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New Zealand *Shigella* typing and antimicrobial resistance summary for 2022 and 2023

May 2024

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EXECUTIVE SUMMARY

New Zealand (NZ) shigellosis case numbers are rebounding following the opening of NZ's borders in July 2022.

Detections of extensively drug resistant (XDR) strains are increasing and there is evidence of local XDR transmission.

Routine genomic analysis of NZ's *Shigella* isolates has made a significant, positive contribution to our understanding of the epidemiology of *Shigella* in NZ, including XDR strains.

This report summarises the results of the genomic surveillance of *Shigella species* isolated from shigellosis cases in NZ, principally from 2022 and 2023 and builds on a previous report circulated in November 2023 and an updated version is reproduced here as Appendix A. An in-depth analysis of the epidemiology of these cases is outside of the scope of this report.

The purpose of this report is to disseminate information for action on shigellosis in New Zealand in a timely manner and ESR recommends that:

- Health agencies and professionals are aware of the continuing emergence of XDR *Shigella* in NZ and the clinical and public health implications of these organisms.
- NZ prepares for increasing numbers of XDR shigellosis cases, particularly among returning international travellers and men who have sex with men (MSM) communities and that there is comprehensive and timely epidemiological data collection on these risk factors to support outbreak detection and investigation.
- Clinical laboratories all report susceptibility to the following antimicrobials to assist in recognising XDR both at the local level; and also in their e-notifications for national surveillance purposes: Ampicillin, Azithromycin, Ceftriaxone, Ciprofloxacin, and Co-trimoxazole.
- Clinical laboratories refer all *Shigella* isolates to ESR in a timely manner for epidemiological typing.
- This report is followed up by regular quarterly reports but that interim alerts should be prepared as the need arises.

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1. Background

Shigellosis is an acute gastrointestinal illness characterised by fever, abdominal cramps, blood or mucus in the stools and a high secondary attack rate among contacts. *Shigella* are unusual among enteric bacteria for the following reasons:

- There are no known animal reservoirs.
- Transmission is via direct or indirect faecal-oral transfer. Food or water may become contaminated and act as vehicles; and transmission can also occur through sexual contact, with men who have sex with men (MSM) particularly at risk.
- A very low dose (approximately 10 organisms) is required to cause an infection, therefore person-to-person transmission is common.
- Shigellosis can be a life-threatening disease, with older and younger people particularly at risk and some *Shigella species* are more likely to cause more severe disease than others.
- Antibiotic treatment is usual in severe cases.
- Antimicrobial resistance is a present and growing concern for this species, with extensively drug resistant (XDR) and multiple drug resistant (MDR) lineages increasingly reported worldwide.

The genus *Shigella* comprises four species: *Shigella dysenteriae* (*S.*), *S. flexneri*, *S. boydii* and *S. sonnei*. Historically these species have been further phenotypically differentiated by serotyping or biotyping (*S. sonnei*).

Shigellosis is a notifiable disease in New Zealand (NZ) requiring specific public health actions and follow up. Confirmed cases require an isolate to be culture confirmed as *Shigella species* – a culture independent diagnostic test yielding a positive *Shigella*/Entero Invasive *Escherichia coli* (*E. coli*) result is insufficient for confirmation[1]. Furthermore, clinical laboratories are required to refer all isolates to ESR for epidemiological typing[2].

Public Health Services follow up notified cases and record epidemiological and risk information in the EpiSurv notifiable disease surveillance system.

In New Zealand, most shigellosis cases are overseas acquired, or are diagnosed in people in direct contact with overseas travellers, although local outbreaks have occurred within the MSM community.

Shigellosis case numbers were markedly impacted by NZ's border closure as part of the national COVID 19 response; and, as seen in Figure 1, are now rebounding towards pre COVID levels.

Epidemics of shigellosis due to drug resistant *Shigella* lineages are being reported internationally, predominantly among MSM, and is a serious emerging public health issue (Charles et al., 2022) (Mason et al., 2023).

Antimicrobial resistance in *Shigella* has previously been reported in NZ (Heffernan et al., 2018) (Tiong et al., 2022).

[1] https://www.tewhatuora.govt.nz/for-the-health-sector/health-sector-guidance/communicable-disease-control-manual/shigellosis/
[2] Appendix 4: Direct laboratory notification of communicable diseases flowcharts – Health New Zealand | Te Whatu Ora

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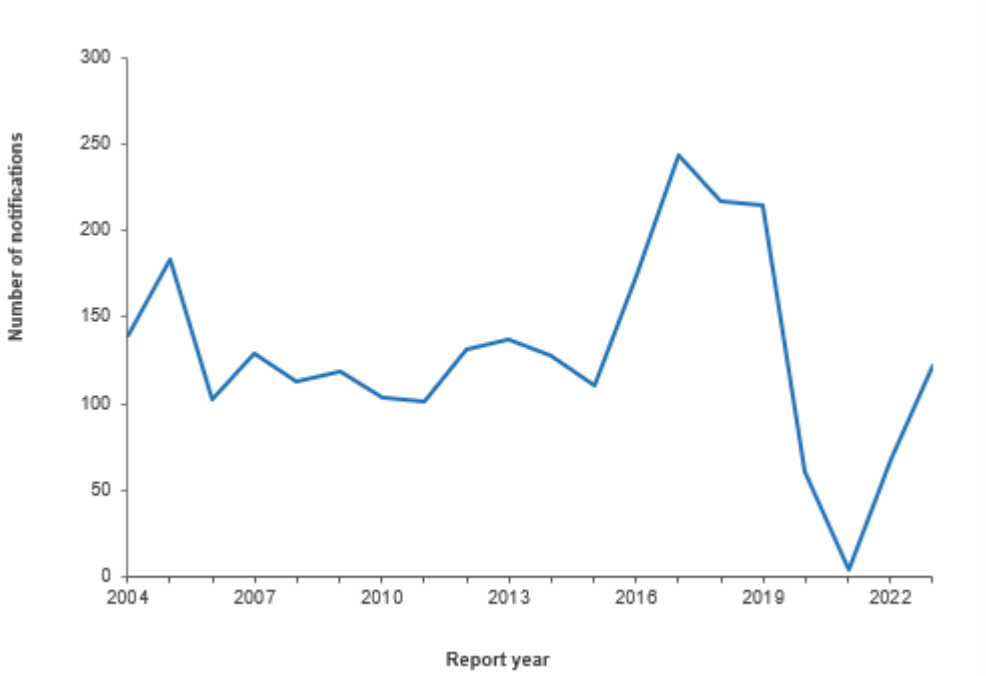
The re-opening of NZ's borders following COVID-19 travel restrictions has led to increased *Shigella* notifications, including XDR and MDR isolates.

Figure 1. Shigellosis notifications by year, 2004–2023. Data is based on notifications recorded in EpiSurv as at 23 February 2024.



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2. Definitions and explanations

2.1 EXTENSIVELY DRUG RESISTANT (XDR)

XDR *Shigella* are defined here as resistant to all commonly recommended empiric and alternative antibiotics — Ampicillin, Azithromycin, Ceftriaxone, Ciprofloxacin (quinolone), and Trimethoprim-Sulfamethoxazole (TMP-SMX)[3].

In most cases, resistance to these treatments is encoded by specific genes, that can be detected from results generated via whole genome sequencing (WGS) analysis as follows:

- **Ampicillin***: blaOXA* genes or blaTEM-1, and any of the genes named below under Ceftriaxone must be recognised as ampicillin resistant when interpreting WGS AMR results.
- **Azithromycin**: *ermB* and/or *mphA*
- **Ceftriaxone** (Kayama et al., 2023)**:**

1. ESBL: any *blaCTX-M*; any *VEB*; any *SHV* or *TEM* shown to confer ceftriaxone resistance
2. AmpC: any *blaDHA* or *blaCMY* genes (acknowledging that *blaDHA-1* results in clinical resistance only 60% of the time)
3. and any potential carbapenem resistance conferring genes including: *GES*, *IMP*, *NDM*, *OXA*, *VIM* genes.

- **Co-trimoxazole (**Trimethoprim-Sulfamethoxazole, TMP-SMX**):** *dfrA* **and** *sul*
- **Quinolone:** (including ciprofloxacin) is more complex, as this can be encoded by:
  1. plasmid-mediated quinolone-resistance regions (*qnr* genes)
  2. and/or mutations in quinolone resistance-determining regions (QRDR denoted by *gyr* and *par* genes)

  The presence of multiple genes indicates unequivocal quinolone resistance.
  The presence of some genes in isolation appears more likely to confer clinical resistance than others, such as *qnr*S1 compared with *qnr*B19; and some are more likely to indicate Intermediate for surveillance purposes than others, such the S83L mutation in *gyrA* compared with the S83A mutation in that gene.

2.2 MULTI DRUG RESISTANT (MDR)

MDR *Shigella* bacteria are defined here as those resistant to any three of the following: Azithromycin, Ceftriaxone, Ciprofloxacin, Co-trimoxazole[4].

