Antimicrobial susceptibility and molecular epidemiology of extended-spectrum β-lactamase producing Enterobacteriaceae in New Zealand, 2013

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SUMMARY

Resistance to third-generation cephalosporins, particularly due to extended-spectrum β-lactamase (ESBL) production, has become endemic among Enterobacteriaceae, such as *Escherichia coli* and *Klebsiella pneumoniae*, in several regions of the world. While the incidence of ESBL-producing Enterobacteriaceae (ESBL-E) is relatively low in New Zealand, rates are increasing. This survey was undertaken to provide contemporary information on the antimicrobial susceptibility and molecular epidemiology of ESBL-E in New Zealand.

A random sample of 352 clinical ESBL-E isolates was selected from isolates referred to the Institute of Environmental Science and Research (ESR) as part of the 2013 annual ESBL-E survey. Basic patient demographic data and hospitalisation history data was provided by the referring laboratories or obtained from the National Minimum Dataset. Antimicrobial susceptibility was determined by agar dilution, the ESBL type identified by PCR and sequencing, and molecular typing of *E. coli* and *K. pneumoniae* was performed by multiple-locus variable tandem repeat analysis (MLVA). *E. coli* were further characterised to identify phylogenetic groups and isolates belonging to multilocus sequence type 131 (ST131).

63.6% (224) of the 352 ESBL-E isolates were *E. coli*, 31.5% (111) were *Klebsiella* spp., and the remaining 4.8% (17) were other Enterobacteriaceae species. The majority (88.6%) of isolates were from urine. 75.6% of patients were female and 64.1% of patients were ≥65 years of age. Patients with ESBL-producing *E. coli* (ESBL-*E. coli*) were almost evenly split between community patients and those in a healthcare facility, whereas 83.3% of patients with ESBL-producing *Klebsiella* spp. (ESBL-*Klebsiella* spp.) were healthcare facility patients.

The majority of ESBL-E were multiresistant, with the most common resistance pattern being resistance to amoxicillin-clavulanate, ciprofloxacin, co-trimoxazole/trimethoprim and gentamicin. 93.3% of ESBL-*E. coli* were susceptible to nitrofurantoin, although most ESBL-*Klebsiella* spp. were resistant. 84.9% and 96.3% of ESBL-E were susceptible to the oral agents mecillinam and fosfomycin, respectively. All ESBL-E were susceptible to

continued

meropenem, 98.9% were ertapenem susceptible, 94.9% piperacillin/tazobactam susceptible, and 98.0% tigecycline susceptible. Generally, ESBL-*Klebsiella* spp. were less susceptible than ESBL-*E. coli* to most antimicrobials.

97.2% of ESBLs identified were CTX-M types, with the remainder being SHV or VEB types. While both group 1 and group 9 CTX-M ESBLs were common in ESBL-*E. coli*, only group 1 CTX-Ms were common among ESBL-*Klebsiella* spp. There were no clear associations between patient ethnic groups and CTX-M type.

71.4% of the ESBL-*E. coli* belonged to phylogenetic group B2, 20.1% belonged to group D, 6.3% to group A, and 2.2% to group B1. The *E. coli* ST131 clone accounted for an estimated 54.4% of ESBL-*E. coli*. All ST131 *E. coli* had a CTX-M-type ESBL, with an almost equal split between group 1 and group 9 CTX-M types. Ciprofloxacin resistance was more prevalent among ST131 ESBL-*E. coli* than other ESBL-*E. coli*.

Based on MLVA typing, there was considerable strain diversity among both the 224 ESBL-*E. coli*, with 62 MLVA types identified, and the 107 ESBL-producing *K. pneumoniae*, with 36 MLVA types identified. However, the Simpson's index of diversity suggested that the ESBL-*K. pneumoniae* were less clonally diverse than the ESBL-*E. coli*.

In conclusion, this survey showed that ESBL-E in New Zealand are usually multiresistant but generally retain susceptibility to several antimicrobial classes including carbapenems, piperacillin/tazobactam, tigecycline, the oral agents mecillinam and fosfomycin, and in the case of ESBL-*E. coli*, also nitrofurantoin. In line with global trends, CTX-M-type ESBLs are dominant in New Zealand and the pandemic *E. coli* ST131 clone accounted for just over half of the ESBL-*E. coli*.

We recommend that surveys to provide contemporary information on the antimicrobial susceptibility and molecular epidemiology of ESBL-E should be repeated at regular intervals or preferably become a routine part of the regular national ESBL-E surveys.

RECOMMENDATIONS

1 Surveys such as this one should be repeated periodically or preferably become an integral part of the regular national extended-spectrum β-lactamase-producing Enterobacteriaceae (ESBL-E) surveys to enhance the usefulness of the regular surveys.

2 Future surveys could be further enhanced by:

- obtaining patient hospitalisation history data from the National Minimum Dataset to enable a more accurate description of the epidemiology of ESBL-E in healthcare vs community settings;
- full identification of the ESBL types;
- identification of sub-clones of ST131 ESBL-producing *Escherichia coli*; and
- using DNA microarrays to identify virulence factors in ESBL-E in New Zealand.

1 INTRODUCTION

Enterobacteriaceae, particularly *Escherichia coli* and *Klebsiella pneumoniae*, are major human pathogens, in both community and healthcare settings. Over the past decade, the prevalence of antimicrobial-resistant Enterobacteriaceae has increased considerably in many parts of the world.[1] Most notably, resistance to third-generation cephalosporins (eg, ceftriaxone, ceftazidime), particularly due to extended-spectrum β-lactamase (ESBL) production, has become endemic in several regions. For example, data from a recent World Health Organization report described resistance rates of over 50% to third-generation cephalosporins in *E. coli* isolates from several countries in the South-East Asia and Western Pacific regions.[1] In New Zealand, data from annual national surveys demonstrate an increase in the incidence of ESBL-producing Enterobacteriaceae (ESBL-E) over the past decade, with marked geographic variation in incidence rates.[2] However, based on aggregate national susceptibility data, the overall prevalence of ESBL production in *E. coli* bloodstream isolates has remained stable in New Zealand over the past decade at <5%, whereas the reported prevalence of ESBL production in *K. pneumoniae* bloodstream isolates is higher at 10–15%.[3]

Throughout the 1980s and 1990s, the most common globally reported types of ESBL were either TEM- or SHV-type ESBLs.[4] However, during the 2000s, the CTX-M-type ESBLs rapidly emerged and spread to become the most commonly identified ESBL type worldwide.[5] The genes encoding CTX-M enzymes are usually located on mobile genetic elements such as plasmids, thus facilitating their widespread dissemination.[6] Based on amino acid similarity, CTX-M enzymes cluster into five main groups, with the vast majority belonging to CTX-M groups 1 and 9. The two most common CTX-M types described to date are CTX-M-15 (belonging to group 1) and CTX-M-14 (belonging to group 9). In particular, CTX-M-15 has emerged to become the most globally prevalent CTX-M enzyme, and a strong association has been described between CTX-M-15-producing *E. coli* and a global 'pandemic' clone of *E. coli* known as sequence type (ST) 131.[6] The ST131 clone is noted for its ability to harbour numerous genes associated with both antimicrobial resistance and virulence.[7] In addition to β-lactam resistance, ST131 *E. coli* isolates are also commonly resistant to fluoroquinolones.[6,7] A 2006 molecular epidemiological study of ESBL types in New Zealand identified CTX-M-type ESBLs as the most common ESBL types in New Zealand. In this study, 81/83 (98%) of ESBL-E

isolates produced CTX-M enzymes, with the most common CTX-M types being CTX-M-15 (78%, 63/81) and CTX-M-14 (14%, 11/81).[8]

Importantly, because the genes that encode ESBL production are often associated with genes conferring resistance to other antimicrobials, such as fluoroquinolones and aminoglycosides,[7] therapeutic options for serious infections caused by ESBL-E are often limited to antimicrobial agents such as carbapenems and amikacin. In addition, spread of ESBL-E in community settings poses a challenge to oral antimicrobial treatment of infections caused by such organisms, and suitable options may be limited to agents such as fosfomycin and mecillinam. To date however, there are no national data on susceptibility of ESBL-E to these agents, nor to newer agents such as tigecycline.

To provide contemporary information on antimicrobial resistance patterns and molecular epidemiology of ESBL-E in New Zealand, we undertook extended phenotypic and genotypic characterisation of nationally representative clinical isolates sent to the Institute of Environmental Science and Research (ESR) as part of the 2013 annual ESBL-E survey.[2] Specifically, the aims of this study were to:

- Provide contemporary information on antimicrobial resistance amongst ESBL-E in New Zealand, including resistance to agents such as fosfomycin, mecillinam and tigecycline.
- Determine the relative prevalence of specific ESBL types in clinical ESBL-E isolates in New Zealand.
- Identify the major clones of ESBL-producing *E. coli* (ESBL-*E. coli*) and ESBL-producing *K. pneumoniae* (ESBL-*K. pneumoniae*) circulating in New Zealand, including ST131 *E. coli.*

2 METHODS

2.1 Isolates and patient information

Among the 793 isolates received for the 2013 annual ESBL-E survey, 458 were from clinical or diagnostic specimens rather than screening specimens.[2] This total excludes repeat ESBL-producing isolates of the same species from the same patient.

From the total 458 non-duplicate clinical isolates, a simple random sample of 352 isolates was selected for this survey using the SAS survey select procedure, and stratified by referring diagnostic laboratory and bacterial species.

When referring isolates for the 2013 annual ESBL-E survey, diagnostic laboratories supplied epidemiological data including patient age, geographic location, hospitalisation status, body site from which the ESBL-E was isolated, and if the isolate was obtained from a diagnostic specimen or screen. Laboratories also supplied data on the susceptibility of the isolates to several antibiotics, including co-trimoxazole and trimethoprim.

