Science for Communities

2016 survey of extended-spectrum β-lactamase-producing Enterobacteriaceae

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Up until 2005, national surveillance of extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae (ESBL-E) was based on diagnostic laboratories referring all isolates to 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.

Commencing with this 2016 survey, several changes have been made to the national surveillance of ESBL-E. Surveys will no longer be conducted annually and consequently there was no survey in 2015. Only isolates from clinical specimens will be collected and included in the surveys. An extended range of analyses will be undertaken, including more analysis of the demographics of patients and greater analysis of the ESBL-E isolates using whole genome sequencing (WGS). The change to include only clinical isolates means that data in this report is not necessarily comparable with that presented in reports for earlier ESBL-E surveys, which are available at https://surv.esr.cri.nz/antimicrobial/esbl.php.

Methods

For the 2016 ESBL-E survey, hospital and community microbiology laboratories in New Zealand were asked to refer all non-duplicate ESBL-E isolated from clinical specimens during August 2016 to ESR. Laboratories that do not test for ESBL production were asked to refer all Enterobacteriaceae isolates that were not susceptible to 3rd-generation cephalosporins. Medlab Central and Medlab South Nelson Marlborough referred isolates during a 31-day period between late August and late September 2016. All remaining laboratories referred ESBL-E during August 2016.

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 antibiotics: amoxicillin-clavulanic acid, cefoxitin, ciprofloxacin or norfloxacin, co-trimoxazole, ertapenem, fosfomycin, gentamicin, imipenem, meropenem, 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 DHB domicile information and hospitalisation history information was also obtained from the Ministry of Health's datasets. 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 an ESBL-E. Initially all isolates were tested by PCR for the presence of genes encoding a CTX-M-type ESBL.[1] Any isolates in which a CTX-M gene was not detected were tested phenotypically for ESBL production by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) combination disc test using both cefotaxime and ceftazidime as substrates in combination with clavulanic acid.[2]

The whole genomes of a subset of 394 ESBL-E were sequenced. 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 MiSeq platform (Illumina) with 2- by 250-bp paired-end chemistry. WGS data was 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 sequence of the *gyrA* and *parC* genes in the assemblies; 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).

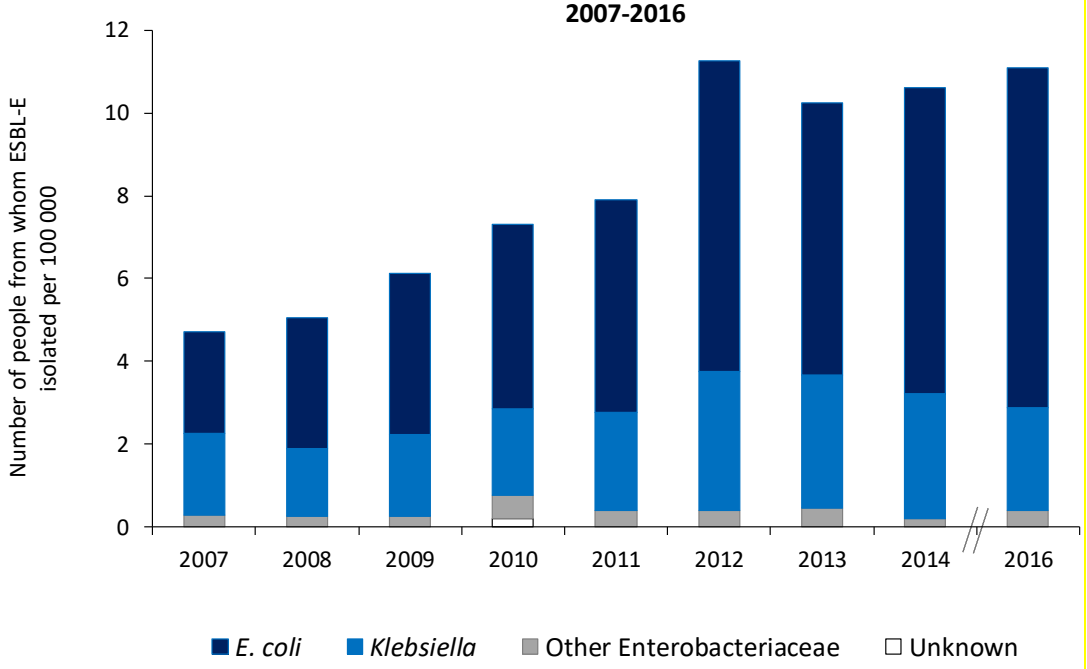
The multilocus sequence types (MLSTs) of *Escherichia coli* and *Klebsiella pneumoniae* were determined *in silico* using direct query of the PubMLST database using the MLST schema described respectively at https://enterobase.warwick.ac.uk/species/index/ecoli and http://bigsdb.pasteur.fr/klebsiella. The *H*30 and *H*30Rx subclones of ST131 *E. coli* were determined using *in silico* PCR, based on the identification of allele 30 of the *fimH* gene,[9] and the presence of the *ybbW* G264A and *smbA* C200T single nucleotide polymorphisms (SNPs) in the *in silico* PCR products.[10]