2.3 EMERGING XDR (EXDR)

As described above, the genetic basis of quinolone resistance is complex with several different genes and mutations able to combine to confer clinical resistance. Review of NZ data has identified an additional category of isolates resistant to Azithromycin, Ceftriaxone, and Co-trimoxazole and with decreased susceptibility to quinolones (MIC ≥0.12 mg/L). As the acquisition of quinolone resistance can be stepwise (with each new mutation or gene decreasing susceptibility further), for the purposes of this document ESR has labelled this category “emerging XDR” (eXDR).

[3] emergency.cdc.gov/han/2023/han00486.asp
[4] https://wwwnc.cdc.gov/eid/article/22/6/15-2088_article

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3. METHODOLOGY

3.1 ESR *SHIGELLA* IDENTIFICATION PROCESS USING WGS

The ESR genomic *Shigella* analysis platform was validated and implemented into routine use in 2023[5].

In 2024 the ESR methodology received accreditation from International Accreditation NZ (IANZ)[6][7]. The methodology is summarised as follows:

Genomic DNA is extracted via the Chemagic™ 360 extraction platform (PerkinElmer, Waltham, MA, USA) and genomes are sequenced on the Illumina NextSeq platform (Illumina, San Diego, CA, USA) plexWell96 library kit (prior to routine sequencing of Shigella some isolates were prepared using the Nextera XT kit). Outputs are initially analysed via an in-house WGS read quality assessment pipeline and assembled using open source tools[8][9][10][11][12].

All isolates that passed ESR's pre-set quality parameters (including number of contigs <450 and sequencing depth >45) proceeded to the ESR in-house WGS *Shigella* typing pipeline, based on published (Wu et al., 2019), (Sherry et al., 2023), and open source tools[13][14][15][16]. For each isolate the WGS data is used to infer the species and identify key genes and mutations associated with antibiotic resistance. Additionally, *S. sonnei* isolates are assigned to an internationally recognized genotype following the framework of Hawkey (Hawkey et al., 2021).

Finer genomic typing analysis methodology is detailed subsequent sections.

3.2 REPORT DATA

Case information on shigellosis notifications (including clinical laboratory e-notifications for the years 2022 and 2023) were extracted from EpiSurv on March 11, 2024.
Notification data were merged with ESR laboratory data, which included historic phenotype results in addition to the newly implemented WGS pipeline data.

[5] Validation Report: Shigella Whole genome sequencing analysis, ESR internal report 2023
[6] https://www.ianz.govt.nz/
[7] ISO 15189:2022(en), Medical laboratories — Requirements for quality and competence
[8] https://github.com/OpenGene/fastp
[9] http://www.ccb.jhu.edu/software/centrifuge/
[10] https://github.com/ncbi/SKESA/releases
[11] https://github.com/tseemann/mlst
[12] https://github.com/ablab/quast
[13] https://github.com/MDU-PHL/abritamr
[14] https://github.com/MDU-PHL/abritamr
[15] https://github.com/tseemann/cgmlst-dists
[16] https://github.com/achtman-lab/GrapeTree

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4. New and changed reporting information from WGS analysis

4.1 IMPLEMENTATION OF GENOMIC BASED TYPING

Genomically, the genus *Shigella* is viewed as a subgroup of *E. coli* with a common core set of genes despite the differences in disease presentation in human hosts. Thus, genomic typing tools used for *E. coli* are also used for *Shigella.*

From 2023 ESR has reported on the seven gene multi locus sequence type (MLST) result (Wirth et al., 2006) (ST) on all patient reports. To better understand fine-scale relationships between sequenced isolates all genome sequences undergo fine-scale genomic clustering using a 2513 locus core genome (cgMLST)[17] scheme.

In addition, isolates of ST245 (the vast majority of *S. flexneri*) and ST152 (the vast majority of *S. sonnei*) are tracked at the Single Nucleotide Polymorphism (SNP)[18] level. The relationships between all *S. sonnei* are estimated using Snippy v4.3.6[19] to identify shared polymorphisms, and iqTree2[20] to infer phylogeny, with *S. sonnei* Ss046[21] used as a reference genome. The relationships between *S. flexneri* ST245 are also identified using Snippy v4.3.6 using reference genome *S. flexneri* 2a str. 2457T. ESR is using a 5-SNP cut-off in the first instance to determine if isolates are likely to be epidemiologically linked. These results are initially viewed using the online visualisation platform, Microreact[22].

Genome based typing is further explained in Appendix B.

4.2 DISCONTINUANCE OF REPORTING OF *S. FLEXNERI* SEROTYPING

An extensive validation process was undertaken prior to ESR moving to real-time WGS based analysis of *Shigella* in late 2023 which revealed disparities in 13/68 (19%) phenotypic/genotypic *S. flexneri* serotyping comparisons[23]. These discrepancies were most likely due to the presence or absence of temperate bacteriophages (Bengtsson et al., 2022; Puzari et al., 2018). This led to the decision to not report *S. flexneri* serotypes inferred from WGS analysis as they could be misleading in an epidemiological investigation.

There is no international genotyping scheme for *S. flexneri* but a review of the ST245 SNP tree (Figure 2) shows that four clades are present – and with various historically conferred serotypes group within each.

[17] https://pubmlst.org/bigsdb?db=pubmlst_escherichia_seqdef
[18] https://github.com/tseemann/snippy
[19] Seemann, T. snippy. https://github.com/tseemann/snippy.
[20] http://www.iqtree.org/
[21] https://www.ncbi.nlm.nih.gov/nuccore/NC_007384.1
[22] https://microreact.org/
[23] Validation Report: Shigella Whole genome sequencing analysis, ESR internal report 2023

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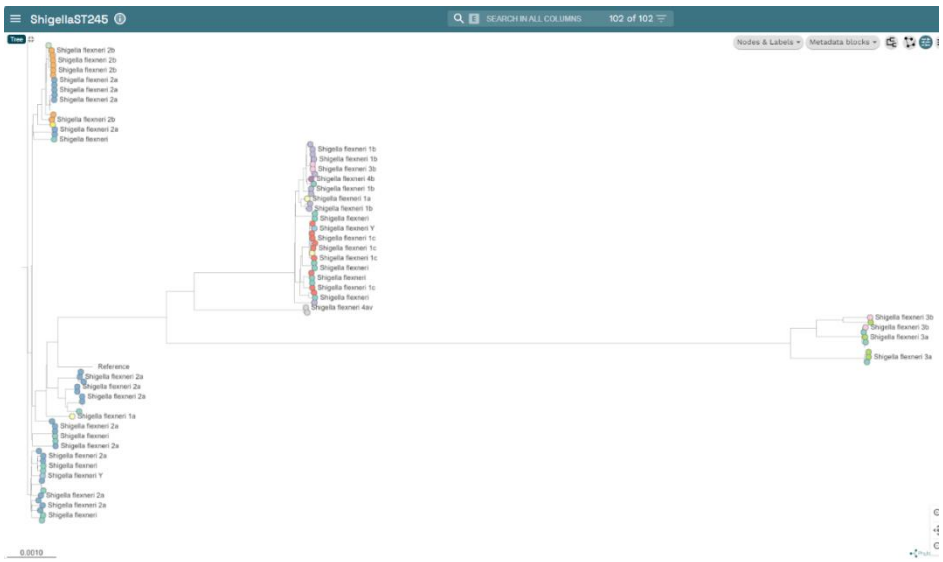
Figure 2. SNIPPY SNP analysis showing historical *S. flexneri* serotype designations.



4.3 DISCONTINUANCE OF REPORTING OF *S. SONNEI* BIOTYPING

As part of the WGS validation process it also became apparent that biotyping of *S. sonnei* did not readily translate to reportable WGS parameters. To-date all *S. sonnei* are ST152 or very closely related STs.

Comparative analysis of NZ data shows *S. sonnei* biotype a are antimicrobially susceptible and invariably fall within the genotype 2 clade (Hawkey et al., 2021) comprising clusters 2.4, 2.4.2, 2.6.1, 2.6.2, 2.7.3, 2.7.4. Whereas biotypes g and f cluster together in two additional clades – the 3.6 clade and the 3.7 clade as shown in Figure 3.

Genotype clade data could be added to individual laboratory reports if Public Health Services consider this a useful addition.

