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ORIGINAL ARTICLE

# Genomic surveillance of *Salmonella* spp. in the Philippines during 2013–2014

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**Background:** Increasing antimicrobial resistance (AMR) in *Salmonella* has been observed in the Philippines. We aimed to characterise the population and AMR mechanisms of *Salmonella* with whole genome sequencing (WGS) and compare it with laboratory surveillance methods.

**Methods:** The serotype, multilocus sequence type, AMR genes and relatedness between isolates were determined from the genomes of 148 *Salmonella* Typhi (*S.* Typhi) and 65 non-typhoidal *Salmonella* (NTS) collected by the Antimicrobial Resistance Surveillance Program during 2013–2014. Genotypic serotypes and AMR prediction were compared with phenotypic data.

**Results:** AMR rates in *S.* Typhi were low, with sparse acquisition of mutations associated with reduced susceptibility to fluoroquinolones or extended-spectrum beta-lactamases (ESBL) genes. By contrast, 75% of NTS isolates were insusceptible to at least one antimicrobial, with more than half carrying mutations and/or genes linked to fluoroquinolone resistance. ESBL genes were detected in five genomes, which also carried other AMR determinants. The population of *S.* Typhi was dominated by likely endemic genotype 3.0, which caused a putative local outbreak. The main NTS clades were global epidemic *S.* Enteritidis ST11 and *S.* Typhimurium monophasic variant (I,4,[5],12: i: -) ST34.

**Conclusion:** We provide the first genomic characterisation of *Salmonella* from the Philippines and evidence of WGS utility for ongoing surveillance.

**Keywords:** antimicrobial drug resistance, epidemiology/surveillance, genomics, salmonella, typhoid fever, whole genome sequencing

## Introduction

*Salmonella enterica* is a common cause of gastroenteritis and bacteraemia worldwide.[1] Although *S. enterica* comprises >2600 serovars, most human infections are caused by a limited number of serovars with different clinical presentations. The typhoidal *Salmonella* include *S.* Typhi and *S.* Paratyphi A, B and C, and are human host-restricted organisms that cause enteric fever, a systemic disease that disproportionally affects children in south-central and southeast Asia and sub-Saharan Africa and is treated with antibiotics. Other serovars are grouped as non-typhoidal *Salmonella* (NTS) and usually cause self-limiting gastroenteritis not requiring antimicrobial treatment. Less commonly, complicated invasive NTS infections that require antibiotic treatment are seen in specific populations, like the immunocompromised.[2]

In the Western Pacific Region, invasive infectious disease agents account for 22% of the foodborne disease burden, with *S.* Typhi and *S.* Paratyphi A as the leading causes. Diarrhoeal disease agents account for 14% of the foodborne disease burden, with NTS the second leading cause after *Campylobacter* spp.[3] In the Philippines, the most common NTS serovars are *S.* Enteritidis and *S.* Typhimurium,[4] which parallels trends in the Western Pacific Region and worldwide.[5,6]

Antimicrobial resistance (AMR) in foodborne pathogens, including *S. enterica*, is a major concern for public health globally. In recent years, rising rates of fluoroquinolone and third-generation cephalosporin resistant *S. enterica* in humans have been reported.[1] In the Philippines, resistance rates of *S.* Typhi against first- and second-line antibiotics remained <10% and without significant variations in the last 10 y.[4] By contrast, resistance rates of NTS against first- and second-line antibiotics >10% were recorded, with resistance to ceftriaxone (third-generation cephalosporin) and ciprofloxacin oscillating around this value in recent years. Resistance to third-generation cephalosporin generally arises via the acquisition of extended-spectrum beta-lactamases (ESBL) or AmpC hydrolytic enzymes.[1] Resistance to fluoroquinolones such as ciprofloxacin may be due to mutations in the quinolone-resistance determining region (QRDR) of the *gyr* and *par* genes or the acquisition of plasmid-mediated quinolone resistance (PMQR) genes.[7]

Until recently, AMR surveillance by the Philippine Department of Health Antimicrobial Resistance Surveillance Program (DOH-ARSP) had involved exclusively phenotypic methods. In this study, we sequenced the whole genomes of *Salmonella* isolates collected by the ARSP during 2013–2014 using WGS to describe their population, identify AMR determinants and to determine the concordance between laboratory tests and genotypic predictions of serotype and resistance.

## Materials and Methods

### Bacterial isolates

A total of 258 *S.* Typhi and 326 NTS isolates were collected by the Philippine DOH-ARSP in 2013 and 2014 (Table 1), and 171 *S.* Typhi and 68 NTS isolates were referred to the ARSRL for confirmation of bacterial identification and resistance profile. Out of these, 153 *S.* Typhi and 65 NTS isolates successfully resuscitated from the biobank were submitted for whole-genome sequencing (WGS).

### Antimicrobial susceptibility testing

Isolates were tested for antimicrobial susceptibility to seven antimicrobial agents, ampicillin (AMP), ceftriaxone (CRO), cefotaxime(CTX), chloramphenicol (CHL), ciprofloxacin (CIP), nalidixic acid (NAL) and trimethoprim-sulfamethoxazole (SXT) with the Vitek 2 Compact automated system (bioMérieux, Marcy-l'Étoile, France) and interpretive criteria and breakpoints from the Performance Standards for Antimicrobial Susceptibility Testing (26th edition) of the Clinical and Laboratory Standards Institute (CLSI).[8] The ESBL phenotype and insusceptibility to quinolones were confirmed using E-test (bioMérieux). Multi-drug resistant (MDR) organisms were those resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole.[9]

### Serotyping

Serological serotyping was performed using the Sven-Gard method for slide agglutination with antisera from Denka Seiken (Tokyo, Japan) and S&A serotest (Thailand). *Salmonella* serotypes were determined with the White–Kauffmann classification scheme.[10]

### DNA extraction and WGS

Isolates were grown on tryptic soy broth overnight at 35°C. DNA was extracted from single colonies using Wizard Genomic DNA Purification Kit (Promega). The DNA extracts were shipped to the Wellcome Sanger Institute for sequencing on the Illumina HiSeq platform (Illumina, San Diego, CA, USA) with 100-bp paired-end reads. Raw sequence data were deposited in the European Nucleotide Archive under the study accession PRJEB17615. Individual run and sample accessions are provided through the links to Microreact projects in the figure legends.