SNPs in the core genome were identified and aligned using snippy and snippy-core.[11] For *E. coli* isolates, reads were mapped to the NCBI Reference Sequence Database (RefSeq) *E. coli* O157:H7 strain Sakai genome, accession number GCF_000008865.1. For the analysis of *E. coli* ST131 isolates, reads were mapped to the genome of ST131 strain EC958, accession number GCF_000285655.3.[12] For the analysis *K. pneumoniae* isolates, reads were mapped to the genome of RefSeq *K. pneumoniae* strain HS11286, accession number GCF_000240185.1. Cladograms and phylogenetic trees were constructed using FastTreeDbl under the maximum-likelihood method and GTR substitution model.[13,14] Bootstrapping (100 X), using a combination of seqboot,[15] FastTreeDbl and Fast Tree-Comparison Tools,[16] was used to estimate statistical support for the phylogeny constructed.

Results

During the 1-month period of the 2016 survey, 521 non-duplicate, distinct ESBL-E were isolated, which equates to a national period-prevalence rate of 11.1 people with ESBL-E from a clinical specimen per 100 000 population. Figure 1 shows the prevalence rates of ESBL-E from clinical specimens over the 10 years 2007 to 2016, and the distribution of ESBLs among *E. coli*, *Klebsiella* species and other Enterobacteriaceae. A notable trend over these years has been a decrease in the proportion of ESBL-E that are *Klebsiella* species, and a concomitant increase in the proportion that are *E. coli*. In 2007, 52.0% of ESBL-E from clinical specimens were *E. coli* and 42.0% were *Klebsiella*. By 2016 these proportions had changed to 74.1% *E. coli* and 22.3% *Klebsiella*.

Figure 1. ESBL-producing Enterobacteriaceae period-prevalence rates, 2007-2016



The rates presented in this graph are period-prevalence rates based on the number of isolates received during the 1-month duration of the surveys, that is, the rates are not annualised. The rates are based on ESBL-producing Enterobacteriaceae (ESBL-E) isolated from clinical specimens only. As data was not specifically collected on which ESBL-E were from clinical specimens as opposed to screening/
surveillance specimens for the surveys conducted between 2007 and 2012 inclusive, all ESBL-E isolates in these years from specimens other than faecal specimens or rectal swabs have been used as a proxy for clinical specimens. There was no survey conducted in 2015. 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 521 ESBL-E isolates referred in 2016 comprised:

- 386 (74.1%) *E. coli*
- 116 (22.3%) *Klebsiella* species
- 8 (1.5%) *Enterobacter* species
- 4 (0.8%) *Proteus mirabilis*
- 2 (0.4%) *Citrobacter freundii*
- 2 (0.4%) *Morganella morganii*
- 2 (0.4%) *Salmonella* species
- 1 (0.2%) *Shigella sonnei*

Patient demographics

The age and ethnicity of the patients from whom ESBL-E were isolated is shown in Table 1. The age-standardised rates were highest in the Asian, Pacific peoples and Middle Eastern/Latin American/African (MELAA) ethnic groups, with the rates in the Asian group twice those in the Māori and European or Other groups. The prevalence of ESBL-E was highest in the ≥65 years age group for all ethnic groups except for the MELAA group. ESBL-producing *E. coli* accounted for over two-thirds of the ESBL-E in all ethnic groups. The proportion of the ESBL-producing *Klebsiella* (71.6%) that were isolated from patients ≥65 years of age (71.6%) was much larger than the proportion of the ESBL-producing *E. coli* that were from patients in this age group (47.4%).

Table 1. Age and ethnicity of patients with an extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae from a clinical specimen, 2016

Age group (years)	Māori		Pacific peoples		Asian		MELAA[1]		European or Other		Total[2]	
	No.	Rate[3]	No.	Rate	No.	Rate	No.	Rate	No.	Rate	No.	Rate
<15	5	2.2	2	2.3	8	8.0	2	17.2	8	1.6	26	2.8
15-64	27	6.3	19	10.2	65	16.0	8	20.6	98	4.9	220	7.2
≥65	14	35.8	9	54.2	17	52.2	0	-	232	38.1	275	39.4
Total cases and crude rate	46	6.6	30	10.4	90[4]	16.7	10	19.1	338	10.8	521	11.1
Age-standardised rate[5]		9.9		15.2		19.8		16.9		9.2		

1 Middle Eastern/Latin American/African.
2 Ethnicity not known for 7 (1.3%) patients: 1 patient in the <15 years age group, and 3 patients in each of the 15-64 and ≥65 years age groups.
3 Period prevalence rate per 100 000 population. 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 census population applied to the 2016 mid-year population estimates from Statistics New Zealand. 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 These 90 patients of Asian ethnicity included 45 of Indian ethnicity and 27 of Chinese ethnicity.
5 The age-standardised rates are direct standardised to the age distribution of the total New Zealand population.

