Is the family pet a risk for multidrug resistant infections?

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ABSTRACT

Risk factors for community-acquired urinary tract infections (UTI) caused by extended-spectrum beta-lactamase-(ESBL) and AmpC beta-lactamase-(ACBL) producing Enterobacteriaceae were investigated in a prospective case-control study conducted between August 2015 and September 2017. Both cases and controls were from the Auckland and Northland regions of New Zealand. A telephone questionnaire was delivered to participants, and the results analysed for putative risk factors for human infections. Analysis was performed using regression models, including factors around pet ownership and any other animal contact. Faecal samples were submitted from some households; this included samples from both people and companion animals. Isolates collected from index case urine samples and ESBL- or ACBL-producing faecal samples were sequenced and subsequently analysed through a bioinformatics pipeline. Pet ownership was not found to be a risk for human ESBL- or AmpC-producing infections in this study. Another important finding of this research was that *E. coli* ST-131 was the most commonly found bacteria associated with the UTI from people recruited into the case-control study. The strains of this sequence type were likely to have entered New Zealand in multiple introductions over the last 20 years. Transmission of ESBL-/ACBL-producing *E. coli* was also suspected to have occurred within households where a person had been recently infected with the same bacteria (in the form of a UTI) caused by an ESBL-/ACBL-producing Enterobacteriaceae. The results of this study as a whole indicate that while pets may not be a major risk for acquisition of ESBL/ACBL-producing bacteria, they are likely to play a role in the transmission of bacteria within homes and the community, and therefore warrant attention in future work.

PRESENTATIONS & PUBLICATIONS

Oral presentation: "Exploring pet ownership as a risk factor for community-acquired extended spectrum β-lactamase and AmpC β-lactamase infection in humans", One Health Aotearoa Symposium: Wellington, New Zealand, December 2018

Poster presentation: "The 'resist-home': household transmission of antimicrobial resistant *E. coli*", One Health Aotearoa Symposium: Wellington, New Zealand, December 2018

Oral presentation: "Exploring pet ownership as a risk factor for ESBL infection in humans", ANZCVS Science Week: Gold Coast, Australia July 2018

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ABBREVIATIONS

ACBL: AmpC beta-lactamase(s)

ACBL-E: AmpC beta-lactamase-producing Enterobacteriaceae

AIC: Akaike information criterion

AmpC: Ampicillin hydrolysing beta-lactamase

AMR: Antimicrobial resistant / antimicrobial resistance

DANMAP: Danish Integrated AMR Monitoring and Research Program

DNA: Deoxyribonucleic acid

ESBL: Extended-spectrum beta-lactamase(s)

ESBL-E: Extended-spectrum beta-lactamase -producing Enterobacteriaceae

LASSO: Least absolute shrinkage and selection operator

MALDI-TOF MS: Matrix-assisted laser desorption/ionization time of flight mass spectrometry

MDR: Multidrug resistant / multidrug resistance

MELAA: Middle Eastern, Latin American, and African

MLST: Multi-locus sequence type

wgMLST: Whole genome multi-locus sequence type

rMLST: Ribosomal multi-locus sequence type

(P)AF: (Population) attributable fraction

PCR: Polymerase chain reaction

PFGE: Pulse-field gel electrophoresis

ROC: Receiver operating characteristic

SNP: Single nucleotide polymorphism

ST: Sequence type

UPEC: uropathogenic *E. coli*

UTI: urinary tract infection

WHO: World Health Organization

1 Introduction

1.1 Overview

The research presented in this thesis focusses on risks for bacterial infections caused by antimicrobial resistant Enterobacteriaceae in the New Zealand community. Some of these bacteria produce enzymes able to break down third and fourth generation cephalosporin antimicrobials, and these drugs are usually reserved for second-line treatment and considered critically important by the World Health Organisation (Anonymous 2014b; Weese *et al.* 2015). Community-acquired infections (i.e. those not acquired in healthcare) caused by multidrug resistant bacteria [e.g. extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae] worldwide are a public health concern, and in New Zealand these are predominantly urinary tract infections (Heffernan *et al.* 2018). Therefore, assessment of risk factors not associated with healthcare is important to reducing spread of these bacteria. Companion animals in the home are one such exposure that could play a role in community transmission of these bacteria.

The family pet plays a central role in the lives of many people. In New Zealand, over 60% of homes report having a companion animal, which is one of the highest rates in the world (Anonymous 2016a). Some antimicrobials used for treatment in pets are similar to those reserved for second-line therapies in people. For example, third generation cephalosporins (cefpodoxime and cefovecin) are available for use in companion animals and include a long-acting injectable formulation (Anonymous 2016b). Resistance to these antimicrobials has been reported to increase in tandem with their use in a population (Burow *et al.* 2014), and other studies have implicated pets in multidrug resistant bacterial

carriage or infection in people (Meyer *et al.* 2012; Johnson *et al.* 2016). Therefore, this is an area worth investigating further in this pet-loving country.

1.2 An introduction to antimicrobial resistance

Almost as soon as antimicrobials (i.e. antibiotics) were used to treat bacterial infections in people, instances of bacterial resistance to those antimicrobials were described (Abraham and Chain 1940; Barber 1947). Barber observed *Staphylococcus* resistance to penicillin from clinical samples in the mid-1940s, the same decade that penicillin became widely available (Barber 1947; Quinn 2013). Many therapeutic antimicrobials aimed at treating or preventing bacterial infection originated from antibacterial compounds produced by organisms such as *Penicillium* or *Streptomyces* to kill or inhibit bacterial growth. As such, antimicrobial resistance (AMR) developed in the absence of therapeutic antimicrobial use for millennia and has been observed in ancient soil samples and uninhabited parts of the world (D'Costa *et al.* 2011; Perron *et al.* 2015). Since the 1940s, AMR in bacterial pathogens has increased and this increase can be interpreted as a reflection upon the use of antimicrobials (MacFadden *et al.* 2018).

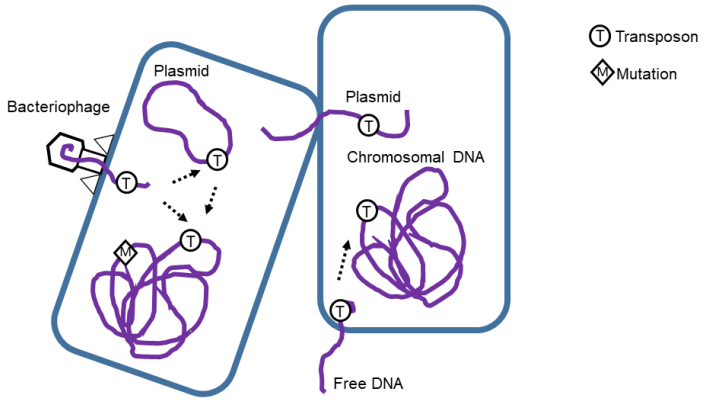

Figure 1.1 Acquisition of antimicrobial resistance in bacteria through mutation and horizontal transmission via bacteriophage, plasmid, and free DNA [adapted from Levy and Marshall (2004)]

The acquisition of antimicrobial resistance in bacteria occurs through two main mechanisms: (1) mutation in bacterial chromosomal DNA, and (2) through horizontal gene transfer. A summary of these mechanisms can be found in **Figure 1.1**. Genetic mutations change the efficacy of an antimicrobial in various ways, for example, a mutation may change the target site for the antimicrobial. Selection pressure in favour of these mutations occurs when bacterial population become exposed to antimicrobials, such as *gyrA/B* mutations subsequent to ciprofloxacin exposure (Martinez and Baquero 2000). Horizontal transfer of antimicrobial resistant genes between bacteria (as shown in **Figure 1.1**) can be through the incorporation of free DNA into a bacterial chromosome (transformation and recombination), bacteriophages (transduction and transposition), and plasmids (conjugation) (Alekshun and Levy 2007). The last of these, plasmids, are self-replicating units of DNA and may remain discrete in the bacterial cytoplasm or become incorporated into the chromosomal DNA through transposition and recombination (Alekshun and Levy 2007; Carattoli 2009). The rate of horizontal gene transmission can also be accelerated by external factors (such as exposure of bacteria to antimicrobials), and in these instances AMR plasmids may spread faster through populations and allow bacteria to survive future challenges (Beaber *et al.* 2004).

In **Table 1.1**, AMR mechanisms for different types of antimicrobial treatment for urinary tract infections are outlined. Importantly, many of the antimicrobial resistance mechanisms outlined in **Table 1.1** are mediated by plasmids, such as fluoroquinolone resistance (e.g. *qnr*), colistin resistance (e.g. *mcr*), resistance to third generation cephalosporins (bla_{ESBL}), and fosomycin resistance (e.g. *fosA).*

Table 1.1 Overview of antimicrobials used to treat urinary tract infections (UTI) and key resistance mechanisms

Antimicrobial Group	Examples for treatment of UTI	Key bacterial resistance mechanism(s) in Enterobacteriaceae	Reference
Beta-lactams:			
-Penicillins	Amoxicillin Ampicillin Mecillinam	Target site mutation, drug modification (ACBL and ESBL enzymes, other beta-lactamase enzymes)	(Alekshun and Levy 2007)
-Cephalosporins	Cephalexin Cefaclor Cefuroxime	Drug modification (ESBL enzymes)	(Iredell *et al.* 2016)
	Cefpodoxime	Drug modification (ESBL enzymes)	
-Cephamycins	Cefoxitin	Drug modification (ACBL enzymes)	
-Carbapenems	Meropenem Impipenem Ertapenem	Drug modification (Carbapenemases)	
Fluoroquinolones	Ciprofloxacin Norfloxacin	Target site mutation, efflux pumps, gyrase protection, drug acetylation	(Jacoby *et al.* 2014)
Aminoglycosides	Gentamicin Amikacin	Target site mutation, efflux pumps, reduced uptake, drug deactivation	(Garneau-Tsodikova and Labby 2016)
Polymixins	Colistin	Reduced drug affinity for outer membrane	(MacNair *et al.* 2018)
Others	Fosfomycin	Target site mutation, decreased transport, drug modification (FosA enzyme)	(Ito *et al.* 2017)
	Nitrofurantoin	Target site mutation, efflux pumps	(Sekyere 2018)
	Trimethoprim (+/- Sulphonamide)	Resistant target site	(Sköld 2001)

ACBL – AmpC beta-lactamase; ESBL – Extended spectrum beta-lactamase

The specific antimicrobial resistance mechanisms which are the focus of this thesis are enzymes that act by hydrolysing the beta-lactam ring, similar to the beta-lactamases described by Abraham and Chain (1940). Cephalosporins are a broad-spectrum antimicrobial class used to treat many types of infection in both humans and animals. They also share molecular heritage with penicillin as they too contain a beta-lactam ring. The enzymes that inactivate third and fourth generation cephalosporins are termed extended-spectrum beta-lactamase (ESBL) enzymes. Bacterial transmission and propagation of ESBL enzymes [along with other similar resistance enzymes such as AmpC beta-lactamase (ACBL) type enzymes] have been facilitated by horizontal gene transmission via plasmids (Carattoli 2009). The role these ESBL- and/or ACBL-producing Enterobacteriaceae play in human and animal health will be further discussed in **Chapter 2** and **3**.

1.3 Why is antimicrobial resistance important?

Antimicrobials revolutionized medicine in the 20th century, and allowed medical research to focus on non-infectious diseases (Quinn 2013). The importance of these advances to the quality and quantity of human life that resulted from the discovery of antimicrobials cannot be overstated. The corollary of this means that bacterial infection once again becomes a serious threat when antimicrobials no longer work. In the 2016 O'Neill report, a review on global AMR, it was estimated that the human cost of AMR would be 10 million lives per year by 2050, just over a century after antimicrobials came into general use (Anonymous 2016c). This death rate is attributable to both AMR in pathogenic bacteria, as well as the increased risk of routine procedures. It is therefore important to try to slow or reverse AMR in bacteria through changing use (and therefore selection pressure) or create a new paradigm of infectious disease prevention or

treatment. Part of this process is through understanding the role that different elements play in the propagation of AMR, hence the research question central to this dissertation: *is the family pet a risk for multidrug resistant infections?*

Since the early 2000's, ESBL enzymes have become increasingly associated with pathogenic bacteria such as extra-intestinal pathogenic *E. coli* (Carattoli 2009; Nicolas-Chanoine et al. 2014). In a 2001 review, it was estimated that 3% of Enterobacteriaceae causing clinical disease in the USA were resistant to third generation cephalosporins (Bradford 2001). A decade later, the Centres for Disease Control reported this to be 14% for *E. coli*, and 23% for *Klebsiella* spp. cultured from clinical infections (Anonymous 2013b). Carriage of ESBL-producing Enterobacteriaceae in the gastrointestinal tract of human populations has been reported at over 50% in some countries (Woerther et al. 2013). An increase in both treatment failure and cost will be inevitable if the trend towards increasing colonisation (and therefore increasing infection) of people with ESBL-producing bacteria continues (Smith and Coast 2013; Anonymous 2016c).

1.4 Antimicrobial resistance and animals

Overall, food-producing animals have been the primary focus of work done in AMR in animals (Chatterjee *et al.* 2018). Pig farmers who manage herds where ESBL-producing Enterobacteriaceae or methicillin resistant *Staphylococcus aureus* were detected are more likely to be carriers of these strains (Schmithausen et al. 2015; Nüesch-Inderbinen and Stephan 2016). Poultry workers have also been found to carry the multidrug resistant bacterial strains present in their flocks (Dorado-García et al. 2017). Likewise, when attributing relative risks of sources of AMR bacteria, production animals have been the focus of much research. Cattle in the United States have been found to carry bla_{ESBL} gene-containing *E. coli* (predominantly ST-10 and related STs), with ESBL genes similar to

those predominant in human samples (i.e. *bla*$_{\text{CTX-M-15}/14/27}$) (Afema et al. 2018). No such comparable study has been published from food animals in New Zealand.

Bacteria associated with human disease, and harbouring ACBL and/or ESBL resistance genes, continue to be cultured from companion animals around the world (**Table 1.2**). In the studies from Japan and Switzerland described in **Table 1.2**, ESBL-producing *E. coli* isolates from companion animals were human associated strains (e.g. STs 131, 38, 12, 410, or 1193). The use of antimicrobials (especially those usually reserved for second- or third-line treatment) in companion animals is something that should be carefully considered, as pets have been consistently found to carry bacteria associated with disease both in humans and in animals (Pomba et al. 2017). Increasing selection pressure in disease causing bacteria [e.g. uropathogenic *E. coli* (UPEC)] is undesirable from both human and animal perspectives. It should also be noted that companion animals have been found to share ESBL-producing *E. coli* with their human owners (Johnson et al. 2009; Johnson et al. 2016), and this will be explored in a New Zealand context further in **Chapter 4**.

Table 1.2 Contemporaneous research in antimicrobial resistance (AMR) in Enterobacteriaceae isolated from companion animals

Bacterial species	ESBL/AmpC enzyme type	Most common MLST type(s)	Country	Prevalence/presence	Reference
UPEC (*E. coli*)	CTX-M-15 (49% of ESBL-producers), CTX-M-1/55/14/27 also reported	ST-410 (then ST-131, ST-73, ST361)	Switzerland	ESBL-producing UTI from cats and dogs	(Zogg *et al.* 2018)
E. coli	CTX-M-27 (19% of ESBL/ACBL producers), CMY-2 (19% of ESBL/ACBL producers), CTX-M-15/14/55 also reported	ST-131 (then ST-1193, ST-38, ST-12, ST-372)	Japan	Clinical samples from cats and dogs	(Maeyama *et al.* 2018)
Klebsiella pneumoniae	CTX-M-15 (40% of ESBL-producers), CTX-M-2, CTX-M-14	ST-15 (then ST-655, ST-11, ST-147, ST-307, ST709)	Japan	Clinical samples from cats and dogs	(Maeyama *et al.* 2018)
E. coli	CTX-M group 1 type most common, CTX-M group 9 type and group 2 also detected	Not reported	United Kingdom	Faecal carriage of AMR *E. coli* in dogs pre- and post-antimicrobial treatment	(Schmidt *et al.* 2018)

ACBL – AmpC beta-lactamase; ESBL – Extended spectrum beta-lactamase; UPEC - uropathogenic *E. coli*; UTI – urinary tract infection

1.5 Antimicrobial use and resistance

As already alluded to in this chapter, there are many interconnected exposures and drivers to increase bacterial resistance to antimicrobials. At a basic level, exposure of bacteria to antimicrobials is a key driver; however, the environment and interactions surrounding this exposure can be complex (Holmes *et al.* 2016). Even in cases where antimicrobial use may play a role in the emergence of bacterial resistance, fitness of the bacteria is not necessarily compromised and there may be antimicrobial-independent reasons for spread of an AMR-containing bacteria (Baker *et al.* 2013).

Therapeutic antimicrobial use is a major driver of resistance in bacteria in human and animal populations, especially in pathogenic bacteria in those populations. Specific examples of this are seen when looking at surveillance data from both AMR and antimicrobial use in respective human and animal populations (Williamson *et al.* 2013; Andersen *et al.* 2015). Antimicrobial use in animals does not commonly spill over into AMR in disease causing bacteria in humans, however examples of this have occurred (Anonymous 2015). For example, the increase in vancomycin resistant *Enterococcus* in people has been widely attributed to the use of avoparcin as a growth promoter in food animals (Smith *et al.* 2002). Other instances of this include the recent emergence of colistin-resistant *E. coli* in extra-intestinal infections in people, subsequent to years of prophylactic polymyxin use in food animals (Liu *et al.* 2016). On the other hand, community use of antimicrobials in humans is a predictor for prevalence of bacteria AMR in a country (MacFadden *et al.* 2018). Although large scale studies in New Zealand have not examined the relationship between use of antimicrobials and resistance in *E. coli*, community-use of antimicrobials in New Zealand has been estimated to be more than 70%

of OECD countries, so this should be considered a putative risk for AMR here (Thomas *et al.* 2014; Williamson *et al.* 2016).

1.6 The role of genomics in understanding antimicrobial resistance

Molecular tools, whether specific genes or the whole of the bacterial DNA is used, are an important part of the science around AMR (McArthur and Wright 2015), and these are utilised throughout the analytic chapters in this thesis. New sequencing technologies have become part of clinical microbiologic workup as well as having utility in outbreaks and epidemiologic research (Bertelli and Greub 2013). Resistance gene characterisation using sequenced reads (or assembled genomes) can be done through extensive databases such as the Comprehensive Antibiotic Resistance Database (CARD) or ResFinder (Zankari et al. 2012; Jia et al. 2017).

Whole genome sequencing has also changed source attribution research, where nucleotide-level differences between isolates can highlight differences in bacteria that would not be evident otherwise. For example, identification of poultry associated *E. coli* ST-131 cultured from human UTI would not be discernible if only examining seven genes as in MLST (Liu et al. 2018). This is in contrast to low-resolution methods such as multiplex PCR, where only (pre-determined) primers matching genes will be detected in tested samples (Pérez-Pérez and Hanson 2002; Cattoir et al. 2007). Although they are not explored in the work presented in this thesis, PCR-based methods are still used for screening for known epidemic types of ESBL genes. For example, where an important characteristic is known (i.e. specific bla_{ESBL} genes from cattle isolates), it may be useful to select only isolates with that trait (Afema et al. 2018). There will continue to be many instances where this continues to be true in the broader field of molecular epidemiology.

When using outputs of high throughput platforms (such as Illumina HiSeq) careful quality control needs to be done to ensure the best inferences are made, and genomes will never be as reliably accurate as those from platforms where both sequence and structure of the genome are considered (Minoche et al. 2011; Caporaso et al. 2012; Bertelli and Greub 2013). Tools like bioinformatics pipelines have been developed to facilitate analysis subsequent to the recent boom in the accessibility of whole genome sequencing of bacteria. A bioinformatics pipeline is a standardised sequential way of taking raw genetic data and making it useful, usually by passing that data through a number of different tools in a stepwise manner. This involves processing inputs (e.g. raw sequenced reads) and returning assembled genomes; these genomes may also have genes annotated [e.g. using Prokka (Seemann 2014)] or identified by some other method like ResFinder and VirulenceFinder (Thomsen et al. 2016). The outputs of these pipelines may be used for further analyses. A nucleotide-based analysis is also useful, and may be incorporated into a bioinformatics pipeline, as it is when using Nullarbor (Seemann 2015; Seemann et al. 2016). Single nucleotide polymorphisms (SNP) are variations in the genomic sequence between an isolate (i.e. sequenced reads) and a reference genome (i.e. assembled and annotated), as used by Snippy (Seemann 2015). The abundance and variation of these tools is a reflection on the need to process the outputs of sequencing now commonplace in many studies (Levy and Myers 2016).

Inferences of transmission events between individuals may be inferred from SNP distances (Harris *et al.* 2010), or based upon gene similarity (Zhang *et al.* 2018), or both methods (Weterings *et al.* 2017). This approach appears to be generally supported, although within-host diversity and the effect of the transfer of heterogeneous bacteria in a transmission event means that this is often more complicated than early work suggested

(Worby *et al.* 2014). With this caveat, both SNP-based and gene-based tools are useful, however directionality and likely transmission pathways should be estimated with caution.

1.7 Thesis objectives

The research objectives of this thesis (outlined below) all aim at better understanding community-acquired ESBL-/ACBL-producing infections in New Zealand. While there has been research into this area from a surveillance perspective, this is still relatively unexplored in the New Zealand context (Heffernan *et al.* 2013; Rogers *et al.* 2014; Heffernan *et al.* 2018). The other unique part of this research stems from the central research question around the risks of companion animal contact. As previously mentioned in this introduction, there are many instances of human-associated strains of ESBL-producing *E. coli* cultured from both clinical and non-clinical samples from companion animals (**Table 1.2**). Other observational studies have looked at companion animal exposure as a risk for carriage or infection with ESBL-producing bacteria, however to my knowledge this is the first study to focus on these exposures (Rogers *et al.* 2014; Leonard *et al.* 2018). The genesis of this research was a grant awarded by the Health Research Council of New Zealand in 2014, to fund the question that also lends its name to the title of this thesis "is the family pet a risk for multidrug resistant infections in people?"

As part of understanding the bacteria cultured in this research, modern molecular techniques were used to describe and analyse the bacterial causes of these infections. The results presented in this thesis are the first (to the best of my knowledge) where the molecular relatedness of community-acquired ESBL-/ACBL- Enterobacteriaceae from the New Zealand community are presented in this way.

1.7.1 Research aims:

1. To identify knowledge gaps in AMR in Enterobacteriaceae in both New Zealand's human and animal populations (**Chapter 2**);
2. To describe the bacterial causes of human community-acquired ESBL- and ACBL-producing urinary tract infections in both the New Zealand and global contexts (**Chapter 3**, **Chapter 5**);
3. To assess risk factors for community-acquired ESBL- and ACBL-producing urinary tract infections in New Zealand, with particular reference to pet-related risks (**Chapter 3)**;
4. To explore transmission of ESBL- and ACBL-producing *E. coli* in New Zealand households (**Chapter 4**)

1.8 Thesis structure

Chapter 2, an invited literature review, is aimed at giving the reader an overview of AMR in New Zealand with a particular focus on Enterobacteriaceae such as *Escherichia coli* and *Klebsiella pneumoniae*. This review was published in the New Zealand Veterinary Journal in 2017, and as presented here remains largely unchanged from that published form. Parts of the introduction given in **Chapter 1** are aimed to provide contemporaneous global updates on the literature provided in **Chapter 2**. In **Chapter 3**, the prospective case control study that forms the backbone of this thesis is presented. **Chapter 4**, a cross-sectional study that was nested in the case control study, examines the potential transmission of AMR *E. coli* between members of several households. The final analytic chapter is **Chapter 5**, this chapter describes a group of *E. coli* isolates (ST-131) collected both globally and as part of **Chapter 3**. As indicated, **Chapters 2** to **5** are presented in publication style, with their own introductions and discussions. Inevitably, there will be some overlap of concepts as these chapters are intended to be understood as stand-alone pieces of work. The final part of this dissertation is a general discussion of the themes presented in the thesis as a whole.

2 Multidrug resistant Enterobacteriaceae in New Zealand: a current perspective

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2.1 Introduction

A medical revolution occurred over the last century with the discovery of antimicrobials. However, bacterial resistance to antimicrobials was evident shortly after each discovery, leading to newer and broader spectrum antimicrobials such as potentiated sulphonamides, cephalosporins and fluoroquinolones (Levy and Marshall 2004). Bacterial resistance, especially to commonly used antimicrobials, limits treatment options, which may prolong the severity or length of disease (Weese *et al.* 2015). In addition, resistance to some antimicrobials, defined as critically important by the World Health Organisation (WHO), is concerning in both human and veterinary medicine because these antimicrobials are often last-line therapies and are important in increasing bacterial resistance (Weese *et al.* 2015). Understanding of the mechanisms of bacterial resistance has improved over the last two decades, and molecular investigation of specific mechanisms of resistance has emerged as the predominant tool in understanding antimicrobial resistance (AMR) (Prescott 2014).

Enterobacteriaceae, the group of gram-negative bacteria that include *Escherichia coli* and *Klebsiella pneumoniae*, are omnipresent and form part of the normal flora of the mammalian gastrointestinal tract, and are transmitted between humans and animals

(Ewers *et al.* 2012; Liebana *et al.* 2013). Human and animal gastrointestinal tracts have been identified as prime potential locations for horizontal gene transfer, including the transfer of resistance genes to other pathogens (Broaders *et al.* 2013; Holmes *et al.* 2016).

Most strains of *E. coli* and *K. pneumoniae* are able to be carried as commensal organisms and they have the potential to become opportunistic pathogens. In humans, both these species can also be a cause of urinary tract infections, septicaemia, and pneumonia (Iredell *et al.* 2016). It is the development of multidrug resistance in pathogenic strains of Enterobacteriaceae that is of particular concern with respect to human health (Anonymous 2014b). Infections caused by these strains are often harder to treat, resulting in increased severity and duration of infection (Iredell *et al.* 2016). Globally, some strains of Enterobacteriaceae, which produce extended spectrum beta-lactamases (ESBL), are recognised as playing a substantial role in the treatment failure of many infections in people, including infections of the bloodstream, urogenital tract and abdomen (Anonymous 2014a). In addition to ESBL-production, these strains are often associated with other mechanisms of resistance, causing them to be multidrug resistant (MDR). In New Zealand, the incidence rate of infections in humans associated with Enterobacteriaceae producing ESBL in 2014 was 95.5 per 100,000 people with 176/359(49%) infections being community related (Dyet *et al.* 2014). From 2006 to 2009 there was a four-fold increase in human infections caused by ESBL-producing bacteria (Dyet *et al.* 2014). The animal burden of MDR bacterial disease, particularly that caused by ESBL-producing bacteria, is likely to be much less. However, there is insufficient research in New Zealand to accurately assess that burden; although we know that ESBL-producing Enterobacteriaceae have been isolated from some clinical infections in companion animals (Karkaba *et al.* 2017b).

This review will focus on the mechanisms and potential transmission pathways of multidrug resistance in Enterobacteriaceae, with an emphasis on ESBL production. We aim to illustrate some of the broader challenges associated with AMR, including how the development of AMR in animals, humans and the environment is interlinked. Due to the interconnectedness of the health of animals, humans and the environment, we propose a One-Health approach for controlling AMR with emphasis on antimicrobial stewardship, in addition to the continued surveillance and study of transmission dynamics.

2.2 Antimicrobial resistance mechanisms

The development of AMR can occur via two processes: mutations in certain genes or by the horizontal transfer of genes encoding resistant mechanisms. These resistant genes are often found on genetic mobile elements or on plasmids, which by their nature are able to be transferred between bacteria without being the result of cell division, i.e. they can be horizontally transmitted between bacteria (Broaders *et al.* 2013). They can therefore contribute to the spread of AMR between bacterial species as well as to other members of the same species (Mathers *et al.* 2015). The control of plasmid-mediated AMR is therefore of particular importance in the effort to reduce the prevalence of AMR and the emergence of new, resistant strains.

Antimicrobial resistance in the Enterobacteriaceae family includes resistance to three important classes of antimicrobials: beta-lactams, aminoglycosides and fluoroquinolones. Resistance to the beta-lactam class of antimicrobials, e.g. penicillins and cephalosporins, is mainly driven by the production of beta-lactamase enzymes, which hydrolyse the beta-lactam ring, rendering the antimicrobial ineffective (Pitout and Laupland 2008). The main focus of this review is on the production of ESBL. However, bacteria that produce ESBL enzymes are often resistant to other classes of antimicrobials, making them MDR

(Mathers *et al.* 2015); some of these other mechanisms of resistance will also be described and are summarised in **Table 2.1**.

2.2.1 Extended-spectrum beta-lactamases

The ESBL produced by Enterobacteriaceae are generally defined as those beta-lactamases which confer resistance to third generation cephalosporins, thus resulting in a wider resistance range compared with the penicillinases (the first group of beta-lactamase enzymes to be described), but these bacteria have no carbapenemase activity (Livermore 2008). There are different types of ESBL, for example TEM, SHV, CTX, which are based on the DNA sequence of the gene encoding for these enzymes. The enzyme types are further classified into variants, e.g. CTX-M-15.

The ESBL-coding genes are generally found on plasmids and they emerged from two different evolutionary pathways. The first ESBL types to be described evolved from the mutations in the parent TEM and SHV variants resulting in new TEM and SHV enzymes with a broader resistance spectrum (Pitout and Laupland 2008). More recently, the CTX-M type emerged as a result of horizontal gene transfer, most probably from the chromosome of *Kluyvera* species to become plasmid-borne (Poirel *et al.* 2002; Rodríguez *et al.* 2004). The CTX-M type, especially CTX-M-15, is now the dominant ESBL type globally and has spread rapidly by plasmids in *E. coli* (Woerther *et al.* 2013).

2.2.2 AmpC beta-lactamases

Genes encoding for ampicillin hydrolysing beta-lactamase (AmpC) enzymes can be found on both the bacterial chromosome and on plasmids. In *E. coli* and *Klebsiella* species the genes for AmpC enzymes are generally plasmid encoded and their acquisition is a more recent event compared with the chromosomally encoded AmpC in other members of the

Enterobacteriaceae family such as *Enterobacter*, *Citrobacter* and *Serratia* species (Carattoli 2009). AmpC production is of clinical importance as a mutant-type may develop in-vivo that produces substantially more AmpC compared with the wild-type (Jacoby 2009). AmpC producers (like ESBL-producers) are often also resistant to other classes of antimicrobials (Carattoli 2009; Hasman *et al.* 2015); both of these mechanisms can result in an infection that can be more difficult to treat. Chromosomally encoded AmpC are induced by some beta-lactam antimicrobials, including the beta-lactamase inhibitor clavulanic acid. As a result, AmpC-producers are not inhibited by clavulanic acid. Susceptibility to clavulanic acid is often used for the identification of an ESBL-producing isolate; therefore, the presence of an inducible AmpC encoding gene can mask the ability to detect whether a strain is also an ESBL-producer in a diagnostic laboratory (Paterson and Bonomo 2005). It is important to differentiate ESBL and AmpC-producers from both an epidemiological perspective, i.e. for determining the origin of the strain and its antimicrobial resistant genes, as well as from a clinical perspective (Drinkovic *et al.* 2015).

2.2.3 Carbapenemases

Carbapenem antimicrobials (e.g. imipenem) have classically been reserved as the beta-lactam therapy of last resort, and resistance by Enterobacteriaceae to these drugs is a substantial public health concern (Heffernan *et al.* 2014; Mathers *et al.* 2015). Carbapenemase-producing Enterobacteriaceae can be grouped into three categories: the serine carbapenemases (e.g. the KPC type from *K. pneumoniae*), the metallo-beta-lactamases, and the OXA type carbapenemases. Of clinical concern are those carbapenemase-producing Enterobacteriaceae that have a minimum inhibitory concentration above the resistance breakpoint, referred to as the carbapenem resistant Enterobacteriaceae. The most recent example of a new carbapenemase sub-type is the

NDM-1 metallo-beta-lactamase, which emerged in 2009 in New Delhi, India (Martirosov and Lodise 2015).

2.2.4 Other mechanisms of resistance

An emergent form of resistance in Enterobacteriaceae is plasmid-mediated colistin resistance (*mcr-1*), which was first described in 2015 (Liu *et al.* 2016). Colistin is a polymyxin and until the identification of this plasmid, polymyxin resistance was only associated with chromosomal mutations (Liu *et al.* 2016). Plasmid-mediated colistin resistance was first described in China as a result of surveillance reports showing an increase in colistin resistance in food producing animals, particularly pigs (Liu *et al.* 2016). Plasmid-mediated colistin resistance has now been found in other Asian countries (Kawanishi *et al.* 2017), Europe (Hasman *et al.* 2015), Northern America (McGann *et al.* 2016), Southern America (Fernandes *et al.* 2016), and Africa (Poirel *et al.* 2016). To my knowledge, *mcr-1* has not been identified in New Zealand. The spread of colistin resistance is of clinical concern as, like carbapenem, colistin is an antimicrobial of last resort and used for the treatment of carbapenem resistant Enterobacterial infections.

Fluoroquinolone resistance was reported in >50% of clinical isolates of *E. coli* in one study in the United States of America (Rattanaumpawan *et al.* 2015). In New Zealand, the prevalence appears to be lower, with approximately 10% of clinical human isolates being reported to be resistant to fluoroquinolones in 2014 (Anonymous 2014a). Fluoroquinolone antimicrobials target the enzymes DNA gyrase and topoisomerase IV. Initially fluoroquinolone resistance was associated with mutations in the genes encoding these enzymes. Subsequently plasmid-mediated resistance also developed, with the emergence of a plasmid encoding a gene for QnrA; this protein prevents fluoroquinolones from binding to DNA gyrase (Jacoby 2005).

Table 2.1 Beta-lactamase enzymes involved in antimicrobial resistance of Enterobacteriaceae [adapted from Rubin and Pitout (2014)]

Enzyme	Enzyme type	Antimicrobial resistance spectrum	Inhibitors
Extended spectrum beta-lactamase	CTX-M, TEM (other than parent type TEM–1, 2, and 13), SHV (other than parent type SHV-1)	Penicillins, cephalosporins, monobactams	Clavulanic acid, tazobactam, sulbactam, avibactam
Penicillinase	TEM-1,2, 13, SHV-1	Penicillins and first generation cephalosporins	As above, predominantly clavulanic acid, sulbactam
AmpC beta-lactamase	AmpC, CMY-2	Penicillins, cephalosporins, monobactams, cephamycins	Avibactam, Boronic acids
Carbapenemase	Serine carbapenemase (KPC, GES, SME1)	Carbapenems, penicillins, cephalosporins, aztreonam	Taxobactram boronic acids, avibactam, clavulanic acid (minor inhibition)
	Metallo-carbapenemase (IMP, NDM, VIM, IND)	All beta-lactams except aztreonam	Metal chelators e.g. EDTA, mercaptopurine, dipicolinic acid,
	OXA	Carbapenems, penicillins, cephalosporins, aztreonam	Avibactam, NaCl

2.3 Occurrence and prevalence of ESBL enzymes

The prevalence of human faecal carriage of Enterobacteriaceae that are MDR varies globally, as shown by a meta-analysis of 66 studies that included results from 28, 909 people in total; prevalence in the Americas was 2 (95% CI=2–5%), in Europe was 4 (95% CI=2–5)%, and in the Western Pacific was 46 (95% CI=29–6)% (Karanika *et al.* 2016). It should be noted that while New Zealand falls into the Western Pacific region, no prevalence studies from New Zealand or Australia were included in the meta-analysis.

At this time and to my knowledge, there have been no published prevalence studies on ESBL carriage in healthy communities of human or animal populations in New Zealand. A summary of reports of AMR in Enterobacteriaceae from various New Zealand sources is shown in **Table 2.2**. Amongst isolates obtained from human clinical urine samples in 2006, ESBL-producing Enterobacteriaceae, were found in 86/9,453 (0.9%), compared with 2/1760 (0.1%) isolates in 2000 (Heffernan *et al.* 2009). Infections caused by transmissible AmpC-producing bacteria have also been reported from human urine samples (Drinkovic *et al.* 2015) and in isolates from infection sites in companion animals (Karkaba *et al.* 2017b). Carbapenemase-producing bacterial infections have also reported from humans in this country (Heffernan *et al.* 2014). Isolates of *E. coli* and *Salmonella enterica,* cultured from New Zealand food sources in 2009–2010, showed low-to-no phenotypic resistance to antimicrobials associated with ESBL and AmpC-production (Heffernan *et al.* 2011). The presence and prevalence of resistance genes (notably ESBL) in bacteria from New Zealand pre-slaughter ruminant food animals have not yet been described.

Table 2.2 A summary of results of selected reports of antimicrobial resistant Enterobacteriaceae from New Zealand

Resistance	Bacterial species	Resistance quantification	Host species/ source	Type of sample	Year(s)	Reference
Ampicillin resistance	*E. coli*	9/296 (3%) isolates	Pigs	Prevalence study	2001	(Nulsen *et al.* 2008)
ESBL-production	*E. coli*	57/8,707 (0.7%) isolates	Human	Clinical (urine)	2006	(Heffernan *et al.* 2009)
ESBL-production	*K. pneumoniae*	31/746 (4.2%) isolates	Human	Clinical (urine)	2006	(Heffernan *et al.* 2009)
ESBL-production	*E. coli*	0/407 isolates	Poultry	Surveillance (post-slaughter)	2006	(Pleydell *et al.* 2010)
Cefoxitin resistance	*E. coli*	9/999 (1%; min 0.3, max 2.2%) isolates	Pig, poultry, calves, produce	Surveillance (food products)	2009–2010	(Heffernan *et al.* 2011)
Carbapenem-resistance	Enterobacteriaceae	35 isolates	Human	Clinical (multiple)	2009–2014	(Heffernan *et al.* 2014)
Plasmid-mediated AmpC-production	*E. coli*	101/26,007 (0.4%) isolates	Human	Clinical (urine)	2011	(Drinkovic *et al.* 2015)
Cephalothin resistance	*E. coli*	467/1,107 (42%) isolates	Dogs, cats	Clinical (urine)	2005–2012	(McMeekin *et al.* 2017)
Ceftiofur resistance	*E. coli*	13/24 (54%) isolates	Horses	Clinical	2004–2014	(Toombs-Ruane *et al.* 2015)
ESBL and/or AmpC-production	Enterobacteriaceae resistant to amoxicillin-clavulanic acid	58/95 (61%) isolates	Companion Animals	Clinical (multiple)	2012–2013	(Karkaba *et al.* 2017b)

AmpC=AmpC beta-lactamase; ESBL=extended-spectrum beta-lactamase

When ESBL-producing Enterobacteriaceae were typed using PCR-based genotyping, two types predominated in human urine samples collected in 2006. In ESBL-producing *E. coli* and *K. pneumoniae* the most prevalent type was CTX-M-15 (63/83 (78%) isolates), followed by CTX-M-14 (11/83 (14%) isolates (Heffernan *et al.* 2009). A subsequent study in 2013, found 342/352 (97%) human ESBL isolates were CTX-M types (Heffernan *et al.* 2013). Enterobacteriaceae are also described by multilocus sequence type (ST), and in the 2013 national survey, 122/224 (54.5%) ESBL-producing *E. coli* isolates were ST-131 (Heffernan *et al.* 2013); this sequence type is internationally associated with ESBL-producing *E. coli* (Mathers *et al.* 2015). In ESBL and AmpC-producing Enterobacteriaceae from clinical animal samples from New Zealand, 6/89 *E. coli* isolates were ST-131 (Karkaba *et al.* 2017b).

2.4 Reservoirs and transmission pathways

2.4.1 Environmental transfer

The natural environment has been identified as a potential reservoir of AMR bacteria, including ESBL and AmpC-producing strains (Huijbers *et al.* 2015). To my knowledge, there are no studies on the prevalence of MDR Enterobacteriaceae strains from different New Zealand environments. ESBL-producing Enterobacteriaceae have been detected in a range of environmental samples elsewhere, including from soil, rivers and lakes in Tunisia (Ben Said *et al.* 2016); from coastal waters in Croatia (Maravić *et al.* 2015); from Banani lake in Bangladesh (Haque *et al.* 2014); and from wild birds in Canada (Parker *et al.* 2016). All of these are potential sources of transmission to humans (Huijbers *et al.* 2015). The presence of AMR bacteria in the natural environment can be the result of transfer from human and animal sources, or they may be intrinsically present (Allen *et al.* 2010).

One of the most important routes for the transfer of ESBL-producing Enterobacteriaceae into the natural environment from human sources is through the discharge of waste water, particularly into waterways (Huijbers *et al.* 2015). Overseas, ESBL-producing Enterobacteriaceae have been isolated from hospital wastewater, treated sewage and storm water. Studies carried out in a number of countries [e.g. Tunisia (Ben Said *et al.* 2016), Poland (Korzeniewska and Harnisz 2013) and Brazil (Conte *et al.* 2017)] show that the prevalence of ESBL-producing Enterobacteriaceae in wastewater varies, but as a result of different methods being used it is difficult to compare between studies. However, there is general agreement that routine waste-water treatment processes do not effectively remove ESBL-producing Enterobacteriaceae; for example, in a study carried out in Brazil it was found that both antimicrobial residues and AMR bacteria were persisting after treatment (Conte *et al.* 2017). In New Zealand, treated sewage is generally discharged into a nearby waterway. Although, counts of *E. coli* are required to be below a specified level in compliance with regional council consenting processes, it is unlikely that current New Zealand sewage treatment practices remove all AMR bacteria (Yuan *et al.* 2015; Hocquet *et al.* 2016).

Animal sources may also play an important role in the transfer of AMR to the natural environment. For example, from manure application onto soil and produce, farm run-off into waterways and milk containing antimicrobials. AMR may develop in the microbial community in the environment as a result of antimicrobials still present in these animal sources or from the transfer of AMR bacteria and their genes (Huijbers *et al.* 2015).

Thus, the environment has been identified as an important reservoir of AMR but there is still little known about the role the environment plays in the transmission of AMR genes found in the environment to animals and humans (Allen *et al.* 2010). The natural

environment can contain AMR genes with seemingly little or no selection pressure from anthropogenic sources (Segawa *et al.* 2013). For example, AMR bacteria including Enterobacteriaceae-producing ESBL have been isolated from seemingly untouched environments such as in soil from the Arctic Fjords (Hatha *et al.* 2015). This is not surprising given most antimicrobials are produced innately by bacteria and fungi that reside in the natural environment. Many of the AMR genes identified from natural environmental sources remain largely uncharacterised and could potentially be a reservoir of novel resistance mechanisms (Hatosy and Martiny 2015; Fitzpatrick and Walsh 2016).

2.4.2 Human-animal transfer

The movement of AMR bacteria and/or their genes between humans, animals and their environment is a complex and interdependent system (Holmes *et al.* 2016). Evidence for transfer of ESBL-producing *E. coli* from food animals to people is varied and animal classes that routinely receive antimicrobials to prevent disease (pigs, poultry) are the most likely sources for food-borne animal-to-human transfer (Lazarus *et al.* 2015). Such transmission could occur through direct contact (e.g. pig farmers or abattoir workers), a contaminated environment, or through the food chain. Environmental sampling around pig farms and meat processing plants have detected ESBL-producing *E. coli*; CTX-M type ESBL-producing bacteria were cultured from air samples on six of the 35 farms in a German study, although no pig-to-human transmission was found on any of those farms (Schmithausen *et al.* 2015). Other studies of farmed pigs in Ireland and Denmark have identified that CTX-M-14 and CTX-M-1 were more likely to be present than CTX-M-15; these are the ESBL enzyme variants that predominates in human clinical cases (Hammerum *et al.* 2014; Wang *et al.* 2016). Poultry production is a possible source of AMR Enterobacteriaceae, and human-associated strains of ESBL-producing *E. coli* have

been found in commercial meat samples and poultry-associated strains have been found in human urinary tract infections in Europe following low-resolution genetic analysis (Leverstein-van Hall *et al.* 2011; van Hoek *et al.* 2016). However, evidence for the contribution of these animal sources of bacteria to human disease is inconclusive and the relative risk of different ESBL-producing bacteria is likely to depend upon whether the bacteria is a host-adapted strain, as carriage of human-adapted strains in animals may be transient (Lazarus *et al.* 2015; van Hoek *et al.* 2016).

Multidrug resistant Enterobacteriaceae isolated from some companion and food animals have been shown to share the same sequence types as those isolated from human infections (Ewers *et al.* 2012; Woodford *et al.* 2014). Isolates from infections in horses and companion animals, caused by ESBL-producing Enterobacteriaceae, shared a number of characteristics with contemporaneous human isolates (Schmiedel *et al.* 2014). Those authors suggest that their study provided some evidence for a possible pathway or reservoir for resistant bacteria. AMR bacteria are also found in animals not recently treated with antimicrobials; ESBL and AmpC-producing *E. coli* were found in the faeces of 9/106 (8%) dogs in a quantification prevalence study (Espinosa-Gongora *et al.* 2015). That study did not assess the potential for these bacteria to be direct causes of disease in humans, however, like other studies it shows companion animals as a potential reservoir for mobile resistant genes (Schmiedel *et al.* 2014; Rocha-Gracia *et al.* 2015).

Animals have the potential to carry and disseminate ESBL-producing *E. coli* and *K. pneumoniae* (Donati *et al.* 2014; Ewers *et al.* 2014a; Ewers *et al.* 2014b) and there are examples of transmission incidents of MDR Enterobacteriaceae between humans and animals, however evidence of substantive risk of animals to human health is lacking (Ewers *et al.* 2012; Rubin and Pitout 2014). The modes of development, persistence and

transmission of AMR bacteria in human and animal populations are complex, and dependant on many factors. Some of these known factors include the misuse and overuse of antimicrobials in humans and animals, and hospitalised or healthcare-based transmission of resistant bacteria; other drivers include environmental contamination, ineffective diagnostic testing and lack of proper vaccination and preventative medicine (Holmes *et al.* 2016).

2.5 Discussion

Challenges presented by AMR are complex and best tackled with a One Health approach, due to the interconnectedness of humans, animals and the environment. Previously somewhat neglected as a One Health issue, AMR is now appearing as a platform within One Health institutes, at symposia and in publications. Other key global health and welfare issues such as climate change, and Ebola virus disease in West Africa have readied us to more fully consider the impact of the environment in infectious disease than we may have in the past (McMichael *et al.* 2006). So too, our consideration of the environment has broadened to include the social, cultural and political environment in which clinicians make decisions about sample submission for culture and susceptibility, antimicrobial prescribing, and adherence to stewardship guidelines (McNulty *et al.* 2012; Pleydell *et al.* 2012). Less compartmentalisation, more widespread engagement and effective communication will facilitate shared action plans on surveillance, stewardship and research to reduce global threats such as AMR.

2.5.1 Surveillance of antimicrobial resistant bacteria

Surveillance of antimicrobial susceptibility is a key component of antimicrobial stewardship (Prescott 2014; Weese *et al.* 2015). An example of a successful One Health surveillance programme is the Danish Integrated AMR Monitoring and Research

Program (DANMAP), which was established in 1995 (www.danmap.org). The surveillance component of this programme has provided data for making policy changes and therefore the improved usage of antimicrobials. For example, surveillance data was able to show that the use of third and fourth generation cephalosporins increased considerably from 2001, and in 2005 the first ESBL-producing Enterobacteriaceae were isolated from slaughter pigs (Aarestrup *et al.* 2006). This resulted in the introduction of a voluntary ban on cephalosporins in the Danish pig industry and a significant decrease in the prevalence of ESBL-producing Enterobacteriaceae in pigs at slaughter and in pork for consumption ensued (Agersø and Aarestrup 2013). In 2012, genome sequencing was introduced as a surveillance tool for the programme and has been used for multilocus sequence typing as well as for the detection and analysis of resistant genes from ESBL and AmpC-producing Enterobacteriaceae isolates. This enabled the detection of the first ESBL and AmpC-producing isolates that were also *mcr-1* (colistin resistant) outside China (Hasman *et al.* 2015).

In New Zealand, a human AMR surveillance programme is currently carried out by ESR. This includes a two-yearly (previously carried out annually) surveillance report on ESBL-producing Enterobacteriaceae and Enterobacteriaceae with acquired carbapenemases (Anonymous 2017; Heffernan *et al.* 2018). Both surveys are only undertaken on human isolates from diagnostic laboratories, including isolates from both screening and clinical samples. Routine surveillance of animals entering the food chain for MDR Enterobacteriaceae does not occur; neither is there surveillance of animals under treatment by veterinarians, i.e. of clinical samples submitted for culture and susceptibility testing. An effective New Zealand surveillance system would require routine surveillance at both the national and local level of both human and animal isolates. Dedicated research

surveillance programmes to understand the prevalence of AMR in particular environments are also important as these facilitate the recognition of unusual phenotypes or resistance mechanism that may be missed during routine surveillance. In addition, research surveillance programmes may enable extra information to be collected that would not otherwise be provided with samples submitted to commercial diagnostic laboratories (McDougall *et al.* 2014).

2.5.2 Antimicrobial use in New Zealand

New Zealand was one of the few developed countries to show a per capita increase in human antimicrobial consumption in a global study that analysed sales data as a proxy for determining human consumption (van Boeckel *et al.* 2014). Between 2005 and 2012 community antimicrobial use increased from ~17 to ~25 defined daily doses/1,000 population/day, unlike in many European countries where there was a decrease (van Boeckel *et al.* 2014). This higher relative use is of concern, and has the opportunity to be addressed by stewardship policies (Thomas *et al.* 2014; Williamson *et al.* 2016).

Antimicrobial prescribing habits of New Zealand veterinarians are only partially documented. Antimicrobial use by Waikato dairy farms, and prescribing practices of their veterinarians were evaluated in 2014 (McDougall *et al.* 2017). In this study, farmers rated veterinary advice as most important; however, they also used their own perception of drug efficacy to make antimicrobial choices. Prescribing by veterinarians was predominantly based on diagnosis, expected pathogen and spectrum of activity. However bacterial culture and susceptibility testing was considered of limited use by farmers and was not widely used by veterinarians (McDougall *et al.* 2017). A survey of prescribing practices of companion animal veterinarians was conducted in 2008 (Pleydell *et al.* 2012). Results of that survey highlighted antimicrobial use that potentially provided selection

pressure for the development of AMR, such as use of fluoroquinolones and amoxicillin-clavulanate as first-line therapies (Pleydell *et al.* 2012). It should be noted that both of these treatments are listed as critically important by the WHO (Weese et al. 2015). On a broader scale, antimicrobial use in New Zealand animals, by total kg of antimicrobial sold, is regularly reported (Anonymous 2013c).

The use of antimicrobials in food animals in New Zealand for the year 2012 was compared with use in a selection of other countries by Hillerton *et al.* (Hillerton *et al.* 2017). The results showed that, based on mg active ingredient sold per kg biomass; New Zealand has a relatively low antimicrobial use in food producing animals. However, this study did not determine the use of specific antimicrobials, such as the third generation cephalosporins or fluoroquinolones. In New Zealand between 2009 and 2011, total ceftiofur sales increased 118% (Anonymous 2013c). It should be noted that ceftiofur is only licensed for use in horses, pigs and cattle in New Zealand, and such use could increase selection pressure for the development of resistant Enterobacteriaceae in these animals (Weese *et al.* 2015; Holmes *et al.* 2016). In most countries, including New Zealand, measurement of antimicrobial consumption is generally based on sales data, represented as mg of active substance, or reported as mg active antimicrobial per kg of biomass in food producing animals (Anonymous 2013c; Hillerton *et al.* 2017). This approach has its limitations as sales data may not reflect the actual amount used and there is insufficient data on how a specific type of antimicrobial is used for a particular animal species.

The low total use of antimicrobials in New Zealand food production is likely to reflect the predominance of pastoral, and therefore less-intensive, agricultural systems when compared to many other countries. However, antimicrobials are still used in healthy animals in New Zealand, such as in-feed antimicrobials in some intensive systems like

zinc bacitracin in the poultry industry, which comprises the greatest mass of antimicrobials used (Anonymous 2013c), and provides an opportunity for reducing antimicrobial usage.

2.5.3 Stewardship of antimicrobials

Antimicrobial stewardship, alongside infection-control measures (e.g. biosecurity and hygiene), is important in the reduction and control of infections caused by MDR bacteria. The key elements of antimicrobial stewardship programmes in humans include implementation of specific interventions to improve antimicrobial use, education of professionals and consumers, tracking and reporting antimicrobial prescribing patterns and resistance, and leadership commitment. Education of New Zealand medical prescribers on the appropriate use of antimicrobials, along with selective reporting of susceptibility by laboratories to prescribers, is likely to have caused a reduction in fluoroquinolone use in humans from 2010–2014 (Williamson *et al.* 2016). After the introduction of DANMAP, and a voluntary ban of cephalosporin use in pig farming in 2010, the use of third and fourth generation cephalosporins in pigs across Denmark dropped from ~130 kg in 2008 to <1 kg active in 2011 (Andersen *et al.* 2015). This resulted in a significant decrease in the prevalence of ESBL-producing Enterobacteriaceae in pigs at slaughter, from 11.8 % (48/407) in 2010 to 0 % (0/78) in 2011 (Agersø and Aarestrup 2013).

Education is a cornerstone of antimicrobial stewardship (Weese *et al.* 2015); animal and human healthcare professionals need to have access to continuing professional development, as well as be motivated to educate patients and clients (McNulty *et al.* 2012; McDougall *et al.* 2017). Knowledge of prescribing practice before implementation of stewardship interventions can be useful to benchmark changes in clinician behaviour

(Pleydell *et al.* 2012; Williamson *et al.* 2016). However, considerable and concerted effort is still needed in order to achieve and maintain optimal prescribing practice in a consistent manner across the country. Linked to education, another method of stewardship is to place requirements upon research and publication. For example, the Equine Veterinary Journal's policy requires declarations and justifications from authors on the use of antimicrobials, similar to requirements for ethical approvals (Bowen 2013).

Veterinary student training is also critical and an area that deserves investment (Castro-Sánchez *et al.* 2016). A review of AMR and stewardship teaching in the veterinary curriculum at Massey University (Palmerston North, NZ) was conducted in 2016. Although there were multiple exposures to these topics during the Bachelor of Veterinary Science degree course, there was little vertical integration through the curriculum (unpublished data by the author). An antimicrobial working group has been formed to start to address these deficits.

2.5.4 Research needs

Retrospective studies of veterinary clinical isolates from New Zealand equine and companion animals have demonstrated an increasing amount of resistance to the antimicrobials used to treat infections in young New Zealand horses and in urinary tract infections in New Zealand dogs (Toombs-Ruane *et al.* 2016; McMeekin *et al.* 2017). Although important groundwork, a key limitation of these studies is that cases submitted to the laboratory are unlikely to have come from uncomplicated conditions. Submissions are more likely to occur when first line empirical treatment had failed (Pleydell *et al.* 2012), thus culture results may reflect a more resistant population of bacteria. To date, research on MDR bacteria of veterinary importance has focussed on phenotype, whereas genomic analysis facilitates a greater understanding of transmission and epidemiology,

and better understanding of the virulence and AMR traits of bacteria (Holt *et al.* 2015). The genotypic characteristics of MDR Enterobacteriaceae from infection sites in companion animals between 2012 and 2013 in New Zealand was reported by Karkaba *et al*. and generally reflected the findings of studies conducted in other countries (Karkaba *et al.* 2017b).

There is a deficit of published observational studies in New Zealand addressing the risk factors for the development and carriage of AMR bacteria in animal populations. The evidence base for AMR in animals as a causative agent for AMR in humans is variable, with findings ranging from no association to a strong causal association (Marshall and Levy 2011; Holmes *et al.* 2016). This deficit will be addressed further in **Chapter 3**.

2.6 Conclusions

This review has described the mechanisms and potential transmission pathways of multidrug resistance in Enterobacteriaceae, especially ESBL-producing bacteria. The challenges associated with AMR, including transfer between animals, humans and the environment have been identified, and a One-Health approach for controlling AMR proposed that includes surveillance of resistant bacteria, monitoring antimicrobial use and antimicrobial stewardship. Future research needs include prospective observational studies, as well as understanding risk factors for AMR in New Zealand. In addition to scientific studies, we suggest the veterinary community needs to maintain an on-going commitment to AMR including engagement in a multi-stakeholder One Health oversight committee on AMR, and for this committee to provide advice and guidance at a national level. There also needs to be improved surveillance and centralised reporting of antimicrobial use and resistance of clinical and post-slaughter isolates in animals, coordinated and aligned with similar surveillance of human isolates. Alongside this integrated surveillance, we also suggest that there be requirements for routine screening of Enterobacteriaceae for ESBL and carbapenemase-producing bacteria in veterinary microbiology and pathology laboratories, using methods aligned with the screening of human isolates. Finally, we suggest the development and support for antimicrobial stewardship and improved infection prevention and control guidelines for New Zealand veterinarians. This should include education and extension for both veterinarians and clients.

