Title: Genomic Surveillance of *Salmonella* spp. in the Philippines, 2013-2014

Marietta L. Lagrada^{a,†}, Silvia Argimón^{b,†}, Janice B. Borlasa^a, Jaywarden P. Abad^a, June M. Gayeta^a, Melissa L. Masim^a, Agnetta M. Olorosa^a, Victoria Cohen^b, Benjamin Jeffrey^b, Khalil Abudahab^b, Sonia B. Sia^a, Charmian M. Hufano^a, John Stelling^c, Matthew T.G. Holden^d, David M. Aanensen^{b,*}, and Celia C. Carlos^{a,*} on behalf of the Philippines Antimicrobial Resistance Surveillance Program

^a Antimicrobial Resistance Surveillance Reference Laboratory, Research Institute for Tropical Medicine, Muntinlupa, Philippines.

^b Centre for Genomic Pathogen Surveillance, Big Data Institute, University of Oxford, Oxford, UK.

^c Brigham and Women's Hospital, Boston, MA, USA.

^d University of St Andrews School of Medicine, St Andrews, Scotland.

[†] These authors contributed equally to this work

^{*} These authors contributed equally to this work

Keywords: Antimicrobial resistance, genomic epidemiology, whole-genome sequencing, surveillance, typhoid fever, non-typhoidal *Salmonella*

ABSTRACT

Increasing antimicrobial resistance (AMR) in *Salmonella* has been observed in the Philippines. This study aims to utilize whole genome sequencing (WGS) to characterize the population and AMR mechanisms of *Salmonella* captured by the Philippine Antimicrobial Resistance Surveillance Program (ARSP) and contrast to traditional laboratory methods.

We sequenced the whole genomes of 148 *Salmonella* Typhi (*S. Typhi*) and 65 non-typhoidal *Salmonella* (NTS) collected in the Philippines in 2013–2014. From the genome sequences, we determined the serotype, multilocus sequence type, presence of determinants of antimicrobial resistance and relatedness between isolates. We also compared the genotypic predictions of serotype and AMR to the phenotypic data.

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. In contrast, three quarters of NTS isolates were insusceptible to at least one antimicrobial, with more than half carrying mutations and/or genes linked to resistance to fluoroquinolones. ESBL genes were detected in five genomes that also carried other AMR determinants. The population of *S. Typhi* was dominated by the likely endemic genotype 3.0, which also caused of a putative local outbreak susceptible to antibiotics. The main NTS clades were global epidemic *S. Enteritidis* ST11 and the monophasic variant of *S. Typhimurium* (I 4,[5],12:i:-) ST34, which had frequently been serotyped as *S. Typhimurium* in the laboratory.

This was the first time that *Salmonella* isolated from the Philippines were characterized by WGS and we provide evidence of its utility for ongoing surveillance at the ARSP.

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 disproportionately 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 below 10% since 2004, although resistance rates for nalidixic acid increased slightly starting around 2012. ⁴ In contrast, resistance rates of NTS against first and second-line antibiotics above 10% were recorded since the year 2004, with resistance to ceftriaxone (third-generation cephalosporin) ranging between 10 and 20%. 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 between 2013 and 2014 using WGS to describe their population, identify AMR determinants, and determine the concordance between laboratory tests and genotypic predictions of serotype and resistance.

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 (AST)

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)¹³. The ESBL phenotype and insusceptibility to quinolones were confirmed using E-testTM (bioMérieux; Marcy-l'Étoile, France). Multi-drug resistant (MDR) organisms were those resistant to resistant to ampicillin, chloramphenicol and trimethoprim-sulfamethoxazole.⁸

Serotyping

Serological serotyping was performed using 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.⁹

DNA Extraction and Whole-Genome Sequencing

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 (ENA) 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.¹⁰ Annotated assemblies were produced as described in detail previously.¹¹

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 (Argimon et al 2020). The mobile genetic elements and repetitive sequences in the genome of CT18 previously defined^{12, 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 v2.0.0¹⁴ and the non-recombinant SNPs were used to infer a maximum-likelihood tree with RAxML v. 8.28¹⁵ 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 v3.12.0,¹⁶ using a blastp percentage identity of 95% and a core definition of 99%. SNPs were identified in the core genome alignment with snp-sites v2.4.0¹⁷ and a tree was obtained with RAxML as described above.