The patients from whom ESBL-E were isolated were categorised as public hospital patients, long-term care facility (LTCF) residents or private hospital patients if, respectively, they were in a public hospital (including emergency department or outpatient clinic), LTCF or private hospital when ESBL-E was isolated or had been in such a healthcare facility in the previous three months. All other patients were categorised as community patients.

Information on patient ethnicity was obtained from the Ministry of Health's National Minimum Dataset. The ethnic groups presented are based on a prioritised classification of ethnicity, with the Māori ethnic group at the top of the hierarchy, followed by Pacific Peoples, Asian, Middle Eastern/Latin American/African (MELAA), and European or Other (including New Zealander) ethnic groups. For some analyses, the Asian ethnic group was subdivided into Indian and Other Asian based on level 2 ethnicity coding. More information about ethnicity classification is available on the Ministry of Health web site: http://www.health.govt.nz/publication/ethnicity-data-protocols-health-and-disability-sector.

2.2 Antimicrobial susceptibility testing

All isolates were confirmed to produce ESBL at ESR by the Clinical and Laboratory Standards Institute's (CLSI's) phenotypic confirmatory disc test,[9] or a double-disc synergy test with cefotaxime, ceftazidime, cefpodoxime and cefepime as substrates.[10]

Susceptibility to amoxicillin-clavulanate, ciprofloxacin, ertapenem, fosfomycin, gentamicin, mecillinam, meropenem, nitrofurantoin, piperacillin-tazobactam and tigecycline was determined by agar dilution according to CLSI methods.[11] The tigecycline pure substance and tazobactam used to prepare agar dilution plates were generously donated by Wyeth Research (Monmouth Junction, New Jersey, United States). All other antibiotic pure substances used for agar dilution were obtained from either Sigma-Aldrich (Saint Louis, Missouri, United States) or Toku-E (Bellingham, Washington, United States).

Minimum inhibitory concentrations (MICs) were interpreted according to CLSI guidelines, except for tigecycline MICs which were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints.[9,12] The MIC_{50} and MIC_{90} values were defined as the MICs at which at least 50% and 90%, respectively, of isolates were inhibited.

The co-trimoxazole/trimethoprim susceptibility results presented in this report are those reported by the referring laboratories. For some isolates susceptibility to both cotrimoxazole and trimethoprim was reported. If an isolate was resistant to one agent but intermediate or susceptible to the other, it was recorded as resistant to co-trimoxazole/trimethoprim, and likewise if it was intermediate to one agent but susceptible to the other, it was recorded as intermediate to co-trimoxazole/trimethoprim.

In keeping with previously described definitions, multidrug resistance was defined as non-susceptibility to ≥3 of the following antibiotics or antibiotic classes: amoxicillin-clavulanate, carbapenems (ertapenem or meropenem), cefoxitin, ciprofloxacin, folate pathway inhibitors (co-trimoxazole or trimethoprim), fosfomycin, gentamicin, mecillinam, nitrofurantoin, piperacillin-tazobactam or tigecycline.[13] For this definition, the co-trimoxazole/trimethoprim susceptibility was that reported by the referring laboratories;

cefoxitin susceptibility was that determined by disc susceptibility testing at ESR; and the susceptibility to all other antibiotics/antibiotic classes was that determined by agar dilution susceptibility testing at ESR.

2.3 Preparation of DNA template

Boiled lysis cell suspensions were used as a source of DNA template in all polymerase chain reactions (PCRs). Plate cultures were used to prepare cell suspensions, approximately equivalent to a 0.5 McFarland standard, in DNase/RNase-free water. Suspensions were heated at 95˚C for 9 minutes. The neat supernatants were stored at -20˚C and used as a source of DNA template.

2.4 Determination of ESBL type

Isolates were tested for the genes encoding CTX-M, TEM, SHV and VEB β-lactamases (ie, *bla*$_{\text{CTX-M}}$, *bla*$_{\text{TEM}}$, *bla*$_{\text{SHV}}$ and *bla*$_{\text{VEB}}$) by PCR using the primers listed in Table 1 and the amplification conditions specified in Table 2.

Initially all isolates were tested for the presence of *bla*$_{\text{CTX-M}}$ genes using a multiplex PCR that detects the genes encoding CTX-M groups 1, 2, 8, 9 and 25.[14] Isolates in which no *bla*$_{\text{CTX-M}}$ gene was detected were tested for the presence of *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ genes. TEM and SHV amplicons were sequenced to determine the TEM or SHV subtype. Isolates in which no CTX-M, TEM or SHV ESBL genes were detected were tested for the presence of *bla*$_{\text{VEB}}$.

PCRs were performed in 25 µL reactions using AmpliTaq Gold 360 PCR Master Mix (ATG360, Applied Biosystems, Carlsbad, California, United States) and 1.5 µL DNA template. All primers were used at 0.1 µM, except those used to amplify *bla*$_{\text{TEM}}$ and *bla*$_{\text{SHV}}$ genes which were used at 0.2 µM.

Table 1. Primers used for the detection and sequencing of ESBL genes

Primer name	Target gene	Primer sequence (5´-3´)	Product size (bp)	Reference
CtxMP1F[1] CtxMP1R[1]	*bla*$_{\text{CTX-M group 1}}$	AAA AAT CAC TGC GCC AGT TC AGC TTA TTC ATC GCC ACG TT	415	14
CtxMP2F[1] CtxMP2R[1]	*bla*$_{\text{CTX-M group 2}}$	CGA CGC TAC CCC TGC TAT T CCA GCG TCA GAT TTT TCA GG	552	14
CtxMP8F[1] CtxMP8/25R[1]	*bla*$_{\text{CTX-M group 8}}$	TCG CGT TAA GCG GAT GAT GC AAC CCA CGA TGT GGG TAG C	666	14
CtxMP9F[1] CtxMP9R[1]	*bla*$_{\text{CTX-M group 9}}$	CAA AGA GAG TGC AAC GGA TG ATT GGA AAG CGT TCA TCA CC	205	14
CtxMP25F[1] CtxMP8/25R[1]	*bla*$_{\text{CTX-M group 25}}$	GCA CGA TGA CAT TCG GG AAC CCA CGA TGT GGG TAG C	327	14
TEM-1[2] TEM-2[2]	*bla*$_{\text{TEM}}$	GTA TCC GCT CAT GAG ACA ATA TCT AAA GTA TAT ATG AGT AAA C	966	15
SHV-1[2] SHV-2[2]	*bla*$_{\text{SHV}}$	GCC GGG TTA TTC TTA TTT GTC GC TCT TTC CGA TGC CGC CGC CAG TCA	1017	16
VebF[2] VebR[2]	*bla*$_{\text{VEB}}$	CGA CTT CCA TTT CCC GAT GC GGA CTC TGC AAC AAA TAC GC	605	17

1 PCR amplification primers.
2 PCR amplification and sequencing primers.

Table 2. PCR conditions used for the detection of ESBL genes

Target gene	PCR conditions
bla$_{\text{CTX-M}}$	Denaturation for 5 min at 94ºC; 30 cycles of 94ºC for 30 sec, 52ºC for 30 sec, 72ºC for 50 sec; and final extension of 72ºC for 6 min.
bla$_{\text{TEM}}$	Denaturation for 5 min at 94ºC; 35 cycles of 94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 60 sec; and final extension of 72ºC for 10 min.
bla$_{\text{SHV}}$	Denaturation for 5 min at 94ºC; 35 cycles of 94ºC for 30 sec, 68ºC for 30 sec, 72ºC for 60 sec; and final extension of 72ºC for 7 min.
bla$_{\text{VEB}}$	Denaturation for 5 min at 94ºC; 35 cycles of 94ºC for 30 sec, 55ºC for 30 sec, 72ºC for 60 sec; and final extension of 72ºC for 10 min.

2.5 Molecular typing

2.5.1 Rationale and multiple-locus variable number tandem repeat analysis

Bacterial typing and sub-typing allow the spread of pathogens to be assessed. PFGE is considered the gold standard typing method for both *E. coli* and *Klebsiella* species, although it is a reasonably slow, labour-intensive process that is not suitable for high throughput analysis. Multiple-locus variable number tandem repeat analysis **(**MLVA) has

emerged as an alternative, powerful typing method that is suitable for high-throughput analysis.[18] It distinguishes non-clonal isolates by assessing the variation in multiple tandem-repeat sequences and has been used to characterise a wide range of prokaryotes, including many species within the family Enterobacteriacae.[18]

For *E. coli* isolates, multiplex PCR reactions were used to amplify the MLVA loci O15711, VNTR2, VNTR13 and CCR1 (Table 3). The amplification conditions used were denaturation at 94˚C for 5 min; followed by 30 cycles of 94˚C for 30 sec, 60˚C for 30 sec, and 72˚C for 45 sec; and a final extension at 72˚C for 5 min.

For *K. pneumoniae* isolates, multiplex PCR reactions were used to amplify the MLVA loci K, N1, KPN3, and KPN26 (Table 3). The amplification conditions used were denaturation at 94˚C for 5 min; followed by 30 cycles of 94˚C for 30 sec, 59˚C for 30 sec, and 72˚C for 45 sec; and a final extension at 72˚C for 5 min.

Labelled forward primers were synthesized by BioSearch Technologies (Novato, California, United States) or Applied Biosystems. All MLVA amplifications were performed in 15 μL reactions using ATG360, an additional 2.5 mM $MgCl_2$, primers at concentrations described in Table 3, and 1.0 μL DNA template.