The majority of the ESBL-E (60.8%) were isolated from patients categorised as community patients (Table 2). However, the situation was reversed for ESBL-producing *Klebsiella* with the minority (38.8%) being isolated from community patients. Community patients dominated in the two younger patient age groups (ie, <65 years), but not in the oldest age group (≥65 years). While community patients dominated in all ethnic groups, this dominance was greatest for the Asian ethnic group, with 78.9% of Asian patients with an ESBL-E being categorised as community patients.

Table 2. Comparison of ESBL-producing Enterobacteriaceae from hospital versus community patients, 2016

	Number (row %)	
	Hospital patient[1]	**Community patient[1]**
All	204 (39.2)[2]	317 (60.8)
Species:		
E. coli	123 (31.9)	263 (68.1)
Klebsiella species	71 (61.2)	45 (38.8)
other species	10 (52.6)	9 (47.4)
Age group (years)		
<15	7 (26.9)	19 (73.1)
15-64	67 (30.5)	153 (69.5)
≥65	130 (47.3)	145 (52.7)
Ethnicity[3]		
Maori	19 (41.3)	27 (58.7)
Pacific peoples	12 (40.0)	18 (60.0)
Asian	19 (21.1)	71 (78.9)
MELAA	3 (30.0)	7 (70.0)
European or Other	150 (44.4)	188 (55.6)
NZDep13 quintile[4]		
1	34 (34.7)	64 (65.3)
2	26 (34.7)	49 (65.3)
3	49 (42.6)	66 (57.4)
4	45 (40.9)	65 (59.1)
5	48 (41.7)	67 (58.3)
Isolation site:		
CSF/blood	19 (70.4)	8 (29.6)
urine	158 (34.8)	296 (65.2)
skin and soft tissue	15 (75.0)	5 (25.0)
respiratory tract	5 (62.5)	3 (37.5)
other	7 (58.3)	5 (41.7)

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 201 of the 204 patients categorised as hospital patients, of whom 177 (88.1%) were in a public hospital and 24 (11.9%) were in a long-term care facility.

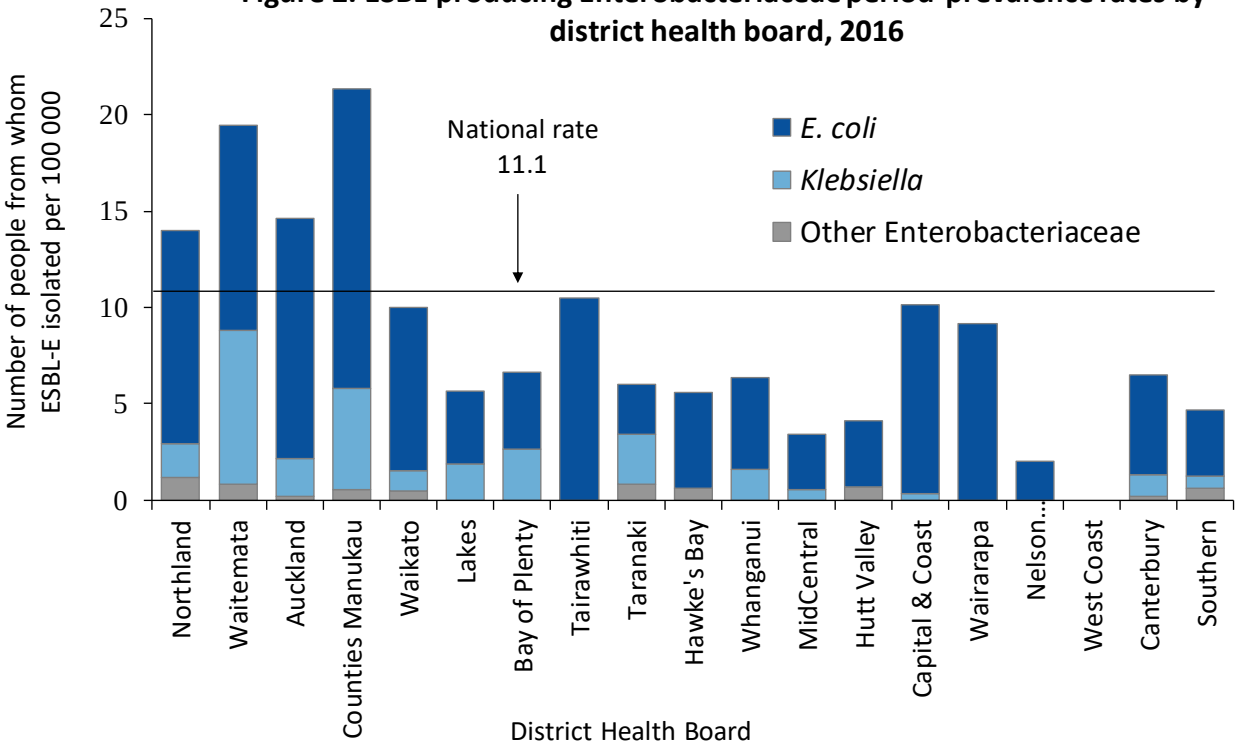
3 Ethnicity not known for 7 patients: 1 hospital and 6 community patients.

