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Comparison of phenotypic and WGS-derived antimicrobial resistance profiles of *Shigella sonnei* isolated from cases of diarrhoeal disease in England and Wales, 2015

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Objectives: Phenotypic and genotypic methods for the detection of antimicrobial resistance (AMR) in *Shigella sonnei* in England and Wales were compared and evaluated.

Methods: WGS data from 341 isolates of *S. sonnei* isolated between June 2015 and January 2016 were mapped to genes known to be associated with phenotypic AMR. Antimicrobial susceptibility testing was performed on all viable isolates ($n = 335$).

Results: Fifteen of 335 isolates had a discrepancy between phenotypic and genotypic testing for 1 of the 10 antimicrobial classes tested, equating to 15 (0.45%) discordant results out of a possible 3350 isolate/antimicrobial combinations. All 15 mismatched results were genotypically resistant but phenotypically susceptible. Eleven of the 15 discrepancies were observed in streptomycin resistance profiles. The most common resistance profile was trimethoprim, sulphonamides, tetracyclines and streptomycin, occurring in 97 (28.4%) isolates. Resistances to ciprofloxacin and the third-generation cephalosporins, not detected in England and Wales prior to 2002, were identified in 18.2% and 12% of isolates, respectively. Three hundred and four (89.1%) isolates were MDR. There was no significant association between any of the AMR determinants tested and recent foreign travel in male or female cases. The number of isolates of *S. sonnei* harbouring bla_{TEM-1} and *ermB/mphA* was significantly higher in men who reported no recent travel outside the UK.

Conclusions: The use of WGS for routine public health surveillance is a reliable method for rapid detection of emerging AMR in isolates of *S. sonnei.*

Introduction

Infectious diarrhoeal disease is usually a self-limiting illness and the standard recommended treatment is oral rehydration therapy.[1] Antimicrobial chemotherapy may be recommended when the patient is at the extremes of age (infant or elderly), has underlying co-morbidities, or if symptoms are severe and/or prolonged.[2] Surveillance of antimicrobial resistance (AMR) in pathogenic gastrointestinal (GI) bacteria is therefore necessary to provide an evidence base for empirical treatment protocols.[3] Additionally, AMR testing of GI bacterial pathogens associated with travellers' diarrhoea, including *Shigella* spp., provides evidence of the global transmission of emerging AMR mechanisms.[4,5]

In England ~1200 cases of *Shigella sonnei* infection are reported each year via the local hospital laboratory surveillance systems. Of these, on average 800 isolates are submitted each year to the Gastrointestinal Bacteria Reference Unit (GBRU) at PHE for confirmation and typing. Historically, phenotypic resistance typing was performed on a subset of isolates for surveillance purposes.[6,7] AMR profiles were also used as epidemiological markers during outbreak investigations.[8]

In June 2015, GBRU implemented WGS as the routine method of surveillance for *S. sonnei*.[9] Known genotypes associated with mechanisms of AMR are detected and the phenotypic AMR profile can be inferred. In a recent report, EUCAST concluded that although available published evidence does not currently support use of WGS-inferred antimicrobial susceptibility to guide clinical decision making, this approach could replace phenotypic testing for surveillance purposes in the near future.[10] Short-read sequencing data derived from GI bacterial pathogens generated at PHE are publicly available and provide a useful resource for monitoring trends in AMR. The aim of this study was to compare and evaluate

phenotypic and genotypic methods for detection of AMR in *S. sonnei*.

Methods

Bacterial isolates

All isolates of *S. sonnei* ($n = 341$) submitted to GBRU for confirmation and typing between June 2015 and January 2016 were included in this study (Table S1, available as Supplementary data at *JAC* Online). The isolates were from 341 different patients reporting to general practitioners or local hospitals with symptoms of GI disease.[9]