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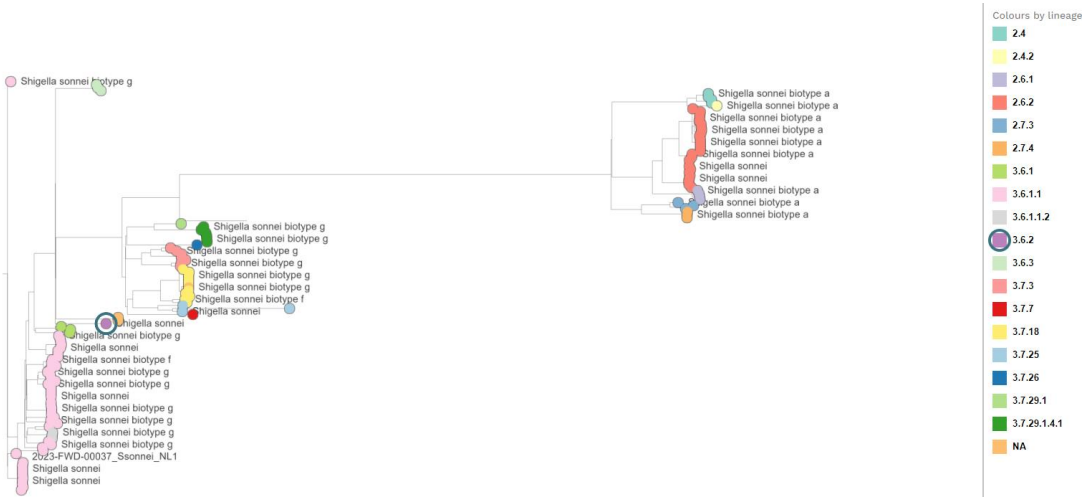
Figure 3. All NZ *S. sonnei* strains analysed via whole genome sequencing to-date and categorised using the genotyping scheme of Hawkey et al., with historical biotype designations shown.



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5. HIGH LEVEL TYPING RESULTS AND EPIDEMIOLOGICAL INFORMATION

During 2022 and 2023 there were 173 confirmed cases of shigellosis for whom an isolate was able to be typed at ESR.

2022: 57 cases – borders opened in July 2022

2023: 116 cases

A breakdown of high-level case/isolate information is shown in Table 1.

TABLE 1. High-level isolate and epidemiological information for the 173 confirmed NZ cases of *Shigella* for the years 2022 and 2023 from whom an isolate was received for typing at ESR.

Species	Case No.	Hospitalised	Overseas Travel	Reported as MSM*	XDR	MDR	eXDR
S. boydii	4	0	3	0	0	0	0
S. dysenteriae	2	0	1	0	0	0	0
S. flexneri	70	30	34	9	1	7	0
S. sonnei	97	18	62	12	16	6	4
Total	173	48	100	21	17	13	4

*MSM status maybe underreported as this field has low completion rates in EpiSurv.

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6. ANTIMICROBIAL RESISTANCE RESULTS

Building on the previous ESR report (Review of Antimicrobial Resistance in Shigellae in Aotearoa New Zealand to 01 September 2023 – updated version reproduced here as Appendix A) whole genome sequencing analysis data generated twice weekly has been streamlined to allow ready recognition of XDR, MDR and eXDR strains.

6.1 XDR SUMMARY

In the previous report four XDR strains were identified during 2022 and a further four in the first eight months of 2023. In the final four months of 2023 a further **nine** XDR *Shigella* were identified bringing the two-year total to 17 cases. The majority of these are *S. sonnei.*

6.2 MDR SUMMARY

In the previous report six MDR strains were identified during 2022 and a further four in the first eight months of 2023. In the final four months of 2024 a further three MDR *Shigella* were identified bringing the two-year total to 13 cases.

6.3 EXDR SUMMARY

In the previous report four eXDR were reported 1 January 2022 – 31 August 2023. No further cases were identified in the remainder of 2023.

Case data for all three categories for the two-year period are tabulated in Table 2 at the end of this report.

6.4 RESULTS OF CLUSTERING OF *S. SONNEI* – EVIDENCE OF LOCAL TRANSMISSION OF XDR AND EXDR

Some closely related genomic clusters were identified and are highlighted in Figures 4 – 9. Isolates relating to the years of this report are circled within each figure and isolate/case information is shown in Table 2.

In some cases, a *S. sonnei* genotype conforms with a cluster as seen in Figures 4 - 6.

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Figure 4. 15-SNP cluster of three isolates of XDR *S. sonnei* genotype 3.6.3. Not all of whom had an overseas travel history. The SNP similarity is suggestive of a recent common ancestor.

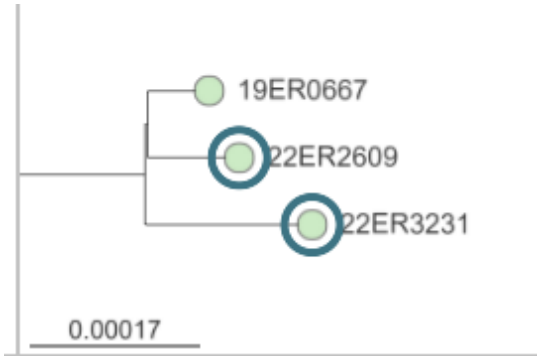


Figure 5. 10-SNP cluster of three isolates of XDR *S. sonnei* genotype 3.6.1.1.2. Not all of whom had an overseas travel history. The SNP similarity is suggestive of a recent common ancestor.

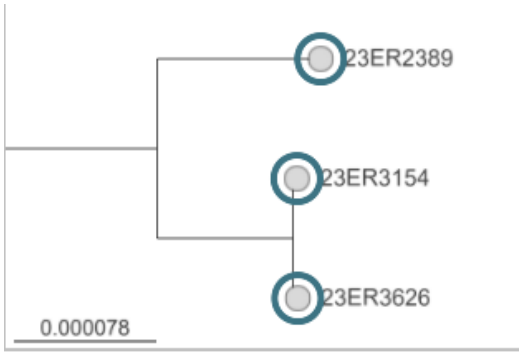
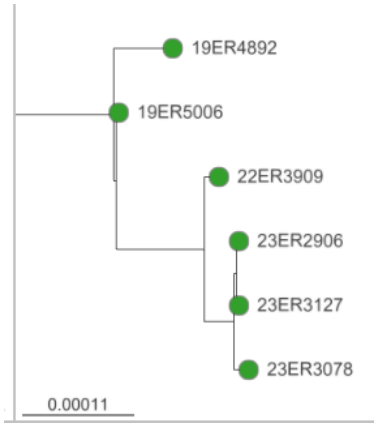


Figure 6. 13-SNP cluster of six isolates of eXDR *S. sonnei* genotype 3.7.29.1.4.1, not all of whom had an overseas travel history. The SNP similarity is suggestive of a recent common ancestor.



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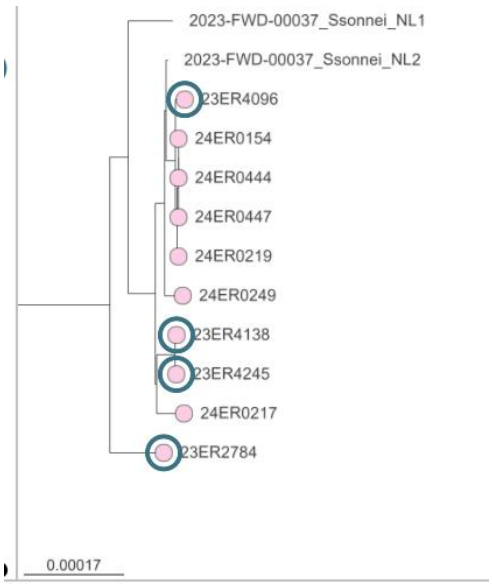
6.5 *S. SONNEI* GENOTYPE 3.6.1.1

6.5.1 Emergent XDR Strain with evidence of local transmission

There is a current growing 5-SNP cluster of XDR *S. sonnei* within genotype 3.6.1.1 (Figure 7). All NZ isolates excluding 23ER2784 fall within 5-SNPs of each other and within 5-SNPs of 2023-FWD-00037_Ssonnei_NL2 – one of two genomic reads uploaded by the Netherlands to the European CDC as part of an outbreak report in 2023 (FWD-00037).

This cluster has been separately alerted in detail via ESR communications to the National Public Health Service and the NZ Microbiology Network (Julianna Lees, Personal Communication, May 2024).

Figure 7. Current and growing 5-SNP cluster of XDR *S. sonnei* within genotype 3.6.1.1. All NZ isolates excluding 23ER2784 fall within 5 SNPs of each other and within 5 SNPs of 2023-FWD-00037_Ssonnei_NL2 – one of two genomic reads uploaded by the Netherlands to the European CDC as part of an outbreak report in 2023. This cluster comprises both overseas and locally acquired cases.



6.5.2 Other 3.6.1.1 clusters

The genotype *S. Sonnei* 3.6.1.1 is a large and diverse group comprising clusters and singletons – some of which are XDR and some of which are not. Two further 5-SNP clusters within this genotype are shown in Figures 8 and 9.

