Author/s:
Ingle, DJ;Andersson, P;Valcanis, M;Wilmot, M;Easton, M;Lane, C;Barden, J;Da Silva, AG;Seemann, T;Horan, K;Ballard, SA;Sherry, NL;Williamson, DA;Howden, BP

Title:
Genomic epidemiology and antimicrobial resistance mechanisms of imported typhoid in australia

Date:
2021-12-01

Citation:
Ingle, D. J., Andersson, P., Valcanis, M., Wilmot, M., Easton, M., Lane, C., Barden, J., Da Silva, A. G., Seemann, T., Horan, K., Ballard, S. A., Sherry, N. L., Williamson, D. A. & Howden, B. P. (2021). Genomic epidemiology and antimicrobial resistance mechanisms of imported typhoid in australia. Antimicrobial Agents and Chemotherapy, 65 (12), https://doi.org/10.1128/AAC.01200-21.

Persistent Link:
https://hdl.handle.net/11343/290131

AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

EPIDEMIOLOGY AND SURVEILLANCE

Genomic Epidemiology and Antimicrobial Resistance Mechanisms of Imported Typhoid in Australia

Danielle J. Ingle,[a] Patiyan Andersson,[b] Mary Valcanis,[b] Mathilda Wilmot,[b] Marion Easton,[c] Courtney Lane,[b] Jessica Barden,[b] Anders Gonçalves da Silva,[b] Torsten Seemann,[b] Kristy Horan,[b] Susan A. Ballard,[b] Norelle L. Sherry,[b,d] Deborah A. Williamson,[a,b,e] Benjamin P. Howden[a,b,f]

[a]Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia
[b]Microbiological Diagnostic Unit Public Health Laboratory, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia
[c]Department of Health, Victoria, Australia
[d]Department of Infectious Diseases, Austin Health, Melbourne, Australia
[e]Royal Melbourne Hospital at the Peter Doherty Institute for Infection and Immunity, Melbourne, Australia
[f]Doherty Applied Microbial Genomics, Department of Microbiology and Immunology at the Peter Doherty Institute for Infection and Immunity, The University of Melbourne, Melbourne, Australia

Deborah A. Williamson and Benjamin P. Howden are joint senior authors.

ABSTRACT Typhoid fever is an invasive bacterial disease of humans that disproportionately affects low- and middle-income countries. Antimicrobial resistance (AMR) has been increasingly prevalent in recent decades in *Salmonella enterica* serovar Typhi (*S. Typhi*), the causative agent of typhoid fever, limiting treatment options. In Australia, most cases of typhoid fever are imported due to travel to regions where typhoid fever is endemic. Here, all 116 isolates of S. Typhi isolated in Victoria, Australia, between 1 July 2018 and 30 June 2020, underwent whole-genome sequencing and antimicrobial susceptibility testing. Genomic data were linked to international travel data collected from routine case interviews. Travel to South Asia accounted for most cases, with 92.2% imported from seven primary countries (the top two were India, $n = 87$, and Pakistan, $n = 12$). A total of 17 *S.* Typhi genotypes were detected in the 2-year cohort, with 48.2% genotyped as part of global AMR lineages. Ciprofloxacin resistance was detected in two lineages, 3.3 and 4.3.1.2, all from cases with reported travel to India. Nearly all multidrug and extensively drug resistant isolates (90%) were from cases with reported travel to Pakistan in genotypes 4.3.1.1 and 4.3.1.1.P1. Extended spectrum beta-lactamases, *bla*CTX-M-15 and *bla*SHV-12, were detected in cases with travel to Pakistan and India, respectively. Linking epidemiological data with genomic studies of *S.* Typhi provides an opportunity to improve understanding of the emergence, spread and risk of drug-resistant *S.* Typhi infections and to better inform empirical treatment guidelines in returned travelers.

KEYWORDS antimicrobial resistance, genomics, typhoid

The prevention, treatment, and control of typhoid fever remains a significant public health challenge in the 21st century (1). *Salmonella enterica* serovar Typhi (*S.* Typhi) is the causative agent of typhoid fever (2) and is estimated to cause 10.9 million infections and 116,800 deaths globally each year (3). This burden of disease disproportionately affects children $<$ 5 years in low-middle income countries (2, 3). In high income countries, cases of typhoid fever most commonly occur as a result of recent international travel to regions where *S.* Typhi is endemic (4–6). Antimicrobial therapy is the mainstay of treatment for typhoid fever, however successive waves of antimicrobial resistance (AMR) in *S.* Typhi raises the specter of untreatable typhoid fever, especially with oral antimicrobials (1, 5). Recently, genomic studies have started to provide critical

Citation Ingle DJ, Andersson P, Valcanis M, Wilmot M, Easton M, Lane C, Barden J, Gonçalves da Silva A, Seemann T, Horan K, Ballard SA, Sherry NL, Williamson DA, Howden BP. 2021. Genomic epidemiology and antimicrobial resistance mechanisms of imported typhoid in Australia. Antimicrob Agents Chemother 65:e01200-21. https://doi.org/10.1128/AAC.01200-21.

Address correspondence to Danielle J. Ingle, danielle.ingle@unimelb.edu.au, or Benjamin P. Howden, bhowden@unimelb.edu.au.

Received 16 June 2021
Returned for modification 17 July 2021
Accepted 10 September 2021
Accepted manuscript posted online 20 September 2021
Published 17 November 2021

insights into the international spread and mechanisms of antimicrobial resistance in *Salmonella* Typhi (6–15).

Multidrug resistance (MDR) to the initial first line drugs for treating typhoid fever, ampicillin, co-trimoxazole, and chloramphenicol, first appeared in the 1960s (5). AMR was a significant driver in the global dissemination of the lineage 4.3.1 (haplotype 58) soon after it emerged in the late 1980s/early 1990s (8, 16). Initially MDR was associated with IncHI1 plasmids and has been detected globally (8, 10, 17). The subsequent integration of a MDR composite transposon into the chromosome at different IS*1* sites is a key feature of sublineage 4.3.1.1 (8).

Resistance to fluoroquinolones emerged in the late 1990s (5). A hallmark of the second sublineage of the global clone, 4.3.1.2, are triple-point mutations in quinolone resistance determining regions (QRDRs) that confer resistance to ciprofloxacin and has been associated with fluoroquinolone treatment failure in South Asia (18, 19). Reduced susceptibility to ciprofloxacin results from single- or double-point mutations in QRDR (19). These point mutations have limited to no fitness cost and so are maintained in populations even once the selective pressure of fluoroquinolone use has ceased (19, 20).

Alarmingly, resistance to extended spectrum beta-lactams and azithromycin has been reported recently in *S.* Typhi (4, 14, 21–23). Extended spectrum beta-lactamases (ESBLs) have been largely associated with mobilization on IncY plasmids, although the recent study of Nair et al. showed different types of chromosomal integration of *bla*CTX-M genes (4). A new sublineage of extensively drug resistant (XDR) *S.* Typhi, 4.3.1.1.P1, emerged from Pakistan (14). This XDR threat is a sublineage of the MDR 4.3.1.1 that has acquired an IncY plasmid carrying *bla*CTX-M-15 and *qnrS1* genes and together with the single QRDR point mutation, *gyrA*-S83F, these confer resistance to ESBLs and ciprofloxacin (4, 14). Azithromycin is the only remaining oral therapeutic option for these XDR *S.* Typhi (5). Further, point mutations in the *acrB* gene have been shown to confer resistance to azithromycin, the last remaining oral therapeutic that is broadly efficacious in South Asia for typhoid fever (12, 22–24). These new resistance profiles are associated with South Asia, however *S.* Typhi isolates resistant to extended spectrum beta-lactams or azithromycin have been detected in returned travelers from these regions (4, 15, 25).

In Australia, typhoid fever is a nationally notifiable disease, and vaccination is recommended for travelers to regions where typhoid is prevalent (26). Moreover, as almost all cases of typhoid in Australia are acquired overseas, the appropriateness of empirical therapy is dependent on the resistance profiles of *S.* Typhi in the region where the infection was acquired. The increasing prevalence of XDR and azithromycin resistant cases of typhoid fever in South Asia highlights the need for enhanced genomic surveillance of *S.* Typhi globally. Here, to better understand the genomic epidemiology and resistance determinants of imported *S.* Typhi we undertook a 2-year study of *S.* Typhi cases reported in Victoria, Australia. Our results inform approaches for optimizing genomic surveillance of *S.* Typhi and empirical treatment approaches based on region of travel.

