# Antimicrobial resistance among *Shigella* in New Zealand

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## ABSTRACT

**AIM:** We undertook a national survey to provide current information on antimicrobial resistance among *Shigella* isolated in New Zealand.

**METHODS:** Diagnostic laboratories are requested to refer all *Shigella* isolates to the Institute of Environmental Science and Research (ESR) for epidemiological typing as part of the national surveillance of shigellosis. The antimicrobial susceptibility of 263 non-duplicate *Shigella* isolates referred to ESR in 2015 and 2016 was tested.

**RESULTS:** The 263 *Shigella* comprised 141 (53.6%) *S. sonnei*, 113 (43.0%) *S. flexneri*, 7 (2.7%) *S. boydii* and 2 (0.8%) *S. dysenteriae*. Among the 141 *S. sonnei*, the majority were either biotype g (90) or biotype a (50). Rates of resistance to the two currently recommended first-line antibiotics, co-trimoxazole and fluoroquinolones, were relatively high at 56.7% and 22.8%, respectively. Azithromycin is considered a second-line treatment option, but 11.0% of *Shigella* were categorised as having a non-wildtype (NWT) azithromycin phenotype (ie, having some mechanism of azithromycin resistance although not necessarily clinically resistant). There were several significant differences in resistance between the two most prevalent *S. sonnei* biotypes, with resistance being significantly more prevalent among biotype g isolates. *Shigella* from patients who had not travelled overseas were significantly more likely to be azithromycin NWT than isolates from patients who had recently travelled (20.7 vs 5.6%). Azithromycin NWT was more prevalent among *Shigella* from males than females (13.9 vs 7.7%).

**CONCLUSIONS:** These results suggest there is an immediate need to revise the currently recommended first-line treatment for shigellosis, especially when treatment is given on an empirical basis. Equally concerning is the fact that resistance to the second-line antibiotic for shigellosis, azithromycin, appears to be emerging in New Zealand. As diagnostic laboratories increase their use of culture-independent testing, it is recommended that they should continue to culture specimens from all shigellosis cases so that isolates are available for susceptibility testing and epidemiological typing.


*Shigella* is a relatively uncommon cause of gastroenteritis in New Zealand with rates of notified shigellosis (3.7 per 100,000 population in 2016) considerably below rates of gastroenteritis due to other enteric pathogens such as *Campylobacter*, *Salmonella*, *Yersinia*, and verotoxin- or Shiga toxin-producing *Escherichia coli* (158.9, 23.2, 18.3 and 8.9 per 100,000, respectively, in 2016).[1]

The majority (61.2% in 2016) of people diagnosed with shigellosis in New Zealand have been overseas during the incubation period for the disease.[1] *Shigella* is easily passed from person to person as the infectious dose is low. While shigellosis is typically a self-limiting infection, appropriate antibiotic treatment can shorten the duration and severity of illness, and reduce the time *Shigella* is excreted. Therefore, to reduce disease transmission, antibiotic treatment is usually recommended for cases of shigellosis in children <6 years of age, people who are institutionalised, men who have sex with men (MSM), people who are immunosuppressed, and food handlers. Antibiotic treatment is also recommended for patients with severe disease to shorten the duration of symptoms.[2] In 2016, 30.2% of shigellosis cases in New Zealand were admitted to hospital.[1]


ISSN 1175-8716
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The Best Practice Advocacy Centre's (BPAC) recommendations for the treatment of shigellosis, which were published in 2009, recommend either co-trimoxazole (first choice if the organism is susceptible) or alternatively a fluoroquinolone (ciprofloxacin or norfloxacin), with a further specific recommendation of ciprofloxacin when the patient is immunocompromised.[3] The current 2014 Australian Therapeutic Guidelines recommend either a fluoroquinolone or co-trimoxazole when treatment of shigellosis is indicated. If an alternative is required due to resistance to these first-line antibiotics, azithromycin is recommended.[2]