4 Quintile of the 2013 New Zealand Deprivation Index (1 = least deprived and 5 = most deprived). Index score not known for 8 patients: 2 hospital and 6 community patients.

Geographic distribution

Figure 2 shows the prevalence of ESBL-E in each district health board (DHB). There are very marked geographic differences, with prevalence rates in Counties Manukau (21.3 per 100 000), Waitemata (19.5), Auckland (14.6) and Northland (14.0) DHBs all being above the national average (11.1). The prevalence of ESBL-E in Waitemata is also notable for the relatively high rate of ESBL-producing *Klebsiella* (8.0 per 100 000) compared to *E. coli* (10.7).

Figure 2. ESBL-producing Enterobacteriaceae period-prevalence rates by district health board, 2016



Data for the Canterbury and South Canterbury DHBs are combined as 'Canterbury'.

Antimicrobial susceptibility

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 and co-trimoxazole/trimethoprim resistance among ESBL-E. While fosfomycin susceptibility was only reported for 371 of the total 521 isolates, the rate of resistance was low at 3.5% for all isolates and 1.1% among ESBL-producing *E. coli*.

Table 3. Antimicrobial susceptibility of ESBL-producing Enterobacteriaceae, 2016[1]

Antimicrobial	Number of isolates with results reported[2]	Percent								
		E. coli			*Klebsiella*			All isolates		
		S[3]	I[3]	R[3]	S	I	R	S	I	R
Amoxicillin-clavulanic acid	510	61.7	3.4	34.9	41.1	1.8	57.1	56.1	2.9	41.0
Piperacillin-tazobactam	206	90.4	2.2	7.4	70.5	18.0	11.5	84.0	7.3	8.7
Cefoxitin	432	91.4	3.5	5.1	90.4	0.0	9.6	89.1	2.6	8.3
Ertapenem	393	98.9	0.4	0.7	97.1	0.0	2.9	98.0	0.3	1.8
Imipenem	47	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Meropenem	250	100	0.0	0.0	100	0.0	0.0	100	0.0	0.0
Ciprofloxacin	461	34.6	2.8	62.6	46.6	9.1	44.3	36.9	4.6	58.6
Norfloxacin	193	21.8	0.8	77.4	41.5	5.7	52.8	28.5	2.1	69.4
Gentamicin	476	57.5	0.3	42.2	44.6	0.9	54.5	53.2	0.6	46.2
Co-trimoxazole	212	38.7	0.0	61.3	11.1	0.0	88.9	32.1	0.0	67.9
Trimethoprim	483	33.3	0.0	66.7	8.6	0.0	91.4	27.1	0.0	72.9
Fosfomycin	371	98.6	0.4	1.1	90.6	0.0	9.4	96.2	0.3	3.5

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 antibiotic reported.
3 S, susceptible; I, intermediate; R, resistant.

ESBL types and other resistance genes

WGS data was available for 394 of the total 521 ESBL-E included in the survey. This subset of 394 ESBL-E isolates included a random sample of 201 of the total 326 ESBL-E referred for the survey from the four laboratories in the Auckland area (Labtests, LabPlus, Middlemore Hospital Laboratory and North Shore Hospital Laboratory) and all isolates from other laboratories.

