different species of *Enterobacteriaceae* have major differences in their plasmid profiles including the likelihood of carrying a plasmid and the number, size and types of plasmid. This suggests that plasmids evolve within the constraints of host cell limitations, which has important implications for their evolution and spread [12].

S. typhimurium antibiotic resistance & virulence plasmids:

Most (67-88%) STM strains carry a virulence plasmid called pSLT [13-14]. It is a self-transmissible, low copy number (estimated 2.75 copies per cell), IncF-type plasmid of approximately 94 kb [5, 14-15]. It encodes genes that increase adhesion to intestinal epithelial cells (*pef*), resistance to complement killing (*rck*) and apoptosis of infected cells (*spv*) [14]. Recently, several pSLT variants that also carry resistance genes have been identified in clinical isolates [16-18]. Some of these can conjugate into various *Salmonella* serovars and displace the natural pSLT in STM (whilst retaining the same virulence properties) [19]. The combination of antibiotic resistance and virulence means that resistant populations will be selected, even in the absence of antibiotic pressures [6]. These shifts in plasmid evolution may lead to greater dissemination of more virulent and resistant salmonellas and may represent an adaptive strategy for these plasmids to spread [5, 19].

A local survey of plasmids in resistant (n=21) and sensitive (n=21) STM isolates, collected by the Enteric Reference Laboratory at CIDM, between January 2009 and October 2010 revealed that all sensitive isolates had pSLT only (on the basis of one MLVA target located on this plasmid, the

presence of the *spv* operon and correct plasmid size and type).

Two resistant isolates had a typical *Enterobacteriaceae* plasmid (IncI1) plus pSLT, seven had only typical *Enterobacteriaceae* plasmids (two IncI1, four IncHI1, one IncL/M and one IncA/C between them) and six had plasmids that were untypable by a PCR-based replicon typing scheme [20]. One resistant isolate had pSLT plus the chromosomal resistance region SGI-1, another had chromosomal resistance regions RR1 and RR2. The location of resistance genes, in two isolates that had pSLT only and two with neither detectable plasmids nor known chromosomal resistance regions, remains unexplained. No IncF plasmids common in other *Enterobacteriaceae* (e.g. those carrying ESBL resistance in *E. coli*) were found. This may be because the pSLT replicon is so similar to the IncF replicons that they are incompatible in the same bacterial cell.

The intracellular life-style of salmonella may limit exposure to and exchange of genetic material with other intestinal pathogens, such as *E. coli* and *K. pneumoniae*. This may significantly influence how salmonella plasmids evolve and acquire resistance determinants, but the acquisition and spread of the common antibiotic resistance plasmids of the *Enterobacteriaceae* may be an indication of things to come.

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Peter Jelfs is the Senior Scientist in-charge of the NSW Mycobacterium Reference Laboratory (MRL) within CIDMLS, Westmead Hospital. A graduate of the University of Western Sydney with a degree in Environmental Health, Peter has had a long career in diagnostic clinical microbiology, molecular biology and bacterial epidemiology.

The NSW MRL performs an important public health role in the characterisation of laboratory confirmed cases of tuberculosis in NSW and is the major contributor to the accumulation of data on the incidence of tuberculosis nationally. The NSW MRL receives around 1500 isolates of Mycobacterium species annually for identification and susceptibility testing, around a third of which are *Mycobacterium tuberculosis*. Peter has overseen the introduction of a wider range of rapid, molecular-based methods into the laboratory, particularly targeting the detection of antibiotic resistance genes. "It's important that we are able to provide clinicians with timely advice on the available treatment options, particularly if there is a high suspicion the patient has contracted a multi-drug or extensively -drug resistant strain" commented Peter. "It is highly probable, given the current trends, that our laboratory will see increasing numbers of difficult to treat cases of tuberculosis in the near future," he added.

Another of Peter's interests has been the ongoing establishment of a range of internationally recognised, molecular epidemiological techniques for genotyping of tuberculosis strains. Peter's work has resulted in an extensive database of tuberculosis genotypes which provide a valuable resource enabling characterisation of outbreak strains of this disease and assisting State TB case investigators in contact tracing.

