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2019 Survey of Extended-spectrum β-lactamase-producing Enterobacterales in New Zealand

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Prepared by: Audrey Tiong, Rosemary Woodhouse, Rhys White, David Winter, and Kristin Dyet

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REVIEWED BY: **Dr Juliet Elvy, Samuel D. Carr, and Dr Xiaoyun Ren**

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1. EXECUTIVE SUMMARY

- ESBL-producing Enterobacterales (ESBL-E) in clinical settings is one of the markers used to assess the *status quo* of antimicrobial resistance in Aotearoa New Zealand. Infections with ESBL-producing Enterobacterales often rely on carbapenems, one of the last line of β-lactam antibiotics available for treatment.
- Data collected as part of this surveillance aims to: (i) define populations in Aotearoa most affected by infections with ESBL-E, (ii) determine rates of antimicrobial resistance to common antibiotics used for treatment of ESBL-E infections, (iii) correlate resistant genotypes to phenotypes, and (iv) describe the genomic diversity of common circulating strains of ESBL-E in Aotearoa.
- Data and ESBL-E were referred to ESR over a 14 day surveillance period in August 2019.
- ESBL-E infections have risen steadily since 2007 with 2019 having the highest reported weekly-prevalence rate of 2.86 per 100 000 population since the inception of ESBL-E surveillance performed at ESR.
- Prevalence of ESBL-E infections were predominantly from community patients (56.5%) and were higher in patients who were ≥65 years of age (52.6%). The most common site of ESBL-E infection was from urine (87.4%).
- The Northern Region represented 50.9% of all cases in Aotearoa followed by 20.1% from Te Manawa Taki, 15.2% from Te Waipounamu, and 13.8% from the Central Region.
- ESBL-E isolates had low rates of resistance to the carbapenems, nitrofurantoin and piperacillin-tazobactam; all with rates of susceptiblity of 90% and above. Genes conferring resistance to antimicrobials had variable correlation with resistant phenotypes.
- The most common ESBL type in this survey was CTX-M which made up 98.9% of genomes available for analysis. ST131 *E. coli* was the most common sequence type (44.9%) among ESBL-producing *E. coli* and clustered into clades that have been typically described overseas. Clade C genomes (43.7%) are frequently reported in nosocomial infections and have the mutations which are likely to confer resistance to fluoroquinolones.
- This report provides valuable baseline information on epidemiologic and genomic surveillance for all future ESBL-E reports. Future surveillance should include whole genome sequencing for all isolates to build on the findings of this report to inform public health strategies and targeted interventions.

2. INTRODUCTION

Extended-spectrum β-lactamases (ESBLs) are enzymes which confer resistance to commonly used β-lactam antibiotics, including the third generation cephalopsorins, and are commonly found in isolates belonging to the Enterobacterales genera. Enterobacterales colonise the gastrointestinal tract but can cause common infections such as uncomplicated urinary tract infections in healthy individuals or serious infections particularly in healthcare-associated patients. Genes conferring resistance to other antibiotic classes are frequently found in ESBL-producing Enterobacterales (ESBL-E) from clinical infections, which further limits available treatment options.

Until 2005, national surveillance of ESBL-E was based on diagnostic laboratories referring all isolates to the Institute of Environmental Science and Research (ESR) for confirmation. Between 2005 and 2014, 1-month long surveys of ESBL-E were undertaken each year. Both the continuous surveillance prior to 2005 and the annual surveys included ESBL-E isolated from both clinical specimens and surveillance/screening specimens.

From 2014 several changes were made to the national surveillance of ESBL-E in New Zealand. Firstly, annual surveys were replaced by surveys every 2 – 3 years. A survey was undertaken in 2016, and the next survey, described in this report, was performed in 2019. While surveys are less frequent, an extended range of analyses is undertaken, including more analysis of the demographics of patients and greater analysis of the ESBL-E isolates using whole genome sequencing (WGS). In 2016 and 2019 surveys, only isolates from clinical specimens were included whereas earlier surveys included isolates from screening specimens. The change to include only clinical isolates means that data in this report is not necessarily comparable with that presented in ESBL-E survey before 2016, which are available at https://www.esr.cri.nz/our-research/nga-kete/infectious-disease-intelligence/antimicrobial-resistance-amr/.

3. METHODS

3.1 ISOLATE AND DATA COLLECTION

For the 2019 ESBL-E survey, hospital and community microbiology laboratories in Aotearoa New Zealand were asked to refer all non-duplicate ESBL-E isolated from clinical specimens isolated for the 14 day period from Sunday 18 August to Saturday 31 August 2019 inclusive. Laboratories that do not test for ESBL production were asked to refer all Enterobacterales isolates that were not susceptible to third generation cephalosporins.

When referring isolates for the survey, laboratories were asked to supply selected epidemiological data, including the patient's date of birth, geographic location, hospitalisation status and history, and body site from which the ESBL-E was isolated. Laboratories were also asked to provide, where available, information on the susceptibility of the ESBL-E isolates to the following antimicrobials: amoxicillin-clavulanic acid, cefoxitin, ciprofloxacin, co-trimoxazole, ertapenem, fosfomycin, gentamicin, meropenem, nitrofurantoin, piperacillin-tazobactam and trimethoprim. Information on the patient's ethnicity and NZDep2013 deprivation index score was obtained from the Ministry of Health's national data collections. Additional district health board (DHB) domicile information and hospitalisation history information was also obtained from the Ministry of Health's datasets. Please note that, although DHBs are referred to in this report, DHBs were disestablished in 2022 and their functions transferred to Te Whatu Ora. The DHBs or districts are grouped into four regions. The Northern region includes Northland, Waitematā, Auckland and Counties Manukau districts; the Te Manawa Taki region includes Waikato, Lakes, Bay of Plenty, Tairawhiti and Taranaki districts; the Central region includes Hawke's Bay, Whanganui, MidCentral, Hutt Valley, Capital & Coast, and Wairarapa districts; and the Te Waipounamu region includes Nelson Marlborough, West Coast, Canterbury, South Canterbury, and Southern districts. The patients from whom ESBL-E were isolated were categorised as hospital patients if they were inpatients in a healthcare facility (including a long-term care facility) when ESBL-E was isolated or had been in a healthcare facility in the previous three months. All other patients were categorised as community patients.

At ESR, all isolates referred for the survey were confirmed as resistant to third generation cephalosporins using a cefotaxime 5 µg disc on the primary culture. All isolates with a ceftazidime zone diameter of less than 17 mm were presumed to be ESBL-producing and included in the survey. All others were excluded from the survey.

3.2 WHOLE GENOME SEQUENCING AND GENOTYPIC ANALYSIS

The genomes of a subset of 188 ESBL-E were sequenced. The subset was stratified by DHB and referring laboratory. All non-duplicate isolates from DHBs that had ≤3 samples were included in the subset. Genomic DNA was extracted using the High Pure PCR Template Preparation kit (Roche), the DNA library was created using the Nextera XT DNA Preparation kit (Illumina), and sequencing performed on the NextSeq platform (Illumina) with 2- by 150-bp paired-end chemistry. WGS data were analysed using an in-house developed pipeline linking together open-source established packages and in-house scripts. The open-source packages used included the nullarbor: 'Reads to report' for public health and clinical microbiology pipeline,[3] SPAdes 3.10,[4] mlst,[5] ABRicate,[6] and isPCR.[7]

Identification of ESBL genes, other acquired resistance genes, and chromosomal mutations in the *gyrA* and *parC* genes associated with fluoroquinolone resistance was based on *de novo* assemblies from the WGS reads. The program ABRicate was used with the ResFinder database to identify acquired resistance genes.[8] Chromosomal fluoroquinolone resistance was determined from the sequences of the *gyrA* and *parC* genes. Resistance was defined as two mutations in the *gyrA* gene (specifically a change from serine (S) at position 83 and aspartic acid (D) at position 87) and at least one mutation in the *parC* gene (specifically a change from serine at position 80).

The multilocus sequence types (MLSTs) of *Escherichia coli* and *Klebsiella pneumoniae* were determined *in silico* using direct query of the PubMLST database using the MLST schema described respectively at https://enterobase.warwick.ac.uk/species/index/ecoli and http://bigsdb.pasteur.fr/klebsiella.

3.3 GENOMIC PHYLOGENETIC ANALYSIS

3.3.1 Predicting regions of recombination

Parsnp v1.7.4[11] was used to generate a core-genome alignment (alignment of the syntenic regions across all genomes). The Genealogies Unbiased By recomBinations In Nucleotide Sequences (Gubbins) algorithm v3.2.1[12] (default settings, 'raxml mode' with the General Time Reversible (GTR) GAMMA correction) was used to assess single-nucleotide variant (SNV) density and/or predicted recombination regions in the core-genome alignment. The identified coordinates for the recombination prediction regions in the core-genome alignments were transformed into their corresponding coordinates in the reference genome.