CTX-M type ESBLs predominated and were identified in 98.2% of the 394 isolates sequenced (Table 4). Among the *E. coli* there were approximately equal numbers of isolates with CTX-M types belonging to group 1 and group 9. In contrast among the *Klebsiella* species with a CTX-M ESBL, 91.4% had a group 1 CTX-M. CTX-M-15 accounted for 211 (91.0%) of the total 232 isolates with a group 1 CTX-M, whereas among the 155 isolates with a group 9 CTX-M, 88

(56.8%) were CTX-M-27 and 55 (35.5%) 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 Enterobacteriaceae, 2016

ESBL type[1]	Number (column %) isolates[1]			
	Escherichia coli **(n=298)**	***Klebsiella*** **(n=81)**	**Other Enterobacteriaceae (n=15)**	**All isolates (n=394)**
CTX-M	295 (99.0)	79 (97.5)	13 (86.7)	387 (98.2)
CTX-M group 1	149[2] (50.0)	74 (91.4)	9 (60.0)	232[2,3] (58.9)
CTX-M group 9	146[2] (49.0)	5 (6.2)	4 (26.7)	155[2,3] (39.3)
SHV	1 (0.3)	2 (2.5)	2[4] (13.3)	5[4,5] (1.3)
TEM	2 (0.7)	0	0	2[6] (0.5)
VEB	0	0	2 (13.3)	2[7] (0.5)

1 Only the 394 ESBL-E whose whole genome was sequenced are included in this analysis of ESBL types.
2 One isolate had both a group 1 CTX-M and a group 9 CTX-M. A further isolate had CTX-M-64, a hybrid of CTX-M-15 and CTX-M-14 β-lactamases. CTX-M-64 is included in the total isolates with a CTX-M ESBL but not included in the CTX-M group 1 or the CTX-M group 9 subtotals.
3 The 232 CTX-M group 1 β-lactamases include 211 CTX-M-15, 11 CTX-M-55, 8 CTX-M-3 and 2 CTX-M-1. The 155 CTX-M group 9 β-lactamases include 88 CTX-M-27, 55 CTX-M-14, 8 CTX-M-24, 3 CTX-M-9 and 1 CTX-M-65.
4 The two Other Enterobacteriaceae isolates (1 *Citrobacter freundii* and 1 *Enterobacter cloacae* complex) with a SHV ESBL (both SHV-12) also had CTX-M-9.
5 The 5 SHV ESBLs include 3 SHV-12, 1 SHV-2 and 1 SHV-55.
6 The 2 TEM ESBLs include 1 TEM-19 and 1 TEM-29.
7 The 2 VEB ESBLs were both VEB-6 in *Proteus mirabilis*.

The prevalence of genes encoding plasmid-mediated fluoroquinolone resistance, 16S rRNA methyltransferases and aminoglycoside modifying enzymes; the *fosA* gene; *dfrA* genes; and chromosomal mutations in the *gyrA* and *parC* genes associated with fluoroquinolone resistance is shown in Table 5. There was a notable difference in the apparent mechanisms of fluoroquinolone resistance in *E. coli* and *Klebsiella*. 60.9% 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 was strongly correlated with the reported ciprofloxacin resistance (p <0.001). While 44.3% of *Klebsiella* were reported to be ciprofloxacin resistant, only 2.5% had these mutations in the *gyrA* and *parC* genes. However, genes associated with plasmid-mediated fluoroquinolone resistance were prevalent in *Klebsiella*, and the combination of *aac(6')-Ib-cr + qnrB + oqxAB* genes was identified in the majority (69.2%) of ciprofloxacin-resistant *Klebsiella* isolates lacking chromosomally mediated fluoroquinolone resistance.

A 16S rRNA methyltransferase gene *(*specifically *armA*) was identified in just one isolate (*Enterobacter cloacae*). Genes for aminoglycoside modifying enzymes were common, in particular *aac(3)-IIa*, *aac(3)-IId*, *aac(6')-Ib-cr* and *aadA5* (Table 5).

While the *fosA* gene was identified in 97.3% of the *Klebsiella*, only 9.1% of the isolates with *fosA*, and for whom susceptibility data was reported, were fosfomycin resistant (Table 5).

A *dfrA* gene was identified 87.9% of the isolates reported to be trimethoprim resistant.

No genes encoding acquired carbapenemases or plasmid-encoded colistin resistance (ie, *mcr-1*) were detected.

Table 5. Distribution of non-β-lactam resistance genes among ESBL-producing Enterobacteriaceae, 2016

Resistance/resistance genes[1]	Number (column %) isolates[1]			
	Escherichia coli **(n=297)[2]**	***Klebsiella*** **(n=81)**	**Other Enterobacteriaceae (n=15)**	**All isolates (n=393)[2]**
Chromosomally mediated fluoroquinolone resistance[3]	181 (60.9)	2 (2.5)	5 (33.3)	188 (47.7)
Plasmid-mediated fluoroquinolone resistance[4]	70 (23.6)	79 (97.5)	8 (53.3)	157 (39.8)
16S rRNA methyltransferases[5]	0	0	1 (6.7)	1 (0.25)
Aminoglycoside modifying enzymes[6]	213 (71.7)	55 (67.9)	12 (80.0)	280 (71.1)
fosA[7]	0	79 (97.3)	6 (40.0)	85 (21.6)
dfrA[8]	197 (66.3)	73 (90.1)	12 (80.0)	282 (71.6)

