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Comparison of phenotypic and WGS-derived antimicrobial resistance profiles of *Salmonella enterica* serovars Typhi and Paratyphi

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Objectives: Surveillance of antimicrobial resistance (AMR) in *Salmonella enterica* serovars Typhi and Paratyphi is essential to provide an evidence base for empirical treatment protocols and to monitor emerging AMR. We sought to compare phenotypic and WGS-based genotypic methods for the detection of AMR in *Salmonella* Typhi and *Salmonella* Paratyphi.

Methods: WGS data from 603 isolates of *Salmonella* Typhi ($n = 332$) and *Salmonella* Paratyphi ($n = 271$) were mapped to genes or chromosomal mutations known to be associated with phenotypic AMR and compared with phenotypic susceptibility data interpreted using breakpoints recommended by EUCAST.

Results: There were two (0.03%) discordant interpretations out of a possible 6030 isolate/antimicrobial class combinations. MDR (resistant to three or more classes of antimicrobial) was detected in 83/332 (25.0%) *Salmonella* Typhi isolates, but was not detected in *Salmonella* Paratyphi. Thirty-six (10.8%) isolates of *Salmonella* Typhi were resistant to ciprofloxacin (MIC >0.5 mg/L), with 33 (9.9%) of 332 exhibiting mutations in *gyrA* and *parC*, and 244 (73.5%) isolates had reduced susceptibility to ciprofloxacin (MIC 0.06–0.25 mg/L). In comparison, 209/227 (92.1%) isolates of *Salmonella* Paratyphi A exhibited resistance to ciprofloxacin (MIC >0.5 mg/L). No resistance to azithromycin or the third-generation cephalosporins was detected.

Conclusions: WGS data provided a robust and informative approach for monitoring MDR and emerging resistance to ciprofloxacin in *Salmonella* Typhi and *Salmonella* Paratyphi. Phenotypic antimicrobial susceptibility testing continues to be performed to guide targeted individual patient treatment, but inferred AMR profiles from WGS data may be used for surveillance and to guide empirical therapy.

Introduction

Salmonella enterica serovars Typhi and Paratyphi A, B and C are highly adapted, human-specific pathogens causing typhoid or enteric fever. Infections are systemic, characterized by fever and gastrointestinal symptoms, and associated with significant morbidity. Death can occur, especially if appropriate antimicrobial therapy is delayed.[1] Globally, there are ∼27 million cases of typhoid fever worldwide and >200000 attributable deaths per year, predominantly among children <5 years old.[2]

Typhoid fever is a significant public health problem throughout the developing world, with particularly high incidence in South-East Asia.[1,3] In the developed world the incidence of typhoid fever has declined over the last century with the provision of clean water and good sanitation, and it is now predominantly a disease associated with travellers returning from high-risk regions.[4] For example, in the USA the annual incidence dropped from 7.5 per 100000 in 1940 to 0.2 per 100000 in the 1990s, whereas the incidence related to foreign travel increased from 33% in 1967–72 to 81% in 1996–97.[4] Between 2006 and 2011, PHE (formerly the HPA) reported ∼500 (range 457–520) cases of enteric fever per year. Since 2012, the number of cases has decreased, with 311 reports in 2014 and 286 in 2015 (https://www.gov.uk/government/publications/typhoid-and-paratyphoid-laboratory-confirmed-cases-in-england-wales-and-northern-ireland). In 2015, >90% of cases were acquired abroad, the majority from travellers returning to the UK from the Indian subcontinent. Travel to sub-Saharan Africa and South America was also documented (https://www.gov.uk/government/uploads/system/uploads/attachment_data/file/618840/Enteric_fever_annual_report_2015.pdf).

The advent of chloramphenicol treatment changed the perception of typhoid fever from a severe, often fatal, disease to a readily