3.3.2 Variant detection and phylogenetic analyses

This report defines a core genome as regions estimated to the nearest 100 bp with ≥95% coverage across one or more genomes in any given population. High-resolution analyses of genetic variants were performed using BWA; BEDTools v2.28.0[13]; seqtk; Trimmomatic;

Pindel[14]; Mosdepth[15]; SAMtools v1.9; Picard v2.7.1[16]; the Genome Analysis Tool Kit v4.3.0.0 (GATK);[17,18] and SNPEff v4.3.1t[19] as implemented in SPANDx v4.0.[20] Any SNVs that occurred within the predicted recombination regions were removed from the alignments. Resulting SNV alignments were used to reconstruct phylogenies. The pairwise SNV distances were determined using snp-dist v0.6.3.[21] Maximum parsimony trees were reconstructed using the heuristic search feature of PAUP v4.0a.[22] The resulting phylogenetic trees were visualised using FigTree v1.4.4.[23]

4. EPIDEMIOLOGY

4.1 DISTRIBUTION OF REFERRED ESBL-PRODUCING ENTEROBACTERALES

During the 14-day period of the 2019 survey, 285 non-duplicate, distinct ESBL-E were isolated. The 285 ESBL-E isolates referred in 2019 comprised of:

- 241 (84.6%) *E. coli*
- 33 (11.6%) *K. pneumoniae*
- 7 (2.5%) *Enterobacter* species
- 3 (1.1%) *Proteus* species
- 1 (0.4%) *Shigella dysenteriae*

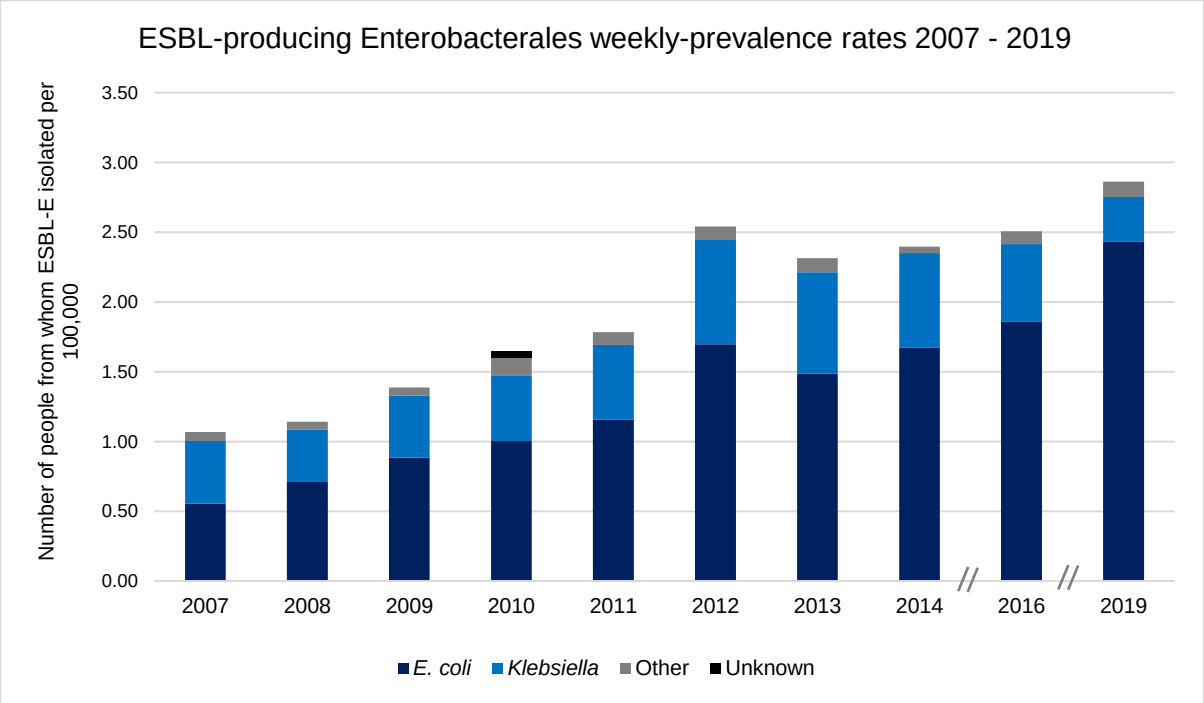

Figure 1: ESBL-producing Enterobacterales weekly-prevalence rates 2007-2019

The rates presented in this graph are period-prevalence rates calculated to reflect a week-long period and based on the number of isolates received during the 1-month duration of the surveys for 2007 - 2016, and during the 2-week duration of the 2019 survey. The rates are based on ESBL-producing Enterobacterales (ESBL-E) isolated from clinical specimens only. Data included in this graph from 2007 - 2012 includes all specimens referred which were not isolated from screening specimens. There were no surveys conducted in 2015, 2017 and 2018. The category 'Unknown' in 2010 represents people identified with an ESBL-E during the survey period but from whom no isolate was referred to ESR and the species was not reported.

The 285 isolates referred equates to a national weekly-prevalence rate of 2.86 people with ESBL-E from a clinical specimen per 100,000 population. Figure 1 shows the adjusted weekly-prevalence rates of ESBL-E from clinical specimens over the last 10 surveillance years from 2007 to 2019, and the distribution of ESBLs among *E. coli*, *Klebsiella* species and other Enterobacterales. ESBL-E prevalence rates have risen steadily since 2007 from a rate of 1.07

to 2.86 in 2019. A notable trend over these years has been an increase in the prevalence of ESBL-producing *E. coli*. Due to this increase in ESBL-producing *E. coli*, the proportion of other ESBL-producing Enterobacterales has decreased despite stable prevalence rates. In 2007, 52.0% of ESBL-E from clinical specimens were *E. coli* and 42.0% were *Klebsiella* species. By 2019 these proportions had changed to 84.9% *E. coli* and 11.2% *Klebsiella* species.

4.2 PATIENT DEMOGRAPHICS

Weekly-prevalence rates for 2016 and 2019 across age and ethnicity of patients from whom ESBL-E were isolated are shown in Table 1. Compared to 2016, prevalence of ESBL-E crude rates have increased across all ethnic groups in 2019. Prevalence of ESBL-E was also higher in the ≥65 years age group for all ethnic groups.

TABLE 1: Weekly-prevalence rates of patients with ESBL-producing Enterobacterales from a clinical specimen for 2016 and 2019 by age and ethnicity

Age group (years)	Māori		Pacific peoples		Asian		MELAA[1]		European or Other		Total[2]	
	2016	2019	2016	2019	2016	2019	2016	2019	2016	2019	2016	2019
<65	1.1[3]	1.6	1.8	2.7	3.3	2.2	4.5	2.9	1.0	1.2	1.4	1.6
≥65	8.1	15.5	12.2	23.2	11.8	15.5	N/A[4]	35.1	8.6	8.3	8.9	19.7
Total crude rate	1.5	3.9	2.4	6.6	3.8	5.2	4.3	6.9	2.5	3.6	2.5	2.9

1 Middle Eastern/Latin American/African.
2 Includes cases where ethnicity is unknown.
3 The denominator data used to determine disease rates for ethnic groups is based on the proportion of people in each ethnic group from the usually resident 2013 and 2018 census population applied to the 2016 and 2019 mid-year population estimates from Statistics New Zealand for 2016 and 2019 rates respectively. Ethnicity is prioritised in the following order: Māori, Pacific peoples, Asian, MELAA, and European or Other ethnicity (including New Zealander). Caution should be used when considering rates based on small numbers of cases.
4 No cases recorded in this category.

In 2019, ESBL-producing *E. coli* accounted for over 80% of ESBL-E in all ethnic groups. The proportion of ESBL-producing *K. pneumoniae* (12.7%) that were isolated from patients ≥65 years of age (52.6%) was much lower than the proportion of ESBL-producing *E. coli* that were from patients in this age group (82.6%) for 2019. This has reversed since 2016 when proportions of ESBL-producing *Klebsiella* spp. were higher than *E. coli* from patients ≥65 years of age.[24]

In 2019 the largest ethnicity group represented is European/Other (56%), followed by Asian (16.7%), Māori (14.2%), Pacific Peoples (9.6%) and Middle Eastern/Latin American/African (MELAA) (2.1%). Median age across ethnicity was calculated for males and females for both 2016 (Figure 2a) and 2019 (Figure 2b). In 2019 all median ages were lower than European/Other except for the male MELAA category, with only one case aged 88 years.

European/Other males and females had median age of 72 and 71 respectively in 2019 and was comparable to 2016 European/Other median age of 76 for both males and females.

Most of the ESBL-E (56.5%) were isolated from patients categorised as community patients (Table 2). However, the situation was reversed for ESBL-producing *K. pneumoniae* with the minority (15.2%) being isolated from community patients. Community patients dominated in all patient age groups. While community patients dominated in all ethnic groups, this dominance was greatest for the MELAA ethnic group and the Asian ethnic group with 66.7% and 61.7% of patients with an ESBL-E being categorised as community patients respectively.