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STATEMENT OF CONTRIBUTION
DOCTORATE WITH PUBLICATIONS/MANUSCRIPTS

We, the candidate and the candidate's Primary Supervisor, certify that all co-authors have consented to their work being included in the thesis and they have accepted the candidate's contribution as indicated below in the *Statement of Originality*.

Name of candidate:	Leah Toombs-Ruane
Name/title of Primary Supervisor:	A/Prof. Jackie Benschop
Name of Research Output and full reference:	
Toombs-Ruane, L. J., Benschop, J., Burgess, S., Priest, P., Murdoch, D. R., & French, N. P. (2017). Multidrug resistant Enterobacteriaceae in New Zealand: a current perspective. New Zealand Veterinary Journal, 65(2), 62-70. doi:10.1080/00480169.20	
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Initial submission was done by candidate (>90%), and revisions were done in collaboration with Dr. Sara Burgess (environmental transfer & AMR mechanisms)	
For manuscripts intended for publication please indicate target journal:	
Candidate's Signature:	Leah Toombs-Ruane Digitally signed by Leah Toombs-Ruane Date: 2019.07.27 07:42:34 -05'00'
Date:	27 July 2019
Primary Supervisor's Signature:	Jackie Benschop Digitally signed by Jackie Benschop Date: 2019.07.28 10:04:10 +12'00'
Date:	28 July 2019

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3 Companion animals as a risk for community-acquired extended-spectrum beta-lactamase and AmpC beta-lactamase producing Enterobacteriaceae infections: a prospective case-control study

3.1 Abstract

Companion animals in the home are a potential source of plasmid-associated multidrug resistant bacteria. With 60% of New Zealand households having at least one pet, the role that pets play in human AMR infections warrants investigation. The objective of this study was to examine risk factors for community-acquired infections, including those associated with having a pet in the home.

A prospective unmatched case-control study was conducted between August 2015 and September 2017. Cases were people with community-acquired urinary tract infection (UTI) caused by newly acquired extended spectrum beta-lactamase (ESBL)- or AmpC beta-lactamase (ACBL)- producing Enterobacteriaceae. Controls (n=525) were people recruited from the community via landline telephone and not matched to cases. A telephone questionnaire was administered and putative risk factors were assessed using regression analyses on two datasets, one with case-reported antimicrobial use (n=141), and the other with independently confirmed antimicrobial use (n=99). A combination of logistic regression, LASSO logistic regression and random forest regression was use to analyse the data.

Pet ownership did not constitute a risk for human urinary tract infections caused by ESBL- or ACBL-producing Enterobacteriaceae in any modelling modality used. Risk factors estimated using multivariate logistic regression on the full dataset included recent antimicrobial treatment (previous 3-months) [adjusted OR = 15.3 (95% CI 6.0 - 43.1)], travel to Asia in the previous year [adjusted OR = 9.9 (95% CI 4.0 - 26.8)], and contact with healthcare in the previous six-months [adjusted OR = 25.0 (7.4 - 107.1)]. Cases were more likely to be female [adjusted OR = 24.7 (95% CI 7.9 - 97.8)] and 65-years or older [adjusted OR = 14.0 (95% CI 4.5 - 49.4)] than the control population.

The whole genomes of ESBL- or ACBL-producing isolates collected from urine were sequenced, and assessed through a bioinformatics pipeline. Isolates with an ESBL-/ACBL-producing phenotype from 132 case people's UTI were available for analysis. Of all isolates, 126/132 (95%) were *E. coli*, 5/132 (4%) were *Klebsiella pneumoniae*, and 1/132 (1%) was *Morganella morganii*. The *bla*$_{\text{CTX-M-15}}$ or *bla*$_{\text{CTX-M-27}}$ genes were found in 90/132 (68%) of ESBL- and/or ACBL-producing isolates from all case people, *bla*$_{\text{CTX-M-27}}$ was found in 48/115 (42%) and *bla*$_{\text{CTX-M-15}}$ was found in 42/115 (37%) of ESBL-producing isolates. For those ESBL-/ACBL-producing isolates identified as *E. coli,* 65/126 (52%) were phylogroup B2, 35/126 (28%) were phylogroup D; 47/126 (37%) were ST-131, and 16/126 (13%) were ST-38.

3.2 Introduction

Extended-spectrum beta-lactamase-producing and AmpC beta-lactamase-producing Enterobacteriaceae (ESBL-E and ACBL-E respectively) are common antimicrobial resistant (AMR) bacteria of importance to people in hospital and healthcare environments, and the community at large (Pitout *et al.* 2005; Heffernan *et al.* 2018; Torres *et al.* 2018). Historically, these bacteria were acquired through hospital exposures, and have since become associated with community-acquired infections (Freeman *et al.* 2008; Heffernan *et al.* 2009; Rogers *et al.* 2015). These ESBL or ACBL enzymes are often expressed from genes on mobile elements (i.e. plasmids), and increasingly are found in bacteria with virulence traits that make the bacteria likely to cause disease (Kaper *et al.* 2004; Nicolas-Chanoine *et al.* 2014). These bacteria are also pathogens of animals, being isolated from clinical samples in companion and production animals around the world (Ewers *et al.* 2012; Hammerum *et al.* 2014; Schmiedel *et al.* 2014; Dohmen *et al.* 2017), including in New Zealand (Karkaba *et al.* 2017b).

As multidrug resistant (MDR) infections become more common in a community, the risk factors for acquisition of bacteria and subsequent infection (via intestinal carriage) are likely to change (Rogers *et al.* 2014; Karanika *et al.* 2016). Travel to high-prevalence areas (such as the Indian subcontinent) was found to be associated with ESBL-E infection in early reports from community-acquired New Zealand infections [e.g. the analysis of isolates from 2004 to 2006 by Freeman *et al.* (2008)]. In 2012, many people infected with an *E. coli* infection resistant to third-generation cephalosporins had not travelled outside of New Zealand, however travel still increased the odds of having a resistant infection (Rogers *et al.* 2014). These findings are consistent with other studies where travel was an

important risk factor for carriage or infection-with multidrug resistant gram-negative infections (Kuenzli *et al.* 2014; Karanika *et al.* 2016; Arcilla *et al.* 2017).

Person-level risk factors for ESBL-E carriage or infection include high healthcare input or contact, recent antimicrobial use, older age, and travel to higher-prevalence countries (Karanika *et al.* 2016; Chatterjee *et al.* 2018). Risk factors for community-level prevalence are predominated by human antimicrobial use, although many countries deemed "high risk" have poor or unreliable recording of community prescriptions of antimicrobials (Woerther *et al.* 2013). High community prescribing of antimicrobials has been associated with an increased risk of bloodstream infections have been identified as risk factors (Lishman *et al.* 2018). Antimicrobial use may therefore be an important risk factor in New Zealand, as community prescribing of antimicrobials is reported to be higher than many other OECD countries (Thomas *et al.* 2014; Williamson *et al.* 2016).

Antimicrobial treatment of companion animals has been identified as a risk factor for AMR in companion animals (Schmidt *et al.* 2018). There are also instances of people becoming ill after coming into contact with companion animals carrying or infected by multidrug resistant pathogens (Pomba *et al.* 2017), or companion animals carrying and/or transmitting MDR bacteria within homes (Johnson *et al.* 2016). Companion animals in New Zealand visiting veterinary clinics may carry human-associated strains of ESBL-E, as well as strains of *mecA*-containing *Staphylococcus* species (Karkaba *et al.* 2017a; Karkaba *et al.* 2017b).

In Europe, human-associated ESBL-E (*E. coli* and *Klebsiella* species) are found in clinical bacterial isolates from companion animals (Dierikx *et al.* 2012; Schmiedel *et al.* 2014; Belas *et al.* 2018). These bacteria may contain plasmid-encoded ESBL genes commonly

associated with human community infections (such as *bla*$_{\text{CTX-M-14}}$, *bla*$_{\text{CTX-M-27}}$ or *bla*$_{\text{CTX-M-15}}$); the bacteria may also be from human associated MLST types (such as ST-131). In some cases, studies have found isolates from companion animals are more likely to be ACBL-producers in contrast to other studies where more isolates are ESBL-producers (Rubin and Pitout 2014). The choice of phenotypic screening and confirmatory tests will also have an impact on the relative proportion of isolates detected in these instances.

Companion animals living in residential care homes (rest homes or elder care facilities) can carry multidrug resistant bacteria (Ewers *et al.* 2014b; Schaufler *et al.* 2015; Pomba *et al.* 2017). In a healthcare environment, this scenario is more likely, especially where nosocomial infections in humans are prevalent (Rogers *et al.* 2014). In the community however, multidrug resistant bacteria are likely to be less prevalent. In New Zealand, pets live in approximately 64% of homes, where 44% of homes have at least one cat and 28% of homes have at least one dog (Anonymous 2016a). Where pet ownership is high, risks associated with this ownership are worth investigating (Meyer *et al.* 2012). However, pet ownership has not been found to be associated with increased risk for infection or carriage of multidrug resistant bacteria in recent studies (Rogers *et al.* 2014; Leonard *et al.* 2018).

While the true or supposed prevalence of intestinal carriage of ESBL-E is not known for New Zealand, the rates are likely to be highest in the Auckland region (Heffernan *et al.* 2018). This is a concentrated population in relation to the rest of the country, with approximately one-third of the population living in the region. The population is more diverse with higher proportions of Asian and Pacifica ethnicities than rest of the country. The Auckland region is also associated with rates of ESBL-producing infections greater than the New Zealand national rate (Dyet *et al.* 2014; Heffernan *et al.* 2018).

The objective of the study presented in this chapter was to determine the risk of companion animals for community-acquired ESBL- and ACBL-producing infections in people. Other risk factors for these community-acquired infections were also assessed. This study also aimed to describe Enterobacteriaceae cultured from these community-acquired ESBL- and ACBL-producing urinary tract infections.

3.3 Materials and methods

3.3.1 Case and control recruitment and sampling

Case and control definitions

A case was defined as a person aged 16-years or older, from whom a urine sample was submitted to the recruiting community laboratory (Labtests Auckland, Healthscope) from which an ESBL-E or ACBL-E was cultured. Participants consented to release of the bacterial isolate for further testing, and to participate in a 20-minute telephone questionnaire. Case participants were ineligible if they had been admitted overnight to hospital for any reason in the 12-months prior to the urine sample collection, or if they lived in a residential care facility such as a rest home. A control participant was eligible to be part of the study if they had not been a patient overnight in hospital in the previous 12-months, did not live in a residential care (rest) home, and were 16-years of age or older.

Sample size

In this study a low prevalence of ESBL-/ACBL-producing bacteria in eligible case people was anticipated, and therefore a high control-to-case ratio was selected. Based on a target of 175 cases (where an estimate of three to four cases per week would be recruited over 50 weeks), 525 controls were recruited. This sample size was considered to be sufficient to detect an odds ratio of >2 for pet-related exposures (assuming approximately 50% of

controls had a pet in the home). Based on the Fleiss case-control sample size calculation, with an α of 0.05 (assuming 60% of controls have a pet) to detect an OR of 2 (pets in the home) with 95% power, 175 cases would need to be recruited (1:3 case-to-control ratio) (Fleiss *et al.* 2013).

Case and control recruitment

Case participants were recruited by a clinical microbiologist through submissions to Labtests Auckland (Healthscope), a community microbiology laboratory serving the Auckland and Northland regional communities. These case participants were recruited between September 2015 and September 2017.

Control recruitment was managed by a research company (UMR Research, Wellington, https://umr.co.nz/) performed via telephone (landline) using a combination of random digit dialling and verified phone numbers in UMR's polling database. Controls were drawn from the Auckland/Northland regions and otherwise unmatched. Control recruitment began in August 2015, with 44 controls recruited each month for the first 6 months of the study (40 from Auckland, 4 from Northland). Due to slow contemporaneous case participant recruitment, control participant recruitment was reduced to 18 participants per month. All control participants were recruited between August 2015 and March 2017.

Questionnaire development and delivery

A 52-question telephone questionnaire was developed to assess risk factor exposures during the six months preceding the multidrug resistant urinary tract infection for cases or the previous six-months for controls. Demographic factors such as age, gender, ethnicity, location (nearest primary school to residence), occupational factors and

household factors such as garden and the number bedrooms/cohabiting people residing were included[1]. The full case questionnaire can be found in **Appendix IV.**

Participants were asked to recall recent antimicrobial treatment; for case participants this was intended to be any treatments prior to their current (i.e. recruited) infection. Other healthcare-related questions included any recent day surgery, and any known chronic disease co-morbidities. Pet related question included exposure, including food, hygiene and contact with pets, vet visits (including any antimicrobial treatment). International travel in the previous year was also assessed.

The questionnaire was pretested by interview (face-to-face) by UMR. This pretesting was done on a cross-section of 10 individuals of varying ages and both male and female, living in Wellington, New Zealand. After pretesting, a pilot of 15 telephone questionnaires was performed on non-cases by a small team at UMR. Language of the questionnaire was modified subsequent to recommendations from UMR.

An optional part of this study was the access to government health data [through the National Health Index (NHI) number] for community prescriptions of antimicrobials and hospitalisations for multidrug resistant infection. This information was requested but not essential for participation in the study.

[1] See https://esblfamilypet.wordpress.com/ for chapter 3 supporting documents including case and control questionnaires

3.3.2 Microbiology

Bacterial culture and selection of isolates

Urine samples were submitted to Labtests Auckland microbiology laboratories and the ESBL-production phenotype ascertained according to EUCAST guidelines using Kirby-Bauer disk diffusion assays (Bauer *et al.* 1966; EUCAST 2018). Mueller-Hinton antimicrobial susceptibility plates from the isolates of people meeting the case definition were sent from Auckland to the ᵐEpiLab. Once received into the ᵐEpiLab, bacterial isolates were subcultured onto Colombia horse blood agar (Fort Richard Laboratories, Auckland, New Zealand), and two colonies were subcultured after 24 hours at 35 (+/- 1) °C (labelled 'a' and 'b'). Purified isolates were stored at -80°C in a nutrient/glycerol suspension [25mg/mL Nutrient broth no. 2 (Oxoid, Basingstoke, U.K.), 15% glycerol (in purified and deionised water)]. Bacterial species identification was performed using matrix-assisted laser desorption/ionisation time of flight (MALDI-TOF) mass spectrometry (biotyper, Bruker, Billerica MA, U.S.A) according to manufacturers' instructions and using the Bruker human pathogen database.

Additional antimicrobial susceptibility testing

Clinical bacterial isolates were re-tested at ᵐEpiLab for an ESBL-producing phenotype using three paired Mastdiscs® [D62C ESBL cefotaxime Paired ID Disks (Mast Group Ltd., Liverpool, U.K.), and D64C ESBL ceftazidime Paired ID Disks (Mast Group Ltd.)], and these isolates were also tested for an ACBL-producing phenotype using a three-disk comparison assay [D69C AmpC disk test (Mast Group Ltd.)]. If the 'a' isolate was not positive for an ESBL- and/or ACBL-producing phenotype, the 'b' isolates was tested likewise. Both isolates were individually suspended in nutrient/glycerol suspension and stored at -80°C. Antimicrobial susceptibility testing using the Kirby-

Bauer disk diffusion assay was performed on isolates included in this study, using EUCAST clinical breakpoints (Kirby & Bauer 1967; EUCAST 2018). Antimicrobials tested against are shown in **Table 3.1** (Mast Group Ltd). Multidrug resistance was defined as resistance to more than two of the following antimicrobial classes: beta-lactam (AMP10, CFX30, CPD10, CRO30, MEC10, ETP10, AUG30, FOX30), aminoglycoside (AK30, GM10), fosfomycin (FOT200), trimethoprim (TM5), nitrofurantoin (NI100), and norfloxacin (NOR10). 'Moderate' or 'intermediate' susceptibilities were defined as those that fell between susceptible and resistance diameters for the following disks: CRO30, GM10, AK30, NOR10, TM5, i.e. they did not meet the definition of 'susceptible' or 'resistant' as given by the EUCAST clinical breakpoints .

Table 3.1 Antimicrobial zone diameters used in Kirby-Bauer disk diffusion phenotypic assays (EUCAST 2018)

Antimicrobial (disk dose)		Susceptible zone size (mm) ≥	Resistant zone size (mm) <
Ampicillin (10ug)	AMP10	14	14
Cephalexin (30ug)	CFX30	14*	14*
Cefpodoxime (10ug)	CPD10	21*	21*
Ceftriaxone (30ug)	CRO30	25	22
Mecillinam (10æg)	MEC10	15	15
Ertapenem (10ug)	ETP10	25	22
Augmentin (amoxicillin 20ug + clavulanic acid 10ug)	AUG30	16*	16*
Cefoxitin (30ug)	FOX30	19†	19†
Gentamicin (10ug)	GM10	17	14
Amikacin (30ug)	AK30	18	15
Norfloxacillin (10ug)	NOR10	22	19
Trimethoprim (5ug)	TM5	18*	15*
Nitrofurantoin (100ug)	NI100	11*‡	11*‡
Fosfomycin (200ug)	FOT200	24*‡	24*‡

* Clinical breakpoint for uncomplicated UTI
† Screening breakpoint for ACBL production
‡ *E. coli* only

3.3.3 Extraction and preparation of genomic DNA for sequencing

Where available, one clinical UTI isolate was sequenced from each case participant. Bacterial isolates were re-cultured from frozen (stored at -80°C) and a single colony subcultured and incubated for 20-24 hours at 35°C (+/- 1°C) , after an initial revival on Colombia horse blood agar (Fort Richard). Extraction of genomic DNA was done using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to a modified and optimised version (for protocol see **Appendix II**) of the tissue/bacterial colony protocol. A PCR-grade water eluate was used, and this was stored at -20°C prior to library preparation. Fluorescent spectrometry (Qubit 2.0, Invitrogen, Carlsbad CA, U.S.A.) was used to assess quality of the extraction, and DNA was normalised to a concentration of

0.16ug/mL prior to preparation of libraries (Nextera XT DNA library preparation kits, Illumina Inc.). Libraries were prepared by Massey Genome service (when sequenced on the Illumina MiSeq platform, by Massey Genome Service), or by the ^{m}EpiLab (when sequenced on the Illumina HiSeq platform at the Otago Genomic and Bioinformatics facility). Assessment of library quality and fragment size distribution was performed by an automated gel fragment analyser prior to pooling of libraries [Labchip GX Touch HT (PerkinElmer, Waltham, U.S.A.); or 2100 BioAnalyzer (Agilent Genomics, Santa Clara, U.S.A.)].)]. Additional information on the protocol used for DNA extraction and library preparation can be found in **Appendix III**.

3.3.4 Next generation sequencing and bioinformatics analyses

Raw pair ended read files were used as the input into the Nullarbor pipeline using 'careful mode' for the *de novo* assembly of genomes via SPAdes (version 3.0) (Bankevich *et al.* 2012; Seemann *et al.* 2016).

Ribosomal multi-locus sequence typing (rMLST) was performed using the outputs from Prokka 3.0 (Seemann 2014), with an in-house perl script and an rMLST database for the analysis. Assembled contiguous nucleotide sequences of genomes (i.e. the output the Nullarbor pipeline) were used for *ad hoc* whole genome MLST of *E. coli* and *Klebsiella pneumoniae* clinical isolates [Fast-GeP, (Zhang *et al.* 2018)]. Whole genome MLST was run using default settings.

The distance matrix tree outputs from whole genome MLST was presented as neighbour-joining trees in SplitsTree (Huson 1998), and subsequently converted to Newick format to allow upload to Evolview (http://www.evolgenius.info/evolview/) for annotating and labelling (He *et al.* 2016).

3.3.5 Data management and statistical analyses

Questionnaire, sample submission, and isolate data were stored in a MySQL (Oracle, Redwood City CA, U.S.A.) database, using Access (Microsoft Corporation, Redmond WA, U.S.A.) as a data entry interface. Statistical analysis of questionnaire data was performed in R-Studio (R version 3.4.3 https://www.r-project.org) and Excel (Microsoft corporation, Redmond WA, U.S.A). Missing variables in this study were not imputed.

Outputs from the case and control questionnaires were used as inputs into a MySQL database and managed through MySQL Workbench (Oracle and Microsoft Access). These data were analysed using Rstudio (rstudio.com), with an open database connectivity (OBC) interface to the data stored remotely and securely on the Massey University Social Sciences server.

R packages "RODBC" (https://cran.r-project.org/web/packages/RODBC/index.html) and "sqldf" (https://cran.r-project.org/web/packages/sqldf/index.html) were used for extraction and manipulation of data from this database through queries in the database programming language SQL, and recoding free-text answers though look-up tables. In this form, data were then recoded to reduce the number of variables. Variables were limited to those with a specific research question (from the questionnaire) and some (such as age) were recoded to ensure factors had non-zero entries and/or were split into groups that made biological sense. Manual recoding of free text answer to "other" answers in the questionnaire included the variables of ethnicity, nearest school, and travel. Where participants answered "unsure" to questions, the answers were coded as "NA" (or missing) for the purposes of analysis.

Within the questionnaire, participants were asked to identify the nearest primary school to their home. The answers to this question were cross-matched to the New Zealand Schools database (http://www.educationcounts.govt.nz/data-services/directories/list-of-nz-schools; accessed 21/2/18) and school latitude and longitude, school decile, NZ 2013 census mesh block with urban area designation extracted and added to the questionnaire dataset.

3.3.6 Regression Modelling

Subset analysis with NHI prescription data

Community prescription data [accessed through National Health Index (NHI)] for the six months preceding the submission of the urine sample of the incident case was available for some case participants (n=99). This included systemic antimicrobial prescriptions, with drug formulation, date and length of course included. For the purposes of using this data in multivariate modelling, the most recent prescription was used if there were multiple recorded prescriptions. This subset was used with the full control dataset for repeated regression modelling as described in the next sections. Sensitivity and specificity of the questionnaire was assessed using NHI prescription data as the true exposure status of cases participants, where the test result was the answer provided in the questionnaire.

Univariate screening of variables

Variables were described first by one-way (count) tables. As no variables in the dataset were continuous (i.e. all were factors, either ordinal, categorical or binary), they were subsequently screened in relation to case and controls using two-way tables. Chi-squared tests and p-values from univariate regressions for odds ratios were used to assess statistical significance of variables in two-way tables. Demographic factors [including decile of nearest primary school, whether that school was in an urban or rural area, region

(Auckland, Northland or Waikato), gender, ethnicity, whether they lived alone, whether they worked in healthcare, and whether they had been a visitor to a hospital or rest-home in the previous six-months] were assessed.

Multivariate regression models

Logistic regression was used to assess the relationship between the outcome variable (case or control) and each variable of interest. All 85 variables were analysed in this way, including variables where more than one method of recoding was used (such as chronic disease). Potential confounders for the exposure of interest (pet ownership) include age, gender, and ethnicity. For healthcare associated exposures, possible confounders include age, chronic disease (i.e. diabetes) and recurrent/multiple urinary tract infections. For travel-associated exposures, possible confounders include age and ethnicity. Hypothesised confounders affecting a model with healthcare, pet, and travel-associated exposures included were age, gender, previous urinary tract infection, and diabetes. A preliminary multivariate model was constructed from variables where at least one factor (or level) in the variable had a p-value ≤ 0.2.

In moving to a final multivariate logistic regression model, variables were excluded from the model using a stepwise approach. Variables were excluded when the covariate did not reach significance of $p<0.05$, provided they were not a confounder or the exposure of interest.

Model fit was assessed using likelihood ratio tests, area-under ROC (c-statistic), and pseudo R-squared (Nagelkerke R-squared) with the R package 'rms' [https://CRAN.R-project.org/package=rms (Miller *et al.* 1991)]. These tests, in addition to p-values,

residual deviance and Akaike information criterion (AIC) were used to describe and assess multivariate model(s) [calculated with the R function 'glm'].

A Least Absolute Shrinkage and Selection Operator (LASSO) logistic regression model was also used. This modelling tool is applied to datasets with a large number of variables of interest, where the effects of multi-collinearity can result in inflated estimates of regression coefficients (and hence odds ratios). Using this approach many variables are reduced to coefficients of zero by the LASSO (Tibshirani 1996), thus, the coefficients from a LASSO logistic regression model tend to be lower than a multivariate logistic regression model, but from these coefficients more conservative estimates of odds ratios can be determined for the variables that emerge (between the range for optimal least absolute shrinkage). This regression modelling was performed in R using the package "glmnet" (https://cran.r-project.org/package=glmnet). Two datasets were analysed using a penalised model, with constraints placed upon the LASSO regression given by a lambda at the minimum (λ_{min}) and minimum plus one standard error (λ_{min} + 1 standard error) (Friedman *et al.* 2010). Supplementary information for LASSO model can be found in **Figure 6.2** in **Appendix V**.

Population attributable fractions

Attributable fractions give an estimation of the population-level effect of exposures on the outcome, accounting for rarity of exposures. Attributable fractions were calculated for variables included in the final multivariate logistic regression model using the R package "AF" (https://CRAN.R-project.org/package=AF), which used adjusted variable odds for the AF analysis. The was done using binary risk factors recoded for the following variables: antimicrobial treatment (reported for <3 month=1, >3months=0), age (>64 years=1, <65years=0), healthcare work (yes=1, or no=0) (Dahlqwist *et al.* 2016).

Random forest regression

Random forest classification is a method of analysing a large number of variables (or large-scale datasets) using machine learning. This method, in comparison to the more commonly used methods of regression in epidemiologic research, does not provide a measure of risk (i.e. odds ratio). However, it does provide robust information on the importance of a variable to the outcome (i.e. case versus control) and further complemented the multivariate logistic regression and LASSO models. Random forest classification also automatically assess potential interactions, as a variable might appear only on one side of a split and it automatically handles missing data through the use of surrogate splits in each of the trees.

Random forest regression was performed in R using the package "randomForest" (https://CRAN.R-project.org/package=randomForest). The same 85 variables screened for use in the multivariate regression model were used in the random forest regression. This was run initially with 2000 trees, and subsequently repeated with 10000 trees. Class weight was changed to reduce class confusion (1000 cases:1 control). The results of the random forest regression are described by the mean decrease in accuracy and the mean decrease in the Gini index. These values are ways of assessing how accurately the trees assign cases and controls at each variable.

3.3.7 Ethics

This case-control study, with a cross-sectional stool-sampling component, was approved by the New Zealand Health and Disability Ethics Committee (HDEC) - central committee (reference 15/CEN/47). Locality agreements were made with the Auckland, Northland, Waitemata, and Counties Manukau district health boards in compliance with HDEC approval.

3.4 Results

3.4.1 Results of questionnaire

Overview of cases and controls

A total of 141 cases and 525 controls were recruited between 1 August 2015 and 30 September 2017. Both cases and controls were predominantly European, 74.1% of controls and 74.5% of cases identified as European or New Zealand European ethnicity. There was a disparity in gender between cases and controls; 94.3% of cases were female compared to 52.2% of controls. The age distribution was also markedly different between cases and controls: for cases 22.0% were 16 to 44 years-old, 37.6% were 45 to 64 years-old, 40.4% were 65-years-or-over. For controls 52.4% were 16 to 44 years-old, 30.7% were 45 to 64 years-old, and 17.0% were 65-years-or-over. Most participants lived with other people, 89.3% and 85.6% of control and case participants respectively. Over 80% of cases and controls lived in main urban or secondary urban areas compared to rural area or minor urban areas (NZ census definitions, 2013 school data), 90.0% and 85.0% of cases and controls lived in urban areas respectively. Further descriptions of the characteristics of cases and controls are described more fully in **Table 3.2**. The distribution of other questionnaire-assessed risk factors are described in **Tables 3.3**, and **3.4.**

Table 3.2 Summary of demographic and heath variables of 141 case and 525 control participants

Variable	Risk factor	Controls (n/N)	Case (n/N)	Crude OR (95%CI)	p-value
Home	Lives alone	56/524	20/139	Ref	
	Lives with others	468/524	119/139	0.7 (0.4 - 1.3)	>0.2
Gender	Male	251/525	8/141	Ref	
	Female	274/525	133/141	15.2 (7.8 - 34.4)	<0.001
Age	Age: 16 to 44 years	275/525	31/141	Ref	
	Age: 45 to 64 years	161/525	53/141	2.9 (1.8 - 4.8)	<0.001
	Age: 65+ years	89/525	57/141	5.7 (3.5 - 9.4)	<0.001
Ethnicity [a]	Maori	39/525	8/141	0.7 (0.3 - 1.6)	>0.2
	Asian	37/525	12/141	1.2 (0.6 - 2.4)	>0.2
	Pacifica	10/525	2/141	0.7 (0.1 - 2.9)	>0.2
	European	389/525	105/141	1.0 (0.7 - 1.6)	>0.2
	MELAA	22/525	5/141	0.8 (0.3 - 2.1)	>0.2
Healthcare work	No	468/525	109/136	Ref	
	Yes: no patients	15/525	15/136	4.3 (2.0 - 9.1)	<0.001
	Yes: patient contact	42/525	12/136	1.2 (0.6 - 2.3)	>0.2
School decile	1 to 3	115/486	18/119	Ref	
	4 to 7	160/486	44/119	1.8 (1.0 - 3.3)	0.065
	8 to 10	211/486	57/119	1.7 (1.0 - 3.1)	0.064
Urban	No	74/486	12/119	Ref	
	Yes	413/486	107/119	1.6 (0.9 - 3.2)	0.16
Overall health	Good to excellent	487/525	113/140	Ref	
	Fair to poor	38/525	27/140	3.1 (1.8 - 5.2)	<0.001
Any chronic disease	No	394/524	60/136	Ref	
	Yes	130/524	76/136	3.8 (2.6 - 5.7)	<0.001
Asthma	None reported	495/524	126/136	Ref	
	Yes	29/524	10/136	1.4 (0.6 – 2.8)	>0.2
Cardiac disease	None reported	506/524	127/136	Ref	
	Yes	18/524	10/136	2.2 (1.0 - 4.8)	0.051
Diabetes	None reported	514/524	126/136	Ref	
	Yes	10/524	10/136	4.1 (1.6 - 10.1)	0.0022
Other chronic disease	None reported	445/524	75/136	Ref	
	Yes	79/524	61/136	4.6 (3.0 - 6.9)	<0.001
Chronic disease score	None reported	394/523	59/136	Ref	
	1 (of 4)	116/523	59/136	3.4 (2.2 - 5.2)	<0.001
	2 (of 4)	10/523	15/136	10 (4.3 - 24)	<0.001
	3 +	3/523	3/136	6.7 (1.2 - 36.8)	0.022
>1 UTI last 6-months	None reported	509/524	64/134	Ref	
	Yes	15/524	70/134	37.1 (20.6 - 71)	<0.001
Doctor visit last 6-months	None reported	260/525	10/138	Ref	
	Yes	265/525	128/138	12.6 (6.8 - 26)	<0.001
Antibiotics last 6-months	>6 months or never	385/521	28/129	Ref	
	3-6 months	72/521	19/129	3.6 (1.9 - 6.8)	<0.001
	<3 months	64/521	82/129	17.6 (10.8 - 29.6)	<0.001
Visitor to hospital last 6-months	None reported	290/523	60/139	Ref	
	Yes	233/523	79/139	1.6 (1.1 - 2.4)	0.01
Visitor to hospital last 6-months:	Lives alone	56/516	19/129	Ref	
- other person	None reported	269/516	74/129	0.8 (0.5 - 1.5)	>0.2
Antibiotics: other person	Lives alone	56/506	19/117	Ref	
	None reported	284/506	48/117	0.5 (0.3 - 0.9)	0.024
	Yes	166/506	50/117	0.9 (0.5 - 1.7)	>0.2
MDR: other person	Lives alone	56/514	19/119	Ref	
	None reported	444/514	96/119	0.6 (0.4 - 1.1)	0.12
	Yes	14/514	4/119	0.8 (0.2 - 2.7)	>0.2

[a] Ethnicity was reported with multiple answers allowed, ethnicities were compared to “not ethnicity” for the purposes of odds ratio screening; MELAA – Middle Eastern, Latin American, and African

Table 3.3 Summary of travel, home, hygiene and food related variables from 141 case and 525 control participants

Variable	Risk factor	Control (n/N)	Case (n/N)	Crude OR (95% CI)	p-value
All travel last year	None reported	271/524	51/140	Ref	
	Yes	253/524	89/140	1.9 (1.3 - 2.8)	0.001
Travel to Africa	None reported	519/524	133/140	Ref	
	Yes	6/524	7/140	4.6 (1.5 - 14.4)	0.007
Travel to EU	None reported	488/524	121/140	Ref	
	Yes	37/524	19/140	2.1 (1.1 - 3.7)	0.016
Travel to Asia	None reported	463/524	97/140	Ref	
	Yes	62/524	43/140	3.3 (2.1 - 5.1)	<0.001
All travel last year:	Lives alone	56/523	19/137	Ref	
- other person	None reported	221/523	48/137	0.6 (0.4 - 1.2)	0.15
	Yes	246/523	70/137	0.8 (0.5 - 1.5)	>0.2
Diet: vegetarian	Yes	20/525	10/139	Ref	
	No	505/525	129/139	0.5 (0.2 - 1.2)	0.093
Diet: meat type	None	20/525	10/139	Ref	
	No homekill or hunted	375/525	99/139	0.5 (0.2 - 1.2)	0.11
	Homekill or hunted	130/525	30/139	0.5 (0.2 - 1.1)	0.077
Diet: raw milk	No	481/525	134/141	Ref	
	Yes	44/525	7/141	0.6 (0.2 - 1.2)	0.18
Diet: vegetables from	None reported	296/525	70/141	Ref	
garden or family/friends	Yes	229/525	71/141	1.3 (0.9 - 1.9)	0.154
Handwashing:	Always	259/525	46/141	Ref	
- before eating	Often	104/525	47/141	2.5 (1.6 - 4.1)	<0.001
	Sometimes	137/525	39/141	1.6 (1 - 2.6)	0.051
	Never	25/525	9/141	2 (0.8 - 4.5)	0.093
Handwashing:	Always	142/523	38/140	Ref	
- after sneezing or blowing nose	Often	145/523	26/140	0.7 (0.4 - 1.2)	0.154
	Sometimes	183/523	50/140	1.0 (0.6 - 1.6)	>0.2
	Never	53/523	26/140	1.8 (1.0 - 3.3)	0.044
Garden at home	No	56/525	8/137	Ref	
	Yes	469/525	129/137	1.9 (0.9 - 4.5)	0.094
Garden: compost	No garden	56/520	7/138	Ref	
	No compost	184/520	78/138	3.4 (1.6 - 8.5)	<0.001
	Yes	280/520	53/138	1.5 (0.7 - 3.8)	>0.2
Garden: cat/dog faeces seen	No garden	56/522	7/140	Ref	
	No	217/522	50/140	1.8 (0.8 - 4.7)	0.155
	Yes	249/522	83/140	2.7 (1.2 - 6.6)	0.020
Garden: feeds wild birds	No	322/522	74/136	Ref	
	Yes	200/522	62/136	1.3 (0.9 - 2.0)	0.12
Garden: bird bath	No	427/525	85/122	Ref	
	Yes	98/525	37/122	1.9 (1.2 - 2.9)	0.005

Table 3.4 Summary of animal-related variables from 141 case and 525 control participants

Variable	Risk factor	Controls (n/N)	Case (n/N)	Crude OR (95%CI)	p-value
Pet in home[a]	None	267/525	71/139	Ref	
	Yes	258/525	68/139	1.0 (0.7 - 1.4)	>0.2
Pet type	None	267/525	71/137	Ref	
	Cat(s) only	95/525	26/137	1.0 (0.6 - 1.7)	>0.2
	Dog(s) only	51/525	16/137	1.2 (0.6 - 2.2)	>0.2
	Cat(s) and dog(s)	44/525	15/137	1.3 (0.7 - 2.4)	>0.2
	Other pets included	68/525	9/137	0.5 (0.2 - 1.0)	0.066
Diet: cat	No cat	386/510	96/135	Ref	
	Commercial diet only	65/510	15/135	0.9 (0.5 - 1.7)	>0.2
	Some raw meat	59/510	24/135	1.6 (1.0 - 2.7)	0.066
Diet: cat hunts	No cat	386/481	96/126	Ref	
	No	26/481	1/126	0.2 (0 - 0.7)	0.069
	Yes	69/481	29/126	1.7 (1.0 - 2.7)	0.035
Person handles pet faeces	No pet	267/497	71/134	Ref	
	No	90/497	14/134	0.6 (0.3 - 1.1)	0.091
	Yes	140/497	49/134	1.3 (0.9 - 2.0)	0.20
Handwashing:	Not applicable[†]	267/521	74/139	Ref	
- before feeding pet	Always	46/521	7/139	0.5 (0.2 - 1.2)	0.16
	Often	33/521	5/139	0.5 (0.2 - 1.3)	>0.2
	Sometimes	61/521	22/139	1.3 (0.7 - 2.2)	>0.2
	Never	114/521	31/139	1.0 (0.6 - 1.6)	>0.2
Handwashing:	Not applicable[†]	267/521	74/139	Ref	
- after feeding pet	Always	146/521	26/139	0.6 (0.4 - 1.0)	0.077
	Often	29/521	12/139	1.5 (0.7 - 3)	>0.2
	Sometimes	47/521	18/139	1.4 (0.7 - 2.5)	>0.2
	Never	32/521	9/139	1.0 (0.4 - 2.1)	>0.2
Handwashing:	Not applicable[†]	267/518	72/139	Ref	
- after petting/stroking pet	Always	72/518	7/139	0.4 (0.1 - 0.8)	0.015
	Often	32/518	17/139	2.0 (1 - 3.7)	0.039
	Sometimes	91/518	21/139	0.9 (0.5 - 1.4)	>0.2
	Never	56/518	22/139	1.5 (0.8 - 2.5)	0.19
Handwashing:	Not applicable[†]	267/467	75/129	Ref	
- after picking up pet faeces	Always	180/467	47/129	0.9 (0.6 - 1.4)	>0.2
	Often	5/467	4/129	2.8 (0.7 - 11)	0.13
	Sometimes	8/467	2/129	0.9 (0.1 - 3.6)	>0.2
	Never	7/467	1/129	0.5 (0 - 2.9)	>0.2
Type of vet visit	No pet	267/517	71/129	Ref	
- last 6-months	None	111/517	26/129	0.9 (0.5 - 1.4)	>0.2
	Short: GP	88/517	23/129	1.0 (0.6 - 1.6)	>0.2
	Short: ER or surgery	38/517	5/129	0.5 (0.2 - 1.2)	0.15
	Long: any	13/517	4/129	1.2 (0.3 - 3.4)	>0.2
Any treatment at vet	No pet	267/513	71/133	Ref	
- last 6-months	No vet	111/513	24/133	0.8 (0.5 - 1.3)	>0.2
	No treatment[‡]	47/513	3/133	0.2 (0.1 - 0.7)	0.019
	Yes treatment[‡]	88/513	35/133	1.5 (0.9 - 2.4)	0.094
Animal contact:	None reported	455/525	130/141	Ref	
- farm	Yes	70/525	11/141	0.6 (0.3 - 1.0)	0.078
Animal contact:	None reported	506/525	129/141	Ref	
- wild or other	Yes	19/525	12/141	2.5 (1.1 - 5.2)	0.018

[a] Included due to research question;[†] Not applicable included if there was no pet in the home, or if the person answering the questionnaire did not engage in activity; [‡] Treatment non-specified medication

3.4.2 Modelling and risk factor analysis

Regression modelling was used to estimate the odds ratios (ORs) for exposure variables. Adjusted ORs for variables included in the multivariate models are shown using the two case datasets where antimicrobial use was self-reported (n=141 cases, **Table 3.5**) and from a subset of this dataset (n=99 cases) where community prescriptions were accessed from the NZ government database (**Table 3.7**). Having a pet in the home was not found to be a significant risk factor in either dataset [adjusted OR: 0.8 (95% CI 0.4 – 1.9)]. Other exposures of interest were found to be associated with an increased risk, such as travel to Asia [adjusted OR: 9.9 (95% CI 4.0 – 26.8)], and antimicrobial treatment in the previous 3-months [adjusted OR: 15.3 (95% CI 6.0 – 43.1)].

Eight animal-related variables not related to having a family pet were examined, and two were associated with significant ORs and remained in the final adjusted questionnaire-only model. These were contact with livestock (farm animals) with a protective OR and contact with wild animals (or other animals not listed) as an increased risk. For contact with wild animals or 'other' animals in the previous six-months, an adjusted OR was 3.4 (95% CI 0.8 – 13.6), and for contact with farm livestock the previous six-months, an adjusted was OR 0.2 (95% CI 0 – 0.8). Other animal-related risks associated with the garden (univariate ORs in **Table 3.3**) were associated with increased risk. These risk factors were: seeing cat/dog faeces in the garden, feeding wild birds in the garden and having a birdbath. While none of the garden-related variables were included in the multivariate logistic regression model(s), cat/dog faeces seen in the garden was one of variable which remained in the LASSO regression model (OR = 1.1) when constrained to λ_{min} + 1 SE (**Table 3.12**)

In univariate screening of 21 pet-associated variables in this study, 10 were associated with the outcome variable with a p-value of ≤0.2 (**Table 3.4**). Protective odds were associated with: having non cat/dog pets (e.g. rodents, fish, birds, livestock), not handling pet faeces, "always" washing hands before feeding pets, "always" washing hands after feeding pets, "always" washing hands after petting cat/dog, pet having a short visit at the vet (surgery or emergency), and a pet visiting the vet but not receiving treatment. Associated with increased odds were including some raw food in cat's diet, cat hunting wildlife, handling of pet faeces, "often" or "never" washing hands after petting cat/dog, "often" washing hands after handling of pet faeces, and a pet visiting the vet and receiving treatment.

From univariate screening of variables, 12 personal healthcare variables were significant to a p-value of ≤0.2. Six of these variables were associated with chronic disease (**Table 3.2**), however only two variables were included in the final model [diabetes (adjusted OR=4.3; 95% CI 0.8 – 24.8) and 'other' chronic disease (adjusted OR=3.0; 95% CI 1.3 – 6.9)].

When adjusted for in the final model, being older and female were associated with having an ESBL-/ACBL-producing UTI [$OR_{age45to65}$=8.8 (95% CI 3.1 - 28.7) and OR_{age65+}=14.0 (95% CI 4.5 - 49.4); OR_{female}=24.7 (95% CI 7.9 - 97.8)]. Visiting a doctor in the previous six-months was associated with an increased risk [adjusted OR=33.6 (95% CI 8.4 - 192.4)], as was having more than one urinary tract in the preceding six months before incident UTI [adjusted OR=142.7 (95% CI 36.8 - 745.4)]. Handwashing was also associated with increased risk ("often" c.f. "always") in the adjusted model [OR=5.5 (95% CI 2.0 - 15.8)].

Five variables were associated with other types of healthcare exposure, including working in healthcare [with patient contact (adjusted OR=1.1; 95% CI 0.3 – 4.0) or without patient contact (adjusted OR=4.1; 95% CI 0.8 – 19.8)], and visiting a hospital or rest home in the previous six-months. Exposures relating to other people in the home that were significant to $p \leq 0.2$ on univariate screening, but were not significant in the final model were: being a visitor to a hospital or rest home in the previous 6-months, taking antimicrobials in the previous 6-months, or having a previous multidrug resistant infection.

Multivariate logistic regression of questionnaire results only

Table 3.5 Odds ratios (OR) for risk factors for urinary tract infections caused by ESBL- or ACBL-producing Enterobacteriaceae in the community, based on questionnaire data from 141 case and 525 control participants

Variable	Risk factor	Controls (n/N)	Case (n/N)	Crude OR (95%CI)	Adjusted OR (95% CI)
Gender	Male	251/525	8/141	Ref	Ref
	Female	274/525	133/141	15.2 (7.8 - 34.4)	24.7 (7.9 - 97.8)
Age	Age: 16 to 44 years	275/525	31/141	Ref	Ref
	Age: 45 - 64 years	161/525	53/141	2.9 (1.8 - 4.8)	8.8 (3.1 - 28.7)
	Age: 65+ years	89/525	57/141	5.7 (3.5 - 9.4)	14.0 (4.5 - 49.4)
Healthcare work	No	468/525	109/136	Ref	Ref
	Yes: no patients	15/525	15/136	4.3 (2.0 - 9.1)	4.1 (0.8 - 19.8)
	Yes: patients	42/525	12/136	1.2 (0.6 - 2.3)	1.1 (0.3 - 4.0)
Diabetes	None reported	514/524	126/136	Ref	Ref
	Yes	10/524	10/136	4.1 (1.6 - 10.1)	4.3 (0.8 - 24.8)
Other chronic disease	None reported	445/524	75/136	Ref	Ref
	Yes	79/524	61/136	4.6 (3.0 - 6.9)	3.0 (1.3 - 6.9)
>1 UTI last 6-months	None reported	509/524	64/134	Ref	Ref
	Yes	15/524	70/134	37.1 (20.6 - 71)	38.7 (12.0 - 150)
Doctor visit <6-months	None reported	260/525	10/138	Ref	Ref
	Yes	265/525	128/138	12.6 (6.8 - 26)	25.0 (7.4 - 107)
Antibiotics <6-months	>6 months or never	385/521	28/129	Ref	Ref
	3 to 6 months	72/521	19/129	3.6 (1.9 - 6.8)	2.6 (0.8 - 7.9)
	<3 months†	64/521	82/129	17.6 (10.8 - 29.6)	15.3 (6.0 - 43.1)
Travel to Asia	No	463/524	98/140	Ref	Ref
	Yes	62/525	43/140	3.3 (2.1 - 5.1)	9.9 (4.0 - 26.8)
Travel to Africa	No	519/524	133/140	Ref	Ref
	Yes	6/525	7/140	4.6 (1.5 - 14.4)	23.2 (0.6 - 520)
Handwashing: - before eating	Always	259/525	46/141	Ref	Ref
	Often	104/525	47/141	2.5 (1.6 - 4.1)	4.6 (1.7 - 12.6)
	Sometimes	137/525	39/141	1.6 (1.0 - 2.6)	2.9 (1.1 - 7.8)
	Never	25/525	9/141	2.0 (0.8 - 4.5)	1.4 (0.3 - 6.8)
Pet in home	No	267/525	71/139	Ref	Ref
	Yes	258/525	68/139	1.0 (0.7 - 1.4)	0.8 (0.4 - 1.9)
Animal contact: farm	No	455/525	130/141	Ref	Ref
	Yes	70/525	11/141	0.6 (0.3 -1.0)	0.2 (0 - 0.8)
Animal contact: wild	No	506/525	129/141	Ref	Ref
	Yes	19/525	12/141	2.5 (1.1 - 5.2)	3.4 (0.8 - 13.6)

Table 3.6 Descriptions and tests for the multivariate regression model in ***Table 3.5***

Model property	Index or score
Nagelkerke R-squared index	0.745
Area under ROC curve	0.966
Likelihood ratio chi-squared (14 d.f.; p-value <0.001)	377.94
AIC	232.19
Null deviance (629 d.f.)	586.62
Residual deviance (611 d.f.)	194.19
Missing observations	36

Multivariate logistic regression of subset where case prescription data was known

Additional prescription information from the national database (through case participant NHI number) was available for a subset of 99 clinical case participants. When reclassified with *antibiotic prescriptions in previous 6 months* as a binary variable, results for 88 individuals were used (both questionnaire and community prescription data was available). The questionnaire test sensitivity for this question was estimated to be 87%, while the specificity was 44% (i.e. >50% false positives where people reported antimicrobial use but no prescriptions were recorded in NZ government database). Results of this subset of data is summarised in **Tables 3.7**, **3.8, 3.9, 3.11 and 3.12**.

Adjusted OR for risk factors of interest (as described for the questionnaire-only dataset) in this model were: having a pet in the home [adjusted OR: 1.3 (95% CI 0.6 – 3.1)], travel to Asia [adjusted OR: 6.9 (95% CI 2.6 – 19.4)], and antimicrobial treatment in the previous 3-months [adjusted OR: 2.8 (95% CI 1.1 – 7.3)]. Of these, antimicrobial treatment in the previous 3-months was the most different to the OR given for the questionnaire-only dataset.

Table 3.7 Self-reporting vs government data (NHI) for systemic antimicrobial community prescriptions (n=99 cases)

Most recent antimicrobial treatment	Reported in questionnaire		Reported though NHI	
	n/N	% (95% CI)	n/N	% (95% CI)
None or >6months	27/88	31 (21 - 40)	57/99†	58 (48 - 67)
3 to 6 months	11/88	13 (6 - 19)	9/99	9 (3 - 15)
<3 months	50/88	57 (46 - 67)	33/99	33 (24 - 43)

† Including prescriptions in government database the week of clinical urinary sample submission

Table 3.8 Summary of characteristics of case-control subset where antimicrobial prescription history was known. Odds ratios (OR) are results of univariate and multivariate analysis.

Variable	Risk factor	Controls (n/N)	Case (n/N)	Crude OR (95%CI)	Adjusted OR (95% CI)
Gender	Male	251/525	8/99	Ref	
	Female	274/525	91/99	10.4 (5.3 - 23.7)	13.3 (4.5 - 48.9)
Age	Age: 16 to 44 years	275/525	16/99	Ref	
	Age: 45 - 64 years	161/525	37/99	3.9 (2.2 - 7.5)	9.2 (2.8 - 35.9)
	Age: 65+ years	89/525	46/99	8.9 (4.9 - 16.9)	17.9 (5.1 - 77.4)
Healthcare work	No	468/525	74/95	Ref	
	Yes: no patients	15/525	13/95	5.5 (2.5 -12.0)	2.8 (0.5 - 15)
	Yes: patients	42/525	8/95	1.2 (0.5 -2.5)	0.4 (0.1 - 1.9)
Diabetes	None reported	514/524	92/97	Ref	
	Yes	10/524	5/97	2.8 (0.9 -8.1)	3.4 (0.5 - 22.7)
Other chronic disease	None reported	445/524	56/97	Ref	
	Yes	79/524	41/97	4.1 (2.6 -6.6)	1.5 (0.6 - 3.6)
>1 UTI last 6-months	None reported	509/524	45/95	Ref	
	Yes	15/524	50/95	37.7 (20.1 - 74.6)	143 (37 - 745)
Doctor visit <6-months	None reported	260/525	7/96	Ref	
	Yes	265/525	89/96	12.5 (6.1 - 30.1)	33.6 (8.4 - 192)
Antibiotics <6-months	>6 months or never	385/521	57/99	Ref	
	3 to 6 months	72/521	9/99	0.8 (0.4 - 1.7)	0.3 (0.1 - 1.2)
	<3 months[†]	64/521	33/99	3.5 (2.1 - 5.8)	2.8 (1.1 - 7.3)
Travel to Asia	No	463/524	69/98	Ref	
	Yes	62/525	29/98	3.1 (1.9 - 5.2)	6.9 (2.6 - 19.4)
Travel to Africa	No	519/524	92/98	Ref	
	Yes	6/525	6/98	5.6 (1.7 - 18.4)	43.6 (2.8 - 493)
Handwashing:	Always	259/525	29/99	Ref	
- before eating	Often	104/525	37/99	3.2 (1.9 - 5.5)	5.5 (2.0 - 15.8)
	Sometimes	137/525	27/99	1.8 (1.0 - 3.1)	2.2 (0.8 - 6.5)
	Never	25/525	6/99	2.1 (0.7 - 5.4)	1.2 (0.2 - 5.5)
Pet in home	No	267/525	51/97	Ref	
	Yes	258/525	46/97	0.9 (0.6 - 1.4)	1.3 (0.6 - 3.1)
Animal contact: farm	No	455/525	90/99	Ref	
	Yes	70/525	9/99	0.7 (0.3 -1.3)	0.6 (0.2 - 2.2)
Animal contact: wild	No	506/525	89/99	Ref	
	Yes	19/525	10/99	3.0 (1.3 -6.5)	2.0 (0.5 - 8.7)

[†]For cases, this time-period is defined as prescriptions between 1 week and 3 months prior to collection of urine sample from which the ESBL/ACBL isolate was cultured. This was to exclude antimicrobials prescribed for incident case infection.

Table 3.9 Descriptions and tests for multivariate regression model in ***Table 3.8***

Model property	Index or score
Nagelkerke R-squared index	0.676
Area under ROC curve	0.957
Likelihood ratio chi-squared (14 d.f.; p-value <0.001)	281.08
AIC	216.96
Null deviance (608 d.f.)	482.91
Residual deviance (595 d.f.)	176.96
Missing observations	22

Population attributable fractions

Attributable fractions give an estimation of the population-level effect of exposures on the outcome, accounting for rarity of exposures. Risk factor variables were expressed as binary variable levels in **Tables 3.10** and **3. 11**.

Table 3.10 Population attributable fractions (AF) for binary outcomes [final model risk factor odds ratios (OR) recoded] from questionnaire data

Risk factor	OR AF model	AF (95% CI)	AF Pr(>|z|)
Pet in home	1.2	0.02 (-0.08 - 0.13)	>0.5
>1 UTI last 6-months	32.2	0.26 (0.18 - 0.35)	<0.001
Female	17.0	0.55 (0.38 - 0.72)	<0.001
Age 65+	3.0	0.11 (0.03 - 0.19)	0.007
Diabetes	6.3	0.03 (-0.01 - 0.06)	0.103
Other chronic disease	3.4	0.13 (0.05 - 0.22)	0.002
Doctor visit last 6-months	17.4	0.53 (0.37 - 0.68)	<0.001
Antibiotics last 3-months	6.4	0.2 (0.11 - 0.3)	<0.001
Healthcare worker	1.6	0.02 (-0.03 - 0.07)	0.406
Travelled to Asia	8.1	0.14 (0.07 - 0.21)	<0.001
Travelled to Africa	7.6	0.01 (-0.01 - 0.02)	0.281
Sometimes/never wash hands before eating	1.1	0.01 (-0.07 - 0.08)	>0.5
No contact with farm animals reported	5.7	0.38 (0.15 - 0.61)	<0.001
Contact with wild or other animals reported	3.8	0.03 (0 - 0.07)	0.066

Table 3.11 Population attributable fractions (AF) for binary outcomes [final model risk factor odds ratios (OR) recoded] from case-control subset where antimicrobial prescription data was known

Risk factor	OR AF model	AF (95% CI)	AF Pr(>|z|)
Pet in home	1.4	0.05 (-0.09 - 0.2)	0.464
>1 UTI last 6-months	43.0	0.35 (0.24 - 0.46)	<0.001
Female	10.4	0.53 (0.33 - 0.73)	<0.001
Age 65+	4.1	0.18 (0.07 - 0.29)	0.002
Diabetes	3.6	0.02 (-0.01 - 0.05)	0.282
Other chronic disease	1.9	0.08 (-0.02 - 0.17)	0.112
Doctor visit last 6-months	15.4	0.58 (0.39 - 0.78)	<0.001
Antibiotics last 3-months†	2.4	0.08 (0 - 0.16)	0.048
Healthcare worker	1.3	0.01 (-0.05 - 0.07)	>0.5
Travelled to Asia	6.1	0.14 (0.06 - 0.22)	<0.001
Travelled to Africa	19.8	0.02 (-0.01 - 0.05)	0.111
Sometimes/never wash hands before eating	0.9	-0.01 (-0.11 - 0.08)	>0.5
No contact with farm animals reported	2.6	0.26 (0.01 - 0.52)	0.045
Contact with wild or other animals reported	3.3	0.04 (-0.01 - 0.08)	0.092

†For case participants, this time-period was defined as prescriptions between 1 week and 3 months prior to collection of urine sample from which the ESBL/ACBL isolate was cultured. This was to exclude antimicrobials prescribed for incident case infection.

LASSO regression modelling

Results of LASSO regression models are described below in **Table 3.12.** The ORs described here are based on coefficients from these regression models with more conservative constraints (λ_{min}+1SE) or less conservative constraints (λ_{min}). As noted in **Table 3.12**, recent antimicrobial use does not appear as a risk factor variable in the conservative prescription dataset model.

