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Antimicrobial susceptibility of *Shigella* in New Zealand, 2019

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Prepared by: Audrey Tiong, Rosemary Woodhouse and Kristin Dyet

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REVIEWED BY: **Juliet Elvy and Jackie Wright**

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DISCLAIMER

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1. Introduction

Shigella infections are a common cause of bacterial diarrhoea worldwide, especially in resource-limited countries.[1] They are estimated to cause over 250 million cases of disease and 210,000 deaths annually.[2] Humans are the only natural host for *Shigella* that are passed from person to person by the faecal-oral route.[1] As *Shigella* has a very low infectious dose, they are very contagious.[1] *Shigella* infections, or shigellosis, are caused by one of four bacterial species: *S. sonnei*, *S. flexneri*, *S. dysenteriae* and *S. boydii,*[1] which are further differentiated by serotyping and biotyping.

Antibiotic resistance has increased in *Shigella* over the last decade, aided by the international dissemination of antibiotic-resistant clones by travellers and in men who have sex with men.[1] The Centers for Disease Control and Prevention and the World Health Organisation have both listed antibiotic-resistant *Shigella* as a serious threat to global health.

Shigellosis is not common in Aotearoa New Zealand and most people who get it have recently travelled to developing countries or have had contact with someone who has.[3] Rates of notified shigellosis (4.5 per 100,000 population in 2019) were considerably below rates of gastroenteritis due to other enteric pathogens such as *Campylobacter*, *Salmonella*, *Yersinia* and Shiga toxin-producing *Escherichia coli* (with rates of 126.1, 24.2, 24.1 and 22.4 per 100,000, respectively, in 2019[3]).

Hospital and community laboratories are requested to refer all *Shigella* isolates to ESR for serotyping and biotyping as part of the laboratory-based surveillance of shigellosis. This report describes the antimicrobial susceptibility of the viable, non-duplicate *Shigella* isolates referred to ESR in 2019. This follows a similar project undertaken in 2015 and 2016.[4]

2. Methods

Shigella species identification and serotyping was undertaken in the Enteric Reference Laboratory, ESR via traditional phenotypic methods using biochemical identification tests, and slide agglutination using commercially supplied antisera.[5-10]

Antimicrobial susceptibility was determined by agar dilution according to the methods of the Clinical and Laboratory Standards Institute (CLSI).[11] Except for azithromycin and tetracycline, minimum inhibitory concentrations (MICs) were interpreted according to the 2021 European Committee for Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints.[12] CLSI breakpoints were used to interpret tetracycline MICs.[11] Azithromycin MICs were categorised using EUCAST 'epidemiological cutoff values' (ECOFFs) for *Shigella* species that 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). Multidrug resistance was defined as resistance (including azithromycin NWT) to ≥3 antibiotic classes.[13]

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.[11] 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.[14] Any isolates with a cefoxitin MIC ≥16 mg/L were tested by PCR for plasmid-mediated AmpC β-lactamase genes.[15]

A sub-sample of isolates were characterised by whole genome sequencing (WGS). They included representatives of every *Shigella* type identified in 2019. Genomic DNA was extracted using the Roche High Pure PCR template preparation kit, the DNA library was created using the Nextera XT DNA preparation kit (Illumina), and sequencing was performed using Illumina technology. WGS data was analysed using an in-house developed pipeline linking together open-source established packages and in-house scripts. Open-source packages used included the Nullarbor2: 'Reads to report' for public health and clinical microbiology pipeline SKESA v.2.3.0 (https://github.com/ncbi/SKESA), and ABRicate (https://github.com/tseemann/abricate) with theResFinder database.

Overseas travel history for shigellosis cases was obtained from information reported in the EpiSurv notifiable disease database and supplemented with any travel information received when the isolate from the case was referred to ESR. Similarly, the EpiSurv database was used to obtain data on males who have had sexual contact with other males. The chi-square test or Fisher's Exact test, as appropriate, were used to determine the significance of any observed differences, with a p value of ≤ 0.05 being considered significant.

