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Short Communication

Azithromycin-resistant *mph*(A)-positive *Salmonella enterica* serovar Typhi in the United States

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ABSTRACT

Objectives: The United States Centers for Disease Control and Prevention (CDC) conducts active surveillance for typhoid fever cases caused by *Salmonella enterica* serovar Typhi (Typhi). Here we describe the characteristics of the first two cases of *mph*(A)-positive azithromycin-resistant Typhi identified through US surveillance.

Methods: Isolates were submitted to public health laboratories, sequenced, and screened for antimicrobial resistance determinants and plasmids, as part of CDC PulseNet's routine genomic surveillance. Antimicrobial susceptibility testing and long-read sequencing were also performed. Basic case information (age, sex, travel, outcome) was collected through routine questionnaires; additional epidemiological data was requested through follow-up patient interviews.

Results: The patients are related and both reported travel to India (overlapping travel dates) before illness onset. Both Typhi genomes belong to the GenoTyphi lineage 4.3.1.1 and carry the azithromycin-resistance gene *mph*(A) on a PTU-FE (IncFIA/FIB/FII) plasmid. These strains differ genetically from *mph*(A)-positive Typhi genomes recently reported from Pakistan, suggesting independent emergence of azithromycin resistance in India.

Conclusions: Cases of typhoid fever caused by Typhi strains resistant to all available oral treatment options are cause for concern and support the need for vaccination of travellers to Typhi endemic regions. US genomic surveillance serves as an important global sentinel for detection of strains with known and emerging antimicrobial resistance profiles, including strains from areas where routine surveillance is not conducted.

1. Introduction

Salmonella enterica serovar Typhi (Typhi) is a globally important human pathogen, although disease burden falls predominantly in south and southeast Asia, and sub-Saharan Africa [2]. The United States is a low-incidence Typhi setting, with cases predominantly associated with international travel to endemic areas [3]. Thus, US Typhi surveillance serves as an important global sentinel for the surveillance of strains with known and emerging antimicrobial resistance (AR) profiles, including strains from areas where routine surveillance is not conducted.

Increasing multidrug resistance (MDR) and global spread of extensively drug resistant strains (XDR) [2] leaves dwindling options for oral antibiotic treatment of Typhi. While azithromycin

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remains a viable oral treatment option for most cases of typhoid fever globally [2,4], azithromycin-resistant strains have recently been reported from India, Nepal and Singapore [5]. The mechanism of resistance is typically a single point mutation in the *acrB* gene, which has arisen in several independent Typhi lineages [6–8]. However, the acquired azithromycin-resistance gene *mph(*A) (encoding a macrolide phosphotransferase) has also been reported in Bangladesh [9], and most recently from a patient in Pakistan [10]. We describe here the first two cases of azithromycin-resistant *mph*(A)-positive Typhi identified in the United States through PulseNet, the US national network for molecular subtyping of enteric bacteria.

2. Material and methods

Isolates PNUSAS349400 and PNUSAS347532 were submitted by clinical diagnostic laboratories to public health laboratories and were sequenced as part of the Centers for Disease Control and Prevention (CDC) PulseNet national passive *Salmonella* surveillance network, per standardized methods (https://www.cdc.gov/nationalsurveillance/salmonella-surveillance.html; https://www.cdc.gov/pulsenet/pdf/PNL38-WGS-on-MiSeq-508.pdf); resistance determinants and plasmids were identified through routine screening, utilizing ResFinder and PlasmidFinder databases, respectively, as previously described [11]. Plasmids were further categorized into plasmid taxonomic units (PTU) using COPLA [12]. Sequenced reads were typed using GenoTyphi (https://github.com/katholt/genotyphi) [13].

For long-read sequencing of both isolates, genomic DNA was extracted (Wizard Genomic DNA Purification Kit, modified manufacturer's protocol, Promega, WI, USA) from cultures incubated on Tryptic Soy Agar-Sheep Blood overnight (37 °C). Libraries were prepared using the Rapid Barcoding Kit (SQK-RBK114.24; Oxford Nanopore Technologies [ONT], Oxford, UK) according to the manufacturer's protocol and sequenced for 72 h on a GridION sequencing platform (R10.4.1 flowcells, ONT). Reads were base-called using the SUP ("Super accurate") model of Guppy v6.5.7, filtered for quality using MinKNOW v23.04.5 (ONT), and filtered for minimum read length (>1000 bp) using Nanoq v0.10.0 [14]. Read sets were downsampled randomly using rasusa v0.7.1 [15] and assembled using flye v2.9 [16] (asm-coverage option set to 10 for PNUSAS349400). Assemblies were rotated to fix start positions of each contig using Circlator v1.5.5 [17], polished using Medaka v1.8.0 (https://github.com/nanoporetech/medaka), and screened for quality using socru v2.2.4 (to ensure expected genomic arrangement) and BUSCO v5.4.6 [18,19]. Long read data are deposited in National Center for Biotechnology Information (NCBI) under the BioSample IDs listed in Table 1.