Amplification products were diluted 1:40 in DNase/RNase-free water and analyzed on an ABI 3130xl Genetic Analyzer (Applied Biosystems) using the GeneScan™ 600 LIZ® size standard (Applied Biosystems) for comparison. Raw data was analysed using the GeneMapper® Software v5.0 (Applied Biosystems) to determine the size and dye label associated with each amplicon and assign an allele number. MLVA results were expressed as a string of four allele numbers: O15711-VNTR2-VNTR13-CCR1 for *E. coli* and K-N1-KPN3-KPN26 for *K. pneumoniae*.

Maximum parsimony trees were constructed in BioNumerics software v6.6 (Applied Maths, Ghent, Belgium). On the maximum parsimony trees, each circle represents a MLVA type, with the number of isolates with the MLVA type proportional to the size of the circle.

Table 3. VNTR loci and primers used for their amplification

VNTR locus	Repeat size (bp)	Primer name	Primer sequence (5′-3′)[1]	Colour channel	Primer concentration in PCR (µM)	Reference
Escherichia coli						
O15711	6	O15711F O15711R	NED - ACCGGCAATCATCGGGCCAACCA GATGCTGGAAAAACTGATGCAGACTCGCGT	Yellow Not labelled	0.06 0.06	19
VNTR2	95	VNTR2F VNTR2R	HEX - CGTCAGTGTATGTCCGAAGG GTGTCTTCTTAGTGTCAGGGGCAGTTTT	Green Not labelled	0.10 0.10	19
VNTR13	39	VNTR13F VNTR13R	FAM - TTTACGCCAATTGTTGAACC GTGTCTTGGTGTCAGCAAATCCAGAGA	Blue Not labelled	0.47 0.47	19
CCR1	28[2]	CCR1F CCR1R	CFR610 - GCTGCAGGAGAATGGGATGGTTTT GGTGAGGTGTCCGAGTGGCTGAAG	Red Not labelled	0.47 0.47	20
Klebsiella pneumoniae						
K	118	KF KR	CFR590 - GAGCTGGCGGCTGGAATA GCAATCTGCCCGGAAATA	Red Not labelled	0.60 0.60	21
N1	116	N1F N1R	NED - CATCAGGTGCAAGATTCCA TGAGCGATTGCTGGCCTA	Yellow Not labelled	0.33 0.33	Turton, pers comm
KPN3	18	KPN3F KPN3R	FAM - GGCCAAACAGTACGATCTGC GAATCGCAGCCTCACACG	Blue Not labelled	0.07 0.07	This study
KPN26	57	KPN26F KPN26R	HEX - GGTCAGTTTCGCCACCAG ATTGCCCAGTTTCTGGAACA	Green Not labelled	0.10 0.10	This study

1 CFR590, Cal FluoroRed 590; CFR610, Cal Fluoro Red 610.
2 Repeat length of 28 bp and an average spacer length of 31 bp.

2.5.2 *E. coli* phylogenetic grouping

E. coli phylogenetic groups were determined using the assay described by Clermont et al and the primers listed in Table 4.[22] This method uses a triplex PCR to determine the phylogenetic group (A, B1, B2, and D), and is based on the presence or absence of amplification products at three loci (Table 5).

PCRs were performed in 25 µL reactions using ATG360, an additional 2.5 mM $MgCl_2$, primers at 0.2 µM, and 1.5 µL DNA template. Amplifying conditions were denaturation for 5 min at 95ºC; 30 cycles of 95ºC for 30 sec, 55ºC for 30 sec, 72ºC for 30 sec; and final extension of 72ºC for 7 min.

Table 4. Primers used for the identification of *E. coli* phylogenetic groups

Primer name	Target gene	Primer sequence (5´-3´)	Product size (bp)	Reference
ChuA.1 ChuA.2	*chuA*	GACGAACCAACGGTCAGGAT TGCCGCCAGTACCAAAGACA	279	22
YjaA.1 YjaA.2	*yjaA*	TGAAGTGTCAGGAGACGCTG ATGGAGAATGCGTTCCTCAAC	211	22
TspE4C2.1 TspE4C2.2	DNA fragment TspE4C2	GAGTAATGTCGGGGCATTCA CGCGCCAACAAAGTATTACG	152	22

Table 5. *E. coli* phylogenetic group assignment based on results of PCR amplification of the *chuA*, *yjaA* and DNA fragment TspE4C2

Phylogenetic group[1]	Target gene:		
	chuA	***yjaA***	**TspE4C2**
A	-	-	+
A	-	-	-
B1	-	+	+
B1	-	+	-
B2	+	+	+
B2	+	+	-
D	+	-	+
D	+	-	-

1 Based on reference 22.

2.5.3 Identification of *E. coli* multilocus sequence type 131

As multilocus sequence type (ST) ST131 *E.coli* strains invariably belong to the B2 phylogenetic group, all group B2 isolates were tested, using a PCR-based method, to

determine whether they were ST131. The PCR-based assay screens for the presence of both a 347 bp fragment of the *pabB* gene found in isolates belonging to the O25-ST131 clone,[23] and ST131-associated single nucleotide polymorphisms (SNPs) in the *mdh* and *gyrB* genes (Table 6).[24] Isolates that generated amplicons for all three loci (O25b *pabB*, *mdh* and *gyrB*) were considered to be ST131.

The O25b *pabB* and *mdh* genes were amplified in a multiplex reaction and the *gyrB* gene was amplified separately in a singleplex reaction. PCRs were performed in 25 µL reactions using ATG360, an additional 2.5 mM $MgCl_2$, primers at 0.1 µM (*pabB* and *mdh*) or 0.2 µM (*gyrB*), and 1.5 µL DNA template. Amplifying conditions were denaturation for 5 min at 95ºC; 30 cycles of 95ºC for 30 sec, xºC for 30 sec (where x is 65˚C for O25 *pabB* and *mdh* PCRs and 60˚C for the *gyrB* PCR), 72ºC for 60 sec; and final extension of 72ºC for 7 min. The *gyrB* allele in 37 isolates and the *mdh* allele in 11 isolates were sequenced, using the MLST primers described in Section 2.5.4, to confirm the ST131 assay results.

Table 6. Primers used for the identification of ST131 *E. coli*

Primer name	Target gene	Primer sequence (5´-3´)	Product size (bp)	Reference
O25pabBspe.F O25pabBspe.R	*pabB*	TCCAGCAGGTGCTGGATCGT GCGAAATTTTTCGCCGTACTGT	347	23
mdh36 F mdh36 R	*mdh*	GTTTAACGTTAACGCCGGT GGTAACACCAGAGTGACCA	275	24
gyrB47 F gyrB47 R	*gyrB*	CGCGATAAGCGCGAC ACCGTCTTTTTCGGTGGAA	132	24

2.5.4 Multilocus sequence typing (MLST)

Isolates representing the more common MLVA profiles, as defined by the MLVA profile being common to >5 isolates, were further characterized using MLST. MLST was carried out using protocols and conditions described on the *E. coli* (http://mlst.warwick.ac.uk/mlst/) and *K. pneumoniae* (http://www.pasteur.fr/recherche/genopole/PF8/mlst/primers_Kpneumoniae.html) MLST websites.[25,26] The loci assessed for *E. coli* MLST were *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA* and *recA*. The loci assessed for *K. pneumoniae* were *gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB* and *tonB*. Sequence types were assigned using the website interface.

2.6 Data analysis

Statistical analyses were performed with SAS software v.9.3 (SAS Institute Inc, Cary, NC, USA).[27] The chi-square test or Fisher's exact test, as appropriate, were used to determine the significance of any observed differences. An associated p value ≤0.05 was used to indicate that a difference was significant.

For the analyses of associations between ST131 *E. coli* and patient demographics, antimicrobial resistance, ESBL types and MLVA types, isolates belonging to phylogenetic groups other than B2 that were not tested in the PCR-based assay for ST131 were assumed to be not ST131.

Simpson's index of diversity was used to assess variation in MLVA types.[28] This index indicates the probability that two isolates randomly selected are different MLVA types. The higher the index, the greater the diversity of MLVA types in a particular population. The index was calculated using the Biodiversity Calculator developed by Danoff-Burg and Xu (http://www.columbia.edu/itc/cerc/danoff-burg/MBD_Links.html). 95% confidence intervals for the index were calculated as previously described.[29]

3 RESULTS

3.1 Isolates and patients

63.6% (224) of the total 352 isolates were *E. coli*, 31.5% (111) were *Klebsiella* spp., and the remaining 4.8% (17) were other Enterobacteriaceae species. The majority (88.6%, 312) of the isolates were from urine. Thirteen (3.7%) isolates were from invasive sites. Details of the bacterial species and source of the isolates are presented in Table 9 and Table 10 in the Appendix.

The majority of patients were female (263/348, 75.6%) and most patients were ≥65 years of age (225/351, 64.1%) (Table 11 in the Appendix). Most (240/339, 70.8%) patients belonged to the European or Other ethnic group (Table 12 in the Appendix). The majority (178/240, 74.2%) of patients belonging to the European and Other ethnic group were ≥65 years of age, whereas the majority of patients in the Māori, Pacific Peoples and Asian ethnic groups were <65 years of age (14/24, 58.3%; 16/24, 66.7%; and 30/47, 63.8%, respectively).

Just over half the patients (166/328, 50.6%) were categorised as public or private hospital patients, 13.1% (43/328) were LTCF residents, and the remaining 36.3% (119/328) were community patients (Table 13 in the Appendix). While patients with ESBL-*E. coli* were almost evenly split between community patients and those in a healthcare facility (public hospital, private hospital or LTCF), the majority (90/108, 83.3%) of patients with ESBL-producing *Klebsiella* spp. (ESBL-*Klebsiella* spp.) were healthcare facility patients.