### Bioinformatics analysis

Genome quality was evaluated based on metrics generated from assemblies, annotation files and the alignment of the reads to the reference genome of strains 08–00436 (accession GCF_002238275.1) or CT18 (accession GCF_000195995.1), as previously described.[11] Annotated assemblies were produced as described in detail previously.[12]

Evolutionary relationships between 148 *S.* Typhi isolates were inferred from single-nucleotide polymorphisms (SNPs) by mapping the paired-end reads to the reference genome of strain CT18 (accession) as described in detail previously.[11] The mobile genetic elements and repetitive sequences in the genome of CT18 previously defined[8,13] were masked in the pseudo-genome alignment with a script available at https://github.com/sanger-pathogens/remove_blocks_from_aln. Recombination regions were removed using Gubbins v. 2.0.0[14] and the non-recombinant SNPs were used to infer a maximum-likelihood tree with RAxML v. 8.28[15] based on the generalised time reversible model with the GAMMA method of correction for among-site rate variation and 500 bootstrap replications. Pairwise SNP differences between genomes were calculated from alignments of SNP positions with a script available at https://github.com/simonrharris/pairwise_difference_count.

Evolutionary relationships between 65 NTS isolates were inferred from core genome SNPs. The core genome was determined with Roary v. 3.12.0,[16] using a blastp percentage identity of 95% and a core definition of 99%. SNPs were identified in the core genome alignment with snp-sites v. 2.4.0[17] and a tree was obtained with RAxML as described above.

Serotype[18] and multi-locus sequence type (MLST[19]) information was derived from all *Salmonella* assembly sequences with Pathogenwatch,[20] as well as genotype information for *S.* Typhi.[21]

Known AMR genes and mutations were identified in the *S.* Typhi assemblies using Pathogenwatch, and in the NTS genomes from sequence reads using ARIBA[22] and the Resfinder[23] (genes) and Pointfinder[24] (mutations) databases. The genotypic predictions of AMR (test) were compared with the phenotypic results (reference), and the concordance between the two methods was computed for seven antimicrobials. Isolates with either a resistant or an intermediate phenotype were considered non-susceptible for comparison purposes.

To contextualise the *S.* Typhi genomes, we compared with global genomes belonging to genotypes 3.0 (n=51), 3.2.1 (n=70) and 4.1 (n=141) available on Pathogenwatch (as of May 2021), which clusters the genomes based on genetic similarity as described in detail previously.[20]

**Table 1.** Number of *Salmonella* isolates analysed by the Antimicrobial Resistance Surveillance Program (ARSP) and referred to the Antimicrobial Resistance Surveillance Reference Laboratory (ARSRL) during 2013 and 2014, isolates submitted for whole-genome sequencing and high-quality genomes obtained, discriminated by sentinel site and AMR profile

| | S. Typhi | | | NTS | | | |
|---|---|---|---|---|---|---|---|
| | 2013 | 2014 | Total | 2013 | 2014 | Total | Grand total |
| Total ARSP | 119 | 139 | 258 | 168 | 158 | 326 | 584 |
| Referred to ARSL | 84 | 87 | 171 | 31 | 37 | 68 | 239 |
| Submitted for WGS | 78 | 75 | 153 | 31 | 34 | 65 | 218 |
| High-quality genomes | 76 | 72 | 148 | 31 | 34 | 65 | 213 |
| *By sentinel site* | | | | | | | |
| BGH | 2 | 3 | 5 | 2 | 2 | 4 | 9 |
| BRT | 4 | 3 | 7 | 0 | 0 | 0 | 7 |
| CMC | 15 | 13 | 28 | 0 | 1 | 1 | 29 |
| CVM | 5 | 2 | 7 | 1 | 1 | 2 | 9 |
| DMC | 3 | 3 | 6 | 1 | 4 | 5 | 11 |
| EVR | 13 | 9 | 22 | 0 | 1 | 1 | 23 |
| FEU | 2 | 1 | 3 | 0 | 2 | 2 | 5 |
| GMH | 5 | 9 | 14 | 0 | 0 | 0 | 14 |
| JLM | 0 | 0 | 0 | 0 | 4 | 4 | 4 |
| MAR | 4 | 6 | 10 | 0 | 3 | 3 | 13 |
| MMH | 1 | 0 | 1 | 0 | 0 | 0 | 1 |
| NMC | 2 | 1 | 3 | 1 | 0 | 1 | 4 |
| RMC | 0 | 0 | 0 | 2 | 0 | 2 | 2 |
| SLH | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| STU | 1 | 1 | 2 | 12 | 10 | 22 | 24 |
| VSM | 18 | 21 | 39 | 11 | 3 | 14 | 53 |
| ZMC | 1 | 0 | 1 | 0 | 3 | 3 | 4 |
| *By AMR profile* | | | | | | | |
| Susceptible | 73 | 69 | 142 | 17 | 17 | 34 | 176 |
| AMP | 0 | 0 | 0 | 4 | 7 | 11 | 11 |
| SXT | 0 | 0 | 0 | 3 | 2 | 5 | 5 |
| AMP CHL | 0 | 0 | 0 | 2 | 2 | 4 | 4 |
| AMP SXT CHL | 0 | 0 | 0 | 1 | 2 | 3 | 3 |
| AMP CIP SXT CHL | 0 | 0 | 0 | 2 | 0 | 2 | 2 |
| AMP CRO | 1 | 0 | 1 | 1 | 1 | 2 | 3 |
| AMP SXT | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| AMP CIP SXT | 0 | 0 | 0 | 1 | 0 | 1 | 1 |
| AMP CIP | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| CHL | 0 | 0 | 0 | 0 | 1 | 1 | 1 |
| CIP NAL | 2 | 3 | 5 | 0 | 0 | 0 | 5 |

## Results

### Demographic and clinical characteristics of the *salmonella* isolates

Out of the 218 *Salmonella* isolates sequenced, 5 were excluded based on genome quality (Table 1). The demographic and clinical characteristics of the remaining 213 isolates (148 *S.* Typhi, 65 NTS) are summarised in Table 2. The majority of the patients were male (126/213, 59.2%), but a pronounced difference in the distribution of patient gender was observed for NTS (64.6% male, 35.4% female). The group aged 0–14 y had the highest percentage of *S.* Typhi (60.1%, 89/148) and NTS (47.7%, 31/65) infections. NTS infections were also frequent in patients aged 45–80 y (38.5%, 25/65), while *S.* Typhi infections were rare in this age group (4.1%, 6/148). The vast majority of the *S.* Typhi isolates were from blood (137/148, 92.6%), while the NTS isolates were recovered from blood and stool in similar proportions (25/65 or 38.5% and 22/65 or 33.8%, respectively).

