Antimicrobial susceptibility of *Shigella*, 2015 and 2016

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Hospital and community laboratories are requested to refer all *Shigella* isolates from cases of shigellosis to ESR for serotyping and biotyping as part of the laboratory-based surveillance of this disease. The antimicrobial susceptibility of viable, non-duplicate *Shigella* isolates referred to ESR in 2015 and 2016 was tested. This is the first antimicrobial susceptibility survey of *Shigella* that ESR had undertaken since 1996.

Methods

Antimicrobial susceptibility was determined by agar dilution according to the methods of the Clinical and Laboratory Standards Institute (CLSI).[1] Except for azithromycin and tetracycline, minimum inhibitory concentrations (MICs) were interpreted according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints.[2] CLSI breakpoints were used to interpret tetracycline MICS.[1] Currently there are no clinical breakpoints to interpret azithromycin MICs for *Shigella*. However for *S. flexneri* and *S. sonnei*, CLSI have defined 'epidemiological cutoff values' (ECVs) for azithromycin MICs.[1] ECVs separate bacterial populations into those with acquired and/or mutational resistance mechanisms (referred to as non-wild type, NWT) and those without such mechanisms (referred to as wild type, WT) (see footnote 1, Table 2 for the azithromycin ECVs). Multidrug resistance was defined as resistance (including azithromycin NWT) to ≥3 antibiotic classes.

Any isolates with a ceftriaxone or ceftazidime MIC ≥2 mg/L were tested for extended-spectrum β-lactamase (ESBL) production using the combination disc test.[1] To identify CTX-M type ESBLs, a multiplex PCR that includes primers to detect the genes for the four CTX-M groups, 1, 2, 8 and 9, was used.[3] Any isolates with a cefoxitin MIC ≥16 mg/L were tested by PCR for plasmid-mediated AmpC β-lactamase genes.[4]

Overseas travel history for shigellosis cases was obtained from information reported in the EpiSurv notifiable disease database supplemented with any additional travel information received when the isolate from the case was referred to ESR. The chi-square test was used to determine the significance of any observed differences, with a *p* value of ≤0.05 being considered significant.

Results

The antimicrobial susceptibility of 263 *Shigella* isolates referred to ESR in 2015 and 2016 was tested. These 263 *Shigella* comprised 141 (53.6%) *S. sonnei*, 113 (43.0%) *S. flexneri*, 7 (2.7%) *S. boydii*, and 2 (0.8%) *S. dysenteriae* (Table 1).

Table 1. Distribution of species, serotypes and biotypes among *Shigella* isolates, 2015 and 2016

Species, biotypes and/or serotype	Number of isolates	Percent of the total 263 isolates
Shigella boydii	**7**	**2.7**
serotype 2	1	0.4
serotype 4	1	0.4
serotype 13	5	1.9
Shigella dysenteriae	**2**	**0.8**
serotype 2	1	0.4
serotype 9	1	0.4
Shigella flexneri	**113**	**43.0**
serotype 1a	2	0.8
serotype 1b	20	7.6
serotype 1c	6	2.3
serotype 2a	31	11.8
serotype 2b	10	3.8
serotype 3a	8	3.0
serotype 3b	1	0.4
serotype 4av	3	1.1
serotype 5b	2	0.8
serotype 6 biotype Boyd 88	10	3.8
serotype 6 biotype Manchester	6	2.3
serotype Y	3	1.1
serotype Y variant	2	0.8
not typable[1]	9	3.4
Shigella sonnei	**141**	**53.6**
biotype a	50	19.0
biotype f	1	0.4
biotype g	90	34.2

1 Agglutinated with polyvalent *S. flexneri* antisera but not with any of the single factor sera.

Resistance to nine of the antimicrobials tested and multidrug resistance is shown in Table 2. There were some significant differences ($p \leq 0.05$) in resistance between *S. flexneri* and *S. sonnei*. *S. flexneri* were significantly more resistant to ampicillin ($p < 0.001$), chloramphenicol ($p < 0.001$) and gentamicin (p 0.026), and more likely to be multiresistant ($p < 0.001$). Conversely, *S. sonnei* were significantly more resistant to co-trimoxazole (p 0.022) (Table 2).

Table 2. Antimicrobial resistance among *Shigella*, 2015 and 2016

Antimicrobial	Percent resistant				
	S. sonnei n = 141	*S. flexneri* n = 113	*S. boydii* n = 7	*S. dysenteriae* n = 2	All species n = 263
Ampicillin	29.1	72.6	14.3	100	47.9
Azithromycin[1]	12.1	9.7	-	-	11.0
Ceftriaxone	7.1	2.7	14.3	50.0	5.7
Chloramphenicol	5.7	59.3	0.0	0.0	28.5
Ciprofloxacin[2,3]	25.5	20.4	14.3	0.0	22.8
Co-trimoxazole	63.8	49.6	14.3	100	56.7
Gentamicin	0.7	5.3	0.0	0.0	2.7
Meropenem	0.0	0.0	0.0	0.0	0.0
Tetracycline	48.9	53.1	0.0	100	49.8
Ciprofloxacin + co-trimoxazole	18.4	14.2	14.3	0.0	16.3
Ciprofloxacin + co-trimoxazole + azithromycin[1]	2.1	0.0	0.0	0.0	1.1
Multiresistant to ≥3 antimicrobials	33.3	62.8	14.3	100	46.0

1 The data given for azithromycin are the percentage that are categorised by the CLSI epidemiological cutoff values (ECVs) as non-wild type (ie, MICs ≥32 mg/L for *S. sonnei* and MICs ≥16 mg/L for *S. flexneri*). There are no CLSI azithromycin ECVs for *S. boydii* or *S. dysenteriae*.