*Shigella* is somewhat notorious for developing antimicrobial resistance and has successively accumulated resistance to most of the antibiotics used for the treatment of infections. There are numerous reports from overseas of high rates of resistance to co-trimoxazole and fluoroquinolones, as well as emerging resistance to azithromycin.[4–6]

A national New Zealand survey in 1996 found that co-trimoxazole resistance was already prevalent among *Shigella* isolated in this country, but no ciprofloxacin resistance was identified and azithromycin susceptibility was not tested.[7] Here we report the results of the first national antimicrobial susceptibility survey of *Shigella* that the Institute of Environmental Science and Research (ESR) has undertaken since the 1996 survey.

# Methods

Diagnostic laboratories are requested to refer all *Shigella* isolates from cases of shigellosis to ESR for serotyping and biotyping as part of the national surveillance of this disease. The antimicrobial susceptibility of viable, non-duplicate *Shigella* isolates referred to ESR in 2015 and 2016 was tested.

Antimicrobial susceptibility was determined by agar dilution according to the methods of the Clinical and Laboratory Standards Institute (CLSI).[8] Except for azithromycin and tetracycline, minimum inhibitory concentrations (MICs) were interpreted according to the European Committee for Antimicrobial Susceptibility Testing (EUCAST) clinical breakpoints.[9] CLSI breakpoints were used to interpret tetracycline MICS.[8] Currently there are no clinical breakpoints to interpret azithromycin MICs. However, for *S. flexneri* and *S. sonnei*, CLSI have defined 'epidemiological cutoff values' (ECVs) for azithromycin MICs.[8] 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).

Any isolates with a ceftriaxone or ceftazidime MIC ≥2 mg/L were tested for extended-spectrum beta-lactamase (ESBL) production using the combination disc test.[8] To identify CTX-M type ESBLs, a multiplex polymerase chain reaction assay (PCR) that includes primers to detect the genes for the four CTX-M groups, 1, 2, 8 and 9, was used.[10] Any isolates with a cefoxitin MIC ≥16 mg/L were tested by PCR for plasmid-mediated AmpC beta-lactamase genes.[11]

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* isolates accounted for 92.3% of the total 285 shigellosis cases notified during these two years. The 263 *Shigella* comprised 141 (53.6%) *S. sonnei*, 113 (43.0%) *S. flexneri*, 7 (2.7%) *S. boydii* and 2 (0.8%) *S. dysenteriae*. Among the 141 *S. sonnei*, the majority were either biotype g (90 isolates) or biotype a (50). There were a wide variety of serotypes among the 113 *S. flexneri*, with the commonest being serotype 2a (31), serotype 1b (20), serotype 2b (10), and serotype 6 biotype Boyd 88 (10).

Resistance to seven of the antimicrobials tested and multiple drug resistance is shown in Table 1. Resistance to the two first-line antibiotics was high, with 56.7% resistance to co-trimoxazole, 22.8% resistance to fluoroquinolones and 16.3% resistance to both co-trimoxazole and a fluoroquinolone. There was complete correlation between resistance to the two fluoroquinolones tested, ciprofloxacin and norfloxacin.