### Concordance between phenotypic and genotypic serotyping and AMR

We determined the serotype of *Salmonella* organisms both by serological methods and genoserotyping. We predicted

**Table 2.** Demographic and clinical characteristics of *Salmonella* culture-positive patients with genomes included in this study (n=213)

| Characteristic | | Number of isolates | |
|---|---|---|---|
| | | SAT | NTS |
| Gender | Male | 84 | 42 |
| | Female | 64 | 23 |
| Age (y) | <1 | 2 | 11 |
| | 1–4 | 16 | 14 |
| | 5–14 | 71 | 6 |
| | 15–24 | 26 | 3 |
| | 25–34 | 20 | 4 |
| | 35–44 | 4 | 0 |
| | 45–54 | 3 | 9 |
| | 55–64 | 2 | 9 |
| | 65–80 | 1 | 7 |
| | >81 | 0 | 1 |
| | Unknown | 3 | 1 |
| Patient type | Inpatient | 133 | 58 |
| | Outpatient | 14 | 7 |
| | Unknown | 1 | 0 |
| Specimen type | Abscess | 1 | 2 |
| | Aspirate | 0 | 2 |
| | Blood[a] | 137 | 25 |
| | Cerebrospinal fluid[a] | 0 | 3 |
| | Stool | 7 | 22 |
| | Urine | 3 | 0 |
| | Fluid | 0 | 1 |
| | Tracheal aspirate | 0 | 1 |
| | Wound | 0 | 9 |

[a]Invasive specimen.

*S.* Typhi only among typhoidal *Salmonella* and 15 different serotypes among the NTS, with *S.* Enteritidis (n=21), and monophasic variant I 4, [5],12: i:—of *S.* Typhimurium (n=16) being the most frequent. The concordance between genoserotyping and serological serotyping was 91.1% overall (194/213), 100% for typhoidal *Salmonella* (148/148 *S.* Typhi) and 70.8% for NTS (46/65). Genoserotyping predicted the monophasic *S.* Typhimurium serovar (I 4, [5],12: i: -) for 16 isolates serotyped in the laboratory as either *S.* Typhimurium (antigenic formula 1,4,[5],12: i:1,2, n=12) or Group B O:4;12; i;—(n=4, Figure 1A). In addition, genoserotyping predicted serovars *S.* Kentucky, *S.* Virchow and *S.* Enteritidis for three isolates reported as *S.* Anatum, *S.* Javiana and *S.* Heidelberg, respectively. *S.* Enteritidis was relatively more frequent than *S.* I 4, [5],12: i:—in invasive isolates (39.9% vs 17.9%, n=28), while their frequencies were comparable in non-invasive isolates (27.0% vs 29.7%, n=37).

We also determined the susceptibilities of *Salmonella* isolates to antimicrobials (Table 3). *S.* Typhi isolates were largely susceptible to five antimicrobials tested. Five isolates presented both decreased susceptibility to ciprofloxacin and resistance to nalidixic acid, explained by the presence of mutations in the QRDR of the *gyrA* gene (D87N, n=3, and D87G, n=2). One isolate was resistant to ampicillin and third-generation cephalosporins (ceftriaxone and cefotaxime), mediated by the presence of the ESBL gene $bla_{\text{CTX-M-15}}$ (Table 3). The overall concordance between phenotypic and genotypic resistance was 100% for *S.* Typhi.

The majority of NTS isolates (73.8%, 48/65) were insusceptible to at least one antimicrobial tested, most commonly to ciprofloxacin (55.4%, 36/65) and ampicillin (38.4%, 25/65). Only five isolates were MDR. Of note, the two *S.* Anatum isolates carried resistance determinants to beta-lactams ($bla_{\text{TEM-1}}$, $bla_{\text{DHA-1}}$), chloramphenicol (*cmlA*, *floR*), trimethoprim-sulfamethoxazole (*sul1*, *sul2*, *dfrA1*), ciprofloxacin (*qnrS1*, *qnrB4*, *oqxA*, *oqxB* and mutation T57S in the *parC* gene) and other antibiotics not tested in the laboratory (*aad2*, *strA-strB*, *tet(A)*, *mphA* and *lnu(F)*). The overall concordance between phenotypic and genotypic resistance was 95.62% for NTS. Chloramphenicol and trimethoprim-sulfamethoxazole exhibited the highest concordances (96.92% and 96.88%, respectively). The concordance for ceftriaxone was 95.31%, and the discordance was due to three false positive results. We identified genes known to confer resistance to third-generation cephalosporins in five genomes, which also carried at least one other AMR determinant. The ESBL gene $bla_{\text{CYM-2}}$ gene was found in the only *S.* Lexington isolate, the ESBL gene $bla_{\text{CTX-M-15}}$ was identified in two *S.* Stanley isolates, only one of which was resistant to ceftriaxone, and the AmpC gene $bla_{\text{DHA-1}}$ was found in the two *S.* Anatum isolates, both of which were susceptible to ceftriaxone. This could be due to low expression of the inducible $bla_{\text{DHA-1}}$ gene.[25]

## In silico genotyping

Multi-locus sequence type and genotype were also derived from the whole-genome sequences. *S.* Typhi isolates were assigned to ST1 (132/148), ST2 (14/148) and ST5215 (2/148), and to genotypes 3.0 (121/148), 3.2.1 (14/148), 3.4 (2/148) and 4.1 (11/148). Sixteen different STs were identified among the NTS isolates and they strongly correlated to genoserotypes (Figure 1A), which supports in silico serotype assignments. Consequently, ST11 (21/65, *S.* Enteritidis) and ST34 (16/65, I 4, [5],12: i: -) were the most prevalent. *S.* Typhimurium isolates were assigned to ST19 (n=1) and ST36 (n=1).

Genotype 3.0 was found in all 14 sentinel sites that referred *S.* Typhi isolates, while genotypes 3.4, 3.2.1 and, in particular 4.1, showed more regional distributions (Table 4 and Figure 2A). *S.* Enteritidis (ST11) and monophasic *S.* Typhimurium (ST34) also showed broad geographic distribution in all three island groups (Luzon in the north, Visayas in the centre and Mindanao in the south of the Philippines; Table 4 and Figure 1A).