Table 3.12 LASSO/elastic net regression model outputs where coefficients for the logistic model were used to calculate odds ratios (OR) for risk factors at the minimum lambda (λ_{min}) and at minimum lambda plus one standard error (λ_{min} + 1SE)

Risk factor variable	OR λ_{min} Questionnaire dataset	OR λ_{min} Prescription dataset	OR λ_{min} + 1SE Questionnaire dataset	OR λ_{min} + 1SE Prescription dataset
>1 UTI last 6-months	28.3	21.4	10.8	12.7
Doctor visit last 6-months	33.0	13.0	2.4	2.4
Travel to Asia	22.0	6.7	2.3	1.7
Other chronic disease	4.4	3.4	2.2	2.2
Antibiotics last 3-months[a]	8.8	1.9[†]	2.0	None[†]
Female	14.8	5.7	1.8	1.5
Contact with wild or other animals reported	1.2	1.8	1.3	1.4
Cat/dog faeces seen in garden	6.2	2.3	1.1	None
Often washes hands before eating	2.3	2.3	1.1	1.1
Travel to Africa	19.4	13.0	None	1.8

[a] Variable different in questionnaire and prescription datasets; [†]For cases in this prescription dataset, this time-period was defined as prescriptions between 1 week and 3 months prior to collection of urine sample from which the ESBL/ACBL isolate was cultured. This was to exclude antimicrobials prescribed for incident case infection.

Random forest regression

Random forest regression was carried out on all 85 examined variables. The top 15 variables from the questionnaire dataset that have the greatest decrease in either accuracy and/or Gini are described in **Table 3.13**. When these variables were examined for the prescription-only dataset, the most important variables for accurately predicting outcomes were: >1 UTI last 6-months, age, chronic disease score, and any chronic disease. The values for the decreases in accuracy were of the same order of magnitude as those shown in **Table 3.13**.

Table 3.13 Random forest regression of questionnaire dataset

Variable	Mean Decrease in Accuracy ($x10^{-2}$)	Mean Decrease in Gini ($x10^{-2}$)
>1 UTI last 6-months	4.63	7.17
Antibiotics last 3-months	2.09	4.04
Chronic disease score	1.62	3.83
Handwashing: after feeding pet	0.39	3.64
Handwashing: after petting/stroking pet	0.30	3.41
Age	0.84	3.27
Type of vet visit: last 6-months	0.50	2.75
Pet type	0.29	2.62
Travel to Asia	0.51	2.41
Any treatment at vet: last 6-months	0.32	1.62
Other chronic disease	0.62	1.39
Doctor visit last 6-months	1.23	1.27
Gender	1.42	1.17
Any chronic disease	1.28	0.99
Handwashing: before eating	0.10	4.52
Handwashing: after sneezing or blowing nose	0.00	4.11
Handwashing: after pet licks hands†	0.16	3.07
Healthcare work	0.15	3.00
Type of animal contact (non-pet)†	0.08	2.95
Handwashing: before handling food†	0.00	2.53

† Not significant ($p \leq 0.2$) on univariate screening of variables

Both the mean decrease in accuracy and the mean decrease in Gini described by the outputs of the random forest regression are measures of variable importance (Archer and Kimes 2008). An interpretation of the mean decrease in accuracy from **Table 3.13** is: when the variable ">1 UTI last 6-months" was removed from the model, the model was less accurate at prediction. For example in **Table 3.13,** having >1 urinary tract had the highest mean decrease in accuracy (4.63×10^{-2}) and was therefore the most important in assigning case vs control status. Random forest models for this dataset were consistently

better at assigning control status when compared to case, and this was described as class error. Class error was 0.38 for cases and 0.03 for controls.

3.4.3 Clinical bacterial isolates

From 141 clinical cases, clinical isolates were available from 132 people. The isolates from nine case people that were not available were reported as *E. coli* with an ESBL-producing phenotype based on results from the clinical microbiology laboratory (Labtests Auckland). Of the isolates that were available for sequencing, 126/132 were *E. coli*, 5/132 were *Klebsiella pneumoniae*, and 1/132 was *Morganella morganii*; 113/132 were ESBL-producing, and 12/132 had plasmid associated ACBL genes (excluding 4/131 ESBL-producing *E. coli* or *Klebsiella pneumoniae* isolates that were also co-producers of ACBL). Species identification reported here are from results of both MALDI-TOF and whole genome sequencing. Seven isolates (all *E. coli*) had non-plasmid associated ACBL genes only [found using CARD (Jia *et al.* 2017)]. Genes for two ESBL enzymes ($bla_{\text{CTX-M-15}}$ or $bla_{\text{CTX-M-27}}$) were found in 68% (90/132) of all clinical bacterial isolates, and 87/106 (82%) of ESBL-containing *E. coli* isolates.

E. coli isolates

A summary of 103/126 (82%) *E. coli* isolates is found in **Table 3.14**, and phylogenies of these bacteria described by ribosomal MLST and whole genome MLST in **Figures 3.1** and **3.2** respectively. Fifteen sequence types had more than one bacterial isolate with that type, and the isolates from these sequence types are described in **Table 3.14**. Clermont extended MLST phylogenetic groupings (A to F) were assigned by Warwick MLST seven-gene scheme for *E. coli* (Clermont *et al.* 2014; Clermont *et al.* 2015). Group B2 accounted for 65/126 (52%) of all *E. coli* isolates, group D accounted for 35/126 (28%) isolates, group B1 accounted for 10/126 (8%) isolates, group A accounted for 7/126 (6%),

and the remaining isolates were E, F, or not assigned. In phylogenetic group B2, there were eleven STs, with ST-12, ST-80, ST-131, ST-998, and ST-1193 all assigned to more than one isolate. In phylogenetic group D, there were seven STs, including ST38 and ST69. Bacterial isolate types not included in **Table 3.14** accounted for 22 MLST (n=23 isolates), and included an un-typed *E. coli* strain.

In silico serotyping of O- :H-antigens and *fimH* adhesion typing was done through the CGE pipeline (Thomsen *et al.* 2016). Two O:H types were found in *E. coli* ST-131 isolates (n=47 isolates), with O25:H4 accounting for 31/47 (66%) of isolates, and O16:H5 accounted for 16/47 (34%) of isolates. For ST-131 O25:H4 isolates, 30/31 (97%) were associated with *fimH*-30. For ST131 O16:H5, 15/16 (94%) were associated with *fimH*-41.

Table 3.14 Fifteen MLST types of E. coli from community acquired urinary tract infections in New Zealand (2015-2017)

Phylogenetic group†	Warwick MLST	Proportion % (95% CI)	*E. coli* isolates n (total =126)	Associated ESBL genes (n isolates)	Associated ACBL genes (n isolates)
A	744	1.6 (0 - 3.8)	2	*bla*$_{CTX-M-8}$ (n=1)	
				bla$_{CTX-M-14}$ (n=1)	
B1	410	1.6 (0 - 3.8)	2	*bla*$_{CTX-M-15}$ (n=1)	
				bla$_{CTX-M-55}$ (n=1)	
B1	648	2.4 (0 – 5.0)	3	*bla*$_{CTX-M-15}$ (n=2)	
				bla$_{CTX-M-55}$ (n=1)	
B1	345	1.6 (0 - 3.8)	2	*bla*$_{CTX-M-14}$ (n=1)	Chromosomal AmpC (n=1)
B1	58	1.6 (0 - 3.8)	2	*bla*$_{CTX-M-14}$ (n=1)	
				bla$_{CTX-M-55}$ (n=1)	
B2	12	2.4 (0 - 5)	3	*bla*$_{CTX-M-15}$ (n=1)	Chromosomal AmpC (n=2)
B2	80	1.6 (0 - 3.8)	2		Chromosomal AmpC (n=2)
B2	131	37.3 (28.9 - 45.7)	47	*bla*$_{CTX-M-14}$ (n=2)	*bla*$_{CMY-2}$ (n=1)
				bla$_{CTX-M-15}$ (n=21)	*bla*$_{CMY-60}$ (n=1)
				bla$_{CTX-M-27}$ (n=24)	
B2	998	1.6 (0 - 3.8)	2	*bla*$_{CTX-M-15}$ (n=2)	
B2	1193	4.0 (0.6 - 7.4)	5	*bla*$_{CTX-M-15}$ (n=2)	*bla*$_{CMY-2}$ (n=2) Chromosomal AmpC (n=1)
D	38	12.7 (6.9 - 18.5)	16	*bla*$_{CTX-M-14}$ (n=1)	
				bla$_{CTX-M-15}$ (n=7)	
				bla$_{CTX-M-24}$ (n=1)	
				bla$_{CTX-M-27}$ (n=6)	
D	69	6.3 (2.1 - 10.6)	8	*bla*$_{CTX-M-14}$ (n=1)	*bla*$_{CMY-2}$ (n=1)
				bla$_{CTX-M-15}$ (n=2)	
				bla$_{CTX-M-27}$ (n=3)	
				bla$_{CTX-M-55}$ (n=1)	
D	349	1.6 (0 - 3.8)	2		*bla*$_{CMY-2}$ (n=1)
					bla$_{DHA-1}$ (n=1)
D	405	2.4 (0 – 5.0)	3	*bla*$_{CTX-M-14}$ (n=1)	Chromosomal AmpC (n=1)
				bla$_{CTX-M-15}$ (n=1)	
D	963	3.2 (0.1 - 6.2)	4		*bla*$_{CMY-2}$ (n=4)
				bla$_{CTX-M-27}$ (n=2)	

†As in Clermont *et al.* (2015)

Non-E. coli isolates

Four unique MLST were found in *K. pneumoniae*, with ST-562 isolated from more than one individual's sample (n=2). Of all the *K. pneumoniae* isolates, 3/5 (60%) had a $bla_{\text{CTX-M-15}}$ gene; 1/5 (20%) was found to have a $bla_{\text{CTX-M-14}}$ gene; and 1/5 (20%) was found to have a $bla_{\text{SHV-27}}$ gene. An ACBL gene ($bla_{\text{DHA-1}}$) was found in 1/5 (20%) of *Klebsiella* isolates. One community-acquired UTI included in this study had an isolate of *Morganella morganii* cultured from a urine sample. This isolate contained both ESBL and ACBL genes, $bla_{\text{VEB-1}}$ and $bla_{\text{DHA-5}}$ respectively, however $bla_{\text{DHA-5}}$ is not considered plasmid associated in *Morganella* as bla_{DHA} genes originate from *Morganella* species (Jacoby 2009).

Antimicrobial susceptibility testing

Combined disk tests (CDT) using cefotaxime and ceftazidime were used for phenotype confirmation of ESBL-production (Anonymous 2013a). ESBL phenotypic CDT assay results for *E. coli* isolates were positive with the ceftazidime (CAZ) test for 87/126 (69%) of isolates, and 103/125 (82%) of isolates were positive with the cefotaxime (CTX) test (one ACBL-producing isolate not tested against CTX). When ACBL isolates were not included, these proportions were 87/106 (82%) and 103/106 (97%) respectively, with three isolates positive on the CAZ test, but not on the CTX test. For *Klebsiella pneumoniae* isolates, all were positive with the CTX test, and 3/5 (60%) were positive with the CAZ test. The *Morganella morganii* isolate was positive with the CAZ test, but not the CTX test.

Cefoxitin is used as a screening test (**Table 3.1**) for ACBL-producing isolates; however, in this study eligible isolates were tested for an ACBL-producing phenotype prior to screening against the panel of antimicrobials, which included cefoxitin. Of *E. coli* isolates resistant to cefoxitin, 22/33 (67%) phenotypically positive for ACBL using the D69C disk

assay (Halstead *et al.* 2012); 2/93 (2%) of *E. coli* isolates that were susceptible to cefoxitin were also were phenotypically positive for ACBL. When an isolate each of *Klebsiella pneumoniae* and *M. morganii* were included, 26/132 of clinical bacterial isolates were phenotypically positive for ACBL. Of these, 17/26 (65%) of isolates were found to have bla_{CMY} or bla_{DHA} genes.

An MDR phenotype was observed in 76/132 (60%) of all clinical isolates. For *E. coli*, 51/126 (40%) of isolates were resistant to beta-lactam, trimethoprim, and fluoroquinolone antimicrobials; 23/126 (18%) of isolates were resistant to beta-lactam, aminoglycoside, trimethoprim, and fluoroquinolone antimicrobials. Individual antimicrobial resistance rates for *E. coli* isolates are shown in **Table 3.15**.

Table 3.15 Antimicrobial susceptibility test results from 126 clinical urinary E. coli isolates with an ESBL- and/or ACBL-producing phenotype from the NZ community (EUCAST 2018)

Antimicrobial (disk dose)	Isolates resistant n	Proportion resistant % (95% CI)
Augmentin (amoxicillin 20ug + clavulanic acid 10ug)	70	55.6 (46.9 - 64.2%)
Ampicillin (10ug)	121	96 (92.6 - 99.4%)
Cephalexin (30ug)	121	96 (92.6 - 99.4%)
Cefpodoxime (10ug)	125	99.2 (97.7 - 100%)
Ceftriaxone (30ug)	115	91.3 (86.3 - 96.2%)
Cefoxitin (30ug)	33	26.2 (18.5 - 33.9%)
Mecillinam (10æg)	12	9.5 (4.4 - 14.6%)
Ertapenem (10ug)	13	10.3 (5.0 - 15.6%)
Amikacin (30ug)	2	1.6 (0 - 3.8%)
Gentamicin (10ug)	45	35.7 (27.3 - 44.1%)
Fosfomycin (200ug)	8	6.3 (2.1 - 10.6%)
Trimethoprim (5ug)	86	68.3 (60.1 - 76.4%)
Nitrofurantoin (100ug)	4	3.2 (0.1 - 6.2%)
Norfloxacillin (10ug)	59	46.8 (38.1 - 55.5%)

For *E. coli* ST131 isolates, 33/47 (70%) of isolates were resistant to norfloxacin, 17/21 (81%) of those with $bla_{CTX\text{-}M\text{-}15}$ genes and 14/24 (58%) of those with $bla_{CTX\text{-}M\text{-}27}$ genes were

resistant to norfloxacin. Resistance to trimethoprim was found in 40/47 (85%) of these isolates, and resistance to gentamicin was found in 22/47 (47%) of ST131 isolates [11/21 (52%) and 9/24 (38%) resistance for $bla_{\text{CTX-M-15}}$ and $bla_{\text{CTX-M-27}}$ containing isolates respectively]. All ST131 isolates were susceptible to nitrofurantoin, and 1/47 (2%) was resistant to fosfomycin. Antimicrobial resistance genes for *E. coli* isolates found through ResFinder are shown in the presence-absence matrix in **Figure 3.2**. All five *K. pneumoniae* isolates were resistant to ampicillin, cephalexin, cefpodoxime, ceftriaxone, and trimethoprim. None of these same isolates were resistant to mecillinam or amikacin. Antimicrobial resistance genes for *K. pneumoniae* isolates found through ResFinder are shown in a presence-absence matrix in **Figure 3.3** (Zankari *et al.* 2012).

Virulence genes

Presence and absence of virulence genes found through VirulenceFinder database is shown in the purple presence-absence matrix in **Figure 3.2** (Kaper *et al.* 2004; Joensen *et al.* 2014). An acid resistant genotype (glutamate decarboxylase) conferred by a *gad* gene was found in 125/126 (99%) of *E. coli* isolates. An increased serum survival genotype conferred by an *iss* gene was found in 78/126 (62%) of *E. coli* isolates. An adherence protein *iha* gene was found in 69/126 (55%) of *E. coli* isolates. A serine protease autotransporter *sat* gene was found in 62/126 (49%) of *E. coli* isolates. A plasmid-associated enterotoxin *senB* gene was found in 61/126 (48%) of *E. coli* isolates.

3.4.4 Phylogenies of clinical bacterial isolates

Describing the relatedness of clinical bacterial isolates from case participants in this study is shown as whole genome MLSTs in **Figures 3.2** and **3.3**. The relationship between *E. coli* isolates are shown in **Figures 3.1** (rMLST) and **3.2**, and for *Klebsiella pneumoniae* in **Figure 3.3**

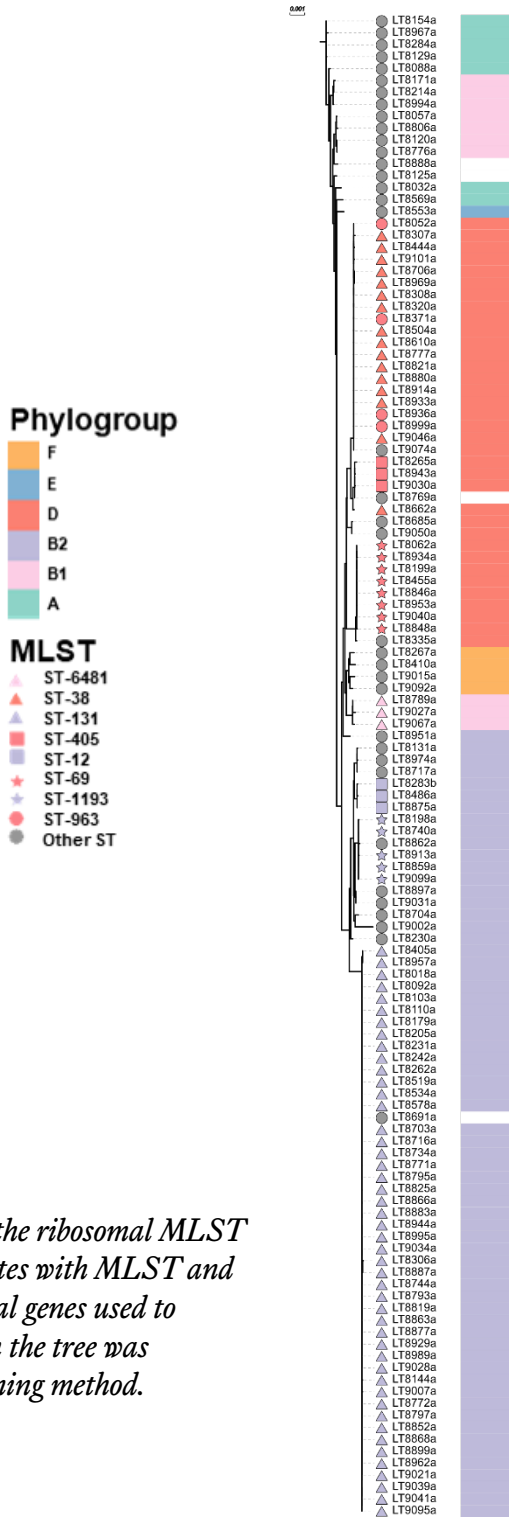

Figure 3.1 Neighbour joining tree of the ribosomal MLST (51 genes) of 126 clinical E. coli isolates with MLST and Clermont phylogrouping. 51 ribosomal genes used to construct distance matrix from which the tree was constructed using the Neighbour Joining method.

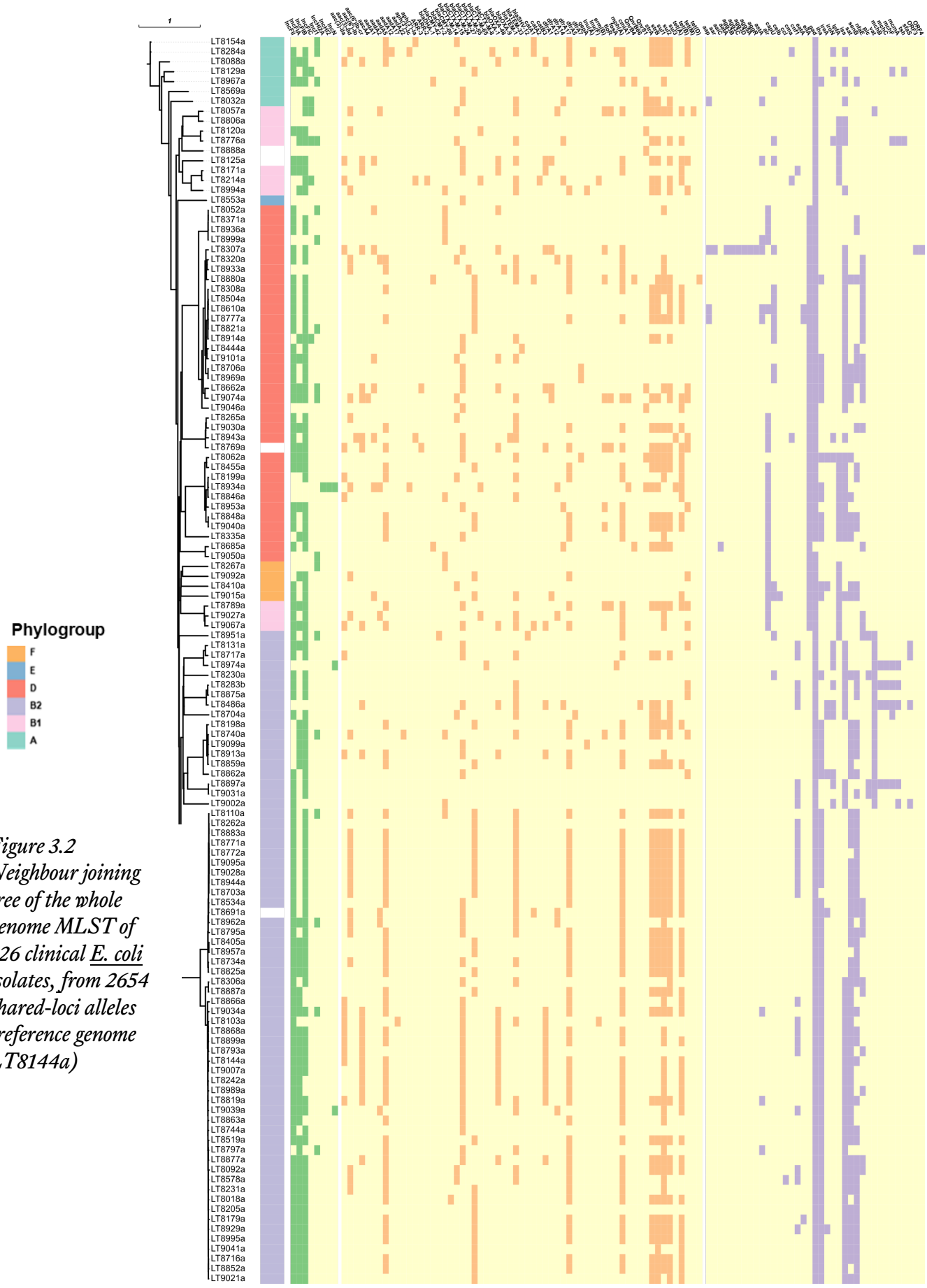

Figure 3.2 Neighbour joining tree of the whole genome MLST of 126 clinical E. coli isolates, from 2654 shared-loci alleles (reference genome LT8144a)

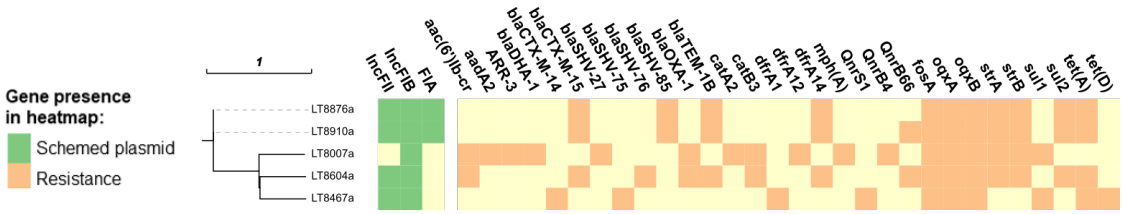

Figure 3.3 Neighbour joining tree of cgMLST of five clinical Klebsiella pneumoniae isolates, from 4254 shared-loci alleles (reference genome LT8007a)

3.5 Discussion

Companion animals in the home were not found to be a risk factor for community-acquired ESBL-/ACBL-producing urinary tract infections in this study. However, previous urinary tract infections, healthcare contact, and travel to Asia or Africa were associated with increased odds of having a community-acquired ESBL-/ACBL-producing urinary tract infection. Gender (being female), being older, and having chronic disease were also associated with having a community-acquired ESBL-/ACBL-producing urinary tract infection. Variables not associated with increased risk included ethnicity, nearest school decile (used as a proxy for social deprivation), and whether or not someone lived in a rural or urban area.

In general, the risk factors associated with increased odds of ESBL infection in this study have been previously described. There are a number of studies where acquisition of antimicrobial resistant bacteria is associated with travel to high-risk global regions, antimicrobial treatment while traveling, and/or gastrointestinal disease while traveling (Freeman *et al.* 2008; Rogers *et al.* 2014; Arcilla *et al.* 2017). Travel to high-risk global regions has been defined in other studies as travel to North Africa, the Indian subcontinent, southeast Asia, the Middle East, and China (Kennedy and Collignon 2010; Arcilla *et al.* 2017). These areas are associated with higher rates of gastrointestinal colonisation with ESBL-producing Enterobacteriaceae (Karanika *et al.* 2016), and is

associated with carriage in travellers returning from visiting such places (Arcilla *et al.* 2017). In the study presented in this chapter, travel to Asia was significantly associated with having a UTI caused by ESBL- and/or ACBL-producing bacteria [adjusted OR = 9.9 (95% CI 4.0 - 26.8); PAF = 0.14 (95% CI 0.07 - 0.21)]. Travel to Africa was only significantly associated with risk in the smaller government prescription dataset (**Table 3.8**).

As noted above, there was some internal disagreement noted between the models within this chapter when examining travel-associated risk factors. LASSO logistic regression modelling found increased odds associated with travel to Asia (all parameters and both datasets described in **Table 3.12**). However, travel to Africa was not associated with increased risk in the conservative model (λ_{min} + 1SE) when using the questionnaire dataset. Travel to Asia (but not travel to Africa) was also among the top ten variables (in terms of effect on outcome) within the random forest regression. The recurrence of Asian travel in all these modelling modalities reinforces the inference of increased risk associated with travel to this continent within this study. As this finding reflects findings from other low-prevalence countries (Rogers *et al.* 2014; Karanika *et al.* 2016; Arcilla *et al.* 2017), it is likely to be widely applicable to the New Zealand population.

Healthcare exposures, including antimicrobial treatment are well described as risk factors for carriage of ESBL-producing bacteria (Karanika *et al.* 2016). They have also been described in a study looking at risks for community-acquired infection, where healthcare exposure in the previous six-months, urinary tract infection in the previous year, and antimicrobial treatment in the previous six-months were all associated with increased risk (Rogers *et al.* 2014). All these risk factors were also identified by multiple modelling methods in the study presented in this chapter. However, antimicrobial treatment in

previous three-months was not found to be a risk factor in the more penalised LASSO regression model (λ_{min} + 1 SE), although it was significantly associated with risks in other models. In multivariate regression models when prescription data was available (for the NHI subset of cases), an adjusted OR of 2.8 (95% CI 1.1 - 7.3) was observed for the risk factor of antimicrobial prescription in the previous three-months. This is less than the adjusted OR of 15.3 (95% CI 6.0 - 43.1) for the same risk factor when case participants self-reported this exposure. This difference shows the importance of validating self-reporting of medication data, especially when it is considered an exposure of interest.

The outputs from random forest regression and LASSO regression, where no differential weighting of variables to test specific hypotheses were imposed upon the models as compared to multivariate regression, varied slightly in the order of the most important variables for predicting the outcome. However, there were areas of crossover between these two model outputs, and the results of the multivariate logistic regression. The consistency of these findings indicates that they are likely non-spurious. The variables important to multivariate logistic regression, LASSO regression, and random forest regression were: (1) being over 65-years old; (2) being female; (3) having more than one urinary tract infection in the previous six-months; (4) travel to Africa and/or Asia in the previous 12-months; (5) contact with healthcare (as a patient) in the previous six-months; (6) contact with wild animals in the previous six-months; (7) having an "other" chronic disease (not asthma, diabetes, COPD, or cardiac disease); (8) not "always" washing hands before eating.

Some variables identified in the random forest regression as important were not detected as significant in either the multivariate or the LASSO regressions. Some of these variables were related to companion animal contact (handwashing behaviour) and animal contact

with veterinary care (**Table 3.13** for results of random forest and **3.4** for animal related univariate ORs). These findings may be spurious, but they also may indicate some low or marginal level of risk associated with animals, or indeed be a result of collinearity or interaction in the LASSO regression. Verification of collinearity/interaction may be possible but was not attempted here. As with other countries, it is likely that the prevalence of these bacteria will increase in both human and animal populations will increase in time, and therefore community-wide risk factors will change as a result (Nicolas-Chanoine *et al.* 2013).

Companion animals were not found to be a risk for ESBL-/ACBL-producing infection. Along with the results of the case control study presented here, other studies have also not found pets to be a risk factor for non-healthcare associated ESBL carriage or infection (Rogers *et al.* 2014; Leonard *et al.* 2018). There was no substantial difference between the OR for having a pet in the home between the questionnaire dataset and the government prescription dataset [OR=0.8 (95% CI 0.4 – 1.9) and OR= 1.3 (95% CI 0.6 - 3.1) respectively], and these results are therefore internally consistent.

Where bacterial isolates from the initial bacterial infection were available, over a third of isolates were *E. coli* ST131 [47/132 (36%) of all isolates and 47/126 (37%) of *E. coli* isolates]. This finding is consistent with previous reports on community-acquired infection in New Zealand (Dyet *et al.* 2014; Drinkovic *et al.* 2015; Heffernan *et al.* 2018). ST131 isolates were more resistant to norfloxacin and trimethoprim when compared to *E. coli* isolates as a whole (70% c.f. 47% for norfloxacin, and 85% c.f. 68% for trimethoprim). As a whole, the *E. coli* isolates in this study contained between one and 15 virulence genes (**Figure 3.2**). Many of these genes promote intestinal colonisation (e.g. *gad* found in 99% of isolates), and allow the bacteria to live commensally prior to invasion/colonisation/infection in the

lower urinary tract (Kaper *et al.* 2004; Schmidt *et al.* 2015). The bacterial factors enabling infection may be related to those contributing to subsequent infection (such as the virulence gene *sat* for an autotransporter toxin that was found in 49% of *E. coli* isolates) (Kaper *et al.* 2004). The findings presented in this chapter show that while there is substantial diversity among *E. coli* isolates causing UTI in the New Zealand community, these bacteria generally contain multiple AMR and virulence genes.

There were a number of limitations within this study. Sources of bias in this study included known information (and possibly recall) biases associated with the questionnaire, non-response bias for cases, and the fact that missing data from the questionnaire was not imputed in this study. The most tangible of these biases was inaccurate reporting of antimicrobial use found for the subset of 70% (99/141) of case participants. Self-reporting of antimicrobial use in case participants provided an over-estimate of the number of antimicrobial treatments in the six-months before their urinary tract infection [44% specificity of this question in questionnaire (**Table 3.7**)]. A comparable test was not performed for control participants, as the same prescription data was not available. Discrepancies in reporting prescription data have been reported elsewhere (West *et al.* 1995). However, the inverse of what was found in this study (i.e. under-reporting of prescriptions) was reported in West *et al.* (1995). This finding is likely due to both the wording of the questions within the questionnaire, and to other factors such as some uncertainty by participants about when their infection occurred (and this information was not available to help prompt them at the time of questionnaire delivery). The questionnaire used in this study asked case recipients to answer questions relating to time prior to their index case infection. However, it can be assumed that the majority of this over-reporting is related to people thinking of the prescription they received for the

index infection. In order to remove these prescriptions for contemporaneous UTI from the NHI prescription dataset, those filled within the seven-days prior to laboratory submission of urine were not included in the regression models. The assumption here was that people would be likely prescribed antimicrobials for symptomatic UTI (or after results of urine dipstick analysis) at the same time, and therefore start treatment before, the lab received a sample.

Another limitation of this study is around the selection of controls. All controls were assumed to be non-carriers of ESBL/ACBL-E, although it is possible that up to 10% had faecal carriage of ESBL-*E. coli* (Woerther *et al.* 2013). In general, reports of carriage of ESBL-E varies between regions (4 - 46%), with a pooled prevalence from 66 studies of 14% (Karanika *et al.* 2016). However, the community carriage prevalence of ESBL/ACBL-E in New Zealand was not known at the time of this research, and too few control participants volunteered to give faecal samples to assess this in the cross-sectional part of the study (described further in **Chapter 4**). The general results of this study indicate that future work with a focus on community-level prevalence of ESBL-ACBL-producing bacteria is needed.

In some studies, length of colonisation of ESBL-producing *E. coli* has been reported up to (or exceeding a year), resulting in a longer window for extra-intestinal colonisation and/or infection (Bar-Yoseph *et al.* 2016). In a study looking at acquisition associated with travel, 11% of people who acquired an ESBL-producing *E. coli* were still carrying the same strain 12-months later (Arcilla *et al.* 2017). The knowledge of time between travel and infection with ESBL-E is valuable information; however, this was not recorded in this study. Additionally, gathering exact details of travel, and any illness or treatments while travelling, would have been valuable in quantifying risk but were not collected as part of

this study but would have been valuable. Recent/prior gastrointestinal distress or medication with proton-pump inhibitors was not investigated in the study presented here, however should be included in future studies investigating community-acquired ESBL-producing infections as they are well-described risk factors (Kuenzli *et al.* 2014; Arcilla *et al.* 2017). Alongside general carriage of these bacteria, an important contributor to multidrug resistance in a population of bacteria are the transferrable elements that allow for horizontal transmission of resistance genes. In the case of ESBL-/ACBL-producing Enterobacteriaceae, plasmids that carry *bla*$_{\text{ESBL}}$ genes. Plasmids were not closely evaluated in this study, and no discrete plasmid isolation and/or sequencing was performed. This should be considered as a priority for future work in this area.

3.6 Conclusions

Although no pet-related factors were found to be significantly associated with ESBL- and/or ACBL-producing UTI in people, some of these factors may be part of a more complex dynamic where domestic animals are part of risk for human infection and/or carriage. It is also possible that pets in the home are a protective factor and their presence dilutes the resistant bacterial population in the home by acting as a source of non-resistant bacteria. The benefits of pet ownership can be immense, especially in older or vulnerable populations, and while zoonotic disease in general should be considered when having close contact with animals, the risk of transmission of ESBL-/ACBL-producing Enterobacteriaceae is low.

4 Transmission of extended spectrum beta-lactamase- and AmpC beta-lactamase-producing *Escherichia coli* between people with a community-acquired urinary infection, their family, and pets in the household: The "resist-home"

4.1 Abstract

The objective of this study was to evaluate transmission of extended spectrum beta-lactamase *Escherichia coli* (ESBL-EC) or AmpC beta-lactamase *E. coli* (ACBL-EC) within households recruited in **Chapter 3**. From cases from these households, a bacterial culture of urine was performed as part of routine diagnostic work-up. In addition, faecal samples from people and pets in the household were collected and screened for ESBL- and/or ACBL-producing Enterobacteriaceae. *E. coli* isolate DNA from these households were sequenced on the Illumina HiSeq and MiSeq platforms. Comparative genomic analysis of *E. coli* genomes from households was conducted using nucleotide, whole genome, and pan-genome analyses.

Twenty-three households were recruited for cross-sectional faecal sampling between December 2015 and January 2017. Twenty of these households submitted samples from pets, and 11 households submitted faecal samples from other people (non-case people) living in the home. Of these, seven households had family members and four had pets' positive for ESBL-EC and/or ACBL-EC. Whole genome sequencing of 125 *E. coli* isolates from these 11 households (7-28 isolates per household) showed eight households (35%)

where more than one person or pet was positive for the same strain of ESBL- and/or ACBL-EC; two of these households included a pet sharing the same strain. In three households, different strains of ESBL- and/or ACBL-EC were cultured from each person or pet.

In a third of households where a person acquired an ESBL-EC or ACBL-EC infection from the community, co-habiting people or pets also had genetically similar *E. coli* in their faeces. This suggests that the AMR bacteria circulate through homes, and contribute to community transmission of ESBL- or ACBL-producing *E. coli*.

4.2 Introduction

The home environment is a place of comfort and security. However, it is also a place for communities of potentially life-threatening microbes to be shared between human and (human or pet) family members. Antimicrobial resistant (AMR) bacteria are one such threat. The known promiscuity of some AMR-containing plasmids means they are found in a variety of bacterial strains, and therefore, may be carried in turn by diverse hosts (Mathers *et al.* 2015).

Escherichia coli is a commensal organism, found both inside and outside the mammalian large intestine. It is commonly used as an indicator of faecal contamination (Edberg *et al.* 2000); however, *E. coli* is also observed (along with other Enterobacteriaceae) as an oropharyngeal coloniser (Sokurenko *et al.* 1998; Lemon *et al.* 2010). Bacterial resistance to antimicrobials, namely resistance to extended spectrum cephalosporins by Enterobacteriaceae, results in infections that are difficult to treat (Levy and Marshall 2004; Livermore *et al.* 2007). Since the turn of the 21st century, enzymes such as AmpC beta-lactamases (ACBL) and extended spectrum beta-lactamases (ESBL) in disease-

causing bacteria have increasingly been reported, as discussed in **Chapter 2** (Bradford 2001; Toombs-Ruane *et al.* 2017). These bacteria are often resistant to multiple classes of antimicrobials, in addition to beta-lactams, and are therefore considered multidrug resistant (MDR) (Magiorakos *et al.* 2012).

ESBL- and AmpC- producing Enterobacteriaceae, predominantly *E. coli* and to a lesser extent *Klebsiella pneumoniae*, are the most common cause of MDR urinary tract infections (UTI) in the New Zealand community (Heffernan *et al.* 2009; Drinkovic *et al.* 2015). Surveillance of these bacterial infections from 2011 to 2014 showed an increasing rate of these infections in New Zealand, especially in the Auckland regional district health board areas (Heffernan *et al.* 2013; Heffernan *et al.* 2018). In comparison to human infections, antimicrobial resistance surveillance data is 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 (Karkaba *et al.* 2017b; McMeekin *et al.* 2017).

Animal to human transmission and human to animal transmission of AMR bacteria, mean that interventions such as therapeutic antimicrobial use (and therefore selective or altered colonisation of the individual's microbiota) in either a pet or a person may affect the microbiome of those they live with. Sharing of genetically similar *Staphylococcus aureus* between pets and people in homes was observed in an MRSA study; carriage of clonal *S. aureus* at the same time was observed in 57% of positive humans and pets (van Balen *et al.* 2017). The association had been also found with *Staphylococcus* species in other studies (Weese *et al.* 2006; Hanselman *et al.* 2009; Walther *et al.* 2012). Reverse zoonotic transfer has also been implicated in MRSA transfer events (van Duijkeren *et al.* 2004; Weese *et al.* 2006).

Human to human transmission within the household is also an important pathway for spread of AMR bacterial spread within the community. Family members can share faecal and oral bacteria (Shaffer and Lozupone 2018), and the likelihood of sharing means that potentially pathogenic bacteria harboured quiescently by one individual could be shared with (and consequently cause disease in) a person (or pet) that they live with (Löhr *et al.* 2013; Holmes *et al.* 2016). In a country with relatively low prevalence of carriage of extended-spectrum beta-lactamase producing *E. coli* such as New Zealand (Rogers *et al.* 2014; Rogers *et al.* 2015), the family may be an important reservoir of antimicrobial resistant bacteria (Karkaba *et al.* 2017b). Isolation of closely related AMR bacteria from co-habiting people has been demonstrated with methicillin resistant *S. aureus* (Miller *et al.* 2012; Knox *et al.* 2015). Transmission of ESBL-producing ST131 *E. coli* within households has also been observed (Johnson *et al.* 2016), in addition to pathogenic urinary tract infection-causing *E. coli* being shared between sexual partners (Ulleryd *et al.* 2015).

4.2.1 Study objective

This study aimed to evaluate transmission of ESBL- or ACBL-producing Enterobacteriaceae, and more specifically *E. coli*, between individuals within households. The particular focus of the extended molecular work presented here is around the genomic relatedness of bacterial isolates from within households where *E. coli* was cultured from multiple individuals (people and/or pets) including an index person with an ESBL- or ACBL-producing urinary tract infection.

4.3 Materials and methods

4.3.1 Study population

Ethics

Human ethics for this study was granted by the New Zealand Health and Disability Ethics Committee (HDEC) under the reference 15/CEN/47; this study was part of a larger prospective case control study looking at risks for community-acquired UTI (**Chapter 3**). Animal ethics for sampling via rectal swab from cats was granted by Massey University Animal Ethics Committee under the reference 15/35.

Study participants

Case participants were recruited into this study from laboratory results submitted to Labtests Auckland (Healthscope) between 28 September 2015 and 5 September 2017 in the Auckland and Northland regions of New Zealand. Prospective case participants were eligible if this was a new infection with an ESBL- or AmpC-producing Enterobacteriaceae, they had no record of being hospitalised overnight in the previous 12-months, and they did not have a residential address in a residential elder care facility or rest home. Case participants were invited to submit faecal samples from themselves, as well as any cat, dogs, or birds that lived with them in the home. Invitations were also given to other members of the household to submit a faecal sample at the same time as case participants, and their pets. Information on age, gender/sex, recent antimicrobial treatment, and recent hospitalisation/veterinary care was also requested with submission of faecal samples.

4.3.2 Microbiologic methods

Bacterial culture and identification

Bacterial isolates from index case people's urine samples were collected as part of **Chapter 3** (see **Section 3.3.3** for methodology). Faecal 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, Mantua, Italy), and by collection of faecal samples by the participant with instructions provided on how they could do this cleanly. Some direction on using cling-film and a sterile faecal collection bottle was provided to participants for sample collection. Only one faecal sample was accepted for each individual; where more than one was provided, the most recent sample was used. Once collected, these samples were stored between approximately 4 and 15 °C for up to 48 hours before being plated onto culture media as described in **Appendix I**. These culture media were: plain MacConkey (BD Difco™ supplied by Fort Richard, Auckland, New Zealand); MacConkey with 1mg/L cefotaxime sodium (Sigma-Aldrich, St. Louis, U.S.A); MacConkey with 1mg/L ceftazidime pentahydrate (Sigma-Aldrich); chromogenic ESBL CHROMagar (CAC, Becton Dickinson, Heidelberg, Germany; supplied by Fort Richard). Rectal swabs taken from cats, and faecal samples from pet birds were enriched in buffered peptone water for approximately 16 hours at 35 (+/- 1) °C before being plated onto culture media. Agar was incubated overnight at 35 (+/-1) °C for 20 to 24 hours and single colonies were selected for subculture onto Columbia horse blood agar (Fort Richard). These colonies were chosen if they were lactose fermenting on MacConkey agar, or pink/purple/blue on ESBL CHROMagar. Up to eight colonies were chosen for subculture from each faecal sample, two from each plate, and labelled 'a' to 'g'. Identification of bacterial species from both urine and faeces was done using matrix

assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry [faecal isolates (bioMérieux, Marcy-l'Étoile, France), urine isolates (Bruker, Billerica, U.S.A.)]. Both faecal and urinary isolates were subsequently stored as pure cultures in glycerol broth suspensions (in-house formulation, ᵐEpiLab) at -80°C prior to subsequent phenotypic tests (where appropriate) and genomic DNA extraction.

Antimicrobial susceptibility tests

Faecal isolates were tested for an ESBL-producing phenotype using three paired disk tests [D62C ESBL cefotaxime Paired ID disks (Mast group, Bootle, U.K.; supplied by Fort Richard, Auckland, New Zealand); D63C ESBL cefepime Paired ID disks (Mast group); D64C ESBL ceftazidime Paired ID disks (Mast group)]. Urine isolates were tested for ESBL-producing phenotype using two paired disk tests [D62C ESBL cefotaxime Paired ID disks (Mast group); D64C ESBL ceftazidime Paired ID disks (Mast group)]. All isolates were also tested for an AmpC-producing phenotype using a three-disk comparison assay [D69C AmpC disk test (Mast group)]. Subsequent antimicrobial susceptibility testing of both urine and faecal isolates was performed using the panel and as described in section **3.3.2**.

4.3.3 Extraction and preparation of genomic DNA for sequencing

Isolates were selected for whole genome sequencing where multiple individuals in a household had at least one ESBL- or AmpC- producing Enterobacteriaceae isolate (**Figure 4.1).** Bacterial isolates were cultured from frozen (stored at -80°C in nutrient/glycerol broth) and a single colony subcultured and incubated for 20-24 hours at 35 (+/- 1) °C, after an initial revival on Colombia horse blood agar. Extraction of genomic DNA was done using a QIAamp DNA mini kit (Qiagen, Hilden, Germany) according to a modified and optimised version of the tissue/bacterial colony protocol as described in

Appendix II. A PCR-grade water eluate was used as part of the final step in DNA extraction, and this was stored at -20 °C prior to library preparation. Fluorescent spectrometry (Qubit 2.0 Fluorometer, Thermo Fisher Scientific, Waltham, U.S.A) was used to assess the quality of the extraction, and DNA was normalised to a concentration of 0.16ug/mL prior to preparation of libraries (Nextera library preparation kits, Illumina Inc., San Diego, U.S.A.). Libraries were prepared at Massey Genome service (when sequenced on Illumina MiSeq, by Massey Genome Service), or at ^{m}EpiLab (when sequenced on Illumina HiSeq at Otago Genomic and Bioinformatics facility). Assessment of library quality and fragment size distribution was performed by an automated gel fragment analyser [Labchip GX Touch HT (PerkinElmer, PerkinElmer, Waltham, U.S.A.); or 2100 BioAnalyzer (Agilent, Santa Clara, U.S.A.)].

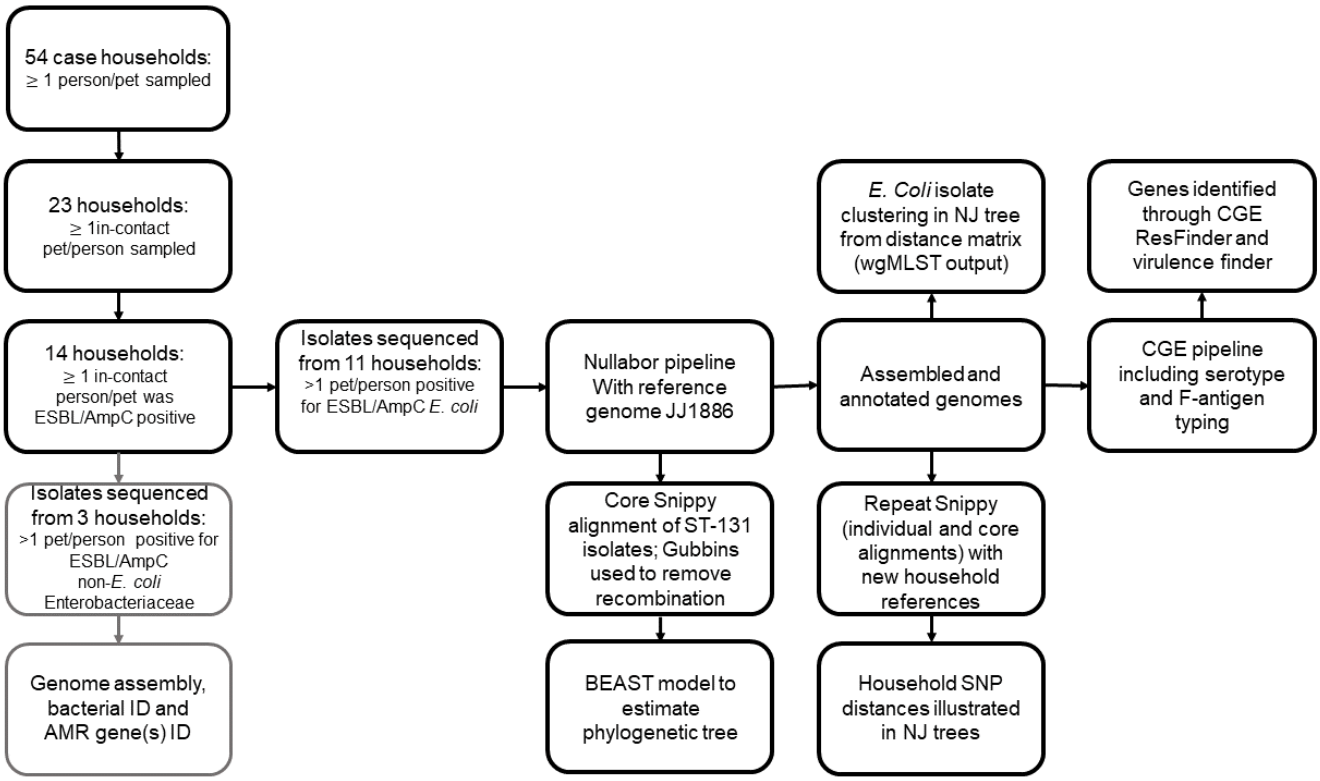

Figure 4.1 Bacterial genome outputs workflow: selection of households and processing of isolates after sequencing

4.3.4 Next generation sequencing

Sequencing of genomic DNA was performed by Massey Genome Services (New Zealand Genomics Limited, Massey University, New Zealand) for Illumina MiSeq; Illumina HiSeq was performed by Otago Genomic and Bioinformatics facility (New Zealand Genomics Limited, Otago University, New Zealand).

4.3.5 Data analysis

Sample and isolate metadata was stored on a MySQL database (Oracle Corporation, CA, USA), and Microsoft Access used for input interface and query management (Microsoft Corporation, WA, USA); data were manipulated in R-Studio, (Boston MA, USA; R version 3.4.3 https://www.r-project.org) and Excel (Microsoft corporation, WA, USA).

4.3.6 Bioinformatics

Raw sequence reads were assessed for quality, and processed [using QCtool: Solexxa, PhiX adapter removal (Truglio 2016)] prior to going into the Nullarbor bioinformatics pipeline (Seemann *et al.* 2016). Assembly of genomes in this pipeline ("careful" mode in Nullarbor) was done using SPAdes assembler (Bankevich *et al.* 2012) and single nucleotide polymorphism (SNP) analysis executed by Snippy (Seemann 2015). All assembled genomes were checked for quality of assembly using the outputs from Nullarbor, and Quast software (Gurevich *et al.* 2013). These assembled genomes were uploaded to the Centre for Genomic Epidemiology pipeline, from which resistance/virulence/plasmid-associated genes were found, and O:H/*fim*-antigen typing was done [http://www.genomicepidemiology.org (Thomsen *et al.* 2016)]. The flow of isolates described here is shown in **Figure 4.1**. A summary of sequencing and assembly of genomes in this chapter can be found in **Appendix III (Table 6.2).**

4.3.6.1 SNP and whole genome trees

Initially, SNP analysis of *E. coli* isolates included in this study was performed using reference genome JJ1886 for *E. coli* isolates (Andersen *et al.* 2013). Individual SNP alignments were subsequently repeated for households, and internal references chosen from the clade with isolates from more than one individual person (see **Table 4.1** for each household's isolates). This isolate was chosen based upon having a low number of contiguous DNA consensus regions (contigs) and a high number of coding sequences (CDS). Whole genome multi-locus sequence typing (wgMLST) was executed using Fast-GeP, gene prediction and comparison software (Zhang *et al.* 2018). A nexus tree output was constructed using Splitstree, then transformed in a Neighbour Joining nexus tree (Huson 1998; Rambaut 2007) for upload to EvolView for annotation and presentation

(He *et al.* 2016). An *E. coli* isolate (LT1090b) was removed from SNP and wgMLST analysis from household HH0039 due to no bla_{ESBL} or bla_{AmpC} beta-lactamase gene detected in ResFinder.

4.3.6.2 Phylogenetic analysis of E. coli ST131 isolates

For ST131 isolates from four households (HH0024, HH0039, HH0040 and HH0086), the reference genome JJ1886 was used for SNP alignment and subsequent BEAST phylogeny; this reference is an *E. coli* isolate originating from a urinary tract infection, and a contains a $bla_{\text{CTX-M-15}}$ gene conferring an ESBL- producing phenotype (Andersen *et al.* 2013).

Using the output of Snippy, a core SNP alignment of *E. coli* from three households (HH0024, HH0039 and HH0040) was prepared using BEAUTi 2, with exact collection dates known for these isolates (Bouckaert *et al.* 2014). Different combinations of substitution models [general time reversible model (GTR), Hasegawa, Kishino and Yano model (HKY)], molecular clocks (strict, relaxed lognormal), and population models (constant coalescent, coalescent extended-Bayesian skyline) were run. The models were compared using Tracer [version 1.6 (Rambaut *et al.* 2018)]. A strict molecular clock, with a general time reversible (GTR) substitution model and coalescent constant population was used for the final BEAST model (Bouckaert *et al.* 2014). Gubbins was used to estimate the effect of recombination that might have occurred between these isolates (Croucher *et al.* 2015). Posterior support, effective sample size (ESS), and model trace stability were used to assess model performance. An ESS of less than 200 for the posterior was considered minimum criteria for model fitness.

Table 4.1 Summary of reference genomes used for intra-household core SNP comparison of isolates [using Snippy 3.0 (Seemann 2015)]

Household	Reference Isolate ID	Read depth	GC content (%)	Assembled genome size (bp)	Assembled contigs (n)	CDS (n)	Pair-wise core-SNP distance (reads to reference)
HH0008	LT1003c	94	50.7	5434577	553	5001	8
HH0015	LT1030h2	61	50.0	5168471	249	4749	2
HH0016	LT1029f	133	49.9	5245042	312	4904	4
HH0024	LT8179a	81	51.1	5160657	178	4808	41
HH0026	LT1044g	118	50.3	4985966	219	4653	7
HH0039	LT1090f	113	50.8	5188047	305	4888	3
HH0040	LT1082f	107	50.3	5164692	181	4840	3
HH0048	LT1097g	129	51.1	5151025	339	4709	1
HH0064	LT8371a	120	50.2	5153473	338	4694	2
HH0065	LT1132e	122	50.0	5118820	288	4715	2
HH0086	LT1173e	199	50.1	5141055	298	4744	8

bp: base pairs
CDS: coding sequences
Read depth expressed as fold-coverage: each base sequenced on average the depth number of times

4.4 Results

4.4.1 Summary

Sixty-eight of the case households in **Chapter 3** owned a pet (68/141; 48%) and 117 had other family members (117/141; 83%). Faecal samples from 23 of these households were collected between December 2015 and January 2017, and were collected between 31 and 180 days after the case UTI sample was collected (median time = 63 days, mean time = 77 days). Twenty of these 23 households submitted samples from pets, and 11 of the 23 households submitted faecal samples from other people (non-case people) living in the home.

In 15 households, at least one cat was sampled and in 11 households, at least one dog was sampled. Of the 11 households with in-contact people sampled, 1/11 had three in-contact people, and 10/11 had one in-contact person sampled. Fourteen households had more than one individual with an Enterobacteriaceae isolate(s) with ESBL-/ ACBL-producing phenotype collected from them, and there were 11 households where ESBL- / ACBL-producing *E. coli* was cultured from more than one individual. An overview of samples submitted from pets and people in these fourteen households is described in **Table 4.3**, with 11 households where *E. coli* was cultured from more than one individual as indicated in the table footnotes. In the14 households described in **Table 4.3**, 13/14 index case persons did not live alone. However, in-contact people were only sampled from 10/14 households. In the 11 households positive for ESBL- / ACBL-producing *E. coli*, no-one lived alone, and in-contact people were sampled from 10/11 (91%) of households.

The index case person (from whom an ESBL- / ACBL-producing bacteria was cultured) in all households were predominantly female (21/23; 91%), and 10/23 (43%) were over 65-years-old. In the households where more than one person/pet was positive for an ESBL-

/ ACBL-producing *E. coli*, 9/11 (82%) of the incident case people were female and 7/11 (64%) were over 65-years-old. The ages of all (n=13) in-contact people sampled ranged (in groups) from 10-to-19 years-old to 70-to-79 years-old; the same age range was observed in ESBL- / ACBL-producing *E. coli* positive in-contact people. Sampled pet ages from all households ranged from 1-to-11 years old for dogs, and 1-to-17 years old for cats; an age range of 1-to-8 years-old was observed for dogs positive for ESBL- / ACBL-producing *E. coli* (no cats were positive).

Table 4.2 Summary of sources of samples from fourteen households

Household	People sampled in household‡	Total ESBL- or AmpC-producing Enterobacteriaceae Isolates	People with ESBL or AmpC phenotype isolates	Pets sampled in household	Pets with ESBL or AmpC phenotype isolates	Total individuals with ESBL or AmpC phenotype isolates
HH0008	2	7	2	3[a]	0	2*
HH0015	2	7	2	None	NA	2*
HH0016	2	14	2	None	NA	2*
HH0024	2	14	2	2[ab]	0	2*
HH0026	1	12	1	1[b]	1[b]	2*
HH0039	2	10	2	1[a]	0	2*
HH0040	4	28	4	3[ab]	0	4*
HH0048	2	9	1	2[b]	1[b]	2*
HH0053	1	11	1	2[ab]	1[a†]	2
HH0064	2	10	1	2[ab]	2[a†, b]	3*
HH0065	2	13	1	1[b]	1[b]	2*
HH0071	1	12	1†	1[a]	1[a]	2
HH0077	1	9	1	1[b]	1[b†]	2
HH0086	2	8	1	2[b]	1[b]	2*

‡ Faecal and/or urine sample; each person counted once; † Not *E. coli;* * Samples cultured ESBL-ACBL- *E. coli* and included in further genomic analysis; [a] cat; [b] dog

From 23 households, 17 case participants submitted a faecal sample (17/23; 74%), and 16/17 reported receiving antimicrobial treatment in the prior six-months including any treatment for index UTI. In all 23 households, 30 people submitted faecal samples; in the 14 households outlined in **Table 4.3,** faecal samples were obtained from 26 people.

