



Carriage of Extended-Spectrum-Beta-Lactamase- and AmpC Beta-Lactamase-Producing *Escherichia coli* Strains from Humans and Pets in the Same Households

Leah J. Toombs-Ruane,^{a*} Jackie Benschop,^a  Nigel P. French,^a  Patrick J. Biggs,^{a,b} Anne C. Midwinter,^a Jonathan C. Marshall,^{a,b} Maggie Chan,^{a*} Dragana Drinković,^c Ahmed Fayaz,^a Michael G. Baker,^d Jeroen Douwes,^e Mick G. Roberts,^f  Sara A. Burgess^a

^amEpiLab, School of Veterinary Science, Massey University, Palmerston North, New Zealand

^bSchool of Fundamental Sciences, Massey University, Palmerston North, New Zealand

^cMicrobiology Department, North Shore Hospital, Auckland, New Zealand

^dDepartment of Public Health, University of Otago, Wellington, New Zealand

^eCentre for Public Health Research, Massey University, Wellington, New Zealand

^fSchool of Natural and Mathematical Sciences, Massey University, Albany, New Zealand

ABSTRACT Extended-spectrum-beta-lactamase (ESBL)- or AmpC beta-lactamase (ACBL)-producing *Escherichia coli* bacteria are the most common cause of community-acquired multidrug-resistant urinary tract infections (UTIs) in New Zealand. The carriage of antimicrobial-resistant bacteria has been found in both people and pets from the same household; thus, the home environment may be a place where antimicrobial-resistant bacteria are shared between humans and pets. In this study, we sought to determine whether members (pets and people) of the households of human index cases with a UTI caused by an ESBL- or ACBL-producing *E. coli* strain also carried an ESBL- or ACBL-producing *Enterobacteriaceae* strain and, if so, whether it was a clonal match to the index case clinical strain. Index cases with a community-acquired UTI were recruited based on antimicrobial susceptibility testing of urine isolates. Fecal samples were collected from 18 non-index case people and 36 pets across 27 households. Eleven of the 27 households screened had non-index case household members (8/18 people and 5/36 animals) positive for ESBL- and/or ACBL-producing *E. coli* strains. Whole-genome sequence analysis of 125 *E. coli* isolates (including the clinical urine isolates) from these 11 households showed that within seven households, the same strain of ESBL-/ACBL-producing *E. coli* was cultured from both the index case and another person (5/11 households) or pet dog (2/11 households). These results suggest that transmission within the household may contribute to the community spread of ESBL- or ACBL-producing *E. coli*.

IMPORTANCE *Enterobacteriaceae* that produce extended-spectrum beta-lactamases (ESBLs) and AmpC beta-lactamases (ACBLs) are important pathogens and can cause community-acquired illnesses, such as urinary tract infections (UTIs). Fecal carriage of these resistant bacteria by companion animals may pose a risk for transmission to humans. Our work evaluated the sharing of ESBL- and ACBL-producing *E. coli* isolates between humans and companion animals. We found that in some households, dogs carried the same strain of ESBL-producing *E. coli* as the household member with a UTI. This suggests that transmission events between humans and animals (or vice versa) are likely occurring within the home environment and, therefore, the community as a whole. This is significant from a health perspective, when considering measures to minimize community transmission, and highlights that in order to manage community spread, we need to consider interventions at the household level.

Citation Toombs-Ruane LJ, Benschop J, French NP, Biggs PJ, Midwinter AC, Marshall JC, Chan M, Drinković D, Fayaz A, Baker MG, Douwes J, Roberts MG, Burgess SA. 2020. Carriage of extended-spectrum-beta-lactamase- and AmpC beta-lactamase-producing *Escherichia coli* strains from humans and pets in the same households. *Appl Environ Microbiol* 86:e01613-20. <https://doi.org/10.1128/AEM.01613-20>.

Editor Charles M. Dozoi, INRS—Institut Armand-Frappier

Copyright © 2020 Toombs-Ruane et al. This is an open-access article distributed under the terms of the [Creative Commons Attribution 4.0 International license](https://creativecommons.org/licenses/by/4.0/).

Address correspondence to Sara A. Burgess, s.burgess1@massey.ac.nz.

* Present address: Leah J. Toombs-Ruane, University of Minnesota, St. Paul, Minnesota, USA; Maggie Chan, Virology Group, Animal Health Laboratory, MPI, Wallaceville, Wellington, New Zealand.

Received 5 July 2020

Accepted 29 September 2020

Accepted manuscript posted online 9 October 2020

Published 24 November 2020

KEYWORDS ESBL, *Enterobacteriaceae*, *Escherichia coli*, antibiotic resistance

Escherichia coli is a commensal microorganism found both inside and outside the mammalian large intestine and is commonly used as an indicator of fecal contamination (1). *E. coli* can cause disease and is responsible for most community-acquired (as opposed to hospital-acquired) urinary tract infections (UTIs) (2). Treatment can be complicated by antimicrobial resistance (3), particularly against extended-spectrum cephalosporins (4). Increasingly, enzymes, such as AmpC beta-lactamases (ACBLs) and extended-spectrum beta-lactamases (ESBLs), have been reported in pathogenic bacteria, including *E. coli* (3). Resistance to beta-lactams is also associated with multidrug resistance (5).

ESBL- and AmpC-producing *Enterobacteriaceae*, predominantly *E. coli* and, to a lesser extent, *Klebsiella pneumoniae*, are the most common cause of multidrug-resistant (MDR) UTIs in the New Zealand community (6). Surveillance from 2011 to 2016 showed an increasing infection rate in New Zealand, with a national period prevalence rate of 11.1 per 100,000 people for ESBL-producing *Enterobacteriaceae* from a clinical sample in 2016 (7). In contrast to human infections, antimicrobial resistance surveillance data are lacking for clinical isolates from animals in New Zealand, although cephalosporin resistance (including ESBL and AmpC production) has been found in bacterial isolates from companion animals (8). The transmission of antimicrobial-resistant (AMR) bacteria between cohabiting animals and humans means that antimicrobial interventions in one individual may indirectly affect the microbiome (and its resistance to antibiotics) of other individuals in the same household. A New Zealand study found that 36 of 83 (43%) patients with a community-acquired UTI associated with plasmid-mediated AmpC beta-lactamase-producing *E. coli* had not been given any antimicrobial treatment in the previous 6 months, nor had they been hospitalized (9). This would suggest that there are other drivers (besides antimicrobial use) for the selection and spread of MDR bacteria in the community, and close contact with household members, including pets, should be considered a potential source.

The family pet plays a central role in the lives of many people, with some behaviors facilitating the transmission of bacteria between people and their pets (10). In New Zealand, over 60% of households have a companion animal, with this rate of pet ownership being among the highest in the world (11). Pets have been shown to share similar *E. coli* and *K. pneumoniae* strains with humans in the same household as well as some of the same clonal lineages associated with UTIs in both humans and pets (12–15). Healthy cats and dogs have also been shown to carry *E. coli* sequence types (STs) that are usually associated with human extraintestinal infection (16). Sharing of other genetically similar bacteria has been observed between cohabiting pets and people. For example, contemporaneous carriage of clonal methicillin-resistant *Staphylococcus aureus* was observed in 57% of positive humans and pets sharing the same household environment (17). A similar association was reported for *Staphylococcus* species in other studies (18, 19). Reverse zoonotic transfer has also been implicated in methicillin-resistant *S. aureus* (MRSA) transfer events (19, 20).

Human-to-human transmission within the household is also an important pathway for the spread of AMR bacteria within the community. Family members can share fecal and oral bacteria (21), potentially resulting in pathogenic bacteria being harbored quiescently by individuals and causing disease in cohabiting persons (or pets) (22, 23). In a country with a relatively low prevalence of carriage of extended-spectrum-beta-lactamase-producing *E. coli*, such as New Zealand (24), the family may be an important reservoir of antimicrobial-resistant bacteria. Isolation of closely related AMR bacteria from cohabiting people has been demonstrated with MRSA (25, 26). The transmission of ESBL-producing sequence type 131 (ST131) *E. coli* strains (the leading cause of urinary tract infections) within households has also been observed (27), in addition to the sharing of urinary tract infection-causing *E. coli* strains between sexual partners (28).

There is a paucity of information on the household carriage of ESBL-producing *E. coli* from countries with high rates of pet ownership, such as New Zealand.

This study aimed to evaluate whether the same strains of ESBL- or ACBL-producing *E. coli* were shared between humans and pets within households. The study focused on the genomic relatedness of ESBL- or ACBL-producing *E. coli* isolates obtained from persons with community-acquired UTIs and their household members (people and/or pets).

RESULTS

Summary of households. Seventy-two human index cases (individuals suspected to have had a UTI) were invited to submit fecal samples from themselves, as well as any other members of the household, including cats or dogs, that lived with them in the home. Sixty-seven (93%) of the invited index cases submitted fecal samples, while 27 (27/67; 40%) of these case households submitted samples from other members of the household (including pets).

In these 27 households that submitted fecal samples from multiple household members, the index case person was predominantly female (24/27; 89%), and 12/27 (44%) were over 65 years old. All the index cases reported receiving antimicrobial treatment in the prior 6 months, including treatment for the index UTI. Twenty-three households (23/27; 85%) submitted samples from pets, 15 households (15/27; 55%) submitted fecal samples from other people, and 11 households (11/27; 41%) submitted samples from both pets and other people (see Table S1 in the supplemental material). Fecal samples from these 27 households were collected between December 2015 and January 2017 and were obtained between 31 and 180 days after the case UTI urine sample was provided (median time, 63 days; mean time, 77 days).