WGS

Genomic DNA was extracted, fragmented and tagged for multiplexing with Nextera XT DNA Sample Preparation Kits, followed by paired-end sequencing on an Illumina HiSeq platform to produce 80–100 bp short-read sequence fragments (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.[11] The data are parsed based on read coverage (100%), basecall agreement (>85%) and nucleotide identity (>90%) to determine the presence of the reference sequence or nucleotide variation within that sequence. The β-lactamase variants were determined with 100% identity using the reference sequences downloaded from the lahey (www.lahey.org) and NCBI β-lactamase data (https://www.ncbi.nlm.nih.gov/pathogens/beta-lactamase-data-resources) 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 were included in the analysis. Reference sequences for acquired resistance genes were curated from those described in the Comprehensive Antimicrobial Resistance Database (http://arpcard.mcmaster.ca) and Resfinder (https://cge.cbs.dtu.dk/services/data.php) datasets. Chromosomal mutations focused on variations in the QRDRs of *gyrA*, *gyrB*, *parC*, *parE* and *rpoB* that are associated with resistance to quinolones and rifampicin.

FASTQ sequences were deposited in the National Center for Biotechnology Information Short Read Archive under the BioProject PRJNA315192.

Antimicrobial susceptibility testing

Susceptibility testing was performed retrospectively on all isolates recovered from the GBRU archive. MICs for all viable isolates (335/341) were determined by agar dilution using Iso-Sensitest agar or Mueller–Hinton agar. The antimicrobials tested were ampicillin, chloramphenicol, colistin, sulphonamide, gentamicin, tobramycin, amikacin, streptomycin, tetracycline, trimethoprim, nalidixic acid, ciprofloxacin, ceftazidime, cefotaxime, cefoxitin, cefpirome, ertapenem and temocillin. Breakpoints used for interpretation were as recommended by EUCAST and the EU Reference Laboratory Antimicrobial Resistance recommended screening guidance (http://www.crl-ar.eu/201-resources.htm#cutoff). Confirmation of MICs of azithromycin, trimethoprim, chloramphenicol and streptomycin was performed by Etest® (bioMérieux, France). Temocillin and cefoxitin were included in the panel to aid detection of OXA-48-like carbapenemases and AmpC production, respectively.

Statistical analysis

Comparisons were made between the AMR profiles associated with cases who reported travel abroad in the 7 days prior to onset of symptoms and those associated with cases who did not report recent travel using the χ^2 test. Results with P values ≤ 0.05 were considered statistically significant. All statistical analyses were performed using STATA 12.0 (Stata Corporation).

Results

Comparison between phenotypic and genotypic AMR

The phenotypic and genotypic AMR results correlated well, with 320 (95.5%) of 335 isolates having concordant results across their complete susceptibility profile, which included 10 classes of antimicrobials. Each of the 15 isolates exhibiting a discrepancy had a mismatch for only one antimicrobial; therefore there were 15 (0.45%) discordant results out of a possible 3350 isolate/antimicrobial class combinations. All were predicted to be resistant from the genome-derived AMR data but were phenotypically susceptible to the corresponding antimicrobial, i.e. all were considered to be major errors (genotypically resistant but phenotypically susceptible), rather than very major errors (genotypically susceptible but phenotypically resistant) (Table 1). Two isolates where CTX-M-3 was detected in the WGS data were phenotypically susceptible to cefotaxime and ceftazidime by MIC. Eleven isolates were predicted to be resistant to streptomycin (*strA-strB*, $n = 9$; *aadA5*, $n = 2$) and one isolate each was predicted to be resistant to trimethoprim (*dfrA1*) or chloramphenicol (*catA*) (Table 1), but all 13 isolates were susceptible to the corresponding antimicrobial using current breakpoints for *S. sonnei*. The MICs for the discrepant isolates were as follows: streptomycin, 4 mg/L ($n = 11$); trimethoprim, 0.25 mg/L ($n = 1$); and chloramphenicol, 4 mg/L ($n = 1$). The breakpoints for streptomycin, trimethoprim and chloramphenicol were 8, 2 and 8 mg/L, respectively.

Resistance to β-lactams

Of the 341 isolates in this study, 103 (30.2%) had genes predicted to confer resistance to ampicillin (Table 2). Specifically, the genes detected encoded the penicillinases TEM-1 ($n = 68$), OXA-1 ($n = 9$) or TEM-117 ($n = 4$), or the ESBLs CTX-M-15 ($n = 26$), CTX-M-27 ($n = 9$), CTX-M-3 ($n = 5$) or CTX-M-14 ($n = 2$) (Table 2). Nineteen isolates with TEM-1 also had a CTX-M variant (CTX-M-15, $n = 10$; CTX-M-27, $n = 9$) and one TEM-117-positive isolate also had CTX-M-3 (Table 3). Carbapenemase or AmpC genes were not detected in any of the 341 isolates.