60.7% of the isolates were from patients in one of the three district health boards (DHBs) in the greater Auckland area (ie, Waitemata, Auckland and Counties Manukau) (Table 14 in the Appendix).

3.2 Antimicrobial susceptibility

Table 7. Antimicrobial susceptibility among clinical isolates of all ESBL-producing isolates, *Escherichia coli* and *Klebsiella* spp.

Antimicrobial	Number (%)						mg/L	
	Susceptible		Intermediate		Resistant		MIC_{50}	MIC_{90}
All isolates (n=352)								
Amoxicillin-clavulanate	91	(25.9)	145	(41.2)	116	(33.0)	16	32
Piperacillin-tazobactam	334	(94.9)	11	(3.1)	7	(2.0)	2	16
Mecillinam	299	(84.9)	20	(5.7)	33	(9.4)	2	16
Ertapenem	348	(98.9)	2	(0.6)	2	(0.6)	0.03	0.12
Meropenem	352	(100)	0	-	0	-	0.03	0.06
Ciprofloxacin	130	(36.9)	34	(9.7)	188	(53.4)	4	≥16
Gentamicin	172	(48.9)	2	(0.6)	178	(50.6)	16	≥64
Tigecycline	345	(98.0)	0	-	7	(2.0)	0.25	1
Nitrofurantoin	237	(67.3)	64	(18.2)	51	(14.5)	16	128
Fosfomycin	339	(96.3)	2	(0.6)	11	(3.1)	2	16
Co-trimoxazole/ trimethoprim[1]	72	(20.5)	1	(0.3)	279	(79.3)	NA	NA
***E. coli* (n=224)**								
Amoxicillin-clavulanate	67	(29.9)	101	(45.1)	56	(25.0)	16	32
Piperacillin-tazobactam	216	(96.4)	5	(2.2)	3	(1.3)	2	8
Mecillinam	205	(91.5)	6	(2.7)	13	(5.8)	1	8
Ertapenem	223	(99.6)	1	(0.5)	0	-	0.03	0.06
Meropenem	224	(100)	0	-	0	-	0.03	0.03
Ciprofloxacin	70	(31.3)	3	(1.3)	151	(67.4)	≥16	≥16
Gentamicin	117	(52.2)	0	-	107	(47.8)	2	≥64
Tigecycline	223	(99.6)	0	-	1	(0.5)	0.25	0.5
Nitrofurantoin	209	(93.3)	11	(4.9)	4	(1.8)	16	32
Fosfomycin	219	(97.8)	0	(0.0)	5	(2.2)	1	2
Co-trimoxazole/ trimethoprim	60	(26.8)	1	(0.5)	163	(72.8)	NA	NA
***Klebsiella* spp. (n=111)**								
Amoxicillin-clavulanate	22	(19.8)	39	(35.1)	50	(45.1)	16	32
Piperacillin-tazobactam	101	(91.0)	6	(5.4)	4	(3.6)	4	16
Mecillinam	83	(74.8)	10	(9.0)	18	(16.2)	4	≥128
Ertapenem	108	(97.3)	1	(0.9)	2	(1.8)	0.03	0.12
Meropenem	111	(100)	0	-	0	-	0.03	0.06
Ciprofloxacin	50	(45.1)	27	(24.3)	34	(30.6)	2	8
Gentamicin	50	(45.1)	0	-	61	(55.0)	32	≥64
Tigecycline	106	(95.5)	0	-	5	(4.5)	1	2
Nitrofurantoin	26	(23.4)	46	(41.4)	39	(35.1)	64	256
Fosfomycin	106	(95.5)	1	(0.9)	4	(3.6)	8	16
Co-trimoxazole/ trimethoprim	9	(8.1)	0	-	102	(91.9)	NA	NA

[1] The co-trimoxazole/trimethoprim susceptibility results are those reported by the referring laboratories when submitting isolates. Consequently MIC_{50} and MIC_{90} values are not available (NA).

Resistance to co-trimoxazole/trimethoprim, ciprofloxacin, gentamicin and amoxicillin-clavulanate was common among ESBL-E (Table 7). There were some significant differences in resistance between *E. coli* and *Klebsiella* spp. Ciprofloxacin resistance was significantly higher ($p < 0.05$) in *E. coli*, but ciprofloxacin intermediate resistance was higher in *Klebsiella* spp. Conversely, resistance to amoxicillin-clavulanate, co-trimoxazole/trimethoprim, mecillinam, nitrofurantoin and tigecycline was significantly more prevalent in *Klebsiella* spp. than *E. coli* (Table 7). Full MIC distribution data is presented in Table 15 in the Appendix.

The majority (303/352, 86.1%) of isolates were multidrug resistant (to ≥3 antibiotic classes), with a significantly higher proportion of *Klebsiella* spp. (105/111, 94.6%) being multiresistant than *E. coli* (183/224, 81.7%). The most common resistance pattern among *E. coli* was amoxicillin-clavulanate, ciprofloxacin, co-trimoxazole/trimethoprim and gentamicin resistance and this pattern accounted for 25.9% (58/224) of *E. coli*. The same pattern, with the addition of nitrofurantoin resistance, was the most common resistance pattern among *Klebsiella* spp. and accounted for 21.6% (24/111) of *Klebsiella* spp. (Table 16 in the Appendix).

3.3 ESBL types

The overwhelming majority (97.2%, 342/352) of ESBLs identified were CTX-M types, with the remainder being SHV or VEB types (Table 8). Notably, while both group 1 and group 9 CTX-M ESBLs were common in *E. coli*, only group 1 CTX-Ms were common among *Klebsiella* spp.

There were no clear associations between ethnicity and CTX-M type. Group 1 CTX-M ESBLs were identified in 70.0% (14/20) of the ESBL-E from patients of Indian ethnicity and 66.7% (18/27) of the ESBL-E from patients of other Asian ethnicities, compared with 67.9% (239/352) of all patients. Group 9 CTX-M ESBLs were identified in 20.0% (4/20) of the ESBL-E from patients of Indian ethnicity and 37.0% (10/27) of the ESBL-E from patients of other Asian ethnicities, compared with 29.6% (104/352) of all patients.

Multidrug resistance was prevalent among both ESBL-E with a group 1 CTX-M (88.3%, 211/239) and isolates with a group 9 CTX-M (81.7%, 85/104).

Table 8. Distribution of ESBL types by species

ESBL type[1]	Number (column %) isolates:			
	Escherichia coli **(n=224)**	***Klebsiella*** **species (n=111)**	**Other Enterobacteriaceae (n=17)**	**All isolates (n=352)**
CTX-M[2]	221 (98.7)	108 (97.3)	13 (76.5)	342 (97.2)
CTX-M group 1	125 (55.8)	104 (93.7)	10 (58.8)	239 (67.9)
CTX-M group 9	97 (43.3)	4 (3.6)	3 (17.6)	104 (29.6)
SHV[3]	3 (1.3)	2 (1.8)	3 (17.6)	8 (2.3)
VEB[4]	0	1 (0.9)	1 (5.9)	2 (0.6)

1 No TEM-type ESBLs were identified.
2 One *E. coli* isolate had both a group 1 and group 9 CTX-M ESBL.
3 The SHV types included SHV-2, SHV-2a, SHV-12 and SHV-28.
4 The VEB types included VEB-1 and VEB-4.

3.4 Molecular types

3.4.1 *E. coli* types

The majority (71.4%, 160) of the 224 ESBL-*E. coli* belonged to phylogenetic group B2, 20.1% (45) belonged to group D, 6.3% (14) to group A, and 2.2% (5) to group B1.

All 160 phylogenetic group B2 isolates were tested in the PCR-based assay to identify isolates that belong to the ST131 clone, and 76.3% (122/160) were positive. This indicates that 54.5% (122/224) of all ESBL-*E. coli* were ST131. *E. coli* ST131 were not significantly associated with any particular patient age group, sex or ethnicity, nor whether the patient was in a healthcare facility or the community.

All 122 ST131 *E. coli* had a CTX-M-type ESBL, with an almost equal split between group 1 CTX-M types (60, 49.2%) and group 9 CTX-M types (62, 50.8%). However, while group 1 CTX-M ESBLs were identified almost as frequently in ST 131 *E. coli* as in other *E. coli* (48.4% vs 51.6%, respectively), group 9 CTX-M ESBLs were more common in ST131 *E. coli* (64.6%, p = 0.014). Ciprofloxacin resistance was more prevalent among ST131 *E. coli* than other *E. coli* (86.9 vs 45.5%, p <0.001).

There was considerable diversity in the MLVA types identified among the 224 ESBL-*E. coli*, with 62 types identified (Table 17 in the Appendix). The Simpson's index of diversity for the *E. coli* MLVA types was 0.880 (95% confidence intervals: 0.844-0.916). There were 10 types that each accounted for ≥2% of the *E. coli* (equivalent to ≥5 isolates), and these 10 types collectively accounted for 64.3% (114) of the 224 isolates (Figure 1 and Table 17 in the Appendix).

None of the 10 most common *E. coli* MLVA types was uniquely associated with a particular healthcare facility or even a DHB. The most common type, 5-2-6-1, which accounted for 31.7% (71/224) of all *E. coli*, was isolated from patients in 13 of the 18 DHBs or combined DHB regions described in Table 14 in the Appendix.

MLVA types with a profile of x-2-6-1 (where x is variable) cluster with the most common MLVA type 5-2-6-1 in the maximum parsimony tree (Figure 2), and isolates with such MLVA profiles accounted for over half all *E. coli* (125/224, 55.8%). Almost all (122/125, 97.6%) isolates with a MLVA profile of x-2-6-1 were ST131 (Table 17 in the Appendix). Full multilocus sequence typing (MLST) of representative isolates of MLVA types 5-2-6-1 and 9-2-6-1 confirmed these isolates were ST131 (Table 17 in the Appendix).