RESULTS

Comprehensive 2-year cohort. Between 1 July 2018 and 30 June 2020, a total of 116 *S.* Typhi isolates were received at the state reference laboratory, MDU PHL in Victoria, Australia (Fig. 1). The 116 *S.* Typhi were subject to routine whole-genome sequencing (WGS), which commenced for all serovars of *Salmonella enterica* from 1 July 2018. The number of cases was consistent between 2 years (first timespan, 1 July 2018 to 30 June 2019, $n = 57$; second timespan, 1 July 2019 to 30 June 2020, $n = 59$). These data represent a sampling fraction of 96.7% of the *S.* Typhi cases notified in Victoria, Australia, over the 2-year study period and provide an unbiased and comprehensive cohort of the *S.* Typhi causing infections.

***Genotypes and epidemiological characteristics of S.* Typhi.** The *S.* Typhi isolates from the 116 cases of typhoid fever were assigned to one of 17 genotypes using GenoTyphi (8, 24), and linked to the reported international travel available for each of

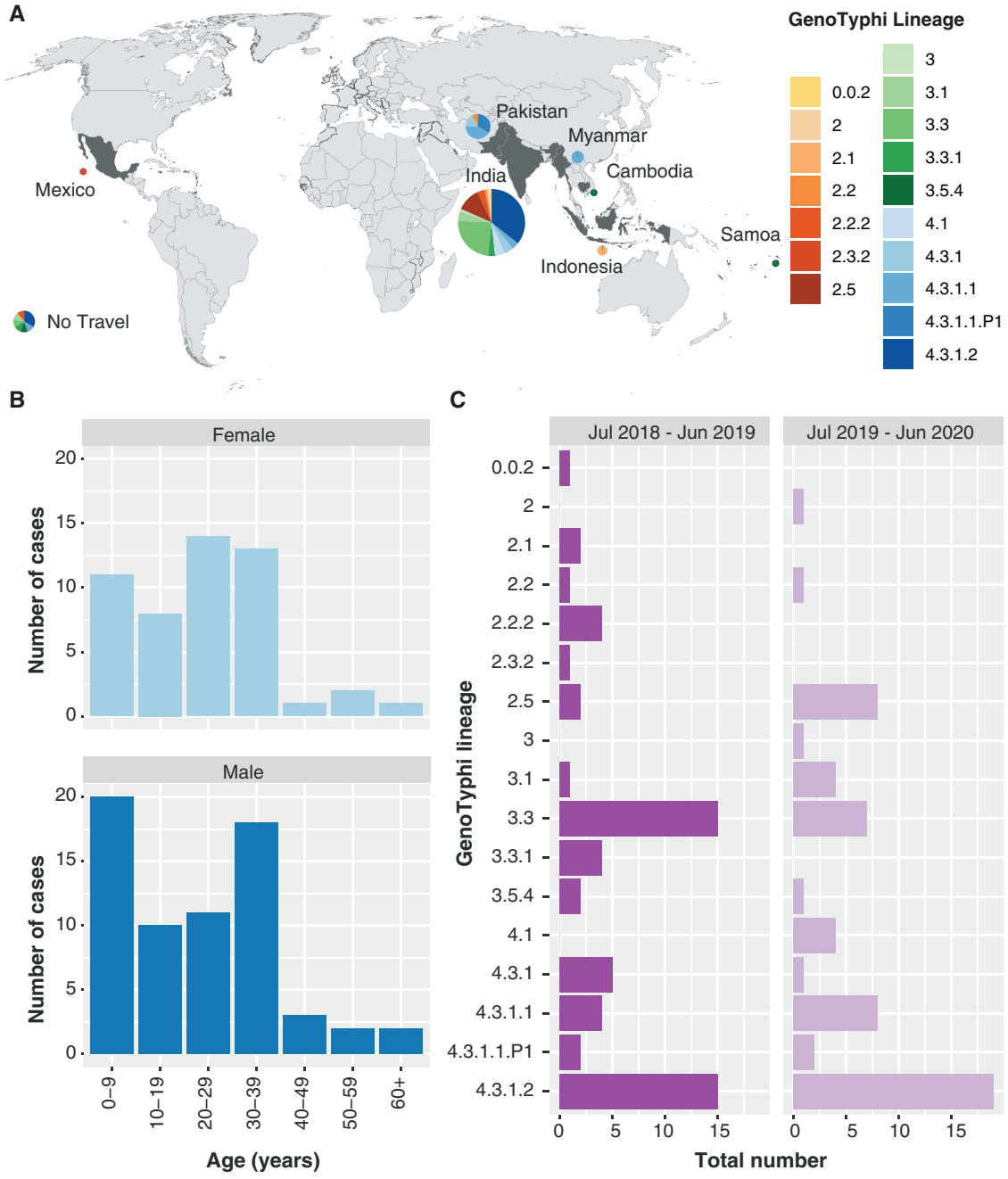

FIG 1 Summary of the 116 *Salmonella enterica* serovar Typhi (*S.* Typhi) isolates from the 2-year study period. A: Distribution of 116 *S.* Typhi isolates with reported international travel. Pie graphs represent the proportion of isolates with reported travel to different countries or no reported travel. The graphs are colored by membership to GenoTyphi lineages. Blank map sourced from https://commons.wikimedia.org/wiki/File:BlankMap-World-Flattened.svg. B: Patient characteristics of individuals which the *S.* Typhi were isolated. The histograms show number of cases of male and female patients, stratified by age (years). C: Membership to the different GenoTyphi lineages over the 2-year study period.

the patients (Fig. 1A, Fig. S1c). Travel was reported to seven countries with the vast majority reporting travel to India (87/116, 75.0%). Of the 87 cases with reported travel to India, the most common genotypes were 4.3.1.2 (n = 31), 3.3 (n = 21) and 2.5 (n = 10) (Table S1). Pakistan was the next most frequent destination for travelers (12/116, 10.4%) with the majority of isolates part of the global sublineages 4.3.1.1 (n = 5) or 4.3.1.1.P1 (n = 4). Fewer than five cases were associated with reported travel to each of Samoa, Cambodia, Mexico, Myanmar and Indonesia. No travel was reported for 9/116

(7.8%) although one case had confirmed contact with a returned traveler from India and another, AUSMDU00019653, confirmed contact with a chronic carrier.

Epidemiological data including age, sex and date of sample collection was available for all 116 cases (Fig. 1B and C). The proportion of cases from males was slightly higher than from females (66/116, 56.9%), however this difference was not significant ($P = 0.16$, two-sided test of proportions). The number of cases from males and females was consistent over the 2 years ($n = 25$ from females each 12-month period and $n = 34$ and $n = 32$ from males in the first and second timespan, respectively). The median age of all cases was 25 years (interquartile range [IQR] 8-32 years. This was consistent between males and females with the median age for males being 24 years (IQR 7.25–33) and for females being 27 years (IQR 10–31). There were some differences in the most common genotypes detected between the two timespans (Fig. 1C). Lineage 4.3.1.2 was the most common in both sampling frames, however lineage 3.3 (associated with returned travelers from India) decreased in prevalence while lineage 2.5 increased (also associated with returned travelers from India).

Investigation of the isolate from the chronic carrier, AUSMDU00017205, and the epidemiologically linked case, AUSMDU00019653, found little genomic difference between the two isolates. The isolate from the chronic carrier was received in early June 2018 with the linked case received 4 months later in September 2018. Both were genotyped as 2.2.2, had 17 pairwise SNPs and with no AMR mechanisms detected, were susceptible to all drugs. Pangenome analysis of the two isolates found they shared 4,546/4,547 genes with the difference a hypothetical protein.

Antimicrobial resistance profiles in *S.* Typhi. Different AMR profiles characterized the *S.* Typhi genotypes detected in the Australian cohort (Fig. 2, Fig. S1 in the supplemental material). A total of 10 out of 116 (8.6%) cases were either MDR ($n = 7$) or XDR ($n = 3$). The XDR isolates were genotyped as 4.3.1.1.P1, while the MDR isolates were either 4.3.1.1 ($n = 6$) or 4.3.1.1.P1 ($n = 1$). No genes associated with carbapenem resistance were detected in any of the *S.* Typhi. Ciprofloxacin resistance resulting from triple point mutations in QRDR was detected in two lineages, 4.3.1.2 (16/34, 47.1%) and 3.3 (2/22, 9.1%).