3. Results

In 2019 222 cases of shigellosis notified.[3] Of these 199 cases were culture confirmed at ESR's Enteric Reference Laboratory, and 192 viable isolates were available for antimicrobial susceptibility testing. The 192 *Shigella* comprised 103 (53.6%) *S. sonnei*, 82 (42.7%) *S. flexneri*, 3 (1.6%) *S. boydii*, 3 (1.6%) *S. dysenteriae,* and one (0.5%) *Shigella* isolate that was unable to be differentiated to species level (Table 1).

Table 1: Distribution of species, serotypes and biotypes among *Shigella* isolated in New Zealand, 2019

Species, biotypes and/or serotypes	Number of isolates	Percent of total isolates
Shigella boydii	**3**	**1.6**
serotype 1	1	0.5
serotype 10	1	0.5
not-typable[a]	1	0.5
Shigella dysenteriae	**3**	**1.6**
serotype 3	1	0.5
not-typable[a]	2	1.0
Shigella flexneri	**82**	**42.7**
serotype 1a	6	3.1
serotype 1b	14	7.3
serotype 1c	8	4.2
serotype 2a	18	9.4
serotype 2b	5	2.6
serotype 3a	5	2.6
serotype 3b	4	2.1
serotype 4av	2	1.0
serotype 4b	3	1.6
serotype 6 biotype Boyd 88	12	6.3
serotype X	1	0.5
serotype Y variant	2	1.0
not-typable[a]	2	1.0
Shigella sonnei	**103**	**53.6**
biotype a	31	16.1
biotype f	1	0.5
biotype g	71	37.0
***Shigella* species[a]**	**1**	**0.5**
Total	**192**	**100.0**

a Not-typable using antisera held by ESR.

Both resistance to the ten 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 ($p < 0.001$) and chloramphenicol ($p < 0.001$), and more likely to be multiresistant (p 0.010). Conversely, *S. sonnei* were significantly more resistant to azithromycin ($p < 0.001$) and co-trimoxazole (p 0.039) (Table 2). Data on the susceptibility to ten antimicrobials tested is shown in Table A6 in the Appendix.

Table 2: Antimicrobial resistance among *Shigella* isolated in New Zealand, 2019

Antimicrobial	Percent resistant					
	S. sonnei **n = 103**	***S. flexneri*** **n = 82**	***S. boydii*** **n = 3**	***S. dysenteriae*** **n = 3**	***Shigella*** **species n = 1**	**All species n = 192**
Ampicillin	39.8	69.5	33.3	100	100	53.7
Azithromycin[a]	29.1	6.1	0.0	33.3	0.0	18.8
Ceftriaxone	12.6	4.9	0.0	33.3	100	9.9
Chloramphenicol	4.9	48.8	0.0	66.7	0.0	24.5
Co-trimoxazole	65.1	50.0	66.7	66.7	0.0	58.3
Fluoroquinolones[b,c]	35.9	32.9	33.3	66.7	100.0	35.4
- Ciprofloxacin[d]	34.0	26.8	0.0	33.3	100	30.7
- Norfloxacin[e]	35.9	32.9	33.3	66.7	100	35.4
Gentamicin	0.0	0.0	0.0	0.0	0.0	0.0
Meropenem	0.0	0.0	0.0	0.0	0.0	0.0
Tetracycline	40.8	43.9	33.3	100	100	43.2
Fluoroquinolone[b] + co-trimoxazole	29.1	25.6	33.3	33.3	0.0	27.6
Fluoroquinolone[b] + co-trimoxazole + azithromycin[a]	23.3	2.4	0.0	0.0	0.0	13.5
Multiresistant to ≥3 antimicrobial classes	40.8	59.8	33.3	100	100.0	50.0