Resistance to quinolones

Mutations in *gyrA* and *parC* resulting in resistance to ciprofloxacin (MIC >0.5 mg/L) were detected in 62 (18.2%) isolates, of which 55 had *gyrA* [83:S-L; 87:D-G] and *parC* [80:S-I] mutations and 7 had *gyrA* [83:S-L;87:D-N] and *parC* [80:S-I] mutations (Table 2). A further 92 isolates exhibited reduced susceptibility (MIC >0.064 to <0.5 mg/L) to ciprofloxacin, 67 (19.6%) had a single *gyrA* mutation, either [83:S-L] ($n = 47$) or [87:D-Y] ($n = 20$), and 25 (7.3%) harboured a plasmid-mediated quinolone resistance (PMQR) determinant (*qnrB19*, $n = 18$; *qnrS1*, $n = 7$) without QRDR mutations (Tables 2 and 3). Nine isolates had a PMQR determinant in combination with a single *gyrA* mutation, either [83:S-L] ($n = 2$) or [87:D-Y] ($n = 2$) or with *gyrA* [83:S-L; 87:D-G] and *parC* [80:S-I] mutations ($n = 3$) or *gyrA* [83:S-L;87:D-N] and *parC* [80:S-I] mutations ($n = 2$) (Tables 2 and 3).

Table 1. Evaluation of genotypic analysis for the prediction of resistance phenotypes of all viable isolates exhibiting resistance to at least one antimicrobial ($n = 335$)

	Phenotype: susceptible		Phenotype: resistant	
Antibiotic	genotype: resistant	genotype: susceptible	genotype: resistant	genotype: susceptible
AMP	0	234 (100)	101 (100)	0
CAZ/CTX	2 (0.67)	293	40 (100)	0
CIP	0	273 (100)	62 (100)	0
STR	11 (23.9)	35	289 (100)	0
GEN	0	328 (100)	7 (100)	0
AZM	0	283 (100)	52 (100)	0
TMP	1 (20)	4	330 (100)	0
TET	0	70 (100)	265 (100)	0
SUL	0	51 (100)	284 (100)	0
CHL	1 (0.3)	326	8 (100)	0

AMP, ampicillin; AZM, azithromycin; CAZ, ceftazidime; CHL, chloramphenicol; CIP, ciprofloxacin (>0.5 mg/L); CTX, cefotaxime; GEN, gentamicin; STR, streptomycin; SUL, sulphonamide; TMP, trimethoprim; TET, tetracycline.
Values shown are the number of isolates, with the percentage in parentheses where the number of isolates is not zero.

Table 2. Resistance genes identified in *S. sonnei*, the predicted resistance phenotype and their prevalence