Carriage of ESBL-/ACBL-producing *Enterobacteriaceae* within households. Among the 23 households (of the 27 total) that submitted fecal samples from pets, eight (8/23; 35%) households had pets that were positive for an *Enterobacteriaceae* strain with an ESBL-/ACBL-producing phenotype, isolated from nine (6/18 dogs and 3/18 cats) individual animals (Table S1). Five of six positive dogs (5/18; 28% of dogs) were positive for ESBL-/plasmid-mediated ACBL-producing *E. coli* (from five households). In previous studies, raw meat diets and prior antimicrobial use have been identified as risk factors for ESBL-producing *Enterobacteriaceae* carriage in companion animals (29, 30). Significantly more dogs (15/18; 83%) than cats (4/18; 22%) were reported to eat raw meat as part of their diet ($P = 0.0006$) (Table S1). However, there was no association between the consumption of raw meat and the carriage of ESBL-producing *Enterobacteriaceae* by pets ($P = 0.23$). Antimicrobial treatment was reported in four of the five dogs positive for ESBL-producing *E. coli* (Fig. 1; Table S1). One dog, with no prior antimicrobial treatment, was positive for other ESBL-/ACBL-producing *Enterobacteriaceae*. There was no association between prior antimicrobial treatment and the presence of ESBL-/ACBL-producing *Enterobacteriaceae* ($P = 0.23$). Significantly more dogs (5/18; 28%) than cats (0/18; 0%) were positive for ESBL-/plasmid-mediated ACBL-producing *E. coli* ($P = 0.02$).

Similarly, among the 15 households that submitted fecal samples from people other than the index case, seven (7/15; 47%) households were positive for ESBL-/ACBL-producing *Enterobacteriaceae*, isolated from nine (9/18; 50%) non-index case individuals. Two of these nine individuals had been treated with antimicrobials in the 6 months prior to the sampling of feces (as reported by the index case). None of the household contacts were reported (by the index case) to have had an MDR infection. In total, 15 households (15/27; 55%) had non-index case individuals (people or pets) that were positive for ESBL-/ACBL-producing *Enterobacteriaceae*, with 11 (11/27; 41%) of these being households positive for ESBL- and/or plasmid-mediated ACBL-producing *E. coli* (Fig. 1). These 11 households formed the basis for the remainder of this study, and the whole genomes of all the ESBL- and/or ACBL-producing *Enterobacteriaceae* isolates from the individuals in these 11 households were sequenced (Table 1; Table S2).

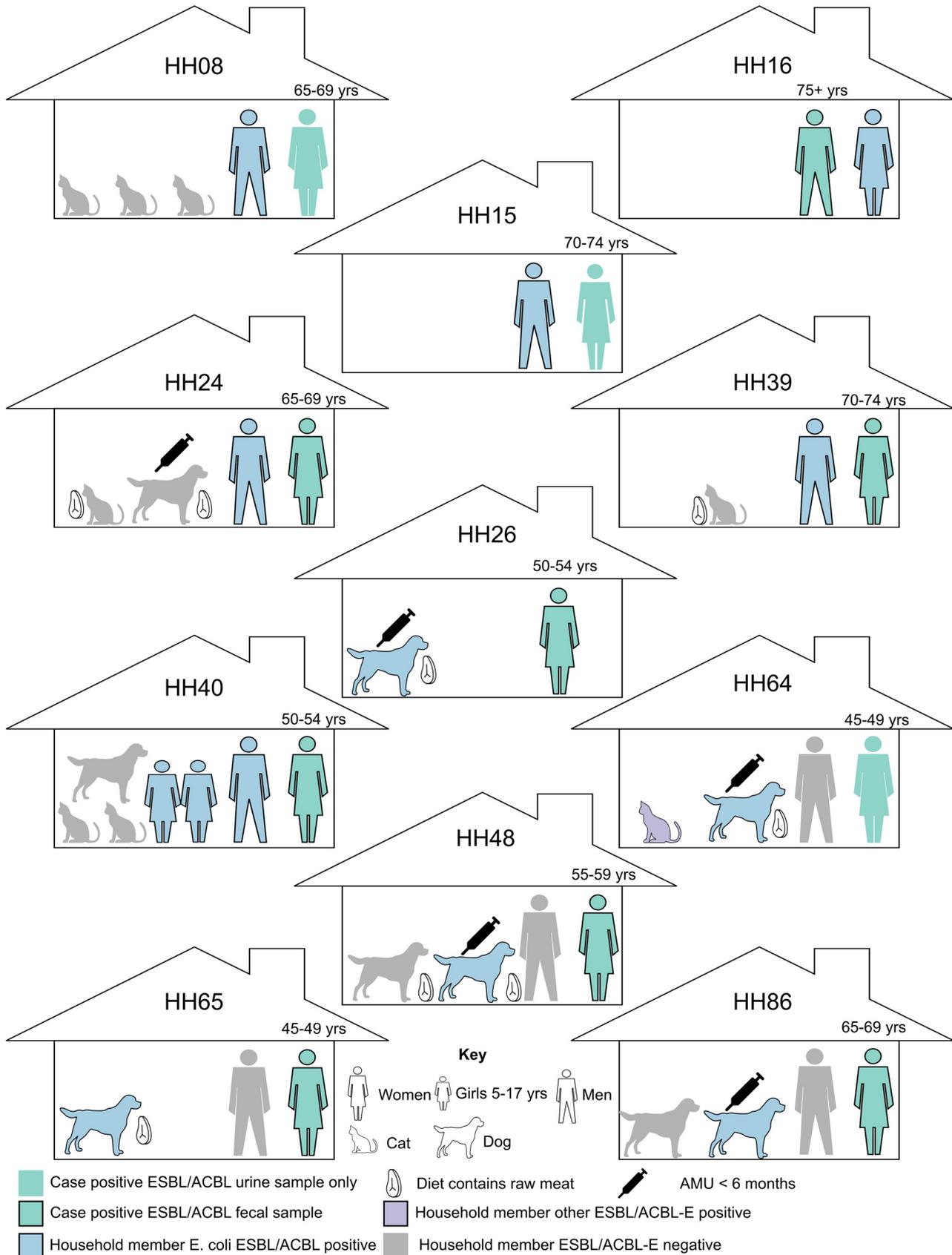


FIG 1 Description of the 11 households where multiple household members were identified to be harboring ESBL-/ACBL-producing *E. coli* strains and whether household members were positive or negative for the carriage of ESBL- and/or ACBL-producing *E. coli*. The age of each case participant is marked above the case. ESBL/ACBL-E, ESBL-/ACBL-producing *Enterobacteriaceae*; AMU, antimicrobial use.

TABLE 1 Summary of the *Enterobacteriaceae* isolates from 11 households

Household	Source of isolates	Bacterial species	Phylogroup(s) ^a
HH08	Urine index case	<i>E. coli</i>	D
	Other person (n = 6)	<i>E. coli</i>	B1 (1/6), D (5/6)
HH15	Urine index case	<i>E. coli</i>	D
	Other person (n = 6)	<i>E. coli</i>	D (6/6)
HH16	Urine index case	<i>E. coli</i>	D
	Fecal index case (n = 8)	<i>E. coli</i>	D
	Other person (n = 5)	<i>E. coli</i>	D
HH24	Urine index case	<i>E. coli</i>	B2
	Fecal index case (n = 7)	<i>E. coli</i>	B2
	Other person (n = 6)	<i>E. coli</i>	B2 (4/6), F (2/6)
HH26	Urine index case	<i>E. coli</i>	A
	Fecal index case (n = 3)	<i>E. coli</i>	B2
	Pet dog (n = 8)	<i>E. coli</i> , <i>Citrobacter</i> sp.	B2 (7/8)
HH39	Urine index case	<i>E. coli</i>	B2
	Fecal index case (n = 4)	<i>E. coli</i>	B2
	Other person (n = 4)	<i>E. coli</i>	B2
HH40	Urine index case	<i>E. coli</i>	B2
	Fecal index case (n = 6)	<i>E. coli</i>	B2
	Other person 1 (n = 6)	<i>E. coli</i>	B2
	Other person 2 (n = 7)	<i>E. coli</i>	B2
	Other person 3 (n = 6)	<i>E. coli</i>	B2
HH48	Urine index case	<i>E. coli</i>	D
	Fecal index case (n = 4)	<i>E. coli</i>	D
	Pet dog (n = 4)	<i>E. coli</i>	D (2/4), B2 (1/4), NT (1/4)
HH64	Urine index case	<i>E. coli</i>	B1
	Pet dog (n = 4)	<i>E. coli</i>	B1 (3/4), D (1/4)
	Pet cat (n = 5)	<i>Enterobacter</i> spp.	
HH65	Urine index case	<i>E. coli</i>	D
	Fecal index case (n = 7)	<i>E. coli</i>	D
	Pet dog (n = 5)	<i>E. coli</i>	A (4/5), B1 (1/5)
HH86	Urine index case	<i>E. coli</i>	B2
	Fecal index case (n = 1)	<i>E. coli</i>	B2
	Pet dog (n = 6)	<i>E. coli</i>	B2

^aValues in parentheses represent the number of isolates of the indicated phylogroup/ total number of isolates from the individual.

Genetic diversity of ESBL-producing *Enterobacteriaceae*. In order to obtain the predominant ESBL-producing *Enterobacteriaceae* strain(s) associated with the households sampled, we cultured up to eight colonies per fecal sample as well as one colony from the index case urine sample. *E. coli* was the predominant species identified (125/131; 95%), with a *Citrobacter* species (1/131; 0.8%) and *Enterobacter* species (5/131; 4%) also being isolated. *E. coli* was the only bacterial species isolated that was shared between the index case and other members of the household (both people and pets). Short-read sequencing was carried out on all of the 125 *E. coli* strains from the 11 households that had an ESBL- or ACBL-producing phenotype (Table S2), and the draft whole-genome sequence assemblies were used to determine the phylogroup and sequence type of each isolate (Table 1), as well as to identify virulence factors, antibiotic resistance determinants, and plasmid types (Fig. 2). The sequence types (determined by use of the Nullarbor bioinformatics pipeline) ST131, ST69, and ST963 were found in more than one household; ST131 was found in four households and accounted for 47/125 (46%) of the isolates described here.