a Azithromycin resistance defined as isolates with an MIC above the EUCAST 2021 ECOFFs (>16 mg/L).
b Fluoroquinolone resistance includes all isolates that were norfloxacin- or ciprofloxacin-resistant.
c Six norfloxacin-resistant isolates that were categorised as susceptible, increased exposure to ciprofloxacin, and three norfloxacin-resistant isolates were ciprofloxacin susceptible.
d 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[16] recommends that fluoroquinolones should not be prescribed for the treatment of shigellosis if the ciprofloxacin MIC is ≥0.12 mg/L. The percentage of isolates that had ciprofloxacin MICs ≥0.12 mg/L were: *S. sonnei* 54.4%, *S. flexneri* 41.5%, *S. boydii* 100%, *S. dysenteriae* 100%, *Shigella* spp. 100% and all species 50.5%.
e The EUCAST clinical breakpoints were used to interpret the norfloxacin MICs, however, these breakpoints are specifically for uncomplicated urinary tract infections.

There were significant differences in resistance between the two prevalent *S. sonnei* biotypes (Table 3). Compared with *S. sonnei* biotype a isolates, *S. sonnei* biotype g isolates were significantly more likely to be azithromycin NWT (p <0.001), more resistant to ampicillin (p < 0.001), ceftriaxone (p 0.011), fluoroquinolones (p <0.001), co-trimoxazole (p <0.001) and tetracycline (p <0.001); and more likely to be multidrug resistant (p <0.001).

Table 3: Antimicrobial resistance among *S. sonnei* biotypes a and g, 2015-16 and 2019

Antimicrobial	Percent resistant			
	***S. sonnei* biotype a**		***S. sonnei* biotype g**	
	2015-16[a] n = 50	**2019 n = 31**	**2015-16[a] n = 90**	**2019 n = 71**
Ampicillin	30.0	9.7	27.8	53.5
Azithromycin[b]	0.0	0.0	17.8	42.3
Ceftriaxone	0.0	0.0	10.0	18.3
Chloramphenicol	2.0	6.5	7.8	4.2
Co-trimoxazole	46.0	25.8	73.3	81.7
Fluoroquinolones[c]	0.0	0.0	38.9	52.1
- Ciprofloxacin	0.0	0.0	38.9	49.3
- Norfloxacin[d]	0.0	0.0	38.9	52.1
Gentamicin	0.0	0.0	1.1	0.0
Meropenem	0.0	0.0	0.0	0.0
Tetracycline	2.0	9.7	75.6	53.5
Fluoroquinolone[c] + co-trimoxazole	0.0	0.0	27.8	42.3
Fluoroquinolone[c] + co-trimoxazole + azithromycin[b]	0.0	0.0	2.2	33.8
Multiresistant to ≥3 antimicrobials classes	2.0	6.5	50.0	56.3

a Data from the 2015-2016 survey[4] were interpreted using the 2016 breakpoint tables. There were differences in azithromycin, norfloxacin and gentamicin breakpoints. For azithromycin isolates were classified NWT in 2015 and 2016 as those with MICs >16 mg/L and >32 mg/L respectively. For gentamicin isolates with an MIC >4 mg/L were resistant in 2016, but this changed to >2 mg/L in 2019. For norfloxacin isolates with an MIC >1 mg/L were resistant in 2016, but this changed to >0.5 mg/L in 2019.
b Azithromycin resistance defined as isolates with an MIC above the EUCAST 2021 ECOFFs (>16 mg/L).
c Fluoroquinolone resistance includes all isolates that were norfloxacin- or ciprofloxacin-resistant.
d The EUCAST clinical breakpoints were used to interpret the norfloxacin MICs, however, these breakpoints are specifically for uncomplicated urinary tract infections.

When compared with results from 2015-2016[4], rates of resistance for *Shigella* spp. from 2019 were higher for ampicillin, ceftriaxone, co-trimoxazole, ciprofloxacin, but lower for tetracycline. Rates of azithromycin NWT isolates were also higher in 2019, even when the

2019 breakpoints were applied to data from 2015-16. The differences in rates of resistance were largely attributable to the increased percentage of resistance found in *S. sonnei* biotype g (Table 3).