Resistance gene	Antibiotic class	Number (%)	Accession number
strA-strB [*aph(3′)-Ib*]	aminoglycosides	267 (78.3)	CP011429
aadA1	aminoglycosides	164 (48.1)	NG_047326
aadA5	aminoglycosides	8 (2.3)	FJ460238
aac(3)-IId	aminoglycosides	6 (1.8)	EU022314
aac(3)-IVa	aminoglycosides	1 (0.3)	X01385
$bla_{\text{TEM-1}}$	β-lactams	68 (19.9)	AF188200
$bla_{\text{TEM-117}}$	β-lactams	4 (1.2)	AY130282
$bla_{\text{OXA-1}}$	β-lactams	9 (2.6)	J02967
$bla_{\text{CTX-M-3}}$	β-lactams	5 (1.5)	EF437434
$bla_{\text{CTX-M-14}}$	β-lactams	2 (0.6)	AF252622
$bla_{\text{CTX-M-15}}$	β-lactams	26 (7.6)	AY044436
$bla_{\text{CTX-M-27}}$	β-lactams	9 (2.6)	AY156923
sul1	folate synthesis inhibitors	25 (7.3)	NG_048086
sul2	folate synthesis inhibitors	281 (82.4)	NG_048110
dfrA1	folate synthesis inhibitors	314 (92.1)	AY963803
dfrA5	folate synthesis inhibitors	3 (0.9)	JF806498
dfrA8	folate synthesis inhibitors	2 (0.6)	U10186
dfrA14	folate synthesis inhibitors	13 (3.8)	Z50805
dfrA17	folate synthesis inhibitors	21 (6.2)	KX573886
mphA	macrolides	6 (1.8)	EF219134
ermB	macrolides	49 (14.4)	JX535233
catA1	phenicols	9 (2.6)	V00622
qnrS1	quinolones	16 (4.7)	GQ336885
qnrB19	quinolones	18 (5.3)	HM146784
gyrA [83:S-L]	quinolones	47 (13.8)	CP009102
gyrA [83:D-Y]	quinolones	20 (5.9)	CP009102
gyrA [83:S-L; 87:D-G]; *parC* [80:S-I]	quinolones	55 (16.1)	CP014225 position 536620 to 533993
gyrA [83:S-L; 87:D-N]; *parC* [80:S-I]	quinolones	7 (2.1)	AE014075 position 3593045 to 3595303
tetA	tetracyclines	271 (79.5)	HQ840942

Table 3. Combinations of AMR phenotypes and genotypes identified in 341 isolates of *S. sonnei* isolated in England and Wales, August 2015 to January 2016