manageable infection.[4] However, outbreaks of chloramphenicol-resistant *Salmonella* Typhi were reported as early as 1972 and by the 1990s outbreaks of typhoid caused by strains resistant to chloramphenicol, sulphonamides, trimethoprim and ampicillin were widespread.[5] Fluoroquinolones became the first-line antimicrobial therapy following their introduction in the 1980s and were initially associated with rapid fever clearance and low rates of both relapse and chronic faecal carriage.[4] However, reduced ciprofloxacin susceptibility (MIC 0.06–0.25 mg/L) has become increasingly prevalent in *Salmonella* Typhi and *Salmonella* Paratyphi and has been associated with clinical failure.[6,7] Reduced ciprofloxacin susceptibility is not detected by standard ciprofloxacin disc diffusion testing. Nalidixic acid resistance has been used as a surrogate marker, but does not detect all cases.[8] In the UK, >90% of *Salmonella* Typhi and *Salmonella* Paratyphi isolates acquired in India between 2006 and 2007 were nalidixic acid resistant.[9] Alternative antimicrobials, including third-generation cephalosporins or azithromycin, are increasingly used as first-line therapies.[3] Reports of emergence of resistance to third-generation cephalosporins and azithromycin have raised concern amongst clinicians.[10,11]

In April 2014, PHE implemented WGS as the routine method of surveillance for *Salmonella* species.[12] Known genotypes associated with mechanisms of antimicrobial resistance (AMR) are detected and phenotypic AMR profiles can be inferred.[13] The aim of this study was to compare and evaluate phenotypic and genotypic methods for detection of AMR in *Salmonella* Typhi and *Salmonella* Paratyphi A, B and C.

Methods

Bacterial isolates

Phenotypic and genotypic testing was performed on 603 isolates of *Salmonella* Typhi ($n = 332$), *Salmonella* Paratyphi A ($n = 227$) and *Salmonella* Paratyphi B ($n = 44$) submitted to PHE between April 2014 and August 2016. No isolates of *Salmonella* Paratyphi C were submitted to PHE during this time frame. The isolates were from 527 patients reporting to general practitioners or local hospitals in the UK with diarrhoea, abdominal pain and/or symptoms consistent with typhoid fever (*Salmonella* Typhi patients, $n = 292$; *Salmonella* Paratyphi A patients, $n = 197$; *Salmonella* Paratyphi B patients, $n = 38$).

WGS

Following extraction at Containment Level 3, genomic DNA was fragmented and tagged for multiplexing with Nextera XT DNA Sample Preparation Kits, followed by paired-end sequencing on an Illumina HiSeq 2500 platform to produce 100 bp paired-end reads (Illumina, Cambridge, UK). AMR determinants were sought using Genefinder, a customized algorithm that uses Bowtie 2 to map reads to a set of reference sequences and Samtools to generate an mpileup file.[14] The data are parsed based on read coverage of the query sequence (100%), consensus base-call variation (>85%) and nucleotide identity (>90%) to determine the presence of the reference sequence or nucleotide variation within that sequence. β-Lactamase variants were determined with 100% identity using the reference sequences downloaded from the Lahey (www.lahey.org) or NCBI (https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources) β-lactamase data resources. Known acquired resistance genes and resistance-conferring mutations relevant to β-lactams (including carbapenems), fluoroquinolones, aminoglycosides, chloramphenicol, macrolides, sulphonamides, tetracyclines, trimethoprim, rifamycins and fosfomycin and acquired genes associated with colistin resistance were included in the analysis.[15,16]

Reference sequences for acquired resistance genes were curated from those described in the Comprehensive Antimicrobial Resistance Database (http://arpcard.mcmaster.ca) and the ResFinder datasets (https://cge.cbs.dtu.dk/services/data.php). Chromosomal mutations focused on previously published variations in the QRDRs of *gyrA*, *gyrB*, *parC* and *parE*, which are associated with resistance to quinolones, and in *rpoB* for rifampicin resistance. FASTQ sequences were deposited in the National Center for Biotechnology Information Short Read Archive (SRA) under the BioProject PRJNA315192 and the SRA numbers are available in Table S1 (available as Supplementary data at *JAC* Online).

ST, eBurst Group (eBG) and serotype were determined from the genome data using MOST.[17,18]

Antimicrobial susceptibility testing

Susceptibility testing was performed retrospectively on all isolates recovered from the PHE archive. MICs were determined in Containment Level 3 by agar dilution using Mueller–Hinton agar for the following antimicrobials: ampicillin, nalidixic acid, ciprofloxacin, streptomycin, gentamycin, trimethoprim, tetracycline, sulphonamides, chloramphenicol, rifampicin, fosfomycin and azithromycin. Breakpoints and screening concentration criteria used for interpretation were as recommended by EUCAST (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf). Confirmation of MICs of azithromycin, trimethoprim, chloramphenicol and streptomycin was undertaken by Etest® (bioMérieux, France). Temocillin and cefoxitin were included in the panel to aid detection of OXA-48-like carbapenemases and AmpC production, respectively.