Serotype ¹⁸ and multi-locus sequence type (MLST, ¹⁹) information were 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 to 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 contextualize the *S. Typhi* genomes, we compared to 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

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 summarized on **Table 2**. The majority of the patients were male overall (126/213, 59.2%), but a pronounced difference in the distribution of patient sex was observed for NTS (64.6% male, 35.4% female). The group aged 0-14 years old 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 45-80 years old (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 genosertotyping. We predicted *S. Typhi* only among typhoidal *Salmonella* and fifteen 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 genosertotyping and serological serotyping was 91.1% overall (194/213), 100% for typhoidal *Salmonella* (148/148 *S. Typhi*) and 70.8% for NTS (46/65). Genosertotyping 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, genosertotyping 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 quinolone resistance determining region (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*_{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*_{TEM-1}, *bla*_{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 lab (*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_{CYM-2}* gene was found in the only *S. Lexington* isolate, the ESBL gene *bla_{CTX-M-15}* was identified in two *S. Stanley* isolates, only one of which was resistant to ceftriaxone, and the ~~and~~ AmpC gene *bla_{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_{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 fourteen 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 center, 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 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. The tree topology and the distribution of pairwise SNPs between genomes showed that the organisms in subclade II were genetically similar

(**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. Within the more diverse subclade I, we observed a group of fifteen tightly clustered isolates 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 3 pairwise SNPs (range 0-8) and carried no known resistance determinants, suggesting an outbreak of enteric fever caused by a pansusceptible 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 fifteen genomes carrying mutation GyrA_D87Y associated with reduced susceptibility to ciprofloxacin formed a discreet, well-supported cluster 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).

S. Typhi from the Philippines in global context

The *S. Typhi* genomes from this study were compared to 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 S or SE Asia. Surprisingly, a group of eight genomes from in Nigeria (2009-2013) also showed close relationships 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 S-SE 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 over 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.

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 lab 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,²⁷ 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,²⁸ 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.²⁹ 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 methods³⁰ 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 2016-2017.³¹ The significance of these findings for public health grant 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 two years. 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 had only been reported before from isolates with travel history to the Philippines.³² Our genomic analysis also showed evidence of a local, persistent outbreak of pansusceptible *S. Typhi*, underscoring the impact of this pathogen even in the absence of resistance.

WGS is currently being utilized for *Salmonella* surveillance in reference laboratories and international networks, and has displaced laboratory methods for both ongoing surveillance and outbreak investigations.³³⁻³⁶ The ARSRL has implemented WGS locally but its routine use continues to be challenged in the setting of a lower middle-income economy. This first study of its utility for *Salmonella* surveillance in the Philippines supports continued application.

ACKNOWLEDGEMENTS

This work was supported by a Newton Fund award from the Medical Research Council (UK) MR/N019296/1 and the Philippine Council for Health Research and Development. This work was also partially supported by research grant U01CA207167 from the U.S. National Institutes of Health. The contents are solely the responsibility of the authors and do not necessarily represent the official views of the funders. The funders had no role in study design, data collection and analysis, or decision to publish, or preparation of the manuscript. S.A. and D.M.A. were additionally supported by the National Institute for Health Research (UK) Global Health Research Unit on genomic Surveillance of AMR (16_136_111) and by the Centre for Genomic Pathogen Surveillance.

REFERENCES

1. Crump JA, Sjolund-Karlsson M, Gordon MA, Parry CM. Epidemiology, Clinical Presentation, Laboratory Diagnosis, Antimicrobial Resistance, and Antimicrobial Management of Invasive Salmonella Infections. Clin Microbiol Rev. 2015;28(4):901-37.
2. Phu Huong Lan N, Le Thi Phuong T, Nguyen Huu H, Thuy L, Mather AE, Park SE, et al. Invasive Non-typhoidal Salmonella Infections in Asia: Clinical Observations, Disease Outcome and Dominant Serovars from an Infectious Disease Hospital in Vietnam. PLoS Negl Trop Dis. 2016;10(8):e0004857.
3. World Health Organization. WHO Estimates of the global burden of foodborne diseases: foodborne disease burden epidemiology reference group 2007-2015. Geneva, Switzerland; 2015.
4. Sia SB, Lagrada ML, Olorosa AM, Limas MT, Jamoralín Jr MC, Macaranas PK, et al. A Fifteen-year Report of Serotype Distribution and Antimicrobial Resistance of Salmonella in the Philippines. Philippine Journal of Pathology. 2020;5(1):19-29.
5. Galanis E, Lo Fo Wong DM, Patrick ME, Binsztein N, Cieslik A, Chalermchikit T, et al. Web-based surveillance and global Salmonella distribution, 2000-2002. Emerg Infect Dis. 2006;12(3):381-8.