1 Includes acquired resistance genes and mutations in the chromosomal *gyrA* and *parC* genes associated with fluoroquinolone resistance.
2 The resistance genes carried by 1 of the 298 *E. coli* isolates sequenced are not included in this analysis.
3 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).
4 The following plasmid-mediated fluoroquinolone resistance genes were identified: *qnrB, qnrD, qnrS, aac(6')Ib-cr, oqxA* and *oqxB*.
5 The only 16S ribosomal methylase gene found was *armA.*
6 The following aminoglycoside modifying enzyme genes were identified: *aac(3)-IIa, aac(3)-IId, aac(3)-IIe, aac(3)-IVa, aac(6′)-Ib, aac(6′)-Ib-cr, aac(6′)-IIa, aadA1, aadA2, aadA5, aadA16, aadB, aph(3′)-Ia, aph(3′)-Ic, aph(3′)-IIa, aph(3′)-Via* and *aph(4)-Ia.*
7 The *fosA* gene codes for a fosfomycin-modifying enzyme and was the only acquired fosfomycin resistance gene identified.
8 The *dfrA* genes mediate trimethoprim resistance.

Molecular epidemiology and population structure

The MLST was able to be derived *in silico* for 293 of the 298 *E. coli* and 76 of the 79 *K. pneumoniae* that underwent WGS. The five *E. coli* and three *K. pneumoniae* isolates unable to be assigned an MLST was due to each of these isolates having 1 allele that was not in the relevant database. The most common types are showed in Table 6.

Table 6. Distribution of multilocus sequence types among ESBL-producing *E. coli* and *K. pneumoniae*, 2016

***E. coli* (n=293)**		***K. pneumoniae* (n=76)**	
MLST[1,2]	**Number (%)**	**MLST[1,2]**	**Number (%)**
ST131	176 (60.1)	ST25	35 (46.1)
ST38	20 (6.8)	ST48	8 (10.5)
ST1193	20 (6.8)	ST14	4 (5.3)
ST12	10 (3.4)	ST323	3 (3.9)
ST69	10 (3.4)		
ST405	7 (2.4)		
ST648	6 (2.0)		
ST73	4 (1.4)		
ST95	4 (1.4)		

1 Only the MLSTs shared by >2 isolates are listed in the table.
2 None of the MLSTs listed belong to the same MLST clonal complex.

ST131 was the predominant MLST among *E. coli*, accounting for 60.1% 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. A core-SNP cladogram for the 298 *E. coli* isolates sequenced is shown in Appendix 1. The clustering of MLSTs was largely in agreement with the core SNP-based phylogeny. There was no obvious clustering of patient demographics with the core SNP-based phylogeny.

69.9% (107/153) of the ST131 *E. coli*, for which the *in silico* PCR typing information was complete, belonged to the *H*30 lineage (which is associated with fluoroquinolone resistance) and 57.0% (61/107) of the ST131 *H*30 *E. coli* belonged to the *H*30Rx subclone (which is associated with the CTX-M-15 ESBL). Among the 107 ST131 *H*30 *E. coli*, all had chromosomal mutations in the *gyrA* and *parC* genes associated with fluoroquinolone resistance, 57.9% had a group 1 CTX-M

ESBL, and 42.1% had a group 9 CTX-M ESBL. All but one the 61 ST131 *H*30Rx *E. coli* had CTX-M-15 ESBL, with the remaining isolate having CTX-M-14. A core-SNP cladogram and phylogenetic tree for the ST131 *E. coli* isolates are shown in Appendix 2A and 2B, respectively. These illustrate the strong association between the *H*30 lineage and chromosomally mediated fluoroquinolone resistance, and the strong association between the *H*30Rx subclone and CTX-M-15 ESBL. In addition, the phylogenetic tree confirms the strong clonal nature of the *H*30 lineage and *H*30Rx subclone, with very limited core-genome variability among isolates belonging to this lineage and subclone.

ST25 was the most prevalent MLST among *K. pneumoniae*, accounting for 46.1% 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. A core-SNP cladogram for the 79 *K. pneumoniae* isolates is shown in Appendix 3. The clustering of MLSTs was largely in agreement with the core SNP-based phylogeny. There was no obvious clustering of patient demographics with the core SNP-based phylogeny.