Results

Of the 527 patients in this study, 242 (45.9%) were female, 283 (53.7%) were male and gender information was not provided for two cases. A travel history was provided for 486 (92.2%) of the 527 cases, with 442 (83.9%) reporting travel within 14 days of onset of symptoms and 44 (8.3%) reporting no travel in that time frame. No travel history was available for 41 (7.8%) cases. The most frequently reported travel destinations from patients with *Salmonella* Typhi and *Salmonella* Paratyphi A infection were India (*Salmonella* Typhi, $n = 108$; *Salmonella* Paratyphi A, $n = 81$), Pakistan (*Salmonella* Typhi, $n = 72$; *Salmonella* Paratyphi A, $n = 52$) and Bangladesh (*Salmonella* Typhi, $n = 20$; *Salmonella* Paratyphi A, $n = 16$). Peru ($n = 9$) and Iraq ($n = 7$) were the most frequently reported travel destinations from patients with Paratyphi B.

Of the 603 isolates from these cases, 358 (59.4%) were from blood cultures (*Salmonella* Typhi, $n = 197$; *Salmonella* Paratyphi A, $n = 141$; *Salmonella* Paratyphi B, $n = 20$), 181 (30.0%) were from faeces (*Salmonella* Typhi, $n = 93$; *Salmonella* Paratyphi A, $n = 67$; *Salmonella* Paratyphi B, $n = 21$) and the infection site was not stated for the remaining 64 (10.6%) (*Salmonella* Typhi, $n = 42$; *Salmonella* Paratyphi A, $n = 19$; *Salmonella* Paratyphi B, $n = 3$). The isolates of *Salmonella* Typhi belonged to eBG 13 (ST1, $n = 243$; ST2, $n = 89$), *Salmonella* Paratyphi A were eBG 11 (ST85, $n = 128$; ST129, $n = 97$; ST1938, $n = 1$; ST1929, $n = 1$) and *Salmonella* Paratyphi B were eBG 5 (ST86, $n = 44$).

Comparison between phenotypic and genotypic AMR

The phenotypic and genotypic AMR results showed high concordance, with 601 (99.7%) of 603 isolates having identical categorical

Table 1. Evaluation of genotypic analysis for the prediction of resistance phenotypes for *Salmonella* Typhi ($n = 332$)

	Phenotype: susceptible		Phenotype: resistant	
Antibiotic	genotype: resistant	genotype: susceptible	genotype: resistant	genotype: susceptible
AMP	0	255	77	0
CIP	0	296	36	0
STR	0	255	76	1
TMP	0	250	82	0
TET	0	326	6	0
SUL	1	248	83	0
CHL	0	253	79	0
RIF	0	332	0	0
FOF	0	332	0	0
CST	0	332	0	0

AMP, ampicillin; CHL, chloramphenicol; CIP, ciprofloxacin; CST, colistin, FOF, fosfomycin; RIF, rifampicin; STR, streptomycin; SUL, sulphonamide; TMP, trimethoprim; TET, tetracycline.

agreement across their complete susceptibility profile, which included 10 classes of antimicrobials. The two isolates exhibiting a discrepancy each had a mismatch for only one antimicrobial; therefore there were only 2 (0.03%) discordant results out of a possible 6030 isolate/antimicrobial class combinations. One isolate was phenotypically resistant to streptomycin (MIC >32 mg/L), but no corresponding resistance determinant was detected in the genome (Table 1). One isolate was predicted to be resistant to sulphonamides from the genome-derived data (*sul1* detected), but was phenotypically susceptible (MIC <16 mg/L) (Table 1).

Resistance to β-lactams, aminoglycosides, sulphonamides, trimethoprim, tetracyclines and phenicols in Salmonella Typhi

Of the 332 isolates of *Salmonella* Typhi, 77 (23.2%) had $bla_{\text{TEM-1}}$, predicted to confer resistance to ampicillin (Table 2). No other β-lactamase genes were detected in any of the isolates.

Seventy-six (22.9%) isolates had genes predicted to confer streptomycin resistance, all of which had *strA-strB*. No phenotypic or genotypic resistance to gentamicin and tobramycin was detected and there were no 16S rRNA methyltransferase genes identified.

