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Comparison of genome-derived and phenotypic AMR profiles of *Shigella* species isolated from patients with symptoms of GI disease in England, 2015-2020

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INTRODUCTION

Shigella* are Gram-negative bacilli belonging to the family *Enterobacteriacae

- Composed of four species: *S. dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei* [1]
- Shigellosis is a gastrointestinal infection which causes bloody and/or mucoid diarrhoea (dysentery)[1] and mainly causes more severe illness in infants and adults (>50 years of age)[2]
- Shigellosis predominantly occurs in developing countries and is a cause of travellers' diarrhoea in the UK – a large number of *Shigella* cases in the UK are associated with foreign travel to Asia and Africa[3]
- Infection is usually self-limiting and the standard recommended treatment for dysentery is oral rehydration therapy[4]

Antimicrobial therapy for *Shigella* infection is recommended for infants and adults (>50 years of age) or for severe and prolonged infection[4]

- The World Health Organisation Guidelines 2005 recommend use of ciprofloxacin as the first-line treatment for shigellosis
- Azithromycin is recommended as an alternative treatment for adults
- Treatment with ceftriaxone or mecillinam is recommended for multi-drug resistant strains of *Shigella* [1]

Surveillance of antimicrobial resistance (AMR) in *Shigella* species (particularly against ciprofloxacin) is necessary to:

- Monitor novel and emerging AMR mechanisms
- Monitor global transmission of emerging travel-associated AMR mechanisms
- Help guide the therapeutic management of shigellosis

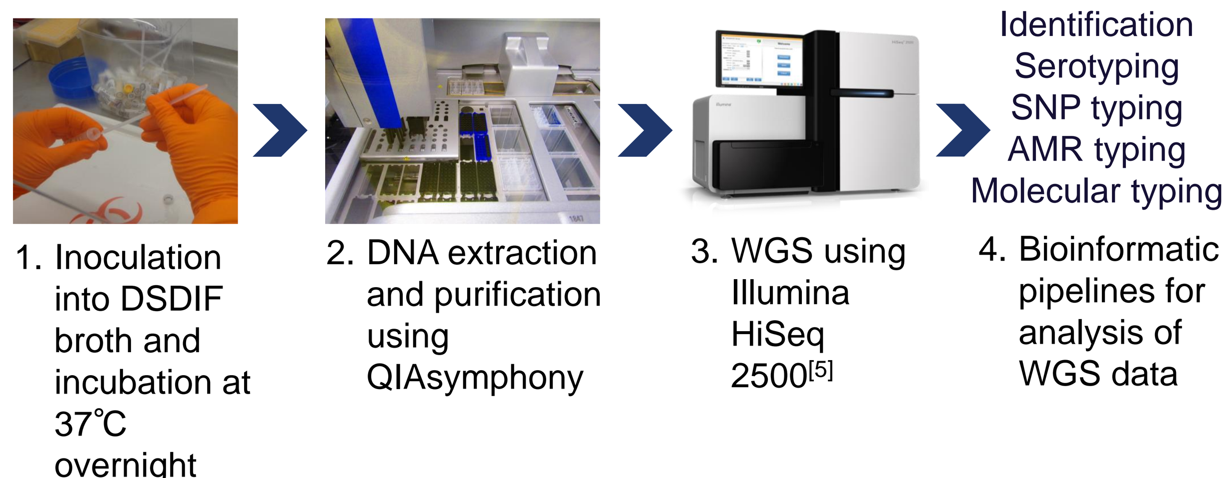
The aim of this study was to compare and evaluate genotypic and phenotypic methods for the detection of AMR in *Shigella* species.