There is no current Best Practice Advocacy Centre (BPAC) guideline for the treatment of *Shigella* in New Zealand. The Australian Therapeutic Guidelines no longer recommend an oral empiric treatment option when treatment of shigellosis is indicated, due to high rates of resistance.[17,18] Instead, prescribers are advised to seek advice from the local public health authority about local susceptibility patterns to guide treatment. In our cohort of isolates, the rates of resistance were relatively high for oral treatment options fluoroquinolones and co-trimoxazole (35.4% and 58.3%, respectively), although, as noted above, rates of resistance in *S. sonnei* to these two antimicrobials were quite variable by biotype (Table 3). 27.6% (53/192) of isolates were resistant to both fluoroquinolones and co-trimoxazole (Table 2), which has increased since 2015-2016 when 16.3% dual resistance was found.[4] Similar proportions of *S. sonnei* (29.1%, 30/103) and *S. flexneri* (25.6%, 21/82) had dual resistance to fluoroquinolones 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 68.9% (71/103) of the *S. sonnei* isolates but 100% (30/30) of the dual-resistant *S. sonnei*.

Thirty-six (18.8%) *Shigella* isolates were categorised as azithromycin NWT (Table 2). All azithromycin NWT isolates were *S. sonnei* biotype g (83.3%, 30/36), *S. flexneri* serotype 2a (13.9%, 5/36), or *S. dysenteriae* not typable (2.8%, 1/36). 42.3% (30/71) of *S. sonnei* biotype g were azithromycin NWT and 27.8% (5/18) of *S. flexneri* serotype 2a were azithromycin NWT. Twenty-four (23.3%) *S. sonnei* isolates were resistant to all three antibiotic classes recommended for treatment, that is azithromycin NWT, fluoroquinolone resistant and co-trimoxazole resistant, and all were *S. sonnei* biotype g. Travel history was recorded for four of the 24 cases, who reported travel to Spain, the UK, Vanuatu and the Cook Islands.

Nineteen isolates (9.9%) were ceftriaxone resistant and all 19 had a CTX-M type ESBL: 11 had a CTX-M group 1 ESBL and eight had a CTX-M group 9 ESBL. 68.4% (13/19) were *S. sonnei* biotype g, four were *S. flexneri*, one was *S. dysenteriae* and one was *Shigella* spp. One isolate was cefoxitin resistant. This *S. dysenteriae* isolate had a DHA-1-like plasmid-mediated AmpC β-lactamase, but was susceptible to ceftriaxone.

There was good correlation between the phenotype and the genotype of the 54 isolates characterised by WGS (Appendix, Table A7). Isolates that were phenotypically resistant to ampicillin, ceftriaxone, chloramphenicol, ciprofloxacin, tetracycline, trimethoprim, and azithromycin NWT were all consistent with their antibiotic resistance genotype. The association between phenotype and genotype was not as well defined for co-trimoxazole and fluoroquinolones. All 27 co-trimoxazole resistant isolates contained at least one trimethoprim resistance gene (*dfrA*), with 25 out of 27 also carrying a sulphonamide resistance gene (*sul*). However, two isolates that contained *dfrA* and *sul* were co-trimoxazole susceptible. The plasmid-mediated *qnr* fluoroquinolone resistance genes were detected in 11 of the 54 isolates. All 11 isolates had MICs greater than 0.12 mg/L, the treatment breakpoint recommended by CDC.[16] This data indicates that the treatment breakpoint assigned by CDC is a good predictor of *qnr* gene carriage. Fluoroquinolone-resistance in isolates without a *qnr* gene is likely due to chromosomal mutations in the *gyrA* or *parC* genes or due to drug-efflux pump mechanisms. One *S. flexneri* isolate containing *tetA* was susceptible to tetracycline (MIC of 1 mg/L). While isolates with *tetA* gene are usually tetracycline resistant, expression of *tetA* may be regulated by a repressor protein in the absence of the antibiotic.[19] Exposure to tetracycline in this case may cause this isolate to revert to tetracycline resistance if used for treatment.