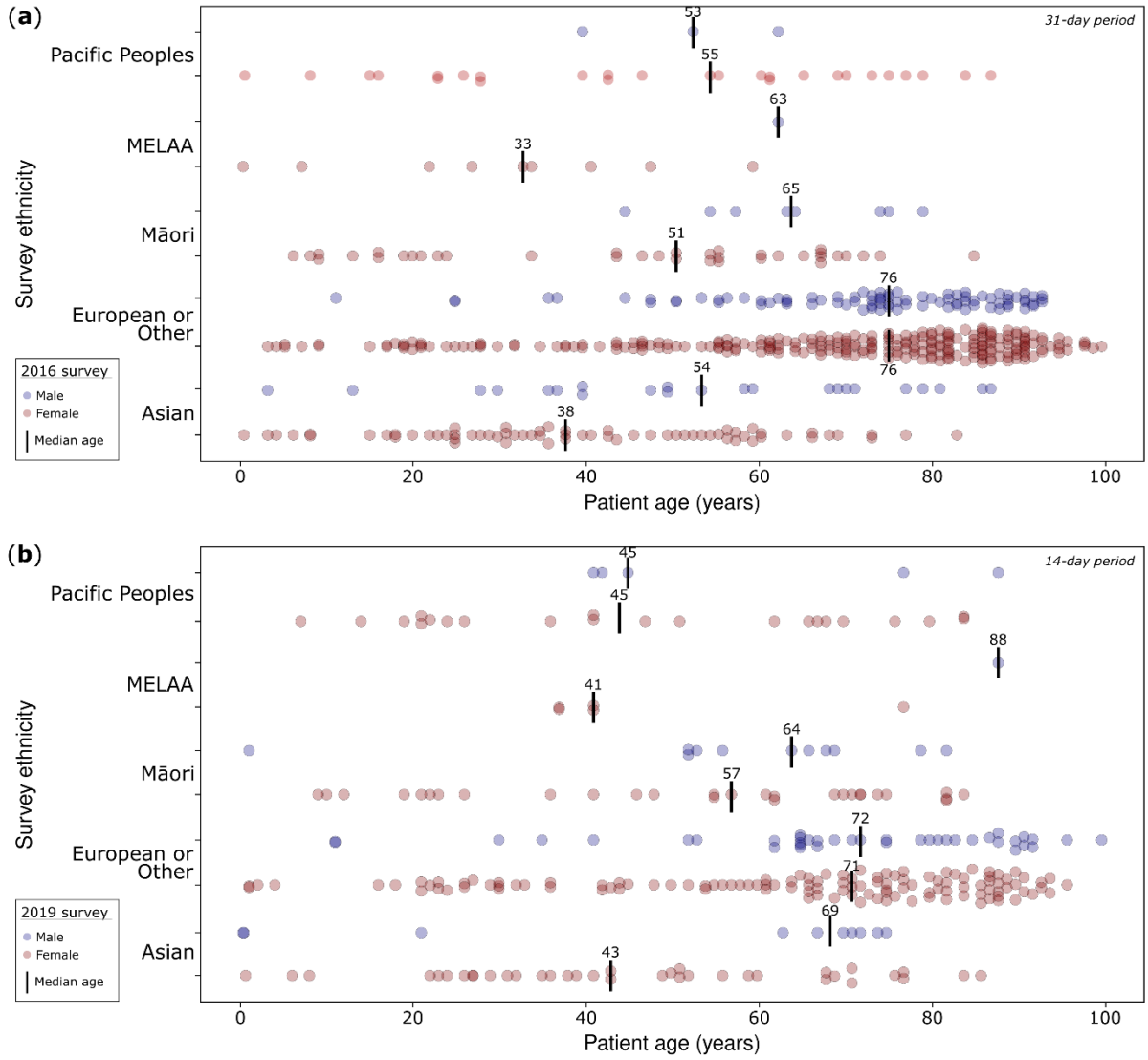

Figure 2: Distribution of patients with ESBL-producing Enterobacterales for 2016 (a) and 2019 (b) by age, sex, and ethnicity

Table 2: Comparison of ESBL-producing Enterobacterales from hospital versus community patients, 2019

	Number (row %)	
	Hospital patient[1]	**Community patient[1]**
All	124 (43.5)[2]	161 (56.5)
Species:		
E. coli	91 (37.8)	150 (62.2)
K. pneumoniae	28 (84.9)	5 (15.2)
other species	5 (45.5)	6 (54.6)
Age group (years)		
<64	51 (37.8)	84 (62.2)
≥65	73 (48.7)	77 (51.3)
Ethnicity[3]		
Māori	17 (42.5)	23 (57.5)
Pacific peoples	11 (40.7)	16 (59.3)
Asian	18 (38.3)	29 (61.7)
MELAA	2 (33.3)	4 (66.7)
European or Other	76 (47.5)	84 (52.5)
NZDep13 quintile[4]		
1	15 (32.6)	31 (67.4)
2	17 (43.6)	22 (56.4)
3	34 (61.8)	21 (38.2)
4	25 (38.5)	40 (61.5)
5	30 (41.1)	43 (58.9)
Isolation site:		
CSF/blood	9 (75.0)	3 (25.0)
urine	98 (39.4)	151 (60.6)
skin and soft tissue	8 (88.9)	1 (11.1)
respiratory tract	3 (60.0)	2 (40.0)
other	6 (66.7)	3 (33.3)

1 Patients were categorised as hospital patients if they were in a healthcare facility (including long-term care facility) when ESBL-E was isolated or had been in a healthcare facility in the previous three months. All other patients were categorised as community patients.
2 The type of healthcare facility these patients were in was known for all 124 patients categorised as hospital patients, of whom 113 (91.1%) were in a public hospital, one (0.8%) was in a private hospital, and 10 (8.1%) were in a long-term care facility.
3 Ethnicity not known for 5 patients, all community patients.
4 Quintile of the 2013 New Zealand Deprivation Index (1 = least deprived and 5 = most deprived). Index score not known for 7 patients: 2 hospital and 5 community patients.

4.3 GEOGRAPHIC DISTRIBUTION

Figure 3 shows the prevalence of ESBL-E for each of the four health regions. Main centres represented a large proportion of the regional data. Notably, ESBL-E referred from the combined Auckland DHBs including Waitematā, Auckland, and Counties Manukau made up a large proportion of the ESBL-E referred for this survey with 92.4% of all 144 Northern Region cases; and 46.7% of all cases across Aotearoa. Both Northern and Te Manawa Taki regions had weekly-prevalence rates higher than the national rate of 2.86 with rates of 3.81 and 2.93 respectively. Central and Te Waipounamu regions had weekly-prevalence rates of 1.83 and 1.72 respectively. These results are consistent with the previous 2016 survey where all Northern Region DHBs period-prevalence rates were above the national rate.

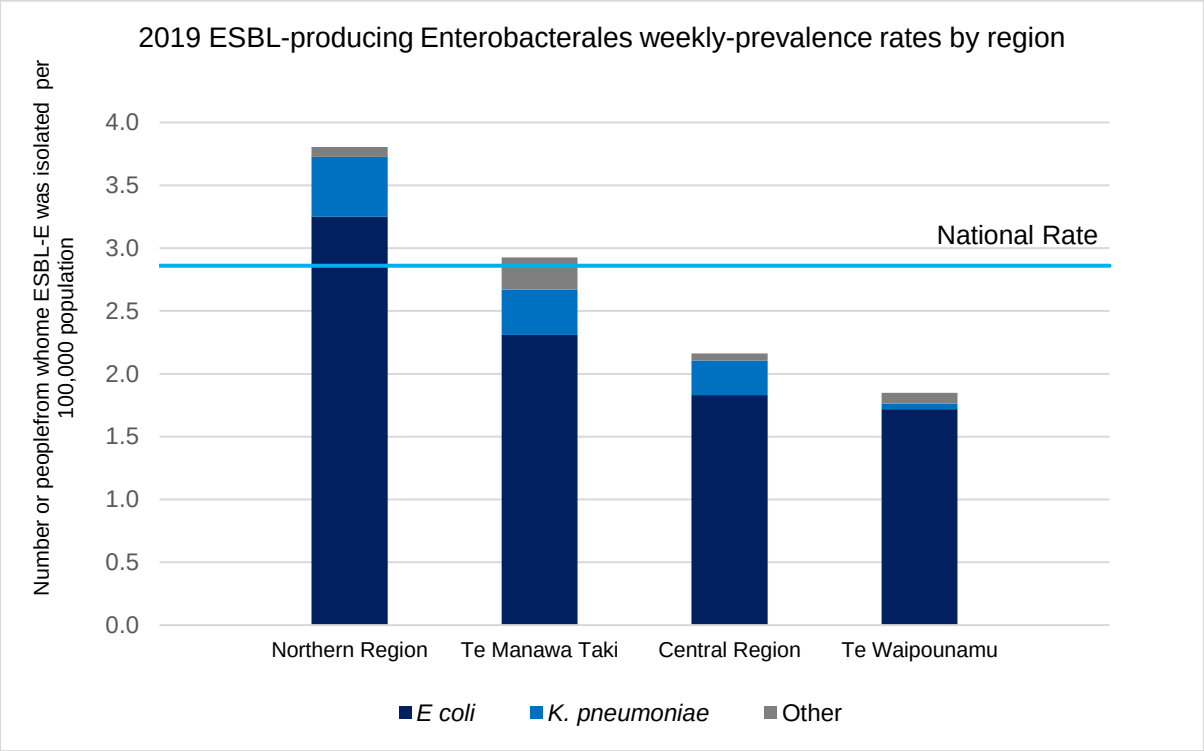

Figure 3: ESBL-producing Enterobacterales by region

The rates presented in this graph are 7-day period-prevalence rates based on the number of isolates received during the 2-week duration of the 2019 survey. The rates are based on ESBL-producing Enterobacterales (ESBL-E) referred from clinical specimens only. Regions were inferred by DHB domicile status. The denominator data used to determine disease rates for the regions are based on the proportion of people in DHB from the usually resident 2018 census population applied to the 2019 mid-year population estimates from Statistics New Zealand for 2019 rates.