No. of AMR phenotypes	No. of isolates (%)	Phenotypic combinations (no. of strains)	Genotypic combinations (no. of strains)
0	2 (0.6)	–	–
1	17 (5.0)	TMP (17)	*dfrA1* (17)
2	18 (5.3)	STR/TMP (7)	*aadA1*/*dfrA1* (7)
		<CIP/TMP (4)	*gyrA*[83:S-L]/*dfrA1* (2)
			gyrA[87:D-Y]/*dfrA1* (2)
		>CIP/TMP (7)	*gyrA*[83:S-L;87:D-N]*parC*[80:S-I]/*dfrA1* (3)
			gyrA[83:S-L;87:D-G]*parC*[80:S-I]/*dfrA1* (3)
			gyrA[83:S-L;87:D-G]*parC*[80:S-I]/*dfrA1*/*dfrA5* (1)
3	16 (4.7)	AMP/STR/SUL (1)	$bla_{TEM-117}$/*strA-strB*/*sul2* (1)
		STR/TMP/SUL (2)	*strA-strB*/*drfA14*/*sul2* (2)
		<CIP/STR/TMP (7)	*gyrA*[83:S-L]/*aadA1*/*dfrA1* (6)
			qnrB19/*aadA1*/*dfrA1* (1)
		STR/TET/SUL (1)	*strA-strB*/*tetA*/*sul2* (1)
		>CIP/TMP/SUL (1)	*gyrA*[83:S-L;87:D-N]*parC*[80:S-I]/*dfrA1*/*dfrA5*/*sul1* (1)
		CTX/STR/TMP (1)	$bla_{CTX-M-3}$/*aadA1*/*dfrA1* (1)
		CTX/<CIP/TMP (2)	$bla_{CTX-M-15}$/*gyrA*[83:S-L]/*qnrS1*/*dfrA1* (2)
		CTX/>CIP/TMP (1)	$bla_{CTX-M-15}$/*gyrA*[83:S-L;87:D-N]*parC*[80:S-I]/*dfrA1* (1)
4	105 (30.8)	STR/TMP/TET/SUL (99)	*strA-strB*/*dfrA1*/*tetA*/*sul2* (17)
			strA-strB/*aadA1*/*dfrA1*/*tetA*/*sul2* (80)
			strA-strB/*aadA1*/*dfrA1*/*dfrA14*/*tetA*/*sul2* (1)
			aadA1/*dfrA1*/*tetA*/*sul2* (1)
		GEN/TMP/TET/SUL (1)	*aac(3)-VIa*/*dfrA1*/*tetA*/*sul2* (1)
		AMP/STR/TMP/SUL (4)	bla_{TEM-1}/*strA-strB*/*dfrA8*/*sul2* (2)
			bla_{TEM-1}/*aadA1*/*dfrA1*/*sul2* (1)
			$bla_{TEM-117}$/*strA-strB*/*dfrA1*/*sul2* (1)
		AMP/AZM/STR/TMP (1)	bla_{TEM-1}/*ermB*/*mphA*/*aadA1*/*dfrA1* (1)
5	117 (34.3)	<CIP/STR/TMP/TET/SUL (51)	*gyrA*[83:S-L]/*strA-strB*/*dfrA1*/*tetA*/*sul2* (16)
			gyrA[83:S-L]/*aadA1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (3)
			gyrA[83:S-L]/*strA-strB*/*dfrA1*/*dfrA5*/*sul1*/*sul2* (1)
			gyrA[87:D-Y]/*strA-strB*/*dfrA1*/*tetA*/*sul2* (16)
			qnrB19/*strA-strB*/*aadA1*/*dfrA1*/*tetA*/*sul2* (15)
		>CIP/STR/TMP/TET/SUL (39)	*gyrA*[83:S-L;87:D-N]*parC*[80:S-I]/*strA-strB*/*dfrA1*/*tetA*/*sul2* (38)
			gyrA[83:S-L;87:D-N]*parC*[80:S-I]/*aadA1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (1)
		AMP/<CIP/AZM/STR/TMP (3)	bla_{TEM-1}/*gyrA*[83:S-L]/*ermB*/*mphA*/*aadA1*/*dfrA1* (3)
		AMP/STR/TMP/TET/SUL (7)	bla_{TEM-1}/*strA-strB*/*dfrA1*/*tetA*/*sul2* (3)
			bla_{TEM-1}/*aadA1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (1)
			bla_{TEM-1}/*aadA1*/*dfrA1*/*t*(A)/*sul2* (1)
			bla_{TEM-1}/*aadA1*/*strA-strB*/*dfrA1*/*drfA14*/*tetA*/*sul2* (1)
			$bla_{TEM-117}$/*strA-strB*/*aadA1*/*dfrA1*/*tetA*/*sul2* (1)
		AMP/TMP/TET/SUL/CHL (1)	bla_{OXA-1}/*dfrA1*/*tetA*/*sul2*/*floR* (1)
		CTX/STR/TMP/TET/SUL (3)	$bla_{CTX-M-3}$/*aadA1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (3)
		CTX/AMP/STR/TMP/SUL (6)	$bla_{CTX-M-3}$/$bla_{TEM-117}$/*aadA1*/*strA-strB*/*dfrA1*/*dfrA14*/*sul2* (1)
			$bla_{CTX-M-15}$/bla_{TEM-1}/*aadA1*/*strA-strB*/*dfrA1*/*dfrA14*/*sul2* (5)
		CTX/STR/TMP/TET/SUL (7)	$bla_{CTX-M-15}$/*qnrS1*/*aadA1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (7)
6	47 (13.8)	AMP/AZM/STR/TMP/TET/SUL (19)	bla_{TEM-1}/*ermB*/*mphA*/*aadA1*/*dfrA1*/*tetA*/*sul2* (10)
			bla_{TEM-1}/*ermB*/*mphA*/*aadA1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (8)
			bla_{TEM-1}/*mphA*/*aadA5*/*strA-strB*/*dfrA1*/*drfA17*/*tetA*/*sul1*/*sul2* (1)
		AMP/>CIP/AZM/STR/TMP/SUL (3)	bla_{TEM-1}/*gyrA*[83:S-L;87:D-N]*parC*[80:S-I]/*ermB*/*mphA*/*aadA5*/*dfrA1*/*drfA17*/*sul1* (3)
		AMP/STR/TMP/TET/SUL/CHL (8)	bla_{OXA-1}/*strA-strB*/*dfrA14*/*tetA*/*sul2*/*catA* (6)
			bla_{OXA-1}/*qnrB19*/*strA-strB*/*dfrA14*/*tetA*/*sul2*/*catA* (2)