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Education Block, Westmead Hospital

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Prospective genotyping of *Salmonella* Typhimurium for public health

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Salmonellosis in New South Wales:

Salmonellosis is a common cause of gastro-intestinal disease in Australia; notifications of human infections increased from 6,191 in 2000 to 12,252 in 2011.(1) This trend is reflected in NSW, with notifications increasing from 1,416 in 2000 to 3,473 in 2011.(2) OzFoodNet estimate that the ~5.4 million cases of food-borne disease per year, is costing Australia A\$1.2billion annually.(3)

Most human *Salmonella* infections are due to *Salmonella enterica* subsp. *enterica*. In NSW, in 2011, 59% of these were identified as serotype *S. Typhimurium* (STM).(4) STM causes apparently sporadic infections, and is frequently identified as the agent responsible in gastro-enteritis outbreaks.(5) The timely surveillance, investigation and management of outbreaks involving contaminated food is imperative for public health investigations. (5)

S. Typhimurium strain typing:

The current method of choice for STM strain typing, for outbreak identification and investigation is multiple locus variable-number tandem-repeat analysis (MLVA). MLVA is a PCR-based method that utilises the principle of amplification and fragment analysis of five variable-number tandem repeat (VNTR) loci of STM. The loci are analysed for fragment size, in the order of STTR9-STTR5-STTR6-STTR10-STTR3. The size of each locus can be determined by the number of repeat units. The last locus is represented as its actual length in base pairs. One of the advantages of this nomenclature is the ability to recognise related, but slightly different MLVA profiles that may be epidemiologically linked.(5) Furthermore, this also assists in distinguishing between outbreaks based on slight differences at one or more loci, and can be used in defining outbreak-related cases. Compared to phage typing and pulse field gel electrophoresis, MLVA data can be easily analysed and shared between laboratories and countries. However, this requires standardisation, which is currently being implemented in Australia, Europe and North America. (See accompanying article by Qinning Wang)

Prospective surveillance of salmonellosis. Aim:

The aim of this project was to describe the role of STM MLVA typing in the surveillance and investigation of food-borne outbreaks. Retrospectively, MLVA data from the

NSW Enteric Reference Laboratory, and epidemiological outbreak/cluster data from the NSW reports, through the NSW Health Enteric team, were collected for the period, January 2009 to June 2011. Individual MLVA patterns were analysed for clusters using the laboratory cluster definition: "5 or more isolates with the same MLVA type over a period of 4 weeks". Isolates were also sent for phage typing.

Results

During the study period there were 4903 STM isolates, which were classified into 732 MLVA patterns. Only 32 (4.4%) of the MLVA patterns were clustered (as defined above); 23 of these clusters were not linked to any food-borne disease investigation. Thirty food-borne disease outbreaks, associated with the remaining 9 clustered MLVA patterns were investigated by public health units, including 2 or more separate clusters associated with each of 7 MLVA patterns.

Altogether, a total of 60 food-borne outbreaks (identified by epidemiological and/or microbiological surveillance) were investigated during the study period; 29 MLVA types were identified for 53 outbreaks, 7 of which were clustered. The 7 clustered MLVA types were 3-9-7-12-523, 3-9-7-13-523, 3-9-7-14-523, 3-9-8-12-523, 3-9-8-13-523, 3-12-9-10-550 and 3-13-11-9-523.

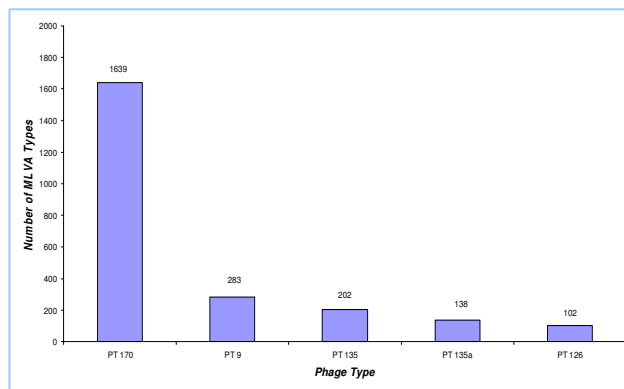


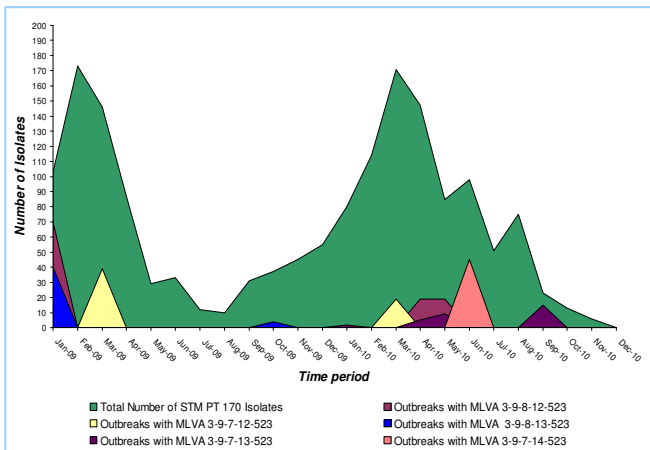
Figure 1. Total Number of MLVA patterns in different phage types