5. ANTIMICROBIAL SUSCEPTIBILITY

5.1 ANTIMICROBIAL SUSCEPTIBILITY DATA FROM REFERRED LABORATORIES

The susceptibility data provided with the ESBL-E isolates submitted for the survey is presented in Table 3. There were high rates of fluoroquinolone, gentamicin, co-trimoxazole and trimethoprim resistance among ESBL-E. While fosfomycin susceptibility was only reported for 197 of the total 285 isolates, the rate of resistance was low at 13.2% for all isolates and 7.8% among ESBL-producing *E. coli*.

Table 3: Antimicrobial susceptibility of ESBL-producing Enterobacterales 2019

Antimicrobial[1]	Number of isolates with results reported[2]	Percent								
		E. coli			*K. pneumoniae*			All isolates		
		S[3]	I[3]	R[3]	S	I	R	S	I	R
Trimethoprim	268	27.6	0.4	71.9	7.7	3.9	88.5	25.1	0.8	74.1
Nitrofurantoin	239	97.2	0.5	2.3	52.6	5.3	42.1	92.9	0.8	6.3
Co-trimoxazole	127	35.0	0.0	65.0	16.7	0.0	83.3	30.7	0.0	69.3
Amoxicillin - clavulanic acid	260	60.6	2.7	36.7	26.7	3.3	70.0	55.0	2.7	42.3
Ciprofloxacin	249	35.5	5.6	58.9	16.7	4.2	79.2	33.7	5.2	61.0
Cefoxitin	195	91.2	1.8	7.1	81.0	0.0	19.1	89.2	2.1	8.7
Fosfomycin	197	92.2	0.0	7.8	25.0	0.0	75.0	86.8	0.0	13.2
Gentamicin	261	66.7	0.5	32.9	31.3	0.0	68.8	60.9	0.8	38.3
Piperacillin-tazobactam	136	95.3	1.9	2.8	81.8	0.0	18.2	91.9	1.5	6.6
Ertapenem	187	99.4	0.6	0.0	100	0.0	0.0	99.5	0.5	0.0
Meropenem	175	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0

1 Based on data supplied by laboratories referring isolates for the survey. No details were collected on which antimicrobial susceptibility testing methods had been used in the referring laboratories.
2 Total number of ESBL-E isolates with susceptibility to the antimicrobial reported.
3 S, susceptible; I, intermediate (CLSI) or susceptible, increased exposure (EUCAST); R, resistant.

5.2 ESBL TYPES

WGS data was available for 188 isolates of the total 285 ESBL-E included in the survey. ESBL genes were detected in 184 of 188 genomes sequenced.

CTX-M type ESBLs predominated and were identified in 98.9% of the 184 ESBL-E isolates sequenced (Table 4). Among the *E. coli* there were approximately equal numbers of isolates with CTX-M types belonging to group 1 (48.1%) and group 9 (51.2%). In contrast among the *K. pneumoniae* with CTX-M, 86.4% had a group 1 CTX-M. CTX-M-15 accounted for 76 (78.3%) of the total 97 isolates with a group 1 CTX-M, whereas among the 85 isolates with a group 9 CTX-M, 64 (75.3%) were CTX-M-27 and 18 (21.2%) were CTX-M-14 (see footnote 3, Table 4). There were no significant associations between CTX-M type and ethnicity.

Table 4: Distribution of ESBL types among ESBL-producing Enterobacterales 2019

ESBL type[1]	Number (column %) isolates[1]			
	E. coli **(n=158)**	***K. pneumoniae*** **(n=21)**	**Other Enterobacterales (n=5)[2]**	**All isolates (n=184)**
CTX-M	157 (99.4)	21 (100)	4 (80.0)	182 (98.9)
CTX-M group 1	76 (48.1)	19 (86.4)	2 (40.0)	97 (52.7)
CTX-M-15	68 (43.0)	6 (28.6)	2 (40.0)	76 (41.3)
Other group 1[3]	8 (5.1)	13 (61.9)	-	21 (11.4)
CTX-M group 9	81 (51.2)	2 (9.5)	2 (40.0)	85 (46.2)
CTX-M-27	64 (40.5)	-	-	64 (34.8)
Other group 9[4]	17 (10.8)	2 (9.5)	2(40.0)	21 (11.4)
SHV[5]	1 (0.6)	-	1 (20.0)	2 (1.1)

1 Only the 184 ESBL-E whose whole genome was sequenced are included in this analysis of ESBL types. Unlike previous years, TEM and VEB ESBL types were not detected in isolates sequenced.
2 Other Enterobacterales included four *Enterobacter cloacae* complex, and one *Proteus mirabilis*.
3 The other CTX-M group 1 β-lactamases include 5 CTX-M-55, and one each of CTX-M-1, CTX-M-3 and CTX-M-123.
4 The other CTX-M group 9 β-lactamases include 18 CTX-M-14, 2 CTX-M-9 and one isolate with both CTX-M-14 and CTX-M-102.
5 Both SHV ESBLs were SHV-12, including an *Enterobacter cloacae* complex.

5.3 OTHER RESISTANCE GENES AND PHENOTYPIC CORRELATION

The prevalence of genes encoding plasmid-mediated fluoroquinolone resistance, aminoglycoside modifying enzymes; *fosA* gene associated with fosfomycin resistance; *dfrA* genes associated with trimethoprim resistance; and chromosomal mutations in the *gyrA* and *parC* genes associated with fluoroquinolone resistance is shown in Table 5.

Table 5: Distribution of non-β-lactam resistance genes among ESBL-producing Enterobacterales, 2019

Resistance/resistance genes[1]	Number (column %) isolates[1]			
	***E. coli* (*n*=158)**	***K. pneumoniae* (*n*=21)**	**Other Enterobacterales (*n*=5)**	**All isolates (*n*=184)[2]**
Chromosomally mediated fluoroquinolone resistance[2]	69 (43.6)	0	0	69 (37.5)
Plasmid-mediated fluoroquinolone resistance[3]	35 (22.1)	21 (100)	4 (80.0)	60 (32.6)
Aminoglycoside modifying enzymes[4]	122 (77.2)	19 (90.5)	4 (80.0)	145 (78.8)
fosA[5]	1 (0.6)	21 (100.0)	4 (80.0)	26 (14.1)
dfrA[6]	113 (71.5)	19 (90.5)	4 (80.0)	136 (73.9)

1 Includes acquired resistance genes and mutations in the chromosomal *gyrA* and *parC* genes associated with fluoroquinolone resistance.
2 Chromosomal-mediated fluoroquinolone resistance is defined as at least two mutations in the *gyrA* gene (specifically a change from serine at position 83 and aspartic acid at position 87) and at least one mutation in the *parC* gene (specifically a change from serine at position 80).
3 The following plasmid-mediated fluoroquinolone resistance genes were identified: *qnrB, qnrD, qnrS*, *aac(6')Ib-cr*, *oqxA* and *oqxB*.
4 The following aminoglycoside modifying enzyme genes were identified: *aac(3)-IIa, aac(3)-IId, aac(3)-IVa, aac(6')-Ib-cr, aadA1, aadA11, aadA12, aadA2, aadA5, ant(2")-Ia, aph(3')-Ib*, *aph(3')-Ia , aph(4)-Ia* and *aph(6)-id*.
5 The *fosA* gene codes for a fosfomycin-modifying enzyme and was the only acquired fosfomycin resistance gene identified.
6 The *dfrA* genes mediate trimethoprim resistance.

No 16S rRNA methyltransferase gene was identified in any isolates that were sequenced. Genes for aminoglycoside modifying enzymes were common, in particular *aph(3')-Ib*, *aph(6)-Id*, *aadA5*, *aac(3)-IIa*, and *aac(3)-IId* (Table 5).