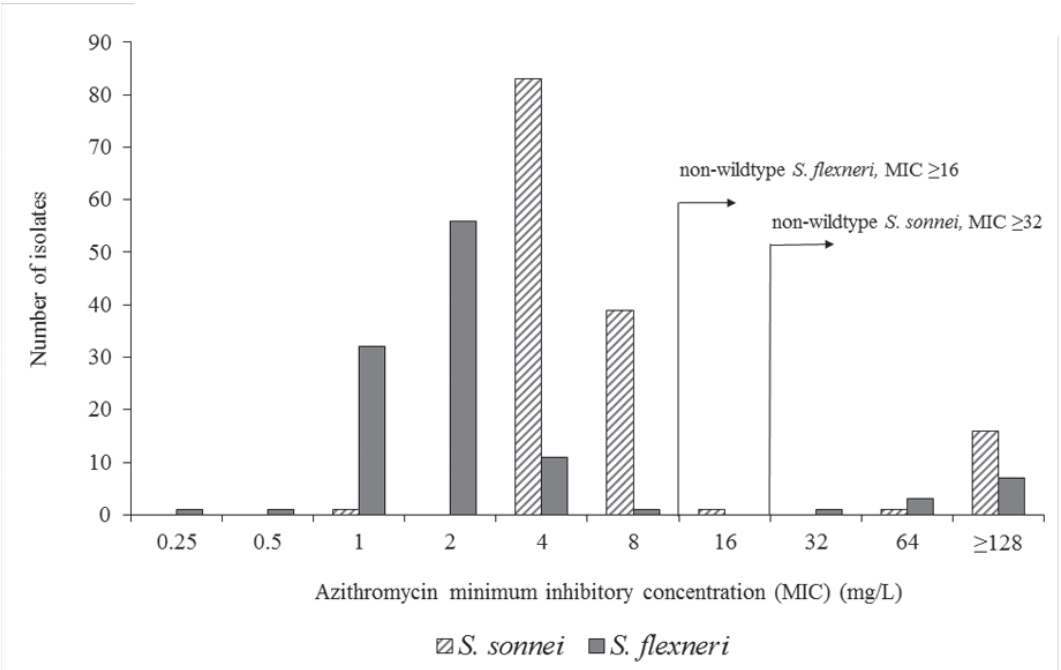
**Table 1:** Antimicrobial resistance among *Shigella* in New Zealand, 2015 and 2016.

| 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 |
| Ciprofloxacin[2] | 25.5 | 20.4 | 14.3 | 0.0 | 22.8 |
| Co-trimoxazole | 63.8 | 49.6 | 14.3 | 100 | 56.7 |
| Norfloxacin | 25.5 | 20.4 | 14.3 | 0.0 | 22.8 |
| 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 |

1. The data given for azithromycin are the percentages 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 azithromycin ECVs for *S. boydii* or *S. dysenteriae*.
2. The rates of ciprofloxacin resistance presented are based on the EUCAST MIC resistance breakpoint of ≥1 mg/L. However, a recent health advisory from the United States Centers for Disease Control and Prevention recommended that fluoroquinolones should not be prescribed for the treatment of shigellosis if the ciprofloxacin MIC is ≥0.12 mg/L (Reference 12). 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%.

Twenty-eight (11.0%) of the *S. sonnei* and *S. flexneri* isolates were categorised as azithromycin NWT (Table 1). The azithromycin MICs of the *S. sonnei* and *S. flexneri* isolates are shown in Figure 1 and demonstrate a typical bimodal distribution. Among the 28 azithromycin NWT isolates, the majority (23) had MICs ≥128 mg/L. Three (2.1%) *S. sonnei* isolates were azithromycin NWT, co-trimoxazole resistant and ciprofloxacin resistant, that is, potentially resistant to all three antibiotic classes recommended for treatment (Table 1). Two of these three *S. sonnei* were biotype g and the remaining isolate was biotype f.

**Figure 1:** Distribution of azithromycin minimum inhibitory concentrations.






NZMJ 22 June 2018, Vol 131 No 1477
ISSN 1175-8716 © NZMA

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

### Comparison of resistance by *Shigella* species and *S. sonnei* biotype

There were some significant differences in resistance between *S. sonnei* and *S. flexneri*. *S. sonnei* were significantly more resistant to co-trimoxazole ($p$ 0.022), whereas *S. flexneri* were significantly more resistant to ampicillin ($p$ <0.001) (Table 1). There were also significant differences in resistance between the two prevalent *S. sonnei* biotypes. Compared with *S. sonnei* biotype a, *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 resistant to both co-trimoxazole and a fluoroquinolone (27.8 vs 0.0%, $p$ <0.001). Notably, *S. sonnei* biotype g accounted for 57.1% (16/28) of all azithromycin-NWT isolates.