Third-generation cephalosporin resistance mediated by ESBLs was rare in the 2-year cohort, detected in 4/116 (3.4%) of *S.* Typhi (Fig. 2). All four of these ESBL isolates were phenotypically resistant to cefotaxime and no other isolates were phenotypically resistant to this drug. The three XDR isolates in 4.3.1.1.P1 with reported travel to Pakistan all had the same ESBL gene, *bla*CTX-M-15, the acquired fluoroquinolone resistance gene, *qnrS1*, a single QRDR mutation, *gyrA*-S83F, and the MDR profile (Fig. 2B). The IncY replicon gene was detected in two of the three XDR isolates, AUSMDU00025222 and AUSMDU00026490. Subsequent alignment of the short-read data of the three XDR isolates to the IncY p60006 plasmid, reported by Klemm et al. (14) from the XDR *S.* Typhi outbreak in Pakistan in 2018, found AUSMDU00025222 and AUSMDU00026490 had $>$ 95% alignment to the reference. In contrast, AUSMDU00044460 only had 55.7% alignment to the IncY plasmid and the absence of the IncY replicon gene, suggestive of integration into the chromosome as has been previously reported by Nair et al. (4). Inspection of assembly graphs in Bandage (27) was unable to confidently infer the integration from short read data alone. The AMR profile of AUSMDU00044460 is different to those previously characterized in integrating into the chromosome with the absence of genes mediating resistance to streptomycin and presence of *bla*TEM-1 (4, 8, 14).

The *S.* Typhi isolate AUSMDU00044634 had a unique ESBL profile in the Australian data. This isolate genotyped as 4.3.1.2 and was from a case with reported travel to India. Only a single AMR gene was detected, *bla*SHV-12, that mediates resistance to extended spectrum beta-lactams. AUSMDU00044634 also had a single point mutation detected, *gyrA*-S83Y, conferring reduced susceptibility to ciprofloxacin; and the IncX3 plasmid replicon. Both AUSMDU00044634 and the *Klebsiella pneumoniae* pIncX-SHV plasmid (the plasmid replicon reference sequence) had $>$92% alignment to the reference IncX plasmid, pLHST2018, from a *S.* Typhi strain collected in India in 2018 (28).

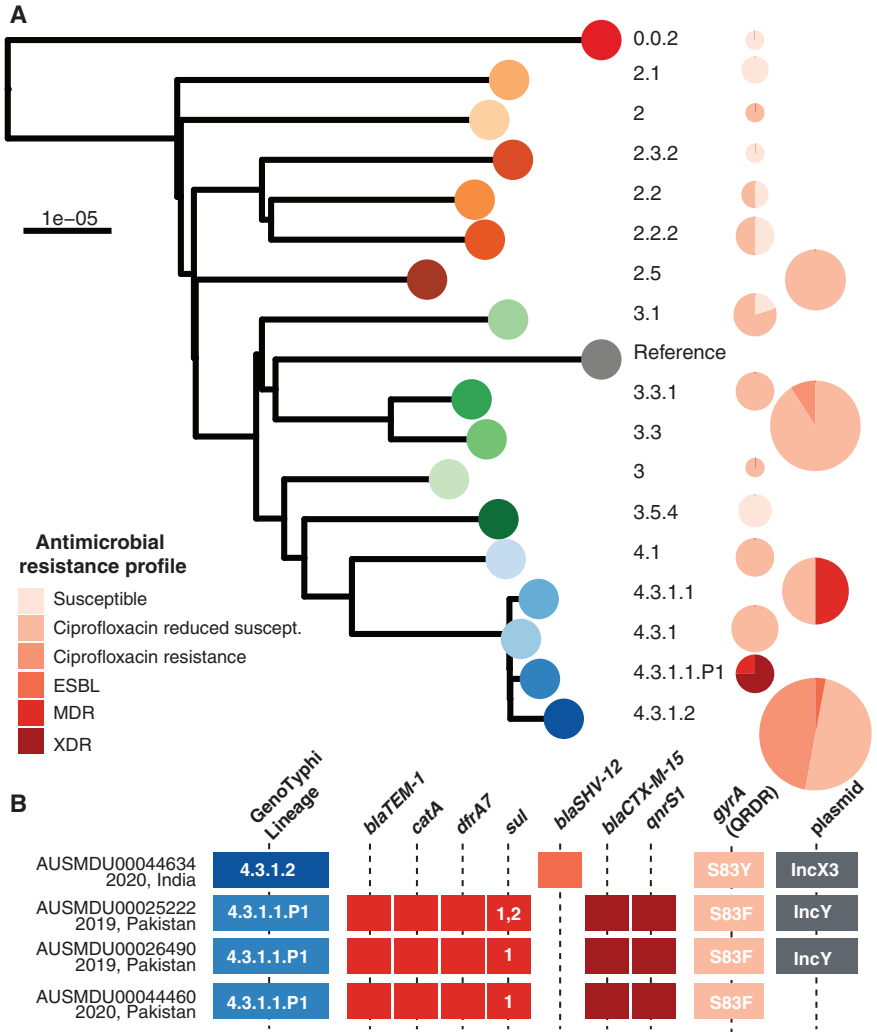

FIG 2 Antimicrobial resistance profiles in *Salmonella enterica* serovar Typhi (*S.* Typhi) data set. A: Framework tree of the 17 different GenoTyphi lineages identified, using reference genome CT18 (accession AL513382). Pie graphs to the right show the number is isolates in each lineage and are colored by the AMR profile: susceptible, reduced susceptibility to ciprofloxacin (1–2 point mutations in QRDRs and no other AMR mechanisms), ciprofloxacin resistant (3-point mutations in QRDRs), ESBL, MDR or XDR. B: Characterization of four *S.* Typhi isolates where ESBL genes were detected. The genotype membership, AMR determinants to therapeutic options are shown (with specific alleles given for *sul* genes and point mutations in QRDRs), as are the presence of plasmid replicons. AMR, antimicrobial resistance; QRDR, quinolone resistance determining region; XDR, extensively drug resistant; ESBL, extended spectrum beta-lactamases.

The pLHST2018 plasmid also carries the *qnrB7* quinolone gene which was absent in the AUSMDU00044634 genome. Further, the IncX plasmid replicon and *bla*SHV-12 gene are rare in *S.* Typhi. This profile has only been reported in three *S.* Typhi isolates on TyphiNET; all from samples collected in India in 2016, although these three public isolates also had triple point mutations in QRDRs and the *qnrB* gene.

Point mutations known to confer resistance to either ciprofloxacin or azithromycin were screened for in all isolates. No known point mutations were detected for azithromycin resistance and no isolates were phenotypically resistant to azithromycin. Only 11 isolates had no point mutations in QRDRs, and with no other AMR determinants detected, were completely susceptible to all drugs (Table S1 in the supplemental material). These were found in isolates from cases with reported travel to Indonesia (n = 2), India (n =2), Cambodia (n = 1), Mexico (n =1), Samoa (n = 1), Pakistan (n =1), or no

travel (*n* =3). None of these 11 isolates genotyped as part of the global clone (4.3.1 and related sublineages).

Nearly all isolates were either classed as resistant to ciprofloxacin (18/116; 15.5%) or to have reduced susceptibility to ciprofloxacin (87/116; 75.0%). The triple mutation profile of *gyrA*-S83F, *gyrA*-D87N and *parC*-S80I was associated with lineage 4.3.1.2 (16/34, 47.1%), whereas the profile *gyrA*-S83F, *gyrA*-D87V, and *parC*-S80I was associated with lineage 3.3 (2/22, 9.1%) (Fig. 2A, Table S1 in the supplemental material). Double point mutations were detected in three isolates all genotyped as 4.3.1.2, *gyrA*-S83F, *parC*-E84G (*n* = 2) and *gyrA*-S83Y, *parC*-E84G (*n* = 1). The remaining 84/116 (72.4%) genomes had a single mutation detected; the most common being *gyrA*-S83F in 65 isolates (Fig. 2, Fig. S1).