No genes encoding acquired carbapenemases were detected. Plasmid-encoded colistin resistance gene *mcr*-1 was detected in an *E. coli* isolate and *mcr*-9 was detected in an *Enterobacter cloacae* isolate.

Genotypic and phenotypic correlation for non-β-lactam resistance genes is summarised in Table 6. Concordance of genotype and phenotype were above 90% for gentamicin, trimethoprim, fosfomycin, and co-trimoxazole.

Genotypic and phenotypic concordance was lowest for ciprofloxacin and is likely due to multiple resistance mechanisms and genotypic combinations that confer resistance to fluroquinolones. There was a notable difference in the apparent mechanisms of fluoroquinolone resistance in *E. coli* and *K. pneumoniae*. 43.6% of *E. coli* had the mutations at the S83 and D87 positions in the *gyrA* gene and S80 position of the *parC* gene, and the presence of these mutations strongly correlated with the reported ciprofloxacin resistance (p <0.001). While 78.3% of *K. pneumoniae* were reported to be ciprofloxacin resistant, none of the isolates that were sequenced had mutations in the *gyrA* and *parC* genes. However, genes associated with plasmid-mediated fluoroquinolone resistance were prevalent in all *K. pneumoniae* that were sequenced, and the combination of *aac(6')-Ib-cr* + *qnrB* + *oqxAB* genes was identified in 47.6% of ciprofloxacin-resistant *K. pneumoniae* isolates.

Fosfomycin had a poor positive predictive value among *E. coli* (11.1%) due to a larger proportion of resistant phenotypes which was not attributed to carriage of the *fosA* gene. While the *fosA* gene was identified in all *K. pneumoniae* that were sequenced (Table 5), only 87.5% of the isolates with *fosA,* and for which susceptibility data was reported, were fosfomycin resistant. Fosfomycin is broadly recommended for the treatment for uncomplicated, *E. coli* urinary tract infections.

Trimethoprim resistance gene *dfrA* was identified in 96.9% of the isolates reported to be trimethoprim resistant. Co-carriage of *dfrA* and sulfamethoxazole resistance gene *sul* were detected 98.2% of the isolates reported to be co-trimoxazole (trimethoprim-sulfamethoxazole) resistant.

Table 6: Antimicrobial resistance genotype and phenotype correlation of non-β-lactam resistance mechanisms among ESBL-producing Enterobacterales 2019

	Phenotype resistant[1]		Phenotype susceptible						
Antimicrobial[2]	**Genotype resistant**	**Genotype susceptible**	**Genotype resistant**	**Genotype susceptible**	**Concordance[3] (%)**	**Sensitivity (%)**	**Specificity (%)**	**PPV[4] (%)**	**NPV[5] (%)**
Gentamicin[6]	62	4	3	100	95.9	95.4	96.2	93.9	97.1
Ciprofloxacin[7]	79	26	10	47	77.8	88.8	64.4	75.2	82.5
Trimethoprim[8]	124	5	4	39	94.8	96.9	88.6	96.1	90.7
Fosfomycin[9]	1	8	0	111	93.3	100	93.2	11.1	100
Co-trimoxazole[10]	55	3	1	24	95.2	98.2	88.9	94.8	96.0

1 Phenotype resistant includes isolates that were interpreted as intermediate or resistant

2 Based on data supplied by laboratories referring isolates for the survey. No details were collected on which antimicrobial susceptibility testing methods had been used in the referring laboratories.

3 Calculated as proportion of correlating results over total number of results available.

4 Positive predictive value.

5 Negative predictive value.

6 Includes only aminoglycoside modifying enzyme genotypes which confer resistance to gentamicin: *aac(3)-IIa, aac(3)-IId, aac(3)-Iva*, and *ant(2'')-Ia*.

7 Includes genotypes for both chromosomal-mediated and plasmid-mediated fluoroquinolone resistance mechanisms as previously defined in Table 5.

8 Includes *dfrA* genotypes which confer trimethoprim resistance.

9 Includes *fosA* genotypes which confer resistance to fosfomycin, and for *E. coli* isolates only.

10 Includes genotypes which have both *dfrA* and *sul* which confers resistance to co-trimoxazole.

6. MOLECULAR EPIDEMIOLOGY

6.1 MULTI-LOCUS SEQUENCE TYPE DISTRIBUTION

WGS data were used to determine an isolate's MLST, with 156 of 158 ESBL-producing *E. coli* and all 21 ESBL-producing *K. pneumoniae* found to have a known sequence type (ST). Two *E. coli* isolates had novel allelic profiles. The most common types, shared by two or more isolates, are shown in Table 7.

Table 7: Distribution of multi-locus sequence types among ESBL-producing *E. coli* and *K. pneumoniae*, 2019

***E. coli* (*n*=158)**		***K. pneumoniae* (*n*=21)**	
MLST[1,2]	**Number (%)**	**MLST[1,2]**	**Number (%)**
ST131	71 (44.9)	ST25	8 (38.1)
ST1193	22 (13.9)	ST307	2 (9.5)
ST38	15 (9.5)		
ST69	8 (5.1)		
ST95	3 (1.9)		
ST141	3 (1.9)		
ST450	3 (1.9)		
ST636	3 (1.9)		
ST998	3 (1.9)		
ST7401	3 (1.9)		
ST10	2 (1.3)		
ST4553	2 (1.3)		

1 Only the MLSTs shared by ≥2 isolates are listed in the table.
2 *E. coli* ST69 and ST7401 both belong to the ST648 MLST clonal complex.

ST131 was the predominant MLST among *E. coli,* accounting for 44.9% of all *E. coli* able to be typed. A comparison of the demographics of the patients with ST131 *E. coli* vs *E. coli* of other MLSTs showed no significant differences in age group distribution, ethnic group distribution or the proportion of patients who were categorised as community patients.

ST25 was the most prevalent MLST among *K. pneumoniae,* accounting for 36.4% of all *K. pneumoniae* able to be typed. All ST25 *K. pneumoniae* had a CTX-M-15 ESBL. A

comparison of the demographics of the patients with ST25 *K. pneumoniae* vs *K. pneumoniae* of other MLSTs showed no significant differences in age group distribution, ethnic group distribution or the proportion of patients who were categorised as community patients.

6.2 GENOMIC DIVERSITY AND PHYLOGENETIC ANALYSIS

Phylogenetic analysis of WGS data can identify lineages associated with specific properties and identify fine-scale transmission patterns. As these methods are most powerful when focusing on a group of related genomes, we focused on most common STs from *E. coli* (ST131) and *K. pneumoniae* (ST25) for these analyses.

6.2.1 ST131 *E. coli*

ST131 is a well-studied group of ESBL-producing *E. coli* that are typically classified into distinct "A", "B" and "C" clades.[25] Clade A is highly divergent, and clade B is described as a precursor to clade C, which has been primarily responsible for the global spread of ST131. Clade C exhibits high levels of fluoroquinolone resistance following the acquisition of chromosomal mutations in the quinolone resistance-determining regions of the *gyrA*, *gyrB*, *parC*, and *parE* genes. The two primary sub-lineages within clade C, C1 (also known as the *H*30R sub-lineage) and C2 (also known as the *H*30Rx sub-lineage), are frequently reported in nosocomial infections. More importantly, clade C2 has been described as an ESBL-producing sub-lineage, carrying $bla_{\text{CTX-M-15}}$. All three clades are represented in the 2019 ESBL-E survey, with clade A (*n* = 38/71, 53.5%) and clade C (*n* = 31/71, 43.7%) most common and clade B rare (*n* = 2/71, 2.8%). All clade C genomes from the 2019 survey have mutations in the *gyrA* and *parC* genes which are likely to confer resistance to fluoroquinolones. Given the large number of ST131 isolates, we produced separate phylogenetic analyses for ST131 clades A, C1 and C2.

ST131 clade A. We investigated the diversity and phylogenetic relationships of the 38 clade A genomes from the 2019 survey. We placed these 38 genomes in context with other clade A genomes obtained from previous New Zealand surveys conducted in 2016 (*n* = 54), 2013 (*n* = 22), 2007 (*n* = 2), and 1999 (*n* = 1). Using the chromosome of MVAST0167 (GenBank: CP014492) as a reference, we constructed a core-genome alignment of 2,550,400 total nucleotides, containing 2,378 variable sites. The high genomic coverage and diversity of this dataset will support reliable inference of relationships between these isolates. This alignment was used to create a maximum parsimony phylogenetic tree containing 118 genomes in total (Figure 4).

The 118 ESBL-producing clade A genomes exhibit substantial genomic variation, with overall median pairwise SNV distance of 93 SNVs (Inter quartile range (IQR): 73 to 108; range: 1 to 181 SNVs). In addition to the diversity observed, the clade also contains several closely

related clusters of isolates. In Figure 4 genomes that are separated by five or few SNVs are connected by red branches. These tightly linked clusters have recently shared a common ancestor and may represent transmission of bacterial lineages, exposure to a common source or endemic clones. The clade A data include two pairs of closely related (separated by three and five SNVs respectively) isolates collected from the same health district but different annual surveys. The persistence of these tightly linked lineages in a single region over several years is evidence for ongoing transmission of the clade A ST131 ESBL-producing *E. coli* within Aotearoa New Zealand.