Eighty-two (24.7%) isolates had *dfrA* alleles (*dfrA1*, $n = 1$; *dfrA7*, $n = 74$; *dfrA14*, $n = 1$; *dfrA15*, $n = 6$), conferring resistance to trimethoprim (Table 2).

Eighty-three (25.0%) isolates had genes predicted to confer sulphonamide resistance, of which 7 (8.4%) had *sul1*, 2 (2.4%) had *sul2* and 74 (89.2%) had both *sul1* and *sul2* (Table 2).

Six (1.8%) isolates had *tetA*(A) (Table 2) and no other tetracycline resistance determinants were detected.

Seventy-nine (23.8%) isolates had *catA1*, predicted to confer chloramphenicol resistance (Table 2).

Resistance to quinolones in Salmonella Typhi

Of the 332 isolates of *Salmonella* Typhi in this study, 36 (10.8%) were resistant to ciprofloxacin (MIC >0.5 mg/L) and exhibited triple mutations at *gyrA*[83:S-F], *gyrA*[87:D-N] and *parC*[80:S-I] ($n = 33$) or double mutations at *gyrA*[83:S-F] and *parE*[460:E-K] ($n = 3$). Two hundred and forty-four (73.5%) isolates were resistant to nalidixic acid and exhibited reduced susceptibility to ciprofloxacin (MIC 0.06–0.25 mg/L). Of these, 229 were associated with single mutations (*gyrA*[83:S-F], $n = 162$; *gyrA*[83:S-Y], $n = 42$; *gyrA*[87:D-N], $n = 9$; *gyrA*[87:D-G], $n = 5$; *gyrA*[87:D-Y], $n = 4$; *gyrB*[464:S-F], $n = 7$) and 15 exhibited double mutations (*gyrA*[83:S-Y]/*parC*[79:D-G], $n = 5$; *gyrA*[83:S-F]/*parC*[84:E-K], $n = 3$; *gyrA*[83:S-Y]/*parC*[78:G-D], $n = 2$; *gyrA*[83:S-F]/*parC*[84:E-G], $n = 2$; *gyrA*[83:S-F]/*parE*[458:S-A], $n = 2$; *gyrA*[83:S-F]/*parE*[502:L-F], $n = 1$) (Tables 2 and 3).

A plasmid-mediated quinolone resistance (PMQR) determinant, *qnrB19*, was detected in just one isolate, in combination with a *gyrA*[83:S-F] mutation.

Resistance to macrolides, rifamycins, fosfomycin and colistin in Salmonella Typhi

None of the isolates was predicted to be resistant to the macrolides, rifamycins or fosfomycin and no acquired genes associated with colistin resistance were detected.

Resistance profiles in Salmonella Paratyphi A and Salmonella Paratyphi B

Of the 227 isolates of *Salmonella* Paratyphi A in this study, 209 (92.1%) were resistant to ciprofloxacin (MIC >0.5 mg/L) and had mutations in both *gyrA* and *parC*. The *parC*[57:T-S] mutation was found in all isolates of *Salmonella* Paratyphi A in this data set. One hundred and sixty-five (72.7%) had double mutations at *gyrA*[83:S-F] and *parC*[57:T-S], 43 (18.9%) had double mutations at *gyrA*[83:S-Y] and *parC*[57:T-S] and 1 had three mutations at *gyrA*[83:S-F], *gyrA*[83:D-G] and *parC*[57:T-S]. One isolate had a *qnrB19* allele as well as mutations in *gyrA*[83:S-F] and *parC*[57:T-S]. Eighteen (7.9%) isolates had a mutation at *parC*[57:T-S], but were susceptible to ciprofloxacin (MIC <0.06 mg/L).

No genotypic or phenotypic resistance was detected in *Salmonella* Paratyphi A to β-lactams, aminoglycosides, macrolides, sulphonamides, chloramphenicol or trimethoprim.

