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Persistent *Salmonella enterica* serovar Typhi sub-populations within host interrogated by whole genome sequencing and metagenomics

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Full article can be found in:
https://journals.plos.org/plosone/article?id=10.1371/journal.pone.0289070

Salmonella enterica subsp. *enterica* serovar Typhi (*S.* Typhi) is the major cause of typhoid fever, a potentially fatal multisystemic illness that is contracted via the faecal-oral route [1]. It remains a global public health problem with an estimated global 10.9 million infections and 116,900 deaths each year [2]. Typhoid fever also disproportionately impact individuals living in developing countries [2, 3]. After resolution of clinical symptoms, some individuals can transition to a carrier state, with intermittent bacterial shedding ranging from three weeks to greater than a year where persistent shedding poses a health risk to other individuals in their vicinity [1]. The key ecological niche for *S.* Typhi carriage within a human host has been determined to be the gallbladder [4-7]. While the gallbladder is a hostile site for survival due to the presence of bile, *S.* Typhi can survive in this niche via the management of oxidative stress, sustained cell envelope component synthesis, and biofilm formation on cholesterol rich gall stones [5, 8-12].

Inside this issue

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In this study, we employed both whole-genome sequencing (WGS) and metagenomics to interrogate within-host dynamics of *S.* Typhi recovered across two household cases (Figure 1). The first patient (Case A) was admitted to hospital with fever of unknown origin. *S.* Typhi was detected in the blood culture of Case A and an isolate was successfully cultured (CIDM-STyphi-A01). As Case A did not have any recent travel to typhoid endemic countries, household contacts were screened for *S.* Typhi. This resulted in *S.* Typhi being recovered from stool sample of one household member (Case B). Case B recalled a previous episode of typhoid fever approximately six years prior in a high incidence country.

However, there were discrepant serotyping results between CIDM-STyphi-A01 and the isolate from Case B (CIDM-STyphi-B01). CIDM-STyphi-A01 was serotyped as *S.* Typhi (Antigenic structure: 9,12,Vi:d:-) while CIDM-STyphi-B01 was serotyped as a rough, monophasic *Salmonella* (Antigenic structure: rough,Vi:d-). When subjected to WGS on the Illumina NextSeq 500 platform (150-cycle mid output), it was determined that both CIDM-STyphi-A01 and CIDM-STyphi-B01 were genetically close with nine core single nucleotide polymorphism (SNP) difference between the two isolates (Table 1). Further interrogation into the genome of CIDM-STyphi-B01 revealed that it had a deletion in a genomic region which consisted of genes responsible for O antigen biosynthesis (Figure 2). This deletion would yield the rough phenotype observed in CIDM-STyphi-B01.

Chronic carriage with a diverse *S.* Typhi population in case B was suspected when CIDM-STyphi-B01 was found to be phenotypically and genomically different from CIDM-STyphi-A01. After 15 days from the first collection of Case B, another isolate of *S.* Typhi was recovered from a stool sample (CIDM-STyphi-B02). When sequenced, CIDM-STyphi-B02 clustered genomically with CIDM-STyphi-A01, showing zero core SNP difference (Table 1). Approximately two months after the isolation of CIDM-STyphi-B01, two other isolates were recovered from stool samples of Case B. These two isolates, CIDM-STyphi-B03 and CIDM-STyphi-B04, genomically clustered with each other (zero core SNPs) but not with the other *S.* Typhi isolates thus far (Table 1). CIDM-STyphi-B05 and CIDM-STyphi-B06, isolated from bile fluid and the gall bladder swab respectively, were obtained during elected cholecystectomy for Case B to treat chronic carriage. Both CIDM-STyphi-B05 and CIDM-STyphi-B06 shared zero SNPs with each other and clustered together with CIDM-STyphi-B01 (Table 1).

Based on the clustering of core SNPs, each genome was subsequently assigned to three different sub-populations with CIDM-STyphi-A01/B02, CIDM-STyphi-B01/B05/B06 and CIDM-STyphi-B03/B04 forming sub-population 01, sub-population 02 and sub-population 03 respectively. Interrogation of the core SNP alignment of all isolates revealed that the presence, or absence, of 11 informative SNPs could be used to differentiate between the three subpopulations (Figure 3). Detection of these 11 SNPs were vital as they provided *a priori* knowledge for the detection of mixed carriage of *S.* Typhi within Case B when metagenomics was applied.