Additionally, these two isolates underwent antimicrobial susceptibility testing (AST) according to CDC National Antimicrobial Resistance Monitoring System's protocol. Specifically, 14 antibiotics were tested (amoxicillin-clavulanic acid, ampicillin, azithromycin, cefoxitin, ceftriaxone, chloramphenicol, ciprofloxacin, colistin, gentamicin, meropenem, nalidixic acid, sulfamethoxazole, tetracycline, trimethoprim-sulfamethoxazole) and resistance was determined using CLSI breakpoints (https://www.cdc.gov/narms/antibiotics-tested.html).

Public health departments routinely submit epidemiologic information for all laboratory-confirmed cases of typhoid fever to CDC, including demographics, clinical outcome details, and travel history. We requested that public health officials perform a supplementary, second interview to collect additional epidemiologic and clinical information (including exposures, clinical course, and treatment information) from the two patients whose isolates carried the *mph*(A) gene.

This project was reviewed by CDC and deemed not to be research (IRB review was not required); the activity was conducted consistently with applicable federal law and CDC policy. Patients provided verbal consent for publication of their case information.

3. Results and discussion

Follow-up interviews revealed that patient one and patient two were related (daughter and mother, respectively). Patient one (with isolate PNUSAS349400) was a healthy woman in her 30 s who became ill in February 2023. She initially reported high fever and was hospitalized for her infection for six days. She failed to respond to multiple antibiotics (fever returned after a week), but subsequently recovered. Patient two (with isolate PNUSAS347532) was a woman in her 60 s with a history of type II diabetes mellitus who became ill in March 2023. Symptoms persisted for three weeks; she was initially evaluated in the emergency department and discharged home. After a second emergency department visit, she was hospitalized for four days and treated with multiple antibiotics over the course of her illness, including amoxicillin-clavulanic acid, azithromycin, ceftriaxone, ciprofloxacin, doxycycline, meropenem, minocycline, moxifloxacin; she ultimately recovered, and underwent laparoscopic cholecystectomy in April 2023.

Both patients spent a portion of their incubation period (defined as 6–30 days before illness onset) in New Delhi, India, where they attended the same wedding. Patient one stayed both at a hotel and with family; she reported consuming only Indian street food with no specific dietary restrictions, and consumed bottled water during her stay, but food may not have been prepared with bottled water. Patient two reported staying with friends and relatives and eating mostly meals prepared in the home; she reported consuming bottled water. Neither patient was vaccinated for typhoid fever before travel.

Isolates PNUSAS349400 (patient one) and PNUSAS347532 (patient two) belong to GenoTyphi lineage 4.3.1.1, a common genetic lineage in India [2,3]. They sit within a cluster of four genomes in a SNP-based phylogenetic tree and differ by a single SNP (Fig. 1) [1]. In addition to azithromycin resistance, they are multidrug resistant (MDR) displaying phenotypic and genotypic resistance to ampicillin, chloramphenicol, trimethoprim-sulfamethoxazole, and ceftriaxone, and decreased susceptibility to ciprofloxacin, due to

Table 1
Molecular and epidemiological characteristics of *mph*(A)-positive Typhi cases.

Patient	Strain ID	SAMN	GenoTyphi	AMR determinants	AST^	Plasmid type	Travel reported	Vaccinated
1	PNUSAS349400	SAMN35155331	4.3.1.1	*aac(6′)-Ib-cr, aadA5,* $bla_{CTX-M-15}$, bla_{OXA-1}, *catA1, dfrA17, dfrA7, gyrA*(S83Y), *mph*(A), *sul1*	ACSuCxCotAzm	PTU-FE (IncFIA/FIB/FII)	India	No
2	PNUSAS347532	SAMN35010716	4.3.1.1	*aac(3)-IIa, aac(6′)-Ib-cr, aadA5,* $bla_{CTX-M-15}$, bla_{OXA-1}, *catA1, dfrA17, dfrA7, gyrA*(S83Y), *mph*(A), *sul1*	ACSuCxGenCotAzm	PTU-FE (IncFIA/FIB/FII)	India	No

^ Antimicrobial susceptibility testing. Antimicrobial abbreviations are as follows: A, ampicillin; Azm, azithromycin; C, chloramphenicol; Cot, cotrimoxazole (trimethoprim-sulfamethoxazole); Cx, ceftriaxone; Gen, gentamicin; Su, sulfamethoxazole.