ST131 clade C1/*H*30 summary. We investigated the diversity and phylogenetic relationships of the 15 clade C1 genomes from the 2019 survey. We placed these 15 genomes in context with other clade C1 genomes obtained from previous surveys conducted in 2016 (*n* = 52), 2013 (*n* = 40), and 2007 (*n* = 5). A previously sequenced clade C0 genome from New Zealand was chosen as an outgroup to root the phylogenetic tree. Using the chromosome of U12A (GenBank: CP035476) as a reference, we constructed a core-genome alignment of 2,548,600 total nucleotides, containing 1,952 variable sites. The high genomic coverage and diversity of this dataset will support reliable inference of relationships between these isolates. This alignment was used to create a maximum parsimony phylogenetic tree containing 114 genomes (Figure 5).

Despite having lower genomic variation when compared to clade A genomes (Figure 4), the 114 clade C1 genomes still exhibit substantial genomic variation, with overall median pairwise SNV distance of 46 SNVs (IQR: 36 to 58; range: 0 to 144 SNVs). As with clade A, this clade contains several closely related clusters of isolates. This includes a pair of genetically indistinguishable genomes representing isolates collected from different patients from the same region in 2016 survey. Clade C1 also contains a cluster of 15 closely related genomes. Most (*n* = 13/15) of these genomes represent isolates from Te Manawa Taki region and the earliest sample (ESR ID: ARL131334) was collected in 2013 and the most recent as part of this survey. These findings underscore the spread and persistence of ESBL-producing clade C1 strains in Aotearoa New Zealand.

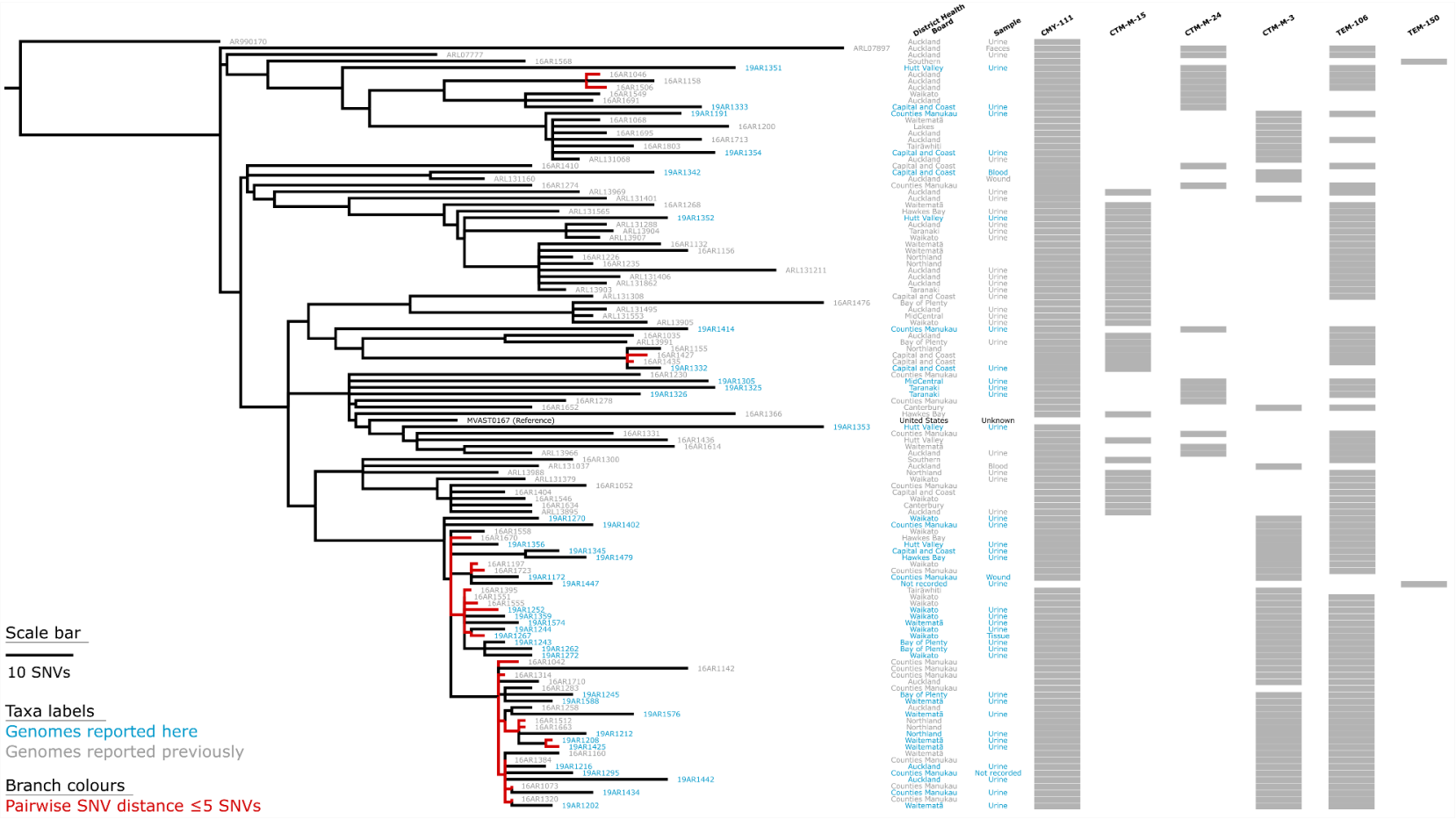

Figure 4: Maximum parsimony phylogeny of *E. coli* sequence type ST131 Clade A isolates, alongside the antimicrobial resistance genotype

Phylogeny was inferred from 2,378 non-recombinant core-genome single-nucleotide variants (SNVs) from 118 genomes. SNVs were derived from a core-genome alignment of approximately 2,550,400 bp and are called against the reference chromosome MVAST0167 (GenBank: CP014492). The phylogenetic tree is rooted according to the genome SE15 (GenBank: AP009378) outgroup, which has been omitted for visual purposes. Grey plots indicate the presence of extended-spectrum beta-lactamase-encoding genes. Notably, genes that are only present in a single isolate are omitted. Red branches shows genomes with a pairwise SNV distance of ≤5 SNVs.

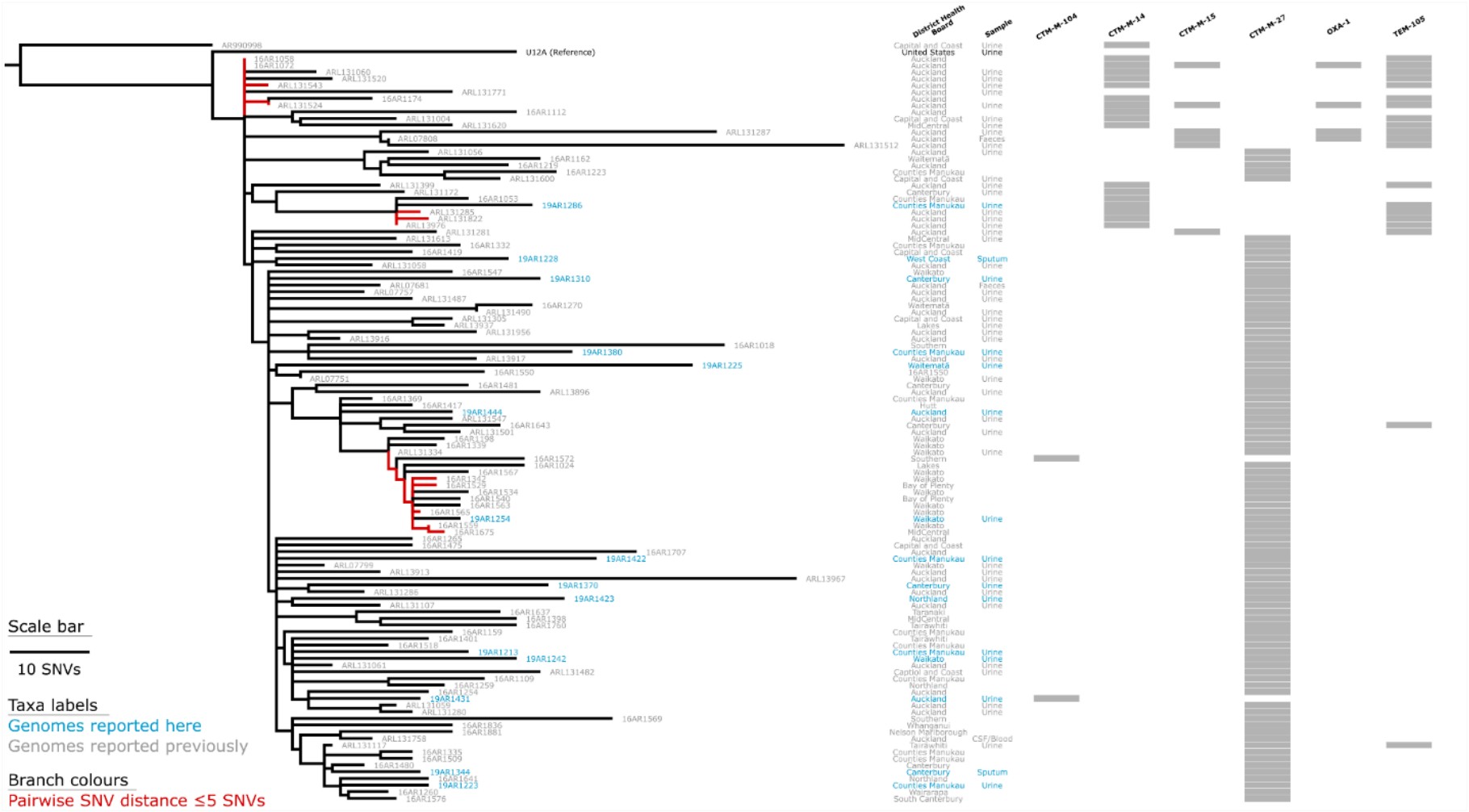

Figure 5: Maximum parsimony phylogeny of *Escherichia coli* sequence type (ST)131 Clade C1 isolates, alongside the antimicrobial resistance genotype