Table 2. Resistance genes identified in *Salmonella* Typhi and *Salmonella* Paratyphi, the predicted resistance phenotype and their prevalence

Resistance gene	Antibiotic class	Number (%), Typhi $n = 332$	Number (%), Paratyphi A $n = 227$	Number (%), Paratyphi B $n = 44$	Accession number
strA-strB	aminoglycosides	76 (22.9)	0	0	CP011429
bla_{TEM-1}	β-lactams	77 (23.2)	0	0	AF188200
sul1	folate synthesis inhibitors	81 (24.4)	0	0	NG_048086
sul2	folate synthesis inhibitors	76 (22.9)	0	0	NG_048110
dfrA1	folate synthesis inhibitors	1 (0.3)	0	0	AY963803
dfrA7	folate synthesis inhibitors	74 (22.3)	0	0	JF806498
dfrA14	folate synthesis inhibitors	1 (0.3)	0	0	U10186
dfrA15	folate synthesis inhibitors	6 (1.8)	0	0	Z50805
catA1	phenicols	79 (23.8)	0	0	V00622
qnrB19	quinolones	1 (0.3)	1 (0.4)	1 (2.3)	HM146784
gyrA[83:S-F]	quinolones	162 (48.8)	0	3 (6.8)	CP009102
gyrA[83:S-Y]	quinolones	42 (12.7)	0	0	
gyrA[87:D-G]	quinolones	5 (1.5)	0	2 (4.5)	
gyrA[87:D-N]	quinolones	9 (2.7)	0	1 (2.3)	
gyrA[87:D-Y]	quinolones	4 (1.2)	0	0	
parC[57:T-S]	quinolones	0	18 (7.9)	0	
gyrA[83:S-F]; *parC*[57:T-S]	quinolones	0	165 (72.7)	0	
gyrA[83:S-Y]; *parC*[57:T-S]	quinolones	0	43 (18.9)	0	
gyrA[83:S-F]; *parC*[78:G-D]	quinolones	2 (0.6)	0	0	
gyrA[83:S-F]; *parC*[84:E-G]	quinolones	2 (0.6)	0	0	
gyrA[83:S-F]; *parC*[84:E-K]	quinolones	3 (0.9)	0	0	
gyrA[83:S-Y]; *parC*[79:D-G]	quinolones	5 (1.5)	0	0	
gyrA[83:S-F]; *gyrA*[87:D-G]; *parC*[57:T-S]	quinolones	0	1 (0.4)	0	
gyrA[83:S-F]; *gyrA*[87:D-N]; *parC*[80:S-I]	quinolones	33 (9.9)	0	0	
gyrB[464:S-F]	quinolones	7 (2.1)	0	0	
gyrA[83:S-Y]; *parE*[460:E-K]	quinolones	3 (0.9)	0	0	
gyrA[83:S-F]; *parE*[458:S-A]	quinolones	2 (0.6)	0	0	
gyrA[83:S-F]; *parE*[502:L-F]	quinolones	1 (0.3)	0	0	
tetA(A)	tetracyclines	6 (1.8)	0	0	HQ840942

Of the 44 isolates of *Salmonella* Paratyphi B, six had a single mutation in *gyrA* ([83:S-F], $n = 3$; [87:D-N], $n = 1$; and [87:D-G], $n = 2$) and had reduced susceptibility to ciprofloxacin (MIC 0.06–0.25 mg/L) and resistance to nalidixic acid (MIC >32 mg/L). One isolate had the *qnrB19* PMQR determinant, but was fully susceptible to ciprofloxacin and nalidixic acid (Tables 2 and 3). No other phenotypic or genotypic resistance was detected.

MDR profiles

Based on the WGS prediction, 292/332 (88.0%) isolates of *Salmonella* Typhi were resistant (or had reduced susceptibility) to at least one antimicrobial on the panel tested, compared with 209/227 (92.1%) *Salmonella* Paratyphi A and 6/44 (13.6%) *Salmonella* Paratyphi B.

Eighty-three (13.8%) of the 603 isolates were MDR (i.e. resistant to three or more classes of antimicrobials) (Table 4).[19] All 83 isolates were *Salmonella* Typhi, representing 25.0% of the 332 isolates of *Salmonella* Typhi tested. The most common genotypic resistance profiles for *Salmonella* Typhi were: (i) *gyrA*[83:S-F] ($n = 106$); (ii) *gyrA*[83:S-F]/bla_{TEM-1}/*strA-strB*/*dfrA7*/*sul1*:*sul2*/*catA1* ($n = 52$); and (iii) *gyrA*[83:S-F]/*gyrA*[87:D-N]/*parC*[80:S-I] ($n = 32$). For *Salmonella* Paratyphi A, the most common resistance profiles were: (i) *gyrA*[83:S-F]/*parC*[57:T-S] ($n = 165$) and (ii) *gyrA*[83:S-Y]/*parC*[57:T-S] ($n = 43$).