Continued

Table 3. *Continued*

No. of AMR phenotypes	No. of isolates (%)	Phenotypic combinations (no. of strains)	Genotypic combinations (no. of strains)
		AMP/AZM/STR/TMP/TET/SUL (8)	bla_{TEM-1}/*gyrA*[83:S-L]/*ermB*/*mphA*/*aadA1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (8)
		CTX/<CIP/STR/TMP/TET/SUL (2)	$bla_{CTX-M-15}$/*gyrA*[87:D-Y]/*qnrS1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (2)
		CTX/>CIP/STR/TMP/TET/SUL (4)	$bla_{CTX-M-15}$/*gyrA*[83:S-L;87:D-N]*parC*[80:S-I]/*qnrS1*/*strA-strB*/*dfrA1*/*tetA*/*sul2* (4)
		CTX/AMP/AZM/STR/TMP/SUL (3)	$bla_{CTX-M-27}$/bla_{TEM-1}/*ermB*/*mphA*/*aadA5*/*drfA1*/*dfrA17*/*sul1* (3)
7	13 (3.8)	AMP/>CIP/AZM/STR/TMP/TET/SUL (6)	bla_{TEM-1}/*gyrA*[83:S-L;87:D-N]*parC*[80:S-I]/*ermB*/*mphA*/*aadA1*/*strA-strB*/*dfrA1*/*dfrA17*/*tetA*/sul1/sul2 (6)
		CTX/>CIP/AZM/STR/TMP/SUL/TET (1)	$bla_{CTX-M-14}$/*gyrA*[83:S-L;87:D-N]*parC*[80:S-I]/*mphA*/*aadA5*/*strA-strB*/*dfrA1*/*dfrA17*/*tetA*/*sul1*/*sul2* (1)
		CTX/AMP/AZM/STR/TMP/TET/SUL (6)	$bla_{CTX-M-27}$/bla_{TEM-1}/*ermB*/*mphA*/*aadA5*/*strB-strA*/*dfrA1*/*dfrA17*/*tetA*/*sul1*/*sul2* (6)
8	6 (1.8)	CTX/<CIP/AZM/GEN/STR/TMP/SUL/TET (1)	$bla_{CTX-M-14}$/*gyrA*[83:S-L]/*mph*(A)/*aac(3)-IId*/*aadA5*/*strA-strB*/*dfrA1*/*dfrA17*/*tetA*/*sul1*/*sul2* (1)
		CTX/AMP/<CIP/GEN/STR/TMP/TET/SUL (5)	$bla_{CTX-M-15}$/bla_{TEM-1}/*gyrA*[83:S-L]/*aac(3)-IId*/*strB-strA*/*dfrA1*/*tetA*/*sul2* (5)

GEN, gentamicin; STR, streptomycin; TMP, trimethoprim; AZM, azithromycin; AMP, ampicillin; CHL, chloramphenicol; <CIP, reduced susceptibility to ciprofloxacin (>0.064 to < 0.5 mg/L); >CIP, ciprofloxacin (>0.5 mg/L); TET, tetracycline, SUL, sulphonamide.

Resistance to aminoglycosides

Three hundred and six (89.7%) isolates had genes predicted to confer streptomycin resistance. Two hundred and sixty-seven had *strA-strB*, of which 112 (32.8%) had *strA-strB* only and 176 had *aadA* variants *aadA1* and/or *aadA5*, with or without *strA-strB* (Tables 2 and 3). Seven had *aac* variants *aac(3)-IId* ($n = 6$) and *aac(3)-IVa* ($n = 1$) with or without *strA-strB*, conferring resistance to a broad range of aminoglycosides, including gentamicin (Tables 2 and 3). There were no 16S rRNA methyltransferase genes detected.

Resistance to sulphonamides, trimethoprim and tetracyclines

Three hundred and thirty-seven (98.8%) isolates had one or more *dfrA* genes (*dfrA1*, $n = 314$; *dfrA17*, $n = 21$; *dfrA14*, $n = 13$; *dfrA5*, $n = 3$; *dfrA8*, $n = 2$), conferring resistance to trimethoprim (Table 2). There were 27 isolates with two different *dfrA* genes (*dfrA1-dfrA17*, $n = 21$; *dfrA1-dfrA5*, $n = 3$; *dfrA1-dfrA14*, $n = 3$). Two hundred and seventy-one (79.5%) isolates had *tetA* (Table 2); no other tetracycline resistance determinants were detected. Two hundred and ninety (85.0%) isolates had genes predicted to confer sulphonamide resistance; 265 (77.7%) had *sul2*, 9 (2.6%) had *sul1* and 16 (4.7%) had both *sul1* and *sul2* (Tables 2 and 3).