Conversely, none of the isolates with a MLVA profile distinct from x-2-6-1 were ST131 (Table 17 in the Appendix). There were three MLVA profiles distinct from x-2-6-1 that were common to >5 isolates: types 2-1.5-5-1, 5-4-6-1 and 7-3-4-2. The multilocus sequence types of representative isolates of these three MLVA types were ST2749, ST493 and ST1193, respectively (Table 17 in the Appendix). ST2749, ST493 and ST1193 do not share any common alleles and are not part of the same clonal complex. They are also in a distinct clonal complex from ST131, which shares one common allele with ST493 and no common alleles with ST2749 and ST1193.

Figure 1. Prevalence of MLVA types found in *E. coli* and *K. pneumoniae*. Each distinct MLVA type is represented by a different colour.

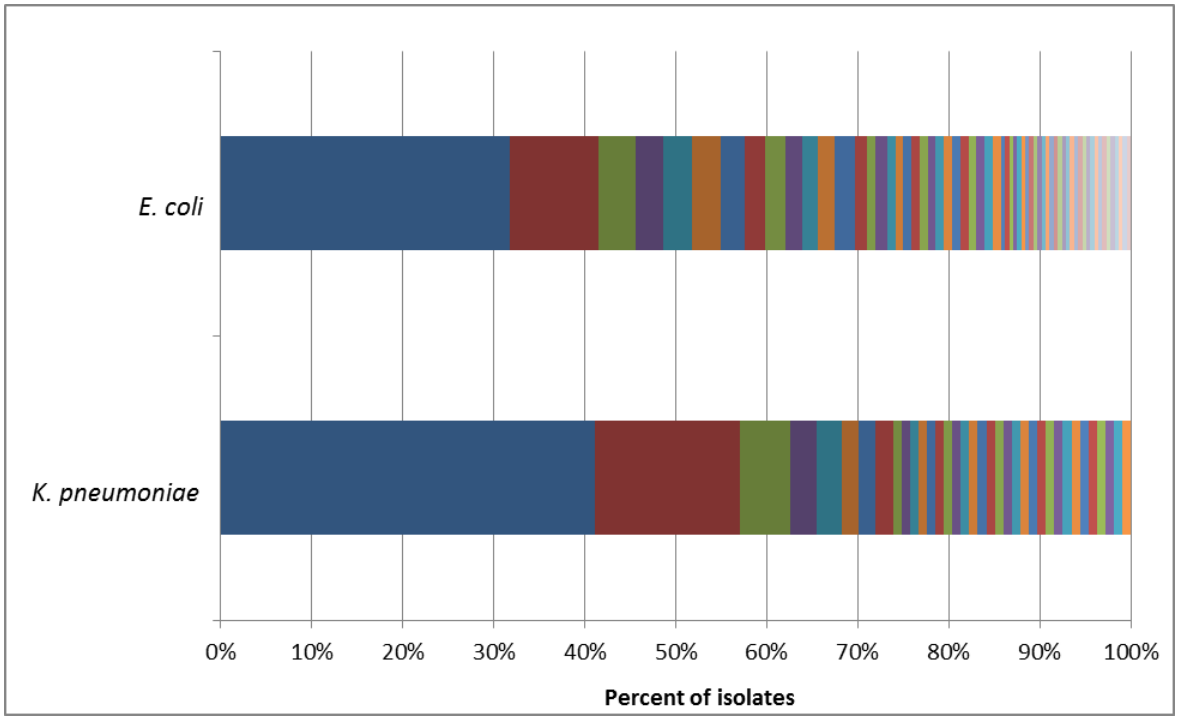
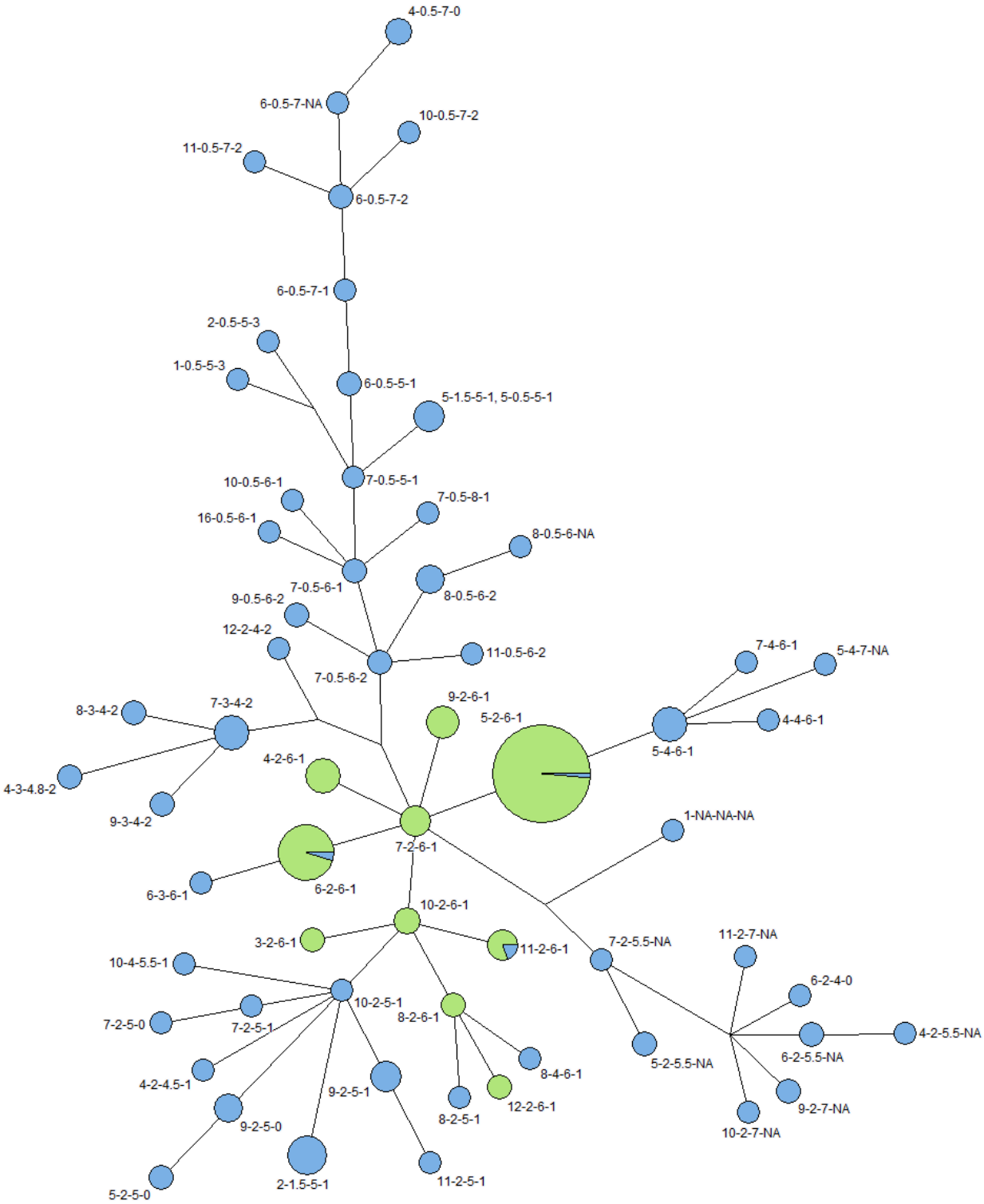


Figure 2. Maximum parsimony tree of the MLVA types among the 224 ESBL-producing *E. coli* isolates. The tree was constructed in BioNumerics. Each node represents a different MLVA type, with the size of the node indicating the number of isolates with a given MLVA type. The length of the line indicates if the MLVA types are single, double, or triple locus variants. ST131 isolates are represented by a green node or partial node.