## Population structure of *Salmonella* in the Philippines

The phylogenetic tree of 148 *S.* Typhi genomes was composed of four well-supported (bootstrap 100%), deep-branching clades that paralleled the genotype calls. However, we observed substantial diversification within the dominant genotype 3.0, which was broadly divided into two major subclades (I and II) in the tree composed by 47 and 74 genomes, respectively, and both with bootstrap support of 100%. The tree topology and the distribution of pairwise SNPs between genomes showed that the organisms in subclade II were genetically similar

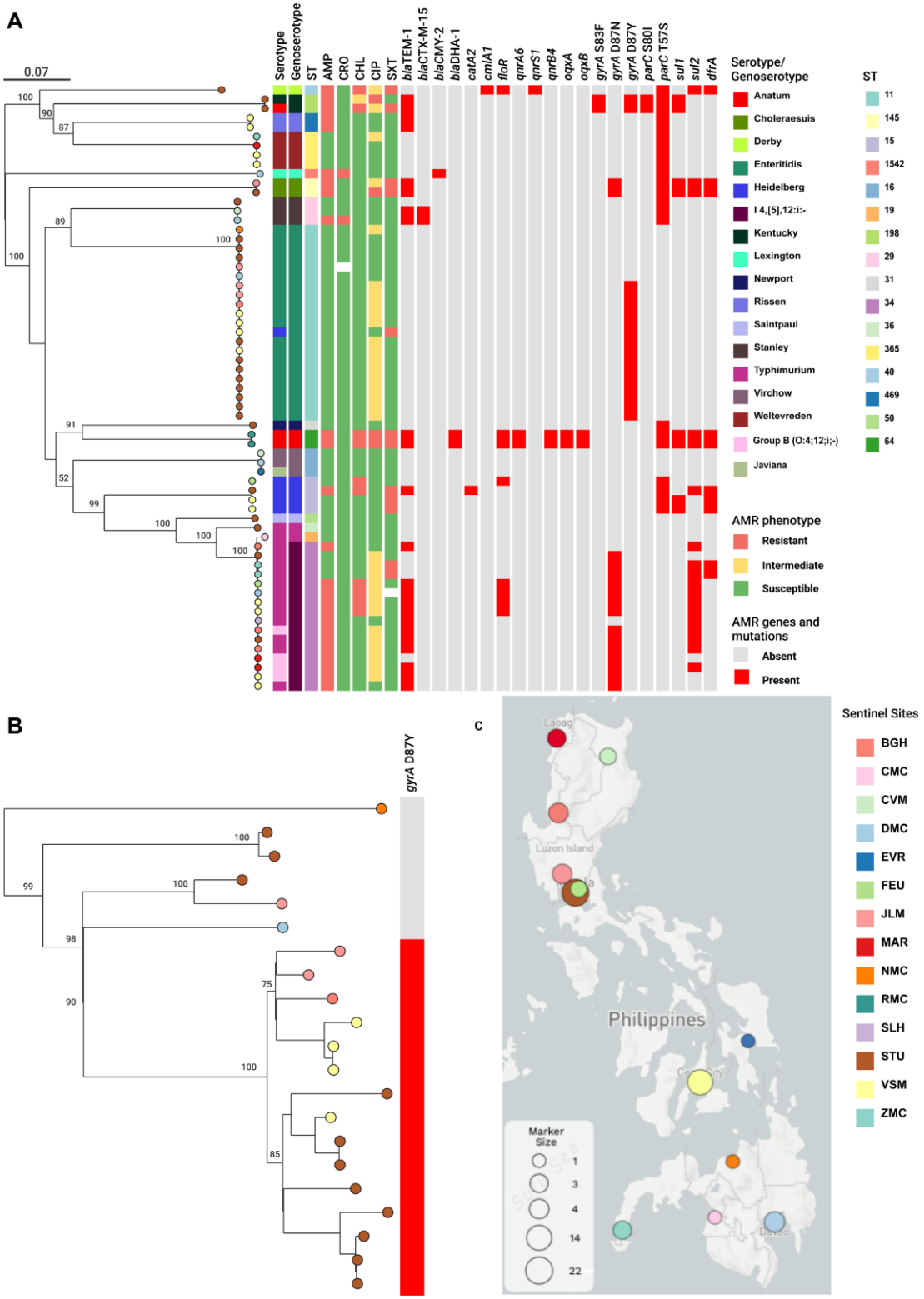
A
0.07
Serotype
Genoserotype
ST
AMP
CRO
CHL
CIP
SXT
blaTEM-1
blaCTX-M-15
blaCMY-2
blaDHA-1
catA2
cmlA1
floR
qnrA6
qnrS1
qnrB4
oqxA
oqxB
gyrA S83F
gyrA D87N
gyrA D87Y
parC S80I
parC T57S
sul1
sul2
dfrA
Serotype/ Genoserotype
Anatum
Choleraesuis
Derby
Enteritidis
Heidelberg
I 4,[5],12:i:-
Kentucky
Lexington
Newport
Rissen
Saintpaul
Stanley
Typhimurium
Virchow
Weltevreden
Group B (O:4;12;i;-)
Javiana
ST
11
145
15
1542
16
19
198
29
31
34
36
365
40
469
50
64
AMR phenotype
Resistant
Intermediate
Susceptible
AMR genes and mutations
Absent
Present
B
gyrA D87Y
C
Laoag
Luzon Island
Philippines
Marker Size
1
3
4
14
22
Sentinel Sites
BGH
CMC
CVM
DMC
EVR
FEU
JLM
MAR
NMC
RMC
SLH
STU
VSM
ZMC

**Figure 1.** Genomic surveillance of NTS from the Philippines, 2013–2014. (A) Phylogenetic tree of 65 isolates inferred from an alignment of 117 371 core genome SNP sites. (B) Subtree of 21 *S.* Enteritidis isolates. The tree leaves are coloured by sentinel site as indicated in (C). The trees are annotated with bootstrap values and the tree blocks indicate the distribution of the serological serotype, genoserotype, sequence types (STs), resistance phenotype for five antibiotics and acquired resistance genes and mutations. AMP: ampicillin; CRO: ceftriaxone; CHL: chloramphenicol; CIP: ciprofloxacin; SXT: sulphamethoxazole-trimetoprim. Origin of isolates. BGH: Baguio General Hospital and Medical Center; CMC: Cotabato Regional Hospital and Medical Center; CVM: Cagayan Valley Medical Center; DMC: Southern Philippines Medical Center; EVR: Eastern Visayas Regional Medical Center; FEU: Far Eastern University Hospital; JLM: Jose B. Lingad Memorial Regional Hospital; MAR: Mariano Marcos Memorial Hospital and Medical Center; NMC: Northern Mindanao Medical Center; RMC: Rizal Medical Center; SLH: San Lazaro Hospital; STU: University of Sto. Tomas Hospital; VSM: Vicente Sotto Memorial Medical Center; ZMC: Zamboanga City Medical Center. The full data are available at https://microreact.org/project/k2BC6hsaxYr1Eo5U9v71iJ-arspnts2013-2014.