METHODS

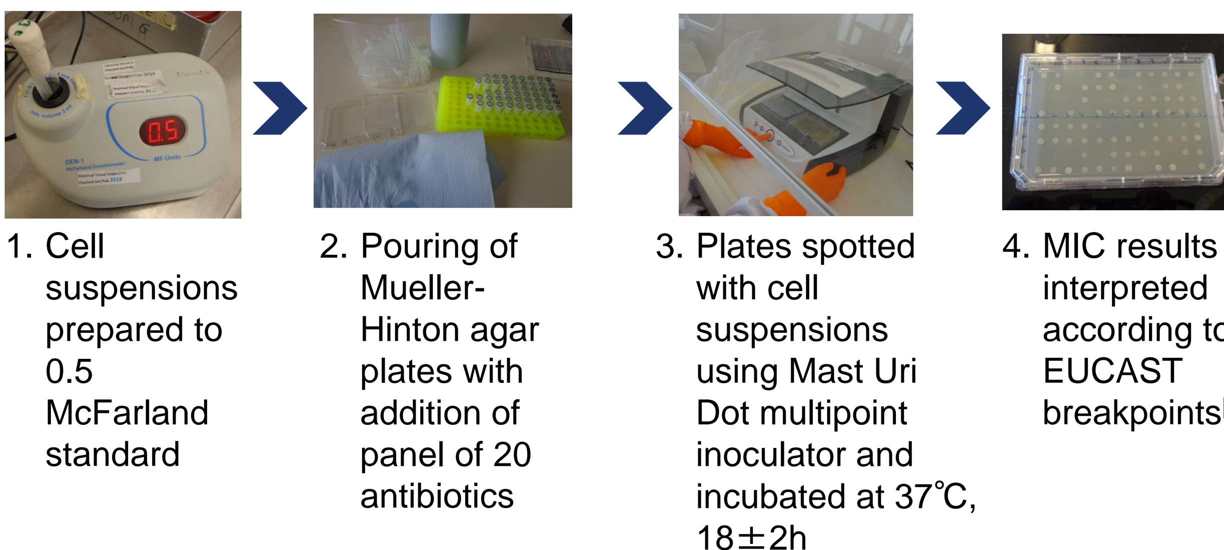
***Shigella* isolates (n=388) received from September 2015 to January 2020 were selected from the GBRU archive for analysis via antimicrobial susceptibility testing (AST).**

Genotypic AST was performed on all 388 isolates. Phenotypic AST was performed retrospectively on all selected viable and pure isolates (n=358). Results from genotypic AST and phenotypic AST were then compared.

Genotypic AST: via whole genome sequencing (WGS) to determine resistance genes (see below)



Phenotypic AST: via agar dilution to determine minimum inhibitory concentration (MIC) (see below)



RESULTS

Out of 358 isolates, 276 (77%) were associated with foreign travel (Figure 1)

- Highest association with Asia (51.8%), followed by Africa (10.6%)
- Top two travel destinations were India (n=83), Pakistan (n=56)
- Geographical distribution of each *Shigella* species varied globally
  - *S. flexneri* and *S. sonnei* predominant in all continents – only two species associated with travel to Europe and South America
  - Much lower prevalence of *S. dysenteriae* and *S. boydii* – only observed in Asia, Africa and North America