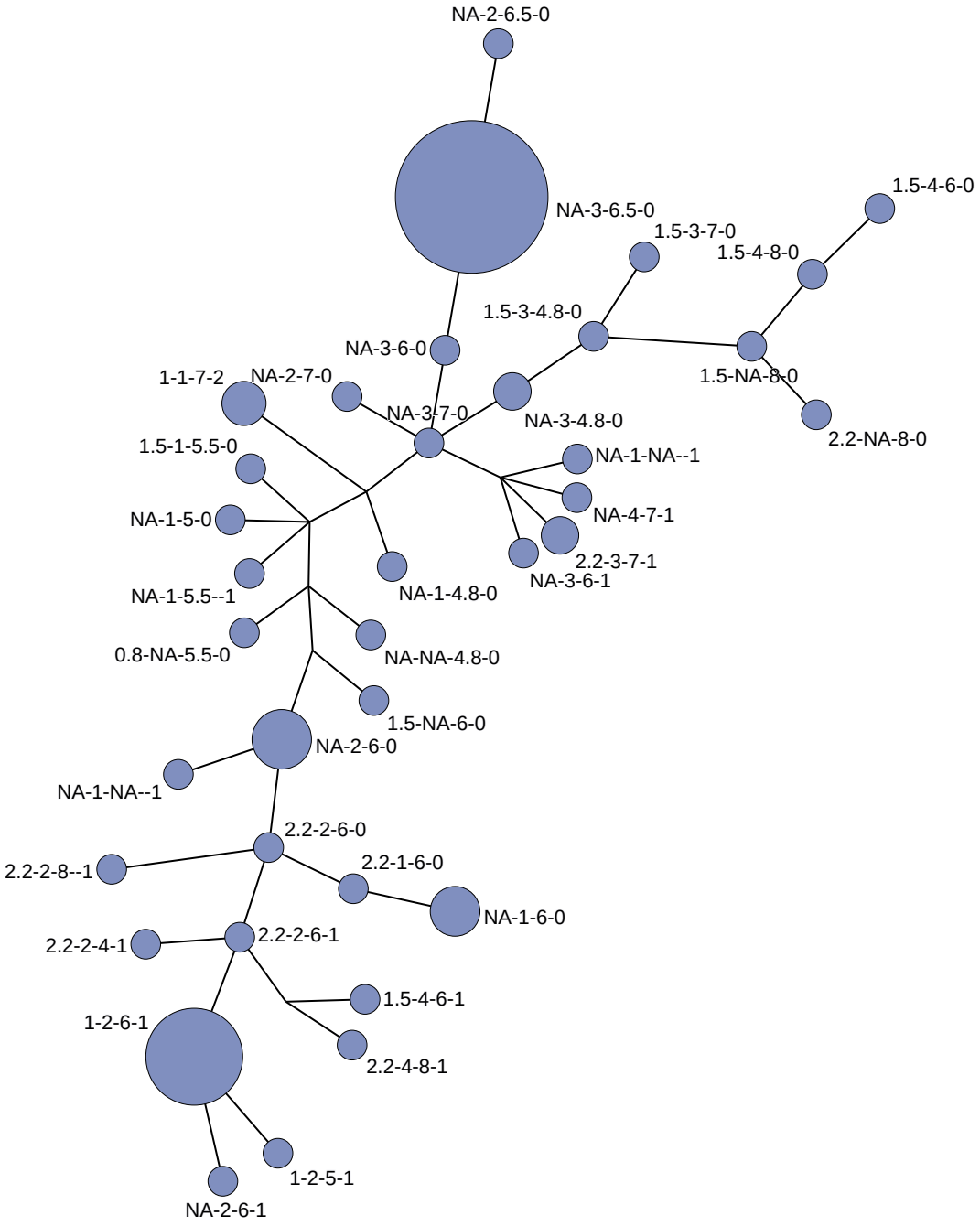
3.4.2 *K. pneumoniae* types

Again there was considerable diversity in the MLVA types identified among the 107 ESBL-producing *K. pneumoniae*, with 36 types identified (Table 18 in the Appendix). The Simpson's index of diversity for the *K. pneumoniae* MLVA types was 0.797 (95% confidence intervals: 0.727-0.867). There were five types that each accounted for ≥2% of the *K. pneumoniae* (equivalent to ≥3 isolates) and these five types collectively accounted for 68.2% of the 107 isolates (Figure 1 and Table 18 in the Appendix).

None of the five most common *K. pneumoniae* MLVA types was uniquely associated with a particular healthcare facility. However, 93.2% (41/44) of the isolates with the most common MLVA type, NA-3-6.5-0, were isolated from patients in the three DHBs in the greater Auckland area, with 70.5% (31/44) being from patients in the Waitemata DHB.

The clustering of the *K. pneumoniae* MLVA types is shown in the maximum parsimony tree in Figure 3. The multilocus sequence types of representative isolates of the three most common *K. pneumoniae* MLVA types, NA-3-6.5-0, 1-2-6-1 and NA-2-6-0, were ST25, ST48 and ST17, respectively (Table 18 in the Appendix). ST25 and ST17 share five alleles in common and are therefore more closely related to each other than ST48.

Figure 3. Maximum parsimony tree of the MLVA types among the 107 ESBL-producing *K. pneumoniae* isolates. The tree was constructed in BioNumerics. Each node represents a different MLVA type, with the size of the node indicating the number of isolates with a given MLVA type. The length of the line indicates if the MLVA types are single, double, or triple locus variants.



4 DISCUSSION

Antimicrobial resistance in Enterobacteriaceae is as a major threat to public health.[1] Increasing resistance in Enterobacteriaceae has resulted in decreasing options for antimicrobial therapy, posing treatment challenges in both hospital and community settings. Furthermore, the global dissemination of specific bacterial clones, and genes encoding antimicrobial resistance determinants in Enterobacteriaceae has greatly facilitated the increase and spread of ESBL-E. In this context, the purpose of this report was to provide a contemporary overview of the antimicrobial susceptibility patterns and molecular epidemiology of ESBL-E in New Zealand.

We found that the majority (86.1%) of ESBL-E were multidrug resistant, with the most common resistance pattern being resistance to amoxicillin-clavulanate, ciprofloxacin, co-trimoxazole/trimethoprim and gentamicin. Of the isolates in this study, 88.6% were from urine, and approximately half (47.8%) of the *E. coli* were from patients in the community. This highlights the therapeutic challenges posed by ESBL-E, particularly in the oral treatment of community-onset *E. coli* urinary tract infections. Notably, the vast majority of ESBL-*E. coli* isolates (93.3%) were susceptible to nitrofurantoin, and this has been recommended as a first-line empiric treatment for uncomplicated UTI in New Zealand (http://www.bpac.org.nz/Supplement/2013/July/antibiotics-guide.aspx). A recent study demonstrated the clinical efficacy of nitrofurantoin in uncomplicated UTI due to ESBL-*E. coli*.[30] However, nitrofurantoin is contraindicated in renal impairment, and has low penetration into tissues, thus limiting its utility beyond uncomplicated cystitis. In addition, the frequency of dosing and duration of treatment may limit patient adherence. Finally, nitrofurantoin has less in vitro efficacy against ESBL-*Klebsiella* spp., with only 23.4% of isolates in this study testing susceptible.

Recently, there has been renewed interest in the use of older agents such as fosfomycin and mecillinam for the oral treatment of infections caused by ESBL-E. Several studies have evaluated the in vitro efficacy of fosfomycin, with reported rates of over 95% susceptibility for ESBL-*E. coli*.[31,32] In keeping with these findings, we observed a high rate of fosfomycin susceptibility among ESBL-*E. coli* (97.8%), although susceptibility among ESBL-*Klebsiella* spp. was somewhat lower at 95.5%. Oral fosfomycin is administered as a single dose, and there have been concerns raised about the theoretical potential for

development of bacterial resistance with repeated dosing, although clinical evidence for this phenomenon is currently limited.[33]

Mecillinam, an oral β-lactam agent widely used in Northern Europe, has demonstrated high in vitro efficacy against ESBL-*E. coli* in several studies.[34,35] We also observed a high rate (91.5%) of mecillinam susceptibility amongst ESBL-*E. coli*, although susceptibility rates were lower for ESBL-*Klebsiella* spp. (74.8%). Both fosfomycin and mecillinam are generally well tolerated, and are safe to use during pregnancy. At present, however, neither are licensed or funded for use in New Zealand.

In serious cases of ESBL-E infection, the treatment of choice is a carbapenem, and it is reassuring to note that 100% of ESBL-*E. coli* and ESBL-*Klebsiella* spp. tested susceptible to meropenem. In addition, there were high rates of susceptibility to ertapenem in both species (99.6% and 97.3%, respectively). Tigecycline is a broad-spectrum antibiotic that has been used for the treatment of infections caused by multidrug-resistant Gram-negative pathogens.[36] It has proven in vitro efficacy against ESBL-E,[37] with previous studies reporting susceptibility rates of >99% for ESBL-*E. coli* and approximately 72-93% for ESBL-*Klebsiella* spp.[37,38] In keeping with these findings, we observed susceptibility rates of 99.6% for ESBL-*E. coli* and 95.5% for ESBL-*Klebsiella* spp.

Similar to the previous molecular epidemiological survey of ESBL-E in New Zealand,[8] there was an overwhelming predominance of CTX-M-type ESBLs, with 97.2% of ESBLs in this study being CTX-M ESBLs. Overall, the majority (69.9%) of CTX-M ESBLs belonged to CTX-M group 1, and the remainder belonged to CTX-M group 9. There were notable differences between species, such that there were similar proportions of group 1 and group 9 CTX-Ms in *E. coli* (55.8% and 43.3%, respectively); however, CTX-Ms in ESBL-*Klebsiella* spp. were dominated by group 1. A previous study from 2006-2007 in Auckland described an association between CTX-M-15 (belonging to CTX-M group 1) and Indian ethnicity, and CTX-M-14 (belonging to CTX-M group 9) and Chinese or South-East Asian ethnicity.[39] However, we did not find an association between distinct ethnic groups and CTX-M types. In particular, CTX-M group 1 ESBLs were not exclusively associated with Indian ethnicity, and CTX-M group 9 ESBLs were no more common among the Asian group (ie, Asians other than those of Indian ethnicity) than other

ethnic groups. It is possible that the lack of association with specific ethnic group reflects the transmission and subsequent endemicity of ESBL types in New Zealand over time.