Of these people from households summarised in **Table 4.3**, 12/26 (46%) were in-contact people, and the remainder were incident case people (n=14). Antimicrobial treatment was reported in two in-contact people, both from households where more than one person/pet was positive for ESBL- / ACBL-producing *E. coli*, however only one of these people were positive for ESBL- / ACBL-producing *E. coli.* Twenty of 23 households (87%) submitted at least one sample from a pet, 7/23 (30%) submitted samples from both cats and dogs, 8/23 (35%) submitted from only cats and 5/23 (16%) submitted from only dogs. Antimicrobial treatment prior to faecal sampling was reported in pets from 14/23 (61%) households; eight individual pets from 7/11 (64%) households where more than one person/pet was positive for ESBL- / ACBL-producing *E. coli*.

Antimicrobial treatment was also reported in households as part of the **Chapter 3** questionnaire. As reported by the incident case person, someone other than the case person was treated with antimicrobials in the six-months prior to the case infection in 5/20 (25%) households. Two of these households were included in the 11 households where more than one person/pet was positive for ESBL- / ACBL-producing *E.* coli. In these same 20 households, no in-contact people (as reported by the case person) had a multidrug resistant infection. Hospitalisation of an in-contact person in the previous year was reported in 6/20 (30%) households, while 4/11 (36%) households where more than one person/pet was positive for ESBL- / ACBL-producing *E. coli* reported the same.

4.4.2 Bacterial isolate descriptions

Eleven households had more than one individual with at least one *E. coli* isolate containing the beta-lactamase genes: *bla*$_{\text{ESBL}}$, *bla*$_{\text{CMY}}$ or *bla*$_{\text{DHA}}$, as determined by whole genome sequencing. A description of the genomics of those isolates (n=125) is found in **Figures 4.2**, **4.3,** and **4.4**. Resistance genes present in the isolates from the 14 households described in **Table 4.3** are summarised in **Table 4.4**. A more complete overview of all resistance genes in the 11 households' *E. coli* isolates is given in **Table 4.6.** Antimicrobial resistance genes are described in **Figure 4.2**, **4.3,** and **4.4** and **Table 4.4** and **4.6**). The multilocus sequence types (determined via Nullarbor) ST-131 and ST-69 were found in more than one household; ST-131 was found in four households and accounted for 47/125 (46%) of isolates described here. Characterisation of *E. coli* isolates is described further in **Tables 4.5.**

Table 4.3 ESBL, ACBL and plasmid-mediated quinolone resistance (PMQR) genes from Enterobacteriaceae in 14 households

Household	Total isolates N	Bacterial isolates (n/N)	ESBL gene(s) (n/N)	ACBL gene(s) (n/N)	PMQR gene(s) (n/N)	Number of people or pets sampled where *E .coli* had ESBL/ACBL genes (n)
HH0008	7	*E. coli* (7/7)	*bla*$_{CTX-M-15}$ (7/7)	none (7/7)	*QnrS1* (7/7)	2
HH0015	7	*E. coli* (7/7)	none (7/7)	*bla*$_{CMY-2}$ (7/7)	none (7/7)	2
HH0016	14	*E. coli* (14/14)	*bla*$_{CTX-M-15}$ (14/14)	none (14/14)	none (14/14)	2
HH0024	14	*E. coli* (14/14)	*bla*$_{CTX-M-14}$ (2/14); *bla*$_{CTX-M-27}$ (12/14)	none (14/14)	none (14/14)	2
HH0026	12	*E. coli* (11/12); *Citrobacter* spp (1/12)	*bla*$_{CTX-M-15}$ (8/12); *bla*$_{CTX-M-27}$ (3/12); none (1/12)	*bla*$_{CMY-83}$ (1/12); none (11/12)	*QnrB10* (1/12); none (11/12)	2
HH0039	10	*E. coli* (10/10)	*bla*$_{CTX-M-27}$ (9/10); none (1/10)	none (10/10)	none (10/10)	2
HH0040	28	*E. coli* (28/28)	*bla*$_{CTX-M-15}$ (28/28)	none (28/28)	none (28/28)	4
HH0048	9	*E. coli* (9/9)	*bla*$_{CTX-M-14}$ (7/9); *bla*$_{CTX-M-15}$ (1/9); none (1/9)	*bla*$_{DHA-1}$ (1/9); none (8/9)	*QnrB4* (1/9); none (8/9)	2
HH0053	11	*E. coli* (11/11)	*bla*$_{CTX-M-15}$ (4/11); none (7/11)	none (11/11)	*QnrB4* (2/11); *QnrS1* (4/11); none (5/11)	1
HH0064	10	*E. coli* (5/10); *Enterobacter* spp (5/10)	none (10/10)	*bla*$_{CMY-2}$ (5/10); none (5/10)	none (10/10)	2
HH0065	13	*E. coli* (13/13)	*bla*$_{CTX-M-15}$ (3/13); *bla*$_{CTX-M-27}$ (8/13); none (2/13)	*bla*$_{CMY-2}$ (2/13); none (11/13)	none (13/13)	2
HH0071	12	*E. coli* (8/12); *Escherichia* spp (4/12)	*bla*$_{CTX-M-15}$ (1/12); *bla*$_{CTX-M-27}$ (7/12); none (4/12)	*bla*$_{CMY-2}$ (4/12); none (8/12)	none (12/12)	1
HH0077	9	*E. coli* (2/9); *Citrobacter* spp (7/9)	bla$_{CTX-M-15}$ (1/9); none (8/9)	*bla*$_{CMY-71}$ (1/9); none (8/9)	*QnrB6* (1/9); none (8/9)	1
HH0086	8	*E. coli* (8/8)	*bla*CTX-M-27 (8/8)	none (8/8)	none (8/8)	2

Table 4.4 A summary of multi-locus sequence type (MLST) and serotying (O- and H- antigen) of 125 E. coli isolates from 11 households

Household	Total *E. coli* isolates N	MLST (n/N)	Serotype (O-antigen)	Serotype (H-antigen)
HH0008	7	ST-69(6/7); ST-58 (1/7)	O15 (6/7); O8 (1/7)	H18 (6/7); H25 (1/7)
HH0015	7	ST-963 (7/7)	NT (7/7)	H18 (7/7)
HH0016	14	ST-500 (14/14)	O8 (14/14)	H5 (14/14)
HH0024	14	ST-131 (12/14); ST-648(2/14)	O25 (12/14); O1 (2/14)	H4 (12/14); H6 (2/14)
HH0026	11	ST-617(8/11); ST-1193 (3/11)	O89 (8/11); O75 (3/11)	H10 (8/11); H5 (3/11)
HH0039	9	ST-131 (9/9)	O25 (9/9)	H4 (9/9)
HH0040	28	ST-131 (28/28)	O25 (28/28)	H4 (28/28)
HH0048	9	ST-38 (7/9); ST-4553 (1/9); ST-538(1/9)	O2 (7/9); O83 (1/9); O13 (1/9)	H30 (7/9); H42 (1/9); H4 (1/9)
HH0064	5	ST-2541 (4/5); ST-963 (1/5)	NT (5/5)	H7 (4/5); H18 (1/5)
HH0065	13	ST-69 (8/13); ST-746 (3/13); ST-10 (1/13); ST-2541 (1/13)	O15 (8/13); NT (5/13)	H2 (8/13); H37 (3/13); H40 (1/13); H7 (1/13)
HH0086	8	ST-131 (8/8)	O16 (8/8)	H5 (8/8)

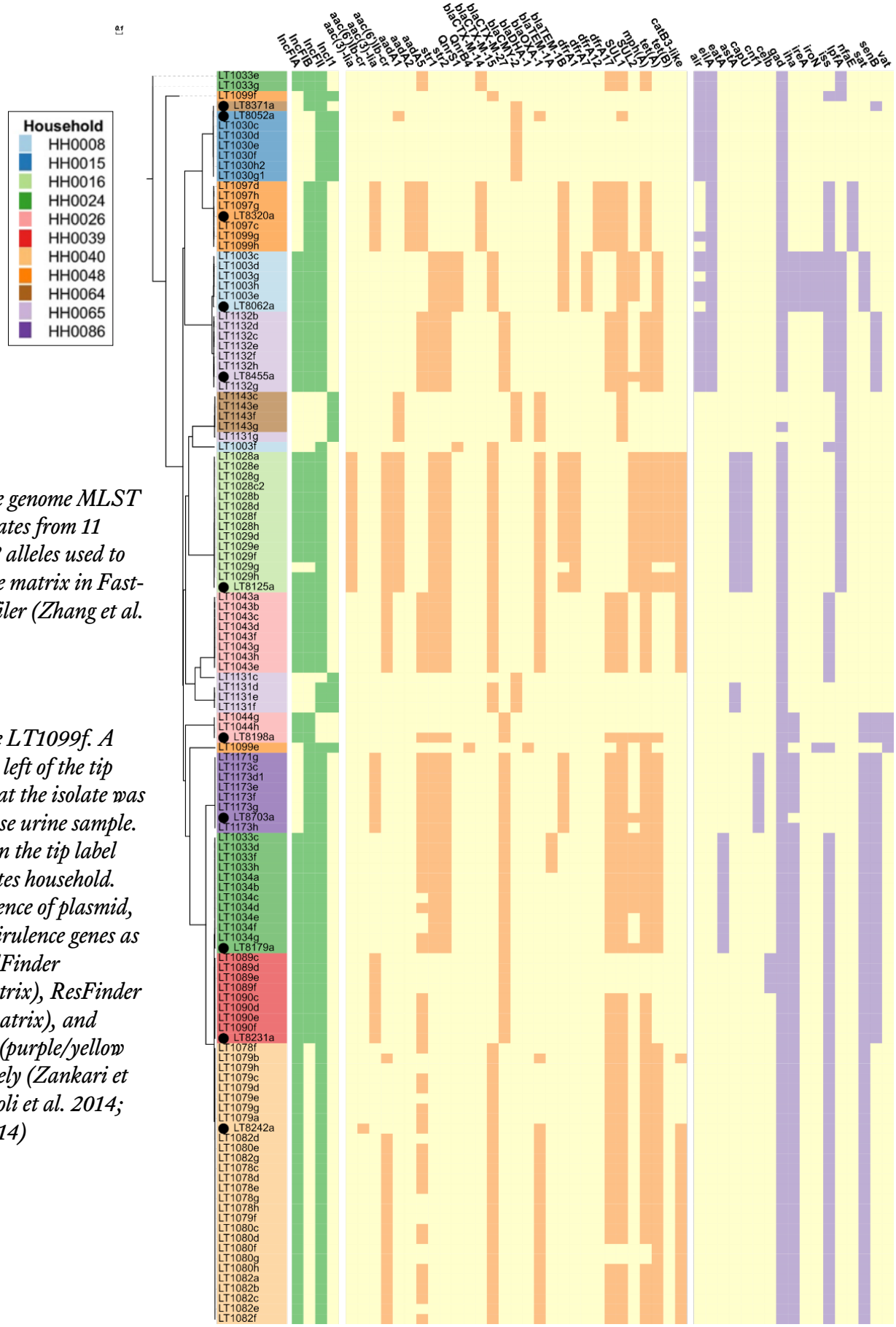

Figure 4.2 Whole genome MLST of 125 E. coli isolates from 11 households; 3022 alleles used to construct distance matrix in Fast-GeP genome profiler (Zhang et al. 2018).

Reference genome LT1099f. A black circle to the left of the tip label indicates that the isolate was from the index case urine sample. The colour strip in the tip label (isolate ID) denotes household. Presence and absence of plasmid, resistance, and virulence genes as found in PlasmidFinder (green/yellow matrix), ResFinder (orange/yellow matrix), and VirulenceFinder (purple/yellow matrix) respectively (Zankari et al. 2012; Carattoli et al. 2014; Joensen et al. 2014)

Table 4.5 Antimicrobial resistance genes in 125 E. coli isolates from 11 households

Resistance gene type	Antimicrobial resistance class	Isolates with resistance genes	
		n/Total isolates	Proportion (95%CI)
aac-type	Aminoglycoside and/or fluoroquinolone	67/125	53.6% (45 - 62%)
aad-type	Aminoglycoside	86/125	68.8% (61 - 77%)
str-type	Aminoglycoside	57/125	45.6% (37 - 54%)
bla_{ESBL}-type	Beta-lactam (incl. 3rd & 4th generation cephalosporins)	109/125	87.2% (81 - 93%)
bla_{AmpC}-type	Beta-lactam (incl. 3rd & 4th generation cephalosporins)	18/125	14.4% (8 - 21%)
bla_{OXA}-type (non-ESBL)	Beta-lactam	49/125	39.2% (31 - 48%)
bla_{TEM}-type (non-ESBL)	Beta-lactam	38/125	30.4% (22 - 38%)
dfrA-type	Trimethoprim	97/125	77.6% (70 - 85%)
sul-type	Sulphonamide	104/125	83.2% (77 - 90%)
Qnr-type	Fluoroquinolone	8/125	6.4% (2 - 11%)
mph-type	Macrolide	92/125	73.6% (66 - 81%)
tet-type	Tetracycline	76/125	60.8% (52 - 69%)
catB-type	Phenicol	43/125	34.4% (26 - 43%)

No colistin or carbapenem resistance genes were found in this dataset. A multidrug resistance genotype was observed in the majority of isolates with 106/125 isolates [84.8%; 95% CI (79 – 91%)] having more than two classes of resistance genes.

Table 4.6 Results of antimicrobial susceptibility testing of 125 E. coli isolates from 11 households

Antimicrobial		Number of isolates (n/Total isolates)	Proportion (95%CI)
Ampicillin	R	122/125	97.6% (95 - 100%)
Cephalexin	R	117/125	93.6% (89 - 98%)
Cefpodoxime	R	125/125	100% (100 - 100%)
Ceftriaxone	M	1/125	0.8% (0 - 2%)
	R	123/125	98.4% (96 - 100%)
Mecillinam	R	4/125	3.2% (0 - 6%)
Ertapenem	M	18/125	14.4% (8 - 21%)
	R	0/125	0% (0 – 0%)
Augmentin	R	42/125	33.6% (25 - 42%)
Cefoxitin	R	32/125	25.6% (18 - 33%)
Gentamicin	R	38/125	30.4% (22 - 38%)
Amikacin	R	0/125	0% (0 – 0%)
Norfloxacillin	M	5/125	4.0% (1 - 7%)
	R	76/125	60.8% (52 - 69%)
Trimethoprim	M	1/125	0.8% (0 - 2%)
	R	96/125	76.8% (69 - 84%)
Nitrofurantoin	R	14/125	11.2% (6 - 17%)
Fosfomycin	R	6/125	4.8% (1 - 9%)

M: moderate susceptibility; R: resistant (see **Table 4.1** for respective zone diameters and disk concentrations)

A multidrug resistant phenotype was observed in 87/125 isolates [69.6%; 95% CI (62 – 78%)]. The overviews given for resistance genotype (**Table 4.6**) and phenotype (**Table 4.7**) do not illustrate the household-level patterns. See **Figure 4.2** for associations of resistance and virulence genotypes for households. This figure also shows the whole genome MLST for all 125 *E. coli* isolates made using 3022 alleles.

Table 4.7 Virulence genes in 125 E. coli isolates from 11 households

Virulence gene	Gene description†	Isolates with gene N	Proportion (95%CI)
gad	Glutamate decarboxylase	121	96.8% (94 - 100%)
iss	Increased serum survival	82	65.6% (57 - 74%)
sat	Secreted autotransporter toxin	67	53.6% (45 - 62%)
iha	Adherence protein	65	52.0% (43 - 61%)
senB	Plasmid associated enterotoxin	41	32.8% (25 - 41%)
lpfA	Long polar fimbriae	37	29.6% (22 - 38%)
eilA	*HilA*–like regulator	32	25.6% (18 - 33%)

†References: (Shin *et al.* 2001; Kaper *et al.* 2004; Sheikh *et al.* 2006; Wiles *et al.* 2008; Joensen *et al.* 2014)

Virulence genes were identified by VirulenceFinder and the seven most common genes are shown in **Table 4.8**; genes *air*, *astA, capU, eatA, cnf1, iroN, nfaE, ireA, vat* and *celb* were also present in isolates at frequencies of less than 25% of isolates. These genes were not investigated in detail, and here serve to provide non-AMR gene similarity between isolates, and as shown in **Figure 4.2** clonal faecal and urinary isolates had a similar virulence gene profile.

Pair ended sequence reads from each *E. coli* isolate in these 11 households was compared to a reference selected from that household (**Table 4.1**). These comparisons were made using SNPs, and the resultant trees are presented in **Figure 4.3** for individual households, and **Figure 4.4** for *E. coli* ST-131 isolates from these households. As shown in **Figure 4.3**, clonal isolates were cultured from more than one person in six households [6/11; 55% (95% CI 25-84%)], and from a person and a pet in two households [2/20; 10% (95% CI 0-23%)].

4.4.3 Phylogenetic analysis of ST-131 isolates

Using isolates from three households' *E. coli* ST-131 isolates, a BEAST analysis on the SNP alignment (with and without recombinant sections removed) was performed using a GTR substitution model. This model identified intra-cluster common ancestors within the year preceding, with an inter-cluster shared common ancestor eight years preceding (95% CI 2.9-13.6 years). A mean posterior for this model was -722.401 (ESS = 71). The resultant tree is not presented here, due to overall low ESS (<200) for all parameters in all tested models. Evolutionary phylogenies were also attempted for each individual household, however as no model was able converge on tree height or posterior trace values, the results are not presented here.

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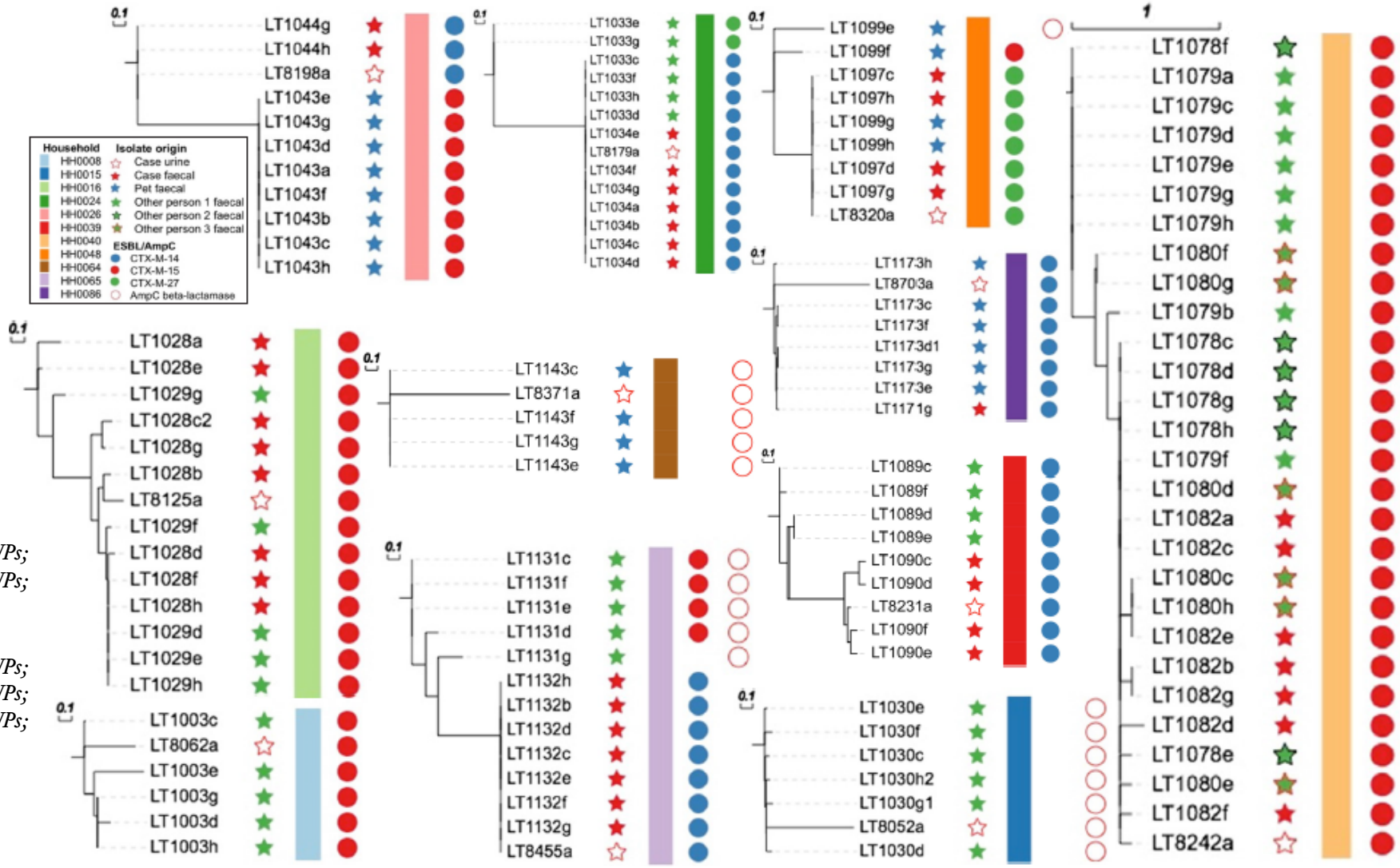


Household HH0008, scale 0.1 = 2 SNPs;
Household HH0015, scale 0.1 = 20 SNPs;
Household HH0016, scale 0.1 = 2 SNPs;
Household HH0024, scale 0.1 = 1 x 10^4 SNPs;
Household HH0026, scale 0.1 =1 x 10^4 SNPs;
Household HH0039, scale 1 = 2 SNPs;
Household HH0040, scale 1 = 10 SNPs,
Household HH0048, scale 0.1 = 1 x 10^4 SNPs;
Household HH0064, scale 0.1 = 1 x 10^4 SNPs;
Household HH0065, scale 0.1 = 2 x 10^4 SNPs;
Household HH0086, scale 0.1 = 10 SNPs

Figure 4.3 Household Neighbour-Joining SNP trees for 125 ESBL- and/or ACBL-producing E. coli isolates from 11 households (range 5-28 per household). Reference genomes for core SNP alignments given in ***Table 4.2*** *and full SNP distances for these figures are in Appendix VI.*

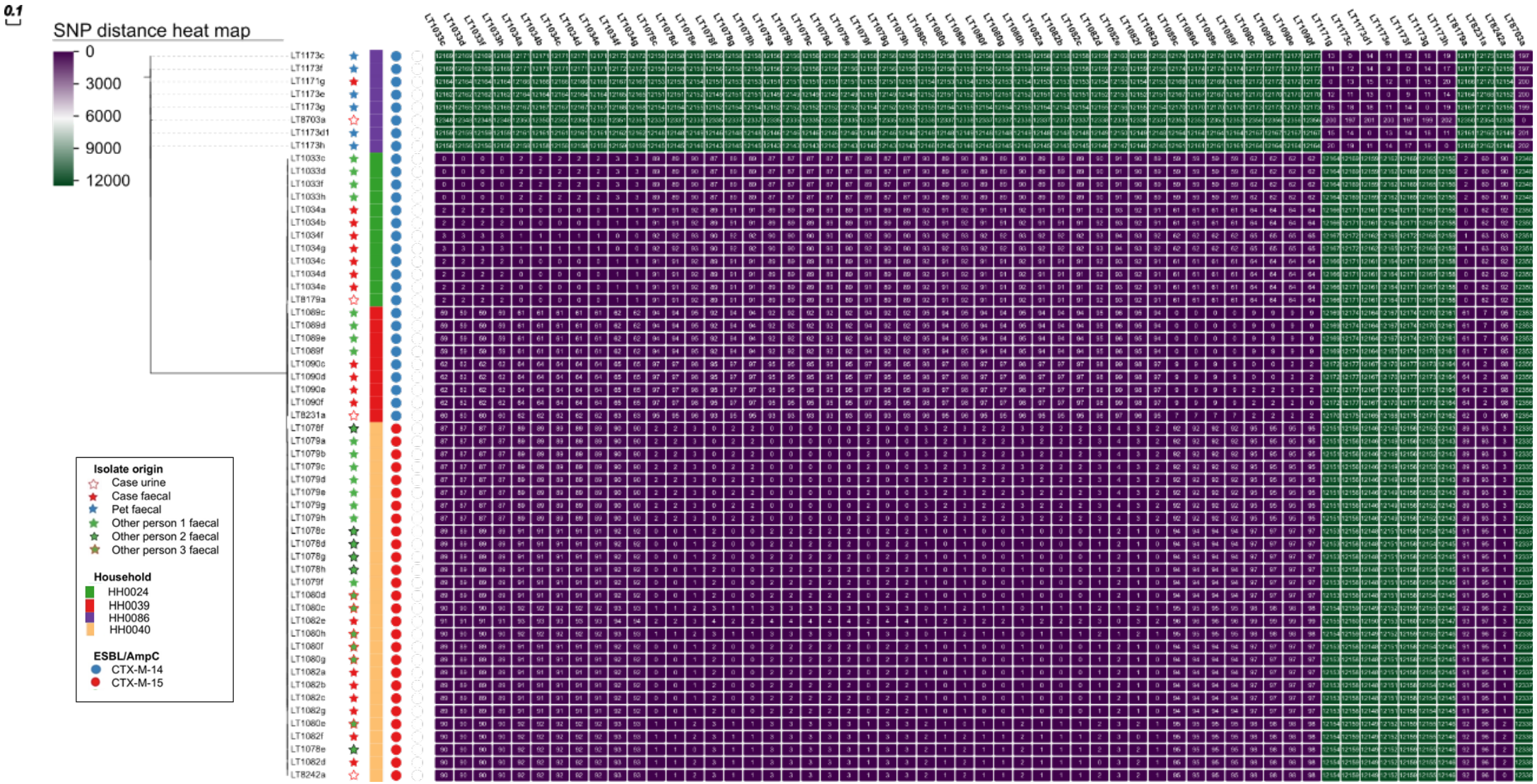


Figure 4.4 Neighbour Joining tree of core SNP phylogeny of E. coli ST-131 isolates from four households. Core SNP alignment using 12,454 SNPs Scale 0.1 = 1.2 x 10^3 reference genome JJ1886; SNP distance matrix and tree generated using Snippy (Seemann 2015).

4.5 Discussion

This study is an important first step in examining household in-contacts of people with clinical ESBL-/ACBL-producing infections in New Zealand. Transmission of ESBL-/ACBL- producing bacteria is likely to have occurred in 8/23 (35%) investigated households. However, this varies whether looking at households where in-contact people were sampled, or where in-contact pets were sampled. In 55% (6/11) of the former, the same strain of ESBL-/ACBL- producing *E. coli* was cultured from more than one person in the household, while this was only true for 10% (2/20) of households where pets were sampled.

The results of this study are comparable to other studies, where carriage of similar AMR genes/bacteria have been found in family members or couples (Alam *et al.* 2015; Johnson *et al.* 2016). However, in a study examining travel-associated acquisition of ESBL-producing Enterobacteriaceae, transmission of bacteria on to other people was found in 8% (13/168) of households (Arcilla *et al.* 2017). Long-term transmission risk was also observed in an outbreak of ESBL-producing *Klebsiella pneumoniae*, where carriage of these bacteria by one individual in a home was a source for the rest of the family (Löhr *et al.* 2013).

Intestinal carriage of MDR Enterobacteriaceae has been described as a significant risk for subsequent infection with MDR bacteria (Woerther *et al.* 2013; Giannella *et al.* 2014). It is very likely that the proportion of New Zealanders who carry ESBL-*E coli* in their faecal microbiota will continue to rise in the coming years, as it has in other countries (Karanika *et al.* 2016). Benchmarking 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 could be noted that at least 11% (2/17) of in-contact people carried an

ESBL-producing *E. coli* un-related to the index UTI in this study. However, the actual prevalence rate is likely to be lower, as prevalence less than 6% has been reported in Europe and the Americas (Karanika *et al.* 2016).

Pets have been described in previous studies as carrying clonal ESBL-*E. coli* along with humans in their house, and in some cases being affected by clinical UTI (Johnson *et al.* 2016). A better understanding of AMR transmission dynamics between people and pets may have been possible if the study design had allowed for sampling at sequential time-points. The dogs that were positive for faecal ESBL-producing *E. coli* may have picked up these bacteria from the environment, or they may have picked it up from their owners. The results presented here show that they are at least a reservoir for infectious AMR pathogens. It is of note that none of the animals positive for the same bacteria as the humans in the household were cats, although more households had a cat sampled (15 households with at least one cat in comparison to 11 households with at least one dog sampled).

Hygiene around the home, between partners, parents and children, and between pets and their owners is likely a factor in the transmission of the MDR bacteria isolated in this study (Ulleryd *et al.* 2015; Johnson *et al.* 2016). In New Zealand, clinical infections with multidrug resistant *E. coli* occur in pets, but at unknown and assumed low rates (Karkaba *et al.* 2017b; McMeekin *et al.* 2017). Isolates of *E. coli* collected from dog faecal samples in two different households were a different MLST from the clinical case, and both dogs carried multiple unique AMR bacteria in their faeces. While the MLST types isolated from the faecal samples of these dogs were not commonly associated with community-acquired infection in New Zealand, the enzyme-coding genes ($bla_{\text{CTX-M-15}}$; $bla_{\text{CTX-M-14}}$)

were those also found predominantly in human infection in New Zealand (Heffernan *et al.* 2013; Heffernan *et al.* 2018).

Genomic similarities between isolates within households are reflected in the allelic profile of the isolates clustered using whole genome MLST, and in the SNP differences when compared to a reference. For example, in household HH0040, less than five SNPs were found between isolates from different individuals, and 12 SNPs were found between all 28 isolates cultured from this household. Other studies examining similar datasets of closely related or clonal ESBL-EC isolates within households have found high similarity between isolates from individuals (Johnson *et al.* 2016; Torres *et al.* 2018). It is of note that four households (where more than one individual was positive for ESBL-/ACBL-producing *E. coli*) were positive for ST-131. Adaptations to support human gut carriage (such as virulence genes like *gad* that improve survival in an acidic stomach) may be a contributor to the global success of certain bacteria (especially epidemic clades such as ST-131), however these may also reflect the relative proportion of these isolates in the clinical case population described in **Chapter 3** (Nicolas-Chanoine *et al.* 2014).

A superficial analysis of the genomics presented here suggests that horizontal gene transfer (via plasmids) between bacteria has occurred in some individuals. This was not explored experimentally within this study, although there is potential for this to have occurred with the ACBL gene $bla_{\text{CMY-2}}$ in household HH0064 (see **Figure 4.3**). This also highlights the value of collecting and sequencing multiple isolates from faecal samples. Discrete plasmids were not assembled through the bioinformatics pipeline; however, large plasmid genes (and related plasmid types) were identified and reported. Long read sequencing of plasmids would be required to confirm and compare plasmids isolated from different bacteria to make any inferences regarding horizontal gene transfer. Evidence of

transmissibility of plasmids from cultured bacteria to a donor organism (via conjugation) would also be required for any assertions around intra-person (and therefore inter-person) transmission of resistance genetics to be made.

A limitation to the study presented here is that transmission within households cannot be examined by a cross-sectional methodology. Consequently, this is often assessed through mathematical modelling in addition to observational studies (Arcilla *et al.* 2017; Haverkate *et al.* 2017). Within a longitudinal study of post-acquisition 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 (Arcilla *et al.* 2017).

The results presented in this chapter identify that some "sharing" of ESBL-/ACBL-producing bacteria within households is likely, even though transmission dynamics cannot be inferred from the cross-sectional design used here. However, evolutionary evidence from three ST-131-containing households suggests a common ancestor for introduction of ESBL-/ACBL- producing *E. coli* into the home within the year before faecal sampling period, and this date does not appear to pre-date the index urinary tract infection by more than three to six months. However, the poor posterior support for this model means that any inference taken from this should be made with caution. Although estimations of common ancestor dates were also attempted for clonal isolates within each household individually, the models were also not sufficiently robust to make inferences. Within a different study design (e.g. longitudinal case study, or cohort study), sampling over a longer time-period may make such inference possible.

Another important limitation to this study is around sample collection. As study participants collected their own faecal samples, it is possible that they unintentionally spiked samples with other people's *E. coli* during collection. This would mean that the results reported here over-represent the proportion of households where individuals carried the same ESBL-/ACBL-producing bacteria. Cases recruited into this study were also likely to have self-selected for submission of faecal samples and may have been specifically concerned about the possibility of pets being a carrier of AMR bacteria. As this cross-sectional study was nested within a larger case control study, some of the limitations affecting that study (see **Chapter 3**) also apply here. This includes a lack of medical histories for participants in this study, with limited oral accounts were only available for case participants.

4.6 Conclusions

In a home environment where one person has a sample positive for ESBL- or ACBL-producing *E. coli* (be it carriage or a clinical infection), other members of the family may also harbour the AMR bacteria. The results presented here suggest that companion animals (specifically dogs) are potential vectors of ESBL-producing *E. coli*, but pets are likely to be less important vectors than other people living in the home. Longitudinal studies are required to examine the relationship between pets and the carriage rates of these bacteria in household environments. Two pets were shown to carry the same bacterial strain as that which caused a UTI in a person living with that pet. However, potential human-to-human spread appears more common in this study, where in 2/20 (10%) of homes, pets carried the same strain as the case person compared with 6/11 (55%) of households were another person (other than index case) carried the same strain. The results of this study suggest that transmission occurs through contact between people (or people and pets) after an introduction event or through exposure to the same source of AMR bacterial isolate, although this does not preclude the presence of other strains/species of AMR bacteria in a person or household. As the prevalence of individual carriage increases, perhaps the household level is the basal unit from which preventative or AMR bacterial depopulation interventions should be targeted.

5 New Zealand community-acquired extended spectrum beta-lactamase-producing *Escherichia coli* ST-131 in a global context

5.1 Abstract

E. coli ST-131 has facilitated the rise and perpetuation of extended spectrum beta-lactamase enzymes (ESBL) around the globe. It has become the predominant ESBL-producing strain in human clinical infections in New Zealand, as it has in many other countries. This study describes community-acquired clinical isolates (n=48) from New Zealand in the context of isolates from around the world. The other isolates examined in this chapter (i.e. not from New Zealand community-acquired urinary-tract infections collected as part of **Chapter 3**) were all *E. coli* ST-131 (n=188) and collected from human and animal sources between 2004 and 2015. The New Zealand community-acquired isolates were collected between 2015 and 2017. Core and accessory genome and core nucleotide alignments were described and analysed using multiple methods including evolutionary phylogenetic analyses. Of isolates from community-acquired UTIs in New Zealand included in this study, 63% were ST-131 serotype O25:H4 (*fimH*-30 clade C). Isolates clustered in clade A (O16:H5-*fimH*-41) were predominantly from New Zealand community sources [**Chapter 3** (64%)], and these isolates were estimated to share a common ancestor between 2003 and 2004. *E. coli* ST-131 appears to have come into New Zealand in multiple introductions and both clade A and clade C appear to be dominant within the New Zealand community.

5.2 Introduction

Escherichia coli ST-131 is an important, dominant, and pervasive pathogenic strain (Nicolas-Chanoine *et al.* 2014; Petty *et al.* 2014). It is a substantial cause of antimicrobial resistant extra-intestinal infections, and often harbours mobile genetic elements with genes encoding extended-spectrum beta-lactamase (ESBL) or AmpC beta-lactamase (ACBL), along with other resistance genes such as those for fluoroquinolone resistance (Petty *et al.* 2014; Kakkanat *et al.* 2017). Since the early 21st century, pathogenic strains of ESBL-producing *E. coli* have risen in prevalence around the globe (Livermore *et al.* 2007; Johnson *et al.* 2010). ST-131 has also been recognised as the cause of more than 50% of the ESBL-producing infections in New Zealand (Heffernan *et al.* 2013; Heffernan *et al.* 2018), and represented 37% of the *E. coli* cultured from clinical urinary tract infections (UTI) from case participants recruited for the case control study described in **Chapter 3**. Along with associations with community-acquired UTIs, this strain is also associated with serious infections such as sepsis (Harris *et al.* 2018).

E. coli ST-131 is classified within the Clermont phylogroup B2, and ST-131 fits within the Warwick MLST scheme (Clermont *et al.* 2015). Isolate types within phylogroup B2 (also called ribotype B2) are often associated with extraintestinal infections such as UTIs (Johnson *et al.* 2001; Bidet *et al.* 2007; Clermont *et al.* 2015). ST-131 can be further subdivided into three clades (A, B and C), and these are defined by different *fimH* pili adhesin and chromosomal quinolone resistance genes alongside phylogenetic clustering (Petty *et al.* 2014). Fluoroquinolone use in the 1980s may have been a driver of the dominance and spread of pathogenic ST-131 around the world, as this appears to have coincided with an increase in point mutations relating to fluoroquinolone resistance

(Petty *et al.* 2014). A common ancestor for all clades of ST-131 is estimated to have been in the late 19th century (Stoesser *et al.* 2016).

In animal populations, ST-131 has been cultured from both clinical and non-clinical samples in companion animals (Johnson *et al.* 2009; Ewers *et al.* 2010; Karkaba *et al.* 2013; Haenni *et al.* 2014; Belas *et al.* 2018). This ST was also cultured from the faecal sample of a dog living in the home of a person who had a community-acquired UTI caused by ST-131 *E. coli* as described in **Chapter 4** of this thesis. More recently, ST-131 has also been found in the poultry supply chain, including in meat (Johnson *et al.* 2017; Liu *et al.* 2018). It has, however, been less commonly cultured from other food-producing animals (Ewers *et al.* 2012; Afema *et al.* 2018). Its importance is therefore largely in human populations, and the ability of the bacteria to be passively carried in the gastrointestinal tract as well as being a pathogen outside the gastrointestinal tract (Woerther *et al.* 2013).

The objective of this study was to describe the genetics of *E. coli* ST-131 from community-acquired infections in New Zealand and to give a global context for these isolates.

5.3 Materials and methods

5.3.1 New Zealand community isolates

Forty-eight New Zealand clinical isolates were selected after multi-locus sequence typing as part of the Nullarbor bioinformatics pipeline (Seemann *et al.* 2016). These isolates were from clinical urinary tract infections and collected as part of a case control study in **Chapter 3** (including metadata) and the ethical approval for that study was granted by the Health and Disability Ethics Committee of New Zealand under the approval number 15/CEN/47.

5.3.2 Search strategy for other isolates

Searches of the NCBI sequence read archive (SRA) database were made between 6-11th December 2017 and 8-11th June 2018 (https://trace.ncbi.nlm.nih.gov/Traces/sra/). Search terms included "O25:H4", "E. coli ST131", "ST 131", "ST-131", and "ST131".

5.3.3 Inclusion criteria

Pre-processing criteria

Isolates were considered eligible for download from the SRA database if they were from projects registered with NCBI where multiple isolates were potentially eligible for inclusion. An attempt was made to sample from across the years available, and to ensure as many isolates as possible were collected near the time the isolates collected and described in **Chapter 3** were collected**.** For all other isolates, pair-ended sequence reads were downloaded via the SRA toolkit at NCBI (SRA toolkit https://github.com/ncbi/sra-tools). Selection of isolates to download was made in an ad-hoc manner for those from reads in BioProject PRJEB4681. Forty isolates were chosen, initially one from each year (2002 to 2012), then 16 isolates were selected from 2012, two each year from 2008 to 2011, and one from each year 2003-2007.

Post-processing criteria

Isolates were excluded if they were not *E. coli*, had low average depth coverage (read depth <40 fold), poor assembly (genome size and Kraken database comparison indicative of not being *E. coli*, >1000 contigs), if the isolate was not ST-131 [as either indicated by SRA database metadata, or as a result of processing with the Nullarbor pipeline (Seemann *et al.* 2016)], if the isolate did not have an ESBL or plasmid-associated AmpC-beta lactamase (Wood and Salzberg 2014). Fifteen isolates were excluded due to poor quality, 72 downloaded isolates were not ST-131, and the remainder (n=90) were excluded due to

absence of AmpC beta-lactamase (ACBL) and/or ESBL gene(s) detected on a ResFinder search as part of the Nullarbor pipeline (Zankari *et al.* 2012; Seemann *et al.* 2016). From the six BioProjects where reads were downloaded from the SRA database (a total of 365 isolates downloaded), 66 isolates from PRJDB3868 were eligible for inclusion (from Japan and Nepal, collected in 2014); 35 isolates from PRJNA297860 were eligible for inclusion (from Thailand, Venezuela, France, Taiwan, Spain, Cambodia, Canada, Laos, and UK collected between 2008 and 2011); 8 from PRJEB23663 were ST-131 (from Germany collected between 2010 and 2014), 13 from PRJEB4681 (from the UK, collected between 2004 and 2012); 24 from PRJNA327820 (USA collected between 2008 and 2014); 42 from PRJNA327820 (Singapore, Australia and New Zealand collected between 2014 and 2015). Forty-eight isolates collected from cases recruited into the case-control study as described in **Chapter 3** were included in these analyses, and further a description of the process from collection to sequencing can be found in **Section 3.3.4**, **Section 4.3.3,** and **Section 4.3.4.**

5.3.4 Bioinformatics

Assembly and annotation

All sequenced pair-ended DNA reads (Illumina HiSeq or MiSeq platforms) were trimmed [Trimmomatic (Bolger *et al.* 2014)], and processed within the Nullarbor bioinformatics pipeline [version 1.20 (Seemann *et al.* 2016)]. Reads were assessed for quality using genome size, read depth and GC% (along with other measures of sequence quality via Nullarbor). Assembly of genomes in this pipeline was done using SPAdes assembler [SPAdes version 3.10.0 (Bankevich *et al.* 2012)] and single nucleotide polymorphism (SNP) analysis executed by Snippy [version 3.1 (Seemann 2015)]. Initial SNP analysis was performed using the reference genome JJ1886 (Andersen *et al.* 2013).

Assembled genomes were uploaded to the Centre for Genomic Epidemiology pipeline (http://www.genomicepidemiology.org, 1 July 2018), from which resistance, virulence, and plasmid-associated genes were identified [using ResFinder, VirulenceFinder, and PlasmidFinder respectively (Zankari *et al.* 2012; Carattoli *et al.* 2014; Joensen *et al.* 2014)], and O:H/*fim*-antigen typing was also done subsequently within this tool (Thomsen *et al.* 2016). Annotation of assembled genomes was done using Prokka [Prokka version 1.11 (Seemann 2014)]. A summary of **Chapter 5** isolates can be found in **Appendix III** (**Table 6.3**).

Gene-based analysis

Serotyping, *fim*-antigen typing, resistance gene identification, virulence gene identification, was carried out using the CGE pipeline (Resfinder, PlasmidFinder, and VirulenceFinder) (Zankari *et al.* 2012; Carattoli *et al.* 2014; Joensen *et al.* 2014; Thomsen *et al.* 2016). Whole genome MLST (wgMLST) using Fast-GeP genome profiler (Zhang *et al.* 2018), using LT8144a (an ST-131 isolates) as the reference genome, the same reference genome as used in whole genome MLST as described in **Chapter 3**. A pan-genome was provided by Roary from the Prokka-annotated genomes (using default settings), and this provided the gene presence/absence outputs for pan-genome heat maps and subsequent trait analysis [Roary version 8.0 and Scoary (Page *et al.* 2015; Brynildsrud *et al.* 2016)].

A Nexus tree output of wgMLST was constructed using SplitsTree, then transformed into a Neighbour Joining tree (Huson 1998; Rambaut 2007) for upload to EvolView (http://www.evolgenius.info/evolview/) for annotation and presentation (He *et al.* 2016).

Clades A, B, and C were determined by isolates clustering in the generated SNP phylogenetic tree with other isolates previously determined as that clade [in Harris *et al.* (2018)], and by clustering of the *fimH*-type where the majority of isolates were *fimH*-41 (clade A), *fimH*-22 (clade B), or *fimH*-30 (clade C) as described in Petty *et al.* (2014).

Statistical association between travel and phylogeny of clade A isolates

Whole genome MLST of clade A isolates from **Chapter 3** was performed using Fast-GeP (Zhang *et al.* 2018). The distance matrix (from alleles) was used, along with binary metadata on whether or not the person from whom the isolate was collected reported travelling outside New Zealand in the previous year (collected as part of the questionnaire in **Chapter 3**). Permutational analysis of variance (PERMANOVA) was performed in Rstudio (r-studio.com; R version 3.4.3 https://www.r-project.org) to compute F-statistics across all groups to compare within group and between group variance (Anderson 2001). PERMANOVA was used to assess whether wgMLST allelic profiles differed between travel and non-travel groups, implemented in Rstudio, to identify if there was a distinct New Zealand community cluster (i.e. a cluster not associated with travel) within the clade A isolates.

Genomics, SNP alignment and evolutionary phylogeny of isolates

Nucleotide variation (core SNP) between genomes was performed as part of the Nullarbor pipeline using the reference genome JJ1886, an ESBL-producing *E. coli* ST-131 isolate (Andersen *et al.* 2013; Seemann *et al.* 2016). For evolutionary phylogenetic models of isolates in clade A, a SNP core alignment to an internal reference (MER102) from this dataset was performed using Snippy (Seemann 2015), and this was used for the Bayesian models to generate an evolutionary phylogeny [BEAST 2 (Bouckaert *et al.* 2014)]. This isolate was selected based upon having a low number of contiguous DNA consensus

regions (contigs) and a high number of coding sequences (CDS). Recombination of genomes was estimated using Gubbins on full SNP alignment outputs from Snippy (Croucher *et al.* 2015). Gubbins was run using default parameters, with a GTR substitution model and FastML used for maximum likelihood tree; these outputs (e.g. variant site estimates) were visualised in Phandango (Ashkenazy *et al.* 2012; Hadfield *et al.* 2018).

The output of Gubbins (full SNP alignment with recombinant sections removed) was the basis for the BEAST models. Model comparison was made using Tracer (Rambaut *et al.* 2018). The models described in **Table 5.1** show the parameters used in generating various models.

Table 5.1 Model parameters for BEAST phylogenetic models for 25 E coli ST-131 clade A isolates

Model	Site model	Clock model	Priors
1	GTR	Strict	Constant coalescent
2	GTR	Relaxed lognormal	Constant coalescent
3	GTR	Strict	Coalescent extended-Bayesian skyline
4	HKY	Strict	Constant coalescent
5	HKY	Relaxed lognormal	Constant coalescent
6	HKY	Strict	Coalescent extended-Bayesian skyline

GTR: general time reversible model, HKY: Hasegawa, Kishino and Yano model

Due to the large number of isolates in clade C (n=201), a selection of 35 isolates were chosen based on distribution across the clade as ascertained by wgMLST in order to represent the diversity within the clade, and also on the quality of reads. These 35 isolates were combined with all isolates collected from New Zealand, and the same methodology used for clade A was used for these isolates. BEAST evolutionary models 1 to 4 were applied to this dataset (as per **Table 5.1**). The date of collection for isolates were given to the nearest date; if only year was known, the date was estimated to be half-way through the given year.

5.4 Results

5.4.1 Summary of isolates

A total of 236 isolates were included in analyses for this study, 24/236 (11%) were from the Americas (collected between 2008 and 2014), 109/236 (46%) were from Asia (collected between 2006 and 2015), 61/236 (26%) were from Australasia (collected between 2014 and 2017), 42/236 (18%) were from Europe (collected between 2004 and 2014). No isolates were from Africa or the Middle East. Twenty-five isolates were collected between the years 2004-2009, 138 isolates were collected between 2010 and

2014, and 73 isolates were collected between 2015 and 2017. The majority of isolates were from human sources (231/236; 98%), and the remainder were from animal sources (either poultry or an unspecified animal). There were 48 isolates included in this study from the case control study described in **Chapter 3.** A brief description of select metadata relating to these isolates is given (where available) in **Table 5.2**.

Table 5.2 Description of select metadata for 48 NZ community-acquired E. coli ST-131 isolates

	Total (n/N)	**Proportion % (95% CI)**
Female	46/47	98 (94 - 100)
Age:		
- 16 to 44 years	12/47	26 (13 - 38)
- 45 to 64 years	16/47	34 (20 - 48)
- 65 years and over	19/47	40 (26 - 54)
Any travel in previous year	27/46	59 (47 - 75)
- Travel to Africa	3/46	7 (0 - 14)
- Travel to Asia	10/46	22 (10 - 34)
Antibiotics:		
- Previous 3 to 6 months	4/31[†]	13 (1 - 25)
- Previous 3 months	12/31[†]	39 (22 - 56)

[†]Missing data described in **Chapter 3**

Travel to Asia or Africa were found to increase the odds of having a urinary tract infection in the case control study (**Chapter 3**), so travel status of the person from whom those 48 isolates was cultured is described here. This travel status was known for 46/48 of the New Zealand community-acquired isolates from **Chapter 3**. Of these, 19/46 (41%) of isolates were from people who had not travelled outside NZ in the previous 12-months. Asian or African travel in the previous 12-months was reported from 28% of people (13/46). Where people lived with others (40/46; 87%), four individuals had not travelled in the previous 12-months, although someone else in their house had travelled outside New Zealand. The

48 isolates from people in New Zealand are highlighted in **Section 5.4.3** with travel metadata included in figures in this section. Records were available for antimicrobial prescriptions for 31/48 (65%) of the people from whom New Zealand community-associated isolates originated. Of these, 15/31 (48%) had no record of antimicrobial prescription in the previous six months, 12/31 (39%) came from people who had their most recent antimicrobial prescription in the previous three months, and 4/31 (13%) had the most recent prescription in the previous three to six months.

5.4.2 Gene based results for *E. coli* ST-131 isolates

Shown in **Figure 5.1** are descriptions of plasmid, ESBL and virulence genes. In this figure, a whole genome MLST (wgMLST) using 3011 genes was used to create a distance matrix and subsequent neighbour joining tree for 256 *E. coli* isolates. In this figure, ESBL and ACBL genes are described alongside other acquired resistance genes found in the assembled genomes. The most commonly found ESBL gene in these isolates was $bla_{\text{CTX-M-15}}$ (171/236; 72%). Other ESBL and ACBL genes found in these isolates were $bla_{\text{CTX-M-14}}$ (12/236; 5%), $bla_{\text{CTX-M-27}}$ (43/236; 18%), $bla_{\text{CMY-2}}$ without a bla_{ESBL} gene (7/236; 3%), a bla_{ESBL} plus $bla_{\text{CMY-2}}$ (4/236; 2%), a bla_{ESBL} plus $bla_{\text{DHA-1}}$ (1/236; 0.4%), and a bla_{ESBL} plus $bla_{\text{CMY-60}}$ (1/236; 0.4%). Of the 48 NZ isolates from **Chapter 3**, 22/48 (46%) had the $bla_{\text{CTX-M-15}}$ ESBL gene. The other two ESBL genes found among these isolates were $bla_{\text{CTX-M-27}}$ (24/48; 50%), and $bla_{\text{CTX-M-14}}$ (2/48; 4%).

Table 5.3 A description of five virulence genes in 236 E. coli ST-131 isolates

Gene	Description	Reference	Association	Isolates with gene N (%)
gad	Glutamate decarboxylase	(Shin *et al.* 2001; Kaper *et al.* 2004)	Survival in acid	235 (99.6)
iha	Adherence protein	(Joensen *et al.* 2014)		227 (96.2)
iss	Increased serum survival	(Joensen *et al.* 2014)		211 (89.4)
sat	Secreted autotransporter toxin	(Kaper *et al.* 2004; Wiles *et al.* 2008)	ExPEC, UPEC	218 (92.4)
senB	Plasmid associated enterotoxin	(Joensen *et al.* 2014)		124 (52.5)
fimH	Type 1 pili	(Wiles *et al.* 2008)	ExPEC, UPEC	235 (99.6)

ExPEC: extra-intestinal pathogenic *E. coli*, UPEC: urinary pathogenic *E. coli*

Three virulence genes (*gad, iss*, and *sat*) associated with extraintestinal pathogenicity were found in the majority of these isolates, with 213/236 having all three of these genes. A description of the function of these genes is found in **Table 5.3.** Five virulence genes (*gad, iha, iss, sat* and *senB*) were found in 94/236 isolates. Presence-absence matrices of all virulence genes found using VirulenceFinder for the 236 isolates is shown in **Figure 5.1.**

A neighbour joining tree of the whole genome MLST is given in **Figure 5.2** with a pan-genome of these isolates where 3603 core genes (identified by Roary) and 13713 total genes are shown in a heat map based on presence and absence of these genes. The isolates cluster into three clades, and these clades (A/B/C) are shown in the colour bar in **Figure 5.2**.

Twenty-five isolates clustered into clade A. The serotypes for this clade were O16:H5 (21/25) and included New Zealand clinical isolates (**Chapter 3**), and isolates from Cambodia, Spain, and Australia. The remaining 4/25 isolates were serotype O25:H4 and all were from Singapore; 20/21 O16:H5 isolates had the *fimH*-41 antigen, and 2/4 each had *fimH*-41 and *fimH*-30 in the O25:H4 isolates.

Clade B and C isolates were initially examined together, then separately for evolutionary analysis. In these two groups were 211 isolates, the majority (201/211) were in clade C. All ten isolates of group B were an O25:H4 serotype; 7/10 were *fimH*-22, 2/10 *fimH*-30 and 1/10 were *fimH*-27. In clade C, 195/201 isolates were O25:H4 *fimH*-30; 3/201 isolates were H4 (O-unknown type) and *fimH*-30; 2/201 isolates were O16:H5 *fimH*-41; 1/201 isolates was O25 H-unknown type *fimH*-30.

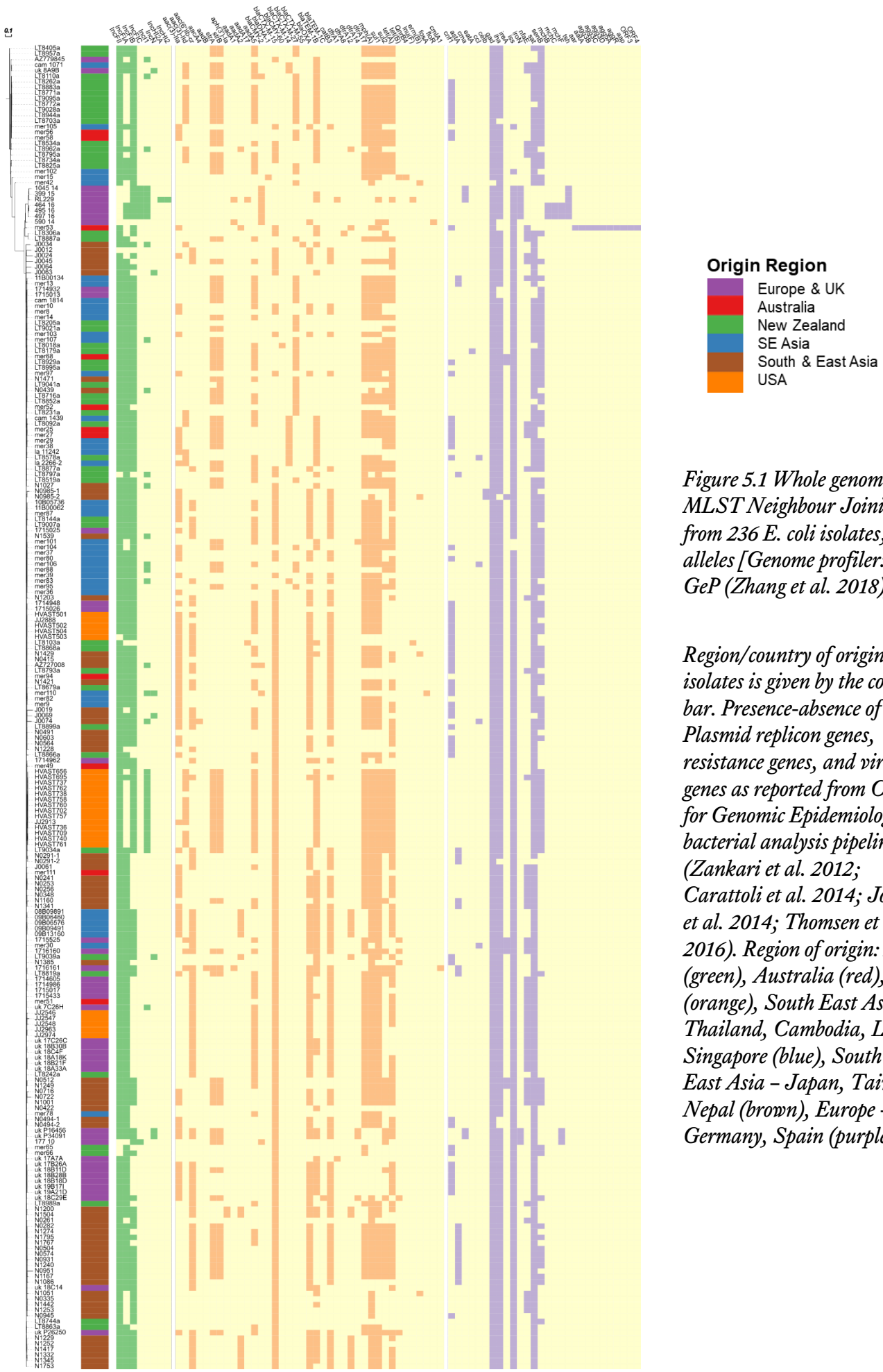


Figure 5.1 Whole genome MLST Neighbour Joining tree from 236 E. coli isolates; 3011 alleles [Genome profiler: Fast GeP (Zhang et al. 2018)].