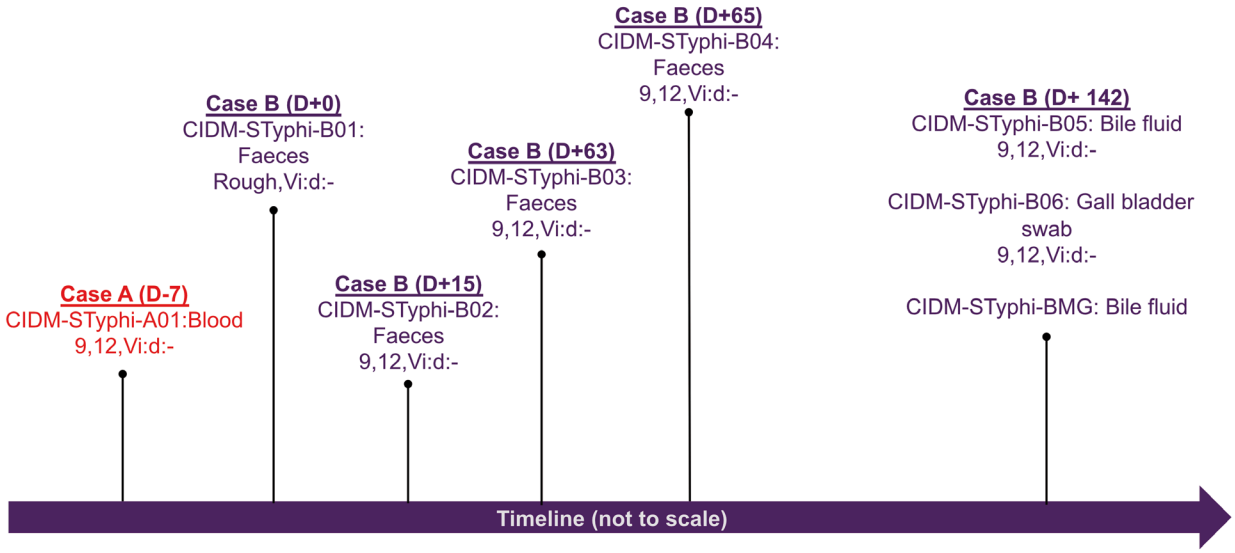
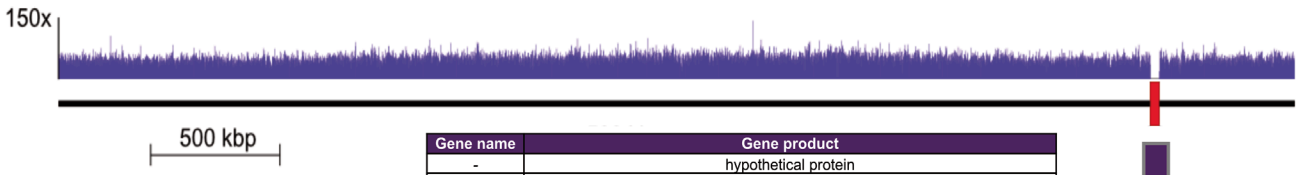


Figure 1: Timeline of *S.* Typhi recovery from Case A and Case B. Timeline measured in days (designated by the letter 'D') relative to the isolation of the first sample from Case B. Sample designation, source and antigenic structure are listed for each isolate.

	CIDM-STyphi-A01	CIDM-STyphi-B01	CIDM-STyphi-B02	CIDM-STyphi-B03	CIDM-STyphi-B04	CIDM-STyphi-B05	CIDM-STyphi-B06
CIDM-STyphi-A01	0	9	0	9	9	8	8
CIDM-STyphi-B01	9	0	9	8	8	1	1
CIDM-STyphi-B02	0	9	0	9	9	8	8
CIDM-STyphi-B03	9	8	9	0	0	7	7
CIDM-STyphi-B04	9	8	9	0	0	7	7
CIDM-STyphi-B05	8	1	8	7	7	0	0
CIDM-STyphi-B06	8	1	8	7	7	0	0

Table 1: SNP distance matrix of the seven isolates used in this study. To be considered a genomic cluster, genomes need to be within five SNP difference to each other.