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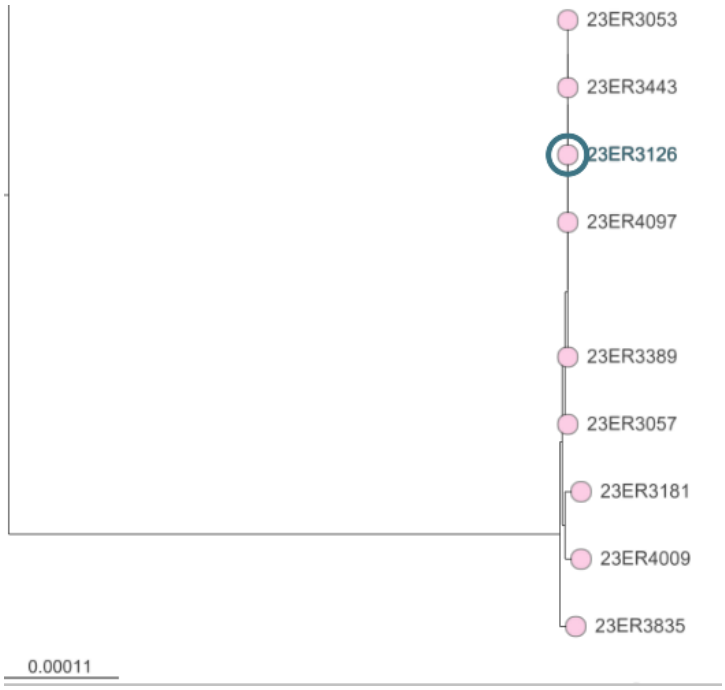
Figure 8. XDR 5-SNP cluster - three isolates of *S. sonnei* genotype 3.6.1.1, not all of whom had an overseas travel history.

Figure 9. 5-SNP cluster - within *S. sonnei* genotype 3.6.1.1 none of which are XDR/MDR/eXDR. This cluster comprises both overseas and locally acquired cases.



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7. WHOLE OF SPECIES CLUSTERING VIA CGMLST

The value of cgMLST is that allows all isolates within the genus to be visualised together. In Figure 10, all the *Shigella* that have been analysed via WGS at ESR to-date are visualised in a minimum spanning tree. The numbers on the branches denote cgMLST differences between isolates. Of note is that:

- *S. sonnei* form a relatively tight group whereas *S. flexneri* is more disparate.
- The phenotypic designation of *S. flexneri* serotype 6 Boyd 88 sits completely separately from the rest of that species.
- When the same information is coloured by ST (Figure 11) it shows the relationship of those without recognised ST designations (novel ST) compared to those with and enables the decision of whether to add samples with novel ST to either the ST152 or ST245 SNP analyses.
- The fact all *Shigella* sequenced isolates undergo cgMLST means ESR is able to provide information on related isolates regardless of ST. This data can also be used to identify new and arising groups of closely related strains that should under SNP based clustering in future.

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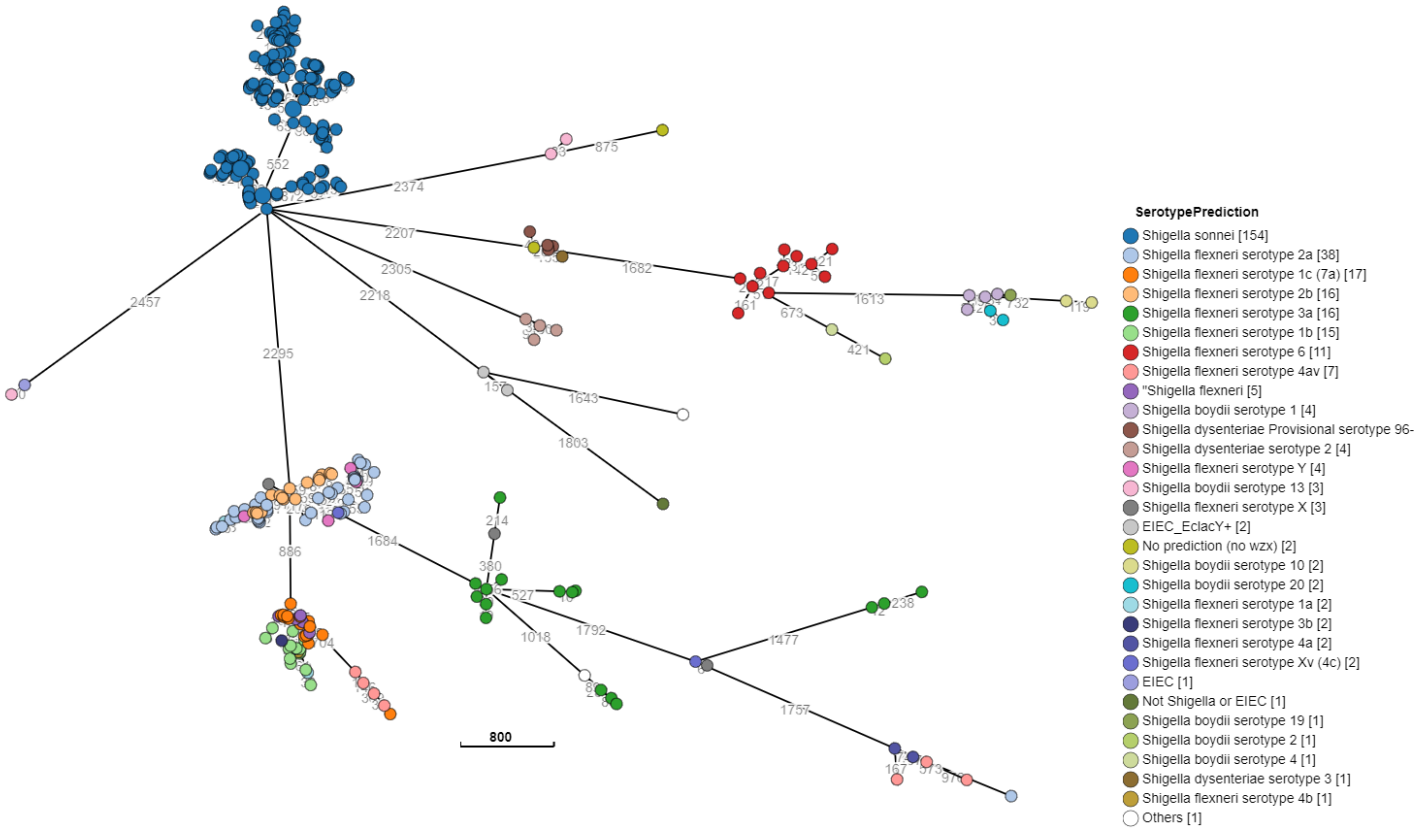

Figure 10. Minimum spanning tree generated from cgMLST analysis of all Shigellae in current ESR database visualised in GrapeTree 2.1. The nodes are coloured by serotype prediction, and cgMLST differences are shown on the branches.

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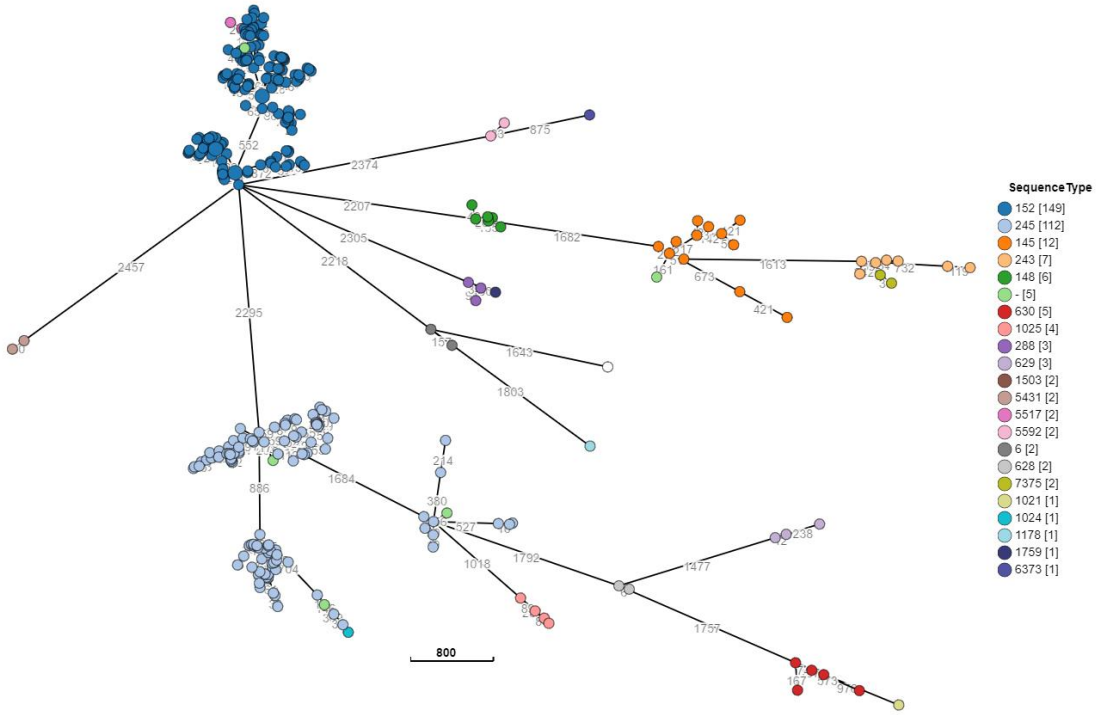
Figure 11. Minimum spanning tree generated from cgMLST analysis of all Shigellae in current ESR database visualised in GrapeTree 2.1. The nodes are coloured by ST, and cgMLST differences are shown on the branches



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8. CONCLUSION

New Zealand (NZ) shigellosis case numbers are rebounding following the opening of NZ's borders in July 2022.