### Comparison of resistance among *Shigella* according to whether the patient had recently travelled outside New Zealand

Among the patients from whom the 263 *Shigella* included in the survey were isolated, 63.1% (166) were reported to have recently travelled overseas. Azithromycin was the only antibiotic for which there was a significant difference in susceptibility depending on whether the patient had recently travelled, with *Shigella* from patients who had not travelled being more likely to be azithromycin NWT than isolates from patients who had recently travelled (20.7 vs 5.6%, $p$ 0.001). Further analysis according to the *Shigella* species and biotype, showed that specifically *S. sonnei* biotype g and *S. flexneri* from patients who had not travelled were significantly more likely to be azithromycin NWT (36.7 vs 8.3%, $p$ 0.001 and 17.8 vs 4.1%, $p$ 0.019, respectively).

### Comparison of resistance among *Shigella* by patient demographics

Except for tetracycline resistance being more common in *Shigella* isolated from males (55.7 vs 43.1%, $p$ 0.041), there were no significant differences in the rates of resistance depending on the sex of the patient. However, the prevalence of azithromycin NWT among *Shigella* from males was nearly twice that among isolates from females (13.9 vs 7.7%, $p$ 0.117). In a further breakdown by age (using age groups of <20, 20–39, 40–59 and ≥60 years), azithromycin NWT was more prevalent among isolates from males in all age groups except the youngest group, 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 nine azithromycin-NWT isolates from females.

## Discussion

Our results suggest there is an immediate need to revise the recommended treatment for shigellosis, especially when treatment is given on an empirical basis. The rates of resistance to the two currently recommended first-line antibiotics are relatively high: 56.7% co-trimoxazole resistance and 22.8% fluoroquinolone (ciprofloxacin or norfloxacin) resistance. Interestingly this rate of co-trimoxazole resistance is almost exactly the same as the rate of 57.0% reported in the last national survey conducted in 1996.[7]

In contrast, during the intervening 20 years between the surveys, fluoroquinolone resistance has emerged (from zero) and risen to a point where nearly a quarter of *Shigella* are resistant. Moreover, it should be noted that the potential for fluoroquinolone treatment failure could be somewhat higher than indicated by the rate of 22.8% resistance we have reported here. We used the EUCAST MIC clinical breakpoint of ≥1mg/L to categorise ciprofloxacin resistance. A recent health advisory from the United States Centers for Disease Control and Prevention (CDC) recommends that fluoroquinolones should not be prescribed for shigellosis if the ciprofloxacin MIC is ≥0.12mg/L, as it is likely *Shigella* with MICs as low as 0.12mg/L

harbour at least one resistance gene known to confer reduced susceptibility to fluoroquinolones in enteric bacteria.[12] However, while CDC is cautioning that fluoroquinolones should not be used to treat infections with *Shigella* with ciprofloxacin MICs as low as 0.12mg/L, it is not yet known if such treatment does actually result in a worse clinical outcome or increase the risk of transmission of the infection to contacts. A third (33.8%) of the *Shigella* in our survey had ciprofloxacin MICs ≥0.12mg/L (see footnote 2, Table 1).

Equally concerning is the fact that resistance to the second-line antibiotic for shigellosis, azithromycin, appears to be emerging in New Zealand. Twenty-eight (11.0%) *Shigella* were categorised as azithromycin NWT (non-wild type), as they had MICs above those of the wild-type population. While this NWT categorisation does not necessarily mean these isolates would be clinically resistant, 23 of the 28 isolates had relatively high azithromycin MICs of ≥128 mg/L and therefore are likely to be clinically resistant.