Gene name	Gene product
-	hypothetical protein
-	hypothetical protein
-	hypothetical protein
gmd	GDP-mannose 4,6-dehydratase
fcl	GDP-L-fucose synthase
gmm	GDP-mannose mannosyl hydrolase
-	hypothetical protein
manC1	Mannose-1-phosphate guanylyltransferase 1
algC	Phosphomannomutase/phosphoglucomutase
wcaJ	UDP-glucose:undecaprenyl-phosphate glucose-1-phosphate transferase
wzxC	Lipopolysaccharide biosynthesis protein WzxC
-	hypothetical protein
-	hypothetical protein
gtfA	UDP-N-acetylglucosamine--peptide N-acetylglucosaminyltransferase GtfA subunit
-	hypothetical protein
galF	UTP--glucose-1-phosphate uridylyltransferase
rfbB	dTDP-glucose 4,6-dehydratase
rfbD_2	dTDP-4-dehydrorhamnose reductase
rmlA	Glucose-1-phosphate thymidylyltransferase
rfbC	dTDP-4-dehydrorhamnose 3,5-epimerase
ascD	CDP-6-deoxy-L-threo-D-glycero-4-hexulose-3- dehydrase reductase
rfbF	Glucose-1-phosphate cytidylyltransferase
rfbG	CDP-glucose 4,6-dehydratase
spnQ	dTDP-4-dehydro-2,6-dideoxy-D-glucose 3-dehydratase
rfbJ	CDP-abequose synthase
rfbE	CDP-paratose 2-epimerase
-	hypothetical protein
rfbV	Abequosyltransferase RfbV
mshA_2	D-inositol-3-phosphate glycosyltransferase
-	hypothetical protein
rfbM	Mannose-1-phosphate guanylyltransferase RfbM
glmM_2	Phosphoglucosamine mutase
-	hypothetical protein

Figure 2: Deletion of the O antigen biosynthesis cluster in CIDM-STyphi-B01. Mapping profile of CIDM-STyphi-B01 across the genome of 129-0238-M (NCBI assembly accession: GCA_001359025.2). Read depths are represented by the blue histogram and the deletion in CIDM-STyphi-B01 is marked up with a red rectangle in its analogous position in 129-0238-M. List of genes and gene products within the deletion are listed in the figure.

During elective cholecystectomy, bile fluid was also collected and subjected to metagenomics for the interrogation of mixed carriage of *S.* Typhi (Figure 1). The DNA extract of the bile fluid was sequenced on a Illumina MiniSeq platform (150-cycle mid output). Sequencing generated 12,498,164 paired-reads where majority were made up of Human DNA (71.38%). However, amongst the reads taxonomically classified as bacteria, *S. enterica* was the most abundant species (Figure 4A). Contaminating human sequences were subsequently removed from CIDM-STyphi-BMG and the host-free sequencing reads were subjected to downstream analysis. At the metagenomic assembly level, three of the eleven SNPs, namely SNP-01, SNP-08 and SNP-11, were assembled to their respectively discriminatory SNPs for sub-population 02 which included the isolates CIDM-STyphi-B01, CIDM-STyphi-B05 and CIDM-STyphi-B06.

When the sequencing reads of host-removed CIDM-STyphi-BMG was interrogated, the three discriminatory SNP positions for sub-population 02 all showed mixed nucleobases (Figure 4B). At these positions, the discriminatory nucleobase made up only 70.10 ± 3.01% of the reads. When the remaining nine SNP positions were interrogated, the discriminatory positions for sub-population 03, namely SNP-02, SNP-05 and SNP-06, also had mixed nucleobases with the discriminatory nucleobase making up 29.89 ± 7.04% of the reads (Figure 4B). There was no evidence for the presence of sub-population 01 in CIDM-STyphi-BMG. Through metagenomics, we showed that *S.* Typhi carriage in case B was indeed associated with at least two sub-populations, sub-population 02 and sub-population 03, with the former being the dominant sub-population.

In conclusion, our study utilised a set of longitudinal isolates of *S*. Typhi recovered from stool and sterile sites of an epidemiologically linked case and genomic diversity was interrogated using both genomic and metagenomic approaches. Our findings highlighted the within-host diversity of *S*. Typhi populations in chronic carriers and will contribute to our understanding of within-host dynamics in cases of persistent infection and inform epidemiological investigations of transmission events associated with chronic carriers. Finally, when chronic carriage of *S.* Typhi is suspected, utilisation of either metagenomic sequencing, WGS of multiple randomly selected colonies from a single sample, or both approaches, should be considered.