**Table 3.** Comparison between antimicrobial susceptibility testing results and genotypic resistance for 213 *Salmonella* isolates

| Antibiotic class | Antibiotic | Isolates tested | Resistant isolates | False positive | False negative | % concordance | Resistance genes/SNPs |
|---|---|---|---|---|---|---|---|
| *S.* Typhi | | | | | | | |
| Penicillin | Ampicillin | 148 | 1 | 0 | 0 | 100 | $bla_{CTX\text{-}M\text{-}15}$ |
| 3rd Generation Cephalosporins | Cefotaxime | 148 | 1 | 0 | 0 | 100 | |
| | Ceftriaxone | 148 | 1 | 0 | 0 | 100 | |
| Fluoroquinolones | Ciprofloxacin | 148 | 5 | 0 | 0 | 100 | *gyrA*_D87G/D87N |
| Quinolone | Nalidixic Acid | 148 | 5 | 0 | 0 | 100 | |
| NTS | | | | | | | |
| Penicillin | Ampicillin | 65 | 25 | 1 | 2 | 95.38 | $bla_{TEM\text{-}1}$, $bla_{CTX\text{-}M\text{-}15}$, $bla_{CMY\text{-}2}$, $bla_{DHA\text{-}1}$ |
| 3rd Generation Cephalosporins | Ceftriaxone | 64 | 2 | 3 | 0 | 95.31 | $bla_{CTX\text{-}M\text{-}15}$, $bla_{CMY\text{-}2}$, $bla_{DHA\text{-}1}$ |
| Fluoroquinolones | Ciprofloxacin | 65 | 36 | 2 | 2 | 93.85 | *gyrA*_S83F/D87N/D87Y, *parC*_S80I/T57S, *qnrA6*, *qnrS1*, *qnrB4*, *oqxA*, *oqxB* |
| Quinolone | Nalidixic Acid | 65 | 35 | 2 | 1 | 95.38 | |
| Folate Pathway Antagonist | Cotrimoxazole | 64 | 12 | 0 | 2 | 96.88 | *sul1*, *sul2*, *dfrA* |
| Phenicols | Chloramphenicol | 65 | 11 | 0 | 2 | 96.92 | *catA2*, *cmIA1*, *floR* |

(Figure 2A and B). Pairs of genomes belonging to subclade II were separated by median of 43 SNPs (IQR=35–51), while pairs in subclade I diverged by a median of 85 SNPs (IQR=67–100), and pairs of genomes belonging to different subclades diverged by a median of 130 SNPs (IQR=123–138). Nevertheless, both subclades were found in all three island groups. The five isolates with decreased susceptibility to ciprofloxacin were all found within subclade II, but at least two independent acquisitions of two different resistance mutations were evidenced on the tree. Importantly, isolates carrying GyrA_D87N disseminated between two different sites in Luzon (Figure 2A). Within the more diverse subclade I, we observed a group of 15 tightly clustered isolates (bootstrap support 100%) from Cotabato Regional and Medical Center (CMC) recovered between May 2013 and July 2014 (Figure 2A). The genomes in this cluster diverged by a median of three pairwise SNPs (range 0–8) and carried no known resistance determinants, suggesting an outbreak of enteric fever caused by a pan-susceptible strain in the population served by this hospital.

NTS isolates belonging to the same genoserotype clustered tightly together on long branches of the phylogenetic tree, thus supporting the genomic predictions. A closer inspection of the *S.* Enteritidis subtree showed that the 15 genomes carrying mutation GyrA_D87Y associated with reduced susceptibility to ciprofloxacin formed a discreet, well-supported cluster (100% bootstrap) of broader geographical distribution (Figure 1B). The remaining six *S.* Enteritidis genomes without any known acquired resistance determinants were found on four different branches of the subtree with narrow geographical distribution. While the distribution of invasive isolates did not significantly associate with the presence of GyrA_D87Y ($p>0.05$), we found relatively more invasive isolates within this successful clone (9/15) than among those without the mutation (2/6).

**Table 4.** Distribution of sequence types (STs), genoserotype and resistance profiles of *Salmonella* across the 17 sentinel sites that referred isolates. Numbers in parentheses indicate the number of isolates

| Site[a] | No. of *S.* Typhi isolates | Prevalent ST | No. of genotypes | Prevalent genotype | AMR resistance profiles | No. of NTS isolates | Prevalent ST[b] | No. of serotypes | Prevalent serotype[b] | AMR resistance profiles[c] |
|---|---|---|---|---|---|---|---|---|---|---|
| BGH | 5 | 1 (5) | 1 | 3.0 (5) | Susceptible (3) CIP, NAL (2) | 4 | 34 (3) | 2 | I 4,[5],12:i:- (3) | AMP (3) |
| BRT | 7 | 1 (7) | 2 | 3.0 (5) | Susceptible (7) | 0 | NA | NA | NA | NA |
| CMC | 27 | 1 (21) | 2 | 3.0 (21) | Susceptible (27) | 1 | 19 (1) | 1 | SAM (1) | Susceptible (1) |
| CVM | 8 | 1 (7) | 2 | 3.0 (7) | Susceptible (8) | 2 | 16 (1) | 2 | SVR (1) | Susceptible (1) |
| DMC | 6 | 1 (5) | 2 | 3.0 (5) | Susceptible (5) AMP, CRO (1) | 5 | 11 (1) | 5 | SEN (1) | Susceptible (1) |
| EVR | 22 | 1 (22) | 2 | 3.0 (21) | Susceptible (22) | 1 | 16 (1) | 1 | SVR (1) | Susceptible (1) |
| FEU | 3 | 1 (3) | 1 | 3.0 (3) | Susceptible (3) | 2 | 34 (1) | 2 | I 4,[5],12:i: (1) | AMP, CHL (1) |
| GMH | 14 | 1 (13) | 3 | 3.0 (8) | Susceptible (14) | 0 | NA | NA | NA | NA |
| JLM | 0 | NA | NA | NA | | 4 | 11 (3) | 2 | SEN (3) | Susceptible (3) |
| MAR | 10 | 1 (9) | 1 | 3.0 (10) | Susceptible (9) CIP, NAL (1) | 3 | 34 (2) | 2 | I 4,[5],12:i:- (2) | Susceptible (1) AMP (2) |
| MMH | 1 | 5215 | 1 | 3.0 (1) | Susceptible (1) | 0 | NA | NA | NA | NA |
| NMC | 3 | 1 (3) | 2 | 3.0 (2) | Susceptible (3) | 1 | 11 (1) | 1 | SEN (1) | Susceptible (1) |
| RMC | 0 | NA | NA | NA | NA | 2 | 64 (2) | 2 | SLA (2) | AMP CIP SXT CHL (2) |
| SLH | 0 | NA | NA | NA | NA | 1 | 34 (1) | 1 | I 4,[5],12:i:- (1) | AMP (1) |
| STU | 2 | 1 (2) | 1 | 3.0 (2) | Susceptible (2) | 22 | 11 (11) | 10 | SEN (11) | Susceptible (11) |
| VSM | 39 | 1 (34) | 4 | 3.0 (30) | Susceptible (37) CIP, NAL (2) | 14 | 11 (4) | 5 | SEN (4) | Susceptible (3) SXT (1) |
| ZMC | 1 | 1 | 1 | 3.0 (1) | Susceptible (1) | 3 | 34 (2) | 2 | I 4,[5],12:i:- (2) | SXT (2) |