Region/country of origin of isolates is given by the colour bar. Presence-absence of Plasmid replicon genes, resistance genes, and virulence genes as reported from Centre for Genomic Epidemiology bacterial analysis pipeline (Zankari et al. 2012; Carattoli et al. 2014; Joensen et al. 2014; Thomsen et al. 2016). Region of origin: NZ (green), Australia (red), USA (orange), South East Asia – Thailand, Cambodia, Laos, Singapore (blue), South and East Asia – Japan, Taiwan, Nepal (brown), Europe – UK, Germany, Spain (purple)

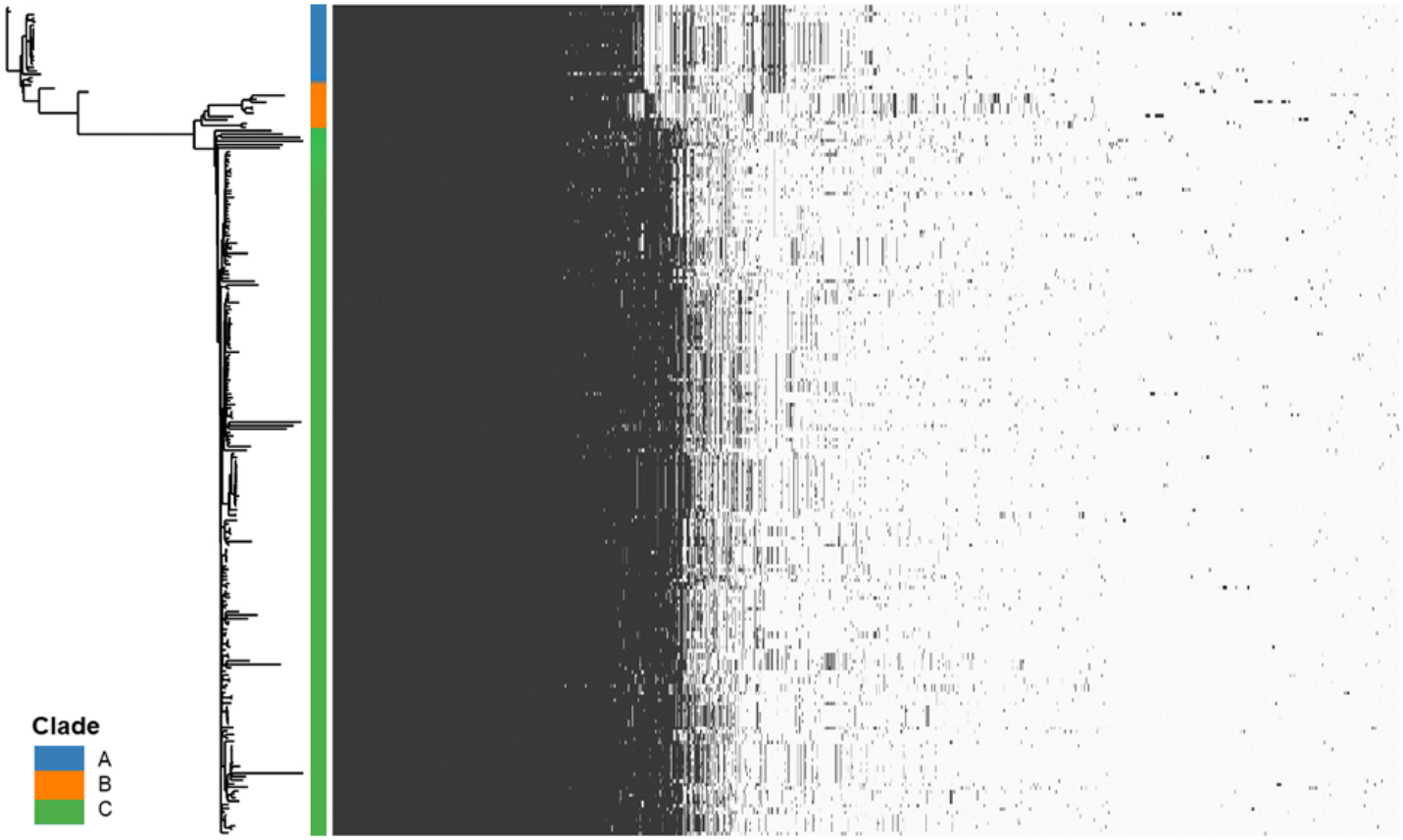


Figure 5.2 Pan-genome of 236 ST-131 E. coli isolates using 13713 genes; 3603 core genes, 224 soft-core genes, 1534 shell genes, 8314 cloud genes. Neighbour-joining tree (unrooted) from whole genome MLST (Figure 5.1 and using 3011 alleles), genome output from pan-genome presence-absence matrix [Roary version 8.0 (Page et al. 2015)]

5.4.3 SNP analysis and evolutionary phylogeny for *E. coli* ST-131 isolates

A core-SNP analysis was generated which clustered isolates into three distinct clades (A, B, and C). Within this dataset using the reference genome JJ1886, 14949 SNPs were found among the 236 isolates. A NeighborNet tree of the core-SNP alignment output is shown in **Figure 5.3**. Individual Snippy core alignments for each separate clade are shown in **Figure 5.4** where 6558 SNPs were used for the NeighborNet of 25 clade A isolates, 7940 SNPs for 10 clade B isolates, and 5505 SNPs for 201 clade C isolates. The same source core SNP phylogeny shown in **Figure 5.3** is also used for **Figure 5.5**, and this core-SNP analysis was used to compare the phylogenetic relationship of New Zealand isolates with other non-New Zealand isolates. However, in Figure 5.5, country of origin and travel metadata of the people from whom the isolate was collected (where known) were included in the figure. A core-SNP alignment of clade A was performed in preparation for evolutionary phylogenetic analysis using an internal reference genome (MER102); 8225 SNPs made up this core alignment and in each isolate ≥94.63% bases aligned to the reference (full alignment used for subsequent BEAST models).

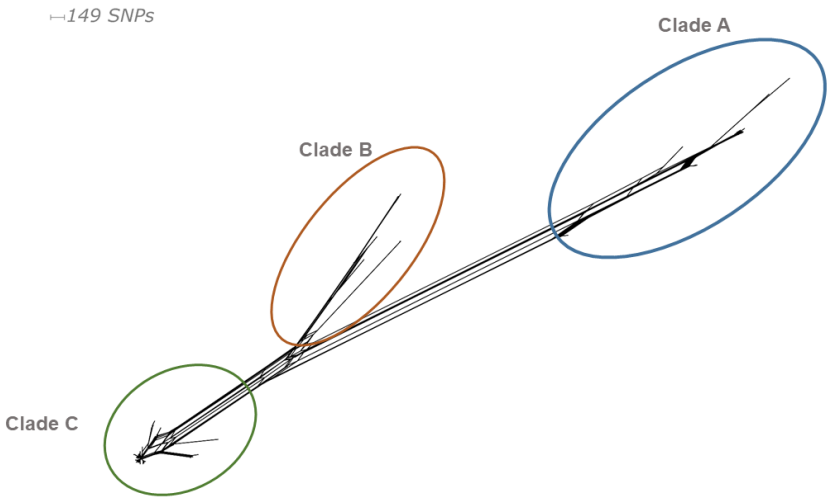


Figure 5.3 NeighborNet trees of Snippy outputs from clades A, B and C combined (using the reference genome JJ1886) of 236 isolates of E. coli ST-131 [Snippy version 3.1 (Seemann 2015)]

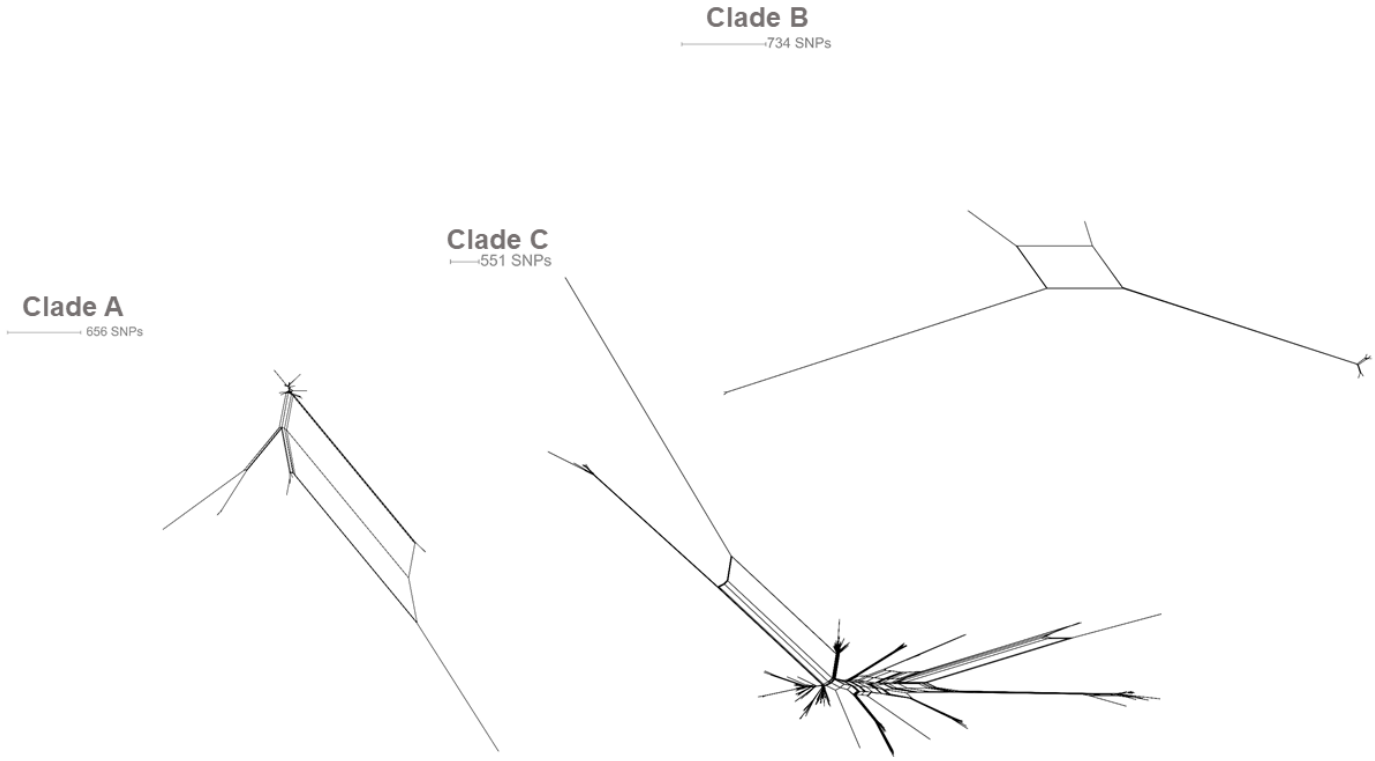


Figure 5.4 NeighbourNet trees of E. coli ST-131 clades A, B and C (using the reference genome JJ1886); 25 isolates in clade A, 10 isolates in clade B, 201 isolates in clade C. Scale is variable, and shown for each clade [Snippy version 3.1 (Seemann 2015)]

6558 SNPs were used for the NeighborNet of 25 clade A isolates, 7940 SNPs for 10 clade B isolates, and 5505 SNPs for 201 clade C isolates.

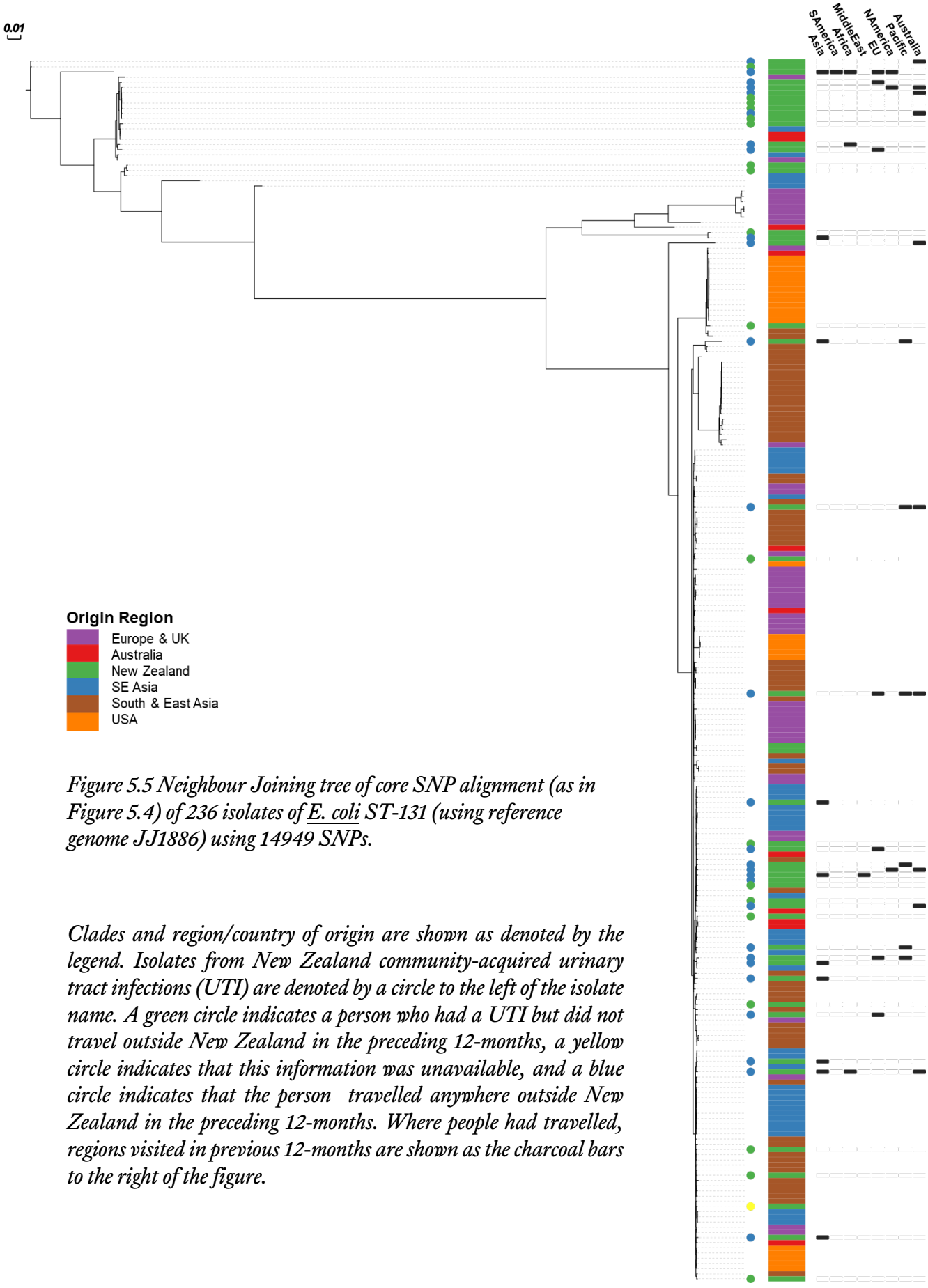


Figure 5.5 Neighbour Joining tree of core SNP alignment (as in Figure 5.4) of 236 isolates of E. coli ST-131 (using reference genome JJ1886) using 14949 SNPs.

Clades and region/country of origin are shown as denoted by the legend. Isolates from New Zealand community-acquired urinary tract infections (UTI) are denoted by a circle to the left of the isolate name. A green circle indicates a person who had a UTI but did not travel outside New Zealand in the preceding 12-months, a yellow circle indicates that this information was unavailable, and a blue circle indicates that the person travelled anywhere outside New Zealand in the preceding 12-months. Where people had travelled, regions visited in previous 12-months are shown as the charcoal bars to the right of the figure.

A common ancestor for the isolates in clade A was estimated using a GTR nucleotide substitution rate with a strict molecular clock and a constant coalescent population in BEAST (Bouckaert *et al.* 2014). The final model ('model 1' in **Table 5.1**) was identified by comparing models using AIC (Akaike information criterion), and measures of posterior support [for 'model 1': mean posterior=-6770.7 (95% highest posterior density -6785.3 to -6757.6; ESS=1178, standard deviation=7.4)]. This model estimated a common ancestor for NZ isolates in this clade between 1984 and 2001 (95% credibility interval), with a common ancestor for 24/25 of all clade A isolates circa 1991 (95% credibility interval between 1978 and 1999). The evolutionary phylogeny estimated by this model is shown in **Figure 5.6**. New Zealand community isolates made up the majority of the isolates examined in this group (16/25; 64%), and a cluster of 12 appear to have a common ancestor around 2008. All model statistics had effective sample size (ESS) support of >200. The mean posterior for this model was -6771 (with an ESS of 1178), the estimated clock rate (prior to adjustment for non-variant sites) was 5.3×10^{-3} [for 1006 SNPs (a core alignment of 8225 SNPs) and MER102 genome size estimate of 5065233 bp] or 1.1×10^{-6} SNPs per site per year.

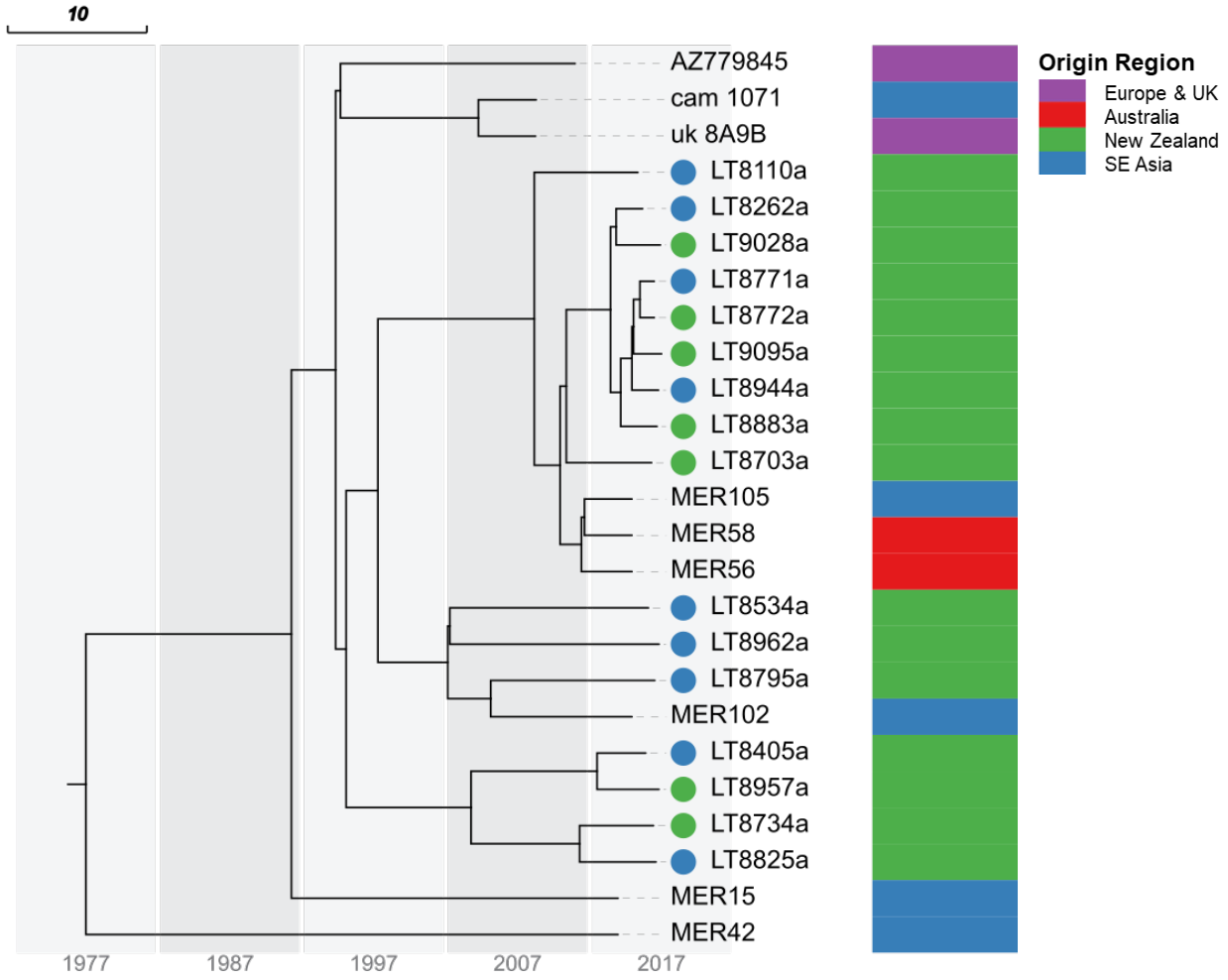


Figure 5.6 Evolutionary phylogeny for E. coli ST-131 'clade A' isolates. SNP alignment with recombinant regions removed used to generate evolutionary trees [BEAST 2.0 TreeAnnotater (Bouckaert et al. 2014); Gubbins was used to remove recombinant regions (Croucher et al. 2015)].

Isolates from New Zealand community-acquired urinary tract infections (UTI) are denoted by a circle to the left of the isolate name. A green circle indicates person who had UTI did not travel outside New Zealand in the preceding 12-months, and a blue circle indicates that they travelled outside New Zealand in the previous 12-months. Reference genome used: MER102 (Harris et al. 2018)

A distance matrix from the whole genome MLST of 16 New Zealand isolates in clade A using the reference genome LT8262a used 3950 alleles. When this was utilised alongside travel status (as in **Figures 5.5** and **5.6**), no statistical association between travel and the tree structure was found [p-value = 0.48 (F-statistic = 0.88)].

Evolutionary analysis for all 236 *E. coli* ST-131 isolates did not result in phylogenies that met the quality criteria outlined in the methods and therefore the results are not given here. Isolates for the smaller clade C group did likewise not meet the quality criteria, and all models had low posterior support (ESS <10).

5.5 Discussion

There are multiple strains of ESBL-producing *E. coli* circulating in the New Zealand community as demonstrated in **Chapter 3**. ST-131 was the most commonly found in cases of community-acquired ESBL-/ACBL-producing urinary tract infections in that study (37% of all *E. coli* isolates), although the actual proportion may be higher, as 50% (of ESBL-producing *E. coli* infections) was reported to be ST-131 in the 2016 national surveillance report (Heffernan *et al.* 2018). The distribution of the isolates from these people shown in **Figure 5.5** indicates that there are multiple sub-types of ST-131 circulating in the Auckland and Northland regions of New Zealand. Some of these strains (as in those in ST-131 clade A) appear to be more closely related to other isolates from NZ when compared to international isolates, although isolates described here were overrepresented in the clade A isolates used in this study.

Travel from a global region with high ESBL-carriage prevalence to a low-prevalence region has become a well-recognised risk factor for carriage of ESBL-producing Enterobacteriaceae (Rogers *et al.* 2014; Karanika *et al.* 2016; Armand-Lefèvre *et al.* 2018).

Travel to Asia was identified in **Chapter 3** as a risk factor that increased the odds of being a case in the case control study. In contrast, it appears from **Figure 5.4** that isolates collected from the New Zealand community (**Chapter 3**) were overall not associated with a particular global region and within clade A, travel was not significantly associated with isolate clustering (using wgMLST). However, of people with a UTI caused by ESBL-producing ST-131 in the case control study described in **Chapter 3,** 30% reported no one in their home had travelled internationally in the previous year (including themselves). It is also important to note that this study was not intended to be a systematic representation of geographic and temporal ST-131 *E. coli*, as not all regions were included (notably no isolates were from Africa, or from Central and South America), and possibly reflects submission patterns to the SRA database as a whole.

The diversity within all isolates included in this study was represented by Figures **5.2, 5.3,** and **5.5**. The presence of a large accessory genome within the isolates in this dataset (approximately 10,000 non-core shell/cloud genes identified) indicates substantial diversity within this sequence type. This aspect is worth investigating in future work, especially around the role of non-AMR aspects of mobile genetic elements. A high proportion (99%) of the isolates included in this study had a combination of the large plasmids IncFIA/IncFIB/IncFII, as shown in **Figure 5.1**. These plasmids have been found associated with bla_{ESBL} carriage in *E. coli* , particularly $bla_{\text{CTX-M-15}}$, so this is not a novel finding (Carattoli 2009). However, it is used here as a proxy for confirmation that it is likely that the majority of bla_{ESBL} in isolates in this study were associated with large plasmids. Long read sequencing of plasmids in these isolates would be needed to confirm and identify plasmids, and genetic associations between plasmids (and between plasmid and bacterial chromosome) should be the focus of future work.

Some differences were noted in this study when compared to similar studies performed outside New Zealand. Unlike the isolates in Matsumura *et al.* (2015), where 46% of isolates carried the $bla_{\text{CTX-M-14}}$ gene, only 5% (12/236) of all isolates [4% (2/48) for the New Zealand isolates] presented in this chapter had that gene. Like Petty *et al* (2014) and Stoesser *et al* (2016), $bla_{\text{CTX-M-15}}$ was the predominant bla_{ESBL} identified in ST-131 in this chapter (Petty *et al.* 2014; Stoesser *et al.* 2016). In comparison, for the clinical ST-131 isolates from **Chapter 3**, 50% (24/48) of isolates carried the $bla_{\text{CTX-M-27}}$ gene and 46% (22/48) carried $bla_{\text{CTX-M-15}}$. These differences suggest that there may be a unique make-up to the ESBL-containing plasmids circulating in New Zealand when compared to other countries (Petty *et al.* 2014; Matsumura *et al.* 2015; Stoesser *et al.* 2016).

Virulence genes were identified as part of the CGE pipeline from assembled genomes (Thomsen *et al.* 2016). The virulence-associated genes identified in over 50% of ST-131 isolates in this chapter were *sat*, *iha*, *gad*, and *senB* (as described in **Table 5.3**). These genes have been identified with pathotypes of *E. coli* associated with extraintestinal infections (e.g. urinary tract infections) and generally are associated with survival (*gad*), adhesion (*iha, fimH*), and cytotoxicity (*sat, senB*) (Guyer *et al.* 2000; Kaper *et al.* 2004; Mao *et al.* 2012; Barrios-Villa *et al.* 2018). As ST-131 is associated with extraintestinal infection, this finding is unsurprising (Nicolas-Chanoine *et al.* 2014).

Lastly and in addition to the descriptive work, evolutionary phylogenies of clade A, B, and C isolates were analysed here. In the work of Stoesser *et al.* (2016) investigating the evolution of ST-131, the 215 isolates used by these investigators were found to have recombination accounting for two thirds of the core nucleotide variant sites. They excluded these regions for subsequent analysis. From their resultant BEAST model, a rate of 2.46×10^{-7} SNPs per site per year was estimated for ST-131 isolates (Stoesser *et al.*

2016). This was similar to the rate estimated in Ben Zakour *et al.* (2016) which was also interested in the evolution of ST-131, where a mutation rate of 4.39×10^{-7} SNPs per site per year was estimated from BEAST models across clades C and B in their dataset. These rates are both lower than that calculated for the clade A isolates presented in this chapter, where a mutation rate of 1.1×10^{-6} SNPs per site per year was estimated, however this difference may be attributed to these being different clades and the intrinsic variability in mutation rate calculation (Sprouffske *et al.* 2018).

Limitations

The isolates included in this chapter do not include any isolates from before 2004, and this was identified as a limitation to common ancestor estimation by Petty *et al.* (2014). Those collected as part of **Chapter 3** and the isolates with a 'MER' prefix were all from samples submitted between 2014 and 2017. All other isolates were collected between 2004 and 2014. Exact dates were also unavailable for many isolates in this dataset, so the temporal inputs into the BEAST models were imprecise. For isolates in clade A, these two factors were less important, as accurate dates were known for all New Zealand isolates (16/25 in this clade), and the sample size was small enough that the full SNP alignment was able to be processed for recombinant regions. Attempts at generating evolutionary phylogenetic models for all 236 isolates included in this study were largely unsuccessful. This may have been attributed to the fact that there was substantial amount of recombination, as suggested by other studies (Stoesser *et al.* 2016), and the available computing power was unable to meet the demands to successfully identify these recombinant regions. Presence of a molecular clock signal was not investigated in this study, however further expansion of this research should look for this (e.g. using Bactdating) to ensure validity of evolutionary phylogeny (Yahara *et al.* 2018).

Another important limitation in this study was around the identification of plasmid replicon genes for large plasmids. These were detected from assembled genomes using PlasmidFinder (Carattoli *et al.* 2014). The decision not to use raw reads was made due to the focus of this study being on the whole genome of *E. coli* ST-131. However, plasmids are an apparatus by which ESBL genes are disseminated between bacteria, and therefore a key part of understanding why particular strains have become globally dominant. Therefore, an approach which uses both short-read (e.g. Illumina HiSeq) and long-read sequencing [e.g. MinION (Oxford Nanopore)] to examine bacteria on a large scale, would be optimal for future investigations of the kind presented in this chapter (San Millan 2018).

5.6 Conclusions

Within the study presented in this chapter, 41% of the community-acquired urinary infections from NZ were cultured from people who had not left NZ in the previous year. All three clades (A, C, and to a lesser extent B) appear to be circulating in the New Zealand community in the absence of recent travel history, and a substantial proportion of clade A isolates were cultured from these people. In the future, it is likely that endemic clones of ST-131, particularly clades A and C, will come to predominate if the prevalence of ESBL-producing bacterial carriage increases in the NZ community.

6 General discussion

6.1 Overview

6.1.1 Summary of findings

The research presented in this thesis was centred on the prospective case control study in **Chapter 3** and the research question: *is the family pet a risk for multidrug resistant infections*?

Companion animals in the home were not found to be risk for community-acquired urinary tract infections caused by ESBL-/ACBL-producing Enterobacteriaceae in this study. However, I did find pets carrying the same strain of bacteria that caused a person to have an ESBL-/ACBL-producing infection in the same household, so pets may have some role in transmission of these bacteria in the home (and therefore the community as a whole).

Other well-recognised risk factors for ESBL-/ACBL-producing infections were found to increase the odds of infection in the case control study. These factors included healthcare contact, recent antimicrobial treatment, and travel to high-risk countries. Other factors that increased the odds of being a case included being female, over 65-years-old, and having more than one urinary tract infection in the previous six-months. *E. coli* isolates from these index urinary tract infections were compared to isolates collected from people and pets in the homes of 23 case people. Faecal samples from 11 of these households cultured ESBL-/ACBL-producing *E. coli* isolates from more than one person or pet, and close genomic relatedness between isolates suggested some form of intra-household transmission was likely to have occurred in eight households. *E. coli* ST-131 was cultured from multiple individuals in four households and was the most commonly cultured strain

from community-acquired infections in case recruits. This strain was examined further for associations with isolates cultured from elsewhere in the globe, and the results suggested that multiple strains are circulating within New Zealand.

In this chapter, I will provide a larger context for the results described in **Chapters 3** to **5**, indicate where future work is needed, and provide recommendations based on this research.

6.1.2 Lessons learned

There were aspects to the research included in this thesis that taught me important lessons in research design. These lessons included maintenance of ethical standards, and the value in treating research ethics as an on-going conversation with oversight bodies and the parties involved in research. Four district health boards were involved in approving this study and negotiating the ethical expectations of each locality gave me the opportunity to reflect on how this study may affect different (albeit geographically close) communities.

Designed to be a prospective case control study, steady contemporaneous case recruitment was an important part of the study design for **Chapter 3**. Slow case recruitment during the initial year of this project resulted in necessary amendments to the study protocol. Efforts put into recruiting cases became successively more active: moving from a letter of invitation, to a follow-up call and incentives, to reminder letters and with multiple follow-up calls to prospective case participants. These changes were vital for the success of the case control study, and although fewer cases were recruited than expected (and over a longer period than initially planned), the resultant study was an important example in how to address challenges in real time.

6.2 Antimicrobial resistance as a societal issue

Antimicrobial resistance is a complex and multifactorial issue. As a clinician, I appreciate the desire to want what is best for your patient. As a scientist, I understand that it is the cumulative actions of many that will lead to or exacerbate antimicrobial resistance in the future. Examining the issues around AMR from "One Health" or "EcoHealth" perspectives, or as a "Wicked Problem", enables one to change perspective and creates the possibly for novel solutions (Signal *et al.* 2013; Roger *et al.* 2016). Mixed-methods approaches to "wicked problems" have been shown to provide a framework for tackling issues where complexity is high so this is something that could be incorporated into action plans in New Zealand (van Woezik *et al.* 2016; Waltner-Toews 2017). The high complexity for issues surrounding AMR originates not only in the nature of the organisms responsible, but also due to the nature of competing interests. Physicians want to use appropriate pharmaceuticals to cure disease, pharmaceutical companies want to stay in business, farmers want to produce products for sale in an economically efficient way, government policy-makers want to preserve market and trade integrity or reduce the impact on the economy that sick people pose, and patients want to be treated for disease and spared disability. Coupled with these human concerns, there are aspects that at much more difficult to control, such as environmental contamination with human waste in both the developed and developing world. This environmental contamination, coupled with other "wicked problems" such as climate change and increased wealth disparity, may lead to the development of hot spots for untreatable human disease. Increased competition for freedom from these areas may add pressure to geopolitical tensions in the future. At a global level, there is regional inequity in the burden of ESBL-producing bacteria (Karanika *et al.* 2016). Gender and racial inequalities in ESBL-producing *E. coli*

infections have been described, although they were not found to be a risk factor in **Chapter 3**, they are worth looking at in any further work done in this country (Thaden *et al.* 2016).

Women, especially post-menopausal women, are more likely to be affected by uncomplicated urinary tract infections (Grigoryan *et al.* 2014). While issues like prostatitis and acute urinary retention (with sequelae of the requirement for catheterisation) will increase the risk for urinary tract infection in men (Emberton and Anson 1999), as shown in **Chapter 3**, women represent a much higher proportion of total community-acquired MDR urinary tract infections [these demographics have been reflected in general cases of ESBL-producing infections in New Zealand (Heffernan *et al.* 2013)].

The social and emotional impacts of urinary tract infections, especially where it is accompanied by incontinence, can be imaginably high (Eriksson *et al.* 2014). The impacts on quality of life for a susceptible urinary tract infection may then be amplified by stigma associated with having a multidrug resistant infection. In addition to the personal implications for the individual emotional health, persistent carriage is also a public health concern. The long-term sequelae of these infections, if carriage status persists or cycling within the household allows for re-carriage, could cause complications and increase morbidity of other unrelated diseases. A proactive approach to this public health challenge is to reduce carriage status at the community level, not only within New Zealand but also as part of the global community.

There are a number of different approaches to reduce the burden of ESBL-*E. coli*. For example, active decolonisation strategies (i.e. using antimicrobial therapies) appear to

yield mixed results, and long term or large scale application of these are not practical (Bar-Yoseph *et al.* 2016). Another approach is within the frameworks of antimicrobial stewardship. Hygiene and biosecurity measures are key to reducing the need for antimicrobials through prevention of bacterial infection and reduction in transmission (Tacconelli *et al.* 2014). As described elsewhere in this thesis, there is also substantial support for the impact that reducing antimicrobial use community (or population) -wide might have on carriage of ESBL-producing bacteria within that population (Agersø and Aarestrup 2013; MacFadden *et al.* 2018). That said, it is important to recognise that this still an area of research requiring further work, and it is unlikely that reducing selection pressure alone will reverse AMR (Johnsen *et al.* 2009; Holmes *et al.* 2016).

6.3 Antimicrobial resistance ecology

6.3.1 Pets in the home

The risk factor of particular interest in the research presented in this thesis was contact with companion animals. As no association between having a pet and acquiring a multidrug resistant infection was found, I could not investigate pet-associated risk mitigation strategies. Despite this negative result, a description of general behaviour around pets in this country can be gleaned from control participants. From these 258 people with a pet in the home, I observed that many people do not wash their hands frequently around contact with their pets. While only 4% of controls with pets reported "never" washing their hands after contact with pet urine or faeces, 19% and 22% of controls did report "never" washing their hands after their pet licks them or after petting their pet respectively. For these two variables, 45% and 29% of control respondents reported "always" washing their hands after these respective exposures (the rest reporting "sometimes" or "often" wash hands). These could be considered as

modifiable behaviours to reduce risks of zoonotic disease. In **Chapter 4**, the bacteria cultured from two dogs in separate households cultured bacteria that was clonally related to isolates causing infection in the clinical sample submitted from the person recruited as a case for the case-control study. This illustrates that transmission events between humans and animals (or vice versa) are likely to be occurring within the home environment. The genomics work from the bacteria collected from faecal samples as part of **Chapter 3** and **4** has been the inspiration for modelling the role that antimicrobial treatment in pets play in the development of antimicrobial resistance.

However, human-to-human, or environment-to-human transfer of ESBL-E are likely to be the predominant mode of transmission, and reverse-zoonotic transmission of AMR bacteria to pets (rather than pets-to-people) may be a more dominant pathway (Messenger *et al.* 2014).

6.3.2 The environment

The role the environment plays in the transmission and propagation of MDR bacteria warrants further examination (Larsson *et al.* 2018). In New Zealand, we still know little about environmental AMR sources, and how these putative sources contribute to transmission pathways. These could be through waterways contaminated by human or animal sources of *E. coli*, by urban environmental transmission, or by contamination of food crops, among others. This knowledge gap was identified in the literature review in **Chapter 2** and has been since followed up by work in the ^{m}Epilab in this area. Other studies related to this work included a pilot study examining ESBL-E in production animals. Future work for exposures should focus on both humans and animals, and the roles that wildlife and the environment may play in providing substrate for (or source of) horizontal gene transmission elements (Larsson *et al.* 2018).

6.3.3 The microbiome

Our understanding of microbiomes has matured substantially over the last decade, and this has been facilitated by advances in metagenomics and computing (Crofts *et al.* 2017). With both new molecular techniques and the reduction in cost of whole genome sequencing, our understanding is likely to increase as the interpretation and utility of the results of these techniques become clearer in the future. It is also likely that our understanding of the role that complex communities of microflora will change, and our ability to manipulate and optimise this population will likewise evolve. Dogs (and to a lesser extent cats) have a similarity in digestive tract physiology and diet to humans, unlike many other domesticated animals. This similarity in combination with the presence of bacteria (such as ESBL-E) in these populations, in addition to evidence provided in **Chapter 4**, means that future work should continue to investigate companion animals' role in community transmission of ESBL-producing *E. coli*.

6.4 Antimicrobial resistance and plasmids

Plasmids are small circular genomes living within a bacterial cell, regulating their own replication and manipulating the host into spreading to conjugated bacteria and as such they might be regarded as an independent living entity (Carattoli 2009). More importantly, plasmids play an integral role in the transmission and dissemination of ESBL-production in disease-causing bacteria. It should be noted however, that plasmid-associated MDR in Enterobacteriaceae includes not only ESBL- and ACBL-producers, but also carbapenemase-producers, fluoroquinolone resistance, and colistin resistance.

Horizontal transmission of antimicrobial resistance is worth investigating further within the dataset curated as part of this project. I found evidence of suspected horizontal gene transmission from individuals as part of the cross-sectional study (**Chapter 4**) between

different bacterial types (e.g. *E. coli* ST-44 and ST-405), as well as between species (e.g. *Klebsiella pneumoniae* and *E. coli*). Low-cost options for obtaining sequences from plasmids include the MinION (Oxford Nanopore Technologies), although ideally PacBio (Pacific Biosciences) sequencing would be used (Levy and Myers 2016; Orlek *et al.* 2017). Obtaining these high-quality plasmid sequences using a long-read technique would therefore be a first step in examining these isolates, and this work is underway in the ^{m}Epilab and includes plasmids from some isolates included in this body of work.

Despite the importance of plasmids, the molecular methodologies used in **Chapters 3**, **4** and **5** did not directly allow investigation of these plasmids as the bioinformatics techniques utilised in those chapters focussed on core genomic differences and found large plasmid genes in bacteria presented in these chapters. Others have examined the factors that make plasmid promiscuous, advancing techniques to explore these factors (Carattoli 2009; Orlek *et al.* 2017). Nevertheless, components of the accessory genome are likely to be key to understanding the implications for horizontal gene transmission dynamics and the determinants of successful colonisation of plasmid-bearing MDR bacteria in multiple mammalian hosts requires larger population size to study (San Millan 2018).

6.5 Recommendations to reduce and manage the community burden of ESBL-producing bacteria

1. Advice to New Zealanders travelling to high-risk regions (such as Asia and Africa) should emphasise hand hygiene and recommend people only use systemic antibiotics while traveling if absolutely necessary
    - See **Chapter 3**
2. Healthcare professionals should consider in-contact people in homes where someone has been positive for ESBL-E or ACBL-E as at risk of carriage of (and therefore becoming infected by) these bacteria
    - See **Chapter 4**
3. Antimicrobial stewardship in general practice is a target for reducing antimicrobial use and thereby reduce AMR selection pressure in the New Zealand community
    - See **Chapter 2**
4. Surveillance screening of ESBL-E should be routine in veterinary diagnostic laboratories, and monitoring these bacteria long-term should be considered a priority
    - See **Chapter 2**
5. More work is required using modern molecular techniques to better understand the role of plasmids in endemic and epidemic AMR strains, as this may lead to modification of risk
    - See **Chapters 4 & 5**
6. Future projects in New Zealand should focus on the role that both urban and rural environments play in AMR transmission
    - See **Chapter 2 & 4**

6.6 Concluding remarks

General applicability and conclusions of the research presented here may not extend beyond New Zealand, however they provide valuable insights. Pets are important companions for many people, including those in vulnerable situations. As companion animals were not found to be a risk for ESBL-/ACBL-E infections, they may not be considered a focus for intervention or regarded as an intrinsic risk factor. Antimicrobial resistance in the New Zealand community warrants further investigation. The results of this thesis indicates that there are various risk factors for ESBL-producing Enterobacteriaceae causing infection in the New Zealand community. Areas that require further research include resistance gene-containing plasmids circulating in the community, and the roles that non-human sources or reservoirs play. Future observational studies should focus on longitudinal carriage and transmission of ESBL-producing *E. coli* within households (including companion animals), utilising whole genome sequencing where intra-person/pet genetic diversity is also captured. Examination of microbiomes and environmental metagenomics could also provide valuable supplemental information, and may shed light on the role that non-disease causing bacterial communities may play in supporting antimicrobial resistance. The results of such work could then be used to inform models of antimicrobial resistance transmission.

While there is still work to be done, this research provides an overview of what is known regarding ESBL-/ACBL-producing Enterobacteriaceae in New Zealand. Methodologies and results presented here have subsequently been used to inform genomic epidemiologic research into AMR in food animals and the environment, and future developments will add to the larger body of work in which the study presented here resides.

APPENDIX I: MICROBIOLOGY

Culture of ESBL and suspected AmpC isolates from Human Laboratory Samples

Day 1

1. Samples (isolates) received from courier (ex-Auckland)
2. Fill in **Clinical Submission** data sheet with date and sample ID (and Labtests ID), comments as required. If possible, get data entry verified by another laboratory member.
3. Inoculate onto labelled blood agar; and incubate at 37 °C for 16-24 hours.
4. Keep Labtests travel plate in chiller. Discard in 7 days.

Day 2

1. Subculture a single colony to a fresh labelled blood agar and incubate as per above; repeat choosing a second colony. Label 'sampleID+a' & 'sampleID+b' respectively.
2. Incubate at 37 °C for 16-24 hours

Day 3

For isolate 'sampleID+a':

1. From blood agar, make a suspension in sterile saline equivalent to a 0.5 McFarland standard with a sterile cotton swab following EUCAST guidelines for antimicrobial susceptibility testing (AST) (Kahlmeter *et al.* 2006). Within 15 mins, dip a sterile cotton swab into the suspension, remove excess inoculum by rotating the swab on the inner wall of the tube and cover a labelled Mueller–Hinton agar plate, swabbing in three directions. Repeat for a total of TWO plates.

2. **Plate 1)** ESBL confirmatory test: use cefotaxime & ceftazidime +/- clav disks (D62C & D64C) as per MAST instructions.
3. Incubate at 37°C in air for 16-20 h
4. **Plate 2)** AmpC phenotypic test: use disks (D69C) from MAST AmpC test (CPD +/- inducer +/- inhibitor) as per MAST instructions. *AST to be performed on isolates (from frozen) at a later date*
5. Incubate at 37°C in air for 16-20 h
6. From blood agar, suspend bacteria into broth/cryoprotectant, gently scraping the surface using a sterile swab and pipette to freezer cryovial. Transfer to -80 °C freezer for storage (prior to consent)
7. Label vial with isolate ID (sample ID + a), and record location in freezer on **Clinical Submission** data sheet

For isolate 'sampleID+b':

1. From blood agar, suspend bacteria into broth/cryoprotectant, gently scraping the surface using a sterile swab and pipette to freezer cryovial. Transfer to -80 °C freezer for storage (prior to consent)
2. Label vial with isolate ID (sample ID + b), and record location in freezer on **Clinical Submission** data sheet

Day 4

1. Measure disk diameters for each plate as per EUCAST protocols using a calliper; record results on **Clinical Submission** data sheet with sampleID+a
2. If AmpC positive (zone difference both ≥5mm), report as AmpC case to be recruited to the clinical microbiologist recruiting cases.

3. If ESBL negative or non-positive, put plate in chiller for repeat ESBL testing using D62C, D64C, D63C on next week (i.e. **Day 5)**

Day 5

1. Subculture onto blood agar

Day 6

2. Repeat **Day 3**, including a D63C plate

Day 7

3. Measure disk diameters for plate as per EUCAST protocols using a calliper; record results on Clinical Submission data sheet with sampleID+a
4. If ESBL and AmpC negative, repeat Days 2 to 4 with frozen 'sampleID+b' the following week

AmpC/ESBL isolation from faecal samples

Day 0 (Sunday)

1. Samples arrive from Auckland (chilled)

Day 1

For bird samples:

1. Fill in Faecal sample data sheet with date and sample ID, comments as required. If possible, get data entry verified by another laboratory member
2. Use a sterile swab to transfer brown/faecal material into 20mL of buffered peptone water (BPW). Incubate for 12-20 hours at 37 °C

For all other samples:

1. Fill in Faecal sample data sheet with date and sample ID, comments as required. If possible, get data entry verified by another laboratory member
2. Aliquot ~1g portion into a labelled container (eppendorf) and store in -80 °C freezer. Record location on Faecal sample data sheet.
3. From a faecal sample use a sterile cotton swab and swab a MacConkey plate, and proceed to streak plate;
4. Using same swab, repeat labelled antimicrobial CAZ (MacConkey plus 1mg/L ceftazidime) plate
5. Using repeat labelled antimicrobial CTX (MacConkey plus 1mg/L cefotaxime) plate
6. Using same swab, repeat procedure on a labelled ESBL selective chromogenic agar plate (CHROMagar™ , Paris France)

7. Incubate plates at 37 °C for 16-24 hours.

Day 2

For bird samples:

1. From a BPW sample, use a sterile cotton swab and swab a MacConkey plate, and proceed to streak plate;
2. Using a fresh swab, repeat labelled antimicrobial CAZ (MacConkey plus 1mg/L ceftazidime) plate
3. Using a new sterile swab, repeat procedure on a labelled antimicrobial FOX (MacConkey plus 1mg/L cefotaxime) plate
4. Using a new sterile swab, repeat procedure on a labelled Chromagar ESBL plate

For all other samples:

1. From plain MacConkey plate record plate growth description on Faecal sample data sheet. Select 2 morphologically distinct Enterobacteriaceae (suspected to be *E coli* or *Klebsiella*) colonies using a sterile loop and subculture onto blood agar. Isolates to be given isolate ID number (sampleID+a/b).
2. From CAZ-MacConkey plate examine for growth and record on Faecal sample data sheet. Using a sterile loop, subculture a maximum of 2 colonies onto labelled blood agar purity plates (one colony per plate). Isolates to be given isolate ID number (sampleID+c/d).
3. Incubate plates at 37 °C for 16-24 hours.
4. From CTX-MacConkey plate examine for growth and record on Faecal sample data sheet. Using a sterile loop, subculture a maximum of 2 colonies onto labelled

blood agar purity plates (one colony per plate). Isolates to be given isolate ID number (sampleID+e/f).

5. Incubate plates at 37 °C for 16-24 hours.
6. From Chromagar ESBL plate examine for growth and record on Faecal sample data sheet. Using a sterile loop, subculture a maximum of 2 colonies onto labelled blood agar purity plates (one colony per plate). Isolates to be given isolate ID number (sampleID+g/h).
7. Incubate plates at 37 °C for 16-24 hours

Day 3

For bird samples:

1. Refer to **Day 2** procedure for **all other samples**

For EACH isolate from non-bird samples:

1. From blood agar, make a suspension in sterile saline equivalent to a 0.5 McFarland standard with a cotton swab. Within 15 mins, dip a cotton swab into the suspension, remove excess inoculum by rotating the swab on the inner wall of the tube and cover a labelled Mueller–Hinton agar plate, swabbing in three directions. Repeat for a total of two plates. *AST and some Day 3/4 sample analysis (ESBL/AmpC testing) to be performed from frozen isolates at a later date.*
    a. **Plate 1)** ESBL confirmatory test: use cefotaxime & ceftazidime +/- clav disks (D62C, D63C & D64C) as per MAST instructions.
    b. **Plate 2**) AmpC phenotypic test: use disks (D69C) from MAST AmpC test (CPD +/- inducer +/- inhibitor) as per MAST instructions
2. Incubate at 37°C in air for 16-20 h

FOR ALL isolates (a-h):

3. From the blood agar purity plate, suspend bacteria into broth/cryoprotectant, gently scraping the surface using a sterile swab and pipette to freezer a labelled cryovial. Transfer to -80 °C freezer for storage. Record location in freezer on Faecal sample data sheet

Day 4

For bird isolates

1. Refer to **Day 3** procedures for **non-bird isolates**

For non-bird isolates

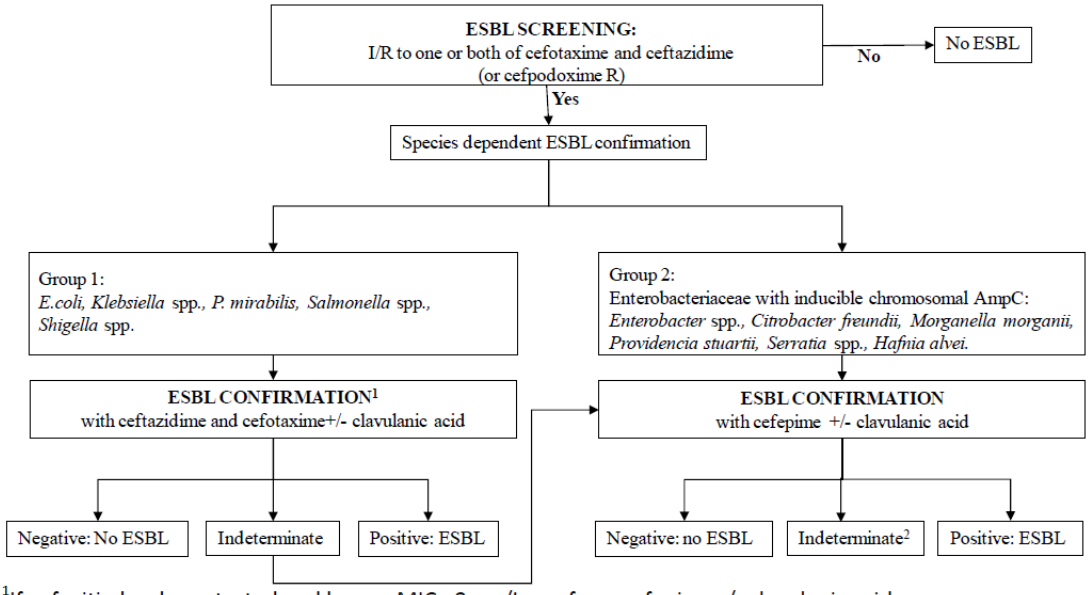
1. Examine ESBL and AmpC plate(s), measure using a calliper; record results in mm on Faecal sample data sheet. Interpret results. If positive for ESBL and/or ACBL phenotype, record as positive, or equivocal on Faecal sample data sheet. *Species ID to be performed at a later date (i.e. MALDI-TOF) for isolated positive for ESBL- or ACBL phenotype*

Day 5

For bird isolates

2. Refer to **Day 4** procedures for **non-bird isolates**

For ESBL equivocal: Measure disk diameters for cefepime plate as per EUCAST protocols using a calliper; record results on Faecal sample data sheet with isolateID



[1]If cefoxitin has been tested and has an MIC >8 mg/L, perform cefepime+/- clavulanic acid confirmation test

[2]Cannot be determined as either positive or negative (e.g. if the strip cannot be read due to growth beyond the MIC range of the strip or no clear synergy in combination-disk and double-disk synergy tests). In case confirmation with cefepime +/- clavulanic acid is still indeterminate genotypic testing is required.

Figure 6.1 Algorithm for detection of phenotypic ESBLs. EUCAST guidelines, and AmpC interpretation from Halstead et al. (2012)

Appendix II: DNA extraction and library preparation

Isolation and purification

Day 1

1. Thawing procedure: using a sterile loop scrape isolate sample from cryovial, and streak onto blood agar. Return vial immediately to freezer
2. Incubate plate(s) in 37°C for approx. 24 hours.
3. Record on Clinical Submission data sheet.