References

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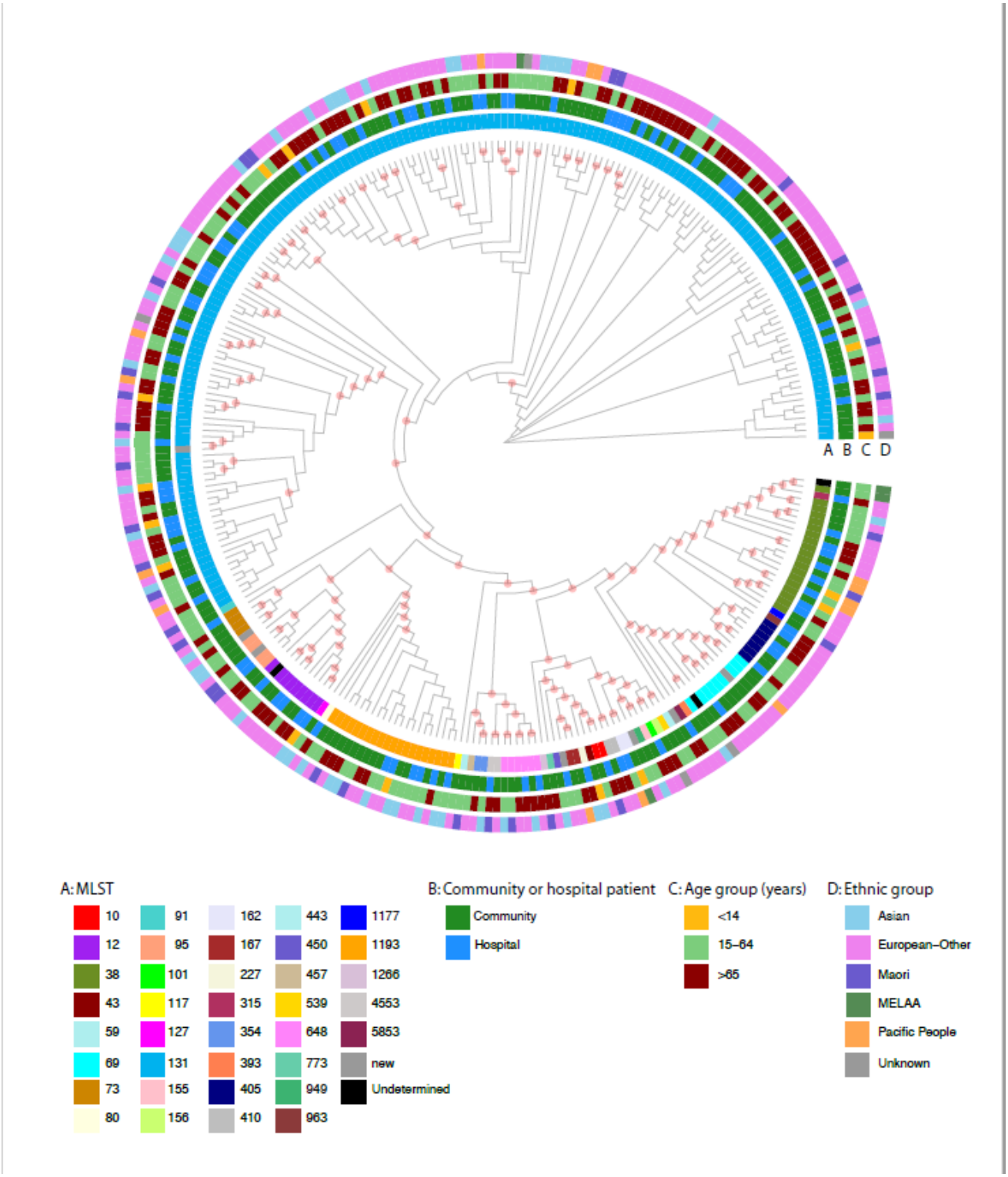
Appendix 1. Core SNP cladogram of the 298 sequenced ESBL-producing *Escherichia coli*

Nodes with greater than 90% bootstrap support are annotated with pink circles.