All isolates were susceptible to gentamicin and no 16S rRNA methyltransferases genes were identified that would likely confer resistance to this antibiotic. However, aminoglycoside modifying enzyme genes *aad* and *aph* were found in 35 of the 54 isolates sequenced. While *aad* and *aph* carriage did not express as gentamicin resistance in our isolates, these genes may be significant as they can confer resistance to other aminoglycosides not included in this surveillance.

Susceptibility among *Shigella* isolates was compared between cases who reported recent travelled overseas, compared with non-travellers. Azithromycin NWT was significantly more prevalent among cases who had not travelled (Table 4). Further analysis according to the *Shigella* species and biotype, showed that *S. sonnei* biotype g isolates from patients who had not travelled were significantly more likely to be ampicillin resistant (77.1 vs 30.5% p <0.001), azithromycin NWT (71.4 vs 13.9% p <0.001) and ciprofloxacin resistant (65.7 vs 33.3% p 0.006). Rate of tetracycline resistance in *Shigella* isolates was significantly higher in cases who had travelled overseas (Table 4). *S. sonnei* biotype g from cases who had

travelled were significantly more likely to be tetracycline resistant (66.7 vs 40% *p* 0.024) and made up 24 of the 55 tetracycline resistant cases who had travelled overseas.

Table 4: Antimicrobial resistance among *Shigella* from cases who had travelled overseas compared with non-travellers, 2019

Antimicrobial	Percent resistant		*p* value for significance of any difference in resistance between travellers and non-travellers
	Cases who had travelled overseas n = 109	Cases who had not travelled overseas n = 83	
Ampicillin	47.7	61.5	0.059
Azithromycin[a]	8.3	32.5	<0.001
Ceftriaxone	10.1	9.6	0.917
Chloramphenicol	26.6	21.6	0.432
Co-trimoxazole	56.9	60.2	0.640
Fluoroquinolone	32.1	39.8	0.272
- Ciprofloxacin	25.7	37.4	0.083
- Norfloxacin	32.1	39.8	0.272
Gentamicin	0.0	0.0	-
Meropenem	0.0	0.0	-
Tetracycline	50.5	33.7	0.021
Fluoroquinolone + co-trimoxazole	21.1	36.1	0.021
Fluoroquinolone + co-trimoxazole + azithromycin[a]	3.7	26.5	<0.001
Multiresistant to ≥3 antimicrobial classes	46.8	54.2	0.308

a Azithromycin resistance defined as isolates with an MIC above the EUCAST 2021 ECOFFs (>16 mg/L).

Antimicrobial resistance among *Shigella* spp., including azithromycin non-susceptibility, has been reported in several other countries (including Australia, North America, England and Asia) to be associated with *Shigella* isolated from men who have sex with men (MSM).[20-23] As part of notifying cases of shigellosis in New Zealand, data has been collected on whether males have had sexual contact with other males since 2017. Data related to sexual contact was available for 29 isolates (9.9%), with ten of these from MSM. Of these seven were *S. sonnei* biotype g, two were *S. flexneri* 2a and one was *S. sonnei* biotype a. Isolates from MSM were more likely to be azithromycin NWT (*p* 0.046) although there were no other significant differences in antibiotic susceptibility or association with overseas travel.

An analysis of total 36 azithromycin-NWT *Shigella* by the age and sex of the patients is presented in Table 5. Prevalence of azithromycin-NWT among isolates from males is higher than that found in females (males 31.3% vs females 5.6%, *p* = <0.001). In a further breakdown by age group, azithromycin NWT isolates were more prevalent in the 20-39 and 40-59 age groups and differences were statistically significant. Twenty-seven (87.1%) of the 31 azithromycin-NWT isolates from males were *S. sonnei* biotype g compared with three (60.0%) of the five azithromycin-NWT isolates from females.

Among the other antimicrobials, only the prevalence of ampicillin resistance was significantly different between the sexes with 62.6% of *Shigella* from males being resistant compared with 43.8% of isolates from females.