Day 2

1. Subculture a single colony onto blood agar. Incubate as above.

Day 3

2. Examine plates for growth, if pure growth of sufficient colonies, proceed to DNA extraction
3. DNA extraction:
    a. Qiagen kit DNA extraction protocol for plasmid retention
    b. Qubit kit for QA of DNA/RNA/Protein
    c. Label, make and freeze DNA at -20°C. Record location on WGS data sheet (ensure isolate ID is recorded)
    d. If DNA is not of sufficient quality, sub colony onto fresh blood agar and incubate for 18-24 hours at 37°C and repeat day 2 procedure

Extraction of genomic DNA (Modified Qiagen protocol for purification from tissues)

Follow directions for prior to start of protocol

1. Dispense 180 uL ATL buffer into Eppendorf vials; remove one loop (i.e. one colony) and suspend in buffer
2. Add 20uL of proteinaseK, mix by vortex and incubate in 37°C overnight and then at 56°C on shaking heat-block for 1-2 hours (OR 3 hours at 56 degrees on shaking heat-block)
3. Briefly centrifuge vials to remove condensation
4. Add 15 uL RNAaseA (100mg/mL) to the vial and incubate at 37°C for 40 minutes on the shaking heat-block
5. Briefly centrifuge to remove condensation
6. Add 200 uL of AL buffer, mix by pulse vortex and incubate at 70°C for 10 minutes on shaking heat-block
7. Add 200uL of (96-100%) ethanol to the sample and mix using pulse vortex
8. Transfer sample to spin column, close cap and centrifuge at 6000xg for 1 minute
9. Add 500 uL of AW1 buffer to spin column and centrifuge at 6000xg for 1 minute
10. Add 500 uL of AW2 buffer to spin column and centrifuge at 14,000rpm for 3 minutes
11. Discard filtrate and centrifuge again at 14,000rpm for 1 minute
12. Use a clean 1.5 microcentrifuge tube (for later freezing), add 75 uL of PCR grade water (37°C) and incubate at room temperature for 5 minutes; then centrifuge at 6000xg for 1 minute
13. This sample should then be checked for quality using Qubit fluorimeter, record [DNA] for later normalisation and store at -20°C

Preparation of libraries from DNA for sequencing

1. Normalise genomic DNA to 0.16ng/uL
2. Tagmentation
    a. Follow Nextera Illumina protocol, substitute 10 minutes with PCR thermocycler for 12 minutes
3. Clean up DNA
    a. Follow Nextera Illumina protocol
    b. Store libraries in clean safe-lock tubes at -20°C prior to pooling of libraries
4. QC of libraries
    a. [DNA] using Qubit
    b. Library fragment analysis using LabChip or Bioanalyzer2100 to determine molarity of each library for accurate pooling of DNA for sequencing
5. If library is of insufficient quality, repeat DNA extraction and library preparation steps

APPENDIX III: ISOLATE SUMMARIES

Table 6.1 Appendix III chapter 3 isolates

Isolate ID	Household	Collection date	Species	MLST	Genome size (bp)	GC%	Read depth	Contigs	N50
LT8007a	HH0001	30/09/2015	Klebsiella pneumoniae	ST-753	5314474	55.8	122	245	188887
LT8018a	HH0005	10/10/2015	Escherichia coli	ST-131	5301563	50.4	137	335	180448
LT8032a	HH0022	13/10/2015	Escherichia coli	ST-484	4946061	50.2	125	384	94478
LT8052a	HH0015	28/10/2015	Escherichia coli	ST-963	5150269	50.5	134	320	115994
LT8057a	HH0003	2/11/2015	Escherichia coli	ST-345	4923090	51	155	236	122675
LT8062a	HH0008	9/11/2015	Escherichia coli	ST-69	5492481	51.5	48	243	285535
LT8088a	HH0010	24/11/2015	Escherichia coli	ST-44	4852736	50.7	139	331	72360
LT8092a	HH0011	23/11/2015	Escherichia coli	ST-131	5266448	51.3	66	199	143508
LT8103a	HH0014	30/11/2015	Escherichia coli	ST-131	5351299	50.1	121	735	162133
LT8110a	HH0012	4/12/2015	Escherichia coli	ST-131	5144652	50.8	117	261	180447
LT8120a	HH0021	15/12/2015	Escherichia coli	ST-58	4916347	51.1	146	280	108516
LT8125a	HH0016	15/12/2015	Escherichia coli	ST-500	5270376	50.5	56	216	163953
LT8129a	HH0023	13/12/2015	Escherichia coli	ST-34	5038369	49.9	153	671	98386
LT8131a	HH0017	22/12/2015	Escherichia coli	ST-998	5329330	50.3	100	140	223116
LT8144a	HH0020	26/12/2015	Escherichia coli	ST-131	5338622	50.8	59	168	191062
LT8154a	HH0025	12/01/2016	Escherichia coli	ST-744	4707466	50	126	470	99952
LT8171a	HH0027	19/01/2016	Escherichia coli	ST-410	5163188	50.6	258	310	88409
LT8179a	HH0024	28/01/2016	Escherichia coli	ST-131	5160657	51.1	81	178	179969
LT8198a	HH0026	5/02/2016	Escherichia coli	ST-1193	5092266	50.3	130	574	162923
LT8199a	HH0035	4/02/2016	Escherichia coli	ST-69	5202027	50.7	139	501	134086
LT8205a	HH0038	8/02/2016	Escherichia coli	ST-131	5128998	50	104	300	160880
LT8214a	HH0036	22/02/2016	Escherichia coli	ST-410	5063940	51	157	648	126665
LT8230a	HH0053	7/03/2016	Escherichia coli	ST-73	5029095	50.4	112	227	186707
LT8231a	HH0039	4/03/2016	Escherichia coli	ST-131	5206381	50.3	99	444	156192
LT8242a	HH0040	20/03/2016	Escherichia coli	ST-131	5215701	51	181	447	170471
LT8262a	HH0052	6/04/2016	Escherichia coli	ST-131	5110787	51	112	178	307664
LT8265a	HH0041	31/03/2016	Escherichia coli	ST-405	5090574	50.5	139	267	90141
LT8267a	HH0044	1/04/2016	Escherichia coli	ST-457	4996121	47.8	78	216	187576
LT8283b	HH0061	20/04/2016	Escherichia coli	ST-12	5173501	50.1	150	564	204846
LT8284a	HH0043	20/04/2016	Escherichia coli	ST-744	4919565	50.4	98	415	118620
LT8306a	HH0045	27/04/2016	Escherichia coli	ST-131	5264432	50.5	110	401	208122
LT8307a	HH0046	30/04/2016	Escherichia coli	ST-38	5422420	50.1	74	439	104397
LT8308a	HH0047	3/05/2016	Escherichia coli	ST-38	5186779	50.5	71	264	152916
LT8320a	HH0048	9/05/2016	Escherichia coli	ST-38	5161032	51	173	381	123639
LT8335a	HH0049	12/05/2016	Escherichia coli	ST-393	5097768	50	81	333	129076
LT8371a	HH0064	31/05/2016	Escherichia coli	ST-963	5153473	50.2	120	338	92286
LT8405a	HH0057	27/06/2016	Escherichia coli	ST-131	4957577	50.7	123	273	178664
LT8410a	HH0059	16/06/2016	Escherichia coli	ST-59	5220194	50.1	138	486	55007
LT8442a	HH0063	13/07/2016	Klebsiella pneumoniae	ST-25	5589238	56.2	78	259	172668
LT8444a	HH0060	21/07/2016	Escherichia coli	ST-38	5215660	50.3	86	345	133028
LT8455a	HH0065	25/07/2016	Escherichia coli	ST-69	5120029	50.2	151	282	164000
LT8467a	HH0066	9/08/2016	Klebsiella pneumoniae	ST-133	5690541	56.6	107	307	165851
LT8486a	HH0068	8/08/2016	Escherichia coli	ST-12	5136947	50.4	104	219	225032
LT8504a	HH0072	23/08/2016	Escherichia coli	ST-38	5152653	50.5	78	257	123936
LT8519a	HH0071	30/08/2016	Escherichia coli	ST-131	5122957	50.9	108	269	166692

Isolate ID	Household	Collection date	Species	MLST	Genome size (bp)	GC%	Read depth	Contigs	N50
LT8534a	HH0070	5/09/2016	Escherichia coli	ST-131	5093878	50.5	129	293	159414
LT8553a	HH0074	21/09/2016	Escherichia coli	ST-57	4995515	50.2	69	170	129752
LT8569a	HH0073	25/09/2016	Escherichia coli	ST-2345	4596790	50.5	132	662	72910
LT8578a	HH0075	3/10/2016	Escherichia coli	ST-131	5292404	50.5	111	346	133277
LT8604a	HH0077	18/10/2016	Klebsiella pneumoniae	ST-405	5668329	56.4	99	362	151889
LT8610a	HH0082	25/10/2016	Escherichia coli	ST-38	5321441	50.5	65	410	111559
LT8662a	HH0091	21/11/2016	Escherichia coli	ST-38	5304785	50.5	91	367	80948
LT8679a	HH0093	2/12/2016	Escherichia coli	ST-131	5290276	50.5	90	257	178374
LT8685a	HH0102	7/12/2016	Escherichia coli	ST-349	5267305	50.9	98	301	115398
LT8691a	HH0080	7/11/2016	Escherichia coli	Unknown ST	5243293	50.9	162	615	178682
LT8703a	HH0086	29/11/2016	Escherichia coli	ST-131	5083072	50.6	131	443	178654
LT8704a	HH0085	13/12/2016	Escherichia coli	ST-95	5021005	49.6	110	281	174711
LT8706a	HH0087	13/12/2016	Escherichia coli	ST-38	5345003	50.6	72	435	104634
LT8716a	HH0104	16/12/2016	Escherichia coli	ST-131	5086836	49.6	135	336	173801
LT8717a	HH0088	21/12/2016	Escherichia coli	ST-998	5202978	50.4	137	297	252929
LT8734a	HH0090	17/01/2017	Escherichia coli	ST-131	5108706	50.5	91	318	181516
LT8740a	HH0142	18/01/2017	Escherichia coli	ST-1193	5157191	50.3	121	285	161155
LT8744a	HH0098	13/01/2017	Escherichia coli	ST-131	5211205	51	100	153	255513
LT8769a	HH0097	7/02/2017	Escherichia coli	ST-7358	5203953	50.5	124	516	88402
LT8771a	HH0114	7/02/2017	Escherichia coli	ST-131	5072572	50.6	139	275	186350
LT8772a	HH0109	8/02/2017	Escherichia coli	ST-131	5049815	50.9	124	255	181591
LT8776a	HH0115	9/02/2017	Escherichia coli	ST-58	5033666	50.6	168	358	111420
LT8777a	HH0096	7/02/2017	Escherichia coli	ST-38	5466893	50.1	93	639	123270
LT8789a	HH0095	21/02/2017	Escherichia coli	ST-648	5304063	50.4	126	372	155339
LT8793a	HH0110	21/02/2017	Escherichia coli	ST-131	5293870	50.4	115	358	162730
LT8795a	HH0113	20/02/2017	Escherichia coli	ST-131	5227381	50.5	118	322	178637
LT8797a	HH0100	17/02/2017	Escherichia coli	ST-131	5163199	50.3	111	369	159114
LT8806a	HH0178	2/03/2017	Escherichia coli	ST-345	4743293	50.6	81	289	151055
LT8819a	HH0105	17/03/2017	Escherichia coli	ST-131	5413553	50.1	167	486	162190
LT8821a	HH0101	28/03/2017	Escherichia coli	ST-38	5457864	49.8	79	461	115082
LT8825a	HH0099	24/03/2017	Escherichia coli	ST-131	5004821	50.5	103	323	180447
LT8846a	HH0139	9/03/2017	Escherichia coli	ST-69	5201815	50.7	135	330	131227
LT8848a	HH0141	4/04/2017	Escherichia coli	ST-69	5234166	50.4	149	357	98524
LT8852a	HH0118	6/04/2017	Escherichia coli	ST-131	5113467	50.7	114	301	154937
LT8859a	HH0117	11/04/2017	Escherichia coli	ST-1193	5062925	50.3	115	196	140191
LT8862a	HH0136	12/04/2017	Escherichia coli	ST-14	5351771	50.4	116	297	104857
LT8863a	HH0145	10/04/2017	Escherichia coli	ST-131	5147584	50.5	118	231	181672
LT8866a	HH0135	12/04/2017	Escherichia coli	ST-131	5208737	50.6	109	243	151001
LT8868a	HH0133	6/04/2017	Escherichia coli	ST-131	5391709	50.3	56	318	166898
LT8875a	HH0138	16/04/2017	Escherichia coli	ST-12	4989088	50.5	108	170	203071
LT8876a	HH0152	14/04/2017	Klebsiella pneumoniae	ST-562	5515023	53.2	80	327	173893
LT8877a	HH0169	17/04/2017	Escherichia coli	ST-131	5200388	49.8	79	377	159114
LT8880a	HH0120	18/04/2017	Escherichia coli	ST-38	5244722	50.5	98	390	110346
LT8883a	HH0144	20/04/2017	Escherichia coli	ST-131	5064306	50.5	125	263	186549
LT8887a	HH0125	24/04/2017	Escherichia coli	ST-131	5140493	50.6	129	270	215233
LT8888a	HH0140	2/05/2017	Escherichia coli	ST-295	4702664	50.6	114	268	161944
LT8897a	HH0119	2/05/2017	Escherichia coli	ST-80	5183283	50.1	128	341	165660
LT8899a	HH0121	30/04/2017	Escherichia coli	ST-131	5293073	50.4	100	349	161706
LT8910a	HH0116	4/05/2017	Klebsiella pneumoniae	ST-562	5544867	52.4	103	442	249057

Isolate ID	Household	Collection date	Species	MLST	Genome size (bp)	GC%	Read depth	Contigs	N50
LT8913a	HH0132	23/05/2017	Escherichia coli	ST-1193	5096054	50.4	132	297	162796
LT8914a	HH0124	24/05/2017	Escherichia coli	ST-38	5303500	50.2	115	274	150170
LT8929a	HH0131	10/05/2017	Escherichia coli	ST-131	5172032	50.3	140	355	170469
LT8933a	HH0134	15/05/2017	Escherichia coli	ST-38	5166554	50.5	121	314	134043
LT8934a	HH0137	13/05/2017	Escherichia coli	ST-69	5325629	49.3	71	369	105754
LT8936a	HH0162	6/06/2017	Escherichia coli	ST-963	5148386	50.7	62	295	92659
LT8943a	HH0171	4/06/2017	Escherichia coli	ST-405	5308992	51.1	70	751	84207
LT8944a	HH0126	30/05/2017	Escherichia coli	ST-131	5120691	50.2	136	413	186427
LT8951a	HH0173	1/06/2017	Escherichia coli	ST-117	5095165	48.9	70	521	97680
LT8953a	HH0165	10/06/2017	Escherichia coli	ST-69	5166376	49.8	72	364	88273
LT8957a	HH0122	10/06/2017	Escherichia coli	ST-131	4943026	50.4	135	316	179239
LT8962a	HH0167	13/06/2017	Escherichia coli	ST-131	5268001	50.8	73	327	174580
LT8967a	HH0177	14/06/2017	Escherichia coli	ST-10	5227291	50.6	69	419	106120
LT8969a	HH0143	13/06/2017	Escherichia coli	ST-38	5479616	50.2	150	454	106014
LT8974a	HH0164	21/06/2017	Escherichia coli	ST-141	4994712	50.1	77	181	302140
LT8989a	HH0123	23/06/2017	Escherichia coli	ST-131	5218304	50.3	124	380	173037
LT8994a	HH0159	2/07/2017	Escherichia coli	ST-90	4971338	50.2	81	261	184340
LT8995a	HH0151	1/07/2017	Escherichia coli	ST-131	5060282	50.5	88	235	172469
LT8999a	HH0146	6/07/2017	Escherichia coli	ST-963	5075554	50.7	81	269	118379
LT9002a	HH0150	10/07/2017	Escherichia coli	ST-127	5104681	50.5	83	402	184777
LT9007a	HH0153	8/07/2017	Escherichia coli	ST-131	5226954	50.5	79	294	161706
LT9015a	HH0127	12/07/2017	Escherichia coli	ST-62	5327794	50	128	395	86846
LT9021a	HH0129	14/07/2017	Escherichia coli	ST-131	5140073	50.1	115	409	135107
LT9024a	HH0128	17/07/2017	Morganella morganii	NOT-TYPED	4065243	52.5	129	293	162188
LT9027a	HH0130	17/07/2017	Escherichia coli	ST-648	5309875	49.7	128	366	116087
LT9028a	HH0157	17/07/2017	Escherichia coli	ST-131	5014608	50.3	74	249	182295
LT9030a	HH0175	19/07/2017	Escherichia coli	ST-405	5186899	50.8	83	303	88999
LT9031a	HH0147	19/07/2017	Escherichia coli	ST-80	5123063	49	91	309	135186
LT9034a	HH0174	20/07/2017	Escherichia coli	ST-131	5391368	50.8	86	348	159143
LT9039a	HH0149	22/07/2017	Escherichia coli	ST-131	5377760	51.1	78	394	166801
LT9040a	HH0166	28/07/2017	Escherichia coli	ST-69	5336519	50.7	84	314	100522
LT9041a	HH0176	28/07/2017	Escherichia coli	ST-131	5047349	50.5	81	220	154568
LT9046a	HH0170	25/07/2017	Escherichia coli	ST-38	4945073	50.3	80	224	166225
LT9050a	HH0158	3/08/2017	Escherichia coli	ST-349	4878614	50.7	80	135	197221
LT9067a	HH0161	9/08/2017	Escherichia coli	ST-648	5322144	50.3	87	298	115632
LT9074a	HH0154	14/08/2017	Escherichia coli	ST-2003	5397428	50.3	85	394	121985
LT9092a	HH0179	21/08/2017	Escherichia coli	ST-354	5051326	50.5	87	185	173398
LT9095a	HH0160	18/08/2017	Escherichia coli	ST-131	5043550	50.6	90	221	180557
LT9099a	HH0163	28/08/2017	Escherichia coli	ST-1193	5013936	50.2	83	180	160714
LT9101a	HH0172	27/08/2017	Escherichia coli	ST-38	5488417	50.7	93	398	111014

Table 6.2 Appendix III chapter 4 isolates

Isolate ID	Source	Household	Collection date	Species	MLST	Genome size (bp)	GC	Read depth	Contigs	N50
LT1003c	Human	HH0008	13/12/2015	Escherichia coli	ST-69	5434577	50.7	94	553	164042
LT1003d	Human	HH0008	13/12/2015	Escherichia coli	ST-69	5445852	50.6	135	575	158266
LT1003e	Human	HH0008	13/12/2015	Escherichia coli	ST-69	5436162	49	73	627	121170
LT1003f	Human	HH0008	13/12/2015	Escherichia coli	ST-58	4864009	50.7	82	248	119956
LT1003g	Human	HH0008	13/12/2015	Escherichia coli	ST-69	5434782	50.4	107	540	150367
LT1003h	Human	HH0008	13/12/2015	Escherichia coli	ST-69	5443523	50.5	109	590	158266
LT1030c	Human	HH0015	12/02/2016	Escherichia coli	ST-963	5174159	50.5	113	288	94030
LT1030d	Human	HH0015	12/02/2016	Escherichia coli	ST-963	5182980	50.1	125	298	97413
LT1030e	Human	HH0015	12/02/2016	Escherichia coli	ST-963	5172726	50.1	83	269	94501
LT1030f	Human	HH0015	12/02/2016	Escherichia coli	ST-963	5176389	50.2	59	279	93422
LT1030g1	Human	HH0015	12/02/2016	Escherichia coli	ST-963	5171168	50	71	267	93422
LT1030h2	Human	HH0015	12/02/2016	Escherichia coli	ST-963	5168471	50	61	249	117931
LT1028a	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5221272	49.2	105	402	112357
LT1028b	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5254279	49.5	89	361	121189
LT1028c2	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5215390	49.6	88	367	104399
LT1028d	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5252332	49.5	114	358	121189
LT1028e	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5207435	49.4	96	304	120914
LT1028f	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5247202	50	144	327	121134
LT1028g	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5216141	50	111	326	112357
LT1028h	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5247855	50	122	345	121134
LT1029d	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5248669	49.9	147	316	119604
LT1029e	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5264378	49.9	141	346	112400
LT1029f	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5245042	49.9	133	312	104398
LT1029g	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5156463	49.7	103	298	121024
LT1029h	Human	HH0016	12/02/2016	Escherichia coli	ST-500	5245579	50.1	158	336	104399
LT1033c	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5161406	50.1	103	254	160632
LT1033d	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5165516	50	85	269	159842
LT1033e	Human	HH0024	11/03/2016	Escherichia coli	ST-648	5274284	49.8	104	266	154029
LT1033f	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5173677	50.1	113	281	160608
LT1033g	Human	HH0024	11/03/2016	Escherichia coli	ST-648	5274564	49.6	100	236	154186
LT1033h	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5150923	50.2	121	246	160462
LT1034a	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5104681	50.3	139	224	160490
LT1034b	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5113673	50.3	110	250	160353
LT1034c	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5118124	50.6	294	248	160736
LT1034d	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5113867	50.5	172	251	160703
LT1034e	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5104204	50.4	101	213	160814
LT1034f	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5108057	50.5	141	228	159114
LT1034g	Human	HH0024	11/03/2016	Escherichia coli	ST-131	5107222	50.5	94	227	160606
LT8052a	Human	HH0015	28/10/2015	Escherichia coli	ST-963	5150269	50.5	134	320	115994
LT1043a	Human	HH0026	8/03/2016	Escherichia coli	ST-617	4897051	50.3	156	344	87883
LT1043b	Human	HH0026	8/03/2016	Escherichia coli	ST-617	4882334	50.2	112	284	90674
LT1043c	Animal	HH0026	8/03/2016	Escherichia coli	ST-617	4888721	50.4	164	294	87883
LT1043d	Human	HH0026	8/03/2016	Escherichia coli	ST-617	4889993	50.3	196	310	88609
LT1043e	Animal	HH0026	8/03/2016	Escherichia coli	ST-617	4887791	50.4	100	282	90674
LT1043f	Animal	HH0026	8/03/2016	Escherichia coli	ST-617	4886047	50.5	157	282	88609
LT1043g	Human	HH0026	8/03/2016	Escherichia coli	ST-617	4887243	50.3	121	303	88130
LT1043h	Human	HH0026	8/03/2016	Escherichia coli	ST-617	4886315	50.3	135	289	87669
LT1044g	Human	HH0026	8/03/2016	Escherichia coli	ST-1193	4985966	50.3	118	219	136965
LT1044h	Human	HH0026	8/03/2016	Escherichia coli	ST-1193	4978130	49.7	95	199	136965

Isolate ID	Source	Household	Collection date	Species	MLST	Genome size (bp)	GC	Read depth	Contigs	N50
LT1089c	Human	HH0039	7/05/2016	Escherichia coli	ST-131	5246570	50.4	116	346	154224
LT1089d	Human	HH0039	7/05/2016	Escherichia coli	ST-131	5245849	50.6	110	373	156060
LT1089e	Human	HH0039	7/05/2016	Escherichia coli	ST-131	5241731	50.7	112	348	154224
LT1089f	Human	HH0039	7/05/2016	Escherichia coli	ST-131	5244117	50.4	109	344	154224
LT1090c	Human	HH0039	7/05/2016	Escherichia coli	ST-131	5195527	51.1	115	375	135260
LT1090d	Human	HH0039	7/05/2016	Escherichia coli	ST-131	5189400	50.6	117	372	154224
LT1090e	Human	HH0039	7/05/2016	Escherichia coli	ST-131	5184690	50.1	90	333	135260
LT1090f	Human	HH0039	7/05/2016	Escherichia coli	ST-131	5188047	50.8	113	305	173795
LT8062a	Human	HH0008	9/11/2015	Escherichia coli	ST-69	5492481	51.5	48	243	285535
LT1078c	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5167731	49.8	57	228	170471
LT1078d	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5163690	50.6	112	191	178679
LT1078e	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5164730	50.3	137	189	178679
LT1078f	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5164284	50.5	129	199	178679
LT1078g	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5165121	50.1	90	210	178679
LT1078h	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5168065	50.4	121	211	178679
LT1079a	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5166581	50.4	102	219	178679
LT1079b	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5169484	50.5	123	206	178679
LT1079c	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5168641	50.4	256	244	170471
LT1079d	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5165281	50.4	136	216	178679
LT1079e	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5165605	50.4	142	205	178679
LT1079f	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5169293	50.5	136	202	178679
LT1079g	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5172039	50.6	161	241	178679
LT1079h	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5162297	50.2	90	204	178679
LT1080c	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5176850	50.4	140	243	178679
LT1080d	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5172358	50.4	133	220	180448
LT1080e	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5172620	50.3	212	233	178679
LT1080f	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5152282	50.4	132	197	178679
LT1080g	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5154204	50.3	58	204	178679
LT1080h	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5190026	50.8	175	312	181987
LT1082a	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5173724	50.7	154	237	180448
LT1082b	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5178377	50.7	180	253	180448
LT1082c	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5170299	50.2	86	219	178679
LT1082d	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5168602	50.5	124	211	180448
LT1082e	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5167410	50.8	114	200	178679
LT1082f	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5164692	50.3	107	181	170471
LT1082g	Human	HH0040	6/05/2016	Escherichia coli	ST-131	5172487	51.3	138	223	178679
LT1097c	Human	HH0048	26/06/2016	Escherichia coli	ST-38	5158294	51	111	349	129502
LT1097d	Human	HH0048	26/06/2016	Escherichia coli	ST-38	5189465	51.2	207	516	127781
LT1097g	Human	HH0048	26/06/2016	Escherichia coli	ST-38	5151025	51.1	129	339	127890
LT1097h	Human	HH0048	26/06/2016	Escherichia coli	ST-38	5163741	51.1	199	371	129722
LT1099e	Animal	HH0048	26/06/2016	Escherichia coli	ST-538	5099672	50.3	116	189	218404
LT1099f	Animal	HH0048	26/06/2016	Escherichia coli	ST-4553	5285110	50	92	173	196514
LT1099g	Animal	HH0048	26/06/2016	Escherichia coli	ST-38	5151070	50.7	104	344	127952
LT1099h	Animal	HH0048	26/06/2016	Escherichia coli	ST-38	5147806	51.1	138	301	127778
LT1143c	Animal	HH0064	24/09/2016	Escherichia coli	ST-2541	4969605	50.2	322	212	183218
LT1143e	Animal	HH0064	24/09/2016	Escherichia coli	ST-2541	4973592	51	90	230	192896
LT1143f	Animal	HH0064	24/09/2016	Escherichia coli	ST-2541	4969026	50.2	97	205	192896
LT1143g	Animal	HH0064	24/09/2016	Escherichia coli	ST-2541	4998195	50.1	103	354	175489
LT1131c	Human	HH0065	24/09/2016	Escherichia coli	ST-746	4914812	49.6	129	313	97240
LT1131d	Human	HH0065	24/09/2016	Escherichia coli	ST-10	4811550	50.7	149	243	147407
LT1131e	Human	HH0065	24/09/2016	Escherichia coli	ST-746	4902883	49.7	130	270	97240

Isolate ID	Source	Household	Collection date	Species	MLST	Genome size (bp)	GC	Read depth	Contigs	N50
LT1131f	Human	HH0065	24/09/2016	Escherichia coli	ST-746	4900803	49.9	119	256	97240
LT1131g	Human	HH0065	24/09/2016	Escherichia coli	ST-2541	5000520	49.4	112	325	175489
LT1132b	Human	HH0065	24/09/2016	Escherichia coli	ST-69	5122019	50.4	127	287	164000
LT1132c	Human	HH0065	24/09/2016	Escherichia coli	ST-69	5127711	50.3	121	318	164000
LT1132d	Human	HH0065	24/09/2016	Escherichia coli	ST-69	5124906	49.8	97	302	164000
LT1132e	Human	HH0065	24/09/2016	Escherichia coli	ST-69	5120564	50	122	271	164000
LT1132f	Human	HH0065	24/09/2016	Escherichia coli	ST-69	5125099	50.1	104	307	158267
LT8125a	Human	HH0016	15/12/2015	Escherichia coli	ST-500	5270376	50.5	56	216	163953
LT1132g	Human	HH0065	24/09/2016	Escherichia coli	ST-69	5118820	50.8	134	288	164000
LT1132h	Human	HH0065	24/09/2016	Escherichia coli	ST-69	5127494	49.8	115	304	163970
LT8179a	Human	HH0024	28/01/2016	Escherichia coli	ST-131	5160657	51.1	81	178	179969
LT8198a	Human	HH0026	5/02/2016	Escherichia coli	ST-1193	5092266	50.3	130	574	162923
LT8231a	Human	HH0039	4/03/2016	Escherichia coli	ST-131	5206381	50.3	99	444	156192
LT1171g	Human	HH0086	14/01/2017	Escherichia coli	ST-131	5175922	50.7	92	449	180446
LT1173c	Animal	HH0086	14/01/2017	Escherichia coli	ST-131	5141055	50	100	301	190943
LT8242a	Human	HH0040	20/03/2016	Escherichia coli	ST-131	5215701	51	181	447	170471
LT1173d1	Animal	HH0086	14/01/2017	Escherichia coli	ST-131	5148741	50.2	116	354	180448
LT1173e	Animal	HH0086	14/01/2017	Escherichia coli	ST-131	5138126	50.1	119	298	180447
LT8320a	Human	HH0048	9/05/2016	Escherichia coli	ST-38	5161032	51	173	381	123639
LT8371a	Human	HH0064	31/05/2016	Escherichia coli	ST-963	5153473	50.2	120	338	92286
LT1173f	Animal	HH0086	14/01/2017	Escherichia coli	ST-131	5165048	50.3	102	422	180447
LT1173g	Animal	HH0086	14/01/2017	Escherichia coli	ST-131	5141995	49.9	100	311	186438
LT8455a	Human	HH0065	25/07/2016	Escherichia coli	ST-69	5120029	50.2	151	282	164000
LT1173h	Animal	HH0086	14/01/2017	Escherichia coli	ST-131	5144385	50.2	128	332	186560
LT8703a	Human	HH0086	29/11/2016	Escherichia coli	ST-131	5083072	50.6	131	443	178654

Table 6.3 Appendix III chapter 5 isolates

Isolate ID	Source	Country	Collection Date	BioProject	Genome size (bp)	GC	Read depth	Contigs	N50
1714605	Human	UK	2008	PRJEB4681	5133863	50.6	59	343	159218
1714932	Human	UK	2012	PRJEB4681	5201547	50.1	65	421	160561
1714948	Human	UK	2012	PRJEB4681	5421497	50.5	70	524	161151
1714962	Human	UK	2012	PRJEB4681	5211171	50.3	88	280	181754
1714986	Human	UK	2012	PRJEB4681	5132718	50.7	63	384	159114
1715013	Human	UK	2012	PRJEB4681	5152824	50.3	92	246	159842
1715017	Human	UK	2012	PRJEB4681	5272881	50.7	86	358	143919
1715025	Human	UK	2012	PRJEB4681	5212490	50.7	81	208	168167
1715026	Human	UK	2012	PRJEB4681	5376100	50.7	63	270	165489
1715433	Human	UK	2004	PRJEB4681	5157361	50.8	69	265	180447
1715525	Human	UK	2005	PRJEB4681	5266568	50.6	83	329	161167
1716160	Human	UK	2011	PRJEB4681	5227618	50.8	66	286	166933
1716161	Human	UK	2011	PRJEB4681	5205254	50.8	74	263	181984
08B09891	Human	Thailand	12/07/2008	PRJNA297860	5213193	50.1	192	220	170358
09B06460	Human	Thailand	6/29/09	PRJNA297860	5214690	50.6	159	223	165175
09B06576	Human	Thailand	7/01/2009	PRJNA297860	5217394	50	165	239	170358
09B09491	Human	Thailand	9/12/2009	PRJNA297860	5230962	50.1	115	279	181896
09B13160	Human	Thailand	12/05/2009	PRJNA297860	5213894	50.7	257	215	172886
1045-14	Human	Germany	2014	PRJEB23663	5305259	49.7	35	155	227318
10B05736	Human	Thailand	7/29/10	PRJNA297860	5369383	50	255	373	162133
11B00062	Human	Thailand	1/11/2011	PRJNA297860	5472686	50.7	68	331	162133
11B00134	Human	Thailand	1/24/11	PRJNA297860	5114200	50.7	69	230	159208
177-10	Human	Germany	2010	PRJEB23663	5166100	51.4	74	160	290704
399-15	Human	Germany	2013	PRJEB23663	5233938	51	66	141	399638
464-16	Animal	Germany	2012	PRJEB23663	5146939	51.1	67	157	268119
495-16	Animal	Germany	2012	PRJEB23663	5141936	51	69	144	254110
497-16	Animal	Germany	2012	PRJEB23663	5392554	51	61	160	278043
590-14	Human	Germany	2014	PRJEB23663	5014253	50.5	74	121	191061
AZ727008	Human	Taiwan	6/01/2011	PRJNA297860	5459365	50.1	205	364	159279
AZ779845	Human	Spain	6/01/2011	PRJNA297860	5150376	50.2	180	237	173571
cam_1071	Human	Cambodia	8/11/2008	PRJNA297860	4989360	51.1	73	246	186350
cam_1439	Human	Cambodia	6/02/2009	PRJNA297860	5255888	50.2	73	242	133643
cam_1814	Human	Cambodia	2/03/2010	PRJNA297860	5089841	50.3	75	290	173686
HVAST501	Human	USA	2013	PRJNA327820	5316389	50	82	254	161706
HVAST502	Human	USA	2013	PRJNA327820	5314683	49.9	57	266	165196
HVAST503	Human	USA	2013	PRJNA327820	5218855	49.9	145	259	166700
HVAST504	Animal	USA	2013	PRJNA327820	5331603	49.9	44	329	161706
HVAST656	Human	USA	Jan-14	PRJNA327820	5322579	50	198	319	165297
HVAST695	Human	USA	Jan-14	PRJNA327820	5457351	49.9	91	381	162981
HVAST702	Human	USA	Jan-14	PRJNA327820	5460866	49.9	73	765	170358
HVAST709	Human	USA	Jan-14	PRJNA327820	5325091	50	65	326	181409
HVAST736	Human	USA	Mar-14	PRJNA327820	5322488	50.2	60	313	180586
HVAST737	Human	USA	Mar-14	PRJNA327820	5322870	50	89	306	181587
HVAST738	Human	USA	Mar-14	PRJNA327820	5317384	50	41	332	189480
HVAST740	Human	USA	Mar-14	PRJNA327820	5325766	50	70	304	180587
HVAST757	Human	USA	May-14	PRJNA327820	5324292	50	79	316	170358
HVAST758	Human	USA	May-14	PRJNA327820	5339470	49.9	67	371	181409
HVAST760	Human	USA	May-14	PRJNA327820	5323983	49.9	51	326	166801
HVAST761	Human	USA	May-14	PRJNA327820	5323070	50	41	327	166801

Isolate ID	Source	Country	Collection Date	BioProject	Genome size (bp)	GC	Read depth	Contigs	N50
HVAST762	Human	USA	May-14	PRJNA327820	5327026	50.1	59	325	170358
J0012	Human	Japan	2014	PRJDB3868	5119860	51.7	26	232	83662
J0019	Human	Japan	2014	PRJDB3868	5336912	51.6	30	266	99181
J0024	Human	Japan	2014	PRJDB3868	5223671	51.7	25	490	29926
J0034	Human	Japan	2014	PRJDB3868	5182010	51.4	32	220	112739
J0045	Human	Japan	2014	PRJDB3868	5436972	51.4	32	320	70616
J0061	Human	Japan	2014	PRJDB3868	5212699	51	54	219	191062
J0063	Human	Japan	2014	PRJDB3868	5230437	51.3	18	240	72584
J0064	Human	Japan	2014	PRJDB3868	5289754	51.8	31	359	72836
J0069	Human	Japan	2014	PRJDB3868	5393050	51.7	24	433	38006
J0074	Human	Japan	2014	PRJDB3868	5454764	51.5	31	373	59621
JJ2546	Human	USA	2008	PRJNA327820	5173366	50.6	32	106	205897
JJ2547	Human	USA	2008	PRJNA327820	5164359	50.3	33	105	191575
JJ2548	Human	USA	2008	PRJNA327820	5165515	50.6	30	107	198590
JJ2888	Human	USA	2013	PRJNA327820	5291916	49.9	59	265	165074
JJ2913	Human	USA	Jan-14	PRJNA327820	5324868	50	44	331	165297
JJ2963	Human	USA	2014	PRJNA327820	5139324	50.5	32	251	159791
JJ2974	Human	USA	2014	PRJNA327820	5141136	50.6	41	253	166801
la_11242	Human	Laos	6/26/08	PRJNA297860	5116079	50.6	55	179	167002
la_2266-2	Human	Laos	5/26/06	PRJNA297860	5176167	50.8	64	226	178793
LT8018a	Human	NZ	10/10/2015	mEpiLab	5301563	50.4	137	335	180448
LT8092a	Human	NZ	23/11/2015	mEpiLab	5266448	51.3	66	199	143508
LT8103a	Human	NZ	30/11/2015	mEpiLab	5351299	50.1	121	735	162133
LT8110a	Human	NZ	4/12/2015	mEpiLab	5144652	50.8	117	261	180447
LT8144a	Human	NZ	26/12/2015	mEpiLab	5338622	50.8	59	168	191062
LT8179a	Human	NZ	28/01/2016	mEpiLab	5160657	51.1	81	178	179969
LT8205a	Human	NZ	8/02/2016	mEpiLab	5128998	50	104	300	160880
LT8231a	Human	NZ	4/03/2016	mEpiLab	5206381	50.3	99	444	156192
LT8242a	Human	NZ	20/03/2016	mEpiLab	5215701	51	181	447	170471
LT8262a	Human	NZ	6/04/2016	mEpiLab	5110787	51	112	178	307664
LT8306a	Human	NZ	27/04/2016	mEpiLab	5264432	50.5	110	401	208122
LT8405a	Human	NZ	27/06/2016	mEpiLab	4957577	50.7	123	273	178664
LT8519a	Human	NZ	30/08/2016	mEpiLab	5122957	50.9	108	269	166692
LT8534a	Human	NZ	5/09/2016	mEpiLab	5093878	50.5	129	293	159414
LT8578a	Human	NZ	3/10/2016	mEpiLab	5292404	50.5	111	346	133277
LT8679a	Human	NZ	2/12/2016	mEpiLab	5290276	50.5	90	257	178374
LT8703a	Human	NZ	29/11/2016	mEpiLab	5083072	50.6	131	443	178654
LT8716a	Human	NZ	16/12/2016	mEpiLab	5086836	49.6	135	336	173801
LT8734a	Human	NZ	17/01/2017	mEpiLab	5108706	50.5	91	318	181516
LT8744a	Human	NZ	13/01/2017	mEpiLab	5211205	51	100	153	255513
LT8771a	Human	NZ	7/02/2017	mEpiLab	5072572	50.6	139	275	186350
LT8772a	Human	NZ	8/02/2017	mEpiLab	5049815	50.9	124	255	181591
LT8793a	Human	NZ	21/02/2017	mEpiLab	5293870	50.4	115	358	162730
LT8795a	Human	NZ	20/02/2017	mEpiLab	5227381	50.5	118	322	178637
LT8797a	Human	NZ	17/02/2017	mEpiLab	5163199	50.3	111	369	159114
LT8819a	Human	NZ	17/03/2017	mEpiLab	5413553	50.1	167	486	162190
LT8825a	Human	NZ	24/03/2017	mEpiLab	5004821	50.5	103	323	180447
LT8852a	Human	NZ	6/04/2017	mEpiLab	5113467	50.7	114	301	154937
LT8863a	Human	NZ	10/04/2017	mEpiLab	5147584	50.5	118	231	181672
LT8866a	Human	NZ	12/04/2017	mEpiLab	5208737	50.6	109	243	151001
LT8868a	Human	NZ	6/04/2017	mEpiLab	5391709	50.3	56	318	166898

Isolate ID	Source	Country	Collection Date	BioProject	Genome size (bp)	GC	Read depth	Contigs	N50
LT8877a	Human	NZ	17/04/2017	mEpiLab	5200388	49.8	79	377	159114
LT8883a	Human	NZ	20/04/2017	mEpiLab	5064306	50.5	125	263	186549
LT8887a	Human	NZ	24/04/2017	mEpiLab	5140493	50.6	129	270	215233
LT8899a	Human	NZ	30/04/2017	mEpiLab	5293073	50.4	100	349	161706
LT8929a	Human	NZ	10/05/2017	mEpiLab	5172032	50.3	140	355	170469
LT8944a	Human	NZ	30/05/2017	mEpiLab	5120691	50.2	136	413	186427
LT8957a	Human	NZ	10/06/2017	mEpiLab	4943026	50.4	135	316	179239
LT8962a	Human	NZ	13/06/2017	mEpiLab	5268001	50.8	73	327	174580
LT8989a	Human	NZ	23/06/2017	mEpiLab	5218304	50.3	124	380	173037
LT8995a	Human	NZ	1/07/2017	mEpiLab	5060282	50.5	88	235	172469
LT9007a	Human	NZ	8/07/2017	mEpiLab	5226954	50.5	79	294	161706
LT9021a	Human	NZ	14/07/2017	mEpiLab	5140073	50.1	115	409	135107
LT9028a	Human	NZ	17/07/2017	mEpiLab	5014608	50.3	74	249	182295
LT9034a	Human	NZ	20/07/2017	mEpiLab	5391368	50.8	86	348	159143
LT9039a	Human	NZ	22/07/2017	mEpiLab	5377760	51.1	78	394	166801
LT9041a	Human	NZ	28/07/2017	mEpiLab	5047349	50.5	81	220	154568
LT9095a	Human	NZ	18/08/2017	mEpiLab	5043550	50.6	90	221	180557
MER10	Human	Singapore	2014	PRJNA398288	5269828	51.3	42	367	159842
MER101	Human	Singapore	2015	PRJNA398288	5029861	50.4	77	188	182402
MER102	Human	Singapore	2015	PRJNA398288	5065233	50.2	80	236	186661
MER103	Human	Singapore	2015	PRJNA398288	5251272	50.4	73	300	163754
MER104	Human	Singapore	2015	PRJNA398288	5128356	50.4	74	228	181788
MER105	Human	Singapore	2015	PRJNA398288	5253144	49.9	82	283	165175
MER106	Human	Singapore	2015	PRJNA398288	5344314	50.5	65	883	117871
MER107	Human	Singapore	2015	PRJNA398288	5208827	50	70	168	164549
MER110	Human	Singapore	2015	PRJNA398288	5388020	50	71	337	154350
MER111	Human	Australia	2015	PRJNA398288	5208675	50.4	68	355	161706
MER13	Human	Singapore	2014	PRJNA398288	5218809	51.4	44	145	127084
MER14	Human	Singapore	2014	PRJNA398288	5090551	51.7	47	177	129226
MER15	Human	Singapore	2014	PRJNA398288	5047310	51.6	48	198	115630
MER25	Human	Australia	2014	PRJNA398288	5240179	51.5	51	178	124268
MER27	Human	Australia	2014	PRJNA398288	5246253	51.3	54	164	147565
MER29	Human	Singapore	2014	PRJNA398288	5277802	51.3	34	196	121450
MER30	Human	Singapore	2014	PRJNA398288	5350647	51.7	56	192	166561
MER36	Human	Singapore	2014	PRJNA398288	5283551	51.5	43	193	155550
MER37	Human	Singapore	2014	PRJNA398288	5286641	51.6	48	229	110569
MER38	Human	Singapore	2014	PRJNA398288	5251674	51.6	55	210	146765
MER39	Human	Singapore	2014	PRJNA398288	5295986	51.6	47	233	129180
MER42	Human	Singapore	2014	PRJNA398288	5005109	51.4	43	114	173937
MER49	Human	Australia	2014	PRJNA398288	5288341	50.4	75	328	159742
MER51	Human	Australia	2014	PRJNA398288	5202477	50.6	79	257	165188
MER52	Human	Australia	2015	PRJNA398288	5164782	50.7	66	273	159741
MER53	Human	Australia	2015	PRJNA398288	5266973	50.3	74	320	112549
MER56	Human	Australia	2015	PRJNA398288	5130224	50.6	64	249	180448
MER58	Human	Australia	2015	PRJNA398288	5179848	50.5	66	315	186350
MER65	Human	NZ	2015	PRJNA398288	5174463	50.4	72	247	167861

Isolate ID	Source	Country	Collection Date	BioProject	Genome size (bp)	GC	Read depth	Contigs	N50
MER66	Human	NZ	2015	PRJNA398288	5150672	50.5	73	225	170471
MER68	Human	Australia	2015	PRJNA398288	5084775	50.5	75	213	160730
MER78	Human	Singapore	2015	PRJNA398288	5216233	50.5	76	300	161677
MER8	Human	Singapore	2014	PRJNA398288	5231284	51.3	39	274	162133
MER80	Human	Singapore	2015	PRJNA398288	5356404	50.1	73	289	168013
MER82	Human	Singapore	2015	PRJNA398288	5253221	50.8	90	310	167861
MER83	Human	Singapore	2015	PRJNA398288	5453864	50.7	86	425	156923
MER87	Human	Singapore	2015	PRJNA398288	5296713	50.4	92	319	159842
MER88	Human	Singapore	2015	PRJNA398288	5203197	50.3	88	162	173827
MER9	Human	Singapore	2014	PRJNA398288	5389991	51.2	40	272	159943
MER94	Human	Australia	2015	PRJNA398288	5165447	50.5	75	267	161164
MER95	Human	Singapore	2015	PRJNA398288	5065070	50	73	213	159056
MER97	Human	Singapore	2015	PRJNA398288	5346843	50.1	73	154	191062
N0241	Human	Nepal	2014	PRJDB3868	5306712	50.6	61	138	198585
N0253	Human	Nepal	2014	PRJDB3868	5311538	50.7	42	164	191062
N0256	Human	Nepal	2014	PRJDB3868	5311234	50.5	40	155	191917
N0261	Human	Nepal	2014	PRJDB3868	5277170	50.7	78	140	200546
N0282	Human	Nepal	2014	PRJDB3868	5325251	50.7	43	162	191062
N0291-1	Human	Nepal	2014	PRJDB3868	5289555	50.7	47	123	229897
N0291-2	Human	Nepal	2014	PRJDB3868	5216278	50.7	49	109	191424
N0335	Human	Nepal	2014	PRJDB3868	5243853	50.6	55	133	187028
N0348	Human	Nepal	2014	PRJDB3868	5384855	50.7	47	154	178652
N0415	Human	Nepal	2014	PRJDB3868	5422539	50.6	50	137	191062
N0422	Human	Nepal	2014	PRJDB3868	5056197	50.7	44	74	229897
N0439	Human	Nepal	2014	PRJDB3868	5107365	50.7	48	105	198380
N0491	Human	Nepal	2014	PRJDB3868	5090341	50.9	63	108	198708
N0494-1	Human	Nepal	2014	PRJDB3868	5207989	50.9	41	111	190119
N0494-2	Human	Nepal	2014	PRJDB3868	5206093	50.8	46	105	276325
N0504	Human	Nepal	2014	PRJDB3868	5276818	50.6	47	119	191062
N0512	Human	Nepal	2014	PRJDB3868	5337898	50.5	62	112	191062
N0564	Human	Nepal	2014	PRJDB3868	5287023	50.6	67	158	181048
N0574	Human	Nepal	2014	PRJDB3868	5297634	50.5	42	139	178853
N0603	Human	Nepal	2014	PRJDB3868	5382640	50.4	86	133	189826
N0716	Human	Nepal	2014	PRJDB3868	5364572	51.1	44	189	124268
N0722	Human	Nepal	2014	PRJDB3868	5364424	51	37	189	111751
N0931	Human	Nepal	2014	PRJDB3868	5351249	51	63	191	191026
N0945	Human	Nepal	2014	PRJDB3868	5252593	50.8	61	138	178749
N0951	Human	Japan	2014	PRJDB3868	5300906	51.1	53	204	171991
N0985-1	Human	Nepal	2014	PRJDB3868	5142191	50.8	43	115	203384
N0985-2	Human	Nepal	2014	PRJDB3868	5227108	50.7	89	146	191062
N1001	Human	Nepal	2014	PRJDB3868	5371583	50.9	68	181	133039
N1027	Human	Nepal	2014	PRJDB3868	5258159	50.6	49	143	174012
N1051	Human	Nepal	2014	PRJDB3868	5259830	50.8	49	198	174445

Isolate ID	Source	Country	Collection Date	BioProject	Genome size (bp)	GC	Read depth	Contigs	N50
N1086	Human	Nepal	2014	PRJDB3868	5273241	50.7	63	176	159190
N1160	Human	Nepal	2014	PRJDB3868	5350836	50.9	53	222	178188
N1167	Human	Nepal	2014	PRJDB3868	5319940	51	51	202	142115
N1200	Human	Nepal	2014	PRJDB3868	5228565	51.1	62	111	178853
N1203	Human	Nepal	2014	PRJDB3868	5214817	51	56	128	173296
N1228	Human	Nepal	2014	PRJDB3868	5199701	50.8	48	145	178643
N1229	Human	Nepal	2014	PRJDB3868	5260469	51.3	54	104	191062
N1240	Human	Nepal	2014	PRJDB3868	5355670	50.9	73	169	190708
N1249	Human	Nepal	2014	PRJDB3868	5333602	50.9	48	139	173945
N1252	Human	Nepal	2014	PRJDB3868	5283262	51.3	70	168	164069
N1253	Human	Nepal	2014	PRJDB3868	5245565	50.9	68	149	178623
N1274	Human	Nepal	2014	PRJDB3868	5290942	51.2	58	162	173945
N1332	Human	Nepal	2014	PRJDB3868	5276883	50.9	47	159	172068
N1341	Human	Nepal	2014	PRJDB3868	5438687	50.9	91	193	191062
N1345	Human	Nepal	2014	PRJDB3868	5271265	51.1	86	126	198708
N1385	Human	Nepal	2014	PRJDB3868	5280387	50.9	72	146	184484
N1417	Human	Nepal	2014	PRJDB3868	5312414	50.8	83	180	191062
N1421	Human	Nepal	2014	PRJDB3868	5315904	51.1	33	168	112552
N1429	Human	Nepal	2014	PRJDB3868	5540733	50.8	83	209	188745
N1442	Human	Nepal	2014	PRJDB3868	5160223	50.9	63	132	187028
N1471	Human	Nepal	2014	PRJDB3868	5048383	51.1	73	118	173830
N1504	Human	Nepal	2014	PRJDB3868	5179466	51	62	124	194593
N1539	Human	Nepal	2014	PRJDB3868	5362469	51.1	64	127	170502
N1753	Human	Nepal	2014	PRJDB3868	5202466	50.6	56	91	208633
N1767	Human	Nepal	2014	PRJDB3868	5264752	51.1	112	123	191062
N1795	Human	Nepal	2014	PRJDB3868	5312932	50.8	59	156	198152
RL229	Animal	Germany	2012	PRJEB23663	5518631	51.2	46	182	139447
uk_17A7A	Human	UK	3/06/2009	PRJNA297860	5296746	50.5	53	251	159742
uk_17B26A	Human	UK	7/05/2009	PRJNA297860	5249169	50.5	54	259	181608
uk_17C26C	Human	UK	9/09/2009	PRJNA297860	5225947	50.5	45	316	157443
uk_18A18K	Human	UK	11/08/2009	PRJNA297860	5129087	51	53	250	159160
uk_18A33A	Human	UK	12/06/2009	PRJNA297860	5228378	50.7	64	286	159646
uk_18B11D	Human	UK	1/03/2010	PRJNA297860	5344511	50.7	48	390	178792
uk_18B18D	Human	UK	1/23/10	PRJNA297860	5259907	50.5	82	283	178792
uk_18B21F	Human	UK	2/01/2010	PRJNA297860	5128269	50.7	56	256	159842
uk_18B28B	Human	UK	2/16/10	PRJNA297860	5248594	50.7	50	249	178792
uk_18B30B	Human	UK	2/20/10	PRJNA297860	5259480	50.3	49	216	180907
uk_18C14	Human	UK	4/09/2010	PRJNA297860	5344204	50.7	42	606	165175
uk_18C29E	Human	UK	5/14/10	PRJNA297860	5458883	50.6	48	256	164451
uk_18C4F	Human	UK	3/16/10	PRJNA297860	5258453	50	64	228	181988
uk_19A21D	Human	UK	7/28/10	PRJNA297860	5262125	50.6	51	271	159742
uk_19B17I	Human	UK	10/13/10	PRJNA297860	5247066	50.8	59	249	159843
uk_7C26H	Human	UK	6/20/08	PRJNA297860	5236119	50.9	57	262	159742

Isolate ID	Source	Country	Collection Date	BioProject	Genome size (bp)	GC	Read depth	Contigs	N50
uk_8A9B	Human	UK	7/26/08	PRJNA297860	5065639	50.7	62	399	178750
uk_P16456	Human	UK	1/01/2005	PRJNA297860	5200246	50.9	41	243	159160
uk_P26250	Human	UK	1/01/2005	PRJNA297860	5217304	50.9	51	226	174535
uk_P34091	Human	UK	1/01/2005	PRJNA297860	5233725	50.8	61	249	159742

Appendix IV: Questionnaire

Questionnaire development

This questionnaire was designed in early 2015, primarily by myself, with assistance from the post-doctoral fellow working on this project (Dr Zoe Grange), my PhD supervisors, and other external collaborators [Professor Jeroen Douwes (Massey University Centre for Public Health), Professor Michael Baker (Otago University Department of Public Health), and Dr Dragana Drinkovic (clinical microbiologist at Waitemata District Health Board)]. I based questions on known or suspected risk factors (e.g. healthcare contact, antimicrobial use, international travel) and questions around the exposure of interest (pet contact). The key resources for designing this questionnaire were the New Zealand and California Health surveys (below). I also used the experiences of Dr Patricia Jaros, who had recently completed her PhD and had conducted a similar prospective case control study (Jaros *et al.* 2013).

- California health interview survey
    - http://healthpolicy.ucla.edu/chis/design/Pages/questionnairesEnglish.aspx
- New Zealand health Survey
    - https://www.health.govt.nz/nz-health-statistics/national-collections-and-surveys/surveys/new-zealand-health-survey

Case questionnaire

"Where you can, please answer all questions for the time-period prior to your diagnosis with a multi-drug resistant infection (ESBL or AmpC). Please remember that for our survey to be accurate, it would be better that you do not answer the question than give an answer you think we want to hear.

"If you have any questions, please call Leah Toombs-Ruane on 06-3569099 ext 84101 or email me at *L.J.Toombs-Ruane@massey.ac.nz*

"Your input is important as it will help us to understand the risk factors associated with these infections, and will provide important information to help reduce future infections. The survey has been approved by the National Human Ethics Committee. The interview is confidential and will take no more than 20 minutes to complete, depending on your answers. By agreeing to take part, you accept that your data will be used in research that may be published. We will never publish your personal information and guarantee that your privacy is protected. In order to comply with the rules for this survey, specified by the National Humans Ethics Committee, can I have your verbal consent to use your answers for research purposes only. As I said before, your personal details and any of your given information will remain anonymous, it will be treated with ethical respect and not be revealed to any person or institute other than the researchers of this survey."

Please tell us your name: _____

What is the date? _____ / _____ / 2017

INTRO1A Are you 16-years or older?

1. Yes

2. No *(If this is your answer: Sorry we are not able to include you in the study. Thank you for your time)*

ETHICS Do you consent for your answers to be used for research purposes only?

1. Yes

No (*If this is your answer: Sorry we are not able to include you in the study. Thank you for your time)*

2. E1 Are you happy to participate in this survey?

1. Yes – proceed (GO TO E2)

2. No – don't proceed

E2 Before we start, I need to confirm a couple of things about you. In the last 12 months (before your diagnosis with a multidrug resistant infection), have you:

A) Been admitted overnight to a hospital for any reason OR

B) Lived in a residential care facility such as a rest home or rehabilitation facility?

1. 'A' or 'B' (If this is your answer: Sorry we are not able to include you in the study. Thank you for your time)
2. Neither
3. Unsure/Refused

HOUSEHOLD DEMOGRAPHICS

Q1 Firstly, has there been more than one person (including yourself) living in your household in the past week?

1. Yes
2. No
3. Unsure
4. Refused

Q1A Thinking about the males and females that lived in your household in the last week including yourself, how many were.....:

-1- Men aged 18 or over? _____

-2- Boys aged 5 to 17 years? _____

-3- Boys under 5 years? _____

-4- Women aged 18 or over? _____

-5- Girls aged 5 to 17 years? _____

-6- Girls under 5 years? _____

Q1B And how many couples live together in your household?

_____ [*ENTER NUMBER - TWO PEOPLE EQUALS ONE COUPLE]*

ANIMAL CONTACT

The following questions are regarding contact with animals. Remember that for our survey to be accurate, it would be better that you do not answer the question than give an answer you think we want to hear.

Q2 Do you have pets that live in, at or with your household?

1. Yes
2. No *(GO TO Q21)*
3. Unsure *(GO TO Q21)*
4. Refused *(GO TO Q21)*

Q3 Has at least one of your pets lived in, at or with your household for the last 6-months?

1. Yes
2. No
3. Unsure
4. Refused

Q4 Please tell me how many of each of the following species of pet(s) you have in your household at present. How many...?

[ENTER NUMBER FOR EACH ANIMAL]

-1- Cats _____

-2- Dogs _____

-3- Chickens _____

-4- Pet birds (not chickens) _____

-5- Rabbits _____

-6- Reptiles _____

-7- Rodents _____

-8- Other animals _____

Q4A Excluding those already mentioned, please tell me the types of other pets you have in your household?

_____ (*WRITE ANSWER)*

Q5 Where did your pet(s) originally come from?

[MULTIPLE RESPONSE OK]

1. Breeder _____
2. Friend or family _____
3. From a previous litter _____
4. Found and adopted _____
5. Pet shop _____

6. SPCA or animal shelter _____
7. Was already here at this address _____
8. Other sources _____
9. Unsure _____
10. Refused _____

Q5A What other sources were these?

_____

Q6 Have any of your current household pet(s) ever travelled outside of New Zealand?

1. Yes
2. No
3. Unsure
4. Refused

Q6A What countries has your pet(s) travelled to or come from?

[MULTIPLE RESPONSE]

1. Australia
2. Canada
3. China
4. Fiji
5. Germany
6. Ireland
7. Japan
8. Samoa
9. Tonga
10. United Kingdom
11. United States of America
12. Unsure
13. Refused
14. Other (specify)

Q6B Did your pet(s) enter New Zealand within the past 12 months?

1. Yes
2. No
3. Unsure
4. Refused

Q7 Do any pet(s) in your household ever sleep indoors?

1. Yes
2. No
3. Unsure
4. Refused

Q7A Do pet(s) in your household sleep in or on the same bed as you (or other people in the household) at any time?

1. Yes
2. No
3. Unsure
4. Refused

Q7A1 And which people do the pet(s) sleep with?

1. Just you (respondent)
2. Other people
3. Both you (respondent) and other people
4. Unsure
5. Refused

Q7A2 Which of the following animal(s) sleep with you (or other people in the household)?