Phylogeny was inferred from 1,952 non-recombinant core-genome single-nucleotide variants (SNVs) from 114 genomes. SNVs were derived from a core-genome alignment of approximately 2,548,600 bp and are called against the reference chromosome U12A (GenBank: CP035476). The phylogenetic tree is rooted according to the clade C0 genome AR990998 outgroup. Grey plots indicate the presence of extended-spectrum beta-lactamase-encoding genes. Notably, genes that are only present in a single isolate are omitted.

ST131 Clade C2/*H*30Rx summary. We investigated the diversity and phylogenetic relationships of the 16 clade C2 genomes from the 2019 survey. We placed these 16 genomes in context with other clade C2 genomes obtained from previous surveys conducted in 2016 (*n* = 68), 2013 (*n* = 61), 2007 (*n* = 41), 2002 (*n* = 29); and 1999 (*n* = 1). We used a clade C0 genome from the 1999 survey as an outgroup to root the phylogenetic tree. Using the chromosome of EC958 (GenBank: HG941718) as a reference, we constructed a core-genome alignment of 2,592,500 total nucleotides, containing 4,483 variable sites. The high genomic coverage and diversity of this dataset will support reliable inference of relationships between these isolates. This alignment was used to create a maximum parsimony phylogenetic tree containing 218 genomes (Figure 6).

Comparing to previous ESBL surveys in Aotearoa New Zealand, the 16 clade C2 genomes from the 2019 survey show sporadic distribution in the phylogeny. No genomes are linked by five or fewer SNVs and isolates sampled in the 2019 survey occur across the tree with no geographical or temporal clustering. It is possible clade C2 is not yet endemic in Aotearoa. Without more detailed epidemiological data it is not possible to determine how many of the infections represented here may be linked to international travel. The clade C2 dataset also contains four isolates assigned to STs other than ST131 but nevertheless part of this clade. Although MLST is frequently used to classify bacterial lineages, it is important to note that ST profiles are not explicitly evolutionary and relying on ST alone to identify closely related isolates is not always reliable. All four of these instances are genomes from previous ESBL surveys conducted in Aotearoa New Zealand.

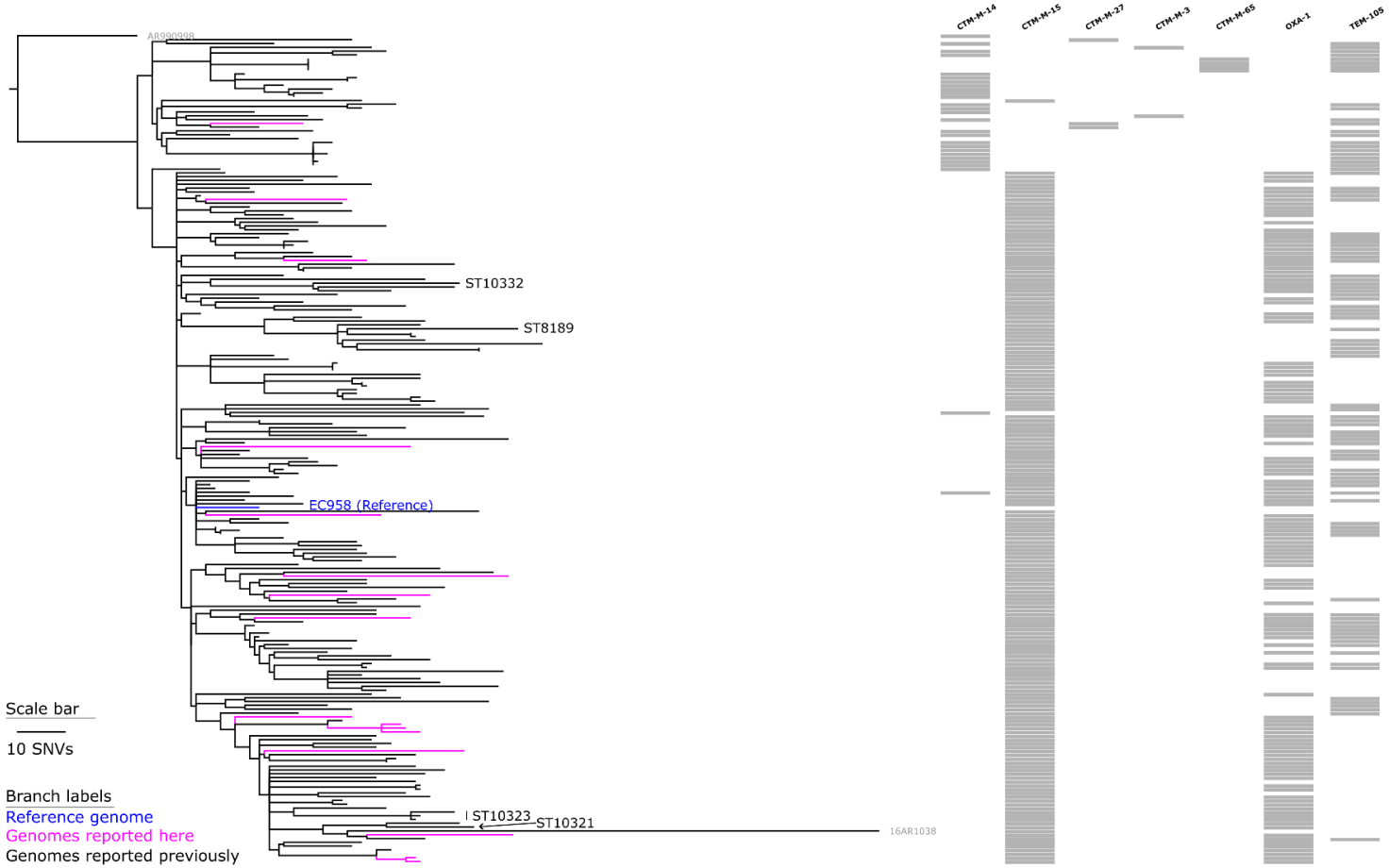

Figure 6: Maximum parsimony phylogeny of *Escherichia coli* sequence type (ST)131 Clade C2 isolates, alongside the antimicrobial resistance genotype

Phylogeny was inferred from 4,483 non-recombinant core-genome single-nucleotide variants (SNVs) from 218 genomes. SNVs were derived from a core-genome alignment of approximately 2,592,500 bp and are called against the reference chromosome EC958 (GenBank: HG941718). The phylogenetic tree is rooted according to the clade C0 genome AR990998 outgroup. Grey plots indicate the presence of extended-spectrum beta-lactamase-encoding genes. Notably, genes that are only present in a single isolate are omitted.

6.2.2 ST25 *K. pneumoniae*

K. pneumoniae ST25 has been sporadically identified as a high-risk clone,[26,27] instances of it carrying *bla*$_{NDM-1}$ and *bla*$_{KPC-2}$ have been linked to its global expansion and clone proliferation.[26,28] The ST25 genomes detected in the 2016 and 2019 surveys carry the *bla*$_{SHV}$ and *bla*$_{CTX-M}$-type beta-lactamases.

We investigated the diversity and phylogenetic relationships of the eight ST25 genomes from the 2019 survey. We placed these eight genomes in context with other ST25 genomes obtained from the 2016 survey (*n* = 35). Using the chromosome of INF014-sc-2279884 (GenBank: CP031810) as a reference, we constructed a core-genome alignment of 2,724,300 total nucleotides, containing 820 variable sites. The high genomic coverage and diversity of this dataset will support reliable inference of relationships between these isolates. This alignment was used to create a maximum parsimony phylogenetic tree containing 44 genomes (Figure 7).