Discussion

A comparison of the phenotypic and genotypic resistance profiles for a large collection of typhoidal salmonellae identified only 2 (0.03%) discordant results out of a possible 6030 isolate/antimicrobial combinations. One isolate had *sul1*, but was phenotypically susceptible to the sulphonamides (MIC <16 mg/L), indicating that the AMR determinant was present but expression was reduced. A second isolate was phenotypically resistant to streptomycin (MIC >32 mg/L), but no streptomycin resistance determinants were detected in the genome.

Previous studies have shown that deriving AMR profiles from the genome data is a robust and accurate approach.[13,15,16,20–22] However, there is a risk of missing novel resistance mechanisms not included in the reference database, which would result in predicting susceptibility for a phenotypically resistant isolate. This is

Table 3. Association between phenotypic resistance to nalidixic acid and low- and high-level ciprofloxacin, PMQR and mutations in *gyrA*, *gyrB*, *parC* and *parE*

	Phenotypic profile			
No. of isolates	NAL	CIP MIC 0.06–0.25 mg/L	CIP MIC >0.5 mg/L	Genetic resistance determinant
Typhi				
33	R	R	R	*gyrA*[83:S-F;87:D-N]; *parC*[80:S-I]
3	R	R	R	*gyrA*[83:S-Y]; *parE*[460:E-K]
161	R	R	S	*gyrA*[83:S-F]
2	R	R	S	*gyrA*[83:S-Y]; *parE*[460:S-A]
42	R	R	S	*gyrA*[83:S-Y]
1	R	R	S	*gyrA*[83:S-Y]; *parE*[502:S-F]
9	R	R	S	*gyrA*[87:D-N]
4	R	R	S	*gyrA*[87:D-Y]
5	R	R	S	*gyrA*[87:D-G]
5	R	R	S	*gyrA*[83:S-Y]; *parC*[79:D-G]
3	R	R	S	*gyrA*[83:S-F]; *parC*[84:E-K]
2	R	R	S	*gyrA*[83:S-F]; *parC*[84:E-G]
2	R	R	S	*gyrA*[83:S-F]; *parC*[78:G-D]
1	R	R	S	*gyrA*[83:S-F]; *qnrB19*
7	R	R	S	*gyrB*[464:S-F]
Paratyphi A				
165	R	R	R	*gyrA*[83:S-F]; *parC*[57:T-S]
43	R	R	R	*gyrA*[83:S-Y]; *parC*[57:T-S]
1	R	R	R	*gyrA*[83:S-F;87:D-G]; *parC*[57:T-S]
18	S	S	S	*parC*[57:T-S]
1	S	S	S	*qnrB19*
Paratyphi B				
1	R	R	S	*gyrA*[87:D-N]
3	R	R	S	*gyrA*[83:S-F]
2	R	R	S	*gyrA*[87:D-G]
1	S	S	S	*qnrB19*

CIP, ciprofloxacin; NAL, nalidixic acid; R, resistant; S, susceptible.

classed as a 'very major error' as it could potentially mean that a patient would be given ineffective treatment. Regular updating of the database to include novel AMR genes and mutations is essential to ensure the ongoing accuracy of AMR profiles derived from WGS data.[23]

Resistance to ampicillin, streptomycin, sulphonamides, trimethoprim and chloramphenicol was detected in 23.2%, 22.9%, 25.0%, 24.7% and 23.8% of isolates of *Salmonella* Typhi, respectively, with 22.9% of isolates exhibiting resistance to all five antimicrobials. Using WGS, Wong *et al.*[24] (2015) identified a single dominant MDR lineage of *Salmonella* Typhi that had emerged and spread throughout Asia and Africa over the last 30 years, displacing antibiotic-susceptible isolates. In this lineage, MDR was mediated either via transmissible IncHI1 plasmids or within multiple chromosomal integration sites corresponding with the loss of the *tetA*(A) determinant encoding resistance to tetracycline.[24] In our study, 6 isolates had *tetA*(A) and a varied combination of bla_{TEM-1}, *strA-strB*, *sul1*, *sul2*, *dfrA15* and *catA1*, whereas 77 isolates exhibited a varied combination of bla_{TEM-1}, *strA-strB*, *sul1*, *sul2*, *dfrA* and *catA1* without *tetA*(A).