-1- Cats

-2- Dogs

-4- Pet birds (not chickens)

-5- Rabbits

-6- Reptiles

-7- Rodents

-8- Other animals

1. Yes
2. No
3. Unsure
4. Refused

Q8 Do the pet(s) in your household lick your face?

1. Yes
2. No
3. Unsure
4. Refused

Q9 Do you personally clean litter trays or collect and dispose of pet faeces or urine (such as handling litter, manure, droppings, or cleaning cage/kennel)?

1. Yes
2. No
3. Not applicable
4. Unsure
5. Refused

Q10 Does another adult or child in the household besides you dispose of pet faeces (poo) or urine (wee) (such as handling litter, manure, droppings, or cleaning cage/kennel)?

1. Adults
2. Children
3. Both children and adults
4. No other person
5. Unsure
6. Refused

Q11 Have your pets visited anywhere outside your home and garden in the past 6-months?

1. Yes
2. No
3. Unsure
4. Refused

Q11A Have your pets visited any of the following places outside of your home and garden in the past 6-months?

[MULTIPLE RESPONSE CIRCLE]

1. Around the local streets
2. Farm or lifestyle block
3. Cattery or kennel
4. Dog park
5. Beach
6. River/fresh waterway
7. Campground
8. Animal day care
9. Hospital or residential care home
10. Park/bush
11. Animal shelter or pound
12. To homes of family or friends
13. School
14. Other places
15. Unsure
16. Refused

Q11A1 What other places did they visit?

_____

Q12 What type or types of food does your cat(s) eat as far as you know?

[MULTIPLE RESPONSE]

1. Commercial canned or sachet cat food
2. Commercial dry cat food
3. Raw meat diet
4. Home-kill (e.g. from own farm: pig, lamb, bull, etc)
5. Hunted prey
6. Raw meat diet
7. Table scraps
8. Unsure
9. Refused
10. Other (specify)

Q13 What type or types of food does your dog(s) eat as far as you know?

[MULTIPLE RESPONSE]

1. Commercial canned dog food
2. Commercial chilled (dog roll)
3. Commercial dry dog food
4. Home-kill (e.g. from own farm: pig, lamb, bull, etc)
5. Hunted meat
6. Raw meat diet
7. Table scraps
8. Raw bones
9. Unsure
10. Refused
11. Other (specify)

Q14 What does your pet bird(s) eat as far as you know?

[MULTIPLE RESPONSE]

1. Commercial bird food
2. Homemade bird food
3. Unsure
4. Refused
5. Other (specify)

Q15 As far as you know, does your cat(s) interact with wildlife (including mice, rats, birds or lizards)?

1. Yes
2. No
3. Unsure
4. Refused

Q16 Do you, or someone in your household, clean your pet's FOOD bowl(s) (or the area they are fed) every day?

1. Yes
2. No
3. Unsure
4. Refused

Q17 If your pet has a WATER bowl, do you, or someone in your household, clean your pet's WATER bowl(s) every day?

1. Yes
2. No
3. Does not have a separate water bowl
4. Unsure
5. Refused

The following questions are regarding pet healthcare. Remember, for our survey to be accurate, it is better that you do not answer a question than give an answer you think we want to hear.

Q18 In the past 6-months has (have) any pet(s) in your household visited a vet?

1. Yes
2. No
3. Unsure
4. Refused

Q18A The following questions relate to any vet visit by any pet in your household during the last six months. What was the longest length of time ANY of your pets spent at a vet clinic (in a single visit)?

1. General vet consultation
2. Less than 1 day
3. 1 to 3 days
4. 4 to 7 days
5. 8 or more days
6. Unsure
7. Refused

Q18B Were any of your pets referred to another clinic/hospital?

1. Yes
2. No
3. Unsure
4. Refused

Q18C Did any of your pets visit the veterinarian as an emergency?

1. Yes
2. No
3. Unsure
4. Refused

Q18D Did any of your pets have surgery in the last six months?

1. Yes
2. No
3. Unsure
4. Refused

Q18E Was medication given to any of your pets by the vet or prescribed to take home?

[MULTIPLE RESPONSE]

1. By the vet
2. Prescribed to take home
3. No medication given by vet or to take home
4. Unsure
5. Refused

Q18E1 What were the medications that was administered by the vet or prescribed to take home?

[MULTIPLE RESPONSE]

1. Antibiotic injection
2. Antibiotic tablet, powder or liquid
3. Ear/skin ointment
4. Other
5. Unsure
6. Refused

Q18E2 What were the name of the medications that was administered by the vet or prescribed to take home?

_____

Q18E3 How many days were the medications prescribed for?

1. 7 days or less
2. 8 days or more
3. Unsure
4. Refused

Q18E4 Was the whole treatment course given as prescribed?

1. Yes
2. No
3. Unsure
4. Refused

Q19 Has (have) any pet(s) in the household ever been diagnosed with an infection which is resistant to lots of different drugs. Your vet may have called it a 'superbug' or an infection resistant to antibiotics?

1. Yes
2. No
3. Unsure
4. Refused

Q19A Can you tell us the name of the pet that was diagnosed with this antibiotic resistant infection?_____

Q20 The following questions are about how often you wash your hands with pets in your household. Please answer as accurately as possible. Do you wash your hands always, often, sometimes, or never...: *CIRCLE WHERE APPROPRIATE*

-1- Before feeding animals?

Always Often Sometimes Never Unsure *N/A*

-2- After feeding animals?

Always Often Sometimes Never Unsure *N/A*

-3- Before petting animals?

Always Often Sometimes Never Unsure *N/A*

-4- After petting animals?

Always Often Sometimes Never Unsure *N/A*

-5- After a pet licks your hands?

Always Often Sometimes Never Unsure *N/A*

-6- After collecting and disposing of faeces (poo) or urine (wee) (e.g. handling litter, manure, droppings, or cleaning cage/kennel)?

Always Often Sometimes Never Unsure *N/A*

Q21 Outside of your household pets, what animal species have you personally had DIRECT or INDIRECT physical contact with in the past 6-months?

(By DIRECT physical contact, we mean you have touched the animal itself.)

(By INDIRECT physical contact, we mean you may have cleaned up their faeces in your garden, bought bags of pony pooh for fertiliser, cleaned a cage, fed or handled feeding bowls or utensils for example but you have not actually touched the animal.) [MULTIPLE RESPONSE]

1. Aviary birds
2. Cats
3. Cattle or cows or calves
4. Chickens or poultry
5. Deer
6. Dogs
7. Fish
8. Horses or ponies or donkeys
9. Llamas or alpacas
10. Pigs or piglets
11. Rodents or rabbits
12. Sheep or lambs
13. Wildlife or zoo animals
14. None
15. Unsure
16. Refused
17. Other (specify)

Q21A What kind of contact did you have with these animals - was it...?

[MULTIPLE RESPONSE]

1. Bathing animals
2. Riding or exercising or walking
3. Petting or stroking or grooming
4. Feeding
5. Cleaning cages or kennels
6. Cleaning stables or pens
7. Job-related (e.g farm worker, veterinarian, trainer, slaughterhouse worker)
8. Picking up manure or faeces
9. Unsure
10. Refused
11. Other (specify)

HOUSEHOLD HEALTH

The following questions are regarding you and your household's health. Please answer for the time prior to you diagnosis with a multi-drug resistant infection (ESBL). Please answer as many as you feel comfortable or are able to. Remember, for our survey to be accurate, it would be better if you do not answer the question than give an answer you think we want to hear.

Q22 How would you describe your overall health in the last 6-months?

1. Excellent
2. Very good
3. Good
4. Fair
5. Poor
6. Unsure
7. Refused

Q23 Do you have any chronic or underlying health conditions?

1. Yes
2. No
3. Unsure
4. Refused

Q23A Please tell us if you have the following...:

[MULTIPLE RESPONSE]

1. Asthma
2. Chronic Obstructive Pulmonary Disase which may include emphysema and chronic bronchitis or C.O.P.D
3. Cardiac disease which may include coronary artery disease, angina or a previous heart attack
4. Diabetes
5. Unsure
6. Refused
7. Other (specify)

Q24 Have you had a medical visit with a G.P. (or with a specialist as an outpatient) in the past 6-months including imaging or radiology? Outpatient means you were admitted and discharged from hospital on the same day.

1. Yes
2. No
3. Unsure
4. Refused

Q25 Have you received treatment as an outpatient in a hospital in the last 6-months? Outpatient means you were admitted and discharged from hospital on the same day.

1. Yes
2. No
3. Unsure
4. Refused

Q26 Have you been to a hospital emergency room (Accident and Emergency) as a patient in the last 6-months?

1. Yes
2. No
3. Unsure
4. Refused

Q27 Have you had day surgery in the past year? This means you were admitted and discharged from hospital on the same day.

1. Yes
2. No
3. Unsure

4. Refused

Q27A Please tell us what the reason or procedure was:

_____

Q28 In the past 6 months have you had more than one urinary tract infection (UTI)?

1. Yes
2. No
3. Unsure
4. Refused

Q29 Have you given birth in the past 6 months?

1. Yes
2. No
3. Unsure
4. Refused

Q30 Have you been prescribed antibiotics in the past 6 months?

1. Yes
2. No
3. Unsure
4. Refused

Q30A Have you been prescribed antibiotics in the past 3 months?

1. Yes
2. No
3. Unsure
4. Refused

Q30B Did you complete the course(s) as prescribed?

1. Yes
2. No
3. Still taking the antibiotics
4. Unsure
5. Refused

Q30C How many days was your most recent prescribed course of antibiotics for?

1. 7 days or less
2. 8 days or more
3. Unsure
4. Refused

Q30D What was the name of your most recent antibiotic medication?

_____

Q31 Have you ever been told by a doctor that you have an infection which is resistant to lots of different antibiotics? Your doctor may have called it a 'superbug' (like an ESBL or MRSA). Or have you been told you are a carrier of a bacteria like MRSA - a bacteria which is resistant to lots of different drugs?

1. Yes
2. No
3. Unsure
4. Refused

Q31A On what date and what diagnosis did your doctor give you? Please answer as fully as possible.

_____

Q31B Were you treated for this antibiotic resistant (superbug) infection?

1. Yes
2. No
3. Unsure
4. Refused

Q31B1 On what date did this treatment end? Please answer accurately aspossible.

_____

Q32 Have any members of the household (other than you) been a patient in a hospital or residential care facility in the past 12-months?

1. Yes
2. No
3. Unsure
4. Refused

Q32A Could you please tell us the reason?

_____

Q32B Can you tell us the longest length of time that any person in your household (other than you) spent in a hospital or residential care facility? This should be the longest single visit.

1. 12 hours or less
2. Overnight (13 to 24 hours)
3. 2 to 3 days
4. 4 to 7 days
5. 8 or more days
6. Unsure
7. Refused

Q33 Have any members of the household (other than you) been prescribed antibiotics in the past 6-months?

1. Yes
2. No
3. Unsure
4. Refused

Q34 Has any other person in your household ever been told by a doctor they have an infection which is resistant to lots of antibiotics. The doctor may have referred to it as a 'superbug' (or an ESBL or MRSA). Or have they been told they are a carrier of a bacteria like MRSA?

1. Yes
2. No
3. Unsure
4. Refused

Q34A Was this person treated for a multidrug resistant bacterial infection?

1. Yes
2. No
3. Unsure
4. Refused

FOOD AND HYGIENE

The following questions are regarding food and hygiene in the household. Please answer as many as you feel comfortable or are able to. Remember for our survey to be accurate, it is better if you do not answer the question than give an answer you think we want to hear.

Q35 Do you eat meat?

1. Yes
2. No
3. Unsure
4. Refused

Q35A What types of meat do you eat, even if you don't eat it often? Do you eat...:

[MULTIPLE RESPONSE]

1. Chicken/poultry
2. Lamb
3. Beef
4. Fish
5. Pork
6. Venison
7. Home-kill (e.g. from own farm, pig, lamb, etc)
8. Hunted meat (e.g. venison, wild pork)
9. Unsure
10. Refused
11. Other (specify)

Q36 Do you drink raw (untreated or unpasteurised) milk?

1. Yes
2. No
3. Unsure
4. Refused

Q37 Where do you get fresh vegetables from?

[MULTIPLE RESPONSE]

1. Supermarket / Cash and Carry
2. Farmers market or farm store
3. Homegrown
4. Fruit and Vegetable Shop (Greengrocer)
5. Friends or family
6. Unsure
7. Refused
8. Other (specify)

Q38 How often do you wash your hands? Please answer as accurately as possible. Do you wash your hands always, often, sometimes, or never...: *CIRCLE WHERE APPROPRIATE*

-1- After going to the toilet?

Always Often Sometimes Never Unsure *N/A*

-2- Before handling food?

Always Often Sometimes Never Unsure *N/A*

-3- Before eating?

Always Often Sometimes Never Unsure *N/A*

-4- After coughing, sneezing or blowing your nose?

Always Often Sometimes Never Unsure *N/A*

HOUSE AND GARDEN

The following questions are regarding your home and garden.

Q39 Would you mind telling us what the nearest primary school to your home is?

[: This question is to help us know what area you are in, and is used as part of a social index for the study.]

_____

Q40 Which of the following best describes your living arrangements during the last week?

1. Lived in a house owned by me or family trust
2. Lived in a house with the owner of the house who is not a family member
3. Lived in a house rented from Housing NZ or Council
4. Lived in a rented house not owned by Housing NZ or Council
5. Unsure
6. Refused
7. Other (specify)

_____

Q41 How many bedrooms are in the house you live in, including any rooms furnished as a bedroom even if no one is using it?

[ENTER NUMBER OF BEDROOMS]

_____

Q42 Does the house you live in have a garden?

[: Garden refers to grass or planted/soil areas within the household surrounds.]

1. Yes
2. No
3. Unsure
4. Refused

Q42A Have you seen cat or dog faeces (poo) in your garden?

1. Yes
2. No
3. Unsure
4. Refused

Q42B Do you have compost in the garden? This includes making compost without having used it yet.

1. Yes
2. No
3. Unsure
4. Refused

Q43 Do you, or someone in your household, put out food for wild birds in your household surrounds?

1. Yes
2. No *(GO TO QUESTION 45)*
3. Unsure
4. Refused

Q43A Where do you (they) put the wild bird food?

[MULTIPLE RESPONSE]

1. On the ground
2. Dish / Plate
3. Bird feeder
4. Bird table
5. Unsure
6. Refused
7. Other (specify)

Q43B How often do you (or someone in your household) clean the items or area you use to feed wild birds?

1. Daily
2. Weekly
3. Monthly
4. Few months a year
5. Never
6. Unsure
7. Refused

Q43C What do you (they) use to clean the items or area you use to feed wild birds?

[MULTIPLE RESPONSE]

1. Brush / Cloth
2. Detergent / soapy water
3. Plain water
4. Disinfectant
5. Unsure
6. Refused
7. Other (specify)

Q44 Do you have a wild bird water bath?

1. Yes
2. No
3. Unsure
4. Refused

Q44A How often do you (or someone in your household) clean the wild bird water bath?

1. Daily
2. Weekly
3. Monthly
4. Few months a year
5. Never
6. Unsure
7. Refused

Q44B What do you (they) use to clean the wild bird water bath?

[MULTIPLE RESPONSE]

1. Brush / Cloth
2. Detergent / soapy water
3. Plain water
4. Disinfectant
5. Unsure
6. Refused
7. Other (specify)

DEMOGRAPHICS

The following questions are about where you and members of your household have travelled in the past year

Q45 Have you travelled outside New Zealand in the past 12 months?

1. Yes
2. No
3. Unsure
4. Refused

Q45A What countries did you visit?

[MULTIPLE RESPONSE]

1. Australia
2. Canada
3. China
4. Fiji
5. Germany
6. Ireland
7. Japan
8. Samoa
9. Tonga
10. United Kingdom
11. United States of America
12. Unsure
13. Refused
14. Other (specify)

Q46 Have any members of the household (other than you) travelled outside New Zealand in the past 12-months?

1. Yes
2. No
3. Unsure
4. Refused

Q47 Are you a healthcare worker?

[This is any job in health care including working as a nurse, doctor, midwife, physiotherapist, occupational therapist, dietician, speech therapist, healthcare assistant, cleaner, orderly, administrator or manager.]

1. Yes
2. No
3. Unsure
4. Refused

Q47A Do you have contact with patients in your work?

1. Yes
2. No
3. Unsure
4. Refused

Q48 Where do you spend most of your time during an average working or school day?

If you do not work, where do you spend most of your day?

1. Preschool childcare facility (including kohanga reo, creche, daycare centre, etc)
2. Educational facility (including primary, secondary, tertiary, etc)
3. Hospital or medical centre
4. Veterinary hospital or clinic
5. Farm
6. Office
7. Medical laboratory
8. Home
9. Vehicle
10. Unsure
11. Refused
12. Other (specify)

Q49 Have you visited a hospital or residential care facility in the past 6-months, as a visitor or volunteer or employee but NOT as a patient?

1. Yes
2. No
3. Unsure
4. Refused

Q50 Have any members of your household (other than you) visited a hospital or residential care facility in the past 6-months, as a visitor or volunteer or employee but NOT as a patient?

1. Yes

2. No
3. Unsure
4. Refused

Q51 Which of the following ethnic groups do you belong to? One or several groups may apply to you.

[MULTIPLE RESPONSE]

1. NZ European
2. NZ Maori
3. Samoan
4. Cook Island Maori
5. Tongan
6. Niuean
7. Chinese
8. Indian
9. Unsure
10. Refused
11. Other (specify)

Just to confirm what was your age is please?

_____

[IF YOU DO NOT WISH TO TELL, US: PLEASE CIRCLE BELOW WHERE APPROPRIATE]

1. 16-17
2. 18-19
3. 20-24
4. 25-29
5. 30-34
6. 35-39
7. 40-44
8. 45-49
9. 50-54
10. 55-59
11. 60-64
12. 65-69
13. 70-74
14. 75+
15. Refused

We are almost at the end of the survey. On behalf of the researchers at Massey University, we thank you very much for taking the time to complete our survey - your input has been very valuable to us. We have a couple of final questions to ask you:

Q52 Do you give permission for a researcher from Massey University to contact you in the near future regarding participation in further research?

1. Yes
2. No / Unsure

That's the end of this survey, thank you again for your time. Please ensure you send us back the consent form as well

Variable coding

Variables were coded as follows: decile, ruralurban, region, gender, eth_maori, eth_asian, eth_pacifika, eth_european, eth_melaa, home, health_work, health_visit. Factors associated with other people in the home were coded as: hh_mdr, hh_hosp, hh_abx, hh_health_visit, hh_travel,

Case/control participant self-reported health information was coded as follows for chronic disease: any, asthma, COPD, cardiac disease, diabetes, other. General health information was coded as follows: uti_6m, cdz_score, age3cat, health, cdz_all, cdz_asthma, cdz_copd, cdz_cardiac, cdz_diabetes, cdz_other, doctorcontact, abx_tx_all.

Pet contact was coded as follows: pet, petcatdog, petsleepinside, petsleepbed, petlickface, cc_handlepoo, pet_visit, cat_food, dog_food, cat_wild, pet_vet, pet_vet, pet_vet_type, pet_vet_tx. Handwashing behaviour around pets was coded as follows: pet_hands_b4food, pet_hands_food, pet_hands_b4pet, pet_hands_b4pet2, pet_hands_pet, pet_hands_lick, pet_hands_poo.

Other (non-pet) animal contact was coded as follows: animal_bird, animal_pet, animal_rodent, animal_fish, animal_farm, animal_equinecamelid, animal_wildother, animal_contact.

International travel in previous year was coded as: travel_all, just_travel_sth_america, just_travel_africa, just_travel_middle_east, just_travel_eu, just_travel_asia, just_travel_africa, just_travel_america, just_travel_pacific, just_travel_aus.

Diet and hygiene were coded as: vegetarian, eats_meat, rawmilk, supermarket, greengrocer, farmersmkt, homegrow_ffam. Handwashing behaviour was coded as:

hands_toilet & hands_toilet2, hands_b4food, hands_b4eat, hands_nose. Garden factors were coded as: garden, cdpoo_garden, compost_garden, feed_birds, birdbath.

APPENDIX V: DATA ANALYSIS

The R code used for data analysis is available at:

- https://esblfamilypet.wordpress.com/2019/01/28/appendix-v-r-code/

Supplementary information for questionnaire dataset modelling of LASSO logisitic regression

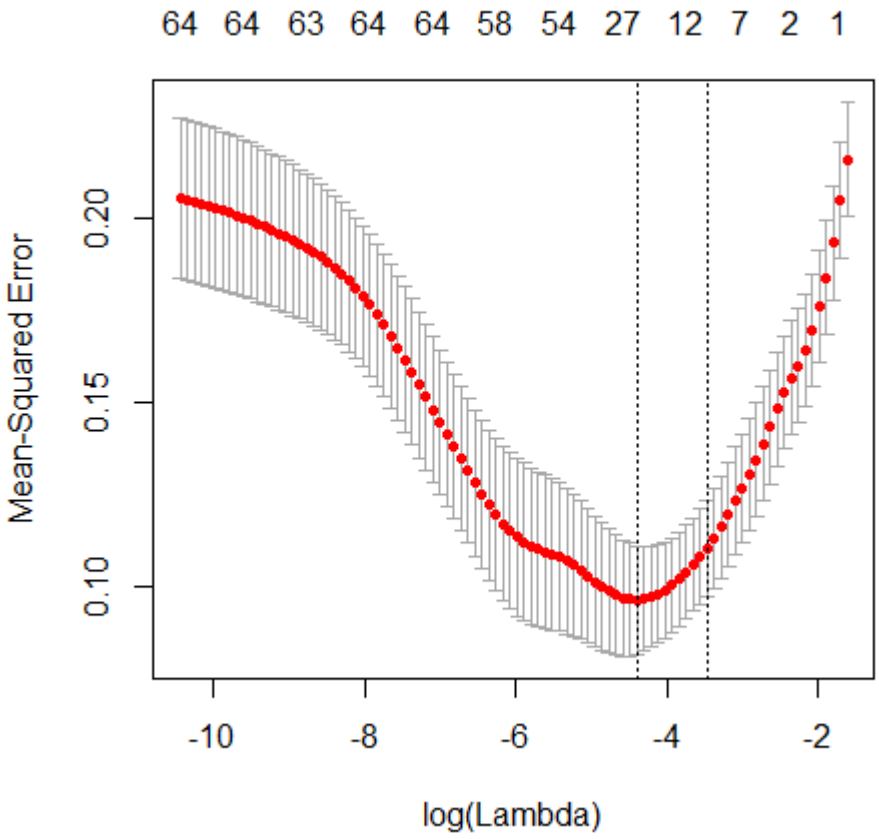


Figure 6.2 Questionnaire dataset LASSO regression modelling (λ_{min} = 0.0123) where ln(λ) dotted lined indicate λ_{min} and $\lambda_{min+1SE}$

Lambda (or λ) is the shrinkage parameter (i.e penalty coefficient) and the closer to 1 [i.e. ln(0)] this parameter is, the more conservative the model. The results of these models for both questionnaire and government (NHI) datasets is shown in **Table 3.12**.

APPENDIX VI HOUSEHOLD SNP DISTANCES IN CHAPTER 4

Table 6.4 Chapter 4 household HH008 SNP distances for Figure 4.3

	LT 1003c	LT 1003d	LT 1003e	LT 1003f	LT 1003g	LT 1003h	LT 8062a	Ref
LT 1003c	0	2	7	89519	0	0	5	8
LT 1003d	2	0	9	89519	2	2	7	10
LT 1003e	7	9	0	89520	7	7	10	13
LT 1003f	89519	89519	89520	0	89519	89519	89520	89516
LT 1003g	0	2	7	89519	0	0	5	8
LT 1003h	0	2	7	89519	0	0	5	8
LT 8062a	5	7	10	89520	5	5	0	9
Ref	8	10	13	89516	8	8	9	0

Table 6.5 Chapter 4 household HH0015 SNP distances for Figure 4.3

	LT 1030c	LT 1030d	LT 1030e	LT 1030f	LT 1030g1	LT 1030h2	LT 8052a	Ref
LT 1030c	0	16	11	18	17	14	123	16
LT 1030d	16	0	15	20	17	14	123	14
LT 1030e	11	15	0	15	16	11	122	13
LT 1030f	18	20	15	0	23	18	129	18
LT 1030g1	17	17	16	23	0	15	124	17
LT 1030h2	14	14	11	18	15	0	121	2
LT 8052a	123	123	122	129	124	121	0	123
Ref	16	14	13	18	17	2	123	0

Table 6.6 Chapter 4 household HH0015 SNP distances for Figure 4.3

ID	LT 1028a	LT 1028b	LT 1028c2	LT 1028d	LT 1028e	LT 1028f	LT 1028g	LT 1028h	LT 1029d	LT 1029e	LT 1029f	LT 1029g	LT 1029h	LT 8125a	Ref
LT 1028a	0	14	9	16	4	15	11	15	15	15	14	6	15	14	18
LT 1028b	14	0	5	4	14	3	7	3	3	3	4	10	3	4	6
LT 1028c2	9	5	0	7	9	6	2	6	6	6	7	13	6	5	9
LT 1028d	16	4	7	0	12	1	5	1	1	1	2	10	1	6	4
LT 1028e	4	14	9	12	0	11	7	11	11	11	10	6	11	14	14
LT 1028f	15	3	6	1	11	0	4	0	0	0	1	9	0	5	3
LT 1028g	11	7	2	5	7	4	0	4	4	4	5	13	4	7	7
LT 1028h	15	3	6	1	11	0	4	0	0	0	1	9	0	5	3
LT 1029d	15	3	6	1	11	0	4	0	0	0	1	9	0	5	3
LT 1029e	15	3	6	1	11	0	4	0	0	0	1	9	0	5	3
LT 1029f	14	4	7	2	10	1	5	1	1	1	0	8	1	6	4
LT 1029g	6	10	13	10	6	9	13	9	9	9	8	0	9	12	12
LT 1029h	15	3	6	1	11	0	4	0	0	0	1	9	0	5	3
LT 8125a	14	4	5	6	14	5	7	5	5	5	6	12	5	0	6
Ref	18	6	9	4	14	3	7	3	3	3	4	12	3	6	0

Table 6.7 Chapter 4 household HH0024 SNP distances for Figure 4.3

ID	LT 1033c	LT 1033d	LT 1033e	LT 1033f	LT 1033g	LT 1033h	LT 1034a	LT 1034b	LT 1034c	LT 1034d	LT 1034e	LT 1034f	LT 1034g	LT 8179a	Ref
LT 1033c	0	2	89773	0	89778	0	1	1	1	1	3	2	3	18	59
LT 1033d	2	0	89775	2	89780	2	3	3	3	3	1	4	5	16	57
LT 1033e	89773	89775	0	89773	114	89773	89774	89774	89774	89774	89776	89775	89776	89791	89829
LT 1033f	0	2	89773	0	89778	0	1	1	1	1	3	2	3	18	59
LT 1033g	89778	89780	114	89778	0	89778	89779	89779	89779	89779	89781	89780	89781	89796	89836
LT 1033h	0	2	89773	0	89778	0	1	1	1	1	3	2	3	18	59
LT 1034a	1	3	89774	1	89779	1	0	0	0	0	2	1	2	17	58
LT 1034b	1	3	89774	1	89779	1	0	0	0	0	2	1	2	17	58
LT 1034c	1	3	89774	1	89779	1	0	0	0	0	2	1	2	17	58
LT 1034d	1	3	89774	1	89779	1	0	0	0	0	2	1	2	17	58
LT 1034e	3	1	89776	3	89781	3	2	2	2	2	0	3	4	15	56
LT 1034f	2	4	89775	2	89780	2	1	1	1	1	3	0	1	18	59
LT 1034g	3	5	89776	3	89781	3	2	2	2	2	4	1	0	17	58
LT 8179a	18	16	89791	18	89796	18	17	17	17	17	15	18	17	0	41
Ref	59	57	89829	59	89836	59	58	58	58	58	56	59	58	41	0

Table 6.8 Chapter 4 household HH0026 SNP distances for Figure 4.3

	LT 1043a	LT 1043b	LT 1043c	LT 1043d	LT 1043e	LT 1043f	LT 1043g	LT 1043h	LT 1044g	LT 1044h	LT 8198a	Ref
LT 1043a	0	54	52	54	85	45	77	60	96093	96094	96092	96094
LT 1043b	54	0	42	52	87	51	71	46	96073	96074	96072	96074
LT 1043c	52	42	0	32	69	35	67	34	96093	96094	96092	96094
LT 1043d	54	52	32	0	79	41	55	48	96095	96096	96094	96096
LT 1043e	85	87	69	79	0	86	94	91	96066	96067	96065	96067
LT 1043f	45	51	35	41	86	0	76	45	96092	96093	96091	96093
LT 1043g	77	71	67	55	94	76	0	75	96054	96055	96053	96055
LT 1043h	60	46	34	48	91	45	75	0	96077	96078	96076	96078
LT 1044g	96093	96073	96093	96095	96066	96092	96054	96077	0	3	1	7
LT 1044h	96094	96074	96094	96096	96067	96093	96055	96078	3	0	2	6
LT 8198a	96092	96072	96092	96094	96065	96091	96053	96076	1	2	0	8
Ref	96094	96074	96094	96096	96067	96093	96055	96078	7	6	8	0

Table 6.9 Chapter 4 household HH0039 SNP distances for Figure 4.3

ID	LT 1089c	LT 1089d	LT 1089e	LT 1089f	LT 1090b	LT 1090c	LT 1090d	LT 1090e	LT 1090f	LT 8231a	Ref
LT 1089c	0	2	2	0	51790	10	9	11	11	9	14
LT 1089d	2	0	0	2	51792	12	11	9	9	7	12
LT 1089e	2	0	0	2	51792	12	11	9	9	7	12
LT 1089f	0	2	2	0	51790	10	9	11	11	9	14
LT 1090b	51790	51792	51792	51790	0	51794	51793	51795	51795	51793	51798
LT 1090c	10	12	12	10	51794	0	1	5	5	5	8
LT 1090d	9	11	11	9	51793	1	0	4	4	4	7
LT 1090e	11	9	9	11	51795	5	4	0	2	2	5
LT 1090f	11	9	9	11	51795	5	4	2	0	2	3
LT 8231a	9	7	7	9	51793	5	4	2	2	0	5
Ref	14	12	12	14	51798	8	7	5	3	5	0

Table 6.10 Chapter 4 household HH0040 SNP distances for Figure 4.3

	LT 1078c	LT 1078d	LT 1078e	LT 1078f	LT 1078g	LT 1078h	LT 1079a	LT 1079b	LT 1079c	LT 1079d	LT 1079e	LT 1079f	LT 1079g	LT 1079h	LT 1080c	LT 1080d	LT 1080e	LT 1080f	LT 1080g	LT 1080h	LT 1082a	LT 1082b	LT 1082c	LT 1082d	LT 1082e	LT 1082f	LT 1082g	LT 8242a	Ref
LT 1078c	0	0	1	4	0	0	4	2	4	4	4	0	4	4	1	0	1	2	2	1	0	1	0	2	1	1	1	1	4
LT 1078d	0	0	1	4	0	0	4	2	4	4	4	0	4	4	1	0	1	2	2	1	0	1	0	2	1	1	1	1	4
LT 1078e	1	1	0	5	1	1	5	3	5	5	5	1	5	5	2	1	2	3	3	2	1	2	1	3	2	2	2	2	5
LT 1078f	4	4	5	0	4	4	0	2	0	0	0	4	0	0	5	4	5	2	2	5	4	5	4	6	5	5	5	5	8
LT 1078g	0	0	1	4	0	0	4	2	4	4	4	0	4	4	1	0	1	2	2	1	0	1	0	2	1	1	1	1	4
LT 1078h	0	0	1	4	0	0	4	2	4	4	4	0	4	4	1	0	1	2	2	1	0	1	0	2	1	1	1	1	4
LT 1079a	4	4	5	0	4	4	0	2	0	0	0	4	0	0	5	4	5	2	2	5	4	5	4	6	5	5	5	5	8
LT 1079b	2	2	3	2	2	2	2	0	2	2	2	2	2	2	3	2	3	4	4	3	2	3	2	4	3	3	3	3	6
LT 1079c	4	4	5	0	4	4	0	2	0	0	0	4	0	0	5	4	5	2	2	5	4	5	4	6	5	5	5	5	8
LT 1079d	4	4	5	0	4	4	0	2	0	0	0	4	0	0	5	4	5	2	2	5	4	5	4	6	5	5	5	5	8
LT 1079e	4	4	5	0	4	4	0	2	0	0	0	4	0	0	5	4	5	2	2	5	4	5	4	6	5	5	5	5	8
LT 1079f	0	0	1	4	0	0	4	2	4	4	4	0	4	4	1	0	1	2	2	1	0	1	0	2	1	1	1	1	4
LT 1079g	4	4	5	0	4	4	0	2	0	0	0	4	0	0	5	4	5	2	2	5	4	5	4	6	5	5	5	5	8
LT 1079h	4	4	5	0	4	4	0	2	0	0	0	4	0	0	5	4	5	2	2	5	4	5	4	6	5	5	5	5	8
LT 1080c	1	1	2	5	1	1	5	3	5	5	5	1	5	5	0	1	2	3	3	0	1	2	1	3	0	2	2	2	5
LT 1080d	0	0	1	4	0	0	4	2	4	4	4	0	4	4	1	0	1	2	2	1	0	1	0	2	1	1	1	1	4
LT 1080e	1	1	2	5	1	1	5	3	5	5	5	1	5	5	2	1	0	3	3	2	1	2	1	3	2	2	2	2	5
LT 1080f	2	2	3	2	2	2	2	4	2	2	2	2	2	2	3	2	3	0	0	3	2	3	2	4	3	3	3	3	6
LT 1080g	2	2	3	2	2	2	2	4	2	2	2	2	2	2	3	2	3	0	0	3	2	3	2	4	3	3	3	3	6
LT 1080h	1	1	2	5	1	1	5	3	5	5	5	1	5	5	0	1	2	3	3	0	1	2	1	3	0	2	2	2	5
LT 1082a	0	0	1	4	0	0	4	2	4	4	4	0	4	4	1	0	1	2	2	1	0	1	0	2	1	1	1	1	4
LT 1082b	1	1	2	5	1	1	5	3	5	5	5	1	5	5	2	1	2	3	3	2	1	0	1	3	2	2	0	2	5
LT 1082c	0	0	1	4	0	0	4	2	4	4	4	0	4	4	1	0	1	2	2	1	0	1	0	2	1	1	1	1	4
LT 1082d	2	2	3	6	2	2	6	4	6	6	6	2	6	6	3	2	3	4	4	3	2	3	2	0	3	3	3	3	6
LT 1082e	1	1	2	5	1	1	5	3	5	5	5	1	5	5	0	1	2	3	3	0	1	2	1	3	0	2	2	2	5
LT 1082f	1	1	2	5	1	1	5	3	5	5	5	1	5	5	2	1	2	3	3	2	1	2	1	3	2	0	2	2	3
LT 1082g	1	1	2	5	1	1	5	3	5	5	5	1	5	5	2	1	2	3	3	2	1	0	1	3	2	2	0	2	5
LT 8242a	1	1	2	5	1	1	5	3	5	5	5	1	5	5	2	1	2	3	3	2	1	2	1	3	2	2	2	0	5
Ref	4	4	5	8	4	4	8	6	8	8	8	4	8	8	5	4	5	6	6	5	4	5	4	6	5	3	5	5	0

Table 6.11 Chapter 4 household HH0048 SNP distances for Figure 4.3

ID	LT 1097c	LT 1097d	LT 1097g	LT 1097h	LT 1099e	LT 1099f	LT 1099g	LT 1099h	LT 8320a	Ref
LT 1097c	0	2	1	0	100205	75728	2	1	2	0
LT 1097d	2	0	1	2	100205	75728	4	3	2	2
LT 1097g	1	1	0	1	100204	75727	3	2	1	1
LT 1097h	0	2	1	0	100205	75728	2	1	2	0
LT 1099e	100205	100205	100204	100205	0	89636	100205	100206	100205	100205
LT 1099f	75728	75728	75727	75728	89636	0	75730	75729	75728	75728
LT 1099g	2	4	3	2	100205	75730	0	1	4	2
LT 1099h	1	3	2	1	100206	75729	1	0	3	1
LT 8320a	2	2	1	2	100205	75728	4	3	0	2
Ref	0	2	1	0	100205	75728	2	1	2	0

Table 6.12 Chapter 4 household HH0064 SNP distances for Figure 4.3

	LT 1143c	LT 1143e	LT 1143f	LT 1143g	LT 8371a	Ref
LT 1143c	0	81	82	83	95176	95176
LT 1143e	81	0	91	76	95181	95181
LT 1143f	82	91	0	87	95196	95196
LT 1143g	83	76	87	0	95189	95189
LT 8371a	95176	95181	95196	95189	0	2
Ref	95176	95181	95196	95189	2	0

Table 6.13 Chapter 4 household HH0065 SNP distances for Figure 4.3

	LT 1131c	LT 1131d	LT 1131e	LT 1131f	LT 1131g	LT 1132b	LT 1132c	LT 1132d	LT 1132e	LT 1132f	LT 1132g	LT 1132h	LT 8455a	Ref
LT 1131c	0	25126	48	43	48286	84380	84380	84379	84378	84377	84377	84377	84377	84378
LT 1131d	25126	0	25130	25123	48015	83245	83245	83244	83243	83242	83242	83242	83242	83243
LT 1131e	48	25130	0	53	48284	84372	84372	84371	84370	84369	84369	84369	84369	84370
LT 1131f	43	25123	53	0	48293	84383	84383	84382	84381	84380	84380	84380	84380	84381
LT 1131g	48286	48015	48284	48293	0	83704	83704	83703	83702	83701	83701	83701	83701	83702
LT 1132b	84380	83245	84372	84383	83704	0	2	1	2	3	3	5	3	4
LT 1132c	84380	83245	84372	84383	83704	2	0	1	2	3	3	5	3	4
LT 1132d	84379	83244	84371	84382	83703	1	1	0	1	2	2	4	2	3
LT 1132e	84378	83243	84370	84381	83702	2	2	1	0	3	3	3	3	2
LT 1132f	84377	83242	84369	84380	83701	3	3	2	3	0	0	2	0	5
LT 1132g	84377	83242	84369	84380	83701	3	3	2	3	0	0	2	0	5
LT 1132h	84377	83242	84369	84380	83701	5	5	4	3	2	2	0	2	5
LT 8455a	84377	83242	84369	84380	83701	3	3	2	3	0	0	2	0	5
Ref	84378	83243	84370	84381	83702	4	4	3	2	5	5	5	5	0

Table 6.14 Chapter 4 household HH0086 SNP distances for Figure 4.3

	LT 1171g	LT 1173c	LT 1173d1	LT 1173e	LT 1173f	LT 1173g	LT 1173h	LT 8703a	Ref
LT 1171g	0	5	4	4	6	4	8	104	8
LT 1173c	5	0	3	3	1	3	9	101	9
LT 1173d1	4	3	0	2	4	0	6	104	6
LT 1173e	4	3	2	0	4	2	6	104	8
LT 1173f	6	1	4	4	0	4	10	100	10
LT 1173g	4	3	0	2	4	0	6	104	6
LT 1173h	8	9	6	6	10	6	0	102	12
LT 8703a	104	101	104	104	100	104	102	0	108
Ref	8	9	6	8	10	6	12	108	0

BIBLIOGRAPHY

Aarestrup FM, Hasman H, Agersø Y, Jensen LB, Harksen S, Svensmark B. First description of blaCTX-M-1-carrying *Escherichia coli* isolates in Danish primary food production. *Journal of Antimicrobial Chemotherapy* 57, 1258-9, doi:10.1093/jac/dkl109, 2006

Abraham EP, Chain E. An Enzyme from Bacteria able to Destroy Penicillin. *Nature* 146, 837, doi:10.1038/146837a0, 1940

Afema JA, Ahmed S, Besser TE, Jones LP, Sischo WM, Davis MA. Molecular epidemiology of dairy cattle-associated *Escherichia coli* carrying *bla*CTX-M genes in Washington State. *Appl Environ Microbiol*, doi:10.1128/aem.02430-17, 2018

Agersø Y, Aarestrup FM. Voluntary ban on cephalosporin use in Danish pig production has effectively reduced extended-spectrum cephalosporinase-producing *Escherichia coli* in slaughter pigs. *Journal of Antimicrobial Chemotherapy* 68, 569-72, doi:10.1093/jac/dks427, 2013

Alam MT, Read TD, Petit RA, Boyle-Vavra S, Miller LG, Eells SJ, Daum RS, David MZ. Transmission and Microevolution of USA300 MRSA in U.S. Households: Evidence from Whole-Genome Sequencing. *mBio* 6, doi:10.1128/mBio.00054-15, 2015

Alekshun MN, Levy SB. Molecular Mechanisms of Antibacterial Multidrug Resistance. *Cell* 128, 1037-50, doi:http://dx.doi.org/10.1016/j.cell.2007.03.004, 2007

Allen HK, Donato J, Wang HH, Cloud-Hansen KA, Davies J, Handelsman J. Call of the wild: antibiotic resistance genes in natural environments. *Nature Reviews Microbiology* 8, 251, doi:10.1038/nrmicro2312, 2010

Andersen PS, Stegger M, Aziz M, Contente-Cuomo T, Gibbons HS, Keim P, Sokurenko EV, Johnson JR, Price LB. Complete Genome Sequence of the Epidemic and Highly Virulent CTX-M-15-Producing H30-Rx Subclone of *Escherichia coli* ST131. *Genome Announcements* 1, doi:10.1128/genomeA.00988-13, 2013

Andersen VD, Jensen VF, Vigre H, Andreasen M, Agersø Y. The use of third and fourth generation cephalosporins affects the occurrence of extended-spectrum cephalosporinase-producing *Escherichia coli* in Danish pig herds. *The Veterinary Journal* 204, 345-50, doi:http://dx.doi.org/10.1016/j.tvjl.2015.03.014, 2015

Anderson MJ. A new method for non-parametric multivariate analysis of variance. *Austral Ecology* 26, 32-46, doi:doi:10.1111/j.1442-9993.2001.01070.pp.x, 2001

Anonymous. EUCAST guidelines for detection of resistance mechanisms and specific resistances of clinical and/or epidemiological importance. EUCAST, Basel, Switzerland, 2013a

Anonymous. Antibiotic resistance threats in the United States, 2013. https://www.cdc.gov/drugresistance/threat-report-2013/pdf/ar-threats-2013-508.pdf Centers for Disease Control and Prevention, Atlanta, Georgia, 2013b

Anonymous. Antibiotics Sales Analysis: 2009-2011 MPI Technical Paper No: 2013/62. Ministry for Primary Industries, Wellinton, New Zealand, 2013c

Anonymous. Surveillance report: Enterobacteriaceae with acquired carbapenemases, 2009-2014. https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/ACE/2014Carbap.pdf , Porirua, New Zealand, 2014a

Anonymous. *Antimicrobial resistance: global report on surveillance.* World Health Organization, 2014b

Anonymous. EMA/ECDC/EFSA/EMA first joint report on the integrated analysis of the consumption of antimicrobial agents and occurrence of antimicrobial resistance in bacteria from humans and food-producing animals. *EFSA Journal* 13, doi:10.2903/j.efsa.2015.4006, 2015

Anonymous. *Companion animals in New Zealand 2016.* Cox Inall Communications Pty Ltd, Auckland, New Zealand, 2016a

Anonymous. 2011-2014 Antibiotic Sales Analysis: MPI Technical Paper No: 2016/65. Ministry for Primary Industries, 2016b

Anonymous. Tackling drug-resistant infections globally: final report and recommendations. *Review on antimicrobial resistance.* http://amr-review.org/sites/default/files/160518_Final%20paper_with%20cover.pdf, London, U.K., 2016c

Anonymous. Enterobacterales with acquired carbapenemases 2017. https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/ACE/2017Carbap.pdf, Wellington, New Zealand, 2017

Archer KJ, Kimes RV. Empirical characterization of random forest variable importance measures. *Computational Statistics & Data Analysis* 52, 2249-60, doi:https://doi.org/10.1016/j.csda.2007.08.015, 2008

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. 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, doi:10.1016/s1473-3099(16)30319-x, 2017

Armand-Lefèvre L, Andremont A, Ruppé E. Travel and acquisition of multidrug-resistant Enterobacteriaceae. *Médecine et Maladies Infectieuses* 48, 431-41, doi:https://doi.org/10.1016/j.medmal.2018.02.005, 2018

Ashkenazy H, Penn O, Doron-Faigenboim A, Cohen O, Cannarozzi G, Zomer O, Pupko T. FastML: a web server for probabilistic reconstruction of ancestral sequences. *Nucleic Acids Research* 40, W580-W4, doi:10.1093/nar/gks498, 2012

Baker S, Duy PT, Nga TVT, Dung TTN, Phat VV, Chau TT, Turner AK, Farrar J, Boni MF. Fitness benefits in fluoroquinolone-resistant *Salmonella* Typhi in the absence of antimicrobial pressure. *eLife* 2, e01229, doi:10.7554/eLife.01229, 2013

Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. SPAdes: A New Genome Assembly Algorithm and Its Applications to Single-Cell Sequencing. *Journal of Computational Biology* 19, 455-77, doi:10.1089/cmb.2012.0021, 2012

Bar-Yoseph H, Hussein K, Braun E, Paul M. Natural history and decolonization strategies for ESBL/carbapenem-resistant Enterobacteriaceae carriage: systematic review and meta-analysis. *Journal of Antimicrobial Chemotherapy*, doi:10.1093/jac/dkw221, 2016

Barber M. Staphylococcal infection due to penicillin-resistant strains. *British Medical Journal* 2, 863-5, 1947

Barrios-Villa E, Cortés-Cortés G, Lozano-Zaraín P, Arenas-Hernández MMdlP, Martínez de la Peña CF, Martínez-Laguna Y, Torres C, Rocha-Gracia RdC.

Adherent/invasive *Escherichia coli* (AIEC) isolates from asymptomatic people: new *E. coli* ST131 O25:H4/H30-Rx virotypes. *Ann Clin Microbiol Antimicrob* 17, 42, doi:10.1186/s12941-018-0295-4, 2018

Bauer AW, Sherris JC, Turck M, Kirby WMM. Antibiotic Susceptibility Testing by a Standardized Single Disk Method. *American Journal of Clinical Pathology* 45, 493-6, doi:10.1093/ajcp/45.4_ts.493, 1966

Beaber JW, Hochhut B, Waldor MK. SOS response promotes horizontal dissemination of antibiotic resistance genes. *Nature* 427, 72-4, doi:http://www.nature.com/nature/journal/v427/n6969/suppinfo/nature02241_S1.html, 2004

Belas A, Marques C, Aboim C, Pomba C. Emergence of *Escherichia coli* ST131 H30/H30-Rx subclones in companion animals. *Journal of Antimicrobial Chemotherapy*, 381-, doi:10.1093/jac/dky381, 2018

Ben Said L, Jouini A, Alonso CA, Klibi N, Dziri R, Boudabous A, Ben Slama K, Torres C. Characteristics of extended-spectrum β-lactamase (ESBL)- and pAmpC beta-lactamase-producing Enterobacteriaceae of water samples in Tunisia. *Science of The Total Environment* 550, 1103-9, doi:https://doi.org/10.1016/j.scitotenv.2016.01.042, 2016

Ben Zakour NL, Alsheikh-Hussain AS, Ashcroft MM, Khanh Nhu NT, Roberts LW, Stanton-Cook M, Schembri MA, Beatson SA. Sequential Acquisition of Virulence and Fluoroquinolone Resistance Has Shaped the Evolution of *Escherichia coli* ST131. *mBio* 7, e00347-16, doi:10.1128/mBio.00347-16, 2016

Bidet P, Metais A, Mahjoub-Messai F, Durand L, Dehem M, Aujard Y, Bingen E, Nassif X, Bonacorsi S. Detection and Identification by PCR of a Highly Virulent Phylogenetic Subgroup among Extraintestinal Pathogenic *Escherichia coli* B2 Strains. *Appl Environ Microbiol* 73, 2373, 2007

Bolger AM, Lohse M, Usadel B. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30, 2114-20, doi:10.1093/bioinformatics/btu170, 2014

Bouckaert R, Heled J, Kühnert D, Vaughan T, Wu C-H, Xie D, Suchard MA, Rambaut A, Drummond AJ. BEAST 2: A Software Platform for Bayesian Evolutionary Analysis. *PLOS Computational Biology* 10, e1003537, doi:10.1371/journal.pcbi.1003537, 2014

Bradford PA. Extended-Spectrum β-Lactamases in the 21st Century: Characterization, Epidemiology, and Detection of This Important Resistance Threat. *Clinical Microbiology Reviews* 14, 933-51, doi:10.1128/cmr.14.4.933-951.2001, 2001

Broaders E, Gahan CGM, Marchesi JR. Mobile genetic elements of the human gastrointestinal tract. *Gut Microbes* 4, 271-80, doi:10.4161/gmic.24627, 2013

Brynildsrud O, Bohlin J, Scheffer L, Eldholm V. Rapid scoring of genes in microbial pan-genome-wide association studies with Scoary. *Genome Biology* 17, 238, doi:10.1186/s13059-016-1108-8, 2016

Burow E, Simoneit C, Tenhagen BA, Kasbohrer A. Oral antimicrobials increase antimicrobial resistance in porcine *E. coli:* a systematic review. *Preventive Veterinary Medicine* 113, 364-75, doi:10.1016/j.prevetmed.2013.12.007, 2014

Carattoli A. Resistance Plasmid Families in Enterobacteriaceae. *Antimicrob Agents Chemother* 53, 2227-38, doi:10.1128/aac.01707-08, 2009

Carattoli A, Zankari E, Garcìa-Fernandez A, Volby Larsen M, Lund O, Villa L, Aarestrup FM, Hasman H. PlasmidFinder and pMLST: in silico detection and typing of plasmids. *Antimicrob Agents Chemother*, doi:10.1128/aac.02412-14, 2014

Castro-Sánchez E, Drumright LN, Gharbi M, Farrell S, Holmes AH. Mapping Antimicrobial Stewardship in Undergraduate Medical, Dental, Pharmacy, Nursing and Veterinary Education in the United Kingdom. *PLoS One* 11, e0150056, doi:10.1371/journal.pone.0150056, 2016

Chatterjee A, Modarai M, Naylor NR, Boyd SE, Atun R, Barlow J, Holmes AH, Johnson A, Robotham JV. Quantifying drivers of antibiotic resistance in humans: a systematic review. *Lancet Infect Dis*, doi:https://doi.org/10.1016/S1473-3099(18)30296-2, 2018

Clermont O, Christenson JK, Daubié A-S, Gordon DM, Denamur E. Development of an allele-specific PCR for *Escherichia coli* B2 sub-typing, a rapid and easy to perform substitute of multilocus sequence typing. *Journal of Microbiological Methods* 101, 24-7, doi:https://doi.org/10.1016/j.mimet.2014.03.008, 2014

Clermont O, Gordon D, Denamur E. Guide to the various phylogenetic classification schemes for *Escherichia coli* and the correspondence among schemes. *Microbiology* 161, 980-8, doi:doi:10.1099/mic.0.000063, 2015

Conte D, Palmeiro JK, da Silva Nogueira K, de Lima TMR, Cardoso MA, Pontarolo R, Degaut Pontes FL, Dalla-Costa LM. Characterization of CTX-M enzymes, quinolone resistance determinants, and antimicrobial residues from hospital sewage, wastewater treatment plant, and river water. *Ecotoxicology and Environmental Safety* 136, 62-9, doi:https://doi.org/10.1016/j.ecoenv.2016.10.031, 2017

Crofts TS, Gasparrini AJ, Dantas G. Next-generation approaches to understand and combat the antibiotic resistome. *Nature Reviews Microbiology* 15, 422, doi:10.1038/nrmicro.2017.28

https://www.nature.com/articles/nrmicro.2017.28#supplementary-information, 2017

Croucher NJ, Page AJ, Connor TR, Delaney AJ, Keane JA, Bentley SD, Parkhill J, Harris SR. Rapid phylogenetic analysis of large samples of recombinant bacterial whole genome sequences using Gubbins. *Nucleic Acids Research* 43, e15-e, doi:10.1093/nar/gku1196, 2015

D'Costa VM, King CE, Kalan L, Morar M, Sung WWL, Schwarz C, Froese D, Zazula G, Calmels F, Debruyne R, Golding GB, Poinar HN, Wright GD. Antibiotic resistance is ancient. *Nature* 477, 457, doi:10.1038/nature10388, 2011

Dahlqwist E, Zetterqvist J, Pawitan Y, Sjölander A. Model-based estimation of the attributable fraction for cross-sectional, case–control and cohort studies using the R package AF. *European Journal of Epidemiology* 31, 575-82, doi:10.1007/s10654-016-0137-7, 2016

Dierikx CM, van Duijkeren E, Schoormans AHW, van Essen-Zandbergen A, veldman K, Kant A, Huijsdens XW, van der Zwaluw K, Wagenaar JA, Mevius DJ. Occurrence and characteristics of extended-spectrum- β -lactamase- and AmpC-producing clinical isolates derived from companion animals and horses. *Journal of Antimicrobial Chemotherapy* 67, 1368-74, 2012

Dohmen W, Dorado-García A, Bonten MJM, Wagenaar JA, Mevius D, Heederik DJJ. Risk factors for ESBL-producing *Escherichia coli* on pig farms: A longitudinal study in the context of reduced use of antimicrobials. *PLoS One* 12, e0174094, doi:10.1371/journal.pone.0174094, 2017

Donati V, Feltrin F, Hendriksen RS, Svendsen CA, Cordaro G, García-Fernández A, Lorenzetti S, Lorenzetti R, Battisti A, Franco A. Extended-Spectrum-Beta-

Lactamases, AmpC Beta-Lactamases and Plasmid Mediated Quinolone Resistance in *Klebsiella* spp. from Companion Animals in Italy. *PLoS One* 9, e90564, doi:10.1371/journal.pone.0090564, 2014

Dorado-García A, Smid JH, van Pelt W, Bonten MJM, Fluit AC, van den Bunt G, Wagenaar JA, Hordijk J, Dierikx CM, Veldman KT, de Koeijer A, Dohmen W, Schmitt H, Liakopoulos A, Pacholewicz E, Lam TJGM, Velthuis AG, Heuvelink A, Gonggrijp MA, van Duijkeren E, van Hoek AHAM, de Roda Husman AM, Blaak H, Havelaar AH, Mevius DJ, Heederik DJJ. Molecular relatedness of ESBL/AmpC-producing *Escherichia coli* from humans, animals, food and the environment: a pooled analysis. *Journal of Antimicrobial Chemotherapy*, dkx397-dkx, doi:10.1093/jac/dkx397, 2017

Drinkovic D, Morris AJ, Dyet K, Bakker S, Heffernan H. Plasmid-mediated AmpC beta-lactamase-producing *Escherichia coli* causing urinary tract infection in the Auckland community likely to be resistant to commonly prescribed antimicrobials. *The New Zealand medical journal* 128, 50-9, 2015

Dyet K, Woodhouse R, Heffernan H. Annual survey of extended-spectrum b-lactamase (ESBL)-producing Enterobacteriaceae, 2014. https://surv.esr.cri.nz/antimicrobial/esbl.php, Wellington, NZ, 2014

Edberg SC, Rice EW, Karlin RJ, Allen MJ. *Escherichia coli*: the best biological drinking water indicator for public health protection. *J Appl Microbiol* 88, 106S-16S, doi:doi:10.1111/j.1365-2672.2000.tb05338.x, 2000

Emberton M, Anson K. Acute urinary retention in men: an age old problem. *British Medical Journal* 318, 921-5, 1999

Eriksson I, Olofsson B, Gustafson Y, Fagerstrom L. Older women's experiences of suffering from urinary tract infections. *Journal of Clinical Nursing* 23, 1385-94, doi:10.1111/jocn.12422, 2014

Espinosa-Gongora C, Shah SQA, Jessen LR, Bortolaia V, Langebæk R, Bjørnvad CR, Guardabassi L. Quantitative assessment of faecal shedding of β-lactam-resistant *Escherichia coli* and Enterococci in dogs. *Veterinary Microbiology* 181, 298-302, doi:http://dx.doi.org/10.1016/j.vetmic.2015.10.004, 2015

EUCAST. Clinical breakpoints version 8.1. http://www.eucast.org/clinical_breakpoints/ (accessed June 2018, 2018