6. Herikstad H, Motarjemi Y, Tauxe RV. Salmonella surveillance: a global survey of public health serotyping. *Epidemiol Infect.* 2002;129(1):1-8.
7. Cuypers WL, Jacobs J, Wong V, Klemm EJ, Deborggraeve S, Van Puyvelde S. Fluoroquinolone resistance in Salmonella: insights by whole-genome sequencing. *Microb Genom.* 2018;4(7).
8. Parry CM, Threlfall EJ. Antimicrobial resistance in typhoidal and nontyphoidal salmonellae. *Curr Opin Infect Dis.* 2008;21(5):531-8.
9. Grimont P, Weill F-X. Antigenic Formulae of the Salmonella serovars, (9th ed.) Paris: WHO Collaborating Centre for Reference and Research on Salmonella. Institute Pasteur. 2007:1-166.
10. Argimon S, Masim MAL, Gayeta JM, Lagrada ML, Macaranas PKV, Cohen V, et al. Integrating whole-genome sequencing within the National Antimicrobial Resistance Surveillance Program in the Philippines. *Nat Commun.* 2020;11(1):2719.
11. Page AJ, De Silva N, Hunt M, Quail MA, Parkhill J, Harris SR, et al. Robust high-throughput prokaryote de novo assembly and improvement pipeline for Illumina data. *Microb Genom.* 2016;2(8):e000083.
12. Holt KE, Parkhill J, Mazzoni CJ, Roumagnac P, Weill FX, Goodhead I, et al. High-throughput sequencing provides insights into genome variation and evolution in Salmonella Typhi. *Nat Genet.* 2008;40(8):987-93.
13. Ingle DJ, Nair S, Hartman H, Ashton PM, Dyson ZA, Day M, et al. Informal genomic surveillance of regional distribution of Salmonella Typhi genotypes and antimicrobial resistance via returning travellers. *PLoS Negl Trop Dis.* 2019;13(9):e0007620.
14. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, et al. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Res.* 2015;43(3):e15.
15. Stamatakis A. RAxML version 8: a tool for phylogenetic analysis and post-analysis of large phylogenies. *Bioinformatics.* 2014;30(9):1312-3.
16. Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MT, et al. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics.* 2015;31(22):3691-3.

17. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, et al. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. *Microb Genom.* 2016;2(4):e000056.
18. Yoshida CE, Kruczkiewicz P, Laing CR, Lingohr EJ, Gannon VP, Nash JH, et al. The Salmonella In Silico Typing Resource (SISTR): An Open Web-Accessible Tool for Rapidly Typing and Subtyping Draft Salmonella Genome Assemblies. *PLoS One.* 2016;11(1):e0147101.
19. Zhou Z, Alikhan NF, Mohamed K, Fan Y, Agama Study G, Achtman M. The Enterobase user's guide, with case studies on Salmonella transmissions, Yersinia pestis phylogeny, and Escherichia core genomic diversity. *Genome Res.* 2020;30(1):138-52.
20. Argimon S, Yeats CA, Goater RJ, Abudahab K, Taylor B, Underwood A, et al. A global resource for genomic predictions of antimicrobial resistance and surveillance of Salmonella Typhi at pathogenwatch. *Nat Commun.* 2021;12(1):2879.
21. Wong VK, Baker S, Connor TR, Pickard D, Page AJ, Dave J, et al. An extended genotyping framework for Salmonella enterica serovar Typhi, the cause of human typhoid. *Nat Commun.* 2016;7:12827.
22. Hunt M, Mather AE, Sanchez-Buso L, Page AJ, Parkhill J, Keane JA, et al. ARIBA: rapid antimicrobial resistance genotyping directly from sequencing reads. *Microb Genom.* 2017;3(10):e000131.
23. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, et al. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemoth.* 2012;67(11):2640-4.
24. Zankari E, Allesoe R, Joensen KG, Cavaco LM, Lund O, Aarestrup FM. PointFinder: a novel web tool for WGS-based detection of antimicrobial resistance associated with chromosomal point mutations in bacterial pathogens. *J Antimicrob Chemother.* 2017;72(10):2764-8.
25. Jacoby GA. AmpC beta-lactamases. *Clin Microbiol Rev.* 2009;22(1):161-82, Table of Contents.
26. Banerji S, Simon S, Tille A, Fruth A, Flieger A. Genome-based Salmonella serotyping as the new gold standard. *Sci Rep.* 2020;10(1):4333.