Resistance to phenicols and macrolides

Nine isolates (2.6%) had *catA1*, predicted to confer chloramphenicol resistance (Table 2). Genes predicted to confer macrolide resistance were found in 52 (15.2%) isolates; 49 had both *ermB* and *mphA*, and three had *mphA* only. Genotypic resistance to azithromycin was confirmed phenotypically in all isolates carrying *ermB* and/or *mph*A.

Resistance to rifamycins and fosfomycin

None of the isolates was predicted to be resistant to the rifamycins and fosfomycin.

MDR AMR profiles

Based on the WGS prediction, 339 (99.4%) of 341 isolates were resistant to at least one antimicrobial on the panel tested. Seventeen (5.0%) were resistant to trimethoprim only, 18 (5.3%) were resistant to two antimicrobials and 304 (89.1%) were MDR (resistant to three or more classes of antimicrobials) (Table 3).[12] The most common genotypic resistance profiles were: (i) *strA-strB*, *aadA1*, *dfrA1*, *sul2*, *tetA* ($n = 80$, 23.5%) and (ii) *gyrA*[83:S-L;87:D-N] *parC*[80:S-I], *strA-strB*, *dfrA1*, *tetA*, *sul2* ($n = 38$, 11.1%) (Table 3).

Association between AMR and travel

Of the 341 isolates in this study, 191 (56%) were from males and 150 (44%) were from females; of these, 70 (21%) isolates were from children under the age of 16 years. Travel histories were available for 207 (60.7%) of the 341 cases; 125 (36.7%) patients reported travel 7 days prior to onset of symptoms, 82 (24.0%) reported that they did not travel and no travel data were available for 134 (39.3%) patients (Table S1).

Of the 42 (12.3%) patients with isolates harbouring bla_{CTX-M} genes, travel histories were available for 19 cases and, of these, 11 reported recent travel abroad prior to onset of symptoms (CTX-M-3, $n = 2$; CTX-M-14, $n = 2$; CTX-M-15, $n = 7$). Eight cases with ESBL-producing *S. sonnei* reported that they had not travelled in the 7 days prior to onset of symptoms (CTX-M-15, $n = 6$; CTX-M-27, $n = 2$) and travel data were not available for 21 (50%) of the 42 cases. Of the 62 (18.2%) cases with ciprofloxacin-resistant *S. sonnei*, 29 (46.8%) reported travel abroad within 7 days of onset of symptoms, 13 (21.0%) reported that they had not travelled during that time frame and travel data were not available for

20 (32.3%) cases. Of the 29 cases reporting travel, 21 (72.4%) had travelled to India (Table S1). Of the 52 cases with azithromycin-resistant *S. sonnei*, 9 (17.3%) reported recent travel abroad, 19 reported they had not travelled recently and 24 cases did not provide a travel history.

There was no significant association between any of the AMR determinants tested and recent foreign travel (7 days prior to onset of symptoms) in male or female cases. The number of isolates of *S. sonnei* harbouring bla_{TEM-1} and *ermB* and/or *mphA* was significantly higher in men who did not report recent foreign travel ($P = 0.002$).

Discussion

The implementation of high-throughput WGS for all isolates of *S. sonnei* for routine public health surveillance has facilitated rapid and comprehensive AMR surveillance in this group of pathogens. A comparison of the phenotypic and genotypic resistance profiles identified 15 (0.45%) discordant results out of a possible 3350 isolate/antimicrobial class combinations. All 15 mismatched results were for isolates predicted to be genotypically resistant to specific antimicrobials but found to be phenotypically susceptible, indicating that the AMR determinant was present but not expressed or expressed poorly. Silent resistance genes have been described previously.[13] Resistance genes are often plasmid mediated, and discrepancies could be due to the loss of plasmids during storage and sub-culture as the phenotypic testing was performed retrospectively. For 13 of the discrepant results, the MICs were just below the respective breakpoints, and the tolerated technical variation that is inherent in the agar dilution method was a possible cause.