2 Norfloxacin susceptibility was also tested and was very similar to ciprofloxacin susceptibility: 59 of the total 60 ciprofloxacin-resistant isolates were also norfloxacin resistant, the remaining ciprofloxacin-resistant isolate had intermediate resistance to norfloxacin, and there was one norfloxacin-resistant isolate that had intermediate resistance to ciprofloxacin. The EUCAST clinical breakpoints were used to interpret the norfloxacin MICs, however, these breakpoints are specifically for uncomplicated urinary tract infections.

3 The rates of ciprofloxacin resistance are based on the EUCAST resistance breakpoint of >0.5 mg/L. However, a recent health advisory from the United States Centers for Disease Control and Prevention recommends that fluoroquinolones should not be prescribed for the treatment of shigellosis if the ciprofloxacin MIC is ≥0.12 mg/L (see Reference 6). The percentage of isolates that had ciprofloxacin MICs ≥0.12 mg/L were: *S. sonnei* 37.6%, *S. flexneri* 29.2%, *S. boydii* 14.3%, *S. dysenteriae* 100%, and all species 33.8%.

There were also significant differences in resistance between the two prevalent *S. sonnei* biotypes. Compared with *S. sonnei* biotype a isolates, *S. sonnei* biotype g isolates were significantly more likely to be azithromycin NWT (17.8 vs 0.0%, *p* 0.002); more resistant to ceftriaxone (10.0 vs 0.0%, *p* 0.021), ciprofloxacin (38.9 vs 0.0%, *p* <0.001), co-trimoxazole (73.3 vs 46.0%, *p* 0.001) and tetracycline (75.6 vs 2.0%, *p* <0.001); and more likely to be multidrug resistant (50.0 vs 2.0% *p* <0.001).

The current Australian Therapeutic Guidelines recommend either a fluoroquinolone (ciprofloxacin or norfloxacin) or co-trimoxazole when treatment of shigellosis is indicated.[5] If an alternative is required due to resistance to these first-line antibiotics, azithromycin is recommended. The overall rates of resistance were relatively high for both ciprofloxacin and co-trimoxazole (22.8% and 56.7%, respectively), although, as noted above, rates of resistance to these two antimicrobials were quite variable by species and/or *S. sonnei* biotype. Although not shown in Table 2, norfloxacin susceptibility was also tested and was very similar to ciprofloxacin susceptibility (see footnote 2, Table 2). While the EUCAST breakpoint for ciprofloxacin resistance is >0.5 mg/L, a recent health advisory from the United States Centers for Disease Control and Prevention recommends that fluoroquinolones should not be prescribed for the treatment of shigellosis if the ciprofloxacin MIC is ≥0.12 mg/L.[6] The percentage of isolates that had ciprofloxacin MICs ≥0.12 mg/L were: *S. sonnei* 37.6% (*S. sonnei* biotype g 57.8% and *S. sonnei* biotype a 0.0%), *S. flexneri* 29.2%, *S. boydii* 14.3%, *S. dysenteriae* 100%, and all species 33.8%.

16.3% (43) of isolates were resistant to both ciprofloxacin and co-trimoxazole (Table 2). Similar proportions of *S. sonnei* (18.4%, 26/141) and *S. flexneri* (14.2%, 16/113) had dual resistance to ciprofloxacin and co-trimoxazole. However, *S. sonnei* biotype g was over-represented among the *S. sonnei* isolates with this dual resistance, as this biotype accounted for 63.8% (90/141) of the *S. sonnei* isolates but 96.2% (25/26) of the dual-resistant *S. sonnei*.

Twenty-eight (11.0%) of the *S. sonnei* and *S. flexneri* isolates were categorised as azithromycin NWT (Table 2). The prevalence of azithromycin NWT was significantly higher among *S. flexneri* serotype 2b (30.0%, 3/10) and *S. sonnei* biotype g (17.8%, 16/90), with the latter accounting for 57.1% (16/28) of all azithromycin-NWT isolates. Three (2.1%) *S. sonnei* isolates were azithromycin NWT, ciprofloxacin resistant and co-trimoxazole resistant, that is, resistant to all three antibiotic classes recommended for treatment. Two of these three *S. sonnei* were biotype g and the remaining isolate was biotype f. The travel

history for two of the three cases was known and both (one with biotype g and one with biotype f) had travelled to India.