Ewers C, Grobbel M, Stamm I, Kopp PA, Diehl I, Semmler T, Fruth A, Beutlich J, Guerra B, Wieler LH, Guenther S. Emergence of human pandemic O25:H4-ST131 CTX-M-15 extended-spectrum-β-lactamase-producing *Escherichia coli* among companion animals. *Journal of Antimicrobial Chemotherapy* 65, doi:10.1093/jac/dkq004, 2010

Ewers C, Bethe A, Semmler T, Guenther S, Wieler LH. Extended-spectrum beta-lactamase-producing and AmpC-producing *Escherichia coli* from livestock and companion animals, and their putative impact on public health: a global perspective. *Clinical Microbiology and Infection* 18, 646-55, doi:10.1111/j.1469-0691.2012.03850.x, 2012

Ewers C, Bethe A, Stamm I, Grobbel M, Kopp PA, Guerra B, Stubbe M, Doi Y, Zong Z, Kola A, Schaufler K, Semmler T, Fruth A, Wieler LH, Guenther S. CTX-M-15-D-ST648 *Escherichia coli* from companion animals and horses: another pandemic clone combining multiresistance and extraintestinal virulence? *Journal of Antimicrobial Chemotherapy* 69, 1224-30, doi:10.1093/jac/dkt516, 2014a

Ewers C, Stamm I, Pfeifer Y, Wieler LH, Kopp PA, Schonning K, Prenger-Berninghoff E, Scheufen S, Stolle I, Gunther S, Bethe A. Clonal spread of highly successful ST15-CTX-M-15 *Klebsiella pneumoniae* in companion animals and horses. *Journal of Antimicrobial Chemotherapy* 69, 2676-80, doi:10.1093/jac/dku217, 2014b

Fernandes MR, McCulloch JA, Vianello MA, Moura Q, Pérez-Chaparro PJ, Esposito F, Sartori L, Dropa M, Matté MH, Lira DPA, Mamizuka EM, Lincopan N. First Report of the Globally Disseminated IncX4 Plasmid Carrying the mcr-1 Gene in a Colistin-Resistant *Escherichia coli* Sequence Type 101 Isolate from a Human Infection in Brazil. *Antimicrob Agents Chemother* 60, 6415-7, doi:10.1128/aac.01325-16, 2016

Fitzpatrick D, Walsh F. Antibiotic resistance genes across a wide variety of metagenomes. *FEMS Microbiology Ecology*, doi:10.1093/femsec/fiv168, 2016

Fleiss JL, Levin B, Paik MC. *Statistical methods for rates and proportions.* John Wiley & Sons, 2013

Freeman JT, McBride SJ, Heffernan H, Bathgate T, Pope C, Ellis-Pegler RB. Community-Onset Genitourinary Tract Infection due to CTX-M-15–Producing *Escherichia coli* among Travelers to the Indian Subcontinent in New Zealand. *Clinical Infectious Diseases* 47, 689-92, doi:10.1086/590941, 2008

Friedman JH, Hastie T, Tibshirani R. Regularization Paths for Generalized Linear Models via Coordinate Descent. *Journal of Statistical Software* 33, 22, doi:10.18637/jss.v033.i01, 2010

Garneau-Tsodikova S, Labby KJ. Mechanisms of resistance to aminoglycoside antibiotics: overview and perspectives. *MedChemComm* 7, 11-27, doi:10.1039/C5MD00344J, 2016

Giannella M, Trecarichi EM, De Rosa FG, Del Bono V, Bassetti M, Lewis RE, Losito AR, Corcione S, Saffioti C, Bartoletti M, Maiuro G, Cardellino CS, Tedeschi S, Cauda R, Viscoli C, Viale P, Tumbarello M. Risk factors for carbapenem-resistant *Klebsiella pneumoniae* bloodstream infection among rectal carriers: a prospective observational multicentre study. *Clinical Microbiology and Infection* 20, 1357-62, doi:doi:10.1111/1469-0691.12747, 2014

Grigoryan L, Trautner BW, Gupta K. Diagnosis and Management of Urinary Tract Infections in the Outpatient Setting A Review. *Journal of the American Medical Association* 312, 1677-84, doi:10.1001/jama.2014.12842, 2014

Gurevich A, Saveliev V, Vyahhi N, Tesler G. QUAST: quality assessment tool for genome assemblies. *Bioinformatics* 29, 1072-5, doi:10.1093/bioinformatics/btt086, 2013

Guyer DM, Henderson IR, Nataro JP, Mobley HLT. Identification of Sat, an autotransporter toxin produced by uropathogenic *Escherichia coli*. *Molecular Microbiology* 38, 53-66, doi:doi:10.1046/j.1365-2958.2000.02110.x, 2000

Hadfield J, Croucher NJ, Goater RJ, Abudahab K, Aanensen DM, Harris SR. Phandango: an interactive viewer for bacterial population genomics. *Bioinformatics* 34, 292-3, doi:10.1093/bioinformatics/btx610, 2018

Haenni M, Saras E, Métayer V, Médaille C, Madec J-Y. High Prevalence of blaCTX-M-1/IncI1/ST3 and blaCMY-2/IncI1/ST2 Plasmids in Healthy Urban Dogs in France. *Antimicrob Agents Chemother* 58, 5358-62, doi:10.1128/aac.02545-14, 2014

Halstead FD, Vanstone GL, Balakrishnan I. An evaluation of the Mast D69C AmpC Detection Disc Set for the detection of inducible and derepressed AmpC β-

lactamases. *Journal of Antimicrobial Chemotherapy* 67, 2303-4, doi:10.1093/jac/dks170, 2012

Hammerum AM, Larsen J, Andersen VD, Lester CH, Skytte TSS, Hansen F, Olsen SS, Mordhorst H, Skov RL, Aarestrup FM, Agerso Y. Characterization of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* obtained from Danish pigs, pig farmers and their families from farms with high or no consumption of third-or fourth-generation cephalosporins. *Journal of Antimicrobial Chemotherapy* 69, 2650-7, doi:10.1093/jac/dku180, 2014

Hanselman BA, Kruth SA, Rousseau J, Weese JS. Coagulase positive staphylococcal colonization of humans and their household pets. *The Canadian Veterinary Journal* 50, 954-8, 2009

Haque A, Yoshizumi A, Saga T, Ishii Y, Tateda K. ESBL-producing Enterobacteriaceae in environmental water in Dhaka, Bangladesh. *Journal of Infection and Chemotherapy* 20, 735-7, doi:http://dx.doi.org/10.1016/j.jiac.2014.07.003, 2014

Harris PNA, Ben Zakour NL, Roberts LW, Wailan AM, Zowawi HM, Tambyah PA, Lye DC, Jureen R, Lee TH, Yin M, Izharuddin E, Looke D, Runnegar N, Rogers B, Bhally H, Crowe A, Schembri MA, Beatson SA, Paterson DL, investigators MT. Whole genome analysis of cephalosporin-resistant *Escherichia coli* from bloodstream infections in Australia, New Zealand and Singapore: high prevalence of CMY-2 producers and ST131 carrying blaCTX-M-15 and blaCTX-M-27. *Journal of Antimicrobial Chemotherapy* 73, 634-42, doi:10.1093/jac/dkx466, 2018

Harris SR, Feil EJ, Holden MTG, Quail MA, Nickerson EK, Chantratita N, Gardete S, Tavares A, Day N, Lindsay JA, Edgeworth JD, de Lencastre H, Parkhill J, Peacock SJ, Bentley SD. Evolution of MRSA During Hospital Transmission and Intercontinental Spread. *Science* 327, 469, doi:10.1126/science.1182395, 2010

Hasman H, Hammerum A, Hansen F, Hendriksen R, Olesen B, Agersø Y, Zankari E, Leekitcharoenphon P, Stegger M, Kaas R, Cavaco L, Hansen D, Aarestrup F, Skov R. Detection of mcr-1 encoding plasmid-mediated colistin-resistant *Escherichia coli* isolates from human bloodstream infection and imported chicken meat. *Eurosurveillance* 20, doi:http://dx.doi.org/10.2807/1560-7917.ES.2015.20.49.30085, 2015

Hatha AAM, Neethu CS, Nikhil SM, Rahiman KMM, Krishnan KP, Saramma AV. Relatively high antibiotic resistance among heterotrophic bacteria from arctic fjord sediments than water – Evidence towards better selection pressure in the fjord sediments. *Polar Science* 9, 382-8, doi:https://doi.org/10.1016/j.polar.2015.10.002, 2015

Hatosy SM, Martiny AC. The Ocean as a Global Reservoir of Antibiotic Resistance Genes. *Appl Environ Microbiol* 81, 7593-9, doi:10.1128/aem.00736-15, 2015

Haverkate MR, Platteel TN, Fluit AC, Cohen Stuart JW, Leverstein-van Hall MA, Thijsen SFT, Scharringa J, Kloosterman RC, Bonten MJM, Bootsma MCJ. Quantifying within-household transmission of extended-spectrum beta-lactamase-producing bacteria. *Clin Microbiol Infect* 23, 46.e1–.e7, doi:10.1016/j.cmi.2016.08.021, 2017

He Z, Zhang H, Gao S, Lercher MJ, Chen W-H, Hu S. Evolview v2: an online visualization and management tool for customized and annotated phylogenetic trees. *Nucleic Acids Research* 44, W236-W41, doi:10.1093/nar/gkw370, 2016

Heffernan H, Wong TL, Lindsay J, Bowen B, Woodhouse R. MAF Technical Paper No: 2011/53. A baseline survey of antimicrobial resistance in bacteria from selected New Zealand foods, 2009-2010. MAF Technical Paper No: 2011/53. Wellington, New Zealand, 2011

Heffernan H, Dyet K, Woodhouse R, Williamson D. Antimicrobial susceptibility and molecular epidemiology of extended-spectrum beta-lactamase producing Enterobacteriaceae in New Zealand, 2013. https://surv.esr.cri.nz/antimicrobial/esbl.php, Wellington, New Zealand, 2013

Heffernan H, Dyet K, Munroe S, Creighton J, Chan S, Taylor S, Mansell C. First cases of KPC-type carbapenemase-producing bacteria in patients in New Zealand hospitals. *Journal of Global Antimicrobial Resistance* 2, 330-3, doi:http://dx.doi.org/10.1016/j.jgar.2014.07.002, 2014

Heffernan H, Woodhouse R, Draper J, Ren X. 2016 survey of extended-spectrum beta-lactamase-producing Enterobacteriaceae. https://surv.esr.cri.nz/PDF_surveillance/Antimicrobial/ESBL/ESBL_2016.pdf, Wellington, New Zealand, 2018

Heffernan HM, Woodhouse RE, Pope CE, Blackmore TK. Prevalence and types of extended-spectrum β-lactamases among urinary *Escherichia coli* and *Klebsiella spp*. in New Zealand. *Int J Antimicrob Agents* 34, 544-9, doi:http://dx.doi.org/10.1016/j.ijantimicag.2009.07.014, 2009

Hillerton JE, Irvine CR, Bryan MA, Scott D, Merchant SC. Use of antimicrobials for animals in New Zealand, and in comparison with other countries. *New Zealand Veterinary Journal* 65, 71-7, doi:10.1080/00480169.2016.1171736, 2017

Hocquet D, Muller A, Bertrand X. What happens in hospitals does not stay in hospitals: antibiotic-resistant bacteria in hospital wastewater systems. *Journal of Hospital Infection* 93, 395-402, doi:http://dx.doi.org/10.1016/j.jhin.2016.01.010, 2016

Holmes AH, Moore LSP, Sundsfjord A, Steinbakk M, Regmi S, Karkey A, Guerin PJ, Piddock LJV. Understanding the mechanisms and drivers of antimicrobial resistance. *The Lancet*, doi:http://dx.doi.org/10.1016/S0140-6736(15)00473-0, 2016

Holt KE, Wertheim H, Zadoks RN, Baker S, Whitehouse CA, Dance D, Jenney A, Connor TR, Hsu LY, Severin J, Brisse S, Cao H, Wilksch J, Gorrie C, Schultz MB, Edwards DJ, Nguyen KV, Nguyen TV, Dao TT, Mensink M, Minh VL, Nhu NT, Schultsz C, Kuntaman K, Newton PN, Moore CE, Strugnell RA, Thomson NR. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. *Proceedings of the National Academy of Sciences*, doi:10.1073/pnas.1501049112, 2015

Huijbers P, Blaak H, de Jong MCM, Graat EAM, Vandenbroucke-Grauls CMJE, De Roda Husman AM. Role of the environment in the transmission of antimicrobial resistance to humans: A review. *Environmental Science & Technology*, doi:10.1021/acs.est.5b02566, 2015

Huson DH. SplitsTree: analyzing and visualizing evolutionary data. *Bioinformatics* 14, 68-73, doi:10.1093/bioinformatics/14.1.68, 1998

Iredell J, Brown J, Tagg K. Antibiotic resistance in Enterobacteriaceae: mechanisms and clinical implications. *British Medical Journal* 352, doi:10.1136/bmj.h6420, 2016

Ito R, Mustapha MM, Tomich AD, Callaghan JD, McElheny CL, Mettus RT, Shanks RMQ, Sluis-Cremer N, Doi Y. Widespread Fosfomycin Resistance in

Gram-Negative Bacteria Attributable to the Chromosomal *fosA* Gene. *mBio* 8, e00749-17, doi:10.1128/mBio.00749-17, 2017

Jacoby GA. Mechanisms of Resistance to Quinolones. *Clinical Infectious Diseases* 41, S120-S6, doi:10.1086/428052, 2005

Jacoby GA. AmpC β-Lactamases. *Clinical Microbiology Reviews* 22, 161-82, doi:10.1128/cmr.00036-08, 2009

Jacoby GA, Strahilevitz J, Hooper DC. Plasmid-Mediated Quinolone Resistance. *Microbiology Spectrum* 2, doi:doi:10.1128/microbiolspec.PLAS-0006-2013, 2014

Jaros P, Cookson AL, Campbell DM, Besser TE, Shringi S, Mackereth GF, Lim E, Lopez L, Dufour M, Marshall JC, Baker MG, Hathaway S, Prattley DJ, French NP. A prospective case–control and molecular epidemiological study of human cases of Shiga toxin-producing *Escherichia coli* in New Zealand. *Bmc Infectious Diseases* 13, 450, doi:10.1186/1471-2334-13-450, 2013

Jia B, Raphenya AR, Alcock B, Waglechner N, Guo P, Tsang KK, Lago BA, Dave BM, Pereira S, Sharma AN, Doshi S, Courtot M, Lo R, Williams LE, Frye JG, Elsayegh T, Sardar D, Westman EL, Pawlowski AC, Johnson TA, Brinkman FSL, Wright GD, McArthur AG. CARD 2017: expansion and model-centric curation of the comprehensive antibiotic resistance database. *Nucleic Acids Research* 45, D566-D73, doi:10.1093/nar/gkw1004, 2017

Joensen KG, Scheutz F, Lund O, Hasman H, Kaas RS, Nielsen EM, Aarestrup FM. Real-Time Whole-Genome Sequencing for Routine Typing, Surveillance, and Outbreak Detection of Verotoxigenic *Escherichia coli*. *J Clin Microbiol* 52, 1501-10, doi:10.1128/jcm.03617-13, 2014

Johnsen PJ, Townsend JP, Bøhn T, Simonsen GS, Sundsfjord A, Nielsen KM. Factors affecting the reversal of antimicrobial-drug resistance. *Lancet Infect Dis* 9, 357-64, doi:https://doi.org/10.1016/S1473-3099(09)70105-7, 2009

Johnson JR, Delavari P, Kuskowski M, Stell AL. Phylogenetic Distribution of Extraintestinal Virulence-Associated Traits in *Escherichia coli*. *The Journal of Infectious Diseases* 183, 78-88, doi:10.1086/317656, 2001

Johnson JR, Miller S, Johnston B, Clabots C, DebRoy C. Sharing of *Escherichia coli* Sequence Type ST131 and Other Multidrug-Resistant and Urovirulent *E. coli* Strains among Dogs and Cats within a Household. *J Clin Microbiol* 47, 3721-5, doi:10.1128/jcm.01581-09, 2009

Johnson JR, Johnston B, Clabots C, Kuskowski MA, Castanheira M. *Escherichia coli* Sequence Type ST131 as the Major Cause of Serious Multidrug-Resistant *E. coli* Infections in the United States. *Clinical Infectious Diseases* 51, 286-94, doi:10.1086/653932, 2010

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. Household Clustering of Escherichia coli Sequence Type 131 Clinical and Fecal Isolates According to Whole Genome Sequence Analysis. *Open Forum Infectious Diseases* 3, ofw129-ofw, doi:10.1093/ofid/ofw129, 2016

Johnson JR, Porter SB, Johnston B, Thuras P, Clock S, Crupain M, Rangan U. Extraintestinal Pathogenic and Antimicrobial Resistant *Escherichia coli*, Including Sequence Type 131 (ST131), from Retail Chicken Breasts: United States, 2013. *Appl Environ Microbiol*, doi:10.1128/aem.02956-16, 2017

Kahlmeter G, Brown DFJ, Goldstein FW, MacGowan AP, Mouton JW, Odenholt I, Rodloff A, Soussy CJ, Steinbakk M, Soriano F, Stetsiouk O. European Committee on Antimicrobial Susceptibility Testing (EUCAST) Technical Notes on antimicrobial susceptibility testing. *Clinical Microbiology and Infection* 12, 501-3, doi:10.1111/j.1469-0691.2006.01454.x, 2006

Kakkanat A, Phan M-D, Lo AW, Beatson SA, Schembri MA. Novel genes associated with enhanced motility of *Escherichia coli* ST131. *PLoS One* 12, e0176290, doi:10.1371/journal.pone.0176290, 2017

Kaper JB, Nataro JP, Mobley HLT. Pathogenic *Escherichia coli*. *Nature Reviews Microbiology* 2, 123-40, doi:10.1038/nrmicro818, 2004

Karanika S, Karantanos T, Arvanitis M, Grigoras C, Mylonakis E. Fecal Colonization With Extended-spectrum Beta-lactamase–Producing Enterobacteriaceae and Risk Factors Among Healthy Individuals: A Systematic Review and Metaanalysis. *Clinical Infectious Diseases* 63, 310-8, doi:10.1093/cid/ciw283, 2016

Karkaba A, Hill K, Benschop J, Grinberg A, Pleydel lE. *Investigating strains of multidrug resistant Enterobacteriacae and Staphylococcus aereus that are causing clinical infections in companion animals in New Zealand.* VetLearn Foundation, 2013

Karkaba A, Benschop J, Hill KE, Grinberg A. Characterisation of methicillin-resistant *Staphylococcus aureus* clinical isolates from animals in New Zealand, 2012–2013, and subclinical colonisation in dogs and cats in Auckland. *New Zealand Veterinary Journal* 65, 78-83, doi:10.1080/00480169.2016.1222919, 2017a

Karkaba A, Grinberg A, Benschop J, Pleydell E. Characterisation of extended-spectrum β-lactamase and AmpC β-lactamase-producing Enterobacteriaceae isolated from companion animals in New Zealand. *New Zealand Veterinary Journal* 65, 105-12, doi:10.1080/00480169.2016.1271730, 2017b

Kawanishi M, Abo H, Ozawa M, Uchiyama M, Shirakawa T, Suzuki S, Shima A, Yamashita A, Sekizuka T, Kato K, Kuroda M, Koike R, Kijima M. Prevalence of Colistin Resistance Gene *mcr-1* and Absence of *mcr-2* in *Escherichia coli* Isolated from Healthy Food-Producing Animals in Japan. *Antimicrob Agents Chemother* 61, doi:10.1128/aac.02057-16, 2017

Kennedy K, Collignon P. Colonisation with *Escherichia coli* resistant to "critically important" antibiotics: a high risk for international travellers. *European Journal of Clinical Microbiology & Infectious Diseases* 29, 1501-6, doi:10.1007/s10096-010-1031-y, 2010

Knox J, Uhlemann A-C, Lowy FD. *Staphylococcus aureus* infections: transmission within households and the community. *Trends in Microbiology* 23, 437-44, doi:https://doi.org/10.1016/j.tim.2015.03.007, 2015

Korzeniewska E, Harnisz M. Extended-spectrum beta-lactamase (ESBL)-positive Enterobacteriaceae in municipal sewage and their emission to the environment. *Journal of Environmental Management* 128, 904-11, doi:https://doi.org/10.1016/j.jenvman.2013.06.051, 2013

Kuenzli E, Jaeger VK, Frei R, Neumayr A, DeCrom S, Haller S, Blum J, Widmer AF, Furrer H, Battegay M, Endimiani A, Hatz C. High colonization rates of extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in Swiss Travellers to South Asia- a prospective observational multicentre cohort study looking at epidemiology, microbiology and risk factors. *Bmc Infectious Diseases* 14, doi:10.1186/1471-2334-14-528, 2014

Larsson DGJ, Andremont A, Bengtsson-Palme J, Brandt KK, de Roda Husman AM, Fagerstedt P, Fick J, Flach C-F, Gaze WH, Kuroda M, Kvint K, Laxminarayan R, Manaia CM, Nielsen KM, Plant L, Ploy M-C, Segovia C, Simonet P, Smalla K, Snape J, Topp E, van Hengel AJ, Verner-Jeffreys DW, Virta MPJ, Wellington EM, Wernersson A-S. Critical knowledge gaps and research needs related to the environmental dimensions of antibiotic resistance. *Environment International* 117, 132-8, doi:https://doi.org/10.1016/j.envint.2018.04.041, 2018

Lazarus B, Paterson DL, Mollinger JL, Rogers BA. Do Human Extraintestinal *Escherichia coli* Infections Resistant to Expanded-Spectrum Cephalosporins Originate From Food-Producing Animals? A Systematic Review. *Clinical Infectious Diseases* 60, 439-52, doi:10.1093/cid/ciu785, 2015

Lemon KP, Klepac-Ceraj V, Schiffer HK, Brodie EL, Lynch SV, Kolter R. Comparative Analyses of the Bacterial Microbiota of the Human Nostril and Oropharynx. *mBio* 1, doi:10.1128/mBio.00129-10, 2010

Leonard AFC, Zhang L, Balfour AJ, Garside R, Hawkey PM, Murray AK, Ukoumunne OC, Gaze WH. Exposure to and colonisation by antibiotic-resistant E. coli in UK coastal water users: Environmental surveillance, exposure assessment, and epidemiological study (Beach Bum Survey). *Environment International* 114, 326-33, doi:https://doi.org/10.1016/j.envint.2017.11.003, 2018

Leverstein-van Hall MA, Dierikx CM, Stuart JC, Voets GM, van den Munckhof MP, van Essen-Zandbergen A, Platteel T, Fluit AC, van de Sande-Bruinsma N, Scharinga J, Bonten MJM, Mevius DJ. Dutch patients, retail chicken meat and poultry share the same ESBL genes, plasmids and strains. *Clinical Microbiology and Infection* 17, 873-80, doi:http://dx.doi.org/10.1111/j.1469-0691.2011.03497.x, 2011

Levy SB, Marshall B. Antibacterial resistance worldwide: causes, challenges and responses. *Nat Med* 10, S122-9, doi:10.1038/nm1145, 2004

Levy SE, Myers RM. Advancements in Next-Generation Sequencing. *Annual Review of Genomics and Human Genetics* 17, 95-115, doi:10.1146/annurev-genom-083115-022413, 2016

Liebana E, Carattoli A, Coque TM, Hasman H, Magiorakos AP, Mevius D, Peixe L, Poirel L, Schuepbach-Regula G, Torneke K, Torren-Edo J, Torres C, Threlfall J. Public health risks of enterobacterial isolates producing extended-spectrum beta-lactamases or AmpC beta-lactamases in food and food-producing animals: an EU perspective of epidemiology, analytical methods, risk factors, and control options. *Clinical Infectious Diseases* 56, 1030-7, doi:10.1093/cid/cis1043, 2013

Lishman H, Costelloe C, Hopkins S, Johnson AP, Hope R, Guy R, Muller-Pebody B, Holmes A, Aylin P. Exploring the relationship between primary care antibiotic prescribing for urinary tract infections, Escherichia coli bacteraemia incidence and antibiotic resistance: an ecological study. *Int J Antimicrob Agents*, doi:https://doi.org/10.1016/j.ijantimicag.2018.08.013, 2018

Liu CM, Stegger M, Aziz M, Johnson TJ, Waits K, Nordstrom L, Gauld L, Weaver B, Rolland D, Statham S, Horwinski J, Sariya S, Davis GS, Sokurenko E, Keim P, Johnson JR, Price LB. *Escherichia coli* ST131-*H*22 as a Foodborne Uropathogen. *mBio* 9, 2018

Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu L-F, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu J-H, Shen J. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. *Lancet Infect Dis* 16, 161-8, doi:https://doi.org/10.1016/S1473-3099(15)00424-7, 2016

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. CTX-M: changing the face of ESBLs in Europe. *Journal of Antimicrobial Chemotherapy* 59, 165-74, doi:10.1093/jac/dkl483, 2007

Livermore DM. Defining an extended-spectrum β -lactamase. *Clinical Microbiology and Infection* 14, 3-10, doi:10.1111/j.1469-0691.2007.01857.x, 2008

Löhr IH, Rettedal S, Natås OB, Naseer U, Øymar K, Sundsfjord A. Long-term faecal carriage in infants and intra-household transmission of CTX-M-15-producing *Klebsiella pneumoniae* following a nosocomial outbreak. *Journal of Antimicrobial Chemotherapy* 68, 1043-8, doi:10.1093/jac/dks502, 2013

MacFadden DR, Fisman DN, Hanage WP, Lipsitch M. The Relative Impact of Community and Hospital Antibiotic Use on the Selection of Extended-Spectrum Beta-lactamase-Producing *Escherichia coli*. *Clinical Infectious Diseases*, ciy978-ciy, doi:10.1093/cid/ciy978, 2018

MacNair CR, Stokes JM, Carfrae LA, Fiebig-Comyn AA, Coombes BK, Mulvey MR, Brown ED. Overcoming mcr-1 mediated colistin resistance with colistin in combination with other antibiotics. *Nature Communications* 9, 458, doi:10.1038/s41467-018-02875-z, 2018

Maeyama Y, Taniguchi Y, Hayashi W, Ohsaki Y, Osaka S, Koide S, Tamai K, Nagano Y, Arakawa Y, Nagano N. Prevalence of ESBL/AmpC genes and specific clones among the third-generation cephalosporin-resistant Enterobacteriaceae from canine and feline clinical specimens in Japan. *Veterinary Microbiology* 216, 183-9, doi:https://doi.org/10.1016/j.vetmic.2018.02.020, 2018

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. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. *Clinical Microbiology and Infection* 18, doi:10.1111/j.1469-0691.2011.03570.x, 2012

Mao B-H, Chang Y-F, Scaria J, Chang C-C, Chou L-W, Tien N, Wu J-J, Tseng C-C, Wang M-C, Chang C-C, Hsu Y-M, Teng C-H. Identification of *Escherichia coli* Genes Associated with Urinary Tract Infections. *J Clin Microbiol* 50, 449, doi:10.1128/JCM.00640-11, 2012

Maravić A, Skočibušić M, Cvjetan S, Šamanić I, Fredotović Ž, Puizina J. Prevalence and diversity of extended-spectrum- β -lactamase-producing Enterobacteriaceae from marine beach waters. *Marine Pollution Bulletin* 90, 60-7, doi:http://dx.doi.org/10.1016/j.marpolbul.2014.11.021, 2015

Marshall BM, Levy SB. Food Animals and Antimicrobials: Impacts on Human Health. *Clinical Microbiology Reviews* 24, 718-33, doi:10.1128/cmr.00002-11, 2011

Martinez JL, Baquero F. Mutation Frequencies and Antibiotic Resistance. *Antimicrob Agents Chemother* 44, 1771-7, doi:10.1128/aac.44.7.1771-1777.2000, 2000

Martirosov DM, Lodise TP. Emerging trends in epidemiology and management of infections caused by carbapenem-resistant Enterobacteriaceae. *Diagn Microbiol Infect Dis* 85, 266-75, doi:10.1016/j.diagmicrobio.2015.10.008, 2015

Mathers AJ, Peirano G, Pitout JDD. The Role of Epidemic Resistance Plasmids and International High-Risk Clones in the Spread of Multidrug-Resistant Enterobacteriaceae. *Clinical Microbiology Reviews* 28, 565-91, doi:10.1128/cmr.00116-14, 2015

Matsumura Y, Johnson JR, Yamamoto M, Nagao M, Tanaka M, Takakura S, Ichiyama S, Matsumura Y, Yamamoto M, Nagao M, Takakura S, Ichiyama S, Fujita N, Komori T, Yamada Y, Shimizu T, Hayashi A, Ono T, Watanabe H, Fujihara N, Higuchi T, Moro K, Shigeta M, Kida K, Terada H, Tsuboi F, Sugimoto Y, Fukumoto C. CTX-M-27- and CTX-M-14-producing, ciprofloxacin-resistant *Escherichia coli* of the *H*30 subclonal group within ST131 drive a Japanese regional ESBL epidemic. *Journal of Antimicrobial Chemotherapy* 70, 1639-49, doi:10.1093/jac/dkv017, 2015

McDougall S, Hussein H, Petrovski K. Antimicrobial resistance in *Staphylococcus aureus*, *Streptococcus uberis* and *Streptococcus dysgalactiae* from dairy cows with mastitis. *New Zealand Veterinary Journal* 62, 68-76, doi:10.1080/00480169.2013.843135, 2014

McDougall S, Compton CWR, Botha N. Factors influencing antimicrobial prescribing by veterinarians and usage by dairy farmers in New Zealand. *New Zealand Veterinary Journal* 65, 84-92, doi:10.1080/00480169.2016.1246214, 2017

McGann P, Snesrud E, Maybank R, Corey B, Ong AC, Clifford R, Hinkle M, Whitman T, Lesho E, Schaecher KE. *Escherichia coli* Harboring *mcr-1* and *bla*CTX-M on a Novel IncF Plasmid: First Report of mcr-1 in the United States. *Antimicrob Agents Chemother* 60, 4420-1, doi:10.1128/aac.01103-16, 2016

McMeekin CH, Hill KE, Gibson IR, Bridges JP, Benschop J. Antimicrobial resistance patterns of bacteria isolated from canine urinary samples submitted to a New Zealand veterinary diagnostic laboratory between 2005–2012. *New Zealand Veterinary Journal* 65, 99-104, doi:10.1080/00480169.2016.1259594, 2017

McMichael AJ, Woodruff RE, Hales S. Climate change and human health: present and future risks. *The Lancet* 367, 859-69, doi:https://doi.org/10.1016/S0140-6736(06)68079-3, 2006

McNulty CAM, Cookson BD, Lewis MAO. Education of healthcare professionals and the public. *Journal of Antimicrobial Chemotherapy* 67, i11-i8, doi:10.1093/jac/dks199, 2012

Messenger AM, Barnes AN, Gray GC. Reverse Zoonotic Disease Transmission (Zooanthroponosis): A Systematic Review of Seldom-Documented Human Biological Threats to Animals. *PLoS One* 9, e89055, doi:10.1371/journal.pone.0089055, 2014

Meyer E, Gastmeier P, Kola A, Schwab F. Pet animals and foreign travel are risk factors for colonisation with extended-spectrum β -lactamase-producing *Escherichia coli*. *Infection* 40, doi:10.1007/s15010-012-0324-8, 2012

Miller LG, Eells SJ, Taylor AR, David MZ, Ortiz N, Zychowski D, Kumar N, Cruz D, Boyle-Vavra S, Daum RS. *Staphylococcus aureus* colonization among household contacts of patients with skin infections: risk factors, strain discordance, and complex ecology. *Clinical Infectious Diseases* 54, 1523-35, doi:10.1093/cid/cis213, 2012

Miller ME, Hui SL, Tierney WM. Validation techniques for logistic regression models. *Statistics in Medicine* 10, 1213-26, doi:doi:10.1002/sim.4780100805, 1991

Nicolas-Chanoine M-H, Gruson C, Bialek-Davenet S, Bertrand X, Thomas-Jean F, Bert F, Moyat M, Meiller E, Marcon E, Danchin N, Noussair L, Moreau R, Leflon-Guibout V. 10-Fold increase (2006–11) in the rate of healthy subjects with extended-spectrum β-lactamase-producing *Escherichia coli* faecal carriage in a Parisian check-up centre. *Journal of Antimicrobial Chemotherapy* 68, 562-8, doi:10.1093/jac/dks429, 2013

Nicolas-Chanoine M-H, Bertrand X, Madec J-Y. *Escherichia coli* ST131, an Intriguing Clonal Group. *Clinical Microbiology Reviews* 27, 543-74, doi:10.1128/cmr.00125-13, 2014

Nüesch-Inderbinen M, Stephan R. Epidemiology of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in the Human-Livestock Environment. *Current Clinical Microbiology Reports*, 1-9, doi:10.1007/s40588-016-0027-5, 2016

Nulsen MF, Mor MB, Lawton DEB. Antibiotic resistance among indicator bacteria isolated from healthy pigs in New Zealand. *New Zealand Veterinary Journal* 56, 29-35, doi:10.1080/00480169.2008.36801, 2008

Orlek A, Stoesser N, Anjum MF, Doumith M, Ellington MJ, Peto T, Crook D, Woodford N, Walker AS, Phan H, Sheppard AE. Plasmid Classification in an Era of Whole-Genome Sequencing: Application in Studies of Antibiotic Resistance Epidemiology. *Front Microbiol* 8, doi:10.3389/fmicb.2017.00182, 2017

Page AJ, Cummins CA, Hunt M, Wong VK, Reuter S, Holden MTG, Fookes M, Falush D, Keane JA, Parkhill J. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinformatics* 31, 3691-3, doi:10.1093/bioinformatics/btv421, 2015

Parker D, Sniatynski MK, Mandrusiak D, Rubin JE. Extended-spectrum β-lactamase producing *Escherichia coli* isolated from wild birds in Saskatoon, Canada. *Letters in Applied Microbiology* 63, 11-5, doi:10.1111/lam.12589, 2016

Perron GG, Whyte L, Turnbaugh PJ, Goordial J, Hanage WP, Dantas G, Desai MM. Functional characterization of bacteria isolated from ancient arctic soil exposes diverse resistance mechanisms to modern antibiotics. *PLoS One* 10, e0069533-e, doi:10.1371/journal.pone.0069533, 2015

Petty NK, Ben Zakour NL, Stanton-Cook M, Skippington E, Totsika M, Forde BM, Phan M-D, Gomes Moriel D, Peters KM, Davies M, Rogers BA, Dougan G, Rodriguez-Baño J, Pascual A, Pitout JDD, Upton M, Paterson DL, Walsh TR, Schembri MA, Beatson SA. Global dissemination of a multidrug resistant Escherichia coli clone. *Proceedings of the National Academy of Sciences* 111, 5694-9, doi:10.1073/pnas.1322678111, 2014

Pitout JDD, Nordmann P, Laupland KB, Poirel L. Emergence of Enterobacteriaceae producing extended-spectrum β-lactamases (ESBLs) in the community. *Journal of Antimicrobial Chemotherapy* 56, 52-9, doi:10.1093/jac/dki166, 2005

Pitout JDD, Laupland KB. Extended-spectrum β-lactamase-producing Enterobacteriaceae: an emerging public-health concern. *Lancet Infect Dis* 8, 159-66, doi:http://dx.doi.org/10.1016/S1473-3099(08)70041-0, 2008

Pleydell E, Rogers L, Kwan E, French N. Low levels of antibacterial drug resistance expressed by Gram-negative bacteria isolated from poultry carcasses in New Zealand. *New Zealand Veterinary Journal* 58, 229-36, doi:10.1080/00480169.2010.69297, 2010

Pleydell EJ, Souphavanh K, Hill KE, French NP, Prattley DJ. Descriptive epidemiological study of the use of antimicrobial drugs by companion animal veterinarians in New Zealand. *New Zealand Veterinary Journal* 60, 115-22, doi:10.1080/00480169.2011.643733, 2012

Poirel L, Kämpfer P, Nordmann P. Chromosome-Encoded Ambler Class A β-Lactamase of *Kluyvera georgiana*, a Probable Progenitor of a Subgroup of CTX-M Extended-Spectrum β-Lactamases. *Antimicrob Agents Chemother* 46, 4038-40, doi:10.1128/aac.46.12.4038-4040.2002, 2002

Poirel L, Kieffer N, Brink A, Coetze J, Jayol A, Nordmann P. Genetic features of MCR-1-producing colistin-resistant *Escherichia coli* isolates, South Africa. *Antimicrob Agents Chemother*, doi:10.1128/aac.00444-16, 2016

Pomba C, Rantala M, Greko C, Baptiste KE, Catry B, van Duijkeren E, Mateus A, Moreno MA, Pyörälä S, Ružauskas M, Sanders P, Teale C, Threlfall EJ, Kunsagi Z, Torren-Edo J, Jukes H, Törneke K. Public health risk of antimicrobial resistance transfer from companion animals. *Journal of Antimicrobial Chemotherapy* 72, 957-68, doi:10.1093/jac/dkw481, 2017

Prescott JF. The resistance tsunami: antimicrobial stewardship, and the golden age of microbiology. *Veterinary Microbiology* 171, 273-8, 2014

Quinn R. Rethinking antibiotic research and development: World War II and the penicillin collaborative. *American Journal of Public Health* 103, 426-34, doi:10.2105/AJPH.2012.300693, 2013

Rambaut A. FigTree, a graphical viewer of phylogenetic trees. http://tree.bio.ed.ac.uk/software/figtree, 2007

Rambaut A, Drummond AJ, Xie D, Baele G, Suchard MA. Posterior Summarization in Bayesian Phylogenetics Using Tracer 1.7. *Systematic Biology* 67, 901-4, doi:10.1093/sysbio/syy032, 2018

Rattanaumpawan P, Nachamkin I, Bilker WB, Roy JA, Metlay JP, Zaoutis TE, Lautenbach E. Risk factors for ambulatory urinary tract infections caused by high-MIC fluoroquinolone-susceptible *Escherichia coli* in women: results from a large case–control study. *Journal of Antimicrobial Chemotherapy*, doi:10.1093/jac/dku548, 2015

Rocha-Gracia RC, Cortés-Cortés G, Lozano-Zaraín P, Bello F, Martínez Y, Torres C. Faecal *Escherichia coli* isolates from healthy dogs harbour CTX-M-15 and CMY-2 β-lactamases. *The Veterinary Journal*, doi:http://dx.doi.org/10.1016/j.tvjl.2014.12.026, 2015

Rodríguez MM, Power P, Radice M, Vay C, Famiglietti A, Galleni M, Ayala JA, Gutkind G. Chromosome-Encoded CTX-M-3 from *Kluyvera ascorbata*: a Possible Origin of Plasmid-Borne CTX-M-1-Derived Cefotaximases. *Antimicrob Agents Chemother* 48, 4895-7, doi:10.1128/aac.48.12.4895-4897.2004, 2004

Roger F, Caron A, Morand S, Pedrono M, Garine-Wichatitsky Md, Chevalier V, Tran A, Gaidet N, Figuié M, de Visscher M-N, Binot A. One Health and EcoHealth: the same wine in different bottles? *Infect Ecol Epidemiol* 6, 30978, doi:10.3402/iee.v6.30978, 2016

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. Community-Onset *Escherichia coli* Infection Resistant to Expanded-Spectrum Cephalosporins in Low-Prevalence Countries. *Antimicrob Agents Chemother* 58, 2126-34, doi:10.1128/aac.02052-13, 2014

Rogers BA, Ingram PR, Runnegar N, Pitman MC, Freeman JT, Athan E, Havers S, Sidjabat HE, Gunning E, De Almeida M, Styles K, Paterson DL. Sequence type 131 *fim*H30 and *fim*H41 subclones amongst *Escherichia coli* isolates in Australia and New Zealand. *Int J Antimicrob Agents* 45, 351-8, doi:http://dx.doi.org/10.1016/j.ijantimicag.2014.11.015, 2015

Rubin JE, Pitout JDD. Extended-spectrum β-lactamase, carbapenemase and AmpC producing Enterobacteriaceae in companion animals. *Veterinary Microbiology* 170, 10-8, doi:http://dx.doi.org/10.1016/j.vetmic.2014.01.017, 2014

San Millan A. Evolution of Plasmid-Mediated Antibiotic Resistance in the Clinical Context. *Trends in Microbiology* 26, 978-85, doi:https://doi.org/10.1016/j.tim.2018.06.007, 2018

Schaufler K, Bethe A, Lubke-Becker A, Ewers C, Kohn B, Wieler LH, Guenther S. Putative connection between zoonotic multiresistant extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* in dog feces from a veterinary campus and clinical isolates from dogs. *Infect Ecol Epidemiol* 5, 25334, doi:10.3402/iee.v5.25334, 2015

Schmidt JW, Agga GE, Bosilevac JM, Brichta-Harhay DM, Shackelford SD, Wang R, Wheeler TL, Arthur TM. Occurrence of Antimicrobial-Resistant *Escherichia coli* and *Salmonella enterica* in the Beef Cattle Production and Processing Continuum. *Appl Environ Microbiol* 81, 713-25, doi:10.1128/aem.03079-14, 2015

Schmidt VM, Pinchbeck G, McIntyre KM, Nuttall T, McEwan N, Dawson S, Williams NJ. Routine antibiotic therapy in dogs increases the detection of antimicrobial-resistant faecal *Escherichia coli*. *Journal of Antimicrobial Chemotherapy*, dky352-dky, doi:10.1093/jac/dky352, 2018

Schmiedel J, Falgenhauer L, Domann E, Bauerfeind R, Prenger-Berninghoff E, Imirzalioglu C, Chakraborty T. Multiresistant extended-spectrum beta-lactamase-producing Enterobacteriaceae from humans, companion animals and horses in central Hesse, Germany. *BMC Microbiol* 14, 187-Article No.: , doi:10.1186/1471-2180-14-187, 2014

Schmithausen RM, Schulze-Geisthoevel SV, Stemmer F, El-Jade M, Reif M, Hack S, Meilaender A, Montabauer G, Fimmers R, Parcina M, Hoerauf A, Exner M, Petersen B, Bierbaum G, Bekeredjian-Ding I. Analysis of Transmission of MRSA and ESBL-E among Pigs and Farm Personnel. *PLoS One* 10, e0138173, doi:10.1371/journal.pone.0138173, 2015

Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinformatics* 30, 2068-9, doi:10.1093/bioinformatics/btu153, 2014

Seemann T. Snippy: Fast Bacterial Variant Calling from NGS Reads. https://github.com/tseemann/snippy, 2015

Seemann T, Goncalves da Silva A, Bulach D, Schultz M, Kwong J, Howden B. Nullarbor. https://github.com/tseemann/nullarbor, Github, 2016

Segawa T, Takeuchi N, Rivera A, Yamada A, Yoshimura Y, Barcaza G, Shinbori K, Motoyama H, Kohshima S, Ushida K. Distribution of antibiotic resistance genes in glacier environments. *Environ Microbiol Rep* 5, 127-34, doi:10.1111/1758-2229.12011, 2013

Sekyere JO. Genomic insights into nitrofurantoin resistance mechanisms and epidemiology in clinical Enterobacteriaceae. *Future Science OA* 4, FSO293, doi:10.4155/fsoa-2017-0156, 2018

Shaffer M, Lozupone C. Prevalence and Source of Fecal and Oral Bacteria on Infant, Child, and Adult Hands. *mSystems* 3, doi:10.1128/mSystems.00192-17, 2018

Sheikh J, Dudley EG, Sui B, Tamboura B, Suleman A, Nataro JP. *EilA*, a *HilA*-like regulator in enteroaggregative *Escherichia coli*. *Molecular Microbiology* 61, 338-50, doi:doi:10.1111/j.1365-2958.2006.05234.x, 2006

Shin S, Castanie-Cornet M-P, Foster JW, Crawford JA, Brinkley C, Kaper JB. An activator of glutamate decarboxylase genes regulates the expression of enteropathogenic *Escherichia coli* virulence genes through control of the plasmid-encoded regulator *Per*. *Molecular Microbiology* 41, 1133-50, doi:doi:10.1046/j.1365-2958.2001.02570.x, 2001

Signal LN, Walton MD, Ni Mhurchu C, Maddison R, Bowers SG, Carter KN, Gorton D, Heta C, Lanumata TS, McKerchar CW, O'Dea D, Pearce J. Tackling 'wicked' health promotion problems: a New Zealand case study. *Health Promotion International* 28, 84-94, doi:10.1093/heapro/das006, 2013

Sköld O. Resistance to trimethoprim and sulfonamides. *Vet Res* 32, 261-73, 2001

Smith DL, Harris AD, Johnson JA, Silbergeld EK, Morris JG. Animal antibiotic use has an early but important impact on the emergence of antibiotic resistance in human commensal bacteria. *Proceedings of the National Academy of Sciences* 99, 6434-9, doi:10.1073/pnas.082188899, 2002

Smith R, Coast J. The true cost of antimicrobial resistance. *British Medical Journal* 346, doi:10.1136/bmj.f1493, 2013

Sokurenko EV, Chesnokova V, Dykhuizen DE, Ofek I, Wu X-R, Krogfelt KA, Struve C, Schembri MA, Hasty DL. Pathogenic adaptation of *Escherichia coli* by natural variation of the *FimH* adhesin. *Proceedings of the National Academy of Sciences* 95, 8922-6, doi:10.1073/pnas.95.15.8922, 1998

Sprouffske K, Aguilar-Rodríguez J, Sniegowski P, Wagner A. High mutation rates limit evolutionary adaptation in *Escherichia coli*. *PLoS genetics* 14, e1007324, doi:10.1371/journal.pgen.1007324, 2018

Stoesser N, Sheppard AE, Pankhurst L, De Maio N, Moore CE, Sebra R, Turner P, Anson LW, Kasarskis A, Batty EM, Kos V, Wilson DJ, Phetsouvanh R, Wyllie D, Sokurenko E, Manges AR, Johnson TJ, Price LB, Peto TEA, Johnson JR, Didelot X, Walker AS, Crook DW, Group MMMI. Evolutionary History of the Global Emergence of the *Escherichia coli* Epidemic Clone ST131. *mBio* 7, doi:10.1128/mBio.02162-15, 2016

Tacconelli E, Cataldo MA, Dancer SJ, De Angelis G, Falcone M, Frank U, Kahlmeter G, Pan A, Petrosillo N, Rodríguez-Baño J, Singh N, Venditti M, Yokoe DS, Cookson B. ESCMID guidelines for the management of the infection control measures to reduce transmission of multidrug-resistant Gram-negative bacteria in hospitalized patients. *Clinical Microbiology and Infection* 20, 1-55, doi:https://doi.org/10.1111/1469-0691.12427, 2014

Thaden JT, Fowler VG, Sexton DJ, Anderson DJ. Increasing Incidence of Extended-Spectrum β-Lactamase-Producing *Escherichia coli* in Community Hospitals throughout the Southeastern United States. *Infection Control & Hospital Epidemiology* 37, 49-54, doi:10.1017/ice.2015.239, 2016

Thomas MG, Smith AJ, Tilyard M. Rising antimicrobial resistance: a strong reason to reduce excessive antimicrobial consumption in New Zealand. *The New Zealand medical journal* 127, 72-84, 2014

Thomsen MCF, Ahrenfeldt J, Cisneros JLB, Jurtz V, Larsen MV, Hasman H, Aarestrup FM, Lund O. A Bacterial Analysis Platform: An Integrated System for Analysing Bacterial Whole Genome Sequencing Data for Clinical Diagnostics and Surveillance. *PLoS One* 11, e0157718, doi:10.1371/journal.pone.0157718, 2016

Tibshirani R. Regression Shrinkage and Selection via the Lasso. *Journal of the Royal Statistical Society. Series B (Methodological)* 58, 267-88, 1996

Toombs-Ruane LJ, Riley CB, Kendall AT, Bolwell CF, Benschop J, Rosanowski SM. Antimicrobial Susceptibilities of Aerobic Isolates from Respiratory Samples of Young New Zealand Horses. *journal of Veterinary Internal Medicine* 29, 1700-6, doi:10.1111/jvim.13600, 2015

Toombs-Ruane LJ, Riley CB, Kendall AT, Hill KE, Benschop J, Rosanowski SM. Antimicrobial susceptibility of bacteria isolated from neonatal foal samples submitted to a New Zealand veterinary pathology laboratory (2004 to 2013). *New Zealand Veterinary Journal* 64, 107-11, doi:10.1080/00480169.2015.1109006, 2016

Toombs-Ruane LJ, Benschop J, Burgess S, Priest P, Murdoch DR, French NP. Multidrug resistant Enterobacteriaceae in New Zealand: a current perspective. *New Zealand Veterinary Journal* 65, 62-70, doi:10.1080/00480169.2016.1269621, 2017

Torres E, López-Cerero L, Morales I, Navarro MD, Rodríguez-Baño J, Pascual A. Prevalence and transmission dynamics of *Escherichia coli* ST131 among contacts of infected community and hospitalized patients. *Clinical Microbiology and Infection* 24, 618-23, doi:https://doi.org/10.1016/j.cmi.2017.09.007, 2018

Truglio M. QCtool. https://github.com/mtruglio/QCtool/, https://github.com/mtruglio/QCtool/, 2016

Ulleryd P, Sandberg T, Scheutz F, Clabots C, Johnston BD, Thuras P, Johnson JR. Colonization with *Escherichia coli* Strains among Female Sex Partners of Men with Febrile Urinary Tract Infection. *J Clin Microbiol* 53, 1947-50, doi:10.1128/jcm.00579-15, 2015

van Balen JC, Landers T, Nutt E, Dent A, Hoet AE. Molecular epidemiological analysis to assess the influence of pet-ownership in the biodiversity of *Staphylococcus aureu*s and MRSA in dog- and non-dog-owning healthy households. *Epidemiol Infect* 145, 1135-47, doi:10.1017/S0950268816003228, 2017

van Boeckel TP, Gandra S, Ashok A, Caudron Q, Grenfell BT, Levin SA, Laxminarayan R. Global antibiotic consumption 2000 to 2010: an analysis of national pharmaceutical sales data. *Lancet Infect Dis* 14, 742-50, doi:http://dx.doi.org/10.1016/S1473-3099(14)70780-7, 2014

van Duijkeren E, Wolfhagen MJHM, Box ATA, Heck MEOC, Wannet WJB, Fluit AC. Human-to-dog transmission of methicillin-resistant *Staphylococcus aureus*. *Emerg Infect Dis* 10, doi:10.3201/eid1012.040387, 2004

van Hoek AHAM, Stalenhoef JE, van Duijkeren E, Franz E. Comparative virulotyping of extended-spectrum cephalosporin-resistant *E. coli* isolated from broilers, humans on broiler farms and in the general population and UTI patients. *Veterinary Microbiology* 194, 55-61, doi:http://dx.doi.org/10.1016/j.vetmic.2016.04.008, 2016

van Woezik AFG, Braakman-Jansen LMA, Kulyk O, Siemons L, van Gemert-Pijnen JEWC. Tackling wicked problems in infection prevention and control: a guideline for co-creation with stakeholders. *Antimicrobial resistance and infection control* 5, 20-, doi:10.1186/s13756-016-0119-2, 2016

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. Sharing More than Friendship — Nasal Colonization with Coagulase-Positive Staphylococci (CPS) and Co-Habitation Aspects of Dogs and Their Owners. *PLoS One* 7, e35197, doi:10.1371/journal.pone.0035197, 2012

Waltner-Toews D. Zoonoses, One Health and complexity: wicked problems and constructive conflict. *Philosophical transactions of the Royal Society of London. Series B, Biological sciences* 372, 20160171, doi:10.1098/rstb.2016.0171, 2017

Wang J, Gibbons JF, McGrath K, Bai L, Li F, Leonard FC, Stephan R, Fanning S. Molecular characterization of *bla*ESBL-producing *Escherichia coli* cultured from pig farms in Ireland. *Journal of Antimicrobial Chemotherapy*, doi:10.1093/jac/dkw278, 2016

Weese JS, Dick H, Willey BM, McGeer A, Kreiswirth BN, Innis B, Low DE. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in the veterinary clinic and in the household. *Veterinary Microbiology* 115, 148-55, doi:10.1016/j.vetmic.2006.01.004, 2006

Weese JS, Giguère S, Guardabassi L, Morley PS, Papich M, Ricciuto DR, Sykes JE. ACVIM Consensus Statement on Therapeutic Antimicrobial Use in Animals and Antimicrobial Resistance. *journal of Veterinary Internal Medicine* 29, 487-98, doi:10.1111/jvim.12562, 2015

West SL, Savitz DA, Koch G, Strom BL, Guess HA, Hartzema A. Recall Accuracy for Prescription Medications: Self-report Compared with Database Information. *American Journal of Epidemiology* 142, 1103-12, doi:10.1093/oxfordjournals.aje.a117563, 1995

Weterings V, Bosch T, Witteveen S, Landman F, Schouls L, Kluytmans J. Next-Generation Sequence Analysis Reveals Transfer of Methicillin Resistance to a Methicillin-Susceptible Staphylococcus aureus Strain That Subsequently Caused a Methicillin-Resistant Staphylococcus aureus Outbreak: a Descriptive Study. *J Clin Microbiol* 55, 2808-16, doi:10.1128/jcm.00459-17, 2017

Wiles TJ, Kulesus RR, Mulvey MA. Origins and virulence mechanisms of uropathogenic *Escherichia coli*. *Experimental and Molecular Pathology* 85, 11-9, doi:10.1016/j.yexmp.2008.03.007, 2008

Williamson DA, Roberts SA, Ritchie SR, Coombs GW, Fraser JD, Heffernan H. Clinical and molecular epidemiology of methicillin-resistant *Staphylococcus aureus* in New Zealand: rapid emergence of sequence type 5 (ST5)-SCCmec-IV as the dominant community-associated MRSA clone. *PLoS One* 8, e62020, doi:10.1371/journal.pone.0062020, 2013

Williamson DA, Roos R, Verrall A, Smith A, Thomas MG. Trends, demographics and disparities in outpatient antibiotic consumption in New Zealand: a national study. *Journal of Antimicrobial Chemotherapy* 71, 3593-8, doi:10.1093/jac/dkw345, 2016

Woerther P-L, Burdet C, Chachaty E, Andremont A. Trends in Human Fecal Carriage of Extended-Spectrum β-Lactamases in the Community: Toward the Globalization of CTX-M. *Clinical Microbiology Reviews* 26, 744-58, doi:10.1128/cmr.00023-13, 2013

Wood DE, Salzberg SL. Kraken: ultrafast metagenomic sequence classification using exact alignments. *Genome Biology* 15, R46, doi:10.1186/gb-2014-15-3-r46, 2014

Woodford N, Wareham DW, Guerra B, Teale C. Carbapenemase-producing Enterobacteriaceae and non-Enterobacteriaceae from animals and the environment: an emerging public health risk of our own making? *Journal of Antimicrobial Chemotherapy* 69, doi:10.1093/jac/dkt392, 2014

Worby CJ, Lipsitch M, Hanage WP. Within-Host Bacterial Diversity Hinders Accurate Reconstruction of Transmission Networks from Genomic Distance Data. *PLOS Computational Biology* 10, e1003549, doi:10.1371/journal.pcbi.1003549, 2014

Yahara K, Nakayama S-i, Shimuta K, Lee K-i, Morita M, Kawahata T, Kuroki T, Watanabe Y, Ohya H, Yasuda M, Deguchi T, Didelot X, Ohnishi M. Genomic surveillance of Neisseria gonorrhoeae to investigate the distribution and evolution of antimicrobial-resistance determinants and lineages. *Microbial Genomics* 4, -, doi:doi:10.1099/mgen.0.000205, 2018

Yuan Q-B, Guo M-T, Yang J. Fate of Antibiotic Resistant Bacteria and Genes during Wastewater Chlorination: Implication for Antibiotic Resistance Control. *PLoS One* 10, e0119403, doi:10.1371/journal.pone.0119403, 2015

Zankari E, Hasman H, Cosentino S, Vestergaard M, Rasmussen S, Lund O, Aarestrup FM, Larsen MV. Identification of acquired antimicrobial resistance genes. *Journal of Antimicrobial Chemotherapy* 67, 2640-4, doi:10.1093/jac/dks261, 2012

Zhang J, Xiong Y, Rogers L, Carter GP, French N. Genome-by-genome approach for fast bacterial genealogical relationship evaluation. *Bioinformatics*, bty195-bty, doi:10.1093/bioinformatics/bty195, 2018

Zogg AL, Zurfluh K, Schmitt S, Nüesch-Inderbinen M, Stephan R. Antimicrobial resistance, multilocus sequence types and virulence profiles of ESBL producing and non-ESBL producing uropathogenic *Escherichia coli* isolated from cats and dogs in Switzerland. *Veterinary Microbiology* 216, 79-84, doi:https://doi.org/10.1016/j.vetmic.2018.02.011, 2018