Table 5: Age and sex distribution of patients with azithromycin-non-wild type *Shigella,* 2019

Age group (years)	Azithromycin non-wild type (n = 36)		*p* value for significance of any difference in azithromycin susceptibility between females and males
	Number of isolates (%[a])		
	From female cases	From male cases	
<20	3 (20.0)	0 (0)	0.101
20-39	2 (6.1)	17 (42.5)	0.002
40-59	0 (0)	11 (44.0)	<0.001
≥60	0 (0)	2 (11.8)	0.216
Unknown	- -	1 (-)	
Total	**5 (5.6)**	**31 (31.3)**	<0.001

a There were 89 female cases of shigellosis: 15 in the <20 years age group, 33 in the 20-39 years age group, 22 in the 40-59 years age group and 19 in the ≥60 age group. There were 99 male cases of shigellosis: 16 in the <20 years age group, 40 in the 20-39 years age group, 25 in the 40-59 years age group, 17 in the ≥60 age group and one of unknown age. These case numbers were used as the denominators to calculate the percentage of azithromycin NWT among isolates for each sex and age group.

4. Conclusions

Antimicrobial resistance in *Shigella* spp. continues to be a burgeoning problem in Aotearoa New Zealand. Shigellosis notifications increased from 2.4 to 4.5 per 100,000 population between 2015 and 2019.[3,24] Similarly, the percentage of antibiotic resistance found in *Shigella* isolates also increased for a number of antibiotics over the same time period. Isolates that were resistant to the three antibiotics recommended for treatment (azithromycin, fluoroquinolone and co-trimoxazole) were predominantly *S. sonnei* biotype g. Additionally, over half of *S. flexneri* and *S. sonnei* biotype g isolates were multi-resistant to at least 3 or more antimicrobial classes. These resistance profiles will affect empiric treatment, when required.

While more than half of shigellosis infections were from cases who had reported travelling overseas, shigellosis in people who did not report overseas travel were more likely to be azithromycin non-wild type and multi-resistant to the three antibiotic classes recommended for treatment. However, a limitation of this surveillance is that overseas travel may have been under-reported among shigellosis cases. This is highlighted by the observation that only five cases of shigellosis were notified in 2021, which was the first full year with border restrictions imposed by the New Zealand government during the ongoing SARS-CoV-2 pandemic, suggesting travel may play a more important role in shigellosis acquisition than previously thought.

In New Zealand, epidemiological data on shigellosis cases is collected through EpiSurv when each case is notified. This data has highlighted specific risk factors that are associated with antibiotic resistant strains of *Shigella*. However, the large amount of missing data, particularly related to sexual contact, emphasises that data collection could be improved. Without improved data collection it will continue to be difficult to draw conclusions related to shigellosis and sexual contact in New Zealand. It is important to collect data on both sexual contact and travel history, as both have been linked with antibiotic resistance trends observed overseas.

Globally there have been increasing reports of extensively drug-resistant (XDR) *Shigella* infections, most notably among the sexual contact network of MSM.[25-26] XDR Shigella has been defined as resistant to all oral antibiotics available for treatment.[25] In Australia the

increase in XDR shigellosis has been driven by a clonal lineage of *S. sonnei* which was transmitted through the MSM network.[25] In 2022 the UK Health Security Agency reported an outbreak of *Shigella* among MSM that was non-susceptible to the oral antibiotics (azithromycin, quinolones and sulphonamides) and also to third generation cephalosporins, aminoglycosides and tetracycline to the Early Warning and Response System.[26-27] Subsequently, nine EU countries reported cases which was genetically linked to the UK cluster.[26]

The addition of WGS to characterise *Shigella* is an invaluable tool that has the potential to recognise transmission networks and emergence of XDR *Shigella* strains, both locally and internationally, by identifying both resistance genes and clonal lineages in addition to facilitating easy comparison of data with other institutions. Future surveillance of *Shigella* in New Zealand should incorporate routine WGS analysis to provide a more comprehensive, real-time surveillance to inform and improve public health and clinical outcomes for shigellosis cases. The availability of WGS data would ensure that we monitor antibiotic resistance genes present in *Shigella* in near real time, rather than waiting for the periodic surveys that are currently used. The availability of WGS data also means that we able to compare our data with international datasets, to detect lineages associated with XDR *Shigella* if they are imported into New Zealand.