We next investigated the genetic relatedness of these strains by performing whole-genome multilocus sequence typing (wgMLST) for all 125 *E. coli* isolates, using 3,022

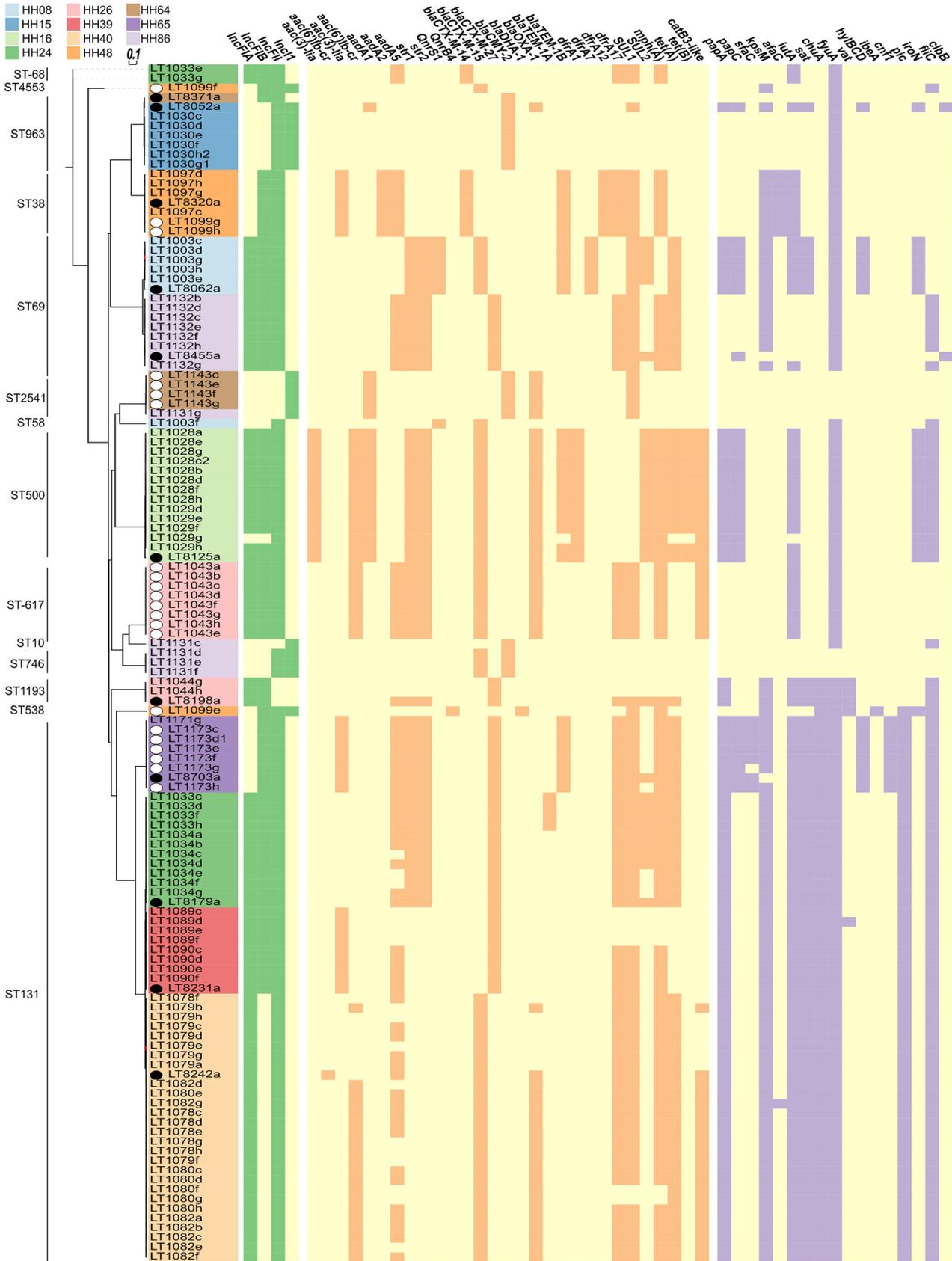


FIG 2 Whole-genome MLST of 125 *E. coli* isolates from 11 households. A total of 3,022 loci were used to construct a distance matrix using the Fast-GeP (v.1.0) genome profiler and the reference genome LT1099f. A black circle to the left of the tip label indicates that the isolate was from the (Continued on next page)

Downloaded from <http://aem.asm.org/> on January 17, 2021 at MASSEY UNIV LIBRARY

loci. The household-level patterns of virulence, antimicrobial resistance, and plasmid genes are also illustrated (Fig. 2). Isolates from five of the households represented distinct monophyletic clades. Analysis of the presence/absence of 25 virulence-associated genes (Fig. 2; Table S3) showed that the within-household clonal fecal and index urine strains had a similar virulence gene profile. Both the index urine and fecal isolates displayed some of the genes typically associated with extraintestinal pathogenic *E. coli* strains, such as *papA* (81/125; 65%), *papC* (30/125; 24%), *sfaC* (7/125; 6%), *afaC* (8/125; 6%), *kpsM* (82/125; 66%), and *iutA* (98/125; 78%) (31), as well as uropathogenic *E. coli* strain-associated genes such as *sat* (67/125; 54%), *vat* (5/125; 4%), *fyuA* (117/125; 94%), and *chuA* (61/125; 49%) (31).

Distribution of antimicrobial-resistant genotypes and phenotypes. All the *E. coli* isolates were resistant to cefpodoxime, in agreement with their ESBL- or ACBL-producing phenotype (Table S4), and they all carried an ESBL gene (*bla*_{CTX-M}) or an ACBL gene (*bla*_{CMY} or *bla*_{DHA-1}) (Table 2 and Table S5). In some individuals and households, the same beta-lactamase gene variant was found across multiple sequence types. For example, the *bla*_{CMY-2} gene was associated with both ST10 and ST746 in one individual from household 65 (HH65), and in HH64, this gene was associated with the index urine ST963 isolate and a dog ST2541 isolate. An MDR phenotype was observed in 87 isolates (87/125; 70%), and an MDR genotype, where genes associated with resistance to three or more classes of antibiotics were detected (including genes that we did not test for phenotypically), was observed in 106 isolates (106/125; 85%) (Table 2). No colistin or carbapenem resistance genes were found.

***E. coli* population within households.** To further explore the genetic relatedness of isolates within households, paired-end sequence reads from each *E. coli* isolate were compared to a draft assembled reference genome selected from that household (Table S6). These comparisons were made using core single nucleotide polymorphisms (SNPs), and the resultant trees for individual households are presented in Fig. 3 and Fig. S1. This demonstrated that in seven of the households (HH08, HH16, HH24, HH39, HH40, HH48, and HH86), clonal strains (strains with a difference of <10 SNPs) were cultured from more than one household member and from a person and a pet in two households (Fig. 3, HH48 and HH86). We also made comparisons using a SNP analysis of the *E. coli* ST131 isolates across four households (Fig. 4), as well as ST69 and ST963 isolates across two households (Table S7 and S8). These results demonstrated that clonal ST131, ST69, and ST963 isolates were cultured from more than one person or pet within a household but not across these households.

DISCUSSION

Fecal carriage of ESBL-producing *Enterobacteriaceae*. This study is an important first step in examining the role of household contacts and pets in the transmission of community-acquired ESBL-/ACBL-producing *E. coli* strains associated with infections. The proportion of household members (9/18; 50% of people) that carried ESBL- or ACBL-producing *Enterobacteriaceae* was higher than that in other case-index carriage studies, which has been reported to range from 8.0% to 36.9% (32). Similarly, the proportion of companion animals that carried ESBL- or ACBL-producing *Enterobacteriaceae* (9/36 animals; 25%) was higher than that reported in previous studies of healthy companion animals, which ranged from 0 to 20.5% (33–35). In New Zealand, there are limited data on the prevalence of ESBL- and ACBL-producing *Enterobacteriaceae* from both healthy companion animals and clinical samples. The prevalence of ESBL-producing *E. coli* from healthy cats and dogs was reported to be 6.5% (30).

Intestinal carriage of MDR *Enterobacteriaceae* has been described to be a significant risk for subsequent infection with MDR bacteria (36, 37), and it is very likely that the

FIG 2 Legend (Continued)

index case urine sample, and a white circle indicates that the isolate originated from a dog fecal sample. The colored strip in the tip label (isolate identifier) denotes the household. The presence or absence of a plasmid, resistance, and the selected virulence genes was found using the PlasmidFinder (green/yellow heat map), ResFinder (orange/yellow heat map), and VirulenceFinder (purple/yellow heat map) databases, respectively.