The increasing incidence of MDR *Salmonella* Typhi globally during the 1990s prompted an increase in the use of fluoroquinolones for the treatment of enteric fever in the UK and elsewhere.[3,4,25] It has been suggested that this change has caused the progressive re-emergence of susceptibility to traditional first-line drugs.[4,26,27] Nevertheless, in the UK, the incidence of resistance to ampicillin, streptomycin, sulphonamides, trimethoprim and chloramphenicol has remained stable over the last decade, at around 22%–23%.[25,28]

The introduction of fluoroquinolones in the 1990s led to an increase in the numbers of isolates that were resistant to nalidixic acid and exhibited reduced susceptibility to ciprofloxacin.[6,29,30] Such isolates were susceptible to ciprofloxacin by disc testing, but were associated with clinical failure.[7,31] Single point mutations, identified in *gyrA* of *Salmonella* Typhi and *Salmonella* Paratyphi A, were associated with reduced susceptibility to ciprofloxacin.[32,33] In this study, comparisons with historical data in England and Wales were confounded by the lack of standardized testing prior to 2014. A local hospital study in England spanning the years 1990–2009 demonstrated a dramatic increase in reduced susceptibility and resistance to ciprofloxacin in both *Salmonella* Typhi and *Salmonella*

Table 4. Combinations of AMR phenotypes and genotypes identified in 602 isolates of typhoidal *Salmonella* isolated in England and Wales, April 2014–August 2016

No. of AMR phenotypes	No. of strains, $n = 603$	Phenotypic combinations (no. of strains in parentheses)	Genotypic combinations (no. of strains in parentheses)
Typhi, $n = 332$			
0	40	susceptible	–
1	209	<CIP (174)	*gyrA*[83:S-F] (106)
			gyrA[83:S-F];*parE*[458:S-A] (2)
			gyrA[83:S-F];*parE*[502:L-F] (1)
			gyrA[83:S-Y] (37)
			gyrA[87:D-G] (1)
			gyrA[87:D-N] (9)
			gyrA[87:D-Y] (2)
			gyrA[83:S-Y];*parC*[79:D-G] (5)
			gyrA[83:S-F];*parC*[78:G-D] (2)
			gyrA[83:S-F];*parC*[84:E-G] (2)
			gyrB[464:S-F] (7)
		>CIP (35)	*gyrA*[83:S-F;87:D-N];*parC*[80:S-I] (32)
			gyrA[83:S-F];*parE*[460:E-K] (3)
3	4	<CIP/SUL/TET (1)	*gyrA*[83:S-Y]/*sul2*/*tetA*(A) (1)
		TMP/SUL/CHL (2)	*dfrA15*/*sul1*/*catA1* (2)
		STR/TMP/SUL (1)	*strA-strB*/*dfrA1*/*sul1*:*sul2* (1)
4	3	AMP/STR/TMP/SUL (1)	bla_{TEM-1}/*strA-strB*/*dfrA14*/*sul2* (1)
		STR/TMP/SUL/TET (1)	*strA-strB*/*dfrA15*/*sul1*/*tetA*(A) (1)
		>CIP/TMP/SUL/TET (1)	*gyrA*[83:S-F;87:D-N];*parC*[80:S-I]/*dfrA15*/*sul1*/*tetA*(A) (1)
5	7	AMP/STR/TMP/SUL/CHL (7)	bla_{TEM-1}/*strA-strB*/*dfrA7*/*sul1*:*sul2*/*catA1* (7)
6	69	AMP/<CIP/STR/TMP/SUL/CHL	*gyrA*[83:S-F]/bla_{TEM-1}/*strA-strB*/*dfrA7*/*sul1*:*sul2*/*catA1* (52)
			gyrA[83:D-G]/bla_{TEM-1}/*strA-strB*/*dfrA7*/*sul1*:*sul2*/*catA1* (4)
			gyrA[87:D-Y]/bla_{TEM-1}/*strA-strB*/*dfrA7*/*sul1*:*sul2*/*catA1* (2)
			gyrA[87:S-Y]/bla_{TEM-1}/*dfrA15*/*sul1*/*tetA*(A)/*catA1* (3)
			gyrA[87:S-Y]/bla_{TEM-1}/*strA-strB*/*dfrA7*/*sul1*:*sul2*/*catA1* (4)
			gyrA[83:S-F];*parC*[84:E-K]/bla_{TEM-1}/*strA-strB*/*dfrA7*/*sul1*:*sul2*/*catA1* (3)
			gyrA[83:S-F]/*qnrB19*/bla_{TEM-1}/*strA-strB*/*dfrA7*/*sul1*:*sul2*/*catA1* (1)
Paratyphi A, $n = 227$			
0	18	susceptible	*parC*[57:T-S] (18)
1	209	>CIP (43)	*gyrA*[83:S-Y]; *parC*[57:T-S] (43)
		>CIP (165)	*gyrA*[83:S-F]; *parC*[57:T-S] (165)
		>CIP (1)	*gyrA*[83:S-F]; *gyrA*[87:D-G]; *parC*[57:T-S] (1)
Paratyphi B, $n = 44$			
0	38	susceptible	
1	6	<CIP (3)	*gyrA*[83:S-F] (3)
		<CIP (3)	*gyrA*[87:D-G] (2)
			gyrA[87:D-N] (1)