Overall, the globally disseminated ST131 clone accounted for 54.4% of all ESBL-*E. coli*, and 76.3% of phylogenetic group B2 *E.coli* isolates. Our ST131 prevalence is in keeping with studies from other geographic regions, where ST131 prevalence among ESBL-*E. coli* reportedly ranges from 23-64%.[7] Although CTX-M-15 is the most commonly described ESBL type associated with ST131,[7] we found that group 1 and group 9 CTX-M types were evenly distributed amongst ST131 (49.2% vs. 50.8%, respectively). However, geographic variation in the distribution of CTX-M types within ST131 has been reported; for example, CTX-M-14 is a relatively common type in ST131 ESBL-*E. coli* in Canada and another group 9 CTX-M type, CTX-M-27, is relatively common in Japan and Switzerland.[40-2]

There was marked diversity among both the 224 ESBL-*E. coli* isolates, with a total of 62 MLVA types identified, and the 107 ESBL-*K. pneumoniae* isolates, with 36 MLVA types identified. However, the Simpson's index of diversity (0.880 vs 0.797 for ESBL-*E.coli* and ESBL-*K. pneumoniae*, respectively) suggests that ESBL-*K. pneumoniae* were somewhat more clonal than ESBL-*E. coli*. This observation of greater clonal restriction among ESBL-*K. pneumoniae* is in keeping with our finding that the majority of ESBL-*Klebsiella* spp. (83.3%) were isolated from patients associated with healthcare facilities, and is consistent with the notion of differential transmission patterns between the two species, with ESBL-*E. coli* circulating both in hospital and community settings, and ESBL-*Klebsiella* spp. largely restricted to healthcare facilities.[43]

In summary, this study provides contemporary information on the antimicrobial resistance patterns and molecular epidemiology of ESBL-E in New Zealand. We recommend that surveys such as this one should be repeated periodically or preferably become an integral part of the regular national ESBL-E surveys to enhance the usefulness of the regular surveys. It is important that current data on the antimicrobial susceptibility of ESBL-E is available for prescribers to ensure the most effective treatment of infections due to these organisms. Data on the current molecular epidemiology and prevalent circulating strains can often be useful background information for infection control personnel when investigating local ESBL-E outbreaks. Future surveys could be further enhanced by (1)

obtaining patient hospitalisation history data from the National Minimum Dataset to enable a more accurate description of the epidemiology of ESBL-E in healthcare vs community settings; (2) full identification of the ESBL types; (3) identification of sub-clones of ST131 ESBL-*E. coli*; and (4) using DNA microarrays to identify virulence factors in ESBL-E in New Zealand. Alternatively, whole genome sequencing could be considered to fully characterise ESBL-E isolates in New Zealand.

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APPENDIX

Table 9. Species distribution among the 352 clinical ESBL-producing Enterobacteriaceae

Species	Number of isolates	Percent of isolates
Citrobacter farmeri	1	0.3
Citrobacter koseri	1	0.3
Enterobacter aerogenes	1	0.3
Enterobacter cloacae complex	9	2.6
Escherichia coli	224	63.7
Klebsiella oxytoca	2	0.6
Klebsiella pneumoniae	107	30.4
Klebsiella species[1]	2	0.6
Proteus mirabilis	2	0.6
Proteus species[1]	1	0.3
Salmonella Stanley	1	0.3
Serratia odorifera	1	0.3

1 Not further identified.

Table 10. Clinical source of the 352 ESBL-producing Enterobacteriaceae

Source	Number of isolates	Percent of isolates
CSF or blood	13	3.7
skin and soft tissue infection	14	4.0
respiratory tract	11	3.1
urine	312	88.6
other sites[1]	2	0.6

1 Includes eye and fluid (not further defined).

Table 11. Age and sex of patients from whom the 352 clinical ESBL-producing Enterobacteriaceae were obtained

Age group (years)	Number (column %) of isolates[1]		
	female	male	total
0-14	10 (3.8)	2 (2.4)	12 (3.4)
15-64	91 (34.7)	23 (27.1)	114 (32.5)
65-84	94 (35.9)	42 (49.4)	137 (39.0)
≥85	67 (25.6)	18 (21.2)	88 (25.1)
total	263	85	

1 Age not recorded for one patient and sex not recorded for four patients.

Table 12. Age and ethnicity of patients from whom the 352 clinical ESBL-producing Enterobacteriaceae were obtained

Age group (years)	Number (column %) of isolates[1]				
	Māori[2]	Pacific Peoples	Asian	MELAA	European or Other
0-14	1 (4.2)	2 (8.3)	4 (8.5)	1 (33.3)	4 (1.7)
15-64	13 (54.2)	14 (58.3)	26 (55.3)	1 (33.3)	58 (24.2)
65-84	9 (37.5)	7 (29.2)	16 (34.0)	1 (33.3)	97 (40.4)
≥85	1 (4.2)	1 (4.2)	1 (2.1)	0	81 (33.8)
total	24	24	47[3]	3	240

1 Age or ethnicity not known for 14 patients.
2 Ethnic groups were prioritised in the following order: Māori, Pacific Peoples, Asian, Middle Eastern/Latin American/African (MELAA), European or Other Ethnicity (including New Zealander).
3 Among the 47 patients in the Asian ethnic group, 20 were Indian and the remaining 27 were of Chinese, South East Asian, or 'Asian ethnicity not further defined'.

Table 13. Categorisation of patients based on their current or recent hospitalisation history and species of ESBL-producing Enterobacteriaceae

Patient categorisation[1,2]	Number (column %) of isolates			
	E. coli **n=203**	***Klebsiella*** **species n=108**	**Other species n=17**	**Total[2]**
Public hospital patient	78 (38.4)	77 (71.3)	9 (52.9)	164 (50.0)
Private hospital patient	2 (1.0)	0	0	2 (0.6)
Long-term care facility resident	26 (12.8)	13 (12.0)	4 (23.5)	43 (13.1)
Community patient	97 (47.8)	18 (16.7)	4 (23.5)	119 (36.3)

1 See the methods section for the description of the patient categorisation.
2 Hospitalisation history not known or recorded for 24 patients.

Table 14. District health board domicile of the patients

District Health Board	Number of isolates[1]	Percent of isolates
Northland	11	3.1
Waitemata	103	29.3
Auckland	63	18.0
Counties Manukau	47	13.4
Waikato	23	6.6
Lakes	6	1.7
Bay of Plenty	6	1.7
Tairawhiti	5	1.4
Taranaki	4	1.1
Hawke's Bay	8	2.3
Whanganui	1	0.3
MidCentral	5	1.4
Capital and Coast/ Hutt Valley	31	8.8
Wairarapa	0	-
Nelson Marlborough	6	1.7
West Coast	0	-
Canterbury/South Canterbury	23	6.6
Southern	9	2.6

1 DHB not recorded for one patient.

Table 15. Distribution of minimum inhibitory concentrations (MICs) among clinical isolates of ESBL-producing *Escherichia coli* (n=224), *Klebsiella* spp. (n=111) and other Enterobacteriaceae (n=17)

Antimicrobial	Source	Percent of isolates with an MIC (mg/L) of:[1]																			
		0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Amoxicillin-clavulanate	*E. coli*												8.0	21.9	45.1	20.1	4.0	0.9			
	Klebsiella											0.9	1.8	17.1	35.1	36.9	8.1				
	Other[3]													11.8	29.4	11.8	11.8	35.3			
	All[4]											0.3	5.7	19.9	41.2	25.0	5.7	2.3			
Piperacillin-tazobactam	*E. coli*									2.2	26.3	37.1	17.9	9.8	3.1	0.9	1.3	0.5	0.0	0.9	
	Klebsiella									2.7	1.8	21.6	25.2	28.8	10.8	4.5	0.9	1.8	0.9	0.9	
	Other									5.9	23.5	23.5	5.9	11.8	29.4						
	All									2.6	18.5	31.5	19.6	15.9	6.8	2.0	1.1	0.9	0.3	0.9	
Mecillinam	*E. coli*							1.3	4.0	25.5	20.5	16.5	19.2	4.5	2.7	2.7	1.3	1.8			
	Klebsiella									2.7	6.3	24.3	29.7	11.7	9.0	1.8	3.6	10.8			
	Other									5.9	17.7	11.8	5.9	23.5	23.5	0.0	5.9	5.9			
	All							0.9	2.6	17.3	15.9	18.8	21.9	7.7	5.7	2.3	2.3	4.8			
Ertapenem	*E. coli*			4.0	34.4	36.2	19.2	2.7	1.3	1.8	0.5										
	Klebsiella			1.8	8.1	40.5	30.6	9.9	4.5	1.8	0.9	0.0	1.8								
	Other			5.9	23.5	29.4	5.9	17.7	11.8	5.9											
	All			3.4	25.6	37.2	22.2	5.7	2.8	2.0	0.6	0.0	0.6								
Meropenem	*E. coli*				31.3	63.0	4.9	0.9													
	Klebsiella				0.9	81.1	14.4	0.9	0.9	0.9	0.9										
	Other				5.9	35.3	35.3	23.5													
	All				20.5	67.3	9.4	2.0	0.3	0.3	0.3										
Ciprofloxacin	*E. coli*	0.5	0.0	8.5	4.0	0.0	0.0	4.0	8.0	4.0	2.2	1.3	0.9	0.9	65.6						
	Klebsiella		0.9	1.8	2.7	5.4	1.8	2.7	4.5	20.7	4.5	24.3	18.0	3.6	9.0						
	Other			5.9	0.0	11.8	0.0	5.9	17.7	11.8	11.8	17.7	0.0	5.9	11.8						
	All	0.3	0.3	6.3	3.4	2.3	0.6	3.7	7.4	9.7	3.4	9.4	6.3	2.0	45.2						
Gentamicin	*E. coli*								1.3	20.1	25.5	4.5	0.9	0.0	0.9	9.8	37.1				
	Klebsiella								1.8	35.1	8.1	0.0	0.0	0.0	0.9	5.4	48.7				
	Other								5.9	17.7	0.0	0.0	5.9	11.8	0.0	0.0	58.8				
	All								1.7	24.7	18.8	2.8	0.9	0.6	0.9	8.0	41.8				
Tigecycline	*E. coli*						3.1	32.1	51.8	11.2	1.3	0.0	0.5								
	Klebsiella							0.9	2.7	17.1	56.8	18.0	4.5								
	Other							5.9	0.0	29.4	58.8	0.0	5.9								
	All						2.0	21.0	33.8	13.9	21.6	5.7	2.0								