High-risk AMR lineages in returned travelers. Reported country of travel was a key marker for high-risk genotypes and AMR profiles (Fig. 3, Fig. S1A to S1D in the supplemental material). Three of the four most prevalent lineages in the Australian data were associated with AMR profile and country, 3.3 and 4.3.1.2 with ciprofloxacin resistance and travel to India, and 4.3.1.1 with MDR and travel to Pakistan. All isolates in the fourth most common lineage 2.5 had a single point mutation *gyrA*-S83F and 6/10 (60%) had the IncFIB plasmid replicon detected. The pangenomes of these four lineages was similar, with plasmids likely to be the key difference in accessory genome content within the lineages (Fig. S1E and Table S1 in the supplemental material). Detailed statistical analysis of stratified data based on genotype prevalence, country, and AMR profile was not able to be conducted due to small numbers in the Australian data. However, previously reported AMR patterns associated with genotype lineage and country of travel were found in the Australian *S.* Typhi cohort.

Triple mutations associated with ciprofloxacin resistance were found only in isolates from cases with reported travel to India. The main sublineage was 4.3.1.2 although genotype 3.3 also had isolates with triple QRDR mutations. This represents 20.7% of all cases with reported travel to India, noting that most had at least a single QRDR mutation. The 4.3.1.2 lineage was the most common genotype in the Australian cohort, with 47.1% having an AMR profile of ciprofloxacin resistant. The 4.3.1.2 lineage was most common in the TyphiNET data for Indian *S.* Typhi genomes from both local and travel-associated cases, with triple point mutations detected in 10.5 and 35.6%, respectively.

In contrast, travel to Pakistan was associated with XDR and MDR isolates of *S.* Typhi that were part of lineage 4.3.1.1.P1 and lineage 4.3.1.1 respectively. Thus, nine of 12 (75.0%) of all returned travelers from Pakistan were at least resistant to chloramphenicol, ampicillin, and co-trimoxazole, and for 25% of cases, the only remaining effective oral therapeutic was azithromycin. The high levels of AMR detected in the Australian data with travel to Pakistan was reflected in the *S.* Typhi genomes associated with Pakistan reported on TyphiNET, with 52.6% being typed as 4.3.1.1.P1 and XDR.

DISCUSSION

In this study we undertook an unbiased 2-year cohort study of *S.* Typhi cases that demonstrated the value of enhanced regional surveillance provided through greater integration of epidemiological and genomic data. We show that high risk lineages, associated with MDR, XDR and ciprofloxacin resistance mechanisms, strongly correlate to country of reported travel. Ongoing integrated analysis will be critical with the prospect of increasing resistance to azithromycin, the last broadly effective oral therapeutic (22), and increasing cases of XDR *S.* Typhi that have been reported globally (9, 14, 24).

The two main travel destinations associated with Australian cases of typhoid fever are India and Pakistan in South Asia, and it is from this region that new AMR patterns in *S.* Typhi are largely emerging (9, 15, 23). The current therapeutic guidelines for typhoid fever in Australia recommend use of ceftriaxone or azithromycin for infections acquired in Southeast Asia or the Indian subcontinent in the first instance, and quinolones if confirmed as susceptible (29). We note the Australian data are biased by local travel patterns and that vaccination status of cases are not routinely collected (vaccination is recommended for travelers to typhoid endemic regions). Despite these limitations, these data

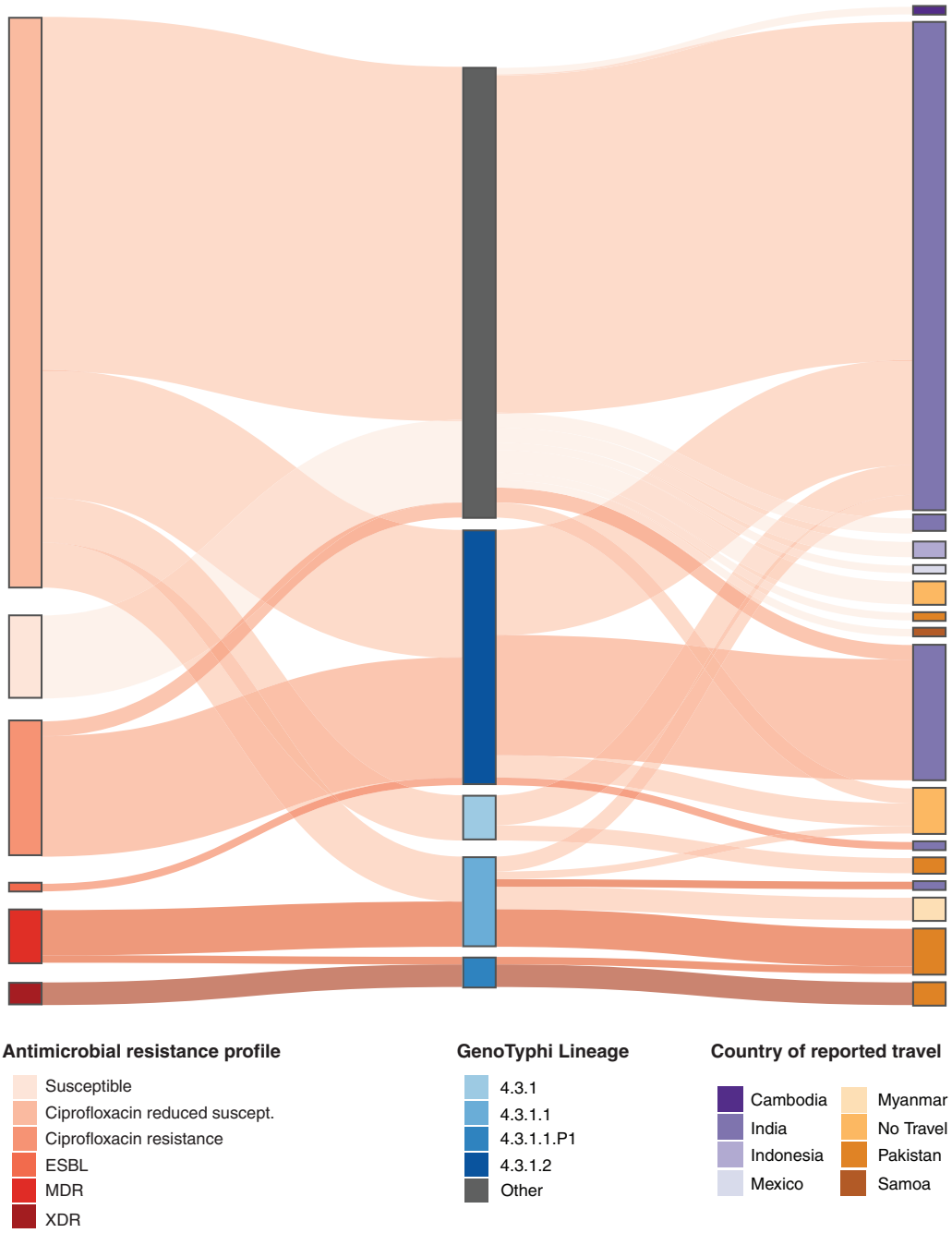

FIG 3 Relationship of AMR profiles with lineage and reported travel. Sankey diagram showing the relationships between three variables; nodes to the left, antimicrobial resistance profile to key treatment options defined by presence of genetic mechanisms resulting in XDR, MDR, ESBL resistance, ciprofloxacin resistance, ciprofloxacin reduced susceptibility or susceptible isolates; center nodes, membership to one of the global GenoTyphi lineages or other; and nodes to the right, country of reported travel. The connections between the nodes are colored by antimicrobial resistance profile. XDR, extensively drug resistant; MDR, multidrug resistance; ESBL, extended spectrum beta-lactamases.

demonstrate that enhanced genomic surveillance of vaccine preventable invasive bacterial pathogens provides opportunities for more targeted treatment guidelines in the future. For example, the ESBL resistant isolate of 4.3.1.2 is not susceptible to one of the two recommended drugs in Australia based upon travel history, but it is susceptible to most oral therapeutics including co-trimoxazole. Further, the relatively high rates of ESBL

resistance in returned travelers from Pakistan, would suggest that a broader-spectrum antimicrobial, such as a carbapenem (for severe disease) or azithromycin, are more appropriate initial antimicrobials for these cases at present.