Phage typing results were available for 63% of the 4903 isolates, which belonged to 82 phage types. The 6 most common phage types (PT) were PT's 170 (54%), 9 (10%), 135 (7%), 135a (5%), 126 (3%), 44 (3%) and 193 (3%)

(Figure 1.), constituting 85% of isolates. The frequencies of MLVA patterns for the STM isolates were examined. The 3 most common MLVA patterns, represented one third of the isolates; they were 3-9-7-13-523 (18%), 3-9-8-12-523 (6%) and 3-9-7-12-523 (6%) and all corresponded to PT170.

The subset of PT170 (N=1639) isolates for the period January 2009 and December 2010, is represented by the green shaded area in Figure 2. The other coloured areas represent separate food-borne outbreaks, attributed to different MLVA patterns, and linked to PT170. Overall the 1639 PT170 isolates were classified into 110 MLVA types, 5 of which accounted for 76% of isolates and caused the clusters shown in Figure 2. They included the 3 most common of all MLVA patterns (shown above), plus 3-9-7-14-523, and 3-9-8-13-523.

Figure 2. Examples of outbreaks of STM phage type 170 characterised by



matching MLVA patterns.

Conclusions:

The use of MLVA for STM strain typing has been widely practised only for approximately the last 5 years or so. As a result, its use in public health surveillance and outbreak detection is somewhat novel, and requires a careful evaluation and evidence synthesis. The results from this study represent only a small set of data, and may be affected by biases and limitations often faced in analysis of small samples. The data does, however, demonstrate the discriminatory nature of MLVA typing of STM, in defining similar versus distinct epidemiological clusters. The more rapid the turn around time of MLVA as compared to phage typing will result in outbreaks being identified much more rapidly. Although the numbers of identifiable clusters (based on the definition used) were limited, it is clear that some MLVA patterns are endemic and may reflect common but unidentified sources.

Acknowledgements

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Molecular approaches to *Salmonella* serotyping and phage typing

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Salmonella infections are a major public health concern in Australia. The genus *Salmonella* comprises two species: *S. enterica* and *S. bongori*, and contains more than 2500 recognized serotypes(1) but only *S. enterica* subspecies *enterica* I (one of 6 subspecies of *S. enterica*) is of clinical relevance to humans and warm-blooded animals. A relatively small number of serotypes cause human salmonellosis ranging in severity from mild gastrointestinal to potentially fatal systemic diseases (including typhoid fever). Rapid, accurate serotype identification is critical for outbreak investigation and surveillance. For common serotypes, such as *S. Typhimurium* (STM), further subtyping is required and, until recently, phage typing has been the method of choice.

Conventional *Salmonella* sero-/phage typing:

Serotyping of *Salmonella enterica* is based on the use of specific antisera to distinguish more than 150 unique surface polysaccharide (O) and flagellar (H) antigens and, for *S. Typhi*, the Vi capsule antigen, according to the Kauffmann-White scheme (1). It is labor-intensive and expensive and may fail because of atypical expression of surface O or H antigens by some isolates.

Phage typing is based on the variable susceptibility, of different strains, to bacteriophages, which either lyse the host bacteria or are incorporated into its DNA (i.e. become lysogenic). The phage type is determined by the lytic and lysogenic patterns produced by a panel of phages (2). In NSW, STM is the most common serotype isolated from humans and animals, which for decades has been routinely subtyped by this method. However, the utility of phage typing is limited by: delayed results due to the need for interstate referral of isolates (because the international phage panel is restricted to one or two reference laboratories in each country), relatively poor discriminatory power and subjectivity of interpretation, leading to some interlaboratory differences.