Antimicrobial	Source	Percent of isolates with an MIC (mg/L) of:[1]																		
		0.002	0.004	0.008	0.016	0.03	0.06	0.12	0.25	0.5	1	2	4	8	16	32	64	128	256	512	1024
Nitrofurantoin	*E. coli*											0.5	0.5	38.8	43.8	9.8	4.9	0.5	0.9	0.5	
	Klebsiella													0.9	3.6	18.9	41.4	18.9	12.6	3.6	
	Other														5.9	5.9	41.2	35.3	11.8		
	All											0.3	0.3	25.0	29.3	12.5	18.2	8.0	5.1	1.4	
Fosfomycin	*E. coli*									17.9	57.1	17.0	4.0	0.9	0.0	0.0	0.9	0.0	0.0	0.0	2.2
	Klebsiella										1.8	27.0	14.4	34.2	16.2	1.8	0.0	0.9	1.8	0.9	0.9
	Other									17.7	0.0	17.7	0.0	5.9	23.5	5.9	11.8	5.9	5.9	0.0	5.9
	All									12.2	36.9	20.2	7.1	11.7	6.3	0.9	1.1	0.6	0.9	0.3	2.0

1 The white fields represent the range of antibiotic concentrations tested. MIC values less than or equal to the lowest concentration tested are presented as this lowest concentration. MIC values greater than the highest concentration tested are presented as the next highest concentration after the highest concentration tested. The vertical bars indicate the breakpoints between the susceptibility categories. For antibiotics where there are two vertical lines, the first line represents the breakpoint between susceptible and intermediate, and the second line represents the breakpoint between intermediate and resistant. For antibiotics where there is one vertical line, the line represents the breakpoint between susceptible and resistant. The breakpoints marked for ciprofloxacin are the CLSI breakpoints for Enterobacteriaceae other than *Salmonella*. The breakpoints marked for fosfomycin are the CLSI breakpoints for urinary *E. coli.*

Table 16. Common antibiotic resistance patterns[1]

	Number (column %) isolates:			
Resistance pattern[1, 2]	***Escherichia coli* (n=224)**		***Klebsiella* species (n=111)**	
Amc Cip Fpi Gm	58	(25.9)	8	(7.2)
Cip Fpi Gm	30	(13.4)	1	(0.9)
Amc Cip Gm	24	(10.7)	0	-
Amc Cip Fpi Gm Nit	5	(2.2)	24	(21.6)
Amc Fpi Gm	23	(10.3)	6	(5.4)
Amc Fpi Gm Nit	0	-	16	(14.4)
Fpi Gm	13	(5.8)	3	(2.7)
Amc Fpi Gm Mec	12	(5.4)	0	-
Cip Gm	12	(5.4)	0	-
Amc Cip Fpi Gm Mec Nit	0	-	10	(9.0)

1 Resistance patterns only recorded when there were ≥10 isolates of either *E. coli* or a *Klebsiella* species with the pattern. There were no instances of ≥10 isolates of any other species having the same pattern.

2 Amc, amoxicillin-clavulanate; Cip, ciprofloxacin; Fpi, co-trimoxazole/trimethoprim; Gm, gentamicin; Mec, mecillinam; Nit, nitrofurantoin.

Table 17. MLVA profiles among *Escherichia coli* isolates and association of MLVA types with phylogenetic group and ST131

MLVA type[1]	Number of isolates	Cumulative percentage	Associated phylogenetic group[2]	Number of isolates within a MLVA type that were ST131[3]
5-2-6-1[4]	71	31.7	B2 (70) and D (1)	70
6-2-6-1	22	41.5	B2	**21**
2-1.5-5-1[5]	9	45.5	A	nt
4-2-6-1	7	48.7	B2	7
5-4-6-1[6]	7	51.8	B2	0
7-3-4-2[7]	7	54.9	B2	0
9-2-6-1[8]	6	57.6	B2	6
7-2-6-1	5	59.8	B2	5
9-2-5-1	5	62.1	D	nt
11-2-6-1	5	64.3	B2	**4**
5-0.5-5-1	4	66.1	D	nt
8-0.5-6-2	4	67.9	D	nt
9-2-5-0	4	69.6	D	nt
4-0.5-7-0	3	71.0	D	nt
10-2-6-1	3	72.3	B2	3
9-0.5-6-2	2	73.2	D	nt
3-2-6-1	2	74.1	B2	2
4-3-4.8-2	2	75.0	B2	0
5-2-5-0	2	75.9	B2	0
5-2-5.5-NA[9]	2	76.8	B2	0
6-0.5-5-1	2	77.7	D	nt
6-0.5-7-2	2	78.6	B1 (1) and D (1)	nt
6-2-5.5-NA	2	79.5	B2	0
7-0.5-6-1	2	80.4	A (1) and B1 (1)	nt
7-0.5-6-2	2	81.3	D	nt
8-2-6-1	2	82.1	B2	2
8-3-4-2	2	83.0	B2	0
9-2-7-NA	2	83.9	D	nt
9-3-4-2	2	84.8	B2	0
12-2-6-1	2	85.7	B2	2
1-0.5-5-3	1	86.2	A	nt
1-NA-NA-NA	1	86.6	A	nt
2-0.5-5-3	1	87.1	A	nt
4-2-4.5-1	1	87.5	D	nt
4-2-5.5-NA	1	87.9	B2	0
4-4-6-1	1	88.4	B2	0
5-1.5-5-1	1	88.8	A	nt
5-4-7-NA	1	89.3	B2	0
6-0.5-7-1	1	89.7	D	nt
6-0.5-7-NA	1	90.2	D	nt
6-2-4-0	1	90.6	B2	0
6-3-6-1	1	91.1	B2	0
7-0.5-5-1	1	91.5	D	nt
7-0.5-8-1	1	92.0	B1	nt
7-2-5-0	1	92.4	D	nt
7-2-5-1	1	92.9	D	nt
7-2-5.5-NA	1	93.3	B2	0

continued

MLVA type[1]	Number of isolates	Cumulative percentage	Associated phylogenetic group[2]	Number of isolates within a MLVA type that were ST131[3]
7-4-6-1	1	93.8	B2	0
8-0.5-6-NA	1	94.2	D	nt
8-2-5-1	1	94.6	D	nt
8-4-6-1	1	95.1	B2	0
10-0.5-6-1	1	95.5	B1	nt
10-0.5-7-2	1	96.0	D	nt
10-2-5-1	1	96.4	D	nt
10-2-7-NA	1	96.9	D	nt
10-4-5.5-1	1	97.3	B2	0
11-0.5-6-2	1	97.8	D	nt
11-0.5-7-2	1	98.2	D	nt
11-2-5-1	1	98.7	D	nt
11-2-7-NA	1	99.1	D	nt
12-2-4-2	1	99.6	B2	0
16-0.5-6-1	1	100	B1	nt

1 Based on variable number tandem repeat loci O15711-VNTR2-VNTR13-CCR1.
2 If all isolates of a MLVA type did not belong to the same phylogenetic group, then the number of isolates belonging to each phylogenetic group is given in parentheses.
3 Only isolates belonging to phylogenetic group B2 were tested for ST131 in the PCR-based assay. A bold entry indicates that some phylogenetic group B2 isolates of the MLVA type were ST131 positive and others were ST131 negative. nt = not tested, as not phylogenetic group B2.
4 One isolate of MLVA type 5-2-6-1 was characterised using MLST and was ST131.
5 Two isolates of MLVA type 2-1.5-5-1 were characterised using MLST and were ST2749.
6 Two isolates of MLVA type 5-4-6-1 were characterised using MLST and were ST493.
7 Two isolates of MLVA type 7-3-4-2 were characterised using MLST and were ST1193.
8 One isolate of MLVA type 9-2-6-1 was characterised using MLST and was ST131.
9 NA denotes that no amplicon was found at the locus.

Table 18. MLVA profiles among *Klebsiella pneumoniae* isolates

MLVA type[1]	Number of isolates	Cumulative percentage
NA-3-6.5-0[2,3]	44	41.1
1-2-6-1[4]	17	57.0
NA-2-6-0[5]	6	62.6
1-1-7-2	3	65.4
NA-1-6-0	3	68.2
2.2-3-7-1	2	70.1
NA-1-NA-(-1)	2	72.0
NA-3-4.8-0	2	73.8
0.8-NA-5.5-0	1	74.8
1-2-5-1	1	75.7
1.5-1-5.5-0	1	76.6
1.5-3-4.8-0	1	77.6
1.5-3-7-0	1	78.5
1.5-4-6-0	1	79.4
1.5-4-6-1	1	80.4
1.5-4-8-0	1	81.3
1.5-NA-6-0	1	82.2
1.5-NA-8-0	1	83.2
2.2-1-6-0	1	84.1
2.2-2-4-1	1	85.0
2.2-2-6-0	1	86.0
2.2-2-6-1	1	86.9
2.2-2-8-(-1)	1	87.9
2.2-4-8-1	1	88.8
2.2-NA-8-0	1	89.7
NA-1-4.8-0	1	90.7
NA-1-5-0	1	91.6
NA-1-5.5-(-1)	1	92.5
NA-2-6-1	1	93.5
NA-2-6.5-0	1	94.4
NA-2-7-0	1	95.3
NA-3-6-0	1	96.3
NA-3-6-1	1	97.2
NA-3-7-0	1	98.1
NA-4-7-1	1	99.1
NA-NA-4.8-0	1	100

1 Based on variable number tandem repeat loci K-N1-KPN3-KPN26.
2 NA denotes that no amplicon was found at the locus.
3 Two isolates of MLVA type NA-3-6.5-0 were characterised using MLST and were ST25.
4 Two isolates of MLVA type 1-2-6-1 were characterised using MLST and were ST48.
5 One isolate of MLVA type NA-2-6-0 was characterised using MLST and was ST17.