TABLE 2 Resistance phenotype and genotype of *E. coli* isolates^a

Household	Isolate(s)	Sequence type	Beta-lactamase phenotype	ESBL gene(s)	ACBL gene(s)	Other resistance phenotype	Other resistance gene(s)
HH08	LT8062a, LT1003d, LT1003e, LT1003g, LT1003h	ST69	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-15}	None	TMP	<i>dfra7</i> , <i>strA</i> , <i>strB</i> , <i>sul1</i> , <i>sul2</i> , <i>terA</i> , <i>qnrS1</i>
	LT1003f	ST58	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-15}	None	NIT	<i>qnrS1</i>
HH15	LT8052a	ST963	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX	None	<i>bla</i> _{GMY-2}	NIT	<i>aadA1</i> -like, <i>sul1</i>
	LT1030c, LT1030d, LT1030e, LT1030f, LT1030g1, LT1030h2	ST963	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX	None	<i>bla</i> _{GMY-2}	None	None
HH16	LT8125a, LT1028a, LT1028b, LT1028c, LT1028d, LT1028e, LT1028f, LT1028g, LT1028h, LT1029d, LT1029e, LT1029f, LT1029g, LT1029h	ST500	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX, MEC	<i>bla</i> _{CTX-M-15}	None	TMP, NIT, NOR, GEN	<i>aac(3)-Ila</i> -like, <i>aac(6')Ib-cr</i> , <i>aadA1</i> , <i>catB3</i> -like, <i>dfra17</i> , <i>mph(A)</i> -like, <i>strA</i> -like, <i>strB</i> -like, <i>sul2</i> -like, <i>terA</i> , <i>terB</i> (13/14)
	LT8179a, LT1034g	ST131	CTX, AMP, LEX, CPD, CRO, MEC	<i>bla</i> _{CTX-M-27}	None	TMP, NOR	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i> , <i>strA</i> , <i>strB</i> , <i>strC</i> , <i>strD</i> , <i>terA</i>
HH24	LT1033c, LT1033d, LT1034a, LT1034b, LT1034c, LT1034d, LT1034e, LT1034f	ST131	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	TMP, NOR	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i> , <i>strA</i> , <i>strB</i> , <i>strC</i> , <i>strD</i> , <i>terA</i>
	LT1033h	ST131	CTX, AMP, LEX, CPD, CRO, MEC	<i>bla</i> _{CTX-M-27}	None	TMP, NOR	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i> , <i>strA</i> , <i>strB</i> , <i>strC</i> , <i>strD</i> , <i>terA</i>
HH26	LT1033f	ST131	CTX, LEX, CPD, CRO, MEC	<i>bla</i> _{CTX-M-27}	None	TMP, NOR	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i> , <i>strA</i> , <i>strB</i> , <i>strC</i> , <i>strD</i> , <i>terA</i>
	LT1033e, LT1033g	ST648	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-14}	None	TMP, NOR	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i>
HH26	LT1043a, LT1043b, LT1043d, LT1043e, LT1043f, LT1043g, LT1043h	ST617	CTX, CAZ, AMP, AMC, LEX, CPD, CRO	<i>bla</i> _{CTX-M-15}	None	TMP, NIT, NOR	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>strA</i> , <i>strB</i> -like, <i>strC</i> , <i>strD</i> , <i>aac(6')Ib-cr</i> , <i>catB3</i> -like
	LT8198a	ST1193	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	TMP, NOR	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>strA</i> , <i>strB</i> -like, <i>strC</i> , <i>strD</i> , <i>terA</i>
HH39	LT1043c	ST617	CTX, CAZ, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-15}	None	TMP, NIT, NOR	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>strA</i> , <i>strB</i> -like, <i>strC</i> , <i>strD</i> , <i>aac(6')Ib-cr</i> , <i>catB3</i> -like
	LT1044g	ST1193	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	NOR	None
HH39	LT1044h	ST1193	CTX, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	NOR	None
	LT8231a, LT1090c, LT1090d, LT1090e, LT1090f	ST131	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	TMP, NOR, GEN	<i>aac(3)-IId</i> -like, <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i>
HH40	LT1089c, LT1089d, LT1089e, LT1089f	ST131	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	NOR, GEN	<i>aac(3)-IId</i> -like
	LT8242a, LT1082a, LT1082b, LT1082d, LT1082e, LT1082f, LT1082g, LT1080c, LT1080d, LT1080e, LT1080h, LT1079f, LT1079g, LT1078g, LT1078h, LT1078c, LT1078d, LT1078e, LT1078f, LT1079a, LT1079c, LT1079d, LT1079e, LT1079g, LT1079h	ST131	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-15}	None	TMP, NOR	<i>aac(6')Ib-cr</i> , <i>aadA5</i> , <i>catB3</i> -like, <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i> , <i>terA</i>
HH48	LT1080f, LT1080g	ST131	CTX, AMP, LEX, CPD	<i>bla</i> _{GMY-1}	None	None	None
	LT1079b, LT1082c	ST131	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-15}	None	TMP, NOR	<i>aac(6')Ib-cr</i> , <i>aadA5</i> , <i>catB3</i> -like, <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i> , <i>terA</i>
HH48	LT1097c, LT1097d, LT1097h, LT1099g, LT1099h	ST38	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-14}	None	TMP, GEN	<i>aac(3)-IId</i> -like, <i>aadA2</i> , <i>aadA5</i> , <i>dfra12</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i>
	LT8320a	ST38	CTX, AMP, AMC, LEX, CPD, CRO	<i>bla</i> _{CTX-M-14}	None	TMP, GEN	<i>aac(3)-IId</i> -like, <i>aadA2</i> , <i>aadA5</i> , <i>dfra12</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i>
HH64	LT1099e	ST538	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX	None	<i>bla</i> _{GMY-1}	None	<i>mph(A)</i> , <i>qnrB4</i> , <i>str1</i>
	LT1099f	ST4553	CTX, AMP, LEX, CPD, CRO	None	None	None	None
HH64	LT1097g	ST38	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-15}	None	NIT, GEN	<i>aac(3)-IId</i> -like, <i>aadA2</i> , <i>aadA5</i> , <i>dfra12</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>str1</i>
	LT8371a	ST963	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX	None	<i>bla</i> _{GMY-2}	NIT	None
HH64	LT1143c, LT1143e, LT1143f, LT1143g	ST2541	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX	None	<i>bla</i> _{GMY-2}	None	<i>aadA1</i> -like, <i>str1</i>
	LT1131d, LT1131e, LT1131f	ST746	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX	<i>bla</i> _{CTX-M-15}	<i>bla</i> _{GMY-2}	None	None
HH65	LT1131c	ST110	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX	<i>bla</i> _{GMY-2}	<i>bla</i> _{GMY-2}	None	None
	LT1131g	ST2541	CTX, CAZ, AMP, AMC, LEX, CPD, CRO, FOX	None	<i>bla</i> _{GMY-2}	None	<i>aadA1</i> -like, <i>str1</i>
HH66	LT8455a, LT1132b	ST69	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	TMP, NIT	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>strA</i> , <i>strB</i> -like, <i>strC</i> , <i>strD</i> , <i>terA</i>
	LT1132c, LT1132d, LT1132e, LT1132f, LT1132g, LT1132h	ST69	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	TMP	<i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>strA</i> , <i>strB</i> -like, <i>strC</i> , <i>strD</i> , <i>terA</i>
HH66	LT8703a	ST131	CTX, AMP, AMC, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	TMP, GEN	<i>aac(3)-IId</i> -like, <i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>strA</i> , <i>strB</i> , <i>strC</i> , <i>strD</i> , <i>terA</i>
	LT1171g, LT1173c, LT1173d, LT1173e, LT1173f, LT1173g, LT1173h, LT1173i	ST131	CTX, AMP, LEX, CPD, CRO	<i>bla</i> _{CTX-M-27}	None	TMP, GEN	<i>aac(3)-IId</i> -like, <i>aadA5</i> , <i>dfra17</i> , <i>mph(A)</i> , <i>strA</i> , <i>strB</i> , <i>strC</i> , <i>strD</i> , <i>terA</i>

^aCTX, cefotaxime; CAZ, ceftazidime; AMP, ampicillin; AMC, amoxicillin/clavulanic acid; LEX, cephalosporin; CRO, cefepime; CPD, cepodoxime; FOX, ceftriaxone; MEC, mecillinam; GEN, gentamicin; NIT, nitrofurantoin; NOR, norfloxacin; GEN, gentamicin.

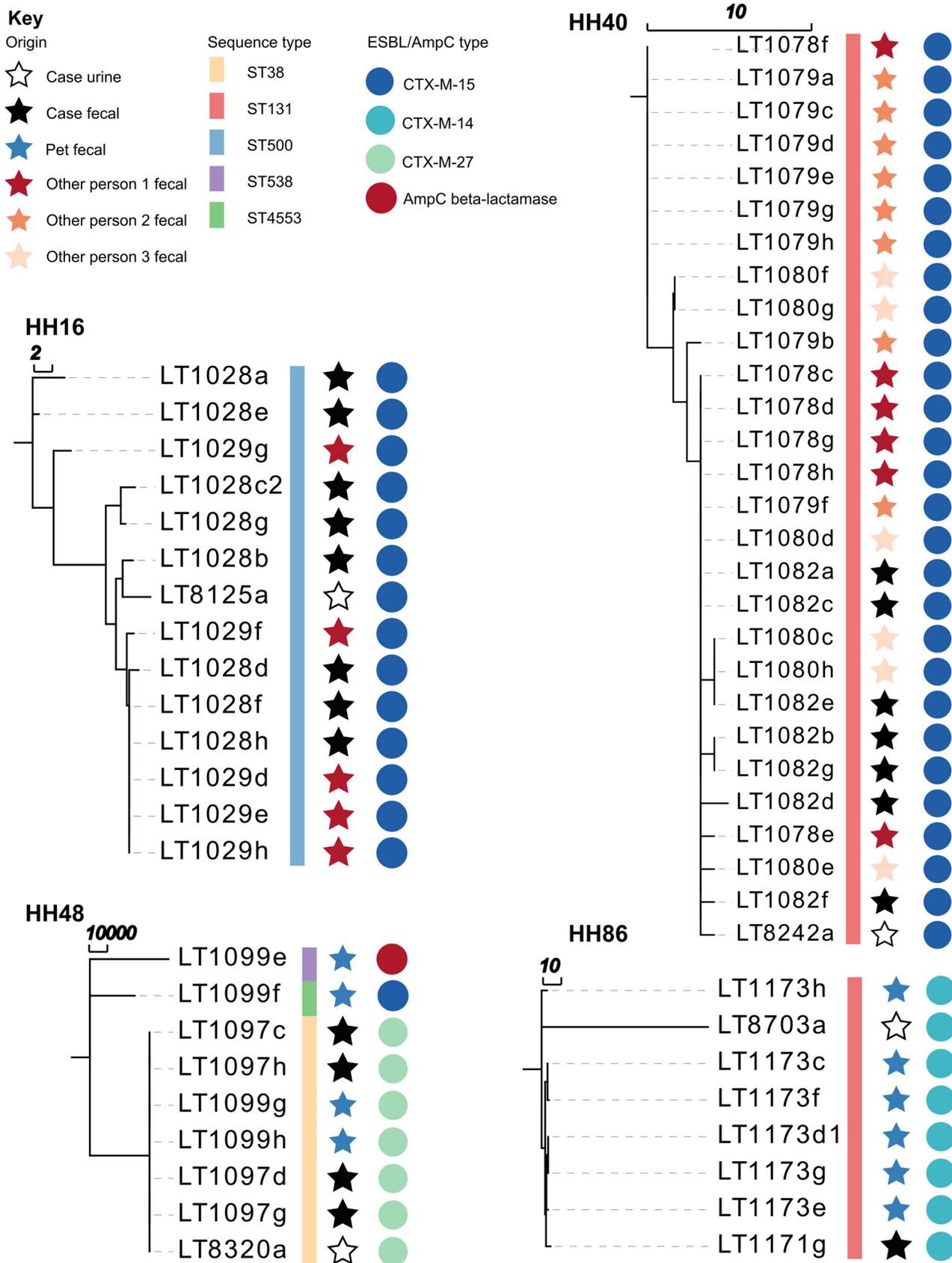


FIG 3 Neighbor-joining core SNP phylogenies, generated using the Snippy program (v.3.0), for ESBL-/ACBL-producing *E. coli* isolates from four households. The scale bar represents the branch length (in number of SNPs).