Deriving AMR profiles from genome data was shown to be a robust and accurate approach for *S. sonnei*. However, WGS data for determining AMR require careful analysis, as the results may be difficult to interpret in terms of phenotypic expression. Although no examples of genotypically susceptible/phenotypically resistant isolates were identified in this study, there is a risk of missing a novel resistance mechanism not currently included in the reference database, which would result in predicting susceptibility in a resistant isolate (a very major error if treatment is needed). Regular updating of the database to include novel AMR genes and mutations, and ongoing phenotypic testing of a subset of 'genotypically susceptible' isolates, will be required to ensure the sensitivity of AMR profiles derived from WGS data remains high.

In this set of isolates, the most common resistances detected were to trimethoprim, streptomycin, sulphonamides, tetracyclines and ampicillin, detected in 98.5%, 85.9%, 84.0%, 79.2% and 30.2% of *S. sonnei*, respectively. A comparison of the results of this study with those from a study in England and Wales in 2002[7] showed an increase in resistance to trimethoprim from 88% to 98.5%, whereas resistance to ampicillin remained stable at ~30%. Over the last decade, an increasing incidence of resistance to trimethoprim, sulphonamides and tetracyclines has been reported worldwide, and regional variations in the incidence of ampicillin resistance have been described.[14] A recent study in China reported similar levels of trimethoprim resistance (98.7%) to those found in this study, and a higher incidence of resistance to streptomycin (98.7%), ampicillin (92.1%) and tetracyclines (86.8%).[15] In Peru, resistance levels were high to trimethoprim (86%), tetracyclines (74%), ampicillin (67%) and chloramphenicol (65%),[16] whereas in Bangladesh resistance to the commonly used antimicrobials trimethoprim, nalidixic acid, ciprofloxacin and ampicillin was 89.5%, 86.5%, 17% and 9.5%, respectively.[17]

Resistance to ciprofloxacin was not detected in England and Wales in the 2002 study[7] but has increased to 18.2% over the last decade and was conferred by triple mutations in *gyrA* and *parC*. Of the 29 cases where a travel destination was documented, 21 reported travel to India. A number of studies report increasing resistance to ciprofloxacin in *S. sonnei* and South Asia has been identified as a reservoir for the global spread of ciprofloxacin resistance in this pathogen.[4,18–20] In our study, a further 27.0% of isolates exhibited reduced ciprofloxacin susceptibility and WGS analysis revealed that single mutations in *gyrA* and/or the PMQR determinants *qnrS1* or *qnrB19* were present in these isolates.

In 2002 in England and Wales, ESBL-producing *S. sonnei* were not detected,[7] whereas in the current study 12% of isolates had CTX-M genes. A number of studies report isolated cases of ESBL-producing *S. sonnei*, but generally the incidence appears to be low.[21–23] Of the 19 cases linked to a documented travel history, 11 reported recent travel prior to onset of symptoms. There is evidence of transmission of domestically acquired ESBL-producing strains harbouring CTX-15 (this study) and CTX-M-27.[24]

The macrolides have only recently been added to the panel of antimicrobials used for phenotypic AMR testing of GI pathogens at PHE in response to WGS data, highlighting increasing resistance to this class of antimicrobial in *Salmonella* and *Shigella*[25,26] and so resistance was not sought prior to 2015. This study detected 15.2% resistance to azithromycin, and this AMR profile has been associated with transmission in MSM.[25,27] Increased resistance to azithromycin has also been observed in the wider community.[20,28]

AMR in *Shigella* spp. is a global public health problem. Growing scientific evidence indicates that it is negatively impacted by antimicrobial usage in the clinical setting, either for the treatment of symptoms caused by *Shigella* or for the treatment of other co-existent infections.[24] The implementation of WGS for routine public health surveillance of GI pathogens will enable PHE to monitor trends in AMR gene content in *S. sonnei* causing travellers' diarrhoea and GI symptoms in the MSM community, and provide real-time data on emerging resistance patterns nationally and internationally.

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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 NIHR, the Department of Health or PHE.

Supplementary data

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

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