AMP, ampicillin; CHL, chloramphenicol; <CIP, reduced susceptibility to ciprofloxacin (MIC 0.125–0.25 mg/L); >CIP, ciprofloxacin MIC >0.5 mg/L; STR, streptomycin; SUL, sulphonamide; TET, tetracycline; TMP, trimethoprim.

Paratyphi A during this period.[28] This was supported by data produced at the national reference laboratory.[9,25]

In the dataset described here, the *parC*[57:T-S] mutation was identified in all isolates of *Salmonella* Paratyphi A and there is evidence that, when present with no additional mutations in the DNA gyrase or topoisomerase genes, this mutation does not confer reduced susceptibility.[34] However, a combination of a single mutation in *gyrA* and *parC*[57:T-S] in *Salmonella* Paratyphi A appears to be associated with full resistance to ciprofloxacin (MIC >0.5 mg/L).

All *Salmonella* Typhi isolates with the triple mutation combination *gyrA*[83:S-F], *gyrA*[87:D-N] and *parC*[80:S-I] had an MIC of ciprofloxacin >0.5 mg/L and this combination of mutations has been described previously.[35] With the exception of three isolates with an MIC >0.5 mg/L harbouring mutations in *gyrA*[83:S-F] *parE*[460:E-K], single mutations in *gyrA* or *gyrB* alone or in combination with a single *parC* or *parE* mutation resulted in an MIC between 0.06 and 0.25 mg/L (Table 3). These findings are consistent with previous studies showing increasing MIC associated with a

higher number of mutations.[36,37] A single mutation in *gyrB* in *Salmonella* Typhi associated with reduced susceptibility to ciprofloxacin has been described previously.[38] There is clinical evidence that treatment with ciprofloxacin may be associated with a poor response in systemic infections caused by *Salmonella* spp. with reduced susceptibility to ciprofloxacin (MIC >0.06 mg/L). The available data relate mainly to *Salmonella* Typhi, but there are case reports of poor response with other *Salmonella* species (http://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_7.1_Breakpoint_Tables.pdf).

PMQR genes in *Salmonella* Typhi and *Salmonella* Paratyphi A and B did not confer reduced susceptibility to ciprofloxacin (MIC >0.06 mg/L) unless they co-existed with *gyrA* QRDR mutations. To avoid missing PMQR determinants, EUCAST recommends the use of perfloxacin discs, which reliably detect all forms of quinolone resistance except resistance caused by *aac(6′)-Ib-cr*.[39] In our study, WGS data showed that PMQR determinants were rarely detected in either *Salmonella* Typhi or *Salmonella* Paratyphi.

Although published evidence does not yet support the use of WGS-inferred antimicrobial susceptibility to guide clinical decision making for infections caused by many bacterial species,[40] this study and others have shown that analysis of WGS data is a robust and informative approach to monitoring MDR and emerging resistance to ciprofloxacin in *Salmonella* Typhi.[41,42] The current trend of AMR seen in imported cases of enteric fever from across the world returning to the UK supports the empirical use of third-generation cephalosporins and/or azithromycin until results of phenotypic susceptibility testing are available. Phenotypic antimicrobial susceptibility testing continues to be performed to guide patient treatment, but inferring AMR profiles from WGS data is appropriate for surveillance to guide empirical therapy.

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Transparency declarations

None to declare.

Disclaimer

The views expressed are those of the authors and not necessarily those of the NHS, the National Institute for Health Research, the Department of Health or PHE.

Supplementary data

Table S1 is available as Supplementary data at *JAC* Online.

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