Molecular approaches to *Salmonella* sero/phage typing:

Recently, there have been alternative approaches to *Salmonella* strain typing, to complement, or potentially replace, traditional serotyping and phage typing. For serotype identification, these include PCR-based methods targeting genes encoding the O, H, and Vi antigens (3, 4) or

other serotype-specific genes (5). Phage type-specific sequences in STM and its phages can be targeted to distinguish phage types by PCR and sequencing (6). Techniques applied in molecular serotype or phage type identification include multiple single or multiplex PCRs, DNA sequencing, amplified fragment length polymorphism, real-time PCR, mass spectrometry and DNA microarrays. Many of these strategies are complicated, labor-intensive and expensive or have limited reproducibility and discriminatory power; so far, none have emerged as widely accepted alternatives to conventional methods.

A new serotype identification system for *Salmonella*:

We have developed a multiplex PCR based reverse line blot (mPCR/RLB) assay (7) to rapidly identify common *Salmonella* serotypes. Comparison of genomic sequences of the reference strain, STM LT2 and non LT2 *Salmonella*, identified 17 *Salmonella*-specific, *S. enterica* subsp. I-specific or *S. enterica* serotype *Typhimurium*-specific targets (8) suitable for mPCR/RLB, for which 38 biotin-labelled primer sets and amino-labelled probes were designed. The method was evaluated by testing 310 randomly selected *S. enterica* subsp. I isolates, representing 38 common and rare serotypes from our culture collection, using single PCR and sequencing to confirm mPCR/RLB results. The assay differentiated 310 *S. enterica* isolates into 208 RLB types. 295 of 310 isolates displayed closely clustered RLB patterns corresponding with serotypes. The rest of 15 isolates were grouped with clusters corresponding to different serotypes to which they had been originally assigned, using antisera. Repeat serotyping showed that the initial results of four isolates had been incorrect and the rest of 11 had the same serotypes as previously assigned. Overall, the serotypes of 299 of 310 isolates (97%) were correctly assigned by mPCR/RLB, based on the RLB patterns (Fig 1). The discriminatory power of the assay is greater than conventional serotyping with a 97% typeability of the isolates. The accuracy of the assay was comparable with that of conventional serotyping and could be improved by the addition of probes targeting more *Salmonella* serotypes, when more sequence data become available. Meanwhile the small proportion of isolates, which were not assigned to correct serotypes by mPCR/RLB, suggests more serotype-specific

CRISPR typing as a surrogate for phage typing

Another mPCR/RLB method has been developed for STM strain typing based on clustered regularly interspersed short palindromic repeats (CRISPRs), which are arrays of short direct repeats, separated by variable, non-repetitive sequences or spacers (10). CRISPRs are widely distributed in bacteria and have been reportedly found in ~ 40% and 90% of bacterial and archaeal genome sequences, respectively (9). Their structures vary greatly among microbial species due to the polymorphisms created by the variable spacers. The direct repeat region of *Mycobacterium tuberculosis* is one of the most extensively studied CRISPR loci from which spoligotyping was developed (11). In our study, three STM CRISPR loci were selected, based on the published whole STM LT2 sequence, available on the CRISPR web-service database (<http://crispr.u-psud.fr/>); two primer sets and 57 probes were designed for the mPCR/RLB assay. 97 randomly selected, sporadic STM isolates, representing 24 phage types, and 42 isolates from three known or suspected outbreaks were analysed. All three CRISPR loci were present in all 139 STM isolates, with varying degrees of polymorphisms, reflecting their different levels of genetic activity. CRISPR-3 was the most active locus, as shown by the greatest number of spacers identified. Overall, 54 different spacers and 48 CRISPR RLB profiles, were