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6. Appendix

Table A6: Antimicrobial susceptibility among *Shigella* isolated in New Zealand, 2019

Antimicrobial	Percent susceptible					
	S. sonnei **n = 103**	***S. flexneri*** **n = 82**	***S. boydii*** **n = 3**	***S. dysenteriae*** **n = 3**	***Shigella*** **species n = 1**	**All species n = 192**
Ampicillin	60.2	30.5	66.7	0.0	0.0	46.4
Azithromycin[a]	70.9	93.9	100.0	66.7	100.0	81.3
Ceftriaxone	86.4	95.1	100.0	66.7	0.0	89.6
Chloramphenicol	95.2	51.2	100.0	33.3	100.0	75.5
Co-trimoxazole	34.0	50.0	33.3	33.3	100.0	41.2
Fluoroquinolones[b,c]	64.1	67.1	66.7	33.3	0.0	64.6
- Ciprofloxacin[c]	65.1	69.5	66.7	33.3	0.0	66.2
- Norfloxacin[d]	64.1	67.1	66.7	33.3	0.0	64.6
Gentamicin	100.0	100.0	100.0	100.0	100.0	100.0
Meropenem	100.0	100.0	100.0	100.0	100.0	100.0
Tetracycline	59.2	56.1	66.7	0.0	0.0	56.8

a Azithromycin susceptibility defined as isolates with an MIC ≤16 mg/L.
b Fluoroquinolone susceptibilities includes all isolates that were norfloxacin- and ciprofloxacin-susceptible.
c Three ciprofloxacin susceptible isolates were norfloxacin resistant
d The EUCAST clinical breakpoints were used to interpret the norfloxacin MICs, however, these breakpoints are specifically for uncomplicated urinary tract infections.

Table A7: Correlation between WGS genotype and phenotype

Antimicrobial	Resistance gene(s)	Genes found in resistant isolates	Genes found in susceptible isolates
Ampicillin	*bla*$_{CTX-M-15}$	7	0
	bla$_{CTX-M-27}$	2	0
	bla$_{DHA}$ & *bla*$_{OXA-1}$	1	0
	bla$_{OXA-1}$	11	0
	bla$_{TEM-1}$	6	0
	bla$_{OXA-1}$ & *bla*$_{TEM-1}$	2	0
	No beta-lactamase genes	0	25
Azithromycin	*ermB* & *mphA*	4	0
	mphA	2	0
	No *erm* or *mph* genes	0	48
Ceftriaxone	*bla*$_{CTX-M-15}$	7	0
	bla$_{CTX-M-27}$	2	0
	bla$_{DHA}$	1	0
	No genes conferring ceftriaxone resistance	0	44
Chloramphenicol	*catA*	15	0
	No *catA* genes	0	39
Ciprofloxacin	*qnrB19*	0	2
	qnrB4	0	1[a]
	qnrS1	5	3[a]
	No *qnr* genes	7	36
Co-trimoxazole	*dfrA*1	2	17
	dfrA14 & *sul2*	4	0
	dfrA17 & *sul1*	2	0
	*dfrA*1 & *dfrA*14 & *sul2*	1	0
	*dfrA*1 & *dfrA*17 & *sul1*	0	1
	*dfrA*1 & *dfrA*5 & *sul1*	0	1
	*dfrA*1 & *sul*2	18	0
	No *dfr* or *sul* genes	0	8
Gentamicin[b]	No 16S rRNA methyltransferase genes	0	54
Meropenem	No carbapenemse genes	0	54
Tetracycline	*tetA*	12	1
	tetB	16	0
	No *tet* genes	0	25
Trimethoprim	*dfrA1*	37	0
	dfrA14	4	0
	dfrA17	2	0
	dfrA1 & dfrA14	1	0
	dfrA1 & dfrA17	1	0
	dfrA1 & dfrA5	1	0
	No *dfr* genes	0	8

a Ciprofloxacin susceptible increased exposure