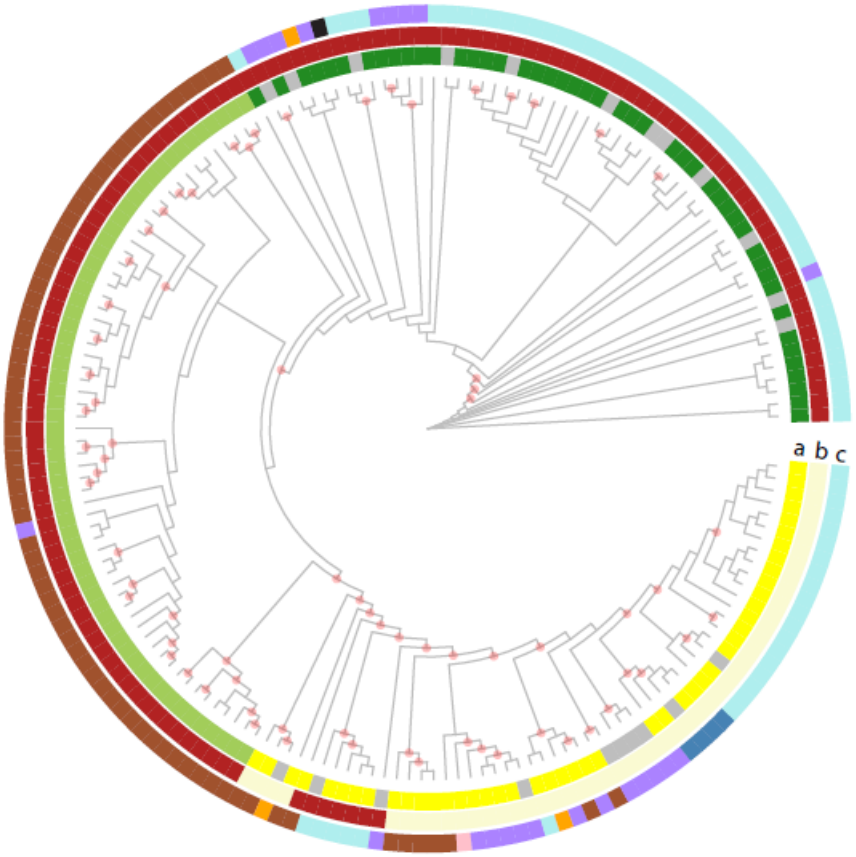
Appendix 2. Core SNP phylogeny of the 175 sequenced ST131 ESBL-producing *Escherichia coli* with (A) a circular cladogram to illustrate the relationship between the isolates and (B) a phylogenetic tree to illustrate the genetic distance between the isolates

Nodes with greater than 90% bootstrap support are annotated with pink circles in the cladogram.

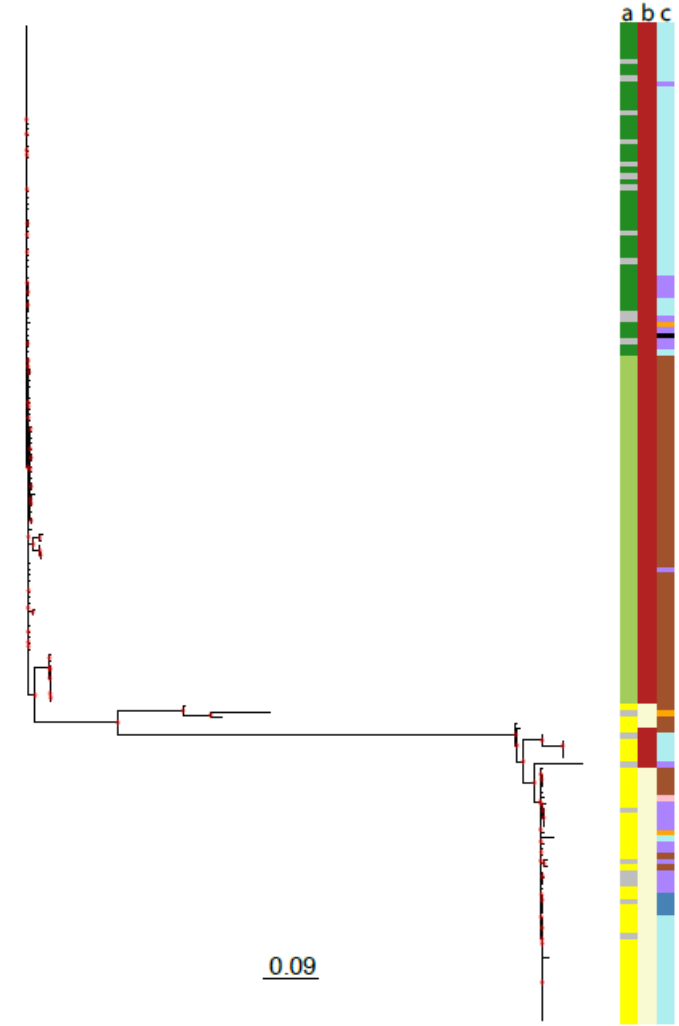
The phylogenetic tree scale bar is the maximum likelihood estimation of the number of substitutions per site.

Note: 1 of the 176 ST131 *E. coli* isolates sequenced was a mixed sequence and therefore excluded from this analysis.

A:



B:



a: H30 subclone
- No
- H30
- H30Rx
- Unable to type

b: Fluoroquinolone susceptibility
- Resistant
- Susceptible

c: ESBL type
- CTX–M–3
- CTX–M–14
- CTX–M–15
- CTX–M–24
- CTX–M–27
- CTX–M–55
- TEM–19

Appendix 3. Core SNP cladogram of the 79 sequenced ESBL-producing *Klebsiella pneumoniae*

Nodes with greater than 90% bootstrap support are annotated with pink circles.