[a]BGH: Baguio General Hospital and Medical Center; BRT: Bicol Regional Training & Teaching Hospital; CMC: Cotabato Regional Hospital and Medical Center; CVM: Cagayan Valley Medical Center; DMC: Southern Philippines Medical Center; EVR: Eastern Visayas Regional Medical Center; FEU: Far Eastern University Hospital; GMH: Governor Celestino Gallares Memorial Hospital; JLM: Jose B. Lingad Memorial Regional Hospital; MAR: Mariano Marcos Memorial Hospital and Medical Center; MMH: Corazon Locsin Montelibano Memorial Regional Hospital; NMC: Northern Mindanao Medical Center; RMC: Rizal Medical Center; SLH: San Lazaro Hospital; STU: University of Sto. Tomas Hospital; VSM: Vicente Sotto Memorial Medical Center; ZMC: Zamboanga City Medical Center.
[b]For simplicity, if two or more STs/serotypes were equally prevalent at a specific site, the most prevalent of the STs/serotypes across the entire study is listed. SLA: *Salmonella* Anatum; SEN: *Salmonella* Enteritidis; SAM: *Salmonella* Typhimurium; monophasic variant of SAM: I 4, [5],12: i: -; SVR: *Salmonella* Virchow.
[c]The resistance profile of the prevalent ST/serotype is listed. NA: not applicable