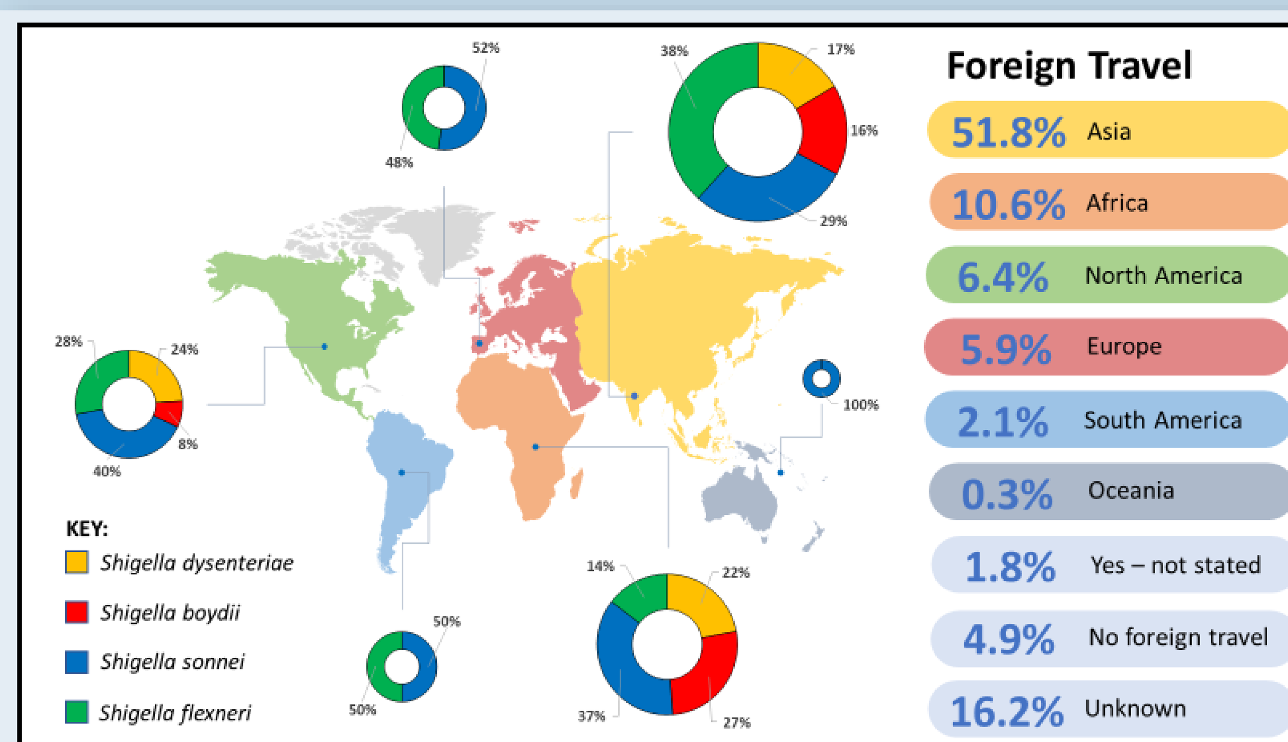


Figure 1. World map showing the geographical distribution of isolates associated with foreign travel (n=358)

Genotypic AMR and phenotypic AMR profiles were compared for 358 isolates (Table 2)

- Out of a possible 2864 isolate/antimicrobial class combinations, there were 147 (5.1%) discrepant results from 119 isolates
- Overall concordance was **94.9%** between the two methods
- There were **54 major errors** (isolates predicted to be genotypically resistant but were phenotypically susceptible)
  - 31/54 (57.4%) associated with tetracycline and trimethoprim-sulfamethoxazole resistance
- There were **93 very major errors** (isolates predicted to be genotypically susceptible but were phenotypically resistant)
  - 32/93 (34.4%) associated with isolates harbouring a single *gyrA* and/or *parC* mutation and exhibiting unexpectedly high MICs to ciprofloxacin
  - 37/93 (29.8%) associated with isolates with no identified AMR determinants but exhibiting high MIC values to fosfomycin

- 335/358 (93.6%) isolates were resistant to at least one antimicrobial
- 222/358 (62%) isolates were multi-drug resistant (resistant to three or more antimicrobial classes)

In total, antimicrobial resistance profiles were analysed for 358 isolates (Table 1)

- Of 358 isolates, 348 (97.2%) carried resistance genes to one or more antimicrobial: *S. dysenteriae* (n=74/81; 91.4%), *S. boydii* (n=80/83; 96.4%), *S. sonnei* (n=95/95; 100%) and *S. flexneri* (n=99/99; 100%)
- A total of 40 different genes were detected conferring resistance to the antimicrobial classes in Table 1:
  - *S. flexneri* isolates were observed to harbour the greatest variety of resistance genes, followed by *S. sonnei*, *S.boydii* and *S. dysenteriae* respectively:
    - *S. flexneri* – 30 resistance genes
    - *S. sonnei* – 27 resistance genes
    - *S. boydii* – 25 resistance genes
    - *S. dysenteriae* – 18 resistance genes

Table 1. Resistance genes identified in all four *Shigella* species, predicted resistance phenotype and prevalence