Downloaded from <http://aem.asm.org/> on January 17, 2021 at MASSEY UNIV LIBRARY

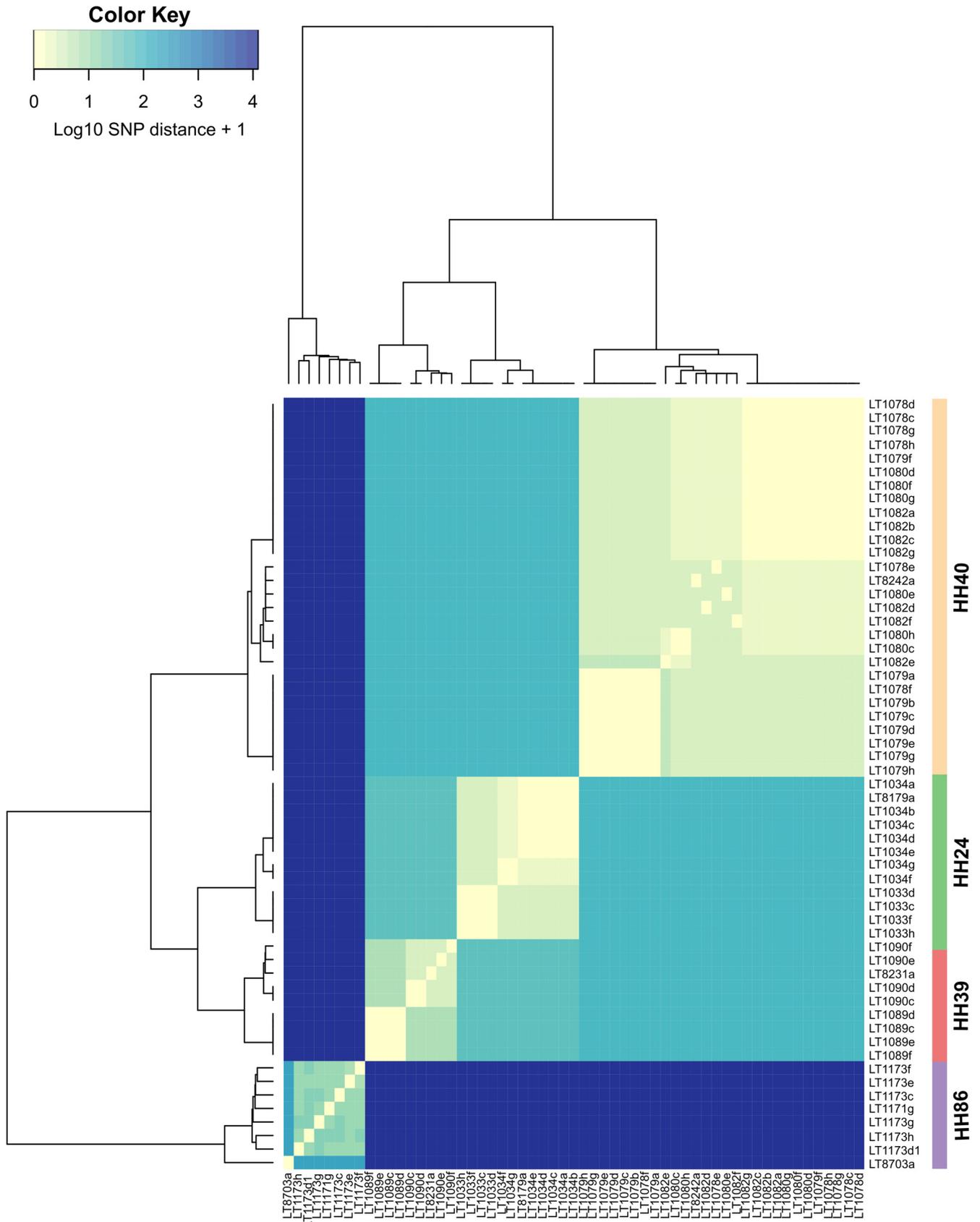


FIG 4 Dendrogram of the core SNP phylogeny of *E. coli* ST131 isolates from four households. The core SNP alignment was determined using 12,454 SNPs. Reference genome JJ1886 was used for core SNP phylogeny, using the Snippy program (v.3.0). The heat map represents the log + 1 of the number of SNPs between each pair of ST131 isolates.

proportion of carriers will continue to rise in the coming years (38). Benchmarking the prevalence of ESBL-producing bacteria in the community could be a useful first step for ongoing surveillance. This was not assessed in the current study, although it should be noted that 25% (3/12) of household human contacts carried an ESBL-producing *E. coli* strain unrelated to the index UTI in this study.

Epidemiology of observed sequenced types. The predominant sequence type of strains isolated from both urine and human fecal samples was ST131. It has been well established that this global lineage is the predominant cause of both UTIs and blood infections; additionally, it is commonly found in other human-derived samples, such as feces and sewage (39–41). Other sequence types found across multiple households were ST69 and ST963. ST69 has frequently been associated with UTIs (35, 36), whereas ST963 is rare in humans but has been detected in wild birds and companion animals (albeit in low numbers) (30, 42, 43). In the two households where ESBL-producing *E. coli* strains were shared by humans and their pet dogs, these strains belonged to ST131 as well as ST38, which, among ESBL-producing strains, is another lineage frequently associated with blood infections in humans (39). To our knowledge, most of the other STs found only in dogs in our study (ST1193, ST4553, ST746, ST2541, ST10) are rare, with the exception of ST10, which has frequently been found in both animals and humans (39, 44).

Household transmission. The sharing of ESBL- or ACBL-producing *E. coli* occurred between multiple household members and/or pets in 7/11 (64%) of the investigated households. However, this proportion varied according to whether the household contacts sampled were people or pets. In 50% (5/10) of the households with multiple people, clonal strains (strains with a difference of <10 SNPs) of ESBL-producing *E. coli* were cultured from more than one person in the household, whereas in 22% (2/9) of the households where pets were sampled, clonal strains were cultured from a person in the household and the pet. The results of this study are comparable to those of other studies, where the carriage of clonally related ESBL- or ACBL-producing *Enterobacteriaceae* has been found in a similar proportion of family members (27, 32, 45).

Pets have been described in previous studies as carrying the same *E. coli* clonal type as that carried by humans in the same household and in some cases being affected by clinical UTIs (27). The dogs that were positive for fecal ESBL-producing *E. coli* isolates in our study may have picked up these bacteria from raw meat, other animals, the environment, or their owners. Transmission may have occurred between dogs and their owners that shared the same strain; however, inference of the directionality of AMR transmission dynamics requires long-term sequential sampling. Another explanation for the sharing of strains is the acquisition of these bacteria from the same source by both the owner and the owner's pet. However, this seems unlikely, given that an earlier New Zealand study did not find ESBL-producing *E. coli* in meat, and the survival of these bacteria on surfaces is limited (46, 47).

The results presented here show that pets are at least transient carriers of ESBL- and ACBL-producing *Enterobacteriaceae*. It is of note that none of the animals positive for the same strain of *E. coli* as the humans in the household were cats. In agreement with the findings of other studies (35), significantly more dogs than cats carried ESBL-/plasmid-mediated ACBL-producing *E. coli* strains. Cats and dogs behave differently with regard to both the type of contact with owners (less hand and face licking by cats) and food (for instance, cats are less likely to eat the same food as humans, including pieces of food handed over by owners during a meal). Although eating raw meat is a risk factor for ESBL-producing *Enterobacteriaceae* carriage in both dogs and cats (29, 35, 48), in our study, significantly more dogs than cats consumed raw meat as part of their diet.

Close contact within the home between partners, between parents and children, and between pets and their owners may be a factor in the transmission of the MDR bacteria isolated in this study. In New Zealand, clinical infections with MDR *E. coli* occur in pets but occur at rates that are unknown and that are assumed to be low (8, 49). Interestingly, isolates of *E. coli* collected from dog fecal samples in two different

households had unique fecal AMR *E. coli* strains, all of which were of an ST different from that of the clinical case. While these STs are not commonly associated with community-acquired infection in New Zealand, the enzyme-coding genes (*bla*_{CTX-M-15}, *bla*_{CTX-M-14}) were those also found predominantly in human infections in New Zealand (7, 50).

Horizontal gene transfer. Analysis of the genome sequences presented here suggests that horizontal gene transfer (via plasmids) between bacteria may have occurred in some individuals. Discrete plasmids were not assembled through the bioinformatics pipeline; however, large numbers of plasmid-associated genes (and related plasmid types) were identified and reported. Although plasmid-mediated gene transfer was not explored experimentally within this study, there is the potential for this to have occurred with the ACBL gene *bla*_{CMY-2}, which was associated with isolates of two different sequence types in HH64. This highlights the value of collecting and sequencing multiple isolates from fecal samples. Long-read sequencing of plasmids would be required to confirm and compare the plasmids isolated from different bacteria to make any conclusions regarding horizontal gene transfer. Evidence of the transmissibility of plasmids from cultured bacteria to a donor organism (via conjugation) would also be required for any assertions around the intraperson (and, therefore, interperson) transmission of resistance genetics to be made.