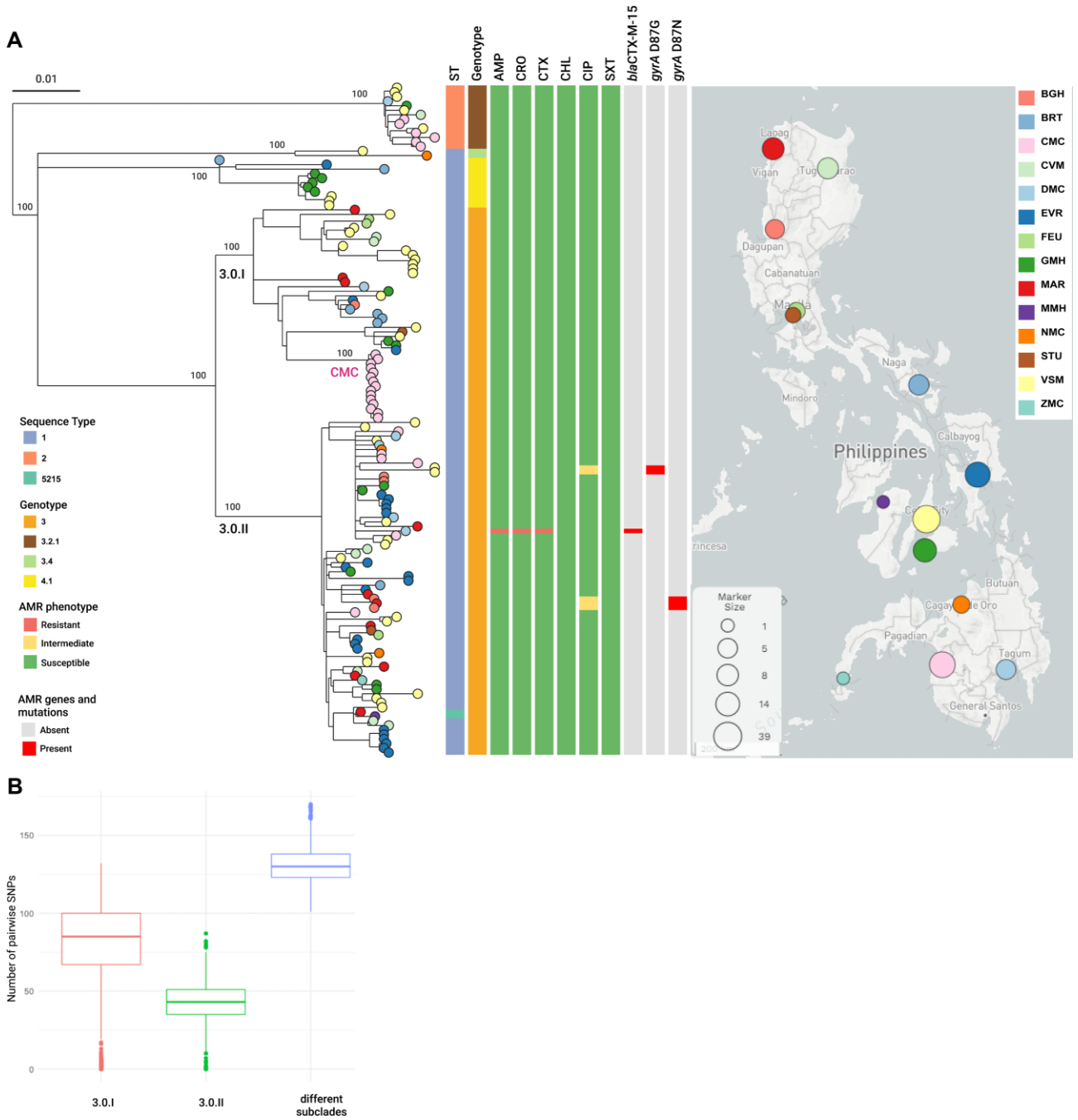


**Figure 2.** Genomic surveillance of *S.* Typhi from the Philippines, 2013–2014. (A) Phylogenetic tree of 148 isolates inferred from an alignment of 2094 SNP sites obtained after mapping the genome sequences to the complete genome of reference strain CT18 and masking regions of mobile genetic elements and recombination. The tree leaves are coloured by sentinel site and indicated on the map. BGH: Baguio General Hospital and Medical Center; BRT: Bicol Regional Training & Teaching Hospital; CMC: Cotabato Regional Hospital and Medical Center; CVM: Cagayan Valley Medical Center; DMC: Southern Philippines Medical Center; EVR: Eastern Visayas Regional Medical Center; FEU: Far Eastern University Hospital; GMH: Governor Celestino Gallares Memorial Hospital; MAR: Mariano Marcos Memorial Hospital and Medical Center; MMH: Corazon Locsin Montelibano Memorial Regional Hospital; NMC: Northern Mindanao Medical Center; STU: University of Sto. Tomas Hospital; VSM: Vicente Sotto Memorial Medical Center; ZMC: Zamboanga City Medical Center. The tree is annotated with subclades within genotype 3.0 (3.0.I and 3.0.II), a putative outbreak cluster (CMC) and bootstrap values on major branches. The tree blocks indicate the distribution of the sequence types (STs), genotype, resistance phenotype for six antibiotics and acquired resistance genes and mutations. AMP: ampicillin; CRO: ceftriaxone; CTX: cefixime; CHL: chloramphenicol; CIP: ciprofloxacin; SXT: sulphamethoxazole-trimetoprim. The data are available at https://microreact.org/project/kRW7Z2TLg3FEM7rmq8sZ1e. (B) Boxplot showing the distribution of the SNP differences between pairs of genomes from genotype 3.0 belonging both to subclade 3.0.I (red), both to subclade 3.0.II (green) or one to each subclade (blue). The horizontal line indicates the median and the box indicates the interquartile range.

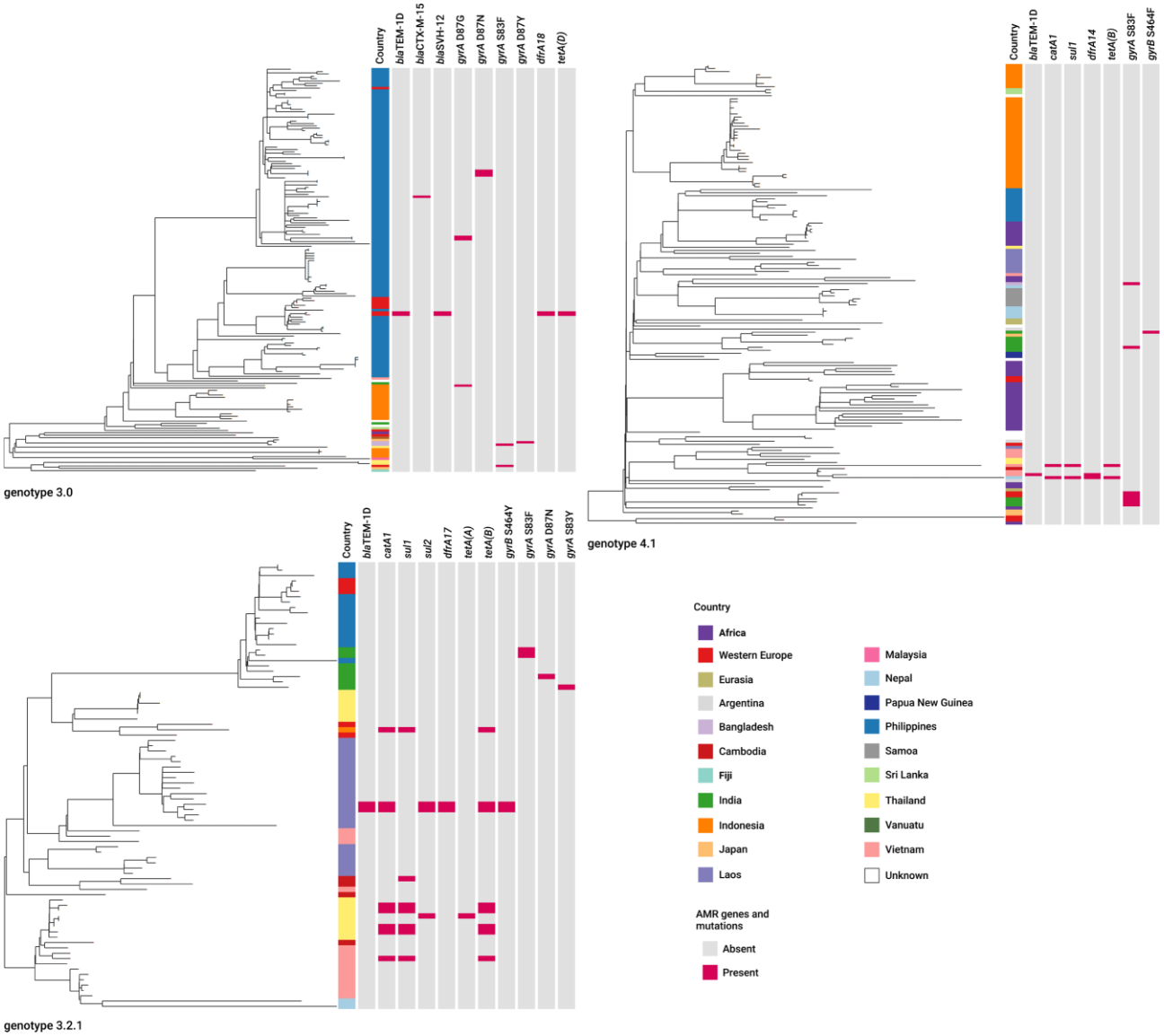


**Figure 3.** *S.* Typhi from the Philippines in global context. Phylogenetic trees of genomes belonging to genotypes 3.0, 3.2.1 and 4.1 from the Philippines and 19 other countries or regions, generated with Pathogenwatch. Genomes from countries sparsely represented but belonging to the same continent/region were grouped to simplify the tree annotation. The trees are also annotated with the distribution of resistance determinants identified by Pathogenwatch. The data are available at https://microreact.org/project/rym1Shfy7, https://microreact.org/project/i5GByUGqNuLR9sGdDRH5hA-global-sat-321 and https://microreact.org/project/pDqxJCq7YzYy6ibxEZ2Rgk-global-sat-41.