Detections of XDR strains are increasing and there is evidence of local XDR transmission.

Routine genomic analysis of NZ's *Shigella* isolates has made a significant, positive contribution to our understanding of the epidemiology of *Shigella* in NZ, including XDR strains.

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TABLE 2. XDR, eXDR and MDR isolate detail for confirmed *Shigella* case isolates from 2022 and 2023 typed at ESR. R = Resistant, S = Susceptible, IS = Intermediate for surveillance purposes inferred from ESR WGS analysis according to the parameters detailed in Section 2 of this report.

ESR Lab#	Identification	ST	Co-trimoxazole	Ceftriaxone	Quinolone	Azithromycin	Date Collected	Health District	Travel History
XDR									
22ER2609	*Shigella sonnei*	152	R	R	R	R	28/07/2022	Taranaki	Australia
22ER3231	*Shigella sonnei*	152	R	R	R	R	14/09/2022	Auckland	No
22ER3595	*Shigella sonnei*	152	R	R	R	R	5/10/2022	Canterbury	Australia/Nepal
22ER3596	*Shigella sonnei*	152	R	R	R	R	4/10/2022	Canterbury	Australia/Nepal
23ER0598	*Shigella flexneri*	630	R	R	R	R	6/02/2023	Counties Manukau	India/Australia
23ER2389	*Shigella sonnei*	152	R	R	R	R	23/06/2023	Auckland	Portugal/Spain/France
23ER2784	*Shigella sonnei*	152	R	R	R	R	1/08/2023	Auckland	No
23ER2876	*Shigella sonnei*	152	R	R	R	R	10/08/2023	Waitemata	Nepal
23ER3154	*Shigella sonnei*	152	R	R	R	R	4/09/2023	Canterbury	No
23ER3626	*Shigella sonnei*	152	R	R	R	R	9/10/2023	Canterbury	Australia
23ER4096	*Shigella sonnei*	152	R	R	R	R	16/11/2023	Waitemata	USA
23ER4138	*Shigella sonnei*	152	R	R	R	R	21/11/2023	Auckland	USA
23ER4245	*Shigella sonnei*	152	R	R	R	R	28/11/2023	Canterbury	No
24ER0036	*Shigella sonnei*	152	R	R	R	R	19/12/2023	Waitemata	No
24ER0037	*Shigella sonnei*	152	R	R	R	R	21/12/2023	Waitemata	No
24ER0039	*Shigella sonnei*	152	R	R	R	R	19/12/2023	Auckland	India
24ER0040	*Shigella sonnei*	152	R	R	R	R	19/12/2023	Waitemata	No

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MDR									
22ER2666	*Shigella flexneri*	245	R	R	S	R	3/08/2022	Auckland	No
22ER2859	*Shigella flexneri*	245	R	R	R	S	15/08/2022	Auckland	Unknown
22ER3283	*Shigella flexneri*	245	R	R	R	S	9/09/2022	Waitemata	No
22ER3766	*Shigella sonnei*	152	R	R	R	S	21/10/2022	Bay of Plenty	UAE/Jordan/Egypt
22ER4382	*Shigella flexneri*	245	R	S	R	R	6/12/2022	Auckland	Canada
22ER4443	*Shigella sonnei*	152	R	S	R	R	13/12/2022	Capital and Coast	Bangladesh
23ER2014	*Shigella flexneri*	245	R	R	R	S	11/05/2023	Hawke's Bay	Pakistan
23ER2185	*Shigella flexneri*	245	S	R	R	R	25/05/2023	Waitemata	Qatar/Germany/England
23ER2802	*Shigella sonnei*	152	R	R	R	S	3/08/2023	Auckland	Singapore/UAE/Jordan
23ER2849	*Shigella sonnei*	152	R	R	R	S	8/08/2023	Capital and Coast	Indonesia
23ER3406	*Shigella flexneri*	245	R	R	R	S	20/09/2023	Waikato	Unknown
23ER3802	*Shigella sonnei*	152	R	R	R	S	18/10/2023	Canterbury	Australia/Singapore
23ER3859	*Shigella sonnei*	152	R	R	R	S	24/10/2023	Hawke's Bay	No
eXDR									
22ER3909	*Shigella sonnei*	152	R	R	IS	R	2/11/2022	Auckland	Australia
23ER2906	*Shigella sonnei*	152	R	R	IS	R	15/08/2023	Auckland	No
23ER3078	*Shigella sonnei*	152	R	R	IS	R	28/08/2023	Auckland	USA
23ER3127	*Shigella sonnei*	152	R	R	IS	R	30/08/2023	Auckland	USA

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APPENDIX A:

A.1 Review of Antimicrobial Resistance in Shigellae in Aotearoa New Zealand to 01 September, 2023

An ESR discussion document prepared by Jackie Wright, David Winter, Kristin Dyet October 2023

Reviewed by Shevaun Paine, Niki Stefanogiannis, Juliet Elvy, Sarah Jefferies.

A.1.1 Summary

Shigellae received at ESR from 2019 to 2023 (n=186) were subjected to whole genome sequencing (WGS) analysis via ESR's newly validated *Shigella* pipeline and outputs were compared with antimicrobial susceptibility (AMR) results reported by diagnostic laboratories in their direct laboratory notifications to 31 August 2023, and with published definitions for extensively drug resistant (XDR) and multidrug resistant (MDR) strains. Following these comparisons a third category was introduced – emerging XDR (eXDR) for MDR isolates with quinolone results that are susceptible for clinical treatment purposes but intermediate for surveillance purposes.

- Nine of 186 (5%) *Shigella* isolates were identified as XDR, one from 2019 and four each from 2022 and 2023 (to 31 August). Eight were *S. sonnei* and one was a *S. flexneri*. Seven cases had reported a recent history of overseas travel and the 2019 case had recent contact with a returning traveller. No risk factors were reported for the remaining case.
- Six isolates (3%) were identified as eXDR. Three had an overseas travel history (two of whom also identified as men who have sex with men (MSM)); two had not travelled overseas but identified as MSM; and one was lost to follow up.
- Sixteen isolates (9%) met the criteria for MDR. Eleven had a history of travel and one of these along with one other identified as MSM. Two cases were lost to follow up, and no risk factors were identified in two cases.

At present XDR is being imported to NZ. However, widespread transmission within NZ has not been identified from notified cases of shigellosis to date.

ESR recommend that:

- Health agencies and professionals are aware of the emergence of XDR *Shigella* in NZ and the clinical and public health implications of these organisms
- NZ prepares for increasing numbers of XDR within our returning international traveller and our MSM communities.
- Clinical laboratories all report susceptibility to the following antimicrobials to assist in recognising XDR at the local level: Ampicillin, Azithromycin, Ciprofloxacin, Ceftriaxone, and Co-trimoxazole, and also in their e-notifications for national surveillance purposes.
- This report is comprehensively updated at the end of 2023 and quarterly thereafter but that interim alerts should be prepared as the need arises.

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A.1.2 Background

Shigellosis is an acute gastrointestinal illness characterised by fever, abdominal cramps, blood or mucus in the stools and a high secondary attack rate among contacts. *Shigella species* are unusual among enteric bacteria for the following reasons[24]:

- There are no known animal reservoirs
- Transmission is via direct or indirect faecal-oral transfer. Food or water may become contaminated and act as vehicles; and transmission can also occur through sexual contact, with men who have sex with men (MSM) particularly at risk.
- A very low dose (approximately 10 organisms) is required to cause an infection, therefore person-to-person transmission is common.
- Shigellosis can be a life-threatening disease, with older and younger people particularly at risk and some *Shigella species* are more likely to cause more severe disease than others.
- Antibiotic treatment is usual in severe cases.
- Antimicrobial resistance is a present and growing concern for this species, with extensively drug resistant (XDR) and multiple drug resistant (MDR) lineages increasingly reported worldwide.

The genus *Shigella* comprises four species: *Shigella* (*S.*) *dysenteriae*, *S. flexneri*, *S. boydii* and *S. sonnei.* Historically these species have been further phenotypically differentiated by serotyping or biotyping (*S. sonnei*). As NZ moves to whole genome sequence (WGS) based analysis, ESR will discontinue reporting serotyping of *S. flexneri* (as it has been shown that inherent phages can impact on serotype designation within a genomically similar group) as well as biotyping of *S. sonnei*. We will instead be reporting the seven gene multi locus sequence type (MLST) result (Wirth et al., 2006) (ST)[25] which is then used as a basis for finer typing methods looking at differences in core genome MLST (cgMLST)[26] and single nucleotide polymorphisms[27].