Notably, we detected known AMR profiles that have been previously found to be associated with different lineages and countries, including when the AMR profiles were rare and newly emerging (14, 15, 21, 28). This is best as exemplified by the characterization of the IncX plasmid with the *bla*SHV-12 gene detected in an isolate from an Australian case with recent travel to India, which was also identified from another isolate collected in India (28). It has only been reported in three isolates on TyphiNET and only a single *S.* Typhi genome in the recent 860 cases from Public Health England was also found to have the *bla*SHV-12 gene (15). This demonstrates the very early detection of a new AMR profile through regional surveillance. It is likely that this profile resulted from a plasmid acquisition event from another member of the *Enterobacterales* circulating in India; most likely *K. pneumoniae*, which most commonly carry *bla*SHV ESBL genes, usually on plasmids (30). As such, it is anticipated that additional plasmid acquisition events will continue to occur within lineages of *S.* Typhi. This has previously been suggested as the means of ESBL resistance for a recent *S.* Typhi isolate collected in the Demographic Republic of the Congo in 2015 that was genotyped as lineage 2.5.1 (31). This demonstrates the role for routine surveillance within public health laboratories to provide an early warning signal of potential new threats.

Importantly, while no carbapenemase resistance genes were detected in the Australian cohort, and have not been detected in *S.* Typhi at all to date, the potential prospect of MDR, azithromycin, or ciprofloxacin resistant *S.* Typhi acquiring a plasmid with carbapenemase genes would be dire. Particularly as resistance to ESBLs and azithromycin have both emerged in the past few years, highlighting the rapid pace at which *S.* Typhi is acquiring AMR mechanisms. Both *Escherichia coli* and *K. pneumoniae* are ubiquitous in the gastrointestinal tract and may harbor carbapenemase resistance plasmids (such as *bla*NDM-carrying *E. coli*, commonly detected in the community in South Asia) (32), and, given the right selective pressures, such plasmids would be retained upon acquisition. Detecting these new AMR profiles as they emerge, characterizing the underlying genetic mechanism and genotype, and linking these data to travel history will be critical for ongoing surveillance and response to *S.* Typhi and may inform public health and clinical practices.

The ongoing pandemic and coincident increase in antimicrobial therapy for patients with severe COVID-19 infections may escalate the levels of AMR in countries such as India, which has high incidence of COVID-19 (33–35), may represent a serious threat to public health both locally and globally. While noting that international travel will be limited in the near future as a result of the ongoing pandemic, efforts can be made to prepare the emergence of these threats. Internationally, Pathogenwatch (9, 36) and TyphiNET, have been developed to analyze and report on all public *S.* Typhi genomes, providing breakdowns of AMR and genotypes by country, and already are valuable resources. In Australia, the newly established AusTrakka platform (https://www.cdgn.org.au/austrakka) is the nationally recognized platform for real-time analysis of integrated pathogen genomic data for public health purposes. AusTrakka provides a central platform for the secure sharing of data at both within and between different state and territory jurisdictions, and *S.* Typhi will be included on the platform. As such, ongoing genomic surveillance efforts on integrated platforms both nationally and internationally will be key for *S.* Typhi.

This study provides a comprehensive baseline for future genomic surveillance of *S.* Typhi in Australia and the surrounding region. Integrating genomic and epidemiological data for prospective surveillance will ensure emerging drug-resistant *S.* Typhi threats are identified early, and treatment guidelines can be appropriately adjusted. Global efforts to address the ongoing threat of typhoid, and emerging drug-resistant clones, remain critically important.

MATERIALS AND METHODS

National surveillance for typhoid fever. The National Notifiable Disease Surveillance System (NNDSS) was established in Australia in 1990. The NNDSS coordinates the surveillance for communicable diseases. Notifications of disease, such as typhoid fever, are made to the appropriate health authority in each jurisdiction in the federated nation and these data are then in turn supplied to the Australian Government of Health. The raw counts of typhoid fever by State and Territory and Year were obtained from http://www9.health.gov.au/cda/source/rpt_4.cfm on 6 January 2021.

Study setting. In Australia, typhoid fever is a notifiable disease and *S.* Typhi isolates in Victoria are forwarded from diagnostic laboratories to the Microbiological Diagnostic Unit Public Health Laboratory (MDU PHL), the bacterial public health reference laboratory for the state of Victoria, for further characterization. Since July 2018, all *Salmonella* isolates received at the MDU PHL have been subject for whole-genome sequencing (WGS). An unbiased sampling approach was taken to include all 116 *S.* Typhi isolates received between 1 July 2018 to 30 June 2020. International travel data was for individual cases was obtained from routine case interviews conducted by the Victorian Department of Health.

Ethics. Data were collected in accordance with the Victorian Public Health and Wellbeing Act 2008. Ethical approval was received from the University of Melbourne Human Research Ethics Committee (study number 1954615.3).

Whole-genome sequencing and quality control. The original sample received at MDU PHL was subcultured to a Nutrient Agar (NA) plate and streaked to achieve single colonies. The NA plate was then incubated 37°C for 18–24 hr. A single colony is harvested, using a 1 μl sterile inoculating loop, and is emulsified into 200 μl lysis buffer. The genomic DNA of 116 isolates was extracted from a single colony using a QIAsymphony^{TM} DSP DNA Virus/Pathogen Kit (Qiagen) according to manufacturer's instructions, and WGS was performed using Illumina NextSeq with 150 bp paired-end reads. Genomes had a phred score of 33 and a depth score of $\geq$50.

Phylogenetic analysis of *S.* Typhi isolates. The 116 *S.* Typhi genomes were mapped to the standard *S.* Typhi reference CT18 (NCBI accession: AL513382) using Snippy (v4.6.0) (https://github.com/tseemann/snippy) using a minimum fraction of 0.9 and a minimum coverage of 10 reads at each base. Phage and repeat regions were masked from the final alignment using coordinates available in Ingle et al. 2019 (6), filtered for recombination using Gubbins (v2.4.1) (37) and the final SNPs extracted with SNP-sites (38). A maximum likelihood (ML) phylogenetic tree was inferred using IQ-Tree (v1.6.12) (39) from the SNP alignment of 3,040 bases using a generalized time-reversible model + constant sites and rapid bootstrapping (40). The final tree was mid-point rooted with phangorn (v2.7.1) (41) and visualized with ggtree (v3.0.2) (42). Ape (v5.5) (43) was used to drop tips from the full tree to generate the framework tree.

S. Typhi isolates were genotyped using GenoTyphi (https://github.com/katholt/genotyphi) (7) using the vcf files from Snippy output. GenoTyphi assigns isolates into the established extended typing framework with the global lineage (associated with haplotype H58) further delineated into sublineages associated with MDR (4.3.1.1), ciprofloxacin resistance (4.3.1.2), and XDR (4.3.1.1.P1) *S.* Typhi (13, 44). GenoTyphi detects known point mutations in the QRDRs in *gyrA* and *parC* genes and also detects the known point mutations (R717Q and R717L) associated with reduced susceptibility to azithromycin in *acrB*. Isolates with 3-point mutations in QRDR regions were defined as ciprofloxacin resistant isolates.

Genome assemblies and screening of accessory genomes. *S.* Typhi genomes were assembled using SPAdes (v 3.14.1) (45). The genome assemblies of all isolates were screened for acquired AMR determinants using the AMRFinder (46) database (https://github.com/ncbi/amr/wiki/AMRFinder-database) as implemented in the abriTAMR tool (https://github.com/MDU-PHL/abritamr). Plasmid replicons were detected using the PlasmidFinder database (47) with ABRicate (https://github.com/tseemann/abricate) using a minimum identify of 90% and minimum coverage of 90%. Isolates were serotyped *in silico* with SISTR (48). Tidyverse (v1.3.1) (49) was used to wrangle the data and ggplot2 (v3.3.5) used to visualize the data.