27. Santos PDM, Widmer KW, Rivera WL. PCR-based detection and serovar identification of *Salmonella* in retail meat collected from wet markets in Metro Manila, Philippines. *PLoS One*. 2020;15(9):e0239457.
28. Hopkins KL, Kirchner M, Guerra B, Granier SA, Lucarelli C, Porrero MC, et al. Multiresistant *Salmonella enterica* serovar 4,[5],12:i:- in Europe: a new pandemic strain? *Euro Surveill*. 2010;15(22):19580.
29. Mather AE, Phuong TLT, Gao Y, Clare S, Mukhopadhyay S, Goulding DA, et al. New Variant of Multidrug-Resistant *Salmonella enterica* Serovar Typhimurium Associated with Invasive Disease in Immunocompromised Patients in Vietnam. *mBio*. 2018;9(5).
30. Allard MW, Luo Y, Strain E, Pettengill J, Timme R, Wang C, et al. On the evolutionary history, population genetics and diversity among isolates of *Salmonella* Enteritidis PFGE pattern JEGX01.0004. *PLoS One*. 2013;8(1):e55254.
31. Chiou CS, Hong YP, Liao YS, Wang YW, Tu YH, Chen BH, et al. New Multidrug-Resistant *Salmonella enterica* Serovar Anatum Clone, Taiwan, 2015-2017. *Emerg Infect Dis*. 2019;25(1):144-7.
32. Hendriksen RS, Leekitcharoenphon P, Mikoleit M, Jensen JD, Kaas RS, Roer L, et al. Genomic dissection of travel-associated extended-spectrum-beta-lactamase-producing *Salmonella enterica* serovar typhi isolates originating from the Philippines: a one-off occurrence or a threat to effective treatment of typhoid fever? *J Clin Microbiol*. 2015;53(2):677-80.
33. Ashton PM, Nair S, Peters TM, Bale JA, Powell DG, Painset A, et al. Identification of *Salmonella* for public health surveillance using whole genome sequencing. *PeerJ*. 2016;4:e1752.
34. Deng X, den Bakker HC, Hendriksen RS. Genomic Epidemiology: Whole-Genome-Sequencing-Powered Surveillance and Outbreak Investigation of Foodborne Bacterial Pathogens. *Annu Rev Food Sci Technol*. 2016;7:353-74.
35. Franz E, Gras LM, Dallman T. Significance of whole genome sequencing for surveillance, source attribution and microbial risk assessment of foodborne pathogens. *Current Opinion in Food Science*. 2016;8:74-9.

36. Nadon C, Van Walle I, Gerner-Smidt P, Campos J, Chinen I, Concepcion-Acevedo J, et al. PulseNet International: Vision for the implementation of whole genome sequencing (WGS) for global food-borne disease surveillance. Euro Surveill. 2017;22(23).

Tables

Table 1. Number of *Salmonella* isolates referred to the 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			Grand Total
	2013	2014	Total	2013	2014	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
<i>By sentinel site</i>							
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
<i>By AMR profile</i>							
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	2
CIP NAL	2	3	5	0	0	0	5

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
Sex	Male	84	42
	Female	64	23
Age (in years)	<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.

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-M-15}
3 rd 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-1} , bla _{CTX-M-15} , bla _{CMY-2} , bla _{DHA-1}
3 rd Generation Cephalosporins	Ceftriaxone	64	2	3	0	95.31	bla _{CTX-M-15} , bla _{CMY-2} , bla _{DHA-1}
Fluoroquinolones	Ciprofloxacin	65	36	2	2	93.85	gyrA_S83F/D87N/D87Y,
Quinolone	Nalidixic Acid	65	35	2	1	95.38	parC_S80I/T57S, qnrA6, qnrS1, qnrB4, oqxA, oqxB
Folate Pathway Antagonist	Cotrimoxazole	64	12	0	2	96.88	sul1, sul2, dfrA
Phenicol	Chloramphenicol	65	11	0	2	96.92	catA2, cmlA1, floR

Table 4. Distribution of sequence types (ST), genosero type and resistance profiles of *Salmonella* across the 17 sentinel sites that referred isolates. Numbers in parenthesis indicate 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

Figures

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 on the map. The trees are annotated with bootstrap values and the tree blocks indicate the distribution of the serological serotype, genoserotype, sequence types (ST), resistance phenotype for five antibiotics and acquired resistance genes and mutations. AMP: ampicillin; CRO: ceftriaxone; CHL: chloramphenicol; CIP: ciprofloxacin; SXT: sulphamethoxazole-trimetoprim. The data are available at

<https://microreact.org/project/k2BC6hsaxYr1Eo5U9v71iJ-arspnts2013-2014>

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. 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 (ST), 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 nineteen 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/pDqxJCq7YzYy6ibxEZ2Rqk-global-sat-41>