Fifteen isolates (5.7%) were ceftriaxone resistant and all 15 had a CTX-M type ESBL: 9 had a CTX-M group 1 ESBL, 4 had a CTX-M group 9 ESBL, 1 had a CTX-M group 8 ESBL, and one had both CTX-M group 1 and group 9 ESBLs. No plasmid-mediated AmpC β-lactamases were identified.

Table 3. Antimicrobial resistance among *Shigella* from cases who had travelled overseas compared with non-travellers, 2015 and 2016

Antimicrobial	Percent resistant		*p* value for significance of any difference in resistance between travellers and non-travellers[1]
	Cases who had travelled overseas n = 166	Cases who had not travelled overseas n = 97	
Ampicillin	44.0	54.6	0.095
Azithromycin[2]	5.6	20.7	<0.001
Ceftriaxone	6.6	4.1	0.398
Chloramphenicol	29.5	26.8	0.638
Ciprofloxacin	24.7	19.6	0.341
Co-trimoxazole	58.4	53.6	0.446
Gentamicin	0.6	6.2	0.007
Tetracycline	50.6	48.5	0.737
Ciprofloxacin + co-trimoxazole	17.5	14.4	0.521
Multiresistant to ≥3 antimicrobial classes	46.4	45.4	0.872

1 Chi-square test.

2 The data given for azithromycin are the percentage of *S. flexneri* and *S. sonnei* that were categorised by the CLSI epidemiological cutoff values (ECVs) as non-wild type. Among the 254 cases who had either of these two species, 162 were reported to have recently travelled overseas and 92 were reported to have not been overseas.

When susceptibility among *Shigella* isolates was compared according to whether the case had recently travelled overseas, azithromycin NWT and gentamicin resistance was significantly more prevalent among cases who had not travelled (Table 3). Further analysis according to the *Shigella* species and biotype, showed that *S. flexneri* from patients who had not travelled were significantly more likely to be azithromycin NWT (17.8 vs 4.1% *p* 0.019) and gentamicin resistant (13.3 vs 0.0% *p* 0.002) than isolates of this species from patients

who had recently travelled. *S. sonnei* biotype a isolates from patients who had not travelled were significantly more likely to be ampicillin resistant (52.9 vs 18.2% *p* 0.012) and *S. sonnei* biotype g isolates from patients who had not travelled were significantly more likely to be azithromycin NWT (36.7 vs 8.3% *p* 0.001). Notably, there were no antibiotics for which the rate of resistance was significantly higher among isolates from patients who had travelled overseas.

Antimicrobial resistance among *Shigella*, including azithromycin non-susceptibility, has been reported in several other countries (including Australia, the United States, Canada, England and Taiwan) to be associated with *Shigella* isolated from men who have sex with men (MSM).[7-11] No information on the sexual practices of cases is currently systematically collected with shigellosis notifications in New Zealand.

An analysis of total 28 azithromycin-NWT *S. flexneri* and *S. sonnei* by the age and sex of the patients is presented in Table 4. While the prevalence of azithromycin NWT among isolates from males (13.9%) was twice that among isolates from females (7.7%), this difference was not significant (*p* 0.117). In a further breakdown by age group, azithromycin NWT was more prevalent among isolates from males in all age groups except the youngest group (<20 years), although the only age group in which the difference reached statistical significance was in the 40-59 years group. Twelve (63.2%) of the 19 azithromycin-NWT isolates from males were *S. sonnei* biotype g compared with four (44.4%) of the 9 azithromycin-NWT isolates from females.

Among the other antimicrobials, only the prevalence of tetracycline resistance was significantly different between the sexes with 55.7% of *Shigella* from males being resistant compared with 43.1% of isolates from females.

Table 4. Age and sex distribution of patients with azithromycin-non-wild type *S. flexneri* and *S. sonnei*, 2015 and 2016

Age group (years)	Azithromycin non-wild type		*p* value for significance of any difference between females and males[2]
	Number of isolates (%[1])		
	From female cases	From male cases	
<20	3 (12.5)	2 (5.6)	0.340
20-39	2 (5.4)	4 (10.3)	0.433
40-59	2 (5.6)	8 (22.2)	0.041
≥60	2 (10.0)	5 (19.2)	0.388
Total	**9 (7.7)**	**19 (13.9)**	**0.117**

1 Only includes cases due to *S. flexneri* or *S. sonnei*. There were 117 female cases of shigellosis due to *S. flexneri* or *S. sonnei*: 24 in the <20 years age group, 37 in the 20-39 years age group, 36 in the 40-59 years age group and 20 in the ≥60 age group. There were 137 male cases of shigellosis due to *S. flexneri* or *S. sonnei*: 36 in the <20 years age group, 39 in the 20-39 years age group, 36 in the 40-59 years age group and 26 in the ≥60 age group. These case numbers were used as the denominators to calculate the percentage of azithromycin NWT among isolates for each sex and age group.

2 Chi-square test.

References

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