### *S.* Typhi from the Philippines in global context

The *S.* Typhi genomes from this study were compared with global genomes from genotypes 3.0, 3.2.1 and 4.1 (Figure 3) available on Pathogenwatch. The Philippine genomes clustered together within each of the three genotypes and were related to genomes from countries in south and southeast Asia. Surprisingly, eight genomes from in Nigeria (2009–2013) were also related to the Philippine genomes within genotype 4.1, separated by between 55 and 139 SNP differences. Genotype 4.1 is widespread in both Africa and south and southeast Asia, but uneven sampling of global isolates curtails our ability to establish sound transmission routes. A small number of genomes from countries in Western Europe (2007–2015) were found interspersed with Philippine genomes from genotypes 3.0 (n=5) and 3.2.1 (n=3). The epidemiological data available confirmed a travel link to the Philippines for two genomes within each genotype.

## Discussion

Our study provided new insights into the *Salmonella* population from the Philippines, with important ramifications for

surveillance. *Salmonella* serotyping is routinely performed at the ARSRL and it is useful for epidemiological investigations, but the serotyping scheme comprises >2500 serovars. The genoserotyping results were largely concordant with the serological serotyping results, and confirmed that the typhoidal and non-typhoidal serovars were accurately discriminated, which is critical for patient management as typhoid fever requires antibiotic treatment. A high concordance (>94%) was reported by larger studies of *Salmonella* combining genoserotyping with MLST information.[18,26] Our study also revealed inaccuracies in the serological serotyping of NTS at ARSRL, notably that most isolates typed as *S.* Typhimurium in the laboratory belonged to the monophasic variant *S.* I 4, [5],12: i: -. MLST information and phylogenetic clustering supported this assignment and highlighted the utility of the genome data. WGS has been used routinely to type *Salmonella* in several high-income countries, and led to the recent proposal of a new naming method based on genome data to remove the need for antibody-based serotyping.[27] The ARSRL has implemented WGS locally but its use continues to be contingent on external funding, an obstacle for its adoption for routine and typing of *Salmonella*.

Overall, the NTS population captured by the ARSP was diverse, with 16 clones defined by serotype and ST. A limitation of our study was that the samples available for retrospective analysis were those referred by sentinel sites to the reference laboratory without a consistent sampling strategy across sites. However, the serotype or ST was not contemplated for sample referral and thus our results should be representative of the population. Twelve of the NTS serotypes identified in this study, including the dominant *S.* Enteritidis and monophasic *S.* I 4, [5],12: i: -, were previously reported from retail meat in the Philippines,[28] suggesting a potential food-chain reservoir. The monophasic variant of *S.* Typhimurium (serovar I 4, [5],12: i: -) ST34 rose in prevalence in Europe since the early 2000s and disseminated across the world likely via the food chain, especially pigs and pig meat,[29] which is the most consumed livestock meat in the Philippines. The low prevalence of MDR *Salmonella* during the survey period is in line with the absence of epidemic MDR *S.* Typhimurium clones, notably, ST313, which is dominant in sub-Saharan Africa, and the biphasic *S.* Typhimurium ST34 clone reported in Vietnam in association with HIV infection.[30] The combination of phylogenetic information and AMR mechanisms extracted from whole genomes led to the identification of a successful lineage of *S.* Enteritidis ST11 carrying mutation GyrA D87Y circulating across the Philippines. The relative genetic uniformity displayed *S.* Enteritidis has challenged epidemiological studies based on conventional subtyping methods[31] and our finding highlights the utility of the genomic data for surveillance in the Philippines beyond the resolution afforded by serotype and MLST. The *S.* Anatum organisms from this study carried the same repertoire of AMR determinants as those reported to have cause a dramatic increase of *S.* Anatum infections in Taiwan during 2016–2017.[32] The significance of these findings for public health merit future, more detailed investigations into these NTS serovars and clones.

The population snapshot of *S.* Typhi showed limited diversity and predominance of genotype 3.0. The relationship between Philippine and global genomes and the diversification within this genotype suggests that this is a clone of local and persistent circulation. A limitation of our study in this respect is that the sampling encompassed only 2 y. We found that AMR was rare in *S.* Typhi and, in agreement with this, the genotypes found in our dataset are not known to be associated with the dissemination of single or multiple resistance,[21] unlike genotype 4.3.1 (haplotype H58), which was absent in our dataset. However, we observed the sporadic acquisition of resistance, notably of ESBL genes, which previously had only been reported from isolates with travel history to the Philippines.[33] Similarly, the independent emergence of insusceptibility to ciprofloxacin linked to two different mutations is likely a reflection of substantial selective pressure imposed by the widespread use of this antibiotic in the Philippines, which calls for strengthening the regulation of rational use. Our genomic analysis also showed evidence of a local, persistent outbreak of pan-susceptible *S.* Typhi, underscoring the impact of this pathogen and the importance of infection prevention and control through hygiene and sanitation, even in the absence of drug resistance.

## Conclusion

WGS is currently being utilised for *Salmonella* surveillance in reference laboratories and international networks, and has displaced laboratory methods for both ongoing surveillance and outbreak investigations.[34–37] The ARSRL has implemented WGS locally but its routine use continues to be challenging in the setting of a lower middle-income economy. This first study of its utility for *Salmonella* surveillance in the Philippines supports continued application.

**Authors' contributions:** MLL and SA contributed equally to this work. DMA and CCC contributed equally to this work. MLL, SA, AMO, VC, BJ, KA, SBS, CMH, JS, MTGH, DMA and CCC conceptualised the study design. All the authors contributed to the study implementation. MLL, SA, JBB, JMG, MLM, AMO, VC, BJ, KA, SBS, CMH, JS, MTGH, DMA and CCC carried out the analysis and interpretation of data. MLL, SA, JBB, JPA, JMG, MLM, AMO, VC, BJ, KA, SBS, CMH, JS, MTGH, DMA and CCC wrote the manuscript draft. MLL, SA, JBB, AMO, VC, BJ, KA, SBS, CMH, JS, MTGH, DMA and CCC contributed to writing and editing the manuscript. All the authors read and approved the final version of the manuscript.

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**Ethical approval:** Ethical approval is not applicable. This study uses archived bacterial samples processed by ARSP. No identifiable data were used in this study.

**Data availability:** All data generated or analysed during this study are included in this published article.

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