References

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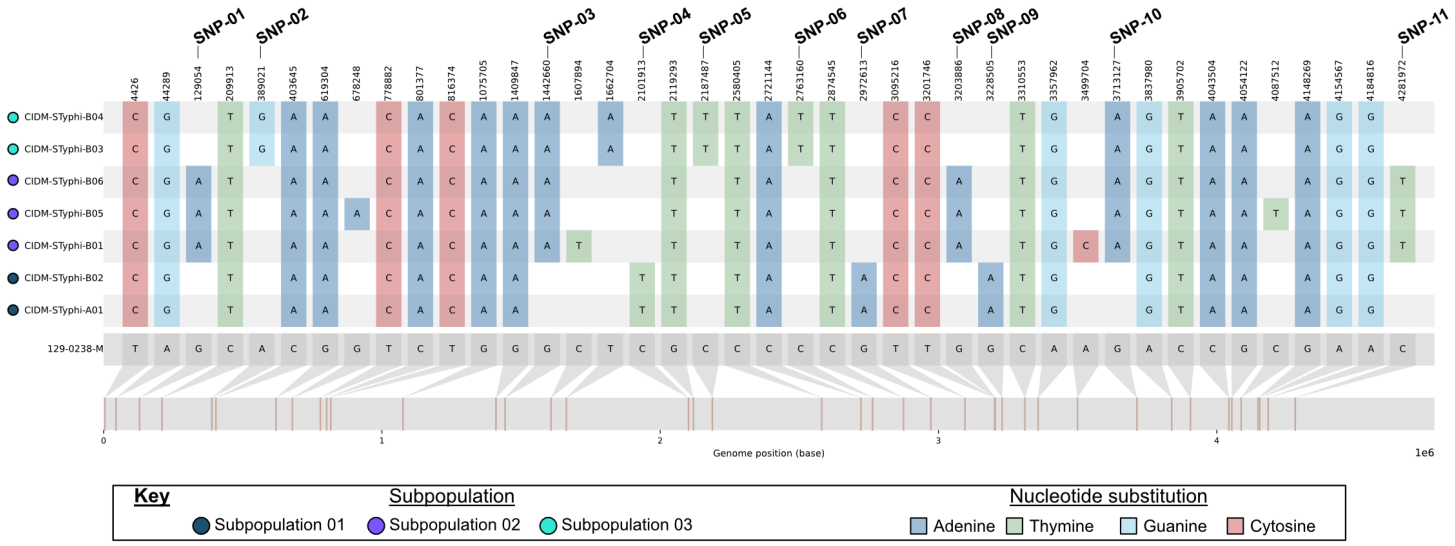


Figure 3: SNPs for the detection of sub-populations. Discriminatory SNP positions are marked in the figure. *S.* Typhi sub-populations of the source genomes are represented next to the isolate name and colour-coded according to the Figure Key. Image was adapted from the published manuscript [13].