Study limitations. A limitation to the study is that transmission within households cannot be examined by a cross-sectional methodology. Consequently, this is often assessed through mathematical modeling, in addition to observational studies (42). Within a longitudinal study of the postacquisition carriage of ESBL-producing *Enterobacteriaceae*, results were used for a transmission model that calculated a 12% probability of household transmission of ESBL-producing *Enterobacteriaceae* from a positive person to a negative person in the home (51). The results presented in this study identify that some sharing of ESBL- and ACBL-producing bacteria within households is likely, even though transmission dynamics cannot be inferred from this cross-sectional study.

Conclusions. The results of this study demonstrate the sharing of the same ESBL-producing *E. coli* strains between household members. This suggests that the transmission of ESBL-producing *E. coli* occurred through contact between people (or people and pets) after an introduction event or through exposure to the same source of AMR bacterial isolate. Although our study suggests that companion animals (specifically, dogs) are carriers of ESBL- and/or ACBL-producing *E. coli*, their importance is hard to assess, and they are likely to be less important vectors than other people living in the home. From a public health perspective, the findings from our study reinforce current guidelines on hygiene practices within households and the importance of considering the entire household in control measures to reduce the spread of AMR enteric pathogens.

MATERIALS AND METHODS

Ethics. Human ethics for this study was granted by the New Zealand Health and Disability Ethics Committee (HDEC) under reference 15/CEN/47; this study was part of a larger prospective case-control study looking at risks for community-acquired UTIs. Animal ethics for sampling via rectal swab from cats was granted by the Massey University Animal Ethics Committee under reference 15/35.

Participant selection. Index cases were recruited based on culture and antimicrobial sensitivity testing results of urine samples collected from persons suspected to have a UTI and submitted to Labtests Auckland (Healthscope) between 28 September 2015 and 5 September 2017. All urine samples came from the Auckland and Northland regions of New Zealand. Additionally, all of these samples had the growth of *E. coli* in the presence of pyuria ($\geq 10 \times 10^6$ white blood cells/liter), and the persons were considered to have a community-acquired UTI.

Index cases were eligible if they were 16 years of age or older, had no previous detection of an ESBL- or ACBL-producing *Enterobacteriaceae* isolate, had no record of being hospitalized overnight in the previous 12 months, and did not reside in an elder care facility or rest home. Seventy-two index cases were invited to submit fecal samples from themselves, as well as from any other members of the household, including cats or dogs that lived with them in the home. Five declined to participate; for the remaining 67, information on age, gender/sex, as well as antimicrobial treatment and hospitalization/veterinary care for the previous 6 months was collected.

Bacterial culture, identification, and susceptibility testing. Bacterial isolates from index case urine samples were collected from Labtests Auckland. Fecal samples were collected by the participants, using a sterile fecal collection bottle. Pet fecal samples were collected either by direct sampling with a rectal

swab (cats only, where litter trays were not used), using an agar transport swab (Copan Diagnostics, Brescia, Italy), or by the participant from the ground or litter box. Samples were processed by plating onto culture media, as follows: plain MacConkey agar (BD Difco, supplied by Fort Richard Laboratories, Auckland, New Zealand), MacConkey agar with 1-mg/liter cefotaxime sodium (Sigma-Aldrich, St. Louis, MO, USA), MacConkey agar with 1-mg/liter ceftazidime pentahydrate (Sigma-Aldrich), and chromogenic ESBL CHROMagar (CAC; Becton, Dickinson, Heidelberg, Germany). Rectal swabs taken from cats were enriched in buffered peptone water for approximately 16 h at 35°C before being plated onto the culture media. The agar plates were incubated overnight at 35°C, and two morphologically different single colonies (from each agar type) were selected for subculture onto Columbia horse blood agar (Fort Richard Laboratories). Up to eight colonies were chosen for subculture from each fecal sample. Identification of bacterial species from both urine and feces was done using matrix-assisted laser desorption ionization–time of flight (MALDI-TOF) mass spectrometry (for fecal strains, the mass spectrometer was from bioMérieux, Marcy-l'Étoile, France; for urine strains, the mass spectrometer was from Bruker, Billerica, MA, USA).

Isolates were tested for antimicrobial susceptibility and an ESBL-producing phenotype according to EUCAST guidelines, using a Kirby-Bauer disk diffusion assay (52, 53). Isolates were also tested for an AmpC-producing phenotype using a three-disk comparison assay (D69C AmpC disk test; Mast Group Ltd., Liverpool, UK). Susceptibility to an additional 14 antibiotics (see Table S9 in the supplemental material) was carried out using the Kirby-Bauer disk diffusion assay, using EUCAST clinical breakpoints (52, 54). “Moderate” susceptibilities were defined as those that fell between the susceptible and resistance diameters for the following antibiotics: ceftriaxone, gentamicin, amikacin, norfloxacin, and trimethoprim.

Genome sequencing and bioinformatics. *Enterobacteriaceae* isolates ($n = 131$) were selected for whole-genome sequencing from 11 households where multiple household members were positive for the carriage of at least one ESBL- or ACBL-producing *Enterobacteriaceae* isolate. Extraction of genomic DNA was conducted using a QIAamp DNA minikit (Qiagen, Hilden, Germany), and libraries were prepared using a Nextera XT DNA library preparation kit (Illumina Inc., San Diego, CA, USA). Sequencing was performed using Illumina MiSeq 2×250 -bp paired-end reads (by the Massey Genome Service, Massey University, Palmerston North, New Zealand) and Illumina HiSeq 2×125 -bp paired-end reads (by the University of Otago Genetic Analysis Service, Dunedin, New Zealand). Raw sequence reads were assessed for quality and processed (using the QCtool Solexa++, PhiX adapter removal [55]), prior to assembly and analysis using the Nullarbor bioinformatics pipeline in the “accurate” mode (56). Assembly of genomes in this pipeline was performed using the SPAdes assembler (v.3.0) (57), while single nucleotide polymorphism (SNP) analysis was executed by the use of the Snippy program (v.3.0) (58). All assembled genomes were checked for quality of assembly using the outputs from Nullarbor (v.1.25) and Quast (v.4.5) software (59). These assembled genomes were uploaded to the Center for Genomic Epidemiology pipeline (<http://www.genomicepidemiology.org>), from which the plasmid type (determined using the PlasmidFinder database [v.2.0]) (60), resistance genes (determined using the ResFinder database [v.3.1]) (61), and virulence genes (determined using the VirulenceFinder database [v.2.0, 23 April 2019]) (62) were identified. Virulence genes were also identified using the ABRicate program (v.0.8.13) with the VirulenceFinder database (23 April 2019) (62).

Initially, SNP analysis of the *E. coli* isolates included in this study was performed using the genome of an ESBL-producing *E. coli* strain (strain JJ1886) originating from a urinary tract infection as the reference genome (63). Individual SNP alignments were subsequently repeated for households with internal references. Whole-genome multilocus sequence typing (wgMLST) was executed using Fast-GeP (v.1.0) gene prediction and comparison software (64). A Nexus tree output was constructed using the SplitsTree program (v.4.14.8) and then transformed in a neighbor-joining Newick tree (65, 66) for upload to the EvolView web server for annotation and presentation (67).

Data availability. The sequence reads generated from this study have been deposited in the NCBI Sequence Read Archive under BioProject accession number [PRJNA600954](https://www.ncbi.nlm.nih.gov/bioproject/PRJNA600954).

SUPPLEMENTAL MATERIAL

Supplemental material is available online only.

SUPPLEMENTAL FILE 1, PDF file, 0.9 MB.

ACKNOWLEDGMENTS

This work was supported by the Health Research Council, New Zealand (grant number 14/331 to N.P.F.).

Thanks go to Jacinda Aplin, Harry Cookson, and Rukhshana Akhter for their contributions in support of this research, as well as the Massey Genome Service and the Otago Genomics Facility for whole-genome sequencing services. Thanks go to the Noun Project for the use of icons created by Gerald Wildmoser, Sewon Park, TripleArt, and Daniel Schroeder.

REFERENCES

1. Edberg SC, Rice EW, Karlin RJ, Allen MJ. 2000. *Escherichia coli*: the best biological drinking water indicator for public health protection. *J Appl Microbiol* 88:106S–116S. <https://doi.org/10.1111/j.1365-2672.2000.tb05338.x>.
2. Schito GC, Naber KG, Botto H, Palou J, Mazzei T, Gualco L, Marchese A. 2009. The ARESC study: an international survey on the antimicrobial resistance of pathogens involved in uncomplicated urinary tract infec-