In New Zealand, most shigellosis cases are travellers or people in direct contact with travellers, although local outbreaks have occurred within the MSM community. Epidemics of shigellosis due to drug resistant *Shigella* lineages are being reported internationally among men who have sex with men (MSM) and is a serious emerging public health issue[28].

The re-opening of New Zealand's borders following COVID-19 restrictions has led to increased *Shigella* detections, including XDR and MDR isolates. This report summarises the results of the AMR surveillance of *Shigella species* isolated from shigellosis cases in NZ, principally from 2022 and 2023.

A.1.3 Methodology

In August 2023 Aotearoa New Zealand (NZ) diagnostic laboratories reported seeing XDR *Shigellae* through their phenotypic testing. At the same time ESR's Enteric and Antimicrobial

[24] https://www.tewhatuora.govt.nz/for-the-health-sector/health-sector-guidance/communicable-disease-control-manual/shigellosis/

[25] https://www.ncbi.nlm.nih.gov/pmc/articles/PMC1557465/

[26] https://pubmlst.org/bigsdb?db=pubmlst_escherichia_seqdef

[27] https://github.com/tseemann/snippy

[28] https://pubmed.ncbi.nlm.nih.gov/30615105/

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Reference laboratories (ERL and ARL) completed the validation of a WGS analysis pipeline for *Shigellae*, which includes detection of antimicrobial resistance (AMR)-associated genes and genomic clustering of isolates. This validation project has generated data for all *Shigella species* isolates with a collection date after 1st January 2022, and a subset of samples detected in 2019 (which were initially analysed using a predecessor pipeline and reported in 2020[29]).

A review has been undertaken comparing antimicrobial susceptibility results reported by diagnostic laboratories in their direct laboratory notifications with the results generated by the ESR'S AMR WGS tool to 31 August 2023. Results for all 186 *Shigellae* subjected to WGS at ESR were then compared with

1. US CDC XDR definition as follows:

   XDR *Shigella* bacteria are resistant to all commonly recommended empiric and alternative antibiotics — azithromycin, ciprofloxacin, ceftriaxone (extended spectrum β lactamase – ESBL – producer), trimethoprim-sulfamethoxazole (TMP-SMX), and ampicillin[30].

In most cases, resistance to these treatments is encoded by specific genes that can be detected from WGS results as follows:

- **Ampicillin***: blaOXA* genes or blaTEM-1, or any of the genes named below under Ceftriaxone must be recognised as ampicillin resistant when interpreting WGS AMR results.
- **Azithromycin**: *ermB* and/or *mphA*
- **Ceftriaxone** (Kayama et al., 2023):

1. ESBL: any *blaCTX-M*; any VEB; any SHV or TEM shown to confer ceftriaxone resistance
2. AmpC: any *blaDHA* or *blaCMY* genes (acknowledging that *blaDHA-1* results in clinical resistance only 60% of the time)
3. and any potential carbapenem resistance conferring genes including: GES, IMP, NDM, OXA, VIM genes.

- **Co-trimoxazole** (Trimethoprim-Sulfamethoxazole, TMP-SMX): *dfrA* and *sul*
- **Quinolone**: (including ciprofloxacin) is more complex, as this can be encoded by:

1. plasmid-mediated quinolone-resistance regions (*qnr* genes)
2. and/or mutations in quinolone resistance-determining regions (QRDR denoted by *gyr* and *par* genes)

   The presence of multiple genes indicates unequivocal quinolone resistance.

   The presence of some genes in isolation appears more likely to confer clinical resistance than others, such as *qnr*S1 compared with *qnr*B19; and some are more likely to indicate Intermediate for surveillance purposes than others, such the S83L mutation in *gyrA* compared with the S83A mutation in that gene.

[29] https://esr2.cwp.govt.nz/assets/Intelligence-Hub-2023/Surveillance-Datasets/Antimicrobial-Resistance-AMR/Shigella/2019.ShigellaAMRreport.pdf

[30] emergency.cdc.gov/han/2023/han00486.asp

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2. MDR definition as follows:

   Multi drug resistant *Shigellae* include those resistant to any three of the following: azithromycin, ceftriaxone, Co-trimoxazole, ciprofloxacin[31].

As described above, the genetic basis of quinolone resistance is complex with several different genes and mutations able to combine to overcome this treatment.

Review of these data identified an additional category of isolates resistant to Azithromycin, Ceftriaxone, and Co-trimoxazole and with decreased susceptibility to quinolones (MIC ≥0.12 mg/L). As the acquisition of quinolone resistance can be stepwise (with each new mutation or gene decreasing susceptibility further) for the purposes of this document ESR has labelled this category "emerging XDR" (eXDR).

More work comparing ciprofloxacin minimum inhibitory concentrations (MICs) with AMR gene presence to assist in confirming reduced quinolone susceptibility for surveillance purposes is planned.

A.1.4 XDR findings

Of the nine XDR cases five cases were *Shigella* (*S.*) *sonnei* biotype g and four of these had a recent history of travel or contact with a traveller (Australia, 1; Pakistan, 1; Asia/Middle East, 1; Europe, 1); three were *S. sonnei* biotype f (Australia/Nepal, 2; Nepal, 1) and one was *S. flexneri* 4av (India).

All eight *S. sonnei* isolates were ST152, one of the sequence types for which ESR routinely performs whole genome sequence based SNP clustering. The relationships between all ST152 were estimated using Snippy v4.3.6[32] to identify shared polymorphisms, and iqTree2[33] to infer phylogeny, with *Shigella sonnei* Ss046[34] used as a reference genome. Overall, ST152 isolates detected in New Zealand fall into three divergent clades; one comprising *S. sonnei* biotype a (which is typically more sensitive to antibiotic treatments and has not been associated with MDR/XDR here) and two other clades each comprising a mixture *S. sonnei* biotype g and biotype f isolates.

The eight *S. sonnei* XDR isolates include two distinct genomic clusters. Both fall within the same biotype g/biotype f clade. One, labelled XDR Cluster 1 (Figure 1), contains three isolates separated by 10-15 SNPs (a degree of separation suggesting a recent shared common ancestor, rather than direct contact between cases). XDR Cluster 2 contains two isolates (from cases reported in Canterbury) separated by a single SNP, suggesting a direct epidemiological link. While not reported as an outbreak, the two cases were partners who lived in the same household, and both had a travel history to Australia and Nepal. A third case reported in Auckland with travel history to Nepal is separated by 30 SNPs (suggesting an indirect link to the other two cases in this cluster). The other three isolates are not closely linked to other cases from New Zealand. However, another Auckland case is indirectly linked (12 SNPs) to cases reported as part of an outbreak in the Netherlands but had no reported travel history.

[31] https://wwwnc.cdc.gov/eid/article/22/6/15-2088_article

[32] Seemann, T. snippy. https://github.com/tseemann/snippy.

[33] http://www.iqtree.org/

[34] https://www.ncbi.nlm.nih.gov/nuccore/NC_007384.1

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None of the XDR cases were reported as identifying as MSM.

Overall, these results suggest XDR shigella currently being detected in New Zealand represent multiple separate introductions from overseas with no community transmission.

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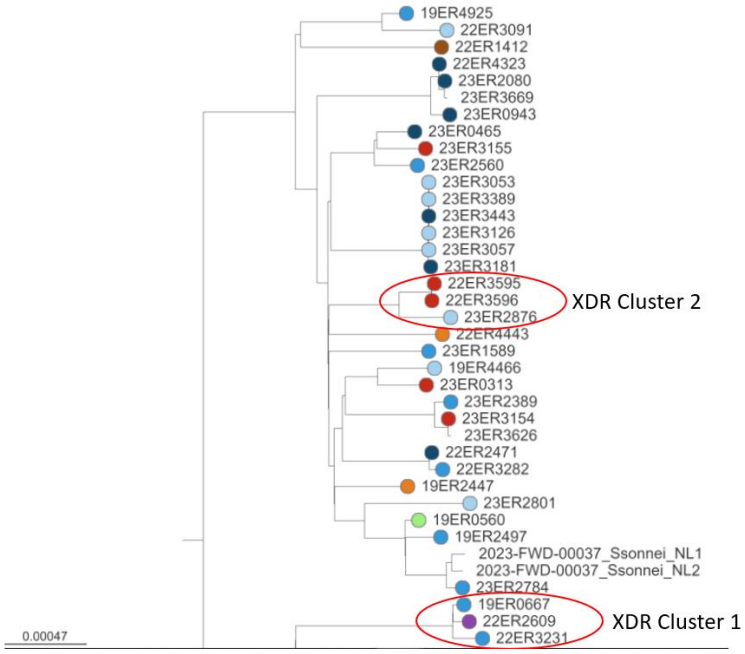
Figure 1. Snippy Tree visualisation of one of the two clades of *S. sonnei* ST152 biotype g/biotype f isolates identified by WGS analysis to date showing the two XDR clusters. Scale shown within the tree indicates nucleotide substitutions per site.