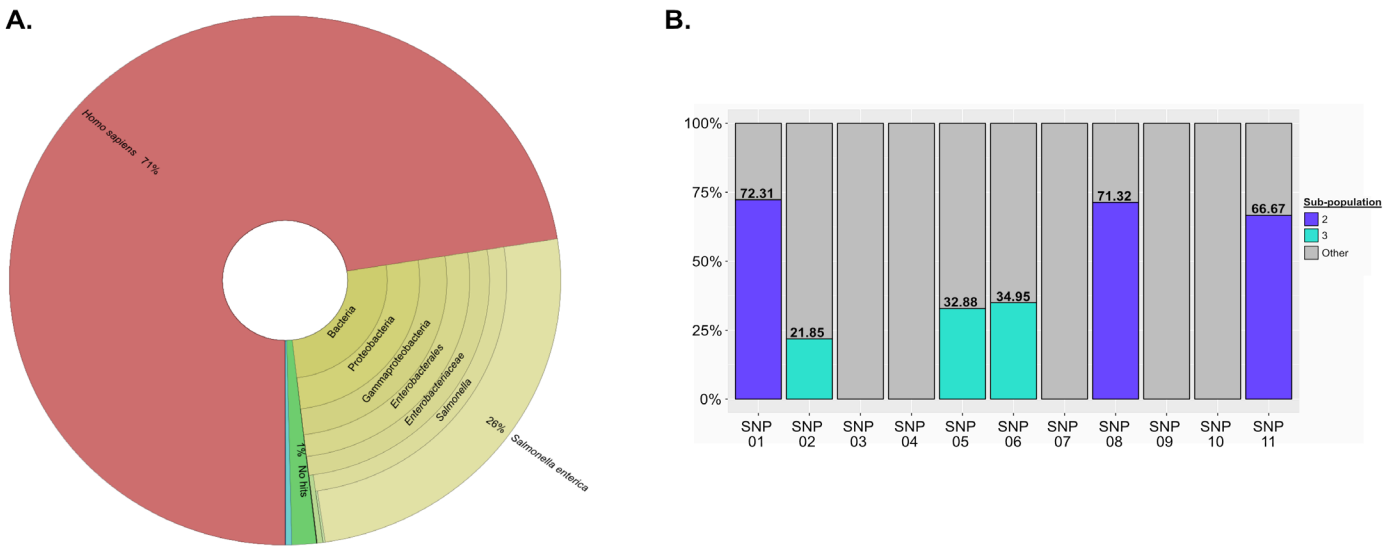


Figure 4: Bile metagenomics (A) Taxonomical classification of sequencing reads from CIDM-STyphi-BMG. (B) Proportions of the three sub-populations in host -removed CIDM-STyphi-BMG, inferred from read mapping data over the 11 discriminatory SNP positions. Stacked histograms are colour-coded according to the figure key. As each SNP position is discriminatory for a single sub-population, reads that do not carry the discriminatory SNP were classified as "Other". Images in both panels were adapted from the published manuscript [13].

News & Events

The CIDM-PH Trial of Genomic Surveillance for Pathogens with Epidemic Potential aims to measure the impact of integrated pathogen genomic surveillance for public health care in New South Wales. As part of this trial, CIDM-PH researchers visited regional public health units (PHUs) to run workshops on the public health applications of pathogen genomics: on 2nd August in Wagga Wagga for Murrumbidgee and Southern NSW LHDs, and on 20th September in Bathurst for Far West and Western NSW LHDs.

At these workshops, researchers talked to PHU staff about communicable disease investigations in regional NSW where genomics could provide additional insight. The workshops identified several potential avenues for research collaboration to develop new genomics capacity in NSW. The trial will continue with ongoing collaboration with participating PHUs and periodic surveys to understand how pathogen genomics is being used.

Trial of Pathogen Genomics Workshops
Above: Bathurst PHU
Below: Wagga Wagga PHU

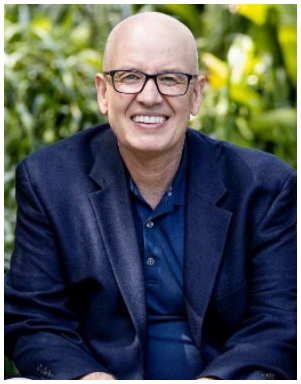
The Centre for Complex Systems, University of Sydney, in collaboration with CIDM-PH presented the **Digital Epidemiology and Pathogen Genomics Workshop** at Westmead. Research in complex systems' modelling approaches to foodborne disease epidemics from non-typhoidal Salmonella epidemiology and pandemic modelling were presented, followed by moderated discussions on the role of disease modelling in informing public health response to infectious diseases.

Digital Epidemiology and Pathogen Genomics Workshop

CONGRATULATIONS!!

Professor Holmes awarded Croonian Medal by Royal Society

Professor Eddie Holmes has been awarded the Croonian Medal and Lecture, one of the most prestigious prizes in the biological sciences. The Croonian Medal and Lecture was awarded to Professor Holmes for being a global authority on virus evolution and emergence, including playing a key role in the discovery and characterisation of SARS-CoV-2 and the first to publicly release the genome sequence.



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UPCOMING EVENTS

CIDM-PH Webinar

Dr Dalong Hu
Research Fellow
National University of Singapore

Pre-emptive epidemiology: a One Health approach to cost-effective precision surveillance of AMR pathogens

28 September 2023

2.00pm – 3.00pm (AEST)

REGISTRATION:
HTTPS://CIDMPHWEBINARDALONGHU.EVENTBRITE.COM.AU

SAVE THE DATE…

PROGRAM & REGISTRATION COMING SOON

CIDM-PH Annual Colloquium

24 November 2023

9.00am – 5:00pm (AEST)

WECC, Westmead Hospital, Westmead NSW

Event Enquiries:

WSLHD-CIDM-PH@health.nsw.gov.au

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