Fig. 1. SNP-based phylogenetic subtree of closely related genomes, exported directly from NCBI pathogen detection, (accessed 30.07.24) [1]. Scale bar indicates number of SNPs. PNUSAS349400 and PNUSAS347532 are highlighted in red.

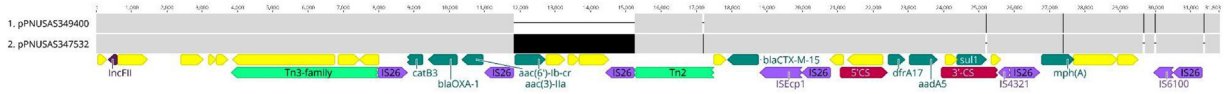

Fig. 2. Pairwise comparison of multiresistance region (~27 kb) of plasmids pPNUSAS349400 and pPNUSAS347532, generated in Geneious Prime v2021.2.2. Plasmids were annotated using Prokka v1.14.6 and ISfinder (https://isfinder.biotoul.fr/blast.php). Complete and partial annotations are denoted in the following colours: coding sequences in yellow, resistance genes in teal, insertion sequences in purple, transposons in light green, and conserved segments of a class 1 integron in fuscia.

a combination of chromosomal and plasmid-mediated determinants (Table 1). Both isolates have a single *gyrA* mutation (S83Y); and a variant of SGI*11* inserted in the chromosome (between *cyaY* and *cyaA*), containing only *catA1, dfrA7* and *sul1* (similar to SGI*11b*, [20]). Resistance genes *catB3* (partial), bla_{OXA-1}, *aac(6′)-Ib-cr,* $bla_{CTX-M-15}$, *dfrA17, aadA5, sul1, qacE* and *mph*(A) were present on a PTU-FE (IncFIA/FIB/FII) plasmid in both genomes, within a large (~27 kb) multiresistance region (Fig. 2). While the multiresistance regions are structurally similar, plasmid pPNUSAS347532 carries *aac(3)-IIa* between two copies of IS*26*, a region that is not present in plasmid pPNUSAS349400 (Fig. 1). Thus, these two strains are closely genetically related, but not identical.

PTU-FE plasmids are common in *Escherichia coli* [21], but have not been reported from India, even in recent efforts to characterize ceftriaxone resistant strains [22], nor have they been detected before in Typhi surveillance in the United States ([23]; https://www.cdc.gov/typhoid-fever/surveillance.html). Since Typhi is prone to carriage of PTUs with the ability to colonize a wide range of bacterial genera [23], novel acquisition of an *E. coli*-associated PTU by a previously circulating Typhi lineage is plausible and, in fact, drove the recent emergence of XDR Typhi [24].

The *mph*(A)-positive Typhi reported here are of a different genotype and carry a different plasmid than recent *mph*(A)-positive genomes from Pakistan [10], indicating independent emergence of plasmid-mediated azithromycin resistance. The epidemiological and molecular evidence is suggestive of local transmission in India, either from a common exposure or person-to-person transmission. While we have not yet detected widespread circulation of this strain in India, the slight genetic variance (both in the chromosome and the plasmid) is worth further contemplation. It is certainly possible that the small variation in these strains occurred in each patient after initial infection; or perhaps there exists a common reservoir from which variants of the original *mph*(A)-positive strain are already evolving.

Strains resistant to penicillin, chloramphenicol, trimethoprim-sulfamethoxazole, fluoroquinolones and third-generation cephalosporins leave few treatment options. The emergence of azithromycin resistance, the only remaining oral treatment option, highlights the need for additional intervention and control measures, including vaccination for travellers and residents of endemic Typhi areas [7]. Because many typhoid infections likely go undetected, and treatment options are increasingly limited, ongoing US-based surveillance is important to detect *mph*(A)-positive Typhi in returning travellers and prevent transmission.

4. Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention, the Mississippi State Department of Health, the California Department of Public Health, or the California Health and Human Services Agency. Names of specific vendors, manufacturers or products are included for public health and informational purposes; inclusion does not imply endorsement of the vendors, manufacturers or products by the Centers for Disease Control and Prevention or the US Department of Health and Human Services.

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Declarations

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