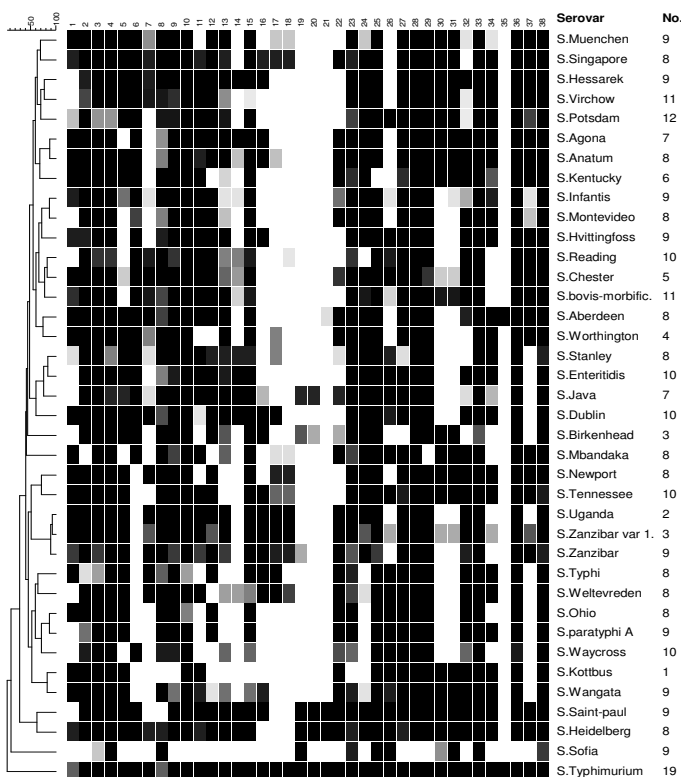


Fig 1. mPCR/RLB identification of *Salmonella* serotypes based on positive percentage rates at each individual probe for isolates from 38 serotypes. No.: number of isolates tested

identified among the 139 isolates tested. The assay distinguished sporadic isolates from each other and, from outbreak strains. Three distinct CRISPR types were identified from the three confirmed or suspected outbreaks. CRISPR typing is more discriminatory than phage typing for individual strains, but strains of most phage types cluster together by the minimum spanning tree analysis (Fig 2), which indicates that the CRISPR typing may be used as surrogate for phage type prediction.

Conclusions.

We developed mPCR/RLB assays to identify a) the most common *Salmonella* serotypes in NSW and b) spacer sequence diversity of STM CRISPR regions, which can be used for phage type prediction and individual strain typing. The specificity, discriminatory power and high throughput of mPCR/RLB techniques promote these molecular alternatives for the identification of *Salmonella* serotypes and STM phage types as routine clinical procedures.

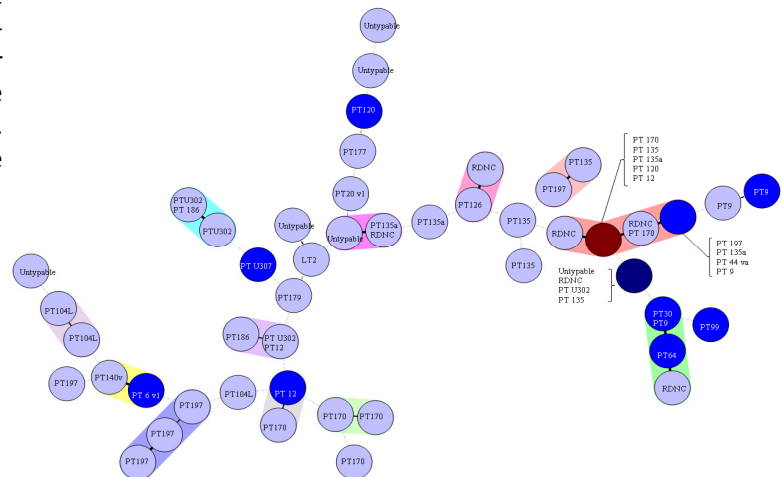


Fig 2. Minimum spanning tree analysis of 97 randomly selected *Salmonella* Typhimurium isolates, representing 24 phage types, based on CRISPR spacer diversity by mPCR/RLB. Coloured cycles

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National Harmonization of MLVA typing scheme for *Salmonella* Typhimurium between Enteric Reference Laboratories in Australia

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Salmonella Typhimurium (STM) is one of more than 2500 *Salmonella* serotypes recognized in the genus *Salmonella*. It is the most common enteric pathogen causing food-borne infection in humans and animals in Australia. Subtyping of STM is required for outbreak investigation and disease surveillance. Historically, phage typing (1), a phenotypic method, has been used to differentiate individual strains of STM. Its limited discriminatory power means that certain phage types predominate for long periods in a geographical area, making outbreak detection difficult.

Recent developments in molecular typing methods have improved their discriminatory power and provided more accurate data for outbreak investigation. In Australia, multilocus variable number tandem repeat analysis (MLVA) has been used for subtyping STM since 2006, by most enteric reference laboratories (ERL). The Queensland Forensic and Scientific Services (QFSS) and Centre for Infectious Diseases & Microbiology (CDIM), New South Wales (NSW) have used MLVA, routinely for all STM isolates for several years. The Institute of Medical and Veterinary Science (IMVS) in South Australia (SA) and Microbiological Diagnostic Unit (MDU), Victoria (Vic), do phage typing routinely and use MLVA selectively and PathWest, Western Australian (WA), currently does pulse field gel electrophoresis (PFGE) typing and refers isolates for MLVA typing, if required, for multi-state outbreak investigations.