Resistance gene	Antibiotic Class	Resistance phenotype	*S. dysenteriae (n=81)* No. (%)	*S. boydii (n=83)* No. (%)	*S. sonnei (n=95)* No. (%)	*S. flexneri (n=99)* No. (%)
bla CMY-4-p	β-lactams	AMP, CTX, CAZ, FEP, FOX	2 (2.47)	1 (1.20)		2 (2.02)
bla CTX-M-3	β-lactams	AMP, CTX, CAZ, FEP, FOX		1 (1.20)	1 (1.05)	
bla CTX-M-14	β-lactams	AMP, CTX, CAZ, FEP, FOX			3 (3.16)	1 (1.01)
bla CTX-M-15	β-lactams	AMP, CTX, CAZ, FEP, FOX	10 (12.35)	7 (8.43)	37 (38.95)	14 (14.14)
bla CTX-M-27	β-lactams	AMP, CTX, CAZ, FEP, FOX			3 (3.16)	4 (4.04)
bla CTX-M-55	β-lactams	AMP, CTX, CAZ, FEP, FOX			3 (3.16)	
bla CTX-M-123[v]	β-lactams	AMP, CTX, CAZ, FEP, FOX			1 (1.05)	
bla OXA-1	β-lactams	AMP	13 (16.05)	7 (8.43)	3 (3.16)	44 (44.44)
bla TEM-1	β-lactams	AMP	25 (30.86)	28 (33.73)	14 (14.74)	34 (34.34)
*bla TEM-117-p**	β-lactams	AMP	2 (2.47)	1 (1.20)		
*bla TEM-191-p**	β-lactams	AMP	3 (3.70)			
mph-(A)	Macrolides	AZM	1 (1.23)	5 (6.02)	33 (34.74)	19 (19.19)
erm(B)[v]	Macrolides	AZM		1 (1.20)	10 (10.53)	9 (9.09)
gyrA_EC2[69:V-I]	Fluoroquinolones	CIP				1 (1.01)
gyrA_EC2[83:S-A]	Fluoroquinolones	CIP				12 (12.12)
gyrA_EC2[83:S-L]	Fluoroquinolones	CIP	17 (20.99)	25 (30.12)	48 (50.53)	45 (45.45)
gyrA_EC2[83:S-X]	Fluoroquinolones	CIP		1 (1.20)		
gyrA_EC2[87:D-G]	Fluoroquinolones	CIP	3 (3.70)	5 (6.02)	26 (27.37)	8 (8.08)
gyrA_EC2[87:D-N]	Fluoroquinolones	CIP		3 (3.61)	12 (12.63)	27 (27.27)
gyrA_EC2[87:D-Y]	Fluoroquinolones	CIP	7 (8.64)	6 (7.23)	23 (24.21)	14 (14.14)
parC_EC2[57:S-R]	Fluoroquinolones	CIP				24 (24.24)
parC_EC2[80:S-I]	Fluoroquinolones	CIP		3 (3.61)	37 (38.95)	29 (29.29)
parC_EC2[80:S-R]	Fluoroquinolones	CIP				1 (1.01)
parC_EC2[84:E-K]	Fluoroquinolones	CIP				6 (6.06)
parC_EC2[96:R-L]	Fluoroquinolones	CIP				1 (1.01)
qepA-1[v]	Fluoroquinolones	CIP				2 (2.02)
qnrB-4	Fluoroquinolones	CIP		2 (2.41)		
qnrB-19	Fluoroquinolones	CIP		1 (1.20)	11 (11.58)	4 (4.04)
qnrS-1	Fluoroquinolones	CIP	30 (37.04)	29 (34.94)	36 (37.89)	34 (34.34)
tet(A)	Tetracyclines	TET	48 (59.26)	53 (63.86)	85 (89.47)	84 (84.85)
dfrA-1	Combination - trimethoprim, sulphonamides	SXT, TMP	35 (43.21)	35 (42.17)	91 (95.79)	75 (75.76)
dfrA-5		SXT, TMP		5 (6.02)	1 (1.05)	1 (1.01)
dfrA-7		SXT, TMP	6 (7.41)	8 (9.64)		
dfrA-8		SXT, TMP			1 (1.05)	
dfrA-12		SXT, TMP			2 (2.11)	
dfrA-14		SXT, TMP	25 (30.86)	26 (31.33)	5 (5.26)	22 (22.22)
dfrA-17		SXT, TMP	5 (6.17)	2 (2.41)	18 (18.95)	9 (9.09)
sul-1	Combination - trimethoprim, sulphonamides	SXT, SMX	5 (6.17)	8 (9.64)	20 (21.05)	9 (9.09)
sul-2		SXT, SMX	53 (65.43)	56 (67.47)	89 (93.68)	62 (62.63)
sul-3		SXT, SMX			1 (1.05)	1 (1.01)

AMP, ampicillin; CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin; AZM, azithromycin; CIP, ciprofloxacin; TET, tetracycline; SXT, trimethoprim-sulfamethoxazole; TMP, trimethoprim; SMT, sulfamethoxazole

Table 2. Evaluation of genotypic analysis for the prediction of resistance phenotypes of all viable and pure isolates exhibiting resistance to at least one antimicrobial (n=358)