- tions. *Int J Antimicrob Agents* 34:407–413. <https://doi.org/10.1016/j.ijantimicag.2009.04.012>.
3. Toombs-Ruane LJ, Benschop J, Burgess S, Priest P, Murdoch DR, French NP. 2017. Multidrug resistant Enterobacteriaceae in New Zealand: a current perspective. *N Z Vet J* 65:62–70. <https://doi.org/10.1080/00480169.2016.1269621>.
 4. Livermore DM, Canton R, Gniadkowski M, Nordmann P, Rossolini GM, Arlet G, Ayala J, Coque TM, Kern-Zdanowicz I, Luzzaro F, Poirel L, Woodford N. 2007. CTX-M: changing the face of ESBLs in Europe. *J Antimicrob Chemother* 59:165–174. <https://doi.org/10.1093/jac/dkl483>.
 5. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, Harbarth S, Hindler JF, Kahlmeter G, Olsson-Liljequist B, Paterson DL, Rice LB, Stelling J, Struelens MJ, Vatopoulos A, Weber JT, Monnet DL. 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. *Clin Microbiol Infect* 18:268–281. <https://doi.org/10.1111/j.1469-0691.2011.03570.x>.
 6. Heffernan HM, Woodhouse RE, Pope CE, Blackmore TK. 2009. Prevalence and types of extended-spectrum β -lactamases among urinary *Escherichia coli* and *Klebsiella* spp. in New Zealand. *Int J Antimicrob Agents* 34:544–549. <https://doi.org/10.1016/j.ijantimicag.2009.07.014>.
 7. Heffernan H, Woodhouse R, Draper J, Ren X. 2016. Survey of extended-spectrum beta-lactamase-producing Enterobacteriaceae, p 1–17. Antimicrobial Reference Laboratory and Health Group, Institute of Environmental Science and Research Limited, Wellington, New Zealand.
 8. Karkaba A, Grinberg A, Benschop J, Pleydell E. 2017. Characterisation of extended-spectrum β -lactamase and AmpC β -lactamase-producing Enterobacteriaceae isolated from companion animals in New Zealand. *N Z Vet J* 65:105–112. <https://doi.org/10.1080/00480169.2016.1271730>.
 9. Drinkovic D, Morris AJ, Dyet K, Bakker S, Heffernan H. 2015. Plasmid-mediated AmpC beta-lactamase-producing *Escherichia coli* causing urinary tract infection in the Auckland community likely to be resistant to commonly prescribed antimicrobials. *N Z Med J* 128:50–59.
 10. Dickson A, Smith M, Smith F, Park J, King C, Currie K, Langdridge D, Davis M, Flowers P. 2019. Understanding the relationship between pet owners and their companion animals as a key context for antimicrobial resistance-related behaviours: an interpretative phenomenological analysis. *Health Psychol Behav Med* 7:45–61. <https://doi.org/10.1080/21642850.2019.1577738>.
 11. Anonymous. 2016. Companion animals in New Zealand 2016. Cox Inall Communications Pty Ltd, Auckland, New Zealand.
 12. Marques C, Belas A, Aboim C, Cavaco-Silva P, Trigueiro G, Gama LT, Pomba C. 2019. Evidence of sharing of *Klebsiella pneumoniae* strains between healthy companion animals and cohabiting humans. *J Clin Microbiol* 57:e01537-18. <https://doi.org/10.1128/JCM.01537-18>.
 13. Ljungquist O, Ljungquist D, Myrenäs M, Rydén C, Finn M, Bengtsson B. 2016. Evidence of household transfer of ESBL-/pAmpC-producing Enterobacteriaceae between humans and dogs—a pilot study. *Infect Ecol Epidemiol* 6:31514. <https://doi.org/10.3402/iee.v6.31514>.
 14. Marques C, Menezes J, Belas A, Aboim C, Cavaco-Silva P, Trigueiro G, Gama LT, Pomba C. 2019. *Klebsiella pneumoniae* causing urinary tract infections in companion animals and humans: population structure, antimicrobial resistance and virulence genes. *J Antimicrob Chemother* 74:594–602. <https://doi.org/10.1093/jac/dky499>.
 15. Johnson JR, Clabots C, Kuskowski MA. 2008. Multiple-host sharing, long-term persistence, and virulence of *Escherichia coli* clones from human and animal household members. *J Clin Microbiol* 46:4078–4082. <https://doi.org/10.1128/JCM.00980-08>.
 16. Bourne JA, Chong WL, Gordon DM. 2019. Genetic structure, antimicrobial resistance and frequency of human associated *Escherichia coli* sequence types among faecal isolates from healthy dogs and cats living in Canberra, Australia. *PLoS One* 14:e0212867. <https://doi.org/10.1371/journal.pone.0212867>.
 17. Van Balen JC, Landers T, Nutt E, Dent A, Hoet AE. 2017. Molecular epidemiological analysis to assess the influence of pet-ownership in the biodiversity of *Staphylococcus aureus* and MRSA in dog- and non-dog-owning healthy households. *Epidemiol Infect* 145:1135–1147. <https://doi.org/10.1017/S0950268816003228>.
 18. Walther B, Hermes J, Cuny C, Wieler LH, Vincze S, Abou Elnaga Y, Stamm I, Kopp PA, Kohn B, Witte W, Jansen A, Conraths FJ, Semmler T, Eckmanns T, Lübke-Becker A. 2012. Sharing more than friendship—nasal colonization with coagulase-positive staphylococci (CPS) and cohabitation aspects of dogs and their owners. *PLoS One* 7:e35197. <https://doi.org/10.1371/journal.pone.0035197>.
 19. Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, Low DE. 2006. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in the veterinary clinic and in the household. *Vet Microbiol* 115:148–155. <https://doi.org/10.1016/j.vetmic.2006.01.004>.
 20. Van Duijkeren E, Wolfhagen MJHM, Box ATA, Heck MEOC, Wannet WJB, Fluit AC. 2004. Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis* 10:2235–2237. <https://doi.org/10.3201/eid1012.040387>.
 21. Shaffer M, Lozupone C. 2018. Prevalence and source of fecal and oral bacteria on infant, child, and adult hands. *mSystems* 3:e00192-17. <https://doi.org/10.1128/mSystems.00192-17>.
 22. Löhr IH, Rettedal S, Natås OB, Naseer U, Øymar K, Sundsfjord A. 2013. Long-term faecal carriage in infants and intra-household transmission of CTX-M-15-producing *Klebsiella pneumoniae* following a nosocomial outbreak. *J Antimicrob Chemother* 68:1043–1048. <https://doi.org/10.1093/jac/dks502>.
 23. Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJV. 2016. Understanding the mechanisms and drivers of antimicrobial resistance. *Lancet* 387:176–187. [https://doi.org/10.1016/S0140-6736\(15\)00473-0](https://doi.org/10.1016/S0140-6736(15)00473-0).
 24. Rogers BA, Ingram PR, Runnegar N, Pitman MC, Freeman JT, Athan E, Havers SM, Sidjabat HE, Jones M, Gunning E, De Almeida M, Styles K, Paterson DL, Australasian Society for Infectious Diseases Clinical Research Network. 2014. Community-onset *Escherichia coli* infection resistant to expanded-spectrum cephalosporins in low-prevalence countries. *Antimicrob Agents Chemother* 58:2126–2134. <https://doi.org/10.1128/AAC.02052-13>.
 25. Miller LG, Eells SJ, Taylor AR, David MZ, Ortiz N, Zychowski D, Kumar N, Cruz D, Boyle-Vavra S, Daum RS. 2012. *Staphylococcus aureus* colonization among household contacts of patients with skin infections: risk factors, strain discordance, and complex ecology. *Clin Infect Dis* 54:1523–1535. <https://doi.org/10.1093/cid/cis213>.
 26. Knox J, Uhlemann A-C, Lowy FD. 2015. *Staphylococcus aureus* infections: transmission within households and the community. *Trends Microbiol* 23:437–444. <https://doi.org/10.1016/j.tim.2015.03.007>.
 27. Johnson JR, Davis G, Clabots C, Johnston BD, Porter S, DebRoy C, Pomputius W, Ender PT, Cooperstock M, Slater BS, Banerjee R, Miller S, Kisiela D, Sokurenko EV, Aziz M, Price LB. 2016. Household clustering of *Escherichia coli* sequence type 131 clinical and fecal isolates according to whole genome sequence analysis. *Open Forum Infect Dis* 3:ofw129. <https://doi.org/10.1093/ofid/ofw129>.
 28. Ulleryd P, Sandberg T, Scheutz F, Clabots C, Johnston BD, Thuras P, Johnson JR. 2015. Colonization with *Escherichia coli* strains among female sex partners of men with febrile urinary tract infection. *J Clin Microbiol* 53:1947–1950. <https://doi.org/10.1128/JCM.00579-15>.
 29. Baede VO, Broens EM, Spaninks MP, Timmerman AJ, Graveland H, Wagenaar JA, Duim B, Hordijk J. 2017. Raw pet food as a risk factor for shedding of extended-spectrum beta-lactamase-producing Enterobacteriaceae in household cats. *PLoS One* 12:e0187239. <https://doi.org/10.1371/journal.pone.0187239>.
 30. Karkaba A, Hill K, Benschop J, Pleydell E, Grinberg A. 2019. Carriage and population genetics of extended spectrum beta-lactamase-producing *Escherichia coli* in cats and dogs in New Zealand. *Vet Microbiol* 233:61–67. <https://doi.org/10.1016/j.vetmic.2019.04.015>.
 31. Johnson JR, Johnston BD, Porter S, Thuras P, Aziz M, Price LB. 2018. Accessory traits and phylogenetic background predict *Escherichia coli* extraintestinal virulence better than does ecological source. *J Infect Dis* 219:121–132. <https://doi.org/10.1093/infdis/jiy459>.
 32. Martischang R, Riccio ME, Abbas M, Stewardson AJ, Kluytmans J, Harbarth S. 2020. Household carriage and acquisition of extended-spectrum beta-lactamase-producing Enterobacteriaceae: a systematic review. *Infect Control Hosp Epidemiol* 41:286–294. <https://doi.org/10.1017/ice.2019.336>.
 33. Kaspar U, von Lutzau A, Schlattmann A, Roesler U, Kock R, Becker K. 2018. Zoonotic multidrug-resistant microorganisms among small companion animals in Germany. *PLoS One* 13:e0208364. <https://doi.org/10.1371/journal.pone.0208364>.
 34. Rubin JE, Pitout JDD. 2014. Extended-spectrum beta-lactamase, carbapenemase and AmpC producing Enterobacteriaceae in companion animals. *Vet Microbiol* 170:10–18. <https://doi.org/10.1016/j.vetmic.2014.01.017>.
 35. van den Bunt G, Fluit AC, Spaninks MP, Timmerman AJ, Geurts Y, Kant A, Scharringa J, Mevius D, Wagenaar JA, Bonten MJM, van Pelt W, Hordijk