Determination of antimicrobial resistance profiles. Isolates with resistance determinants to ampicillin, chloramphenicol, and co-trimoxazole were defined as MDR. Isolates that, in addition to the MDR profile, also had a gene conferring resistance to ESBLs, the presence of *qnrS1* and at least one QRDR point mutation were defined as XDR. The designation ESBL was for *S.* Typhi isolates where an ESBL resistance gene was detected and the absence of other mechanisms (known AMR genes, triple point mutations in QRDRs, or a single point mutation in *acrB*) that would result in resistance. Isolates were classed as ciprofloxacin resistant if 3-point mutations in QRDRs were detected, while 1- or 2-point mutations in QRDRs (where no other AMR mechanisms were detected) resulted in a reduced susceptibility to ciprofloxacin profile. To visualize the relationship of the AMR profile to membership to GenoTyphi global lineage and country of reported travel was visualized as a Sankey plot with networkD3 (v0.4).

Exploration of accessory genome content. Differences in the accessory genome of the genotype lineages with $\geq$10 isolates were explored with Panaroo (v1.2.7) (50). Briefly, the .gff files from the annotated genomes assemblies were used as input to panaroo using the strict clean-mode and default parameters.

The presence of IncY and IncX plasmids were investigated in more detail for the isolates where $bla_{CTX\text{-}M\text{-}15}$ and $bla_{SHV\text{-}12}$ were respectively detected. Three isolates, AUSMDU00025222, AUSMDU00026490 and AUSMDU00044460 were aligned to the IncY plasmid, p60006 (accession: LT906492) of a *S.* Typhi isolate collected in Pakistan (14). AUSMDU00044634 was aligned to IncX plasmid, pLHST2018 (accession: CP052768), of a *S.* Typhi isolate collected in India (28) using snippy (v4.6.0). The publicly available pIncX-SHV (accession: JN247852) from *K. pneumoniae* collected in Italy (30) was also aligned using the –ctgs option in snippy.

Investigation of infection linked to chronic carrier. One isolate, AUSMDU00019653, was epidemiologically linked to a chronic case reported outside the 2-year cohort. The relatedness of AUSMDU00019653

to the isolate from the chronic case, AUSMDU00017205, was investigated first through mapping-based approaches to reference CT18 described above with pairwise SNP-distances determined with snp-dists (v0.7.0) (https://github.com/tseemann/snp-dists). AUSMDU00017205 was assembled as above and the pangenome of the two genomes was explored with Panaroo (v1.2.7) (50). AUSMDU00017205 was characterized for known AMR mechanisms and GenoTyphi lineage as above.

Comparison of Australian *S.* Typhi with public data on TyphiNET. Details on publicly available *S.* Typhi data that have been characterized for GenoTyphi lineage, AMR mechanisms and are accompanied by geographical data were downloaded from TyphiNET on 10th August 2021. The most frequent genotypes from India (the most common destination for travelers in the Australian data), prevalence of the XDR profile with genotype and country, and combination of IncX and *bla*SHV-12 were compared with the Australian data.

Cefotaxime and azithromycin susceptibility testing. Antimicrobial susceptibility testing for cefotaxime and azithromycin were performed for all isolates using agar dilution. Clinical and Laboratory Standards Institute (CLSI) 2019 breakpoints were used for interpretation. Isolates with a MIC $\geq$4 μg/ml defined as cefotaxime resistant. Isolates with a MIC $\geq$32 μg/ml defined as azithromycin resistant.

Data availability. Details and the accession numbers of the sequence data of genomes included in our analysis are available in Table S1 in the supplemental material, and the reads of isolates sequenced at MDU PHL are available on the NCBI Sequence Read Archive (BioProject PRJNA319593).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.1 MB.

SUPPLEMENTAL FILE 2, XLSX file, 0.02 MB.

ACKNOWLEDGMENTS

The Microbiological Diagnostic Unit Public Health Laboratory is funded by the Victorian Government. D.J.I. was supported by a National Health and Medical Research Council (NHMRC) Emerging Leadership Fellowship (GNT1195210). B.P.H. was supported by NHMRC Leadership Fellowship (GNT1196103). D.A.W. is supported by an NHMRC Emerging Leadership Fellowship (GNT1174555). The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

We declare that there are no conflicts of interest.

REFERENCES

1. Levine MM, Simon R. 2018. The gathering storm: Is untreatable typhoid fever on the way? mBio 9:e00482-18. https://doi.org/10.1128/mBio.00482-18.
2. Crump JA, Sjölund-Karlsson M, Gordon MA, Parry CM. 2015. Epidemiology, clinical presentation, laboratory diagnosis, antimicrobial resistance, and antimicrobial management of invasive *Salmonella* infections. Clin Microbiol Rev 28:901–937. https://doi.org/10.1128/CMR.00002-15.
3. Stanaway JD, Reiner RC, Blacker BF, Goldberg EM, Khalil IA, Troeger CE, Andrews JR, Bhutta ZA, Crump JA, Im J, Marks F, Mintz E, Park SE, Zaidi AKM, Abebe Z, Abejie AN, Adedeji IA, Ali BA, Amare AT, Atalay HT, Avokpaho EFGA, Bacha U, Barac A, Bedi N, Berhane A, Browne AJ, Chirinos JL, Chitheer A, Dolecek C, Zaki MES, Eshrati B, Foreman KJ, Gemechu A, Gupta R, Hailu GB, Henok A, Hibstu DT, Hoang CL, Ilesanmi OS, Iyer VJ, Kahsay A, Kasaeian A, Kassa TD, Khan EA, Khang Y-H, Razek HMAE, Melku M, Mengistu DT, Mohammad KA, Mohammed S, Mokdad AH, Nachega JB, Naheed A, Nguyen CT, Nguyen HLT, Nguyen LH, Nguyen NB, Nguyen TH, Nirayo YL, Pangestu T, Patton GC, Qorbani M, Collaborators G 2017 T and P, et al. 2019. The global burden of typhoid and paratyphoid fevers: a systematic analysis for the Global Burden of Disease Study 2017. Lancet Infect Dis 19:369–381. https://doi.org/10.1016/S1473-3099(18)30685-6.
4. Nair S, Chattaway M, Langridge GC, Gentle A, Day M, Ainsworth EV, Mohamed I, Smith R, Jenkins C, Dallman TJ, Godbole G. 2021. ESBL-producing strains isolated from imported cases of enteric fever in England and Wales reveal multiple chromosomal integrations of *blaCTX-M-15* in XDR *Salmonella* Typhi. J Antimicrob Chemoth 76:dkab049.
5. Dyson ZA, Klemm EJ, Palmer S, Dougan G. 2019. Antibiotic resistance and typhoid. Clin Infect Dis 68:S165–S170. https://doi.org/10.1093/cid/ciy1111.
6. Ingle DJ, Nair S, Hartman H, Ashton PM, Dyson ZA, Day M, Freedman J, Chattaway MA, Holt KE, Dallman TJ. 2019. Informal genomic surveillance of regional distribution of *Salmonella* Typhi genotypes and antimicrobial resistance via returning travellers. PLoS Negl Trop Dis 13:e0007620. https://doi.org/10.1371/journal.pntd.0007620.
7. Wong VK, Baker S, Connor TR, Pickard D, Page AJ, Dave J, Murphy N, Holliman R, Sefton A, Millar M, Dyson ZA, Dougan G, Holt KE, Parkhill J, Feasey NA, Kingsley RA, Thomson NR, Keane JA, Weill F-X, Hello SL, Hawkey J, Edwards DJ, Harris SR, Cain AK, Hadfield J, Hart PJ, Thieu NTV, Klemm EJ, Breiman RF, Watson CH, Edmunds WJ, Kariuki S, Gordon MA, Heyderman RS, Okoro C, Jacobs J, Lunguya O, Msefula C, Chabalgoity JA, Kama M, Jenkins K, Dutta S, Marks F, Campos J, Thompson C, Obaro S, MacLennan CA, Dolecek C, Keddy KH, Smith AM, Parry CM, Karkey A, Dongol S, et al. 2016. An extended genotyping framework for *Salmonella enterica* serovar Typhi, the cause of human typhoid. Nat Commun 7:12827. https://doi.org/10.1038/ncomms12827.
8. Wong VK, Baker S, Pickard DJ, Parkhill J, Page AJ, Feasey NA, Kingsley RA, Thomson NR, Keane JA, Weill F-X, Edwards DJ, Hawkey J, Harris SR, Mather AE, Cain AK, Hadfield J, Hart PJ, Thieu NTV, Klemm EJ, Glinos DA, Breiman RF, Watson CH, Kariuki S, Gordon MA, Heyderman RS, Okoro C, Jacobs J, Lunguya O, Edmunds WJ, Msefula C, Chabalgoity JA, Kama M, Jenkins K, Dutta S, Marks F, Campos J, Thompson C, Obaro S, MacLennan CA, Dolecek C, Keddy KH, Smith AM, Parry CM, Karkey A, Mulholland EK, Campbell JI, Dongol S, Basnyat B, Dufour M, Bandaranayake D, Naseri TT, Singh SP, Hatta M, et al. 2015. Phylogeographical analysis of the dominant multidrug-resistant H58 clade of *Salmonella* Typhi identifies inter- and intracontinental transmission events. Nat Genet 47:632–639. https://doi.org/10.1038/ng.3281.
9. Argimón S, Yeats CA, Goater RJ, Abudahab K, Taylor B, Underwood A, Sánchez-Busó L, Wong VK, Dyson ZA, Nair S, Park SE, Marks F, Page AJ, Keane JA, Baker S, Holt KE, Dougan G, Aanensen DM. 2021. A global resource for genomic predictions of antimicrobial resistance and surveillance of *Salmonella* Typhi at pathogenwatch. Nat Commun 12:2879. https://doi.org/10.1038/s41467-021-23091-2.
10. Park SE, Pham DT, Boinett C, Wong VK, Pak GD, Panzner U, Espinoza LMC, von Kalckreuth V, Im J, Schütt-Gerowitt H, Crump JA, Breiman RF, Adu-