Antimicrobial	S. dysenteriae (n=81)				S. boydii (n=83)				S. sonnei (n=95)				S. flexneri (n=99)			
	P : S		P : R		P : S		P : R		P:S		P:R		P:S		P:R	
	G : R	G : S	G : R	G : S	G : R	G : S	G : R	G : S	G:R	G:S	G:R	G:S	G:R	G:S	G:R	G:S
	Major errors			Very major errors	Major errors			Very major errors	Major errors			Very major errors	Major errors			Very major errors
Ampicillin	3[a]	29	45	4	3[b]	38	38	4	3[b]	31	58	3	4[c]	26	68	1
Azithromycin	1	80	0	0	0	78	5	0	0	4	33	58	0	42	19	38
Cephalosporins (CTX/CAZ/FEP/FOX)	3	69	9	0	1	74	8	0	4	49	42	0	1	78	20	0
Ciprofloxacin	0	81	0	0	0	80	3	0	0	43	37	15	0	43	39	17
Tetracycline	2	33	46	0	10[a]	30	43	0	2	10	83	0	1	3	83	12
Ertapenem	0	81[d]	0	0	0	83	0	0	0	95	0	0	0	99	0	0
Trimethoprim-sulfamethoxazole	3[e]	15[d]	63	0	6[f]	13	64	0	3	0	92	0	4[e]	11	84	0
Fosfomycin	0	53[d]	0	28	0	74	0	9	0	95	0	0	0	99	0	0

P:S, phenotype susceptible; P:R, phenotype resistant; G:R, genotype resistant; G:S, genotype susceptible.
CTX, cefotaxime; CAZ, ceftazidime; FEP, cefepime; FOX, cefoxitin.
Breakpoints: ampicillin (>8 mg/L); azithromycin (>16 mg/L); cefotaxime (>2 mg/L); ceftazidime (>4 mg/L); cefepime (>4 mg/L); cefoxitin (>8 mg/L); ciprofloxacin (>0.5 mg/L); tetracycline (>8 mg/L); ertapenem (>0.5 mg/L); trimethoprim-sulfamethoxazole (>4 mg/L); fosfomycin (>32 mg/L).
[a]1 isolate MIC = 8 mg/L; [b]3 isolates MIC = 8 mg/L; [c]2 isolates MIC = 8 mg/L; [d]1 isolate nbg, no bacterial growth; [e]2 isolates MIC = 4 mg/L; [f]1 isolate MIC = 4 mg/L

DISCUSSION

There was a good correlation between genotypic and phenotypic AMR profiles with 94.9% overall concordance between the two methods

- 119 isolates exhibiting 147 discrepant results:
  - **39 isolates with 54 major errors** – possible reasons include:
    - 15 of the major errors for ampicillin, tetracycline and trimethoprim-sulfamethoxazole had MIC values just below the EUCAST breakpoint – technical variation in agar dilution method is a possible cause
    - EUCAST breakpoints used for interpretation are non-specific to *Shigella* species – accuracy of interpretation of phenotypic data may be affected
    - AMR determinant present but not expressed/poorly expressed (silent resistant genes)
    - Loss of plasmids contributing to plasmid mediated resistance genes during storage and subculture (retrospective testing)
  - **86 isolates with 93 very major errors** – possible reasons include:
    - AMR determinants not included in the reference database used for WGS analysis
    - Presence of novel, unknown AMR mechanisms, where resistance has not yet been identified
    - Mixed with another resistant organism due to contamination of isolate during storage (retrospective testing)
    - **32 very major errors associated with ciprofloxacin resistance**
      - Strains with triple mutations or more in *gyrA* and *parC* genes typically confer high levels of ciprofloxacin resistance
      - Strains with double mutations or fewer in *gyrA* and *parC* genes and/or plasmid mediated AMR determinants typically result in reduced susceptibility instead of conferring full resistance
      - Different mutations in QRDR genes do not necessarily confer same level of resistance – some may have greater/lesser effect than others
      - Isolates harbouring the same combinations of resistance genes have showed variable MICs to ciprofloxacin, resulting in some strains classified as either susceptible or resistant
      - Shows analysis and interpretation of genomic data is complex in terms of predicting genotypic expression and resistance

CONCLUSIONS

- High concordance between the two methods indicates that genotypic AMR profiles are a robust and accurate approach for predicting resistance in *Shigella* species
- WGS should be used for continuous monitoring and public health surveillance to monitor emerging AMR
- However, data is complex and requires careful analysis – results can be difficult to interpret in terms of phenotypic expression
- Regular phenotypic monitoring is required to identify novel AMR mechanisms and allow updating of the reference database used for WGS analysis

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NIHR | Health Protection Research Unit in Gastrointestinal Infections at University of Liverpool

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