- J. 2020. Faecal carriage, risk factors, acquisition and persistence of ESBL-producing Enterobacteriaceae in dogs and cats and co-carriage with humans belonging to the same household. *J Antimicrob Chemother* 75:342–350. <https://doi.org/10.1093/jac/dkz462>.
36. Woerther P-L, Burdet C, Chachaty E, Andremont A. 2013. Trends in human fecal carriage of extended-spectrum β -lactamases in the community: toward the globalization of CTX-M. *Clin Microbiol Rev* 26: 744–758. <https://doi.org/10.1128/CMR.00023-13>.
 37. Giannella M, Trecarichi EM, De Rosa FG, Del Bono V, Bassetti M, Lewis RE, Losito AR, Corcione S, Saffiotti C, Bartoletti M, Maiuro G, Cardellino CS, Tedeschi S, Cauda R, Viscoli C, Viale P, Tumbarello M. 2014. Risk factors for carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection among rectal carriers: a prospective observational multicentre study. *Clin Microbiol Infect* 20:1357–1362. <https://doi.org/10.1111/1469-0691.12747>.
 38. Karanika S, Karantanos T, Arvanitis M, Grigoros C, Mylonakis E. 2016. Fecal colonization with extended-spectrum beta-lactamase-producing Enterobacteriaceae and risk factors among healthy individuals: a systematic review and metaanalysis. *Clin Infect Dis* 63:310–318. <https://doi.org/10.1093/cid/ciw283>.
 39. Day MJ, Hopkins KL, Wareham DW, Toleman MA, Elviss N, Randall L, Teale C, Cleary P, Wiuff C, Doumith M, Ellington MJ, Woodford N, Livermore DM. 2019. Extended-spectrum beta-lactamase-producing *Escherichia coli* in human-derived and foodchain-derived samples from England, Wales, and Scotland: an epidemiological surveillance and typing study. *Lancet Infect Dis* 19:1325–1335. [https://doi.org/10.1016/S1473-3099\(19\)30273-7](https://doi.org/10.1016/S1473-3099(19)30273-7).
 40. Manges AR, Geum HM, Guo A, Edens TJ, Fibke CD, Pitout JDD. 2019. Global extraintestinal pathogenic *Escherichia coli* (ExPEC) lineages. *Clin Microbiol Rev* 32:e00135-18. <https://doi.org/10.1128/CMR.00135-18>.
 41. Salipante SJ, Roach DJ, Kitzman JO, Snyder MW, Stackhouse B, Butler-Wu SM, Lee C, Cookson BT, Shendure J. 2015. Large-scale genomic sequencing of extraintestinal pathogenic *Escherichia coli* strains. *Genome Res* 25:119–128. <https://doi.org/10.1101/gr.180190.114>.
 42. Borges CA, Beraldo LG, Maluta RP, Cardozo MV, Barboza KB, Guastalli EAL, Kariyawasam S, DebRoy C, Avila FA. 2017. Multidrug-resistant pathogenic *Escherichia coli* isolated from wild birds in a veterinary hospital. *Avian Pathol* 46:76–83. <https://doi.org/10.1080/03079457.2016.1209298>.
 43. Pietsch M, Irrgang A, Roschanski N, Michael GB, Hamprecht A, Rieber H, Kasbohrer A, Schwarz S, Rosler U, Kreienbrock L, Pfeifer Y, Fuchs S, Werner G, RESET Study Group. 2018. Whole genome analyses of CMY-2-producing *Escherichia coli* isolates from humans, animals and food in Germany. *BMC Genomics* 19:601. <https://doi.org/10.1186/s12864-018-4976-3>.
 44. Ludden C, Raven KE, Jamroz D, Gouliouris T, Blane B, Coll F, de Goffau M, Naydenova P, Horner C, Hernandez-Garcia J, Wood P, Hadjirin N, Radakovic M, Brown NM, Holmes M, Parkhill J, Peacock SJ. 2019. One Health genomic surveillance of *Escherichia coli* demonstrates distinct lineages and mobile genetic elements in isolates from humans versus livestock. *mBio* 10:e02693-18. <https://doi.org/10.1128/mBio.02693-18>.
 45. Tande D, Boisrame-Gastrin S, Munck MR, Hery-Arnaud G, Gouriou S, Jallot N, Nordmann P, Naas T. 2010. Intrafamilial transmission of extended-spectrum-beta-lactamase-producing *Escherichia coli* and *Salmonella enterica* Babelsberg among the families of internationally adopted children. *J Antimicrob Chemother* 65:859–865. <https://doi.org/10.1093/jac/dkq068>.
 46. Heffernan H, Wong TL, Lindsay JA, Bowen B, Woodhouse R. 2011. A baseline survey of antimicrobial resistance in bacteria from selected New Zealand foods, 2009–2010. Antimicrobial Resistance Laboratory, ESR, Wellington, New Zealand.
 47. Warnes SL, Highmore CJ, Keevil CW. 2012. Horizontal transfer of antibiotic resistance genes on abiotic touch surfaces: implications for public health. *mBio* 3:e00489-12. <https://doi.org/10.1128/mBio.00489-12>.
 48. Nuesch-Inderbinen M, Treier A, Zurfluh K, Stephan R. 2019. Raw meat-based diets for companion animals: a potential source of transmission of pathogenic and antimicrobial-resistant Enterobacteriaceae. *R Soc Open Sci* 6:191170. <https://doi.org/10.1098/rsos.191170>.
 49. McMeekin CH, Hill KE, Gibson IR, Bridges JP, Benschop J. 2017. Antimicrobial resistance patterns of bacteria isolated from canine urinary samples submitted to a New Zealand veterinary diagnostic laboratory between 2005–2012. *N Z Vet J* 65:99–104. <https://doi.org/10.1080/00480169.2016.1259594>.
 50. Heffernan H, Dyet K, Woodhouse R, Williamson D. 2013. Antimicrobial susceptibility and molecular epidemiology of extended-spectrum beta-lactamase producing Enterobacteriaceae in New Zealand, 2013, p 1–45. Antimicrobial Reference Laboratory and Health Group, Institute of Environmental Science and Research Limited, Wellington, New Zealand.
 51. Arcilla MS, van Hattem JM, Haverkate MR, Bootsma MCJ, van Genderen PJJ, Goorhuis A, Grobusch MP, Lashof AMO, Molhoek N, Schultsz C, Stobberingh EE, Verbrugh HA, de Jong MD, Melles DC, Penders J. 2017. Import and spread of extended-spectrum beta-lactamase-producing Enterobacteriaceae by international travellers (COMBAT study): a prospective, multicentre cohort study. *Lancet Infect Dis* 17:78–85. [https://doi.org/10.1016/S1473-3099\(16\)30319-X](https://doi.org/10.1016/S1473-3099(16)30319-X).
 52. EUCAST. 2018. Clinical breakpoints version 8.1. http://www.eucast.org/clinical_breakpoints/. Accessed June 2018.
 53. EUCAST. 2013. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. EUCAST, Basel, Switzerland.
 54. Bauer AW, Sherris JC, Turck M, Kirby WMM. 1966. Antibiotic susceptibility testing by a standardized single disk method. *Am J Clin Pathol* 45: 493–496. https://doi.org/10.1093/ajcp/45.4_ts.493.
 55. Truglio M. 2016. QCtool. <https://github.com/mtruglio/QCtool/>.
 56. Seemann T, Goncalves da Silva A, Bulach D, Schultz M, Kwong J, Howden BN. 2016. Nullarbor. <https://github.com/tseemann/nullarbor>.
 57. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Pribelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J Comput Biol* 19:455–477. <https://doi.org/10.1089/cmb.2012.0021>.
 58. Seemann T. 2015. Snippy: fast bacterial variant calling from NGS reads. <https://github.com/tseemann/snippy>.
 59. Gurevich A, Saveliev V, Vyahhi N, Tesler G. 2013. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29:1072–1075. <https://doi.org/10.1093/bioinformatics/btt086>.
 60. Carattoli A, Zankari E, Garcia-Fernandez A, Volby Larsen M, Lund O, Villa L, Aarestrup FM, Hasman H. 2014. PlasmidFinder and pMLST: in silico detection and typing of plasmids. *Antimicrob Agents Chemother* 58: 3895–3903. <https://doi.org/10.1128/aac.02412-14>.
 61. Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. 2012. Identification of acquired antimicrobial resistance genes. *J Antimicrob Chemother* 67:2640–2644. <https://doi.org/10.1093/jac/dks261>.
 62. Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. 2014. Real-time whole-genome sequencing for routine typing, surveillance, and outbreak detection of verotoxigenic *Escherichia coli*. *J Clin Microbiol* 52:1501–1510. <https://doi.org/10.1128/JCM.03617-13>.
 63. Andersen PS, Stegger M, Aziz M, Contente-Cuomo T, Gibbons HS, Keim P, Sokurenko EV, Johnson JR, Price LB. 2013. Complete genome sequence of the epidemic and highly virulent CTX-M-15-producing h30-rx subclone of *Escherichia coli* ST131. *Genome Announcements* 1:e00988-13. <https://doi.org/10.1128/genomeA.00988-13>.
 64. Zhang J, Xiong Y, Rogers L, Carter GP, French N. 2018. Genome-by-genome approach for fast bacterial genealogical relationship evaluation. *Bioinformatics* 34:3025–3027. <https://doi.org/10.1093/bioinformatics/bty195>.
 65. Huson DH. 1998. SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* 14:68–73. <https://doi.org/10.1093/bioinformatics/14.1.68>.
 66. Rambaut A. 2016. FigTree, a graphical viewer of phylogenetic trees. <http://tree.bio.ed.ac.uk/software/figtree>.
 67. He Z, Zhang H, Gao S, Lercher MJ, Chen W-H, Hu S. 2016. Evolvview v2: an online visualization and management tool for customized and annotated phylogenetic trees. *Nucleic Acids Res* 44:W236–W241. <https://doi.org/10.1093/nar/gkw370>.