MLVA detects five different tandem repeat loci on the STM genome (2). Each locus contains variable numbers of repeated sequences which may be duplicated or deleted

as part of organism's adaptation to its environment. This leads to differences, between strains, in the total length of the amplified locus. The lengths of PCR products at the five loci are measured by capillary gel electrophoresis to obtain exact sizes for each locus. MLVA type is designated by reporting these variable lengths arranged in an agreed order (i.e. STTR9-STTR5-STTR6-STTR10-STTR3). Public Health and OzFoodNet epidemiologists in NSW and Qld have found routine, rapid MLVA testing useful for more timely identification and response to outbreaks. However, differences in result reporting from different laboratories make its use in investigations of multi-state or national outbreaks difficult.

The differences in reporting are due to the use of different coding systems, which include: the original Lindstedt coding scheme (2) (used, until recently, by QFSS and MDU) in which allele numbers are allocated randomly to particular locus sizes and, one for each of the five loci form the MLVA type; an in-house system used by the IMVS, which was later replaced by European Centre for Disease Control (ECDC) system (5) and an "Australian" MLVA coding system (used by CIDM and MDU and recently adopted by QFSS), which was accepted, in principle, as the agreed method, but not universally implemented.

The latter is a repeat number-based coding system (3, 4) in which the number of repeated sequences at each locus is inferred by subtracting the length of the known flank-

ing region from the total length of the PCR amplicon and dividing the resulting value by the known length of individual repeat sequences. For locus STTR3, which contains repeat sequences of two different lengths, the actual amplicon size is used as the code. For the other four loci the assigned code is the “repeat number plus 1” (if there are no repeats, and only the flanking region is amplified, the code is “1”; if there is no amplicon [i.e. the flanking region is absent], the code is “0”). These five codes arranged in the agreed order form the MLVA type. The advantage of this system is that it allows an assessment of relationships between different MLVA types and is objective and easily understood.

However, in addition to coding differences, there can be also variations in size-calling of PCR amplicons. This can differ, between laboratories, from 1-2 base pairs (bp) to more than one repeat length difference. Over the past a few years the MDU conducted several inter-laboratory QAP/PTP programs to validate methods between reference laboratories. The size-calling differences between laboratories were persistent and the problem remained unresolved. Various factors were implicated, including differences in genetic analysers, polymers, capillary arrays, size ladders and even primer-labelling dyes, some of which cannot easily be harmonized between labs.

In later 2011, the CIDM and QFSS participated in an ECDC quality assurance program (QAP), which involved testing a calibration set of STM isolates, for which each of the five loci had been sequenced to provide a gold standard for size calling. Accurate sizes were determined by reference to a size compensation table provided by the ECDC, showing how sizes measured by individual laboratories should be adjusted to reflect the true sequenced sizes. The results showed that the “Australian” coding convention, as used by CIDM, produced predictable differences in size lengths for loci STTR5, STTR6 and STTR10 compared with the ECDC system, and can easily be adjusted to give comparable results when different coding systems are taken into account. The ECDC QAP provides the basis for resolution of size-calling differences between Australian laboratories in results of the QAP programs conducted by MDU.

On November 29th, 2011, a MLVA typing harmonization meeting was held in Sydney in conjunction with OzFoodNet. Laboratory scientists from IMVS, QFSS and CIDM and OzFoodNet epidemiologists attended the meeting and a final agreement was reached regarding the MLVA reporting system, whilst acknowledging that MDU and PathWest were not represented. This was summarised in a final report (6) and, in brief, the following decisions were taken without dissent: the coding system currently in use by CIDM, QFSS and MDU (repeat numbers plus one for the first 4 loci and whole amplicon length for the 5th) would be adopted nationally; CIDM will prepare documents outlining the coding system and

standard operating procedures (SOP) for the most commonly used MLVA method (primers, PCR conditions, size marker, equipment etc) with instructions for adjustment of amplicon sizes, when necessary, for laboratories using different methods;

A collaborative QAP system will be established, in which each MLVA testing laboratory, in rotation, will identify a small set of isolates, chosen to cover the range of current MLVA types, with varied copy numbers for each locus. The MLVA loci of these isolates will be sequenced and used as gold standard for analysis of results from all laboratories to which they are sent for testing.

It was proposed that OzFoodNet consider including a laboratory session in future meetings to allow further discussion and updates on strain typing and molecular epidemiology of food-borne pathogens.

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