Sarkodie Y, Owusu-Dabo E, Rakotozandrindrainy R, Soura AB, Aseffa A, Gasmelseed N, Keddy KH, May J, Sow AG, Aaby P, Biggs HM, Hertz JT, Montgomery JM, Cosmas L, Olack B, Fields B, Sarpong N, Razafindrabe TJL, Raminosoa TM, Kabore LP, Sampo E, Teferi M, Yeshitela B, Tayeb MAE, Sooka A, Meyer CG, Krumkamp R, Dekker DM, Jaeger A, Poppert S, Tall A, Niang A, Bjerregaard-Andersen M, Løfberg SV, Seo HJ, Jeon HJ, Deerin JF, Park J, Konings F, Ali M, Clemens JD, Hughes P, Sendagala JN, et al. 2018. The phylogeography and incidence of multi-drug resistant typhoid fever in subSaharan Africa. Nat Commun 9:5094. https://doi.org/10.1038/s41467-018-07370-z.

11. Thanh DP, Thompson CN, Rabaa MA, Sona S, Sopheary S, Kumar V, Moore C, Thieu NTV, Wijedoru L, Holt KE, Wong V, Pickard D, Thwaites GE, Day N, Dougan G, Turner P, Parry CM, Baker S. 2016. The molecular and spatial epidemiology of typhoid fever in rural Cambodia. PLoS Negl Trop Dis 10: e0004785. https://doi.org/10.1371/journal.pntd.0004785.
12. Hooda Y, Sajib MSI, Rahman H, Luby SP, Bondy-Denomy J, Santosham M, Andrews JR, Saha SK, Saha S. 2019. Molecular mechanism of azithromycin resistance among typhoidal *Salmonella* strains in Bangladesh identified through passive pediatric surveillance. PLoS Negl Trop Dis 13:e0007868. https://doi.org/10.1371/journal.pntd.0007868.
13. Rahman SIA, Dyson ZA, Klemm EJ, Khanam F, Holt KE, Chowdhury EK, Dougan G, Qadri F. 2020. Population structure and antimicrobial resistance patterns of Salmonella Typhi isolates in urban Dhaka, Bangladesh from 2004 to 2016. PLoS Negl Trop Dis 14:e0008036. https://doi.org/10.1371/journal.pntd.0008036.
14. Klemm EJ, Shakoor S, Page AJ, Qamar FN, Judge K, Saeed DK, Wong VK, Dallman TJ, Nair S, Baker S, Shaheen G, Qureshi S, Yousafzai MT, Saleem MK, Hasan Z, Dougan G, Hasan R. 2018. Emergence of an extensively drug-resistant *Salmonella enterica* serovar Typhi clone harboring a promiscuous plasmid encoding resistance to fluoroquinolones and third-generation cephalosporins. mBio 9:e00105-18. https://doi.org/10.1128/mBio.00105-18.
15. Chattaway MA, Gentle A, Nair S, Tingley L, Day M, Mohamed I, Jenkins C, Godbole G. 2021. Phylogenomics and antimicrobial resistance of Salmonella Typhi and Paratyphi A, B and C in England, 2016–2019. Microb Genom 7:000633. https://doi.org/10.1099/mgen.0.000633.
16. Kariuki S, Revathi G, Kiiru J, Mengo DM, Mwituria J, Muyodi J, Munyalo A, Teo YY, Holt KE, Kingsley RA, Dougan G. 2010. Typhoid in Kenya is associated with a dominant multidrug-resistant *Salmonella enterica* serovar Typhi haplotype that is also widespread in Southeast Asia. J Clin Microbiol 48:2171–2176. https://doi.org/10.1128/JCM.01983-09.
17. Holt KE, Phan MD, Baker S, Duy PT, Nga TVT, Nair S, Turner AK, Walsh C, Fanning S, Farrell-Ward S, Dutta S, Kariuki S, Weill F-X, Parkhill J, Dougan G, Wain J. 2011. Emergence of a globally dominant IncHI1 plasmid type associated with multiple drug resistant typhoid. PLoS Negl Trop Dis 5: e1245. https://doi.org/10.1371/journal.pntd.0001245.
18. Thanh DP, Karkey A, Dongol S, Thi NH, Thompson CN, Rabaa MA, Arjyal A, Holt KE, Wong V, Thieu NTV, Vinh PV, Thanh TH, Pradhan A, Shrestha SK, Gajurel D, Pickard D, Parry CM, Dougan G, Wolbers M, Dolecek C, Thwaites GE, Basnyat B, Baker S. 2016. A novel ciprofloxacin-resistant subclade of H58 *Salmonella* Typhi is associated with fluoroquinolone treatment failure. Elife 5:e14003. https://doi.org/10.7554/eLife.14003.
19. Britto CD, Dyson ZA, Mathias S, Bosco A, Dougan G, Jose S, Nagaraj S, Holt KE, Pollard AJ. 2019. Persistent circulation of a fluoroquinolone-resistant *Salmonella enterica* Typhi clone in the Indian subcontinent. J Antimicrob Chemoth 75:337–341.
20. Baker S, Duy PT, Nga TVT, Dung TTN, Phat VV, Chau TT, Turner AK, Farrar J, Boni MF. 2013. Fitness benefits in fluoroquinolone-resistant *Salmonella* Typhi in the absence of antimicrobial pressure. Elife 2:e01229. https://doi.org/10.7554/eLife.01229.
21. Godbole GS, Day MR, Murthy S, Chattaway MA, Nair S. 2018. First report of *CTX-M-15 Salmonella* Typhi from England. Clin Infect Dis 66:1976–1977. https://doi.org/10.1093/cid/ciy032.
22. Duy PT, Dongol S, Giri A, To NTN, Thanh HND, Quynh NPN, Trung PD, Thwaites GE, Basnyat B, Baker S, Rabaa MA, Karkey A. 2020. The emergence of azithromycin-resistant *Salmonella* Typhi in Nepal. Jac-Antimicrobial Resist 2:dlaa109. https://doi.org/10.1093/jacamr/dlaa109.
23. Carey ME, Jain R, Yousuf M, Maes M, Dyson ZA, Thu TNH, Nguyen TNT, Dan THN, Nguyen QNP, Mahindroo J, Pham DT, Sandha KS, Baker S, Taneja N. 2021. Spontaneous emergence of azithromycin resistance in independent lineages of *Salmonella* Typhi in Northern India. Clin Infect Dis 72:e120–e127. https://doi.org/10.1093/cid/ciaa1773.
24. Sajib MSI, Tanmoy AM, Hooda Y, Rahman H, Andrews JR, Garrett DO, Endtz HP, Saha SK, Saha S. 2021. Tracking the emergence of azithromycin resistance in multiple genotypes of typhoidal *Salmonella*. mBio 12. https://doi.org/10.1128/mBio.03481-20.
25. Octavia S, Chew KL, Lin RTP, Teo JWP. 2021. Early release-azithromycin-resistant *Salmonella enterica* Serovar Typhi AcrB-R717Q/L. Emerg Infect Dis 27:624–627. https://doi.org/10.3201/eid2702.203874.
26. Australian Government, Department of Health. 2018. Typhoid fever. https://immunisationhandbook.health.gov.au/vaccine-preventable-diseases/typhoid-fever
27. Wick RR, Schultz MB, Zobel J, Holt KE. 2015. Bandage: interactive visualization of de novo genome assemblies. Bioinformatics 31:3350–3352. https://doi.org/10.1093/bioinformatics/btv383.
28. Jacob JJ, Pragasam AK, Vasudevan K, Veeraraghavan B, Kang G, John J, Nagvekar V, Mutreja A. 2021. *Salmonella* Typhi acquires diverse plasmids from other Enterobacteriaceae to develop cephalosporin resistance. Genomics 113: 2171–2176. https://doi.org/10.1016/j.ygeno.2021.05.003.
29. Antibiotic Expert Group. 2021. *Salmonella* Typhi and Paratyphi A, B and C bacteraemia (typhoid and paratyphoid fever), p 1–2. *In* eTG complete digital. Therapeutic Guidelines Limited, West Melbourne, Australia. https://tgldcdp.tg.org.au/viewTopic?topicfile=bloodstream-infections-septic-shock-directed-therapy&guidelineName=Antibiotic#toc_d1e734.
30. García-Fernández A, Villa L, Carta C, Venditti C, Giordano A, Venditti M, Mancini C, Carattoli A. 2012. *Klebsiella pneumoniae* ST258 producing KPC-3 identified in Italy carries novel plasmids and OmpK36/OmpK35 porin variants. Antimicrob Agents Chemother 56:2143–2145. https://doi.org/10.1128/AAC.05308-11.
31. Phoba M-F, Barbé B, Lunguya O, Masendu L, Lulengwa D, Dougan G, Wong VK, Bertrand S, Ceyssens P-J, Jacobs J, Puyvelde SV, Deborggraeve S. 2017. *Salmonella enterica* serovar Typhi producing *CTX-M-15* extended spectrum β-lactamase in the Democratic Republic of the Congo. Clin Infect Dis 65:1229–1231. https://doi.org/10.1093/cid/cix342.
32. Ranjan A, Shaik S, Mondal A, Nandanwar N, Hussain A, Semmler T, Kumar N, Tiwari SK, Jadhav S, Wieler LH, Ahmed N. 2016. Molecular epidemiology and genome dynamics of New Delhi metallo-β-lactamase-producing extraintestinal pathogenic *Escherichia coli* strains from India. Antimicrob Agents Chemother 60:6795–6805. https://doi.org/10.1128/AAC.01345-16.
33. Langford BJ, So M, Raybardhan S, Leung V, Westwood D, MacFadden DR, Soucy J-PR, Daneman N. 2020. Bacterial co-infection and secondary infection in patients with COVID-19: a living rapid review and meta-analysis. Clin Microbiol Infect 26:1622–1629. https://doi.org/10.1016/j.cmi.2020.07.016.
34. Vijay S, Bansal N, Rao BK, Veeraraghavan B, Rodrigues C, Wattal C, Goyal JP, Tadepalli K, Mathur P, Venkateswaran R, Venkatasubramanian R, Khadanga S, Bhattacharya S, Mukherjee S, Baveja S, Sistla S, Panda S, Walia K. 2021. Secondary Infections in Hospitalized COVID-19 Patients: Indian Experience. Infect Drug Resist 14:1893–1903. https://doi.org/10.2147/IDR.S299774.
35. Rawson TM, Moore LSP, Zhu N, Ranganathan N, Skolimowska K, Gilchrist M, Satta G, Cooke G, Holmes A. 2020. Bacterial and fungal co-infection in individuals with coronavirus: A rapid review to support COVID-19 antimicrobial prescribing. Clin Infect Dis 71:2459–2468. https://doi.org/10.1093/cid/ciaa530.
36. Dyson ZA, Holt KE. 2021. Five years of GenoTyphi: updates to the global Salmonella Typhi genotyping framework. Biorxiv 2021.04.28.441766.
37. Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR. 2015. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. Nucleic Acids Res 43:e15. https://doi.org/10.1093/nar/gku1196.
38. Page AJ, Taylor B, Delaney AJ, Soares J, Seemann T, Keane JA, Harris SR. 2016. SNP-sites: rapid efficient extraction of SNPs from multi-FASTA alignments. Microb Genom 2:e000056. https://doi.org/10.1099/mgen.0.000056.
39. Nguyen L-T, Schmidt HA, von Haeseler A, Minh BQ. 2015. IQ-TREE: A fast and effective stochastic algorithm for estimating maximum-likelihood phylogenies. Mol Biol Evol 32:268–274. https://doi.org/10.1093/molbev/msu300.
40. Minh BQ, Nguyen MAT, von Haeseler A. 2013. Ultrafast approximation for phylogenetic bootstrap. Mol Biol Evol 30:1188–1195. https://doi.org/10.1093/molbev/mst024.
41. Schliep KP. 2011. phangorn: phylogenetic analysis in R. Bioinformatics 27: 592–593. https://doi.org/10.1093/bioinformatics/btq706.
42. Yu G, Smith DK, Zhu H, Guan Y, Lam TT. 2017. ggtree: an r package for visualization and annotation of phylogenetic trees with their covariates and other associated data. Methods Ecol Evol 8:28–36. https://doi.org/10.1111/2041-210X.12628.