A.1.5 Emerging XDR findings

Two 2019 cases, one case from 2022 and three cases from 2023 were initially thought to be XDR by WGS. Three cases had an overseas travel history recorded (USA, 2; Australia, 1) and four cases, including two who had travelled, identified as MSM. All were *S. sonnei* biotype g, and all had the same AMR genomic profile including the S83L mutation in *gyrA*, which is associated with quinolone resistance. Despite the presence of this mutation the phenotypic susceptibility testing results from ESR's Antibiotic Resistance Laboratory's 2019 survey[35] indicated both isolates had ciprofloxacin MICs that should be interpreted as susceptible (0.12mg/L). The three 2022/2023 clinical laboratory results were all reported as susceptible to ciprofloxacin.

As described above, the genetic basis of resistance to quinolone is complex, with multiple different genes in the so-called quinolone resistance-determining region (QRDR) contributing to this phenotype. Clinically significant resistance to quinolones likely requires a combination of specific genes and mutations to be present in the genome. However, Baker et al., 2018 report the formation of these resistant clones may involve the stepwise addition of individual mutations such as *gyrA* D87Y and S83L[3637].

[35] https://esr2.cwp.govt.nz/assets/Intelligence-Hub-2023/Surveillance-Datasets/Antimicrobial-Resistance-AMR/Shigella/2019.ShigellaAMRreport.pdf

[36] https://journals.plos.org/plosmedicine/article?id=10.1371/journal.pmed.1002055

[37] https://www.nature.com/articles/s41598-018-25764-3

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In addition, communication with Norelle Sherry[38] indicated:

1) Interpreting quinolone resistance for treatment is different to interpreting resistance/emerging resistance for AMR surveillance purposes.
2) Therefore, an isolate with a ciprofloxacin MIC of ≥ 0.12 and <0.5 mg/L (the EUCAST MIC breakpoint) with any quinolone resistance gene present should be interpreted as Intermediate for surveillance purposes even though ciprofloxacin would be reported as susceptible in the clinical laboratory by phenotypic methods and may well be clinically effective.

To capture strains that may not have clinically relevant resistance to quinolones, but should never-the-less be the focus of surveillance, we have created a new "emerging XDR" classification for samples resistant to Co-trimoxazole, Azithromycin, and Ceftriaxone and with MICs ≥ 0.12 mg/L for Ciprofloxacin. This surveillance interpretation is in-line with 2018 information from the US CDC[39].

Six eXDR cases were detected in our data: three had an overseas travel history (two of whom also identified as MSM); two had not travelled overseas but identified as MSM; and one was lost to follow up.

These six eXDR isolates are genomically distinct from the XDR cases as they cluster in the second biotype g/biotype f clade. Both 2019 isolates in this group had no travel history and were classified by the investigating public health team as being part of an outbreak of three cases (the third case isolate did not undergo WGS) associated with person to person transmission, and cluster within 5 SNPs. The 2022 and 2023 isolates also cluster within 5 SNPs and the two groups are within 10 SNPs of each other suggesting a very recent common ancestor - Figure 2.

Figure 2. Snippy Tree visualisation of the second biotype g/biotype f clade of *S. sonnei* ST152 isolates analysed via WGS to date showing the emerging XDR cluster. Scale shown within the tree indicates nucleotide substitutions per site.

[38] https://www.doherty.edu.au/people/dr-norelle-sherry

[39] https://emergency.cdc.gov/han/han00411.asp

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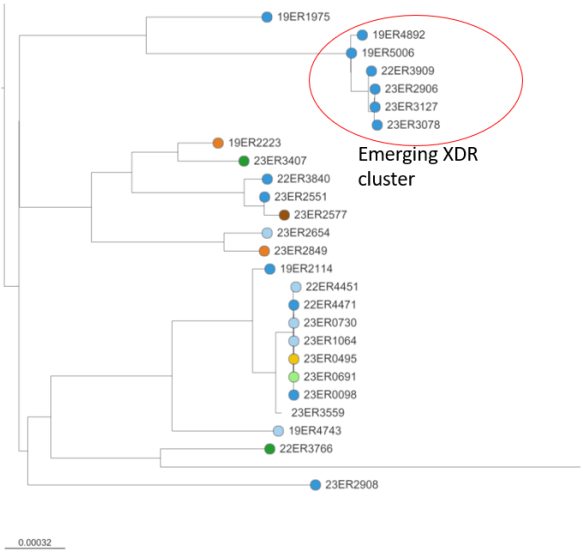

A.1.6 MDR findings

Sixteen isolates met the criteria for MDR. Eleven had a history of travel and one of these along with one other identified as MSM. Two cases were lost to follow up, and no risk factors were identified in two cases.

The case isolates cover a diverse range of *Shigellae* and fell into four categories based on both genotypic and available phenotypic results:

- One was susceptible to Co-trimoxazole but was resistant to all other classes (*S. flexneri* 2a)
- One was susceptible to quinolones but resistant to all other classes (*S. flexneri* 3b)
- Three were ESBL negative but resistant to all other classes (*S. flexneri* 1c, *S. sonnei* biotype g; *S. dysenteriae*)
- Eleven were susceptible to macrolides but were resistant to all other classes (*S. dysenteriae* 3, 1; *S. flexneri,* 2; *S. flexneri* 1b; 1 *S. sonnei* biotype g, 5; *S. sonnei* biotype f, 1; *S. flexneri* 3a, 1)

A.1.7 Clinical laboratory correlation with WGS findings

Laboratory notification data were reviewed for 119 cases from the period 1 July 2022 – 31 August 2023 and 100 had at least a partial clinical lab AMR result reported as part of the electronic direct laboratory notification.

A.1.8 Comments on specific antibiotic correlations

A.1.9 Co-trimoxazole

The presence of *dfrA* and *sul* genes together are associated with Co-trimoxazole resistance.

A clinical laboratory result for Co-trimoxazole susceptibility/resistance was noted for 97 isolates.

All isolates that are positive for both *dfrA* (trimethoprim resistance) and *sul* (sulphonamide) and that had a viewable clinical laboratory antibiotic susceptibility result were Co-trimoxazole R (n=54).

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One isolate that was only *sul2* positive (23ER2849) and three isolates that were only *dfr*A1 positive (23ER1589, 23ER2675 and 23ER3053) and were also reported as Co-trimoxazole R which was unexpected. The *sul* gene is carried on a small plasmid[40] and therefore could have been lost during laboratory processing. Plasmid loss could also occur in the host. Two examples of this are the isolates from 2 Auckland outbreak cases, and two isolates from a single case which have different Co-trimoxazole sensitivities by the clinical laboratory and by WGS (as well as other AMR gene disparities not focussed on in this report).

This is an unresolved potential flaw in the WGS method that will need to be further investigated as it may lead to under reporting of Co-trimoxazole resistance.

The remaining phenotypically susceptible isolates (n = 39) had no sulphur drug resistance genes or only *dfr*A1 detected. No others had only *sul2* detected.

A.1.10 Ampicillin

A clinical laboratory result for ampicillin susceptibility/resistance was noted for 58 isolates.

The presence of either *blaOXA-1* or *blaTEM-1* or any higher level β lactamase gene is associated with ampicillin resistance.

The 55 isolates reported as ampicillin resistant were positive for either *blaOXA-1* or *blaTEM-1* or one of the *blaCTX-M-* ESBL genes. The three isolates reported as susceptible to amoxicillin had none of these genes.

NB isolates negative for *blaOXA* genes and *blaTEM-1* but positive for any of the genes named below under Ceftriaxone must be recognised as ampicillin resistant when interpreting WGS AMR results.

A.1.11 Ceftriaxone

ESBL

In this dataset, the presence of *CTX-M* genes is associated with resistance to extended spectrum β lactamases (ESBL). A clinical laboratory result for ESBL susceptibility/resistance was noted for 40 isolates.

All 18 isolates that were reported as ESBL negative by the clinical laboratories were negative for *blaCTX-M-* genes, and all 22 isolates that were reported as ESBL positive were positive for *blaCTX-M-15* or *blaCTX-M-27*

AMPC

A single isolate (19ER3026) in this dataset was found to be positive for *blaDHA-1.* This isolate had no ESBL genes and was subsequently shown to have a Ceftriaxone MIC of 0.12 mg/L (Kristin Dyet, personal communication, May 2024). As this is less than the EUCAST breakpoint of 1 mg/L[41] it was not classified as Ceftriaxone resistant for the purposes of this report.

A.1.12 Azithromycin

The presence of erm(B) and mph(A) genes is associated with resistance to azithromycin. A clinical laboratory result for azithromycin susceptibility/resistance was noted for 35 isolates.

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[40] https://card.mcmaster.ca/ontology/36551
[41] https://www.eucast.org/fileadmin/src/media/PDFs/EUCAST_files/Breakpoint_tables/v_14.0_Breakpoint_Tables.xlsx, accessed May 2024.

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Absence of both *erm*(B) and *mph*(A) correlated with an azithromycin MIC of ≤16 (n = 23), and presence of either or both genes correlated with reported resistance (n = 12).

A.1.13 Meropenem

On 10 occasions clinical laboratories reported meropenem as susceptible and no genes known to confer meropenem resistance were identified by WGS in any of the Shigella isolates evaluated here.

A.1.14 Quinolones/Ciprofloxacin

A clinical laboratory result for quinolone/ciprofloxacin susceptibility/resistance was noted for 95 isolates. The following genes were associated with clinical quinolone/ciprofloxacin resistance: plasmid-mediated quinolone-resistance regions (*qnr*); mutations in quinolone resistance-determining regions (QRDR) such as *gyrA*_D87N; *gyrA*_S83L; *parC*_S80I.

Of the 37 isolates reported as I or R by the clinical laboratories 26 were carrying multiple quinolone resistance genes.

All four of the isolates that were reported as I (intermediate) had only the *qnr*S1 gene. Six reported as R by the clinical laboratories also only had *qnr*S1. Three with only this gene were reported as S.

Of the nine isolates positive for only the *qnr*B19 gene seven had a clinical laboratory result. All seven were reported by the clinical laboratories as susceptible. However, the remaining two were from 2019 and had been tested by the ARL lab, ESR and both had a ciprofloxacin MICs of 0.25 mg/L.

In all 18 ARL ciprofloxacin MIC results from 2019 were reviewed in conjunction with their WGS results. Two isolates positive for *gyrA*_S83A and five isolates positive for *gyrA*_D87N all had MICs of 0.03 or 0.06 mg/L. One isolate positive for *gyrA*_D87Y and eight isolates positive for *gyrA*_S83L had MICs of 0.12 mg/L putting them in the Intermediate category for surveillance purposes along with the *qnr*B19 isolates above.

Because of this variability between phenotype and genotype for single quinolone resistance gene findings it is proposed that for 12 months ERL performs ciprofloxacin MICs using a gradient strip on all Shigellae in real time (as they are sent for sequencing) so that the MICs can be correlated with the genes for real time Shigella AMR surveillance. The scoping of this proposal has been prepared separately.

A.1.15 Augmentin

On two occasions – 22ER2609 and 23ER0598 – Clinical laboratories reported organisms that met the criteria for XDR as Augmentin susceptible – the clinical validity of these results is uncertain.

A.1.16 Further refinements for the Shigella WGS pipeline

As a result of this review, the ESR Shigella whole genome sequencing pipeline output will be revised to ensure XDR, MDR and eXDR are more readily recognisable within the data reported.

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ESR Lab#	Identification	Co-tri-moxazole	Ceftriaxone	Quinolone	Azithromycin	Date Collected	Health District	Travel history
XDR								
19ER0667	*Shigella sonnei* biotype g	R	R	R	R	7/2/2019	Combined Auckland	No (case had contact with relative who had just returned from Indonesia, UAE, and Afghanistan)
22ER2609	*Shigella sonnei* biotype g	R	R	R	R	28/07/2022	Taranaki	Australia
22ER3231	*Shigella sonnei* biotype g	R	R	R	R	14/09/2022	Combined Auckland	Pakistan
22ER3595	*Shigella sonnei* biotype f	R	R	R	R	5/10/2022	Canterbury	Australia/Nepal
22ER3596	*Shigella sonnei* biotype f	R	R	R	R	4/10/2022	Canterbury	Australia/Nepal
23ER0598	Shigella flexneri 4av	R	R	R	R	6/02/2023	Combined Auckland	India/Australia
23ER2389	*Shigella sonnei* biotype g	R	R	R	R	23/06/2023	Combined Auckland	Portugal/Spain/France
23ER2784	*Shigella sonnei* biotype g	R	R	R	R	1/08/2023	Combined Auckland	No
23ER2876	*Shigella sonnei* biotype f	R	R	R	R	10/08/2023	Combined Auckland	Nepal
eXDR								
19ER4892	*Shigella sonnei* biotype g	R	R	IS	R	2/12/2019	Combined Auckland	Unknown
19ER5006	*Shigella sonnei* biotype g	R	R	IS	R	10/12/2019	Combined Auckland	No
22ER3909	*Shigella sonnei* biotype g	R	R	IS	R	2/11/2022	Auckland	Australia
23ER2906	*Shigella sonnei* biotype g	R	R	IS	R	15/08/2023	Combined Auckland	No
23ER3078	*Shigella sonnei* biotype g	R	R	IS	R		Combined Auckland	United States of America
23ER3127	*Shigella sonnei* biotype g	R	R	IS	R		Combined Auckland	United States of America
MDR								
19ER2250	*Shigella flexneri* 3a	R	R	R	S	4 /6/2019	Combined Wellington	Unknown
19ER2447	*Shigella sonnei* biotype g	R	R	R	S	20/6/2019	Combined Wellington	India
19ER2497	*Shigella sonnei* biotype g	R	R	R	S	24/6/2019	Auckland	Unknown
19ER3026	*Shigella dysenteriae*	R	S	R	R	6/8/2019	Canterbury	India

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19ER3425	*Shigella dysenteriae* 3	R	R	R	S	27/8/2019	Southern	India
19ER4466	*Shigella sonnei* biotype g	R	R	R	S	20/10/2019	Combined Auckland	Middle East nfd
22ER2666	*Shigella flexneri* 3b	R	R		R	3/08/2022	Combined Auckland	No
22ER2859	*Shigella flexneri* 1b	R	R	R	S	15/08/2022	Auckland	Unknown
22ER3283	*Shigella flexneri*	R	R	R	S	9/09/2022	Auckland	No (had contact with another case)
22ER3766	*Shigella sonnei* biotype g	R	R	R	S	21/10/2022	Bay of Plenty	United Arab Emirates/Jordon/Egypt
22ER4382	*Shigella flexneri* 1c	R	S	R	R	6/12/2022	Combined Auckland	Canada
22ER4443	*Shigella sonnei* biotype g	R	S	R	R	13/12/2022	Combined Wellington	Bangladesh
23ER2014	*Shigella flexneri*	R	R	R	S	11/05/2023	Hawkes Bay	Pakistan
23ER2185	*Shigella flexneri* 2a	S	R	R	R	25/05/2023	Combined Auckland	Qatar/Germany/England
23ER2802	*Shigella sonnei* biotype f	R	R	R	S	3/08/2023	Combined Auckland	Singapore/UAE/Jordan
23ER2849	*Shigella sonnei* biotype g	R	R	R	S	8/08/2023	Capital and Coast	Indonesia

Table 1. XDR, eXDR and MDR isolate detail. R = Resistant, S = Susceptible, IS = Intermediate for surveillance purposes (different from intermediate for clinical purposes) inferred from ESR WGS analysis according to the parameters detailed in Section A.1.3 of this report combined with diagnostic laboratory report data in EpiSurv,

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APPENDIX B: GENOME TYPING EXPLAINED

Table 3. Summary of genomic typing methods used for *Shigella* isolates at ESR.

	7-gene MLST	cgMLST	SNP
Method basis	Seven "housekeeping" genes deemed to be conserved within the species are assessed for allelic variation within each gene	2513 genes associated with core *E. coli/Shigella* genome assessed for allelic variation within each gene	Core genome compared with high quality reference genome from a genomically similar organism for base differences (Single nucleotide polymorphisms)
Degree of differentiation	Coarse – similar to serotyping	Fine – suitable for epidemiological investigations	Finest – suitable for epidemiological and outbreak investigations, and phylogeny
Advantages	Low implementation and maintenance costs Reference free, can manage diverse lineages in one project Quick Internationally comparable	Low implementation and maintenance costs Reference free, can manage diverse lineages in one project Quick International comparison possible	Finer resolution, supports outbreak investigation and transmission tracking Off the shelf tools exist, updating new clusters is quite simple Can be used to define stable cluster identifiers Potential for use beyond clustering International reads can be added to local project for comparison
Current usage	Used internationally in conjunction with species and serotype as a component of organism identification	Used internationally as an epidemiological comparison tool	Used internationally for fine comparison within selected ST groups. At ESR used for *S. sonnei* ST152 group and *S. flexneri* ST245 group
Current learnings	Some isolates may have a different ST but fall within a close range of a larger group of a	Highlights similarities and differences between types as described in the preceding column	The choice of reference genome is pivotal to result quality – – *S. sonnei* ST152 relatedness

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	more common ST (by cgMLST). These are deemed a closer entity than isolates with different ST types which do not cluster closely with a common ST		cannot be reliably assessed by SNP analysis if a *S. flexneri* ST245 reference genome is used
Challenges	Understanding your dataset and the relative significance of different STs (as above)	Determining the appropriate comparative method and difference cut-off for a given situation	Determining the appropriate reference strain and difference cut-off for a given situation

Note: The content of table is based on a 2023 evaluation by ESR Enteric and Pathogen Genomics teams and collated by J. Wright for this and other reports (Personal communication David Winter and Jackie Wright, January 2024)

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