Ceftriaxone can be useful to treat severe and invasive shigellosis.[13] Ceftriaxone resistance still appears to be relatively uncommon among *Shigella* isolated in this country, with just 5.7% resistance. However, all ceftriaxone resistance was mediated by CTX-M type extended-spectrum beta-lactamases. The genes for this type of ceftriaxone resistance are readily transmissible between different strains of enteric bacteria and are often transmitted along with genes conferring resistance to several other classes of antibiotics.

The rates of resistance among *Shigella* isolated in New Zealand in 2015–16 are similar to, or even higher than, rates reported elsewhere in the world.[4–6] One of the most distinctive features of our results was that resistance was not more prevalent among *Shigella* apparently acquired overseas. This finding contrasts to the usual situation with antimicrobial resistance in New Zealand. A global review by the World Health Organization, published in 2014, reported that New Zealand had relatively low rates of antimicrobial resistance compared with most other countries and regions.[14] ESR's regular surveillance of resistance among another enteric pathogen, *Salmonella,* has consistently shown that infections acquired overseas are more resistant than those acquired in New Zealand.[15]

However, many countries are reporting both recent increases in the incidence of shigellosis and increasing levels of resistance among *Shigella*. In particular, there are reports from several developed countries (including Australia, the US, Canada and England) of locally-acquired (ie, non-travelled associated) shigellosis being associated in particular with MSM, and that resistance, especially to azithromycin, is prevalent among *Shigella* infections in MSM.[4,5,16–19] It has been suggested that the use of azithromycin to treat sexually transmitted infections, specifically gonorrhoea and chlamydia, may be exerting selective pressure for the emergence and spread of azithromycin-resistant *Shigella* among MSM.[5,16]

During the years covered by this survey, information on sexual practices was not routinely collected for shigellosis cases in New Zealand. However, as shigellosis among MSM is a recognised public health issue, in December 2017 the risk factor information collected for shigellosis notifications was extended to include a question on the sexual practices of male cases.

In the absence of any information on the sexual practices of the shigellosis cases included in this survey, we investigated if there were any significant differences in resistance according to the sex and age of the patient. While at the 95% probability level, the only difference was a higher rate of tetracycline resistance among isolates from males, *Shigella* from males were almost twice as likely to be azithromycin NWT as those from females but, due to the relatively small total number (28) of azithromycin-NWT isolates, this difference by the sex of the patient did not reach statistical significance. The higher rate of azithromycin NWT in males, which was particularly evident in the older age groups, coupled with the fact that azithromycin NWT was significantly more common among *Shigella* from people who had not travelled overseas, suggests azithromycin-NWT *Shigella* infections may also be associated with MSM in New Zealand.

Our results support the need for routine antimicrobial susceptibility testing of *Shigella* isolates from all cases to inform appropriate treatment options for individual patients and also to monitor changes in resistance patterns and guide empiric therapy. Diagnostic laboratories are increasingly introducing so-called 'culture-independent diagnostic testing', such as nucleic acid amplification tests (NAAT), to diagnose pathogens including enteric pathogens like *Shigella*. However, it is important that laboratories continue to also culture specimens from shigellosis cases, as a culture is required to perform susceptibility testing and also to undertake current subtyping methods (such as serotyping and biotyping) to provide epidemiological information.

In conclusion, while shigellosis is usually a self-limiting infection, antibiotic treatment is recommended for severe cases and also to reduce disease transmission among certain patient groups. However, our results show there are high rates of resistance to co-trimoxazole and fluoroquinolones, the antibiotics currently recommended for treatment, and also emerging resistance to the second-line treatment azithromycin, among *Shigella* in New Zealand. ESR plans to monitor trends in resistance among *Shigella* with more regular (three-yearly) national surveys.

**Competing interests:**
Nil.

**Acknowledgements:**
Staff of the ESR Antimicrobial Reference Laboratory for antimicrobial susceptibility testing and the ESR Enteric Reference Laboratory for *Shigella* identification and typing. This survey was funded by the Ministry of Health.

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