43. Paradis E, Claude J, Strimmer K. 2004. APE: Analyses of phylogenetics and evolution in R language. Bioinformatics 20:289–290. https://doi.org/10.1093/bioinformatics/btg412.
44. Britto CD, Dyson ZA, Duchene S, Carter MJ, Gurung M, Kelly DF, Murdoch DR, Ansari I, Thorson S, Shrestha S, Adhikari N, Dougan G, Holt KE, Pollard AJ. 2018. Laboratory and molecular surveillance of paediatric typhoidal *Salmonella* in Nepal: Antimicrobial resistance and implications for vaccine policy. PLoS Negl Trop Dis 12:e0006408. https://doi.org/10.1371/journal.pntd.0006408.
45. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: A new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
46. Feldgarden M, Brover V, Haft DH, Prasad AB, Slotta DJ, Tolstoy I, Tyson GH, Zhao S, Hsu C-H, McDermott PF, Tadesse DA, Morales C, Simmons M, Tillman G, Wasilenko J, Folster JP, Klimke W. 2019. Validating the AMRFinder tool and resistance gene database by using antimicrobial resistance genotype-phenotype correlations in a collection of isolates. Antimicrob Agents Chemother 63:e00483-19. https://doi.org/10.1128/AAC.00483-19.
47. Carattoli A, Zankari E, García-Fernández A, Larsen MV, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. In silico detection and typing of plasmids using plasmidfinder and plasmid multilocus sequence typing. Antimicrob Agents Chemother 58:3895–3903. https://doi.org/10.1128/AAC.02412-14.
48. Yoshida CE, Kruczkiewicz P, Laing CR, Lingohr EJ, Gannon VPJ, Nash JHE, Taboada EN. 2016. The *Salmonella* In Silico Typing Resource (SISTR): An open web-accessible tool for rapidly typing and subtyping draft salmonella genome assemblies. PLoS One 11:e0147101. https://doi.org/10.1371/journal.pone.0147101.
49. Wickham H, Averick M, Bryan J, Chang W, McGowan L, François R, Grolemund G, Hayes A, Henry L, Hester J, Kuhn M, Pedersen T, Miller E, Bache S, Müller K, Ooms J, Robinson D, Seidel D, Spinu V, Takahashi K, Vaughan D, Wilke C, Woo K, Yutani H. 2019. Welcome to the tidyverse. JOSS 4:1686. https://doi.org/10.21105/joss.01686.
50. Tonkin-Hill G, MacAlasdair N, Ruis C, Weimann A, Horesh G, Lees JA, Gladstone RA, Lo S, Beaudoin C, Floto RA, Frost SDW, Corander J, Bentley SD, Parkhill J. 2020. Producing polished prokaryotic pangenomes with the Panaroo pipeline. Genome Biol 21:180. https://doi.org/10.1186/s13059-020-02090-4.