In Aotearoa New Zealand, the genomes of 44 ESBL-producing ST25 exhibit substantial genomic variation, as evidenced by the overall median pairwise SNV distance of 61 SNVs (IQR: 46 to 74; range: 2 to 261 SNVs). Figure 7 displays three clusters of closely related genomes with less than five pairwise SNVs. The first cluster comprises five genomes from 2016, representing different ethnicities and ages. The second cluster includes two genomes from Waitematā in 2016 with a two-SNV difference. Notably, the third cluster involves a 2016 genome differing by four SNVs from a 2019 genome, both from the Northern region. These clusters suggest potential ESBL-producing *K. pneumoniae* ST25 transmission or shared exposure throughout the Northern region.

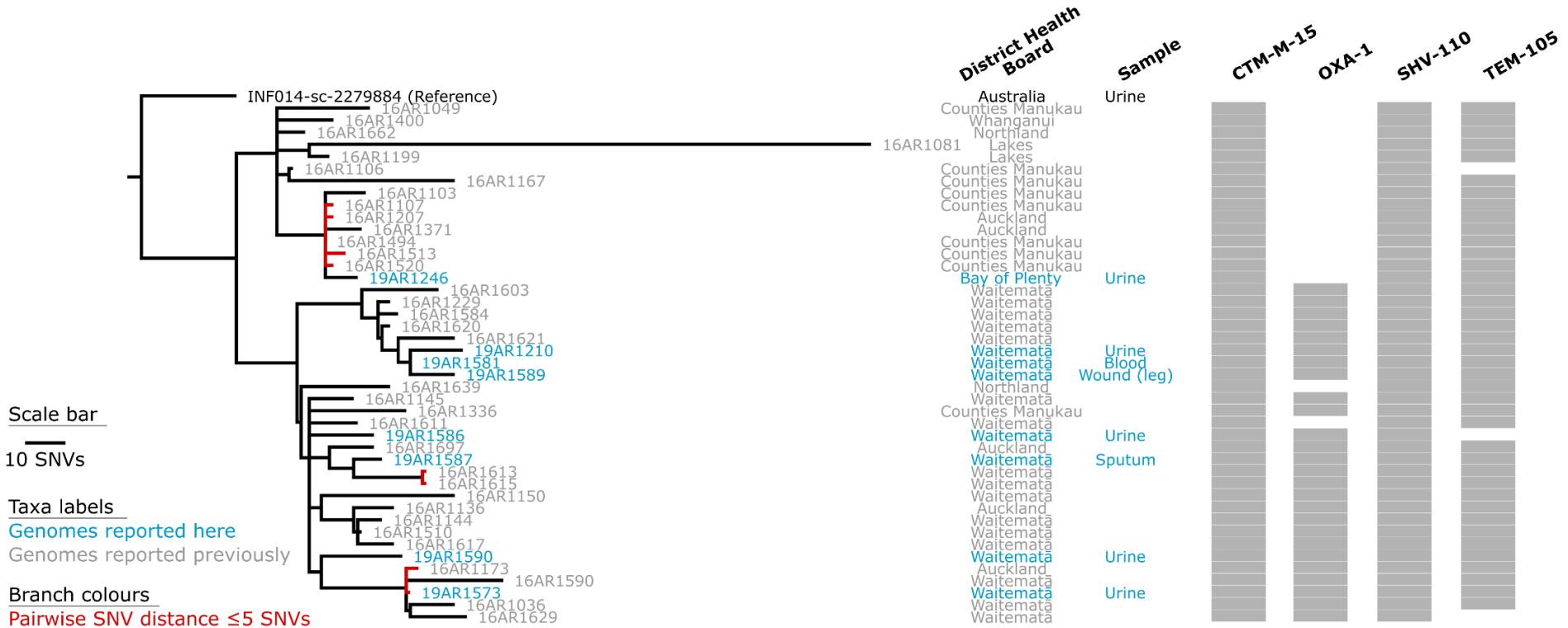

Figure 7: Maximum parsimony phylogeny of *Klebsiella pneumoniae* sequence type (ST)25 isolates, alongside the antimicrobial resistance genotype

Phylogeny was inferred from 820 non-recombinant core-genome single-nucleotide variants (SNVs) from 44 genomes. SNVs were derived from a core-genome alignment of approximately 2,724,300 bp and are called against the reference chromosome INF014-sc-2279884 (GenBank: CP031810). The phylogenetic tree is rooted according to the INF014-sc-2279884 outgroup. Black plots indicate the presence of extended-spectrum beta-lactamase-encoding genes. Notably, genes that are only present in a single isolate are omitted.

7. CONCLUDING REMARKS

The 2019 national survey of ESBL-E in Aotearoa New Zealand is the second to be conducted from clinical specimens alone. The shorter collection period from 31 days in 2016 to two weeks for this survey has disadvantages as it limits the ability to compare national period prevalence rates between surveys. Another disadvantage is the decreased sample size may affect the resolution of the data collected. The 2016 survey had eight different genera of ESBL-E, compared to 2019 where only five different genera were identified. However proportional calculations from this survey still support the trend of increasing ESBL *E. coli* infections from 74.1% in 2016 to 84.9% in 2019.

A reduction in collection period also exaggerated the sampling bias towards the Auckland region, making up almost half of all isolates that were referred for this survey. The large representation for this region may be attributable to Auckland being the largest and most ethnically diverse city in Aotearoa. However, the lower proportion of isolates from surrounding areas are consequently under-represented which can affect the interpretation and comparisons of data particularly for weekly-prevalence rate between regions.

Most referring laboratories rely on European Committee on Antimicrobial Susceptibility Testing (EUCAST) or Clinical Laboratory and Standards Institute (CLSI) standards for their susceptibility testing methods and analysis. Different testing platforms, methods, and adherence to these standards across the different laboratories may affect the antimicrobial susceptibility data presented as part of this survey. For example, the interpretation of the standards for reporting in diagnostic laboratories may be prioritised to promote safer patient management by using a conservative approach to interpretation of susceptibility and report isolates as resistant to deter clinicians from using inappropriate antimicrobials for treatment. Other factors such as changes to susceptibility breakpoints from regular annual updates from EUCAST or CLSI, minor transcription errors, or missing data can affect rates of resistance for the dataset collected for this surveillance.

There will be increasing reliance on WGS to perform surveillance of infectious diseases in the future, including surveys that provide information on antimicrobial resistance. Future ESBL-E surveys should include WGS analysis for all isolates as this will benefit from the comprehensive data that genomes can provide and will be a more cost-effective option for molecular surveillance. For the 2019 survey, a sub-sample of representative isolates were selected for sequencing. This presents as a limitation of this survey where there is no genomic information for isolates that were not sequenced including their ESBL status and other resistance genes which may be of interest.

Correlation of genotype and phenotype of non-β-lactam antimicrobial resistance for the 2019 ESBL-E dataset gave variable results and did not provide sufficient data to infer phenotypic resistance based on gene carriage. Data used to assess phenotypic prediction based on the WGS resistome would benefit from concurrent susceptibility testing for future surveys. As the data used for phenotypic resistance was provided by referring laboratories, this will inadvertently affect the accuracy of correlation data and caution should be used when applying the data presented in this survey to clinical settings.

This report includes the first detailed whole-genome phylogenetic analyses of ESBL-producing organisms in Aotearoa. These analyses provide both an insight into the transmission dynamics of these organisms and a basis for comparison in future. By focusing on the predominant lineages within ESBL-producing *E. coli* and *K. pneumoniae* circulating in New Zealand across all surveys, several genomically linked clusters (separated by 5 or fewer SNVs) were identified that are also clustered in time or geography. These results demonstrate the sustained detection of specific ESBL-producing lineages in clinical settings.

The genomic data produced and analysed for this report will form an invaluable resource for future surveillance of ESBL producing organisms. Isolates collected in future will be able to be compared to this 'baseline' data, with detailed phylogenetic and genomic analysis helping to track trends in both transmission and the genes responsible for ESBL-production. These results will inform public health strategies and enhance understanding of microbial evolution and aiding in targeted interventions.

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INSTITUTE OF ENVIRONMENTAL SCIENCE AND RESEARCH LIMITED

Kenepuru Science Centre
34 Kenepuru Drive, Kenepuru, Porirua 5022
PO Box 50348, Porirua 5240
New Zealand
T: +64 4 914 0700 **F:** +64 4 914 0770

Mt Albert Science Centre
120 Mt Albert Road, Sandringham, Auckland 1025
Private Bag 92021, Auckland 1142
New Zealand
T: +64 9 815 3670 **F:** +64 9 849 6046

NCBID – Wallaceville
66 Ward Street, Wallaceville, Upper Hutt 5018
PO Box 40158, Upper Hutt 5140
New Zealand
T: +64 4 529 0600 **F:** +64 4 529 0601

Christchurch Science Centre
27 Creyke Road, Ilam, Christchurch 8041
PO Box 29181, Christchurch 8540
New Zealand
T: +64 3 351 6019 **F:** +64 